The transmission dynamics of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci in hospital wards

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Declaration

All work in this thesis is the result of original research conducted by myself, except where stated otherwise in the text and acknowledgements. All sources of information used and all individuals who provided data sets have been acknowledged. Some of chapter 2 has been previously published in a modified form (Cooper *et al.*, 1999), and builds on work originally carried out as part of a Masters thesis (Cooper, 1996). The text makes the extent of the new work clear.

No part of this thesis has been submitted for a degree at any other university.

Summary

This thesis presents a study of the transmission dynamics of nosocomial pathogens in hospital wards, with particular reference to methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). The work makes use of mathematical models, and obvservational and epidemiological studies.

Transmission dynamics of a potential pathogen are first explored using a stochastic host-vector epidemic model, where health-care workers' hands are assumed to be the vectors. Consequences of changes in patient management are presented, and stochastic effects are shown to be essential to an understanding of ward dynamics.

Observations of carer handwashing behaviour and carer-patient contact patterns are described, and the factors associated with handwashing compliance and contact rates explored using statistical models. Patient-carer mixing patterns are investigated. Refinements to the host-vector model are used to show how different aspects of observed contact patterns may both increase and decrease the spread of nosocomial pathogens. For contact patterns typical of intensive care units, the model predicts that infection rates will increase as the staff-to-patient ratio decreases, so understaffing may result in more cross-infection even if handwashing levels do not change.

The effect of antibiotic treatment on the spread of resistant strains is studied using a two-strain model. Changing patterns of antibiotic use are shown to be capable of causing large and rapid changes in ward prevalences of resistant strains.

To investigate possible fitness costs, growth kinetics of methicillin-sensitive and methicillin-resistant *S. aureus* strains are compared. No evidence for differences in growth rates is found, though there is a suggestion that MRSA stains may have longer lag periods.

Finally, a Markov chain Monte Carlo (MCMC) approach is developed to enable model parameters to be estimated from the incomplete data typical of ward-based epidemiological studies. The approach is used to estimate parameters using *S. aureus* and VRE transmission data. With the latter data, transmission rates were related to patient antibiotic use. All antibiotic combinations considered were associated with increased acquisition rates, the effect being strongest for cephalosporins.

List of abbreviations

ANOVA Analysis of variance

AP-PCR Arbitrary primed PCR

BHI Brain heart infusion

CCTV Closed circuit television

CFU Colony-forming unit

CI Confidence interval *or* Credible interval

CNS Coagulase negative staphylococci

EDTA Ethylenediamine Tetraacetic acid

HAI Hospital-acquired infection

HCW Health-care worker

ICU Intensive care unit

MCMC Markov Chain Monte Carlo

MQL Marginal quasi-likelihood

MRSA Methicillin-resistant *Staphylococcus aureus*

MSA Mannitol salt agar

MSSA Methicillin-sensitive *Staphylococcus aureus*

NIH National Institutes of Health

OD Optical density

PAS Patient administration system

PCR Polymerase chain reaction

PFGE Pulsed-field gel electrophoresis

PQL Penalized quasi-likelihood

RAPD Randomly amplified polymorphic DNA

RCT Randomized controlled trial

RFLP Restriction fragment length polymorphism

SIR Susceptible → Infected→Removed

SIS Susceptible→Infected→Susceptible

TSA Tryptone soy agar

TSB Tryptone soy broth

VRE Vancomycin-resistant enterococci

Chapter 1

Introduction

1.1 Hospital-acquired infections

It has been estimated that there are in the region of 100,000 hospital acquired infections (HAIs) in England and Wales each year, and about two million in the United States (Glynn *et al.*, 1997; CDC, 1992). At any one time about one patient in ten has an HAI (The Hospital Infection Working Group of the DoH and PHLS, 1995). These infections are not only harmful to the affected patients, but also, indirectly, to others through the financial burdens they place on health services (Casewell, 1995; Mehtar, 1995; Coello *et al.*, 1993). In 1993 the direct excess cost of a methicillin-resistant *Staphylococcus aureus* (MRSA) infection was estimated to be nearly £2,500 per patient due to increased patient stays and antibiotic usage (Mehtar, 1995). A more recent and more detailed study found that patients presenting with HAIs during their stays incurred costs almost three times greater than those who didn't, even after adjusting for possible confounding by age, sex, diagnosis, co-morbidities, and speciality (Plowman *et al.*, 1999). The absolute increase was estimated at over £3000 per case, mostly attributable to the extra overheads and nursing care resulting from the increased length of stay. Applied to the NHS throughout England, this was estimated to represent an

annual cost of just under a billion pounds annually, £930 million of which was incurred during the patients' hospital stays, the rest post-discharge. The same study, again after adjusting for potential confounders, found that patients with an HAI were seven times more likely to die in hospital than uninfected patients. The authors estimated that a 10% reduction in the incidence of HAIs throughout England would translate into the release of resources valued at £93 million and free up over a third of a million bed days.

The problem may not have increased in scale, but in recent years the urgency of the matter has risen following the detection and transmission of strains of MRSA with intermediate vancomycin resistance (Hiramatsu *et al.*, 1997b,a; Hiramatsu, 1998). As antibiotic resistance continues to spread amongst bacterial populations and the range of therapeutic options diminishes, the prevention and control of infection, rather than treatment, must become increasingly important.

1.2 Infection control and Semmelweis

Much of current hospital infection control practice and much of the best evidence of its efficacy descends from the pioneering work of Ignaz Phillip Semmelweis (Carter and Carter, 1994; Rotter, 1997). Working as an assistant in the maternity clinic in Vienna General Hospital between 1846 and 1849, Semmelweis became alarmed by maternal mortality that sometimes amounted to 20% of patients in a month, much of it attributable to what was known as "childbed" or "puerperal fever". The clinic had two sections, but one side—the side Semmelweis worked on—had mortality rates four to five times greater than the other. Confused by this discrepancy, he attempted to make the sections as similar as possible in every respect, even considering the psychological effects of the bell-ringing that accompanied the priest's visits. However, nothing seemed to make any difference until

hospital officials instigated a halving of the number of medical students in Semmelweis's section. This change *was* followed by a marked reduction in mortality, which fell from between 10 and 20% to below 5%. Not until the forensic expert Professor Kolletschka died from septicaemia after a student pricked him with a knife during an autopsy could Semmelweis understand this change. He saw that the disease from which his friend had died "was identical to that from which so many maternity patients died" and guessed that "the autopsy knife had been contaminated by cadaverous particles". The medical students in the first section routinely examined the cadavers before examining patients and must have also spread the lethal particles. The hypothesis explained many aspects of childbed fever, not least the fact that rates seemed to go up when Semmelweis was present. "Only God knows the number of patients who went prematurely to their graves because of me", he declared. "I have examined corpses to an extent equalled by few other obstetricians." Subsequently Semmelweis insisted that everyone in his section wash their hands in a chlorine solution on entry to the labour ward. The change was indeed followed by substantial reductions in mortality. When there was a further outbreak of puerperal fever in which 11 out of 12 exposed patients died, apparently as a result of everyone examining one "highly interesting" patient on the ward before visiting other patients, all patient examinations were required to be preceded by the same handwashing protocol. Similar reductions in mortality were subsequently found at other institutions following the same interventions, and laboratory experiments added further credence to the hypothesis. Unfortunately, this work preceded Koch's studies and the general acceptance of the germ theory of disease by several years, and despite an initial positive reaction and vociferous campaigning by Semmelweis, it fell into disfavour. By 1879, however, Pasteur had collected blood from victims of puerperal fever in Parisian hospital morgues, and identified Leeuwenhoek's animalcules—streptococcal

chains—in the cultures. Attending a lecture that year on puerperal fever he interrupted the obstetrician (who was dismissing the far-fetched idea that micro-organisms could be responsible): "The cause of this disease is doctors who carry a germ from a sick patient to a healthy one...I shall show you the microbe" (Friedman and Friedland, 2000).

1.2.1 Beyond Semmelweis

Since Semmelweis's time, a vast body of knowledge has been accumulated about pathogenic bacteria (the "cadaverous particles"), but there have been only limited gains in our understanding of contact transmission (Wong, 2000) and little progress in assessing the relative importance of different transmission routes and the factors influencing transmissibility. Contemporary discussions of the role of airborne transmission and of fomites in the spread of hospital pathogens are not very different from those taking place in the 1840s (Carter and Carter, 1994; McGowan Jr., 1981; Bauer *et al.*, 1990).

Indeed, in writing the revised national guidelines for the control of MRSA in hospitals, after extensively reviewing the evidence for different interventions, the authors were forced to conclude that few of their recommendations were supported by well-designed experiments or epidemiological studies (Working Party Report, 1998).

Recent systematic reviews of the literature have found the same lack of strong evidence for most infection control activities. While there *is* evidence to support the consensus view that implicates the contaminated hands of carers in the transmission of pathogenic organisms (Larson, 1988; Pratt *et al.*, 2000a; Working Party Report, 1998), specifics—such as choice of handwashing agent, handwashing technique, glove use etc—have only a limited basis in scientific evidence (Pratt *et al.*, 2000b). The importance of staff carriage of MRSA is also often debated, but another systematic review found that

crucial evidence regarding the role of staff carriage was lacking. The available literature did not enable any decisive conclusion about its importance to be reached (Nicholson *et al.*, 1997).

The most important difference between our time and that of Semmelweis is the use of antibiotics. Their introduction in the late 1940s revolutionised medical practice, and led many to think that the problem of infectious diseases had been overcome. That antibiotic resistance might ruin this dream was, however, foreseen from the beginning of the antibiotic era. As early as 1946 Alexander Fleming said:

> It is to be hoped that penicillin will not be abused as were the sulfonamides...There is probably no chemo-therapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring 'fastness' [resistance]. (cited in Mazel and Davies, 1998)

However, though the link between antibiotic use and bacterial drug-resistance might seem incontrovertible, another recent systematic review again had to conclude that there was no strong evidence relating antibiotic use to resistance levels in hospitals (Standing Medical Advisory Committee, sub-group on Antimicrobial Resistance, 1998). The authors found the literature to be dominated by retrospective studies and swamped with unsystematic reviews and editorials.

As the reviews mentioned above suggest, much of the hospital infection literature remains anecdotal in nature and consequently likely to be subject to large reporting and publication biases.

1.3 Directions of future research

1.3.1 Interventional studies

There are a number of obstacles to conducting well-designed experimental studies of the nosocomial transmission of pathogenic bacteria, and some authors have even claimed that randomized controlled trials (RCTs) are not feasible in this field (Standing Medical Advisory Committee, sub-group on Antimicrobial Resistance, 1998). In contrast to Semmelweis's interventions, contemporary studies have to compare different hand decontamination agents or increase handwashing compliance. The latter is hard to do, and the effects of interventions may be difficult to monitor. Changing handwashing agents may also change handwashing compliance (Rotter, 1997), and as the effects are likely to be smaller so sample sizes need to be larger. Interventions are made at the level of the ward or hospital rather than the individual, so cluster-randomized designs are required (Ukoumunne *et al.*, 1999). However, these obstacles can be overcome, as has been shown by a recent cluster-randomized controlled trial of the effect of handwashing interventions in the community (Carabin *et al.*, 1999). In principle, similar studies should be possible in hospital settings. Such interventional studies are in fact essential not only for assessing the control measures, but also for estimating the true socio-economic costs of HAIs. As Plowman *et al.* (1999) admit, their estimates on costs of HAIs may be biased; confounding factors other than those considered may lead to patients who are more likely to acquire infections to have longer stays or greater mortality. Their analysis, as with all other such analyses, provides no way of disentangling the increased length of stay resulting from the infection from the increased chance of an infection resulting from an increased length of stay. A controlled trial that reduced the number of infections in one group would be required to provide an unbiased estimate of the true costs.

1.3.2 Mathematical modelling

The potential value of population-level thinking in tackling the problem of nosocomial infections has recently been emphasised (Starr *et al.*, 1997; Massanari, 1997). Mathematical models are fundamental tools for such an approach, allowing the systematic exploration of the consequences of current knowledge, beliefs and assumptions regarding the transmission process. The UK Standing Medical Advisory Committee sub-group on Antimicrobial Resistance (1998) recently concluded that "Mathematical modelling in bacterial population genetics...has the potential to provide answers unobtainable in any experimental situation." . Equally important, however, may be the role of mathematical modelling in providing questions. By helping to define the problem more precisely, modelling can expose important areas where our knowledge is lacking. Also, by allowing the exploration of the interactions of a number of factors, mathematical models may help to propose interventions most likely to have the greatest impact. They should also aid in the design of the experiments required to confirm or refute the efficacy of such interventions.

1.4 An overview of the thesis

The unifying theme of this thesis is a population-level approach to hospital-acquired infections. This is undertaken through mathematical modelling, observational studies, laboratory investigations, and population-level analyses of hospital transmission data.

Populations may be studied at many different levels: populations of pathogens within a host; patients within a ward or a hospital; individuals within a community. The focus of this thesis is on the transmission dynamics of pathogens, or potential pathogens, within single hospital wards.

Chapter 2 first presents a simple model for the transmission dynamics

of a potential pathogen, such as *Staphylococcus aureus*, in a single hospital ward. Hand-borne spread is assumed, and carer behaviour explicitly modelled. The emphasis is placed on results from simulation studies with the stochastic version of the model. These are used to explore the effects of lengths of patient stays, carer handwashing behaviour, the rate of detection of colonized patients, as well as properties of the organism itself.

Chapter 3 describes an observational study in a surgical/medical ward. The aim of this was to collect data for model parameter estimation, and to assess the need for refinements to the model. Three sets of data are presented:

- Carer hand-washing behaviour
- Patient-carer contact patterns
- *S. aureus* transmission data

The factors associated with handwashes by carers before and after patient contacts and important sources of heterogeneities in patient-carer contact patterns are explored with statistical models. The *S. aureus* cross-infection data are used to measure the amount of normally undetected asymptomatic transmission between patients. Further analysis of these transmission data appears in chapter 8.

Chapter 4 then presents modifications to the basic model motivated by the results of chapter 3. In particular, the model is adapted to allow for non-homogeneous mixing between patients and carers and heterogeneities in patients themselves. The effects of carer-patient contact patterns appropriate to intensive care units (ICUs) are also considered.

Chapter 5 provides an introduction to the second half of the thesis. This is concerned with the problem of antibiotic resistant bacteria and the relation between antibiotic use and their spread. This chapter provides a brief literature review, with emphasis on the nosocomial transmission of

drug-resistant *S. aureus* strains. Where possible, published data are used to estimate parameters required in transmission models.

Chapter 6 then reviews the recent modelling literature relating drug-resistance to drug use and presents a simple two-strain model for the transmission of a potential pathogen such as *S. aureus*. Pre-existing drug-resistant and sensitive strains are assumed, and transmission and persistence of the organisms are related to antibiotic-consumption. Some results for the deterministic model are presented, though the emphasis is again on the stochastic treatment. Future modelling directions are discussed.

Chapter 7 describes a laboratory study intended as a rough assessment of an important model parameter: the cost of resistance from the perspective of the bacterium. This is assessed through a comparison of the growth kinetics of methicillin-resistant and methicillin-sensitive *S. aureus* strains.

One of the biggest problems with stochastic models such as those presented in this thesis lies in estimating parameter values from available data, particularly when the epidemic process is only partially observed and the data leave substantial uncertainty in the true underlying process. Chapter 8 shows how recent developments allow these problems to be overcome. Parameter estimates for transmission rates are obtained using simulated data, transmission data from chapter 3, and data from a large study of the nosocomial transmission of vancomycin-resistant enterococci (VRE). These latter data are used to relate the transmission rate to instantaneous patient antibiotic use.

Finally, chapter 9 summarises the most important results from previous chapters, relates these to the overall state of knowledge about the spread of HAIs, and proposes directions for future research.

Chapter 2

A basic single-ward model

2.1 Introduction

Until recently there had been little attempt to study the transmission dynamics of nosocomial infections within a quantitative framework. Little use has been made of mathematical models in comparison to their role in understanding community-based epidemics (Anderson and May, 1991; Isham and Medley, 1996). In part this may be due to the small populations affected. This means that stochastic effects can be dominant and the usual deterministic approximations are of limited utility.

Although stochastic epidemics have been the subject of much theoretical study (Bailey, 1975b), there have been surprisingly few attempts to relate this work directly to applied situations. The diversity of infection control practice and policies found in hospitals may in part reflect the lack of such a quantitative framework. The development of appropriate, validated models should aid the design of cost-effective control strategies.

What work there has been on modelling nosocomial infection has mostly occurred in the last four years. This has concentrated on the competitive interactions between antibiotic resistant and sensitive strains, and has largely ignored stochastic effects. These types of models are discussed in detail in

chapter 6.

This chapter follows on from earlier work in modelling the nosocomial transmission of a hand-borne pathogen in a hospital ward, and presents a simple mathematical for the spread of an organism such as *S. aureus*. Because the population is small a stochastic approach is adopted: the timing of events is determined by a chance process and only integer values are allowed for the number of patients and carers (Bailey, 1975b). For simplicity a single strain is considered in this chapter.

In the earlier work the mathematical properties of the model were explored, and particular attention was given to the quasi-endemic solution (Cooper, 1996). Here, the implications for the management of nosocomial infections are studied using essentially the same model (there are only minor differences). The effects of changing parameter values relating to both the management of patients and the properties of the pathogen are investigated. This work has been reported in Cooper *et al.* (1999).

2.2 Methods

2.2.1 Model Perspective

There is a large body of evidence to suggest that a high proportion of hospital infections result from the transfer of pathogens on the hands of health-care workers (HCWs) (Larson, 1988; Casewell and Phillips, 1977; Knittle *et al.*, 1975; Cookson *et al.*, 1989; Lingnau and Allerberger, 1994; Wolinsky *et al.*, 1960). Here, a single strain of a hand-borne pathogen, such as *S. aureus*, for which airborne and other transmission routes can be neglected is considered. The model considers two populations: patients and HCWs occupying a single ward. Patients may be admitted and discharged, but bed occupancy is assumed to be 100%. The population of HCWs is also assumed to be constant. Carers are uniquely classified as being either transiently con-

taminated with the pathogen, or entirely free of contamination. Similarly, patients are considered to be either colonized or uncolonized. Patients who are asymptomatic carriers of a potential pathogen and those who have overt infection are grouped into the same compartment; no distinction is made between them. Both are assumed to transmit the organism to HCWs with equal probability on contact.

2.2.2 Model Assumptions

Given the perspective described above, the assumptions which lead to the model are as follows:

1. All transmission from one patient to another is caused by a contact from a HCW transiently colonized with the pathogen. It is assumed that all such contacts between transiently colonized HCWs and uncolonized patients have a given probability, p, of colonizing the patient.

   The possibility of direct patient-to-patient contact is ignored, though in certain special cases—such as paediatric wards—it may be an important transmission route (Riley and Rouse, 1995). However, data collected as part of the study described in chapter 3 suggests that it is unlikely to be so in more typical wards.

   Airborne transmission and transmission from contact with inanimate surfaces are also neglected. For *S. aureus*, airborne transmission appears to be of minor significance (Bauer *et al.*, 1990; Mortimer *et al.*, 1966). Observed patterns of recovery of airborne staphylococci and transmission of strains cannot easily be reconciled with a major role for airborne spread (Lidwell *et al.*, 1970, 1971).

   The importance of environmental contamination for transmission of *S. aureus* remains unclear (Boyce *et al.*, 1997), but for faecal pathogens such as vancomycin-resistant enterococci (VRE) and *Clostridium dif-*

ficile it appears more likely to be a significant reservoir (Weber and Rutala, 1997; Samore *et al.*, 1996). These transmission routes may also become more important in places where there are more susceptible patients such as burns units and ICUs.

2. HCWs acquire transient hand-contamination with the pathogen by touching colonized patients. It is assumed that all such contacts between uncolonized HCWs and colonized patients have a given probability of colonizing the carer, p'.

   Both direct carer-to-carer transmission and the possibility of HCWs becoming colonized from sources outside the ward under consideration are ignored. For staff whose work involves moving between wards the latter may be important, but here only the *within-ward* spread of nosocomial pathogens is under consideration.

3. In the absence of reliable data on the carer-to-patient transmissibility it is assumed to be equal to the patient-to-carer transmissibility. For *S. aureus* survival time on transiently colonized hands of HCWs is unlikely to be a limiting factor in its spread if handwashing is practised at levels typically found in observational studies. Transmissibility can therefore be considered to be largely a function of the rate of shedding, and the number of organisms needed to establish colonization. Host factors are likely to account for much of the variation in these values. For example, there is evidence that patients with heavily infected wounds might be expected to shed more bacteria (Burke and Corrigan, 1961; Selwyn, 1965). This would correspond to an increase in patient-to-carer transmissibility. Similarly, patients with wounds, eczema or taking antibiotics may require far fewer organisms to establish colonization, resulting in an increase in carer-to-patient transmissibility (Foster and Hutt, 1960). Different transmissibilities may therefore re-

flect different wards as much as different strains.

In principle, the probability of transmission from a colonized patient to a HCW can readily be measured by sampling carer hand contamination following patient contacts. Either a hand-wash sampling or a contact-plate technique can be used, though the method chosen will influence the relative values of the carer-to-patient and patient-to-carer transmissibilities obtained (Ayliffe *et al.*, 1979). Since the former is more sensitive it should lead to a higher value for patient-to-carer transmissibility and a hence a lower value for carer-to-patient transmissibility. Handwash sampling typically shows roughly twice the frequency of contamination with *S. aureus* as contact-plate sampling (Ayliffe *et al.*, 1975).

Casewell and Phillips (1977), using a gloved-hand technique, found that in 17 out of 47 contacts with seven colonized patients *Klebsiellae* spp were transferred to nurses hands, giving an overall patient-to-carer transmissibility of 0.36, or 0.22 per-strain if colonization with multiple types is accounted for and contacts assumed to be evenly distributed amongst the patients.

In two other studies HCW hand contamination could be ascribed to specific patient-carer contacts. However, in the first a transmission probability for single organisms could not be calculated due to a lack of typing of the recovered organisms (Sanderson and Weissler, 1992), and in the second, samples were only taken following bed-making and wound dressing in a burns unit (Ojajärvi, 1980).

In other studies where carer hand contamination has been measured it is not possible to associate the acquisition of the organism on a carer's hands with a single patient contact (Ayliffe *et al.*, 1979, 1975; Bauer *et al.*, 1990). To be used for assessing transmissibilities additional as-

sumptions have to be made about contact rates, handwashing rates, prevalence of patient colonization, and the frequency of hand contamination with a carer's own strains. In studies where HCWs' hands are sampled at random the frequency of recovery of *S. aureus* varies between about 10 and 30%, though values as high as 60% and 70% have been recorded in a burns unit and a hospital for skin diseases (Ayliffe *et al.*, 1975).

In contrast to the patient-to-carer transmissibility, estimation of the carer-to-patient transmissibility presents many problems. Studies which attempt to directly measure the chance of patient colonization when inoculated with bacteria cannot easily be related to activities on a real ward (Foster and Hutt, 1960; Shinefield *et al.*, 1963).

It is, however, possible to make an order of magnitude estimate. Studies from the 1960's and 1970's suggest that new strains of *S. aureus* are acquired by hospital patients at a rate of about 5 per 100 patient weeks (Parker *et al.*, 1965; Shooter *et al.*, 1963; Lidwell *et al.*, 1970, 1971). It is assumed that all these arise from contact with a transiently colonized carer, and that about one in ten HCWs have *S. aureus* on their hands at any one time (Ayliffe *et al.*, 1979, 1975). If each patient requires on average five carer contacts per day, and carers decontaminate their hands after 40% of such contacts (Larson and Kretzer, 1995) then a carer-to-patient transmission probability of about 0.01 can be derived. This can be considered to be the mean for all *S. aureus* strains. Acquisition rates for resistant *S. aureus* strains are typically greater than for sensitive strains when the number of sources is accounted for. Lidwell and co-workers found that the acquisition rate of tetracycline resistant strains per source was about ten times greater than that for sensitive strains, and five times greater than that for strains resistant only to penicillin (Lidwell *et al.*, 1966).

What data there are therefore suggest the carer-to-patient transmissibility is likely to be smaller than patient-to-carer transmissibility. However, the former may be expected to vary more between wards than the latter due to different patient susceptibilities. None of the above estimates were derived from wards where patients are particularly susceptible to colonization (burns units, ICUs and neonatal units etc.), and all the relevant studies were carried out in the 1960's and 1970's when risk factors associated with infection (such as use of invasive devices) were probably lower than they are today (Casewell, 1995). Given these uncertainties, for simplicity it was assumed that the patient-to-carer and carer-to-patient transmissibilities were the same in all the simulations.

4. HCWs do not become long-term carriers of the pathogen.

   During investigations of MRSA outbreaks, long-term HCW colonization has repeatedly been found to be rare (Lingnau and Allerberger, 1994; Cox *et al.*, 1995; Thompson *et al.*, 1982), suggesting that it accounts for only a small percentage of patient acquisitions. Lidwell *et al.* (1966) estimated that the rate at which patients acquired tetracycline-resistant *S. aureus* strains from a colonized staff member was only about a fifth of the acquisition rate from a colonized patient. Other researchers have also found that most organisms acquired by patients come from other patients rather than staff (Barber *et al.*, 1960; Witte *et al.*, 1994). The main impact of staff carriage may be to occasionally seed new outbreaks, thus enabling persistence of the organism for longer than would be possible if patients were the only reservoir (Lessing *et al.*, 1996). In special cases, and for pathogens with higher rates of carriage amongst staff (such as the tetracycline-resistant *S. aureus* strains of the 1960s and 1970s), long-term carer colonization may however account for a substantial number of patient

acquisitions (Coovadia *et al.*, 1989; Parker *et al.*, 1965; Lidwell *et al.*, 1970, 1971) and Casewell (1986) considered both patients and staff to be significant sources during MRSA outbreaks. Also, if MRSA has been endemic in a setting for a long time higher levels of staff colonization may be likely. Staff colonization lasting for an intermediate length of time, arising from close-contact activities such as changing dressings of heavily contaminated wounds, may also be important in the case of *S. aureus* (Cookson *et al.*, 1989).

5. The population of patients is considered to be homogeneous.

   Each patient is considered to be equally likely to contact a member of staff in any time interval, equally likely to become colonized, and, if colonized, equally likely to transmit the pathogen to a HCW on contact.

   This restriction is an obvious candidate for exploration in a further refinement of the model. For example, perhaps one quarter of the population appears to be unable to become nasal carriers of *S. aureus* (Williams, 1963), and patients with invasive devices may be both more likely to receive contacts, and more susceptible to infection. A recent study found that such patients were more than seven times more likely to become infected (Glynn *et al.*, 1997). Chapter 4 extends the current framework to situations such as these.

6. The population of HCWs is considered to be homogeneous.

   Variation between HCWs due, for example, to differences in behaviour such as handwashing(Gould, 1994) and in the microbial skin flora (Reybrouck, 1983) are neglected. Though again, chapter 4 presents a framework for addressing this sort of question.

7. The detection of colonized patients is assumed to be a random process, and the mean time to detection to depend only on a constant level of

surveillance activity.

In practice, surveillance activities might increase during an outbreak, and the mean time to detection decrease. There are also likely to be higher detection rates for patients with clinical infections than for asymptomatic carriers. Since the infection-to-colonization ratio may differ in different settings the detection rate may also be expected to vary substantially according to setting (Lidwell *et al.*, 1966; Cox *et al.*, 1995).

8. Once detected, colonized patients are assumed to be removed from the ward and so are no longer a source of infection. Colonized patients who are not detected are removed from the ward at the same rate as uncolonized patients.

9. Each time a HCW washes his or her hands any contamination present is removed.

   In fact real handwashes may vary in their effectiveness at removing potential pathogens, depending on both technique and the disinfectant used (Kjølen and Andersen, 1992). However handwashing, even with less than optimal agents, may be expected to lead to very large reductions in the transient flora. Studies such as those by Bonilla *et al.* (1997), where high levels of hand contamination are observed before handwashing, and only very low levels after, appear to confirm this.

2.2.3 Model Structure

At any point in time the model's state is determined by the number of colonized patients in the ward and the number of contaminated HCWs. The sizes of the carer and patient populations are assumed constant so these also define the number of uncolonized patients and uncontaminated HCWs.

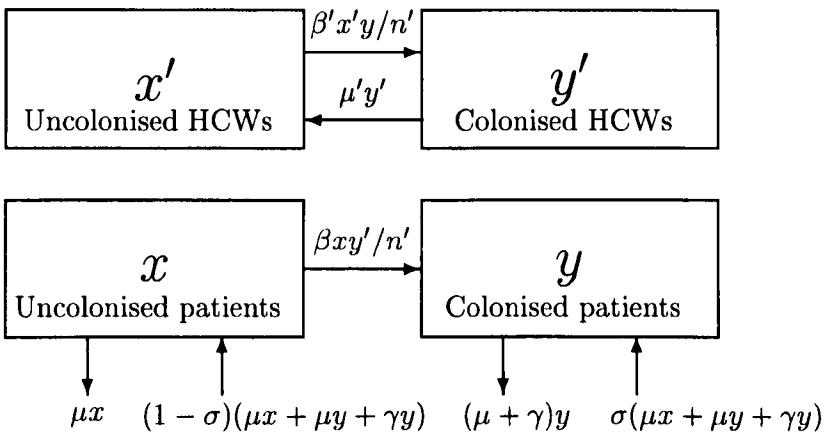

Figure 2.1: Flow diagram of the model illustrating the rates of transitions between the four compartments. See table 2.1 for an explanation of the parameters, and table 2.2 for details of the events represented by the arrows.

Table 2.1 lists the parameters used in the model and their default values, chosen to be realistic, where relevant data existed. For some of the results, the parameter values are varied. Table 2.2 shows the possible events and their rates.

At the start of all the simulations all patients are uncolonized and all HCWs are uncontaminated, so an epidemic can only occur once an infected/colonized patient is admitted.

Mathematical details of the model are given below. The model is illustrated diagrammatically in figure 2.1.

The model is constructed as follows:

- Let x be the number of susceptible patients, and y the number of colonized patients (i.e. those who either have infections or are asymptomatic carriers). Let the total number of patients in the ward be a constant n, so $x + y = n$.

- Similarly, let the total number of carers be n', x' of whom are not car-

rying the pathogen, and y' of whom are temporarily colonized, where $x' + y' = n'$.

- The removal rate for patients not known to be carrying the pathogen is μ. This includes removals due to death, transfers to other wards, and discharges.

- The detection rate of patients colonized with the pathogen is γ. Since colonized patients are assumed to be removed from the ward, this represents the additional removal rate for colonized patients.

- The rate at which temporarily colonized carers are cleared is μ'. This is effectively the handwashing rate of carers.

- It is assumed that each patient requires a contact from a carer in a given time interval with a given probability, and that all these requirements are met and no superfluous contacts are made. Let c be the mean number of contacts required by a patient each day, p the probability that a patient becomes colonized on contact with a colonized HCW, and p' the probability that a HCW becomes colonized on contact with a colonized patient. Taking $\beta = cp$ and $\beta' = cp'$, the rates at which contacts which can potentially result in colonization are made are βx (for patient colonization) and $\beta' y$ (for staff colonization). Since a fraction y'/n' of the former contacts will be with colonized carers, and x'/n' of the latter with susceptible carers, the rates for patient and carer colonization will be $\beta x y'/n'$ and $\beta' x' y/n'$ respectively.

- The proportion of patients newly admitted to the ward who are carrying the pathogen is σ $(0 \leq \sigma \leq 1)$.

Given these rates, the differential equations for the deterministic version of the model can be written:

$$\frac{dy}{dt} = \sigma(\mu x + \mu y + \gamma y) + \beta x \frac{y'}{n'} - (\mu + \gamma)y \tag{2.1}$$

$$\frac{dy'}{dt} = \beta' y \frac{x'}{n'} - \mu' y' \tag{2.2}$$

The basic model is a well-studied host-vector model, and is very similar to the most simple models of the transmission of malaria. In this case, instead of mosquitoes biting humans, the vectors are hospital staff touching patients. The mathematical properties of the deterministic version of this model are well understood and have been extensively studied (Bailey, 1975a; Aron and May, 1982), while the stochastic version continues to be the subject of research (Nåsell, 1991)

For this system of equations, the basic reproduction number, R_0—the mean number of secondary cases arising from one primary case in a completely susceptible population (Anderson and May, 1991)—is given by

$$R_0 = \frac{(n-1)\beta\beta'}{(\mu+\gamma)(n'\mu' + \beta')}. \tag{2.3}$$

In the deterministic version of the model, the introduction of one colonized patient will lead to an outbreak if and only if $R_0 \geq 1$.

2.2.4 Stochastic model

In the stochastic model the numbers of susceptible and colonized patients and susceptible and colonized staff are now represented by discrete random variables $X(t)$, $Y(t)$, $X'(t)$ and $Y'(t)$ respectively. Again, $X(t) + Y(t) = n$ and $X'(t) + Y'(t) = n'$. What were rates in the deterministic model must be interpreted probabilistically in the stochastic model. Thus, the probability of a colonized carer being cleared of the pathogen (i.e. the probability that one such carer washes his or her hands) in a short time interval Δt is given by $\mu' \Delta t$.

The transition probabilities can be written as:

$$Pr\{[Y(t+\Delta t), Y'(t+\Delta t)] = [i+1, j] \mid [Y(t), Y'(t)] = [i, j]\} = \beta \frac{(n-i)}{n'} j\Delta t + \sigma(\mu n + \gamma i)\Delta t + o(\Delta t)$$

$$Pr\{[Y(t+\Delta t), Y'(t+\Delta t)] = [i, j+1] \mid [Y(t), Y'(t)] = [i, j]\} = \beta' i \frac{(n'-j)}{n'} \Delta t + o(\Delta t)$$

$$Pr\{Y(t+\Delta t) = i-1 \mid Y(t) = i\} = (\mu+\gamma)(1-\sigma) i\Delta t + o(\Delta t)$$

$$Pr\{Y'(t+\Delta t) = j-1 \mid Y'(t) = j\} = \mu' j\Delta t + o(\Delta t)$$

All other transitions have probability $o(\Delta t)$.

Note that in the stochastic model even if $R_0 < 1$ there will always be some probability of an outbreak following the introduction of a colonized patient. Conversely, if $R_0 > 1$, the introduction of a colonized patient may result in no secondary cases at all. Consequently the interpretation of R_0 is not as clear as it was in the deterministic model.

Because colonization and decolonization in carers occur on a much shorter timescale than they do amongst patients a good approximation to the process can be made by assuming that the probability of there being a given number of colonized HCWs on the ward at one time depends only on the number of colonized patients at that time. For this approximating process an analogous and closely related value, R_0', can be defined as

$$R_0' = \frac{\beta(n-1)\overline{y'}_1}{n'(\mu+\gamma)} \tag{2.4}$$

where $\overline{y'}_1$ is the mean number of colonized HCWs given that there is one colonized patient. Then it can be shown that the mean number of colonized patients, $\overline{Y}(t)$ increases with time if and only if $R_0' > 1$ (Cooper, 1996).

A Monte Carlo simulation program was written in Turbo Pascal (Borland, 1990) using standard methods (Renshaw, 1991) and run on IBM compatible personal computers (486 and 586 processors) to determine the behaviour of the stochastic model. The random number generator used here

was the ran3 function taken from Press *et al.* (1989). The program listing is available from the author, and is published in an earlier form in Cooper (1996).

2.2.5 Handwashing rate—handwashing frequency relationship

The handwashing frequency of scenario 4 is defined to be the probability than a HCW washes his or her hands before a patient contact. This is derived from the handwashing rate, μ', using the formula:

$$\frac{\mu'}{(\mu' + c\frac{n}{n'})} \tag{2.5}$$

(See, for example, Cox and Miller (1965) for a derivation). Since, in practice, handwashes do not generally occur at random, but are usually associated with patient contacts, this is a more meaningful parameter, and corresponds more directly with the observed hand decontamination frequency.

2.2.6 Model Scenarios

The behaviour of the stochastic version of the basic model was examined by running simulations for different parameter values, where each run simulated transmission on the ward for a period of 365 days. To examine the effects of different management policies on transmission, parameter values were chosen and varied in turn from their default values (table 2.1) within five different scenarios. For each of these, three abstracted results are presented, each calculated as the mean of the 1000 simulation runs:

- The *successful introduction rate* on a ward was defined to be the number of "outbreaks" per year. An outbreak was defined to be an event when a secondary case of patient colonization arose due to cross-infection following the introduction of a colonized patient to a ward in

Parameter	Meaning	Default value
n	Number of patients	20
n'	Number of health care workers (HCWs)	3
μ	Patient removal rate	0.10 /day
μ'	Handwashing rate	14.0 /day
γ	Detection rate of colonized patients	0.10 /day
σ	Proportion of admissions already colonized	0.01
c	Patient-carer contact rate	5/day
p	Carer-patient transmission probability	0.1
p'	Patient-carer transmission probability	0.1
β	Carer-patient transmission rate ($\beta = cp$)	0.5
β'	Patient-carer transmission rate ($\beta' = cp'$)	0.5

Table 2.1: Parameters and their default values. The default values for the parameters n, n', μ, μ' and c are taken to approximate observed values in a general medical ward (Cooper, 1996). The low value for n' is a weighted average across all nursing shifts. The numerous peripatetic HCWs are likely to account for much of the inter-ward transmission, but relatively little intra-ward transmission, and are therefore not included in this estimate of n'. It is far harder to obtain values for p, and p'. The choice of these parameter values is discussed in the text.

Event	Rate of event
Patient removal (when no colonization detected)	$\mu(x+y)$
Detection of colonized patient and removal	γy
HCW handwash	$\mu'(x'+y')$
Removal of contamination from hands of HCW	$\mu' y'$
Carer-patient contact	$c(x+y)$
Carer-patient transmission	$\beta x y'/n'$
Patient-carer transmission	$\beta' y x'/n'$
Admission of uncolonized patient	$(1-\sigma)(\mu x + \mu y + \gamma y)$
Admission of colonized patient	$\sigma(\mu x + \mu y + \gamma y)$

Table 2.2: Events and their rates. Here x represents the number of uncolonized patients and y the number of colonized patients. y' represent the number of HCWs who are carrying the pathogen on their hands, and x' represents the number of HCWs free of hand contamination.

which no patients or HCWs were colonized. This measure is intended to reflect surveillance data.

- The *ward-level prevalence* was calculated as the percentage of days per year on which at least one colonized patient was present on the ward at the beginning of the day.

- Yearly *colonized patient-days* were calculated as a percentage of the total patient-days per year. This measure is most closely related to measures of morbidity and economic cost of infections.

The scenarios are defined as follows.

- Scenario 1: Transmissibility

  In order to assess the impact of changing transmissibility results are given for different values for the patient-to-carer and carer-to-patient transmissibilities. In line with the above discussion, these parameters are varied between 0 and 0.3. This corresponds to changing R_0 from 0 to 5.09, with $R_0 = 1$ when these parameters are 0.133.

  For the remaining scenarios, results are presented for three different transmissibilities. Patient-to-carer and carer-to-patient transmissibilities are taken as 0.07, 0.10 and 0.13. For these parameters, R_0 takes the values 0.28, 0.57, and 0.96 respectively, assuming other parameters take their default values.

- Scenario 2: Probability of Colonization at Admission

  The probability of a patient being colonized on entry to the ward was varied between 0% and 10%. This is meant to reflect the different prevalences of infection and carriage in the community. In terms of management, the introduction of effective screening procedures for patients entering wards (and consequent isolation and control of transmission) would reduce this parameter.

- Scenario 3: Duration of Patient Stay

  The mean lengths of patient stays in the ward were varied between 4 and 20 days. Because of the assumption that bed occupancy is 100%, a shorter duration implies a greater turnover of patients on the ward, each discharge being immediately replaced by an admission. Consequently, increasing patient turnover is likely to increase the likelihood of colonized patients being admitted, but decrease the probability that they will transmit infection during their stay.

- Scenario 4: Handwashing Frequency

  By varying the rate at which HCWs are assumed to wash their hands, the frequency with which decontamination occurs prior to patient contact was varied between 0 and 100%. For a value of 100% there should be no transmission, and transmission should be maximal for 0% handwashing.

- Scenario 5: Infection Detection

  To investigate the effect of different levels of surveillance activities in the ward, the mean time taken for a colonized patient to be detected was varied upwards from three days. Because of the time required for routine microbiological procedures it is unlikely that a shorter time-to-detection could be feasible unless PCR-based methods were used. The greater the expected time to detection, the greater the chance that a colonized patient will be discharged before colonization is detected.

2.2.7 Results

Figure 2.2 shows three sample runs selected from 100 simulation runs with the default parameter set. The runs were selected to illustrate the extremes of behaviour. All three were made using the same parameter values in a ward initially free of colonized patients and carers. The different outcomes

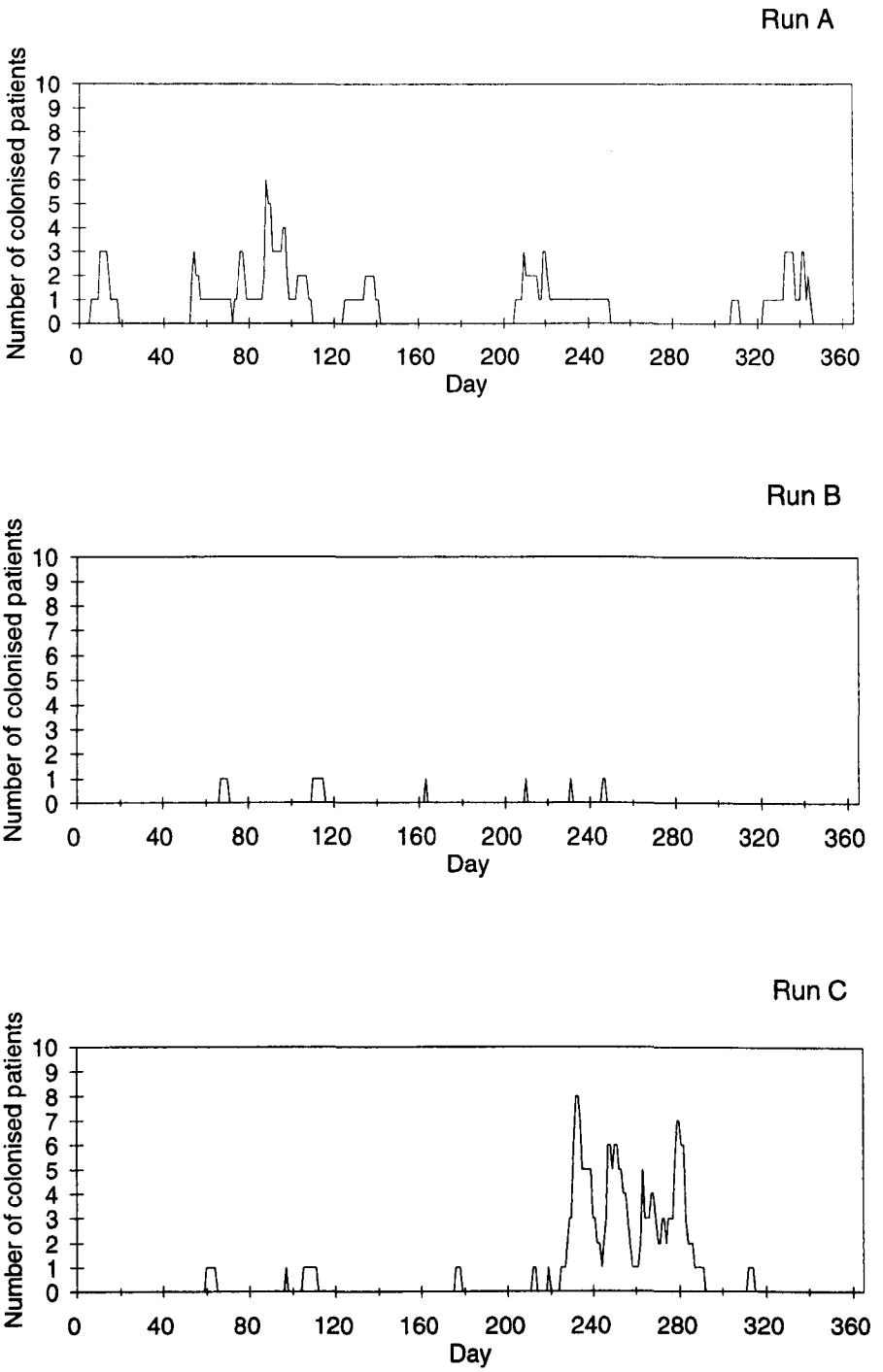

Figure 2.2: Sample simulation runs from the model, all using the default parameter values (given in table I) which give an R_0 value of 0.57. These runs were selected to illustrate the high degree of variability that occurs in the stochastic model. In the following graphs, all parameters are set to their default values, unless otherwise stated.

shown are therefore due entirely to stochastic effects. Although the apparent patterns of colonization in the three wards are completely different, and attempts might be made to explain the differences on behavioural or biological grounds, the properties of the organism and the practices in the wards are identical.

- Scenario 1 : Transmissibility

  Figure 2.3 shows how increasing patient-to-carer and carer-to-patient transmissibilities has a dramatic impact on the successful introduction rate, ward-level prevalence, and colonized patient-days. As the transmissibilities increase, the mean number of outbreaks increases rapidly, and then declines to a value of 1 as the pathogen becomes almost endemic on the ward, as indicated by the prevalence graph. The number of colonized patient-days is also highly sensitive to small changes in the transmissibilities. The non-linear nature of these results indicates that a small change in transmissibility can induce large epidemiological changes, assuming that carer-to-patient and patient-to-carer transmission are equally affected. The error bars show that there is wide variation about these means due to stochastic effects. Thus, for the highest transmissibility, 5% of wards have a prevalence of nearly 100%, while another 5% have a prevalence of less than 40%.

- Scenario 2 : Probability of Colonization at Admission

  The results presented in figure 2.4 show that for relatively low prevalences, the successful introduction rate increases linearly with the probability of admitting a colonized patient. As this probability is increased further, the ward-level prevalence becomes very large and outbreaks tend to merge into each other, so that the successful introduction rate plateaus and decreases. The number of colonized patient-days also increases linearly over the range of values investigated suggesting that

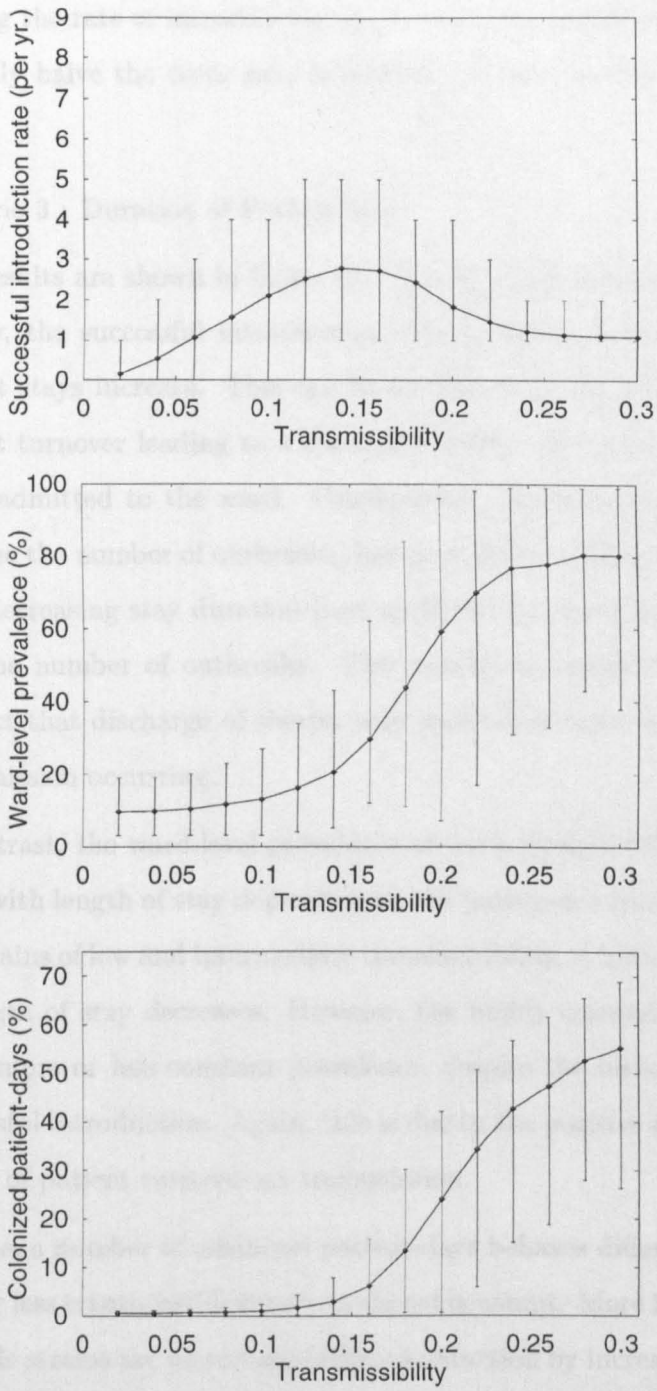

Figure 2.3: Abstracted results as a function of the transmissibility of the pathogen. For each carer-to-patient and patient-to-carer transmissibilities are equal. $R_0 = 1$ when the transmissibility is 0.133. Error bars indicate the range of values obtained by excluding the 5% highest and lowest values.

halving the rate of introduction of the pathogen on any ward will potentially halve the costs associated with infection providing $R_0 < 1$.

- Scenario 3 : Duration of Patient Stay

  The results are shown in figure 2.5. For all three values of transmissibility, the successful introduction rate decreases as the lengths of patient stays increase. This can be accounted for by a lower rate of patient turnover leading to a decreased number of colonized patients being admitted to the ward. Consequently, increasing turnover will increase the number of outbreaks, but note that doubling the turnover (e.g. decreasing stay duration from eight to four days) less than doubles the number of outbreaks. This non-linear behaviour is due to the fact that discharge of shorter stay patients is more likely prior to transmission occurring.

  In contrast, the ward-level prevalence of the pathogen behaves differently with length of stay depending on the pathogen's transmissibility. For strains of low and intermediate transmissibility, it increases slightly as length of stay decreases. However, the highly transmissible strain has a more or less constant prevalence, despite the increased rate of successful introduction. Again, this is due to the positive and negative effects of patient turnover on transmission.

  The mean number of colonized patient-days behaves differently again, and for less transmissible strains is almost constant. More highly transmissible strains are more susceptible to reduction by increasing patient turnover, though for the parameters chosen here the effect is small. If the mean time to detection of colonized patients is considerably larger than the default value used here, then both the colonized patient-days and ward-level prevalence of the more transmissible strains show

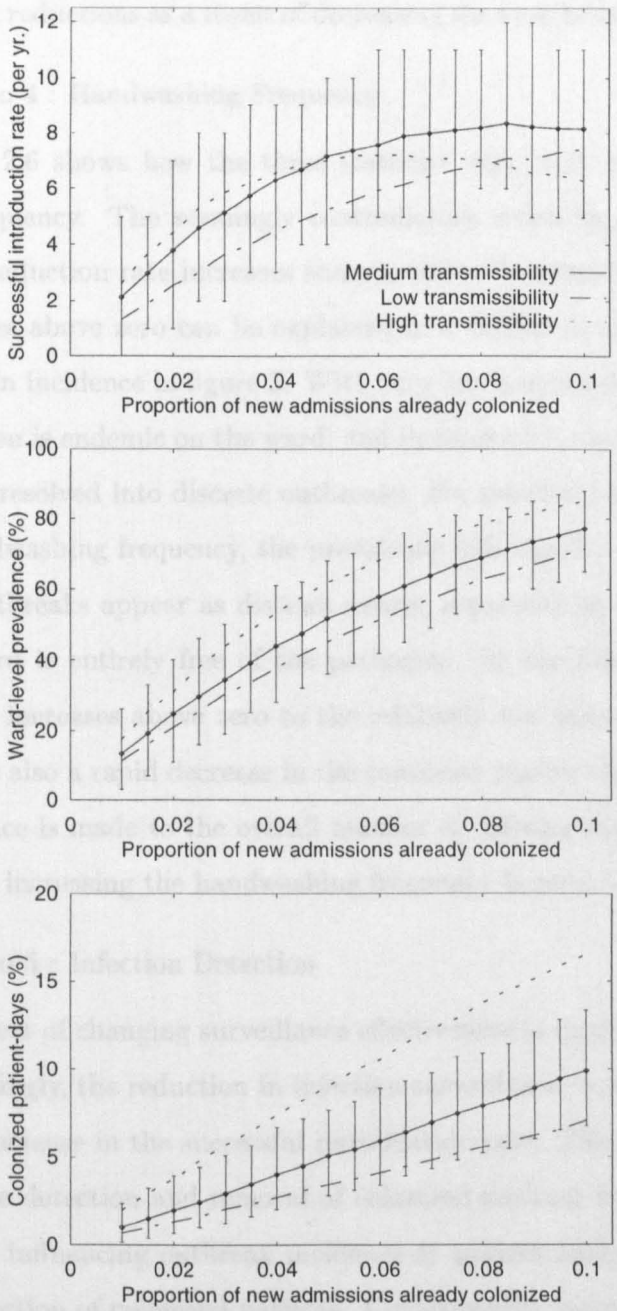

Figure 2.4: Abstracted results as a function of the proportion of patients colonized on admission. Each point represents the mean from 1000 simulation runs. Results for three levels of transmissibility are given: 0.13 (dotted line); 0.10 (solid line); 0.07 (dashed line). Corresponding R_0 values are 0.96, 0.57 and 0.28. Error bars for the medium transmissibility line indicate the range of values obtained by excluding the 5% highest and lowest values. Note the change of the y-axis scales in this and subsequent figures.

marked reductions as a result of decreasing the lengths of patient stays.

- Scenario 4 : Handwashing Frequency

  Figure 2.6 shows how the three statistics vary with the handwashing frequency. The seemingly contradictory result that the successful introduction rate increases sharply as the handwashing frequency increases above zero can be explained in a similar manner to the decrease in incidence in figure 2. With very low handwashing levels, the pathogen is endemic on the ward, and incidents of cross-infection cannot be resolved into discrete outbreaks. For relatively small increases in handwashing frequency, the prevalence falls rapidly to below 20%, and outbreaks appear as distinct events, separated by periods where the ward is entirely free of the pathogen. As the handwashing frequency increases above zero to the relatively low values of 0.2 or 0.3 there is also a rapid decrease in the colonized patient-days. Very little difference is made to the overall number of colonized patient-days by further increasing the handwashing frequency beyond about 0.4.

- Scenario 5 : Infection Detection

  The effect of changing surveillance effectiveness is shown in figure 2.7. Surprisingly, the reduction in infection surveillance, γ, leads to only a small increase in the successful introduction rate. This would suggest that the detection and removal of colonized patients is not as important in influencing outbreak incidence as patient turnover or rate of introduction of colonized patients. Consequently, increasing infection surveillance is expected to have almost no effect on outbreak surveillance data. Ward-level prevalence and colonized patient-days are much more sensitive to changes in infection surveillance, and the effect increases with increasing transmissibility. Consequently, surveillance is expected to have a more dramatic effect on clinically and economically

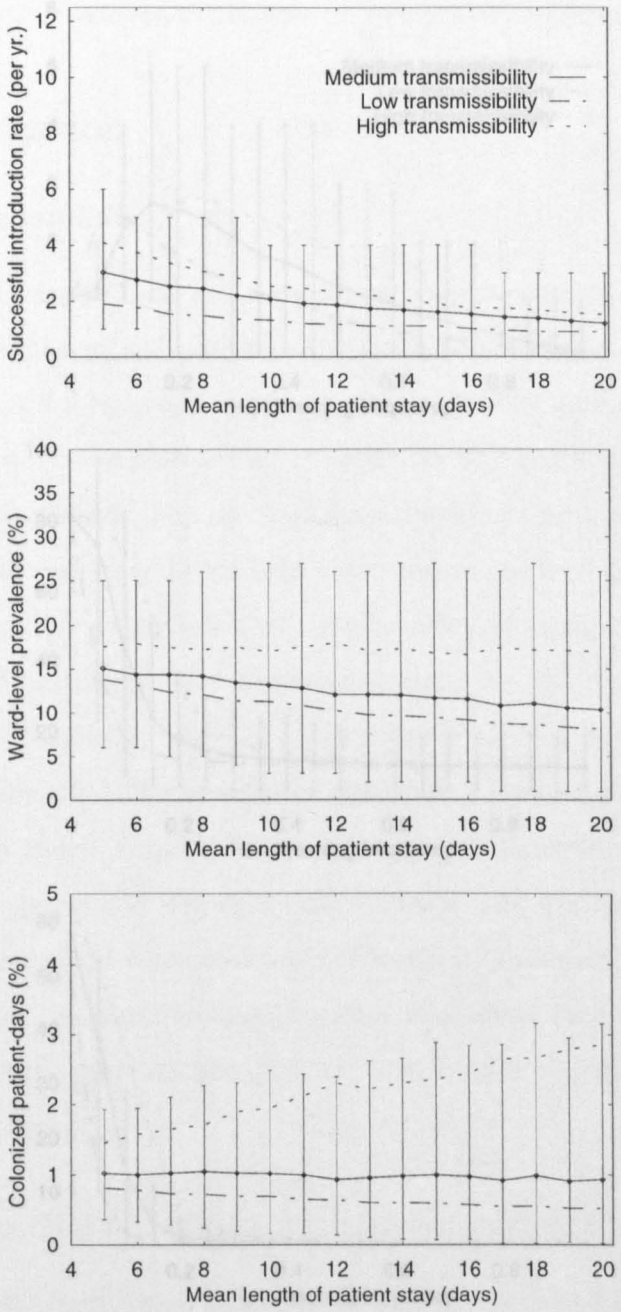

Figure 2.5: Abstracted results as a function of mean length of patient stay. Results for three levels of transmissibility are given: 0.13 (dotted line); 0.10(solid line); 0.07 (dashed line). For the first two of these, $R_0 = 1$ when the mean length of patient stay is 11.0 and 76.4 days respectively. For the lowest level of transmissibility R_0 is always less than one. Error bars for the medium transmissibility line indicate the range of values obtained by excluding the 5% highest and lowest values.

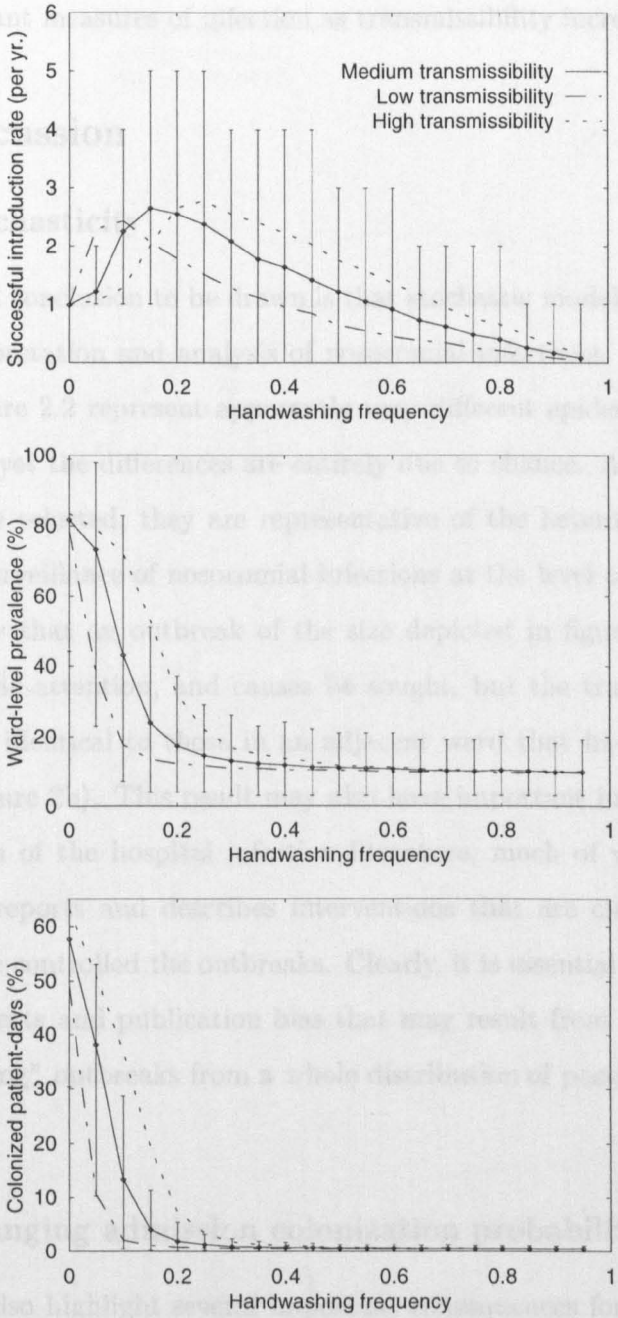

Figure 2.6: Abstracted results as a function of the handwashing frequency, the probability that an HCW handwash precedes a given patient contact. Results for three levels of transmissibility are given: 0.13 (dotted line); 0.10(solid line); 0.07 (dashed line). $R_0 = 1$ when the handwashing frequency is 0.29, 0.19, and 0.10 respectively. Error bars for the medium transmissibility line indicate the range of values obtained by excluding the 5% highest and lowest values.

important measures of infection as transmissibility increases.

2.3 Discussion

2.3.1 Stochasticity

The principal conclusion to be drawn is that stochastic models are essential for the interpretation and analysis of nosocomial infections. The patterns shown in figure 2.2 represent apparently very different epidemiological situations, and yet the differences are entirely due to chance. Although these patterns were selected, they are representative of the heterogeneity introduced into surveillance of nosocomial infections at the level of the ward. It is quite likely that an outbreak of the size depicted in figure 2.2C would attract specific attention, and causes be sought, but the transmission parameters are identical to those in an adjacent ward that had no infection problem (Figure 2b). This result may also have important implications for interpretation of the hospital infection literature, much of which consists of outbreak reports and describes interventions that are claimed (or implied) to have controlled the outbreaks. Clearly, it is essential to consider of stochastic effects and publication bias that may result from selecting only the "interesting" outbreaks from a whole distribution of possible outbreaks patterns.

2.3.2 Changing admission colonization probability

The results also highlight several important consequences for management of organisms such as *S. aureus*. In particular, alterations in patient management and infection control can have non-linear, and sometimes counter-intuitive, effects on different measures of infection. It was found that reducing the proportion of patients who are already colonized on admission to be an effective way of controlling the spread of infections providing R_0

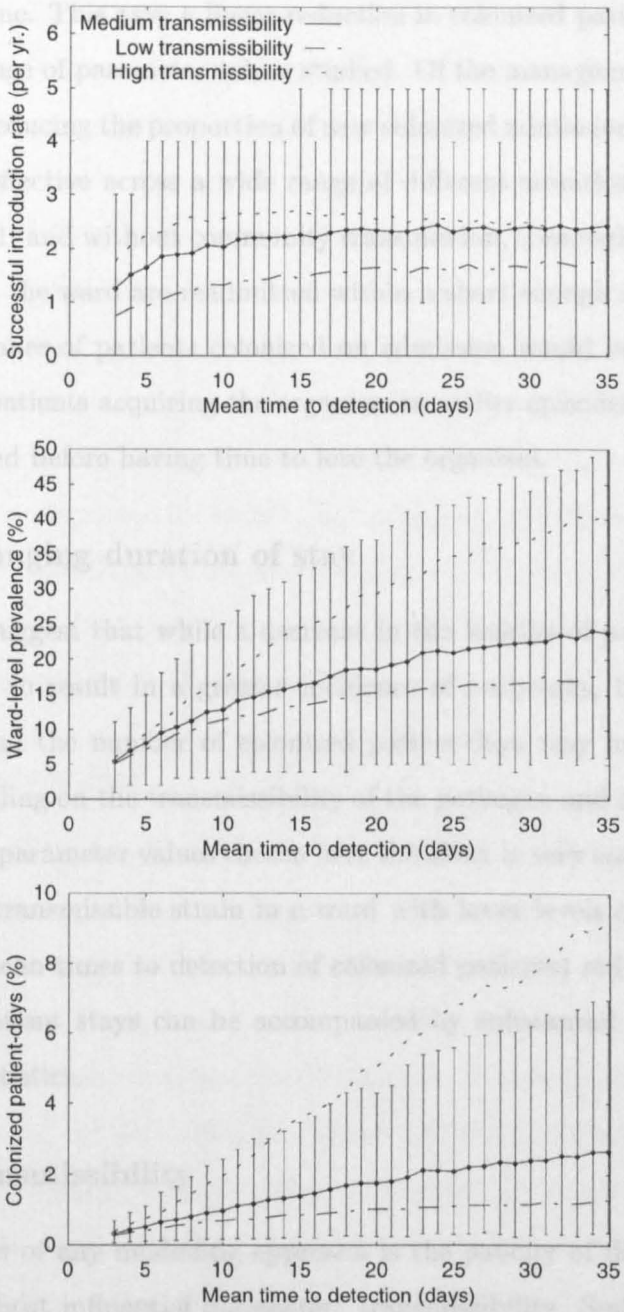

Figure 2.7: Abstracted results as a function of the mean time to detection of a colonized patient. Results for three levels of transmissibility are given: 0.13 (dotted line); 0.10(solid line); 0.07 (dashed line). For the first two of these, $R_0 = 1$ when the mean time to detection is 11.0 and 76.4 days respectively. For the lowest level of transmissibility R_0 is always less than one. Error bars for the medium transmissibility line indicate the range of values obtained by excluding the 5% highest and lowest values.

is less than one. This gave a linear reduction in colonized patient-days over the whole range of parameter values studied. Of the management strategies considered, reducing the proportion of new colonized admissions to the ward is the most effective across a wide range of different situations. Note that even if $R_0 < 1$, and without community transmission, if enough patients discharged from the ward are readmitted within a short enough space of time, then the number of patients colonized on admission would be expected to increase, as patients acquiring the organism in earlier episodes on the ward are readmitted before having time to lose the organism.

2.3.3 Changing duration of stay

The results suggest that while a decrease in the lengths of patient stays is always likely to result in a greater incidence of outbreaks, the ward-level prevalence and the number of colonized patient-days may increase or decrease depending on the transmissibility of the pathogen and other parameters. For the parameter values chosen here the effect is very small. However, for a highly transmissible strain in a ward with lower levels of surveillance (i.e. larger mean times to detection of colonized patients) reductions in the lengths of patient stays can be accompanied by substantial reductions in these two statistics.

2.3.4 Transmissibility

One weakness of any modelling approach is the paucity of direct information on the most influential parameter: transmissibility. Small changes in the transmissibility of a pathogen can result in large changes in the observed prevalence and in the total number of colonized patient-days. Such changes could be due to changes in hospital procedures, or due to the evolution of the pathogen, perhaps as the result of the adaptation of bacteria to their resistance genes (Schrag and Perrot, 1996; Lenski, 1997). Note that unlike

the transmissibility of the pathogen, the susceptibility of the patient population is, at least to some extent, a management decision. The tendency to hospitalise only the most severely ill could be expected to result in a more susceptible population due to reduced immune response, higher antibiotic usage, higher levels of catheterisation, higher levels of usage of invasive devices and so on. All of these factors should result in an increase in the value the carer-to-patient transmission probability. However, it is not clear how the probability of transmission from a patient to a carer should be affected, although it seems likely that individuals with infections lead to more transmission than asymptomatic carriers. In the absence of reliable information on these two parameters it was assumed they were equal in all our simulations. As a result the graphs cannot be considered as exploring the results of changing patient susceptibilities alone.

2.3.5 Handwashing

The importance of handwashing in the control of hospital infections has been repeatedly emphasised (Larson, 1988). The model results support this. Since it was assumed that all transmission occurs via the transiently colonized hands of HCWs and handwashing was assumed to reduce carriage to zero this result is not unexpected. What may be surprising is how large the reductions in the ward-level prevalence and colonized patient-days are as the hand decontamination frequency is increased above zero to relatively low values. For the parameter values chosen here, increasing the hand decontamination frequency above about 40% makes very little difference to the prevalence and intensity.

Note, however, that the handwashing frequency used in the model cannot be taken straight from values observed in observational studies. In the model it is assumed that each handwash is 100% effective at removing pathogens. This is clearly not the case in practice (Kjølen and Andersen, 1992). To be

realistic, the handwashing frequency in the model should perhaps be much lower than frequencies found in studies, which are typically in the region of 40% (Larson and Kretzer, 1995).

2.3.6 Infection surveillance

Finally, the effect on transmission of the level of surveillance activities on the ward (measured as the mean time taken to detect a colonized patient) was considered. In contrast to the handwashing graph (scenario 4), where increased levels of handwashing cause rapidly diminishing reductions in colonized patient days as the handwashing frequency increases above fairly small values, colonized patient-days and ward-level prevalence varied almost linearly over the whole range of parameter values examined. Note that it has been assumed that the mean time to detection is constant. In practice one may expect it to decrease in an outbreak situation as surveillance activities increase. This effect is likely to mean that surveillance activities are in practice much more effective than the results presented here suggest. The patterns shown suggest that once handwashing rates are at some reasonable level, increasing resources should be directed at improving detection for greatest effect.

2.3.7 Measurement of infection

Three indices of infection on a ward over one year have been considered. It clearly matters how infection is measured, in that some management tools had opposite effects on different measurements (e.g. successful introduction rate and colonized patient-days in Figure 5). In particular, it is apparent that the successful introduction rate becomes meaningless if a pathogen becomes endemic on wards; if all patients are infected the incidence of new infection is zero. Other measures may be more meaningful in different situations. For example, in an ICU, where colonization is more likely to result

in clinically important consequences, the probability of a patient becoming colonized during their stay may be a more accurate measure of the problem.

2.3.8 Extensions

Here, a highly simplified model for the transmission of a single pathogen within a single ward has been presented. The intention has been to make the model as simple as possible, whilst retaining some of the essential aspects of transmission. To this end a number of simplifying assumptions in the construction of the model were made. Clearly, a set of different assumptions will produce a different model, with potentially different results.

Mathematical models are seldom realistic enough to allow explicit predictions to be made. The value of this type of model lies rather in improving understanding of the non-linear interactions between a small number of processes. Improved correspondence between model and reality requires the inclusion of the next set of important processes. Thus the complicating factors of patient heterogeneities, non-homogeneous mixing patterns and antibiotic use are considered later in chapters 4 and 6.

Chapter 3

An observational study

This chapter describes a one month prospective observational study aimed at collecting data for estimation of model parameters. The study was conducted in a 15 bed mixed medical/surgical ward. Detailed observations of patient-carer contacts and carer handwashing behaviour were made and analysed using Poisson and logistic regression models. To measure transmission of MSSA and MRSA between patients, consenting patients were swabbed for *S. aureus* and a combination of typing methods used to identify cases of cross-infection. Carer hand contamination with *S. aureus* was assessed using finger-plate sampling following patient contacts.

3.0.9 Background

Transmission

Between the late 1950s and early 1970s a number of prospective studies were carried out in which all patients in a given ward were swabbed at regular intervals, enabling asymptomatic and symptomatic transmission to be detected (see chapter 5). Such large-scale prospective ward-level studies do not appear to have been repeated since the early 1970s. Recent prospective studies that have been published have lacked sufficient detail for parame-

ter estimation (see chapter 5). Instead, the hospital infection literature has been dominated by outbreak reports or retrospective surveys. The former are unlikely to be representative of the normal state of affairs in hospital wards, and both usually have insufficient reporting detail to be of much use (denominators are rarely reported, and the reasons and frequency of screening are not usually given). As a consequence, it is not clear how much normally undetected transmission of *S. aureus* occurs on wards.

Both hospital populations and standard practices have changed substantially over the past 20-30 years and hospital *S. aureus* strains may exhibit very different behaviour to earlier strains (Casewell, 1995). There is a need to conduct similar studies again, but now with the benefit of more accurate and reliable typing methodologies, together with more sophisticated statistical analysis.

Handwashing and contact patterns

If the assumption that most transmission of *S. aureus* between patients occurs via the transiently colonized hands of health-care workers is justified, then the hand decontamination behaviour of the carers must be crucial to the transmission dynamics.

Although there have been many observational studies on hospital wards aimed at collecting data relevant to infection control, most of these have concentrated solely on the handwashing practices of health-care workers (Larson and Kretzer, 1995), usually with the objective of producing an overall handwashing "rate". Prior to this study, there appears to have been little attempt to relate handwashing compliance to factors associated with carers, patients and contact-types using a statistical model.

Heterogeneity in the rates at which patients are contacted by carers and associations of contact rates with other patient risk factors for the acquisition of *S. aureus* strains can have important impacts on the pathogen's

transmission dynamics (see chapter 4). The transmission dynamics will also be affected by who makes the contacts. For example, transmission patterns on wards where required patient contacts are repeatedly made by the same carer would be expected to differ from those on wards where they are evenly distributed amongst the different carers. However, literature searches failed to reveal any studies that attempted to measure patient-carer contact rates and contact patterns.

3.0.10 Aims

The aims of this study were therefore:

- To investigate how much transmission of *S. aureus* (MSSA and MRSA) actually occurs between patients during the normal running of a typical medical/surgical ward.
- To measure handwashing compliance in carers, and to determine the factors associated with poor compliance and sources of heterogeneity in handwashing rates.
- To measure carer-patient contact rates, the epidemiologically important factors associated with high contact rates, and to explore heterogeneities in these rates.
- To investigate carer-patient contact patterns and look for departures from the assumption of homogeneous mixing made in chapter 2.
- The current study was intended as a pilot, and an important aim was to evaluate the effectiveness of data collection methods, and to determine the form larger scale studies should take.

3.1 Method

3.1.1 Study Environment

Prior to the study, approval from ethics committee and ward staff was obtained and the broad aims of the study were explained to the permanent ward staff.

The study was based on a 15-bed mixed medical surgical ward and lasted for 1 month. After a preliminary period of two weeks where different data recording techniques were assessed, patient data and swabs were collected between the 6th of July and the 7th of August 1998. Observations of patient-carer contacts and handwashes on the ward were all carried out between the 13th of July and the 6th of August 1998.

The ward was divided into three areas: one bay of four beds containing exclusively female patients (beds 1-4); one bay of three beds directly opposite the nurses station containing both male and female patients (beds 5-7); one side room (bed 8); and one bay of seven beds containing only male patients (beds 9-16, there was no bed 13).

Two sets of data were collected: patient-carer contact data and associated handwashing and transmission data. A nurse was employed for the duration of the study to take swabs, obtain patient consent, and jointly conduct ward observations with the author.

3.1.2 Contact pattern data

The contact pattern data consisted of recordings of direct contacts between patients and health care workers (HCWs). Patient-carer contacts were recorded in observation periods that lasted for lengths of time decided beforehand; typically 1.5 hours, but going up to 7.5 hours for overnight observation periods. Observation periods are illustrated in figure 3.1.

Contact details were recorded on form CR1 (see appendix A for this and

other forms) and included the purpose of the contact, the patient and carer involved, the time of the contact, whether or not gloves were worn, whether or not an apron was worn, and whether or not it was preceded or followed by handwashing. All contacts were classified as one of the following:

- Contact type I — Simple Touch

  This included casual contacts between the patient and carer, and taking pulse, blood pressure, temperature, etc.

- Contact type II —Extensive Touch

  More prolonged contacts between the patient and carer including physical examination, physiotherapy, being helped to wash etc.

- Contact type III—Complex Touch

  All contacts (short and long) between the patient and the carer where an invasive or semi-invasive procedure was carried out. This included: changing a wound dressing; inserting or adjusting a catheter; inserting or adjusting a drip.

Where curtains were drawn the nature of the contact was assessed either by noting the equipment taken behind the curtain or by asking staff. In most cases there was no ambiguity as to the nature of the contact. In a few cases a guess as to the extent of the contact had to be made, and in such cases this was indicated on the recording form. A contact was considered to end when the carer left the patient or contacted a potentially contaminated site outside the patient's immediate environment. Carers present on the ward during the observation period were listed in form C1 and were identified by unique codes. Where possible this was completed from ward records before the observation period, though changes during the observation period were often required. It was found to be possible to observe contacts on patients in all 15 beds in the ward by moving around the ward, rather than viewing from a fixed location, except during the busiest observation periods.

3.1.3 Patient data

For all patients entering the ward during the study period, or present on the ward at the beginning of the study, details were recorded on form P1. Additional data were collected during each day of the study period for each patient on the ward. These data were entered directly into form P2.

3.1.4 Carer data

Details of which nurse was on which shift and in which nursing team were kept for each day. The named-nurse[1] for each patient was also recorded. These data were entered in form C1.

3.1.5 ICU observations

11.5 hours of ward observations were also conducted in the Intensive Care Unit (ICU) on the 17th and 18th of June, 1999. In this case only contact details between patients under observation and HCWs were recorded. No other patient or carer details were noted. In contrast to observations based in the medical/surgical ward, consistently identifying staff members presented some problems: staff were not known to the observer beforehand and observations were carried out over only two days. It was found that these problems could be overcome by associating a unique ID code with a description of staff footwear, thus allowing identification of staff even when they were behind drawn curtains.

3.1.6 Transmission data

Staphylococcus aureus isolates (both sensitive and resistant strains) were collected from those patients who had provided written consent and agreed to be swabbed. The aim was to collect swabs from patients on admission

[1]The nurse with primary responsibility for that patient's care (Dooley, 1999)

to the ward, twice per week, and immediately prior to discharge or transfer. Nasal swabs were requested from all the nursing staff who were present on the ward during the study period, and as many of the other carers who worked on the ward during this period as was practicable. Transmission of *S. aureus* from patients to the hands of carers was investigated by taking finger streaks immediately before and after different procedures. No swabs were taken in the ICU.

Transmission data were analysed by fitting them to a transmission model using an MCMC approach. This is described in chapter 8.

Cultivation and identification

Patient swabs were taken from the nose, axillae and throat. Carer swabs were taken only from the nose, usually by the carers themselves by circling the swab through both nostrils consecutively while applying even pressure. All swabs were taken using sterile synthetic-tipped transport swabs (F108C, Bibby Sterilin, Staffordshire) which were used to inoculate salt broths (CM435, Oxoid, Basingstoke), pooling nose, throat and axillae swabs taken at the same time from a patient.

After overnight incubation at 37°C the broths were used to inoculate mannitol salt agar (MSA) plates (CM85, Oxoid), and MSA plates supplemented with 5 mg/L oxacillin. These were incubated at 37°C and examined after 24 and 48 hours. *S. aureus* colonies were identified by colonial morphology, ability to ferment mannitol, and coagulase production. Further confirmation of their identity was later provided by detection of the coagulase gene and from colony morphology on Baird Parker medium. *S. aureus* colonies were then stored temporarily on nutrient agar slopes before being transferred onto tryptone soy agar (TSA, labM, Lancs) plates supplemented with 5% defibrinated horse blood (CS Microbiology, Buckingham). Colonies with distinct morphologies from each plate were subcultured and stored in

Tryptic Soy Broth (TSB, Difco laboratories, Detroit) with 10% glycerol at -70^{o}C. Isolates obtained from the ward during the period of study as a result of routine hospital procedures were processed similarly. Initial culturing, identification of *S. aureus* colonies, and transferring to slopes was carried out jointly with clinical microbiology staff at the hospital.

Finger streaks were taken from both hands of carers before and after they touched patients believed to be carrying *S. aureus* by pressing fingers and thumbs firmly onto a blood agar plate (Oxoid). After overnight incubation at 37^{o}C, colonies resembling *S. aureus* were subcultured onto MSA and MSA supplemented with oxacillin, and then processed as for the rest of the isolates described above.

Antibiotic susceptibility testing

All isolates were tested for antimicrobial susceptibility by the disk diffusion method on Mueller-Hinton agar according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (1997). The following antibiotics were used: penicillin; sulphamethoxazole; vancomycin; teicoplanin (30μg); ciprofloxacin; doxycycline (30U); hydrochloride; gentamicin; tobramycin (10μg); neomycin (30μg); kanamycin (30μg); streptomycin (10 U); chloramphenicol; spiramycin (100μg); fusidic acid (10μg); rifampicin; mupirocin (5μg); tetracycline; minocycline (30μg); clindamycin; erythromycin; and lincomycin (15μg) (all from Oxoid, UK). NCCLS breakpoints were used (1997), except in the cases of doxycycline,tobramycin, kanamycin, streptomycin, spiramycin, fusidic acid, minocycline, and lincomycin for which the French breakpoints were used (Courvalin and Soussy, 1996). Swedish breakpoints were used for teicoplanin (Ringertz *et al.*, 1997). A zone diameter of less than 20mm around the 5μg mupirocin disk was considered to indicate at least low-level resistance, while strains that grew without a zone of inhibition around a 200μg mupirocin disk were considered to possess high-level

resistance.

Oxacillin resistance (equivalent to methicillin-resistance) was determined by screening on TSA plates supplemented with 10mg/L oxacillin (Sigma Chemicals Co., St Louis) (NCCLS, 1997) and confirmed by the detection of the *mecA* gene as previously described (Murakami *et al.*, 1991).

Molecular typing

Molecular typing was based on polymorphisms of the coagulase and protein A genes, and randomly amplified DNA (RAPD).

DNA preparation

Genomic DNA was isolated using a Wizard Genomic DNA Purification Kit (Promega, WI), with a number of modifications to the manufacturer's instructions. In short, tryptic soy broth (TSB, Difco, Detroit) was inoculated with a single colony, and incubated overnight with vigorous shaking at 37^{o}C. 1 ml of the culture was transferred to a 1.5 ml microcentrifuge tube and centrifuged at 13000 $\times$ g for three minutes. Supernatant was removed and cells resuspended in 480μl of 50mM EDTA before adding 120μl of 40mg/ml lysozyme and incubating at 37^{o}C for 20 minutes. The sample was centrifuged at 13000g for three minutes, the supernatant removed and the cells resuspended in 600μl of nuclei lysis solution, then incubated at 80^{o}C for 5 minutes. 3μl of RNase solution was added to the samples, mixed by inversion, and incubated for 15 minutes at 37^{o}C. After cooling to room temperature, adding 200 μl of protein precipitation solution, vortexing, and incubating on ice for 5 minutes, the samples were centrifuged at 13000 $\times$ g for three minutes in the cold room and the supernatant removed and added to 600 μl of room temperature isopropanol. These samples were then mixed by inversion, centrifuged at 13000 $\times$ g for two minutes and the supernatant discarded. The DNA pellet was washed with 70% ethanol which was aspi-

rated off after centrifuging for 2 minutes at $13000 \times g$. Samples were vacuum dried for 30 minutes before rehydrating by incubating with 100 μl of DNA Rehydration Solution for one hour, and stored at -20^oC.

Arbitrary primed PCR (AP-PCR)

Typing based on Randomly Amplified Polymorphic DNA (RAPDs) closely followed a previously described protocol (Murchan *et al.*, 1998). 50 μl of reaction mixture was used, containing 0.5μl of *Taq* DNA polymerase; 2.5μl of $MgCl_2$; 0.1μl of each of dATP, dCTP, dGTP and dTTP; and 5μl of *Taq* PCR buffer, all as supplied by Gibco BRL (Gaithersburg, MD). To this 2μl of DNA template and 0.5mM of primer were added. Primers used were: 5'-TCA CGA TGC A-3' (AP4) and 5'-GTG GAT GCG A-3' (AP7). Cycling was performed in a Touchdown thermocycler (Hybaid, Middlesex) and PCR reaction conditions were: predenaturation at 94^oC for five minutes; 5 cycles of 94^oC for 20s, 35^oC for 30s, 72^oC for 90s; 30 cycles of 94^oC for 20s, 55^oC for 30s and 72^oC for 30s; then one minute at 72^oC. Amplified DNA was stored at -20^oC. PCR-products were separated by electrophoresis on 1.5% agarose gels (Gibco BRL) with ethidium bromide using a 1kb molecular weight marker and photographed under UV light.

Detection of protein A and coagulase gene polymorphisms

Typing based on restriction fragment length polymorphism (RFLP) of the protein A gene was similar to previously described methods (van Belkum *et al.*, 1997; Frénay *et al.*, 1994). The reaction mixture differed from that used for the AP-PCR only in the following respects: only 1μl of each DNA template was used; only 1μl of $MgCl_2$ was used; the primers used were 5'-GCT AAA AAG CTA AAC GAT GC-3' and 5'-CCA CCA AAT ACA GTT GTA CC-3' (Gibco BRL). PCR conditions were as follows: 35 cycles of denaturation at 95^oC for one minute, annealing at 50^oC for one minute, and

chain extension at 72°C for one minute 30 seconds; followed by 5 minutes at 72°C. PCR products were digested using the restriction endonuclease *Rsa*I (Gibco BRL) according to the manufacturer's instructions, and examined using electrophoresis with a 1.5% agarose gel and a 1KB molecular weight marker.

RFLP analysis of the coagulase gene closely followed the methods of Goh *et al.* (1992), but used only the two primers: COAG3, 5'-AAA GAA AAC CAC TCA CAT CA-3'; and COAG2, 5'-CGA GAC CAA GAT TCA ACA AG-3'. Otherwise, the reaction mixture was as for the protein A gene. PCR conditions were: 40 cycles of 95°C for 30s, 55°C for 2 minutes, 72°C for four minutes; followed by 5 minutes at 72°C. PCR products were digested using the restriction endonuclease *Alu*I (Gibco BRL) according to the manufacturer's instructions, and then analysed as described above.

3.1.7 Statistical analysis

Handwashing data

Handwashing data were recorded as a binary responses associated with each carer-patient contact, so logistic regression was used to construct a descriptive model of the probability of any particular carer washing their hands for a given contact. Two distinct models were constructed: one for pre- and one for post-contact hand decontamination.

The data collected can be considered to have a hierarchical structure: at the lowest level are observations of handwashing behaviour associated with each contact. These can be considered to be clustered within two overlapping higher levels: the different observations periods; and the different carers.

If such clustered observations are correlated, then the assumptions of a standard logistic regression are violated: the residuals are no longer independent, and type I errors are more likely than the α value would suggest (Hox, 1995). One potential solution would be to include a dummy vari-

able for each observation period and each carer. However, a more economical and informative approach is to use the multilevel modelling framework, which considers the higher level units themselves to be samples from a larger population (Goldstein, 1995). Thus, when constructing the model, both intercepts and slopes were allowed to vary randomly across the levels, and estimates for both fixed and random effects were obtained.

MLwiN v.1.0.2 (Goldstein *et al.*, 1998) was used for initial model exploration and fitting using second order penalized quasilikelihood (PQL) when this converged, and first order PQL or marginal quasilikelihood (MQL) otherwise. Second order PQL has been shown to provide much better estimates than 1st order PQL or MQL when fitting multilevel models with binary responses (Goldstein and Rasbash, 1996), but recent simulation studies have shown that it still underestimates the true values of higher order variances in such random effects logistic regression models, and gives standard errors which are too low (Draper and Browne, 2000). The final parameter estimates were therefore obtained using Markov Chain Monte Carlo (MCMC) methods, which have been shown to outperform the quasilikelihood based methods for this type of model (Draper and Browne, 2000). In particular, Metropolis sampling as implemented in the program WinBUGS v.1.2 (Spiegelhalter *et al.*, 1999) was used to obtain posterior distributions to the parameters for the previously selected models. Diffuse priors were used throughout.

Explanatory variables considered in the regression are listed in table 3.1.

Following data exploration with a standard logistic regression analysis the following interactions were also considered in the regression: [carer type] * [contact type]; [carer type]* [activity level]; [carer type]*[day].

During model construction variables significant at or near to the five per cent level were added to the model in a stepwise fashion. The approximate Wald hypothesis test was used to determine significance. Random effects

Level	Variable	Coded as
Contact	contact type	baseline category is for type 1 contacts binary variables for type 2 and type 3 contacts
	MRSA status of patient	0 - not known to be colonized 1 - known to be colonized
	sex of patient	0 - female; 1 - male
	sink adjacent to bed	0 - no; 1 - yes
Carer	carer type	baseline category is nurse binary variables for: doctor health care assistant student nurse student doctor other (eg. physiotherapist, phlebotomist etc)
Observation period	time since start of study	time in days, mean-centred
	Activity level 1	mean-centred, continuous value
	Activity level 2	mean-centred, continuous value
	Activity level 3	mean-centred, continuous value
	time of day	1 - observation period stopped before midday 0 otherwise
	observer	Baseline category is data recorded by observer 1 binary variables for two observers and observer 2
	ward sister present	baseline category is for no sister present binary variables for: ward sister present and active acting ward sister present and active

Activity levels 1, 2, and 3 give three distinct measures of the level of ward activity, and are defined as follows:

- *Activity level 1* The nurse-to-patient ratio, defined as the ratio of the number of patients seen to be contacted during the observation period to the number of nurses observed to make contacts.
- *Activity level 2* The ward contact rate of a given observation period, defined as the number of contacts made by nurses per hour.
- *Activity level 3* The ward contact rate per nurse. i.e. activity level 2 divided by the number of nurses making the observed patient contacts.

Table 3.1: Variables considered for inclusion in the logistic regression.

were also retained only if significant at or near to the five per cent level.

Contact data

Contact rates: Count data corresponding to the number of contacts received by each patient in each observation period were analysed using a Poisson regression model. Since some patients might be expected to require more contacts than others, and some observation periods might be expected to be busier than others (reflecting the time of day, ward rounds, day of week, staffing levels etc), the multilevel framework is again appropriate. In this case there are again two overlapping classifications. The response variable (number of contacts of one patient in one observation period) can be considered to be clustered within higher level units corresponding to either the patients or the observation periods.

Again, MLwiN was used for model selection, and WinBUGS for final parameter estimates, using diffuse priors.

Model construction was carried out in a similar manner to that for the logistic regressions. Explanatory variables considered for inclusion in the model were chosen for epidemiological relevance and are listed in table 3.2.

Contact patterns: To formally test the assumption that patient contacts are not preferentially met by certain nurses, patient×carer contact matrices for each of the observation periods where all beds were observed were formed. Cell ij of such a table records the number, c_{ij}, of contacts made by nurse j on patient i in the observation period. The null hypothesis is that the $\sum_j c_{ij}$ contacts received by patient i are distributed across the columns (the carers) according to multinomial distribution, where each contact has a probability of $\frac{\sum_i c_{ij}}{\sum_{ij} c_{ij}}$ of being met by carer j. Because the c_{ij} were mostly ones and twos, asymptotic techniques were not appropriate. Instead, exact tests of the null-hypothesis were performed using StatXact (1997). This program

Level	Variable	Coded as
Patient× Obs. period	Patient taking antibiotics	0 – no; 1 – yes
	Operation in last 24 hours	0 – no; 1 – yes
	Operation in last week	
	(and not in last 24 hrs)	0 – no; 1 –yes
	length of ward stay to date	time in days (1–89)
	Catheter present	0 – no; 1 – yes
	Other line present†	0 – no; 1 – yes
Patient	sex	0 – female; 1 – male
	ln(age)	in years, mean-centred (26-91)
	admitted from home	0 – no; 1 – yes
	transferred from another ward	0 – no; 1 – yes
	ln(days in hospital in last year)	(1–186)
Observation period	time of day	1 - obs. period stopped before midday 0 otherwise

Table 3.2: Variables considered for inclusion in the patient contact Poisson regression. Ranges of non-categorical variables (before taking the logarithm) are shown in parentheses.

† Any line excluding catheter, oxygen line, or nasogastric tube.

enumerates all possible contingency tables consistent with the marginal totals, and returns the probability (the exact p-value) of getting a likelihood less than or equal to that of the actual data.

3.2 Results

3.2.1 Data collected

Of the 685 carer-patient contacts observed, carers were seen to decontaminate their hands before the contacts on 36.1% of the occasions, and after the contacts 39.1% of the time.

A total of 88 hours and 40 minutes of ward observation were conducted over 45 observation periods. The number of beds under observation varied between observation periods: for 66.7 hours (and 30 observation periods) all 15 beds were observed; for 1.5 hours and 1 observation period 14 beds were observed; for 11.8 hours and 8 observation periods 9 beds were observed; for 8.4 hours and five observation periods only 8 beds were observed; and for 0.25 hours and one observation period two beds were observed. In total, the 45 observation periods give a total of 1195.6 observed patient hours. Figure 3.1 shows the distribution of observation periods throughout the day. Over the one month study period there were 54 patients recorded on the ward (some patients who stayed less than 24 hours may not have been included). The mean age was 65 (range: 26 to 91). Over the same period 71 different HCWs were observed to make contact with patients. Of these 26 were nurses, student nurses or health-care assistants. The others—the peripatetic HCWs—were not known to the observers, and it is likely that some of these may have been counted twice in the above figure.

Patients were not necessarily in their beds during the observed hours. In many cases, though the beds were theoretically occupied, the patients were physically absent. Often they were in the operating theatre, on weekend

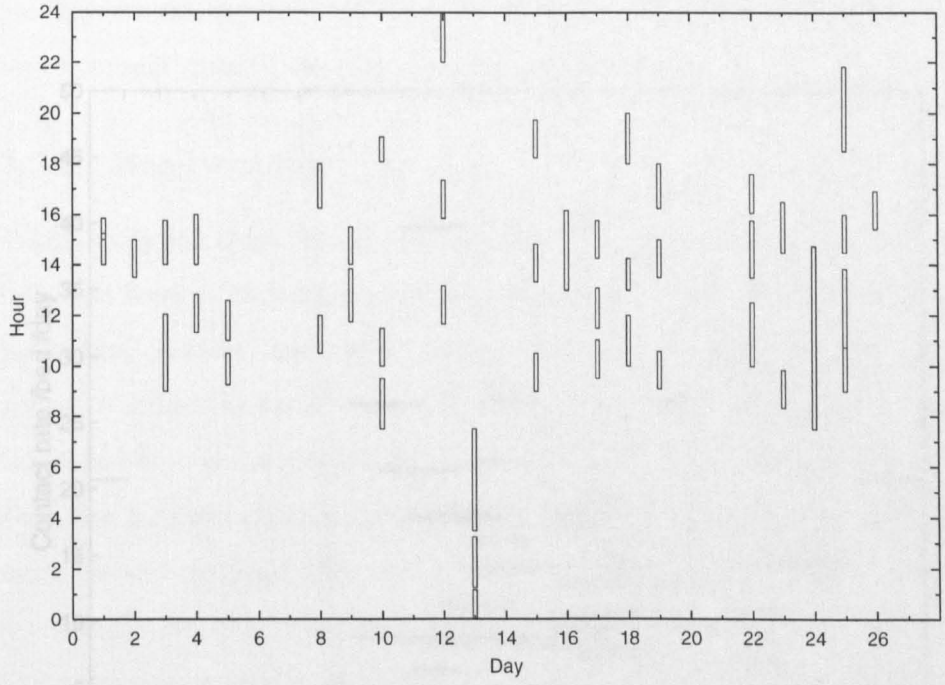

Figure 3.1: Distribution of ward observation periods.

leave, smoking in the corridor, having limbs fitted, or undergoing physiotherapy. In the analysis (as only *within-ward* transmission is under consideration), such beds were considered to be occupied. A bed was considered to be empty only if it could have been occupied by a newly-admitted patient.

During these observation periods a total of 690 contacts were observed. These comprised 483 type 1 contacts, 118 type 2 contacts, and 89 type three contacts. Only 5 patient-to-patient contacts where observed, though it is likely that some may have gone unobserved during the observation periods as a number of the patients would often smoke together in an area just outside the ward.

Table 3.3 gives a breakdown of contact types made by three carer categories: doctors (including medical students); nurses (including student nurses and health-care assistants); and others.

Patient-carer contact rates obtained in the different observation periods are illustrated in figure 3.2. Contact rates showed considerable variation

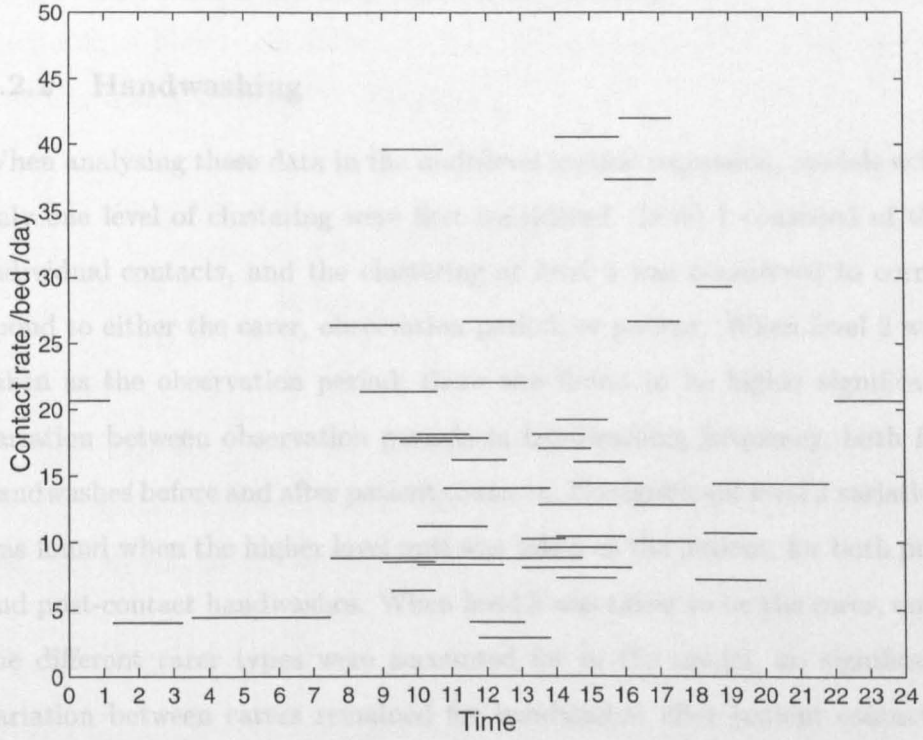

Figure 3.2: Distribution of observed contact rates by time of observation period. One observation period was undertaken to assess the feasibility of conducting observations during a ward round. This gave a contact rate of 240 /patient /day, and is not shown in the figure. See also section 3.2.3.

	Contact type		
	Type 1	Type 2	Type 3
Doctors	64	19	15
Nurses	394	93	61
Other	25	6	13

Table 3.3: Frequency of contacts by carer category. Note that medical students and student nurses are included in the doctors and nurses categories respectively

throughout the day, with peaks at about 9am and 4pm, and considerably lower contact rates in the early hours of the morning.

3.2.2 Handwashing

When analysing these data in the multilevel logistic regression, models with only one level of clustering were first considered. Level 1 consisted of the individual contacts, and the clustering at level 2 was considered to correspond to either the carer, observation period, or patient. When level 2 was taken as the observation period, there was found to be highly significant variation between observation periods in handwashing frequency, both for handwashes before and after patient contacts. No significant level 2 variation was found when the higher level unit was taken as the patient, for both pre- and post-contact handwashes. When level 2 was taken to be the carer, once the different carer types were accounted for in the model, no significant variation between carers remained for handwashes after patient contacts. However, the variation between carers for handwashes before contacts was just significant at the five per cent level. Because of this, a cross-classified model for pre-contact handwashes was considered (Goldstein, 1995): level-1 contacts were considered to be embedded within both carers and observation periods, which constituted overlapping level 2 units. After fitting such a cross-classified model in MLwiN with 2nd order PQL, the variation between carers was found not to be significant ($P > 0.1$), while that between observation periods was significant at the 10%, but not 5% level. As a result of this, only level 2 clustering corresponding to the different observation periods is considered in the following analysis.

The model for pre- and post-contact handwashes is then given by equations 3.1 to 3.3, where i corresponds to the patient-carer contacts, j corresponds to the observation periods, and y_{ij} is 1 if there was a handwash for the ijth contact, and 0 otherwise. $\mathbf{b}$ is the vector of slope parameters cor-

responding to the vector of explanatory variables $\mathbf{x_{ij}}$. u_j is the departure of the intercept for the jth observation period from the mean, a. Normal score plots of these level 2 residuals indicated good agreement with the assumption that they follow a normal distribution.

$$y_{ij} \sim \text{binomial}(\pi_{ij}, 1) \tag{3.1}$$

$$\text{logit}(\pi_{ij}) = a + u_j + \mathbf{bx_{ij}} \tag{3.2}$$

$$u_j \sim N(0, \sigma_u^2) \tag{3.3}$$

Final parameter estimates, obtained using WinBUGS, are given in table 3.4. Those variables listed in table 3.1, but not appearing in table 3.4, were not significant in the final model.

3.2.3 Contact rates

When constructing the multilevel Poisson regression model for the contact-count data, two different 2-level hierarchies were first considered. In both cases level 1 corresponded to observations on one patient in one observation period. Level 2 was taken as the patient in the first case, and the observation period in the second. In both cases, even after including all the significant explanatory variables, there was found to be significant level 2 variation in the intercepts (though not in any of the slopes). Both models showed fairly good agreement with the assumption that level 2 residuals are normally distributed, though the former was a little leptokurtotic. Both models also exhibited large and significant extra-Poisson variation, indicating that neither classification alone could suffice, and that instead both classifications should be used.

Level-2 residual plots also indicated that one observation period was clearly an outlier (residual equal to 4.9 standard deviations of the level 2 observation period residuals) . This corresponded to the shortest observation

Parameter	Estimate	95% credible interval	
Pre-contact handwashes			
intercept (a)	-1.10	{-1.48,	-0.74}
contact type 2	2.13	{1.62,	2.65}
contact type 3	3.08	{2.44,	3.76}
doctor	-1.51	{-2.21,	-0.83}
health care assistant	-1.04	{-1.70,	-0.41}
other	-1.20	{-2.26,	-0.18}
(day since start of study)× nurse †	-0.07	{-0.12,	-0.04}
σ_u^2 (level 2 intercept variance)	0.57	{0.17,	1.24 }
Post-contact handwashes			
intercept (a)	-0.99	{-1.34 ,	-0.66}
contact type 2	2.42	{1.89,	2.98}
contact type 3	3.07	{2.41 ,	3.76}
doctor	-1.98	{-2.71 ,	-1.29}
health care assistant	-0.74	{ -1.34,	-0.16}
(day since start of study)× nurse †	-0.06	{-0.10 ,	-0.02}
(day since start of study)× other †	0.16	{0.05 ,	0.29}
σ_u^2 (level 2 intercept variance)	0.45	{0.11 ,	1.03}

Table 3.4: Coefficients from the random effects logistic regression. † For computational reasons, the "day since start of study" variable was mean-centred before performing the analysis by subtracting 13.90. Intercept estimates thus correspond to nurses washing their hands before or after type I contacts on this day of the study. Estimates were obtained using WinBUGS, using a run of 44,000 samples and burn-in of 1000 for the pre-contact data and 50,000 samples with a burn-in of 10,000 for post-contact data. Diffuse priors were used: ($N(0, 10^{-6})$ for slope parameters, and $\Gamma(0.001, 0.001)$ for precessions.

period (25 minutes), where only two beds were being observed, both at the time being visited by a ward round. This observation period in fact fell on the first day of the study, and was conducted to assess the feasibility of observing contacts during a ward round. It was therefore excluded from the subsequent analysis.

A three-level cross-classified model was therefore constructed, including all those explanatory variables that were significant in the two-level models. This three-level model is defined by equations 3.4 to 3.8.

$$c_{jk} \sim \text{poisson}(\mu_{jk}) \tag{3.4}$$

$$\log \mu_{jk} = a + \mathbf{bx_{jk}} + \log t_j + u_j + u_k + \lambda_{jk} \tag{3.5}$$

$$u_j \sim N(0, \sigma^2_{u_j}) \tag{3.6}$$

$$u_k \sim N(0, \sigma^2_{u_k}) \tag{3.7}$$

$$\lambda_{jk} \sim N(0, \sigma^2_{\lambda}) \tag{3.8}$$

Here c_{jk} represents the number of contacts received by patient k during the jth observation period. $\mathbf{b}$ is again the vector of slope parameters corresponding to the explanatory variables $\mathbf{x_{jk}}$. u_j and u_k are the random effects for the observation periods and patients respectively. λ_{jk} is the patient by observation period random effect which models extra-Poisson variability. Because different observation periods had different lengths, offsets $\log(t_j)$ are included, where t_j is the duration in minutes of the jth observation period.

Estimates for the elements of $\mathbf{b}$ significant in either of the 2-level models are given in table 3.5.

The rate ratios shown in table table 3.5 indicate how the per-patient contact-rates change with the explanatory variables. For categorical variables the contact rate is multiplied by the rate ratio: thus patients taking antibiotics are estimated to be contacted at 1.89 times the rate of patients

Parameter	Estimate	95% credible interval	Rate ratio
Fixed effects			
intercept (a)	-5.39	{-5.92, -4.90 }	
patient taking antibiotics	0.64	{0.31 , 0.98 }	1.89
Operation in last 24 hours	0.55	{ 0.07, 1.02 }	1.74
ln(days on ward)	-0.12	{ -0.28 , 0.03 }	0.88
Sex	0.34	{-0.10 , 0.80 }	1.40
ln(Age) (mean centred, mean=4.16)	0.71	{-0.02 , 1.42 }	2.03
Catheter	0.04	{-0.43 , 0.51 }	1.04
Other Line	0.40	{0.03 , 0.77 }	1.49
Random effects			
$\sigma^2_{u_j}$ (observation period)	0.26	{0.09, 0.53 }	
$\sigma^2_{u_k}$ (patient)	0.15	{0.01, 0.41 }	
σ^2_λ (extra-Poisson)	0.60	{0.36, 0.90 }	

Table 3.5: Parameter estimates for the multilevel Poisson regression model of patient-carer contact rates. Variables that were not significant in either of the 2-level models are not shown. Rate ratios are defined as *Exp(parameter estimate)*, and are discussed in the text. Estimates were obtained using WinBUGS, using a run of 100,000 samples and burn-in of 10,000. Diffuse priors were used: $N(0, 10^{-6})$ for slope parameters, and $\Gamma(0.001, 0.001)$ for precisions.

not taking antibiotics, other factors being equal. For non-categorical variables, the rate ratios give the factor by which the contact rate changes for a proportional change in the variable. Thus, increasing the number of days a patient has spent on the ward by a factor m is estimated to result in an increase in the contact rate by a factor equal to the rate ratio raised to the power $ln(m)$. So, for example, doubling the time spent on the ward is estimated to decrease the contact rate by a factor of 0.92 ($0.88^{ln(2)}$). Similarly, doubling the age is estimated to increase the contact rate by a factor 1.63. In fact the 95% credible intervals for both age and time spent on the ward contain zero. However both might be expected to be determinants of the contact rate *a priori*, and both intervals only just include zero, so they have been left in the model.

3.2.4 Contact patterns

Nurse-patient associations

Of the 25 patient-carer contact tables examined (corresponding to the observation periods where all beds in the ward were being observed and with sufficient contacts to make an assessment), six showed departures from homogeneity significant at the 5% level. An upper bound for the probability of this given that the null hypothesis can be calculated from the binomial distribution to be 0.0012. Thus, there is very strong evidence to suggest that a patient's contacts are not equally likely to come from each nurse, even when allowing for different nurses' contact rates.

Two potential sources of this heterogeneity were considered: nursing teams and named nurses. Firstly, all nurses were assigned to one of two teams (known as the red and blue teams). The blue team were primarily responsible for the care of patients in beds 1–7, and the red team for beds 8–16. Though all patients were in fact cared for by members of both teams, this team structure clearly provided a significant source of heterogeneity.

	Beds 1–7	Beds 8–16	Total
Blue Team	103	56	159
Red Team	93	156	249
Unknown team	4	0	4
Totals	200	212	412

Table 3.6: Patient-nurse contacts classified by team membership of nurses

Table 3.6 gives breakdown of the distribution of contacts to different beds by team membership. Thus, blue team nurses made just over 1.8 time as many patients contacts on beds 1–7 as on beds 8–16, while red team nurses made almost 1.7 times more contacts on beds 8–16.

To see how much of the heterogeneity this team structure accounted for, patient×nurse contact tables were again constructed as described above. However, data from each observation period were split into two tables: contacts on beds 1–7; and contacts on beds 8–16. When analysed as described above for the whole ward data the null hypothesis (the multinomial distribution) could be rejected in only 3 out 43 cases. The upper bound for the probability of this is now (from the binomial distribution) 0.167, and no longer provides sufficient evidence to reject the null hypothesis The exact likelihoods of each of the 43 tables could be combined, and a Monte Carlo sampling strategy used to estimate the chance of getting this or a lower likelihood. Unfortunately, while StatXact does support Monte Carlo sampling, sampling across multiple tables has not been implemented so no attempt to detect residual heterogeneity was made.

The other possible cause of heterogeneity considered arises from the recently introduced practice of "named nursing". A patient's named nurse is allocated when the patient is admitted to the ward, and is considered to be responsible for that patient from admission to discharge (Dooley, 1999).

Fifty-five patient-named nurse pairings were identified from the hospital computer systems (PAS), and 39 of these were confirmed at ward-level. Of

these, 23 pairs were seen to be present on the ward during the same observation periods. Two of these pairs corresponded to a long-stay patient whose named nurse changed mid-way through the study. To avoid the considerable complications this would have caused, all observations for this patient were excluded from the analysis.

Every patient's named nurse belonged to the team with primary responsibility for that patient's care. The patient-carer contact rates of the 21 named-nurse–patient pairs were therefore compared with contact rates for the 120 nurse–patient pairs for nurses who weren't named nurses, but who nonetheless belonged to the same team. In both cases, only observation periods where the presence of both patient and carer was confirmed and the patient was under observation were used. Contact rates were then calculated as the ratio of the total number of contacts observed between each pair, and the total time they were present during the same observation periods. Mean daily contact rates (and standard errors) for named-nurse–patient pairs, and non-named-nurse-patient pairs were 5.71(1.30) and 6.24 (0.88) respectively . Clearly these differences are not significant. Variances however did differ significantly between the two groups (P=0.007). However, the non-named-nurse—patient contact rates did contain one obvious outlier (with a contact rate of 64.0 $days^{-1}$). When this point was excluded the non-named-nurse—patient contact rate was reduced to 5.75, and the difference between the variances of the two groups was no longer significant at the 5% level (P=0.056). Overall, no evidence was found to suggest that named-nurses contacted their patients at rates differing from those of other members of the same nursing team.

Contacts in the ICU

Patient-carer contact rates recorded in the ICU are shown in table 3.7. The overall mean contact rate per patient was 144 contacts/day (total number

of contacts observed/total patient-days observed).

In contrast with the situation in the medical/surgical ward, a large proportion of each patient's contacts in the ICU came from a nurse specifically assigned to that patient's care. The last column in table 3.7 gives the proportion of contacts for each patient that came from this nurse. Of all the patient-carer contacts observed, 60% came from the patient's assigned nurse The remaining contacts were divided between other nurses (principally for procedures requiring more than one nurse), and other carers (doctors, physiotherapists etc).

3.2.5 Transmission data

Patient carriage

Details of all the patient swabs are given in tables 3.8 and 3.9. Of the 46 swabs that were taken as part of routine microbiological screening, the 18 routine screens for carriage usually only looked for MRSA strains, and would have failed to detect MSSA carraige.

Of the 50 patients who stayed on the ward during the observation period, swabs could not be obtained from 32 due to either refusal or inability of the patients to provide informed consent to take part in the study. No swabs at all (including routine swabs) were obtained from 18 of these patients.

Staff carriage

Table 3.10 gives the breakdown of the number of staff working in the ward during the study who were swabbed. Those carers present on the ward during the study who weren't swabbed either refused to take part in the study, were not present long enough for there to be an opportunity to swab them, or were present on the ward for too short a period time to merit swabbing. None of the four carers found to be carrying *S. aureus* were colonized with MRSA.

Patient	Observation Period	Duration of ob. period (hrs)	# Contacts from assigned nurse	#Contacts from others	Total contact rate (per day)	Proportion of contacts from assigned nurse
1	1	2.5	14	5	182.4	0.74
2	1	2.5	5	3	76.8	0.63
3	1	2.5	12	8	192	0.60
4	1	2.5	9	0	86.4	1.00
5	2	3.0	8	4	96	0.67
6	2	3.0	5	6	88	0.45
7	2	3.0	6	10	128	0.38
8	2	3.0	14	9	184	0.61
9	3	3.0	9	9	144	0.50
10	3	3.0	5	4	72	0.56
11	3	3.0	10	6	128	0.63
12	3	3.0	15	9	192	0.63
13	4	1.5	7	10	272	0.41
14	4	1.5	9	2	176	0.82
15	4	1.5	8	5	208	0.62
16	5	1.5	4	3	112	0.57
17	5	1.5	5	5	160	0.50
18	5	1.5	9	7	256	0.56
19	5	1.5	6	2	128	0.75

Table 3.7: Contact rates in the ICU

	Site swabbed		
	Carriage site	Wounds	All sites
Routine swabs	18 (3)	28 (11)	46 (14)
Study swabs	47 (10)	–	47 (10)
Total	65 (13)	28 (11)	93 (24)

Table 3.8: Number of swabs taken, both as part of the study, and for routine purposes, as part of normal hospital procedure. Numbers in brackets indicate *S. aureus* positive swabs. The carriage sites swabbed as part of the study were nose, throat and axillae. Routine swabs included swabs of throat, nose, axillae, ear, hairline, perineum, penis, sputum, or combinations of these.

	Site swabbed		
	Carriage site	Wounds	All sites
Routine swabs	7 (3)	15 (6)	20 (8)
Study swabs	18 (4)	–	18 (4)
Total	24 (5)	15 (6)	32 (10)

Table 3.9: Number of patients swabbed, both as part of the study, and for routine purposes, as part of normal hospital procedure. Numbers in brackets indicate the number of patients from whom *S. aureus* was recovered.

	Nurse	Student nurse	Doctor	Med. student	Other
Number on ward	19	4	30	3	24
Number swabbed	14(3)	3(0)	1(0)	1(1)	0 (0)

Table 3.10: Number of carers swabbed. Numbers in brackets indicate the number from whom *S. aureus* was recovered. No carer was swabbed more than once.

RFLP type	Approximate fragment size (bp) 500	450	400	320	240	160
A			+		+	+
B						
C		+	+	+	+	+
D			+		+	
E	+					
F			+	+		
G	+		+		+	
H	+				+	
I		+		+		
J			+			

Table 3.11: RFLP patterns of *Alu*I-digested coagulase gene PCR products (ignoring undigested fragments).

3.2.6 Typing of strains

From the 24 *S. aureus* positive swabs, after separating strains growing with distinct morphologies from each swab (or each pooled nose, throat and axillae swab) 50 isolates identified as *S. aureus* were selected for typing. Four of these (3b, 6, 22b and 36a) subsequently failed the Baird Parker test, and are not included in the rest of the analysis here.

Coagulase gene polymorphisms

Each *S. aureus* isolate produced a coagulase gene PCR product between approximately 500 and 900 bp. After digestion with *Alu*I nine distinct RFLP patterns were identified (table 3.11 and figure 3.3).

Protein A polymorphisms

45 of the isolates produced an amplicon ranging from about 340 to 660 bp following the protein A PCR. The number of 24bp repeat elements in the amplicon was estimated by subtracting 53bp (the unrepeated part of the amplicon (Uhlén *et al.*, 1984)) from the estimated size of the variable

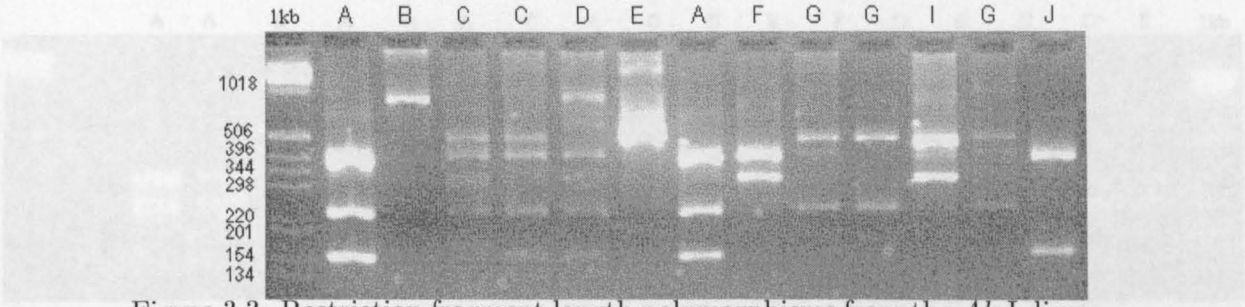

Figure 3.3: Restriction fragment length polymorphisms from the *Alu*I digestion of coagulase gene amplicons obtained from *S. aureus* strains isolated from patients and carers. Nine distinct patterns were observed. Before digestion amplicons were between approximately 500 and 900bp.

RFLP type	approx. restriction fragment sizes (bp)	estimated number of 24bp repeats
A	**450**	17
B	**240**	7
C	**330**	12
D	**300**	10
E	**150**	4
F	**260**	9
G	**130**	3

Table 3.12: RFLP patterns of *Rsa*I-digested protein A PCR products obtained from *S. aureus* strains isolated from patients and carers. The amplicon sizes correspond to fragments containing the 24-bp repeats in the X region of the *spa* gene.

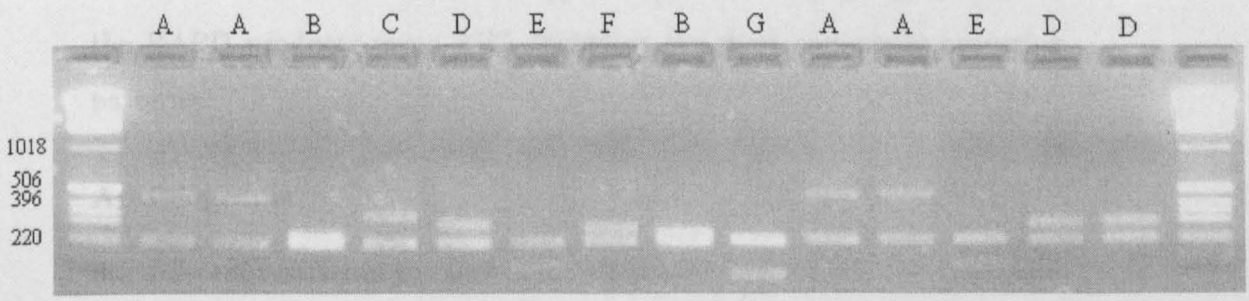

Figure 3.4: RFLPs from the *Rsa*I digestion of protein A gene amplicons obtained from *S. aureus* strains isolated from patients and carers. Seven distinct patterns were observed, containing between three and 17 24bp repeats in the variable size fragment. Three isolates produced no PCR products.

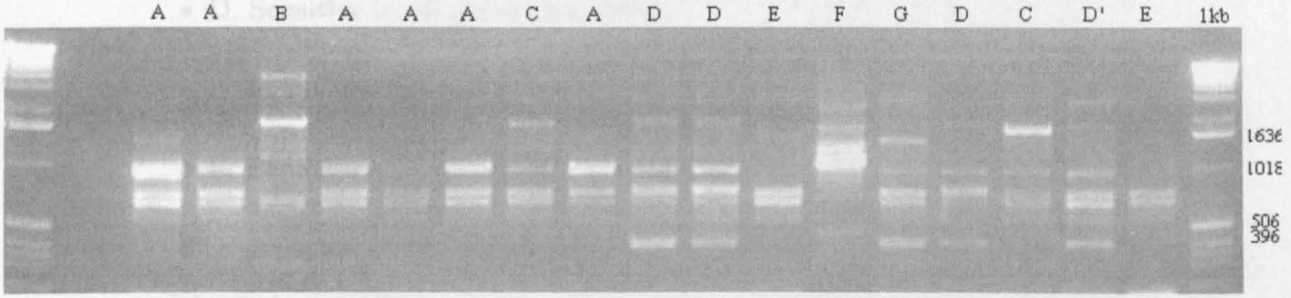

Figure 3.5: RAPD products obtained using the AP7 primer with *S. aureus* strains isolated from patients and carers. Eight distinct patterns were observed and seven isolates produced no products.

size fragment produced by the *Rsa*I digestion of protein A amplicon and dividing by 24. The was always a fragment of about 220bp. The seven distinct patterns observed are detailed in table 3.12 and shown in figure 3.4.

RAPDs

Eight distinct RAPD patterns were observed. These have been labelled A–H. All of these came from PCRs using the AP7 primer. AP4 could not reliably be made to produce RAPD products.

Seven of the isolates produced no discernible RAPD products, or bands too faint to determine a pattern type. Figure 3.5 displays a selection of the RAPD products using AP7, including the most commonly occurring patterns.

Antibiograms

Six different resistance patterns were found:

- A. Penicillin, Ciprofloxacin, Spiramycin, Erythromycin, Oxacillin
- B. Penicillin, Ciprofloxacin, Erythromycin, Oxacillin

- C. Penicillin
- D. Sensitive to all antibiotics tested
- E. Penicillin, Erythromycin
- F. Penicillin, Erythromycin (intermediate), Oxacillin

The presence of the *mecA* gene was confirmed in all strains showing resistance to oxacillin.

Summary of typing results

Table 3.13 summarises the phenotyping and genotyping results for the whole ward.

The results show that patients 1 and 2 were carrying indistinguishable MRSA strains. Isolates 19b and 8 also gave identical types under all four typing methods, suggesting that strains carried by patients 10 and 7 were related. Isolates 35 (from a nurse's hands) and 36b (from a different nurse's nose) also typed identically under all methods, suggesting a common source. Isolate 4a shared a coagulase type, RAPD type and antibiogram with 8 and 19b though differed in the protein A type. The number of protein A repeats has been reported to occasionally vary between strains otherwise typing identically (Hoefnagels-Schuermans *et al.*, 1997). It therefore seems likely that isolates 8 and 19b are epidemiologically related.

Isolate 11 differs from isolates recovered from patients 1 and 2 only in the protein A type and antibiogram. However, unlike those isolates, it was not an MRSA, but was in fact sensitive to all antibiotics tested. Poston *et al.* (1993) have reported that *mec* and *spa* are linked, and can sometimes be co-eliminated. It is conceivable that in this case *mec* could have been eliminated and a mutation in the *spa* locus occurred, but without further information they should be assumed to be different strains.

	Type codes							
Isolate #	coag RFLP	protein A RFLP	RAPD	antibiogram	swab date	patient #	swab sites	strain ID
1a	A	A	-	A	8/7/98	1	NTA	1
14a	A	A	A	B	8/7/98	1	NTA	1
14b	A	A	A	B	8/7/98	1	NTA	1
23	A	A	A	B	15/7/98	1	NTA	1
24a	A	A	-	B	15/7/98	1	NTA	1
24b	A	A	A	B	15/7/98	1	NTA	1
24c	A	A	A	-	15/ 7/98	1	NTA	1
26	A	A	A	B	21/ 7/98	1	NTA	1
27b	A	A	A	B	21/ 7/98	1	NTA	1
28a	A	A	A	B	21/ 7/98	1	NTA	1
27a	A	A	-	B	21/ 7/98	1	NTA	1
28b	A	A	-	B	21/7/98	1	NTA	1
15a	G	-	B	F	8/7/98	1	NTA	2
2	A	A	A	B	6/8/98	2	NTA	1
5a	A	A	A	B	28/7/98	2	W	1
9	A	A	A	B	6/8/98	2	NTA	1
17a	A	A	A	B	8/7/98	2	NTA	1
17b	A	A	A	B	8/7/98	2	NTA	1
18a	A	A	-	B	8/7/98	2	NTA	1
29	A	A	A	B	21/7/98	2	NTA	1
31a	A	A	A	B	28/7/98	2	NTA	1
32b	A	A	A	B	30/7/98	2	NTA	1
33a	A	A	A	B	30/7/98	2	NTA	1
25a	A	A	A	B	15/7/98	2	NTA	1
32a	A	A	A	B	30/7/98	2	NTA	1
33b	A	A	A	-	30/7/98	2	NTA	1
34	A	A	A	B	30/7/98	2	NTA	1
3a	B	B	C	C	5/8/98	3	W	3
11	A	C	A	D	24/7/98	4	W2	4
4a	B	D	D	C	29/7/98	4	W1	5
8	B	E	D	C	28/7/98	7	W	5
10	E	F	E	C	06/7/98	8	W1	6
13	E	F	E	C	28/7/98	8	W2	6
12	F	F	E	E	28/7/98	8	W2	7
16a	D	-	F	D	08/7/98	9	TA	8
19b	B	E	D	C	13/7/98	10	NTA	5
19a	F	-	G	C	13/7/98	10	NTA	9
21	I	B	C	C	14/7/98	11	NTA	10
7	D	G	D'	D	21/7/98	11	S	11
22a	G	B	E	C	14/7/98	M1	N	12
20a	G	B	D'	C	13/7/98	N1	N	13
30a	F	D	A	C	21/7/98	N2	N	14
30b	F	D	-	C	21/7/98	N2	N	14
35	J	D	H	C	5/8/98	N3	N	15
36b	J	D	H	C	5/8/98	N4	N	15
36c	J	D	-	C	5/8/98	N4	N	15

Table 3.13: Phenotyping and genotyping results for all isolates. Codes for swab sites are: N (nose), T (throat), A (axillae), W (wound/ulcer), S(sputum). RAPD types followed by a prime had the same bands, but in different relative intensities to the type without the prime.

Figure 3.6 provides a schematic illustration of all the ward strains and *S. aureus* colonization and transmission episodes on the ward during the study. For each day of each patient's stay on the ward, patients are classified as being either colonized with *S. aureus*, uncolonized, or of unknown colonization status. The colonization status was arrived at by making the following assumptions

- Once colonized, a patient is assumed to remain colonized with the same strain in the absence of treatment.

- If the first swab of a patient gives a *S. aureus* strain type not found among patients or staff on the ward prior to that date, then the patient is assumed to have been colonized with that strain on admission to the ward. Similarly, positive swabs taken within three days of the patient's admission or of the start of the study are assumed to be present when the patient entered, or at the beginning of the study if that occurred first.

- If the first swab of a patient gives a strain previously found among patients or staff, it is assumed that the strain was acquired by cross-infection.

- Negative swabs of carriage sites (nose, throat and axillae) are assumed to indicate that the patient was not colonized with any strain up to the date when the swab was taken, when other swabs (wound swabs) don't contradict this.

- Negative wound swabs, or routine MRSA screening swabs are assumed to provide no evidence for non-carriage of *S. aureus*.

- For patients for whom MRSA was successfully eradicated, it is assumed that patients are of unknown colonization status between the last negative and first positive swab. Two consecutive negative swabs

	Patient colonization status		
	colonized	uncolonized	unknown
carer colonized	0	1	3
carer uncolonized	5	11	4 (1)
carer status unknown	1	1	0

Table 3.14: Numbers of finger streaks taken from carers by colonization status of patients and carers. Plates were taken before and after all contacts, and from the left and right hands. *S. aureus* positive numbers are given in parenthesis.

following earlier positive swabs were assumed to indicate the loss of the carriage.

Finger streaks

For logistic reasons it proved impossible to take as many finger streaks as had been hoped from carers following contacts with patients known to be colonized with *S. aureus*. Instead, additional finger streaks were taken before and after contacts with patients of unknown colonization status, as well as with those who had screened negative for *S. aureus* carriage. Table 3.14 details these swabs. Of the 104 finger plates made (26 occasions, before and after contact, left and right hands) only one yielded *S. aureus*. This was obtained from a nurse who had screened negative for *S. aureus* carriage, following an assisted wash (contact type 2) of a patient of unknown colonization status. From table 3.13 it can be seen that this strain (isolate 35) has an identical type to one carried by nurse N3 (isolate 36). Interestingly, nurse N3 had helped the same patient take their tablets one hour earlier. Since the finger plates taken before the contact were negative, and finger plates were taken immediately after the patient contact, it appears that the patient became transiently contaminated from N3, and then passed on the organism to N4.

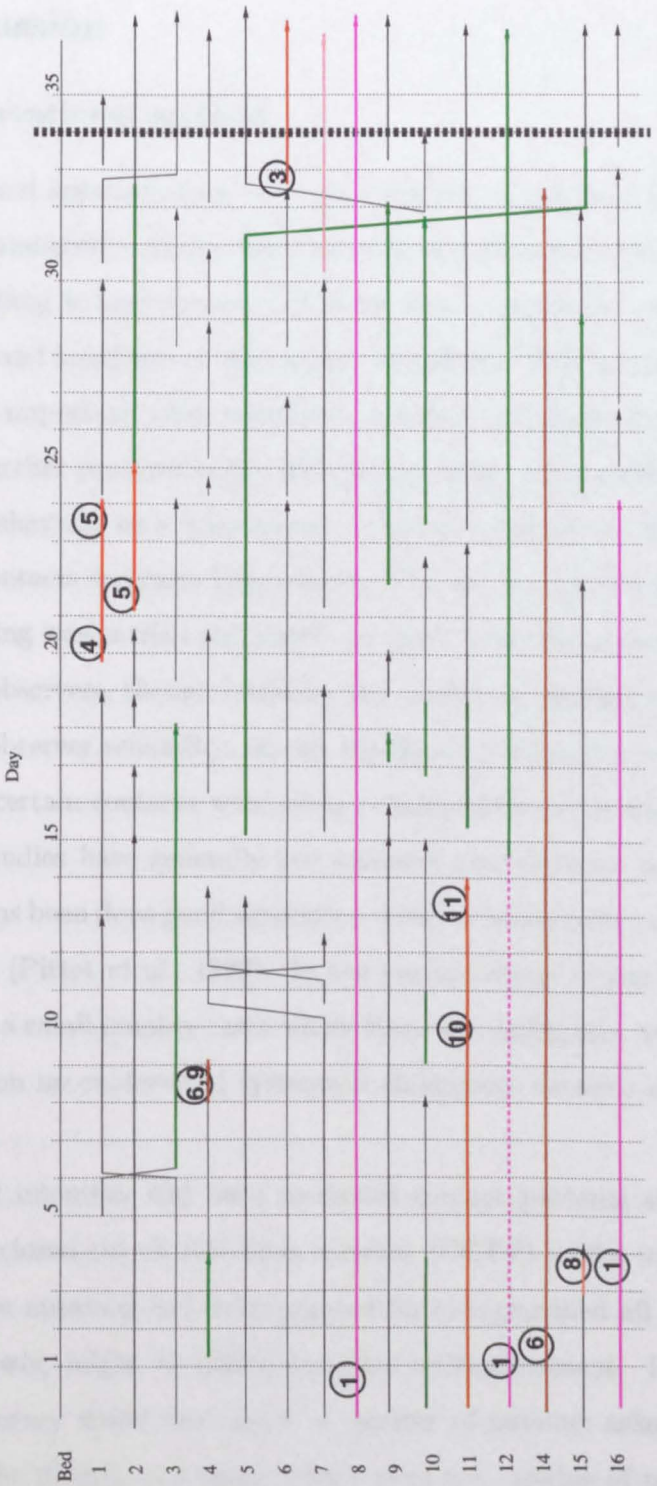

Figure 3.6: Ward stays and colonization status of patients. Arrows indicate patient stays. Green line segments indicate periods where patients were uncolonized. Other colours indicate carriage of particular strains (indicated by the circled numbers). Black and broken line segments indicate periods of unknown carriage status.

3.3 Discussion

3.3.1 Observational method

The observational approach used here has a number of potential problems. There may be ambiguity about when one contact ends and another begins, when handwashing is appropriate, and what level a particular contact is. Some contacts and handwashes may simply be missed. This last point may be particularly important when wards are crowded, and lines of vision are obscured. A further problem is the Hawthorne effect: the modification of the subject's behaviour as a consequence of being observed. In this study carer-patient contacts and carer handwashes were recorded by two observers. Initially recording was carried out jointly in order to ensure agreement between the two observers, though following this period no attempt was made to check inter-observer reliability, though checks on consistency were made to ensure that certain contacts were always classified the same way. Other handwashing studies have generally not assessed inter-observer reliability, but when this has been done good agreement between independent observers has been found (Pittet *et al.*, 1999). In the current study it was felt that there were only a small number cases where there was ambiguity, and in the logistic regression no evidence of systematic differences between observers was found.

The original intention had been to record contact patterns and ward activities using closed circuit television cameras (CCTV) in the ward, and ethics committee approval had been granted for this provided all patients and ward staff who might be filmed provided written consent. However, a preliminary survey found that about a quarter of patients asked would not consent to be filmed, and many others were not capable of providing informed consent. Also, one nurse out of the 19 asked would not consent to be filmed. Patients and carers could not be filmed without their consent, so it

would have only been possible to film for about a third of the time (less if the non-consenting nurse was present). Due to these difficulties, it was decided to abandon the use of CCTV for the current study. CCTV has, however, been used previously to conduct ward observations (Brown *et al.*, 1996), though solely for the purpose of recording carer handwashing practices. In that case the ward was a neonatal unit and patient privacy therefore not a major concern. Despite the privacy problems, CCTV does offer many advantages over direct observations: observations can be conducted in less time by fast-forwarding; data extracted from tapes can easily be validated at a later date by other investigators; it eliminates the extra risk of missing contacts during the busiest periods; carer behaviour is perhaps less likely to be affected as it easier to forget about the presence of a camera than a person (and carers may be misled as to the purpose of the cameras, as in the study by Brown and co-workers); potential biases due to the fact that not all beds could be observed during the busiest periods will be eliminated. Careful positioning of cameras should also be able to eliminate the problem with direct observations that a mass of bodies in the ward obscures the view to patient beds. For situations where consent is not required for CCTV use, this may therefore be preferable to direct recording of contacts.

Asking the patients to record the contacts themselves was also attempted, but it was immediately obvious that this would introduce large biases into the results. Those patients who required the most care were those least able and willing to record their own contacts, and patients who initially recorded their own contacts were unable to continue doing so post-operatively. The alternative of asking carers to record contacts was also rejected. It proved extremely unpopular with the nurses, who said they wouldn't have time, didn't think they would be able to accurately recall the numbers of patients contacted, and were unwilling to record contacts using simple push-button counting devices (which may also have provided a potential cross-infection

source). Other researchers have relied on self-reporting by those making the contacts (Edmunds *et al.*, 1997). However, reported contact rates were not verified by other means, and the reliability of such methods may be open to question.

One other possibility, that may in the future be the best way to collect these sorts of data, is to use electronic devices attached to each carer and patient that transmit information between each other using the conductivity of the skin. These could be used to record contacts, the identity of the person making the contact, contact duration, time etc, while maintaining patient and carer privacy. Such devices work on changes in capacitances, and so should still work if latex gloves are used (and could possibly even detect their use). However, at the moment such devices are in the prototype stage (Zimmerman, 1996), but could in the near future prove invaluable for such contact-tracing studies.

3.3.2 Patient consent

A major problem with this study was the very low number of consenting patients. Only 18 out of 50 patients consented to be swabbed. Reasons for non-consent were: too ill (3 patients); lack of English and no translators available (4); unconscious (3); too confused (5); lack of opportunity to request consent (5); psychotic (1); consent refused (11).

13 of the consenters were male, compared to 18 of the non-consenters, and the mean age of consenters was 63.3 compared to 65.4 for non consenters. Neither of these differences are statistically significant at the 5% level, but such a low consent rate does suggest that bias due to underlying illness etc may be important. Fortunately, this will only affect the transmission results, as contact patterns were recorded regardless of consent.

The requirement of patient consent may represent one of the reasons why studies where all patients entering a ward are systematically swabbed at

regular intervals have rarely been carried out in recent years. This represents a major problem for assessing the amount of transmission and hence the impact of any control measures. One solution may be to implement routine admission and weekly swabbing as part of the standard practice for hospital wards being studied, thus circumventing the need for patient consent. An alternative approach appropriate to intensive care settings—that adopted by Talon *et al.* (1995)—is to obtain consent from relatives rather than patients themselves.

3.3.3 Handwashing

In contrast to previous studies (reviewed by Larson and Kretzer, 1995), data were recorded and analysed to allow handwashing to be related to contact type, carer details, and factors related to the observation period. One recent study has presented a comparable analysis, using a logistic regression model to explore the factors associated with handwashing (Pittet *et al.*, 1999). Unlike that study, in this study HCWs were assigned unique IDs, allowing heterogeneity in handwashing frequencies between carers to be explored.

The estimated handwashing frequency of about 40% was similar to that reported elsewhere. However, it was found to vary enormously according to the type of contact made: simple contacts had the lowest handwashing frequency, while more prolonged contacts and invasive or semi-invasive procedures the highest, both before and after patient contacts. These effects were large and highly significant. The finding that doctors washed their hands far less frequently than nurses was also in accordance with previous studies. Again, this effect was large and highly significant. An important difference to previous studies is that in this case the difference cannot simply be attributed to different types of contacts being made, as these are included in the regression. In fact, it was found that doctors and nurses made similar proportions of type 1, 2 and 3 contacts. More surprising is the

finding that health care assistants were less likely to wash their hands before and after contacts than nurses. However, since there were only three health care assistants observed, this difference should be treated with caution. The finding that other HCWs were less likely to wash their hands before (but not after) contacts is also surprising. However, since none of these HCWs were based on the ward it is possible that handwashes occurred before they entered the ward and were therefore unobserved.

The handwashing frequency of nurses was found to decrease slightly with time from the start of the study. One possible explanation of this is that nurses washed their hands more often at the start of the study, knowing that they were being observed, but as they became used to the observers, levels fell. It is interesting that the opposite pattern was seen in other HCWs (physiotherapists, phlebotomists etc): there was a suggestion that these carers increased their handwashing frequency during the study. Since these carers weren't informed that a study was taking place at the beginning, but may have become aware of the fact later on, this observation is consistent with the hypothesis that the presence of observers accounted for the difference.

After accounting for the type of HCW, the type of contacts made, and the observation period, no further variation in handwashing frequency was found between individuals. This is also somewhat surprising, but should not be taken to mean that such variation does not exist. It does however suggest that poor handwashing compliance is a problem amongst HCWs in general, rather than a few poorly-performing individuals. Interventions aimed at increasing compliance should not be aimed only at the individuals considered to be the worst offenders, but rather at groups of carers as a whole. There was, however, found to be significant variation in handwashing frequency according to the observation period. In an attempt to find plausible sources for this heterogeneity the following factors were considered: time of

observation period (AM or PM); observers present; presence or absence of influential staff members (ward sisters); activity levels on the ward. None were found to approach significance. Since there was some evidence of heterogeneity between carers when heterogeneity between observation periods was not considered, it seems likely that the composition of the staff on duty during different observation periods may in fact account for some of this heterogeneity.

Some of the negative findings in this study are particularly interesting (though again must be interpreted cautiously, as effects may have been real and important but too small to detect): carers were found to be no more likely to wash their hands if a sink was adjacent to the bed, or if the patient was known to be MRSA positive. Furthermore, during the study it became known to the staff that there were three MRSA positive patients on the ward. Alcohol handscrub was placed next to each bed, and there was a visit from the infection control team. Despite these measures, handwashing frequencies before and after these interventions did not differ significantly. This is consistent with other studies based on didactic interventions (Larson and Kretzer, 1995). In contrast to the findings of Pittet *et al.* (1999), handwashing frequency was not found to be influenced by the activity level on the ward. This was true for all three measures of activity level used. This has a direct bearing on the discussion of staff-patient ratios, and is discussed further in chapter 4.

3.3.4 Contact patterns

This study represents one of the first attempts to systematically record and analyse patient-carer contact rates, and factors associated with these rates. Of particular interest are the results that patients who were taking antibiotics had contact rates almost twice that of those who weren't. For pathogens transmitted by direct contact this could represent one previously

unconsidered explanation for the association of antibiotic use with the acquisition of such organisms. If antibiotics provide an additional risk themselves, this observation can have important implications for the transmission dynamics (see chapter 4). Note that some of the additional contacts of patients taking antibiotics may be related to the administration of the antibiotics themselves. Patients having had an operation in the last 24 hours also had substantially higher contact rates. After these two factors had been accounted for, the presence of a line (apart from a catheter, nasal-gastric tube and oxygen line) was found to be associated with a more modest increase in contact rate. There was also a suggestion that men had higher contact rates than women, and that contact rates increased with age. These observations may partly explain some of the reported risk factors for acquiring hospital pathogens (for example Asensio *et al.*, 1996).

After accounting for these factors, there remained significant heterogeneity in contact rates associated with the observation period, suggesting that other factors not included in the model affected the contact rate. Clearly time of day is likely to be one factor, as patterns of ward activity change greatly throughout the day.

About half as much heterogeneity again could be explained by remaining variation amongst the patients due to factors not included above. However, by far the largest source of additional variation in contact rates was associated with term for extra-Poisson variation. Factors likely to lead to the aggregation of contacts that this extra variation represents may include the fact that some procedures require more than one contact, or may involve more than one contact if the carer is interrupted (as was sometimes observed). Also, some procedures may require more than one carer, again leading to aggregation. The most important factor for aggregation, however, is likely to be underlying illness, which may clearly vary considerably within individual patients from day to day. For some periods patients were

not actually present in the ward during the observations, and contact rates were then recorded as zero, again leading to aggregation.

3.3.5 Hand contamination

Measurements of hand contamination can enable the estimation of the patient-to-carer transmissibility, an important model parameter (see chapter 2, section 2.2.2). Consequently the possibility of using random sampling of carers' hands to plot the change in contamination levels as a function of time was considered, thus enabling an estimation of the parameter based on a knowledge of the contact rate and handwashing frequency. However, simulation studies suggested several hundred swabs would be required to obtain even a very rough estimate of the parameter. Because of this it was decided to sample hands directly after contacts with *S. aureus* colonized patients instead. The frequency of detectable hand contamination found this way appears to be low when compared to other studies with different organisms (Casewell and Phillips, 1977), but in fact only six streaks were taken following contact with known *S. aureus* carriers, so the sample is very small. Also, the finger streak method used is known to be about half as sensitive as the gloved hand method (Ayliffe *et al.*, 1975).

Hand-sampling following individual contacts should, however, be far more informative than the more common practice of taken random hand samples (Ayliffe *et al.*, 1979, 1975; Bauer *et al.*, 1990) Indeed, it has been pointed out elsewhere that the low prevalences of hand contamination typically found when carers' hands are sampled at random follows from a consideration of the transmission dynamics, a result confirmed by the above-mentioned simulation studies (Austin *et al.*, 1999b). However, lack of time amongst carers can make the collection of such data difficult.

3.3.6 Typing

The utility of the protein A gene for typing may be questioned, based on the above observation that organisms apparently coming from a single strain appeared to change their protein A type on one occasion. Indeed, while some researches have reported the number of 24bp repeats to be stable over long periods of time (Frénay *et al.*, 1994), others have found it to be variable in otherwise identical strains (Trzciński *et al.*, 1997; van Belkum *et al.*, 1997; Hoefnagels-Schuermans *et al.*, 1997). In this study, the protein A type was interpreted as providing evidence of a common source for strains when isolates had the same type, but different protein A types were not assumed to provide evidence of different sources.

The ward MRSA strain in this study had a surprisingly large number of 24bp repeats (17). This is potentially interesting in light of the link reported by Frénay *et al.* (1994) between the number of repeats and epidemicity. However, van Belkum *et al.* (1997) found no correlation between protein A repeats and the ability of a strain to colonize the nose, and suggested that no single lineage has an increased propensity for nasal colonization. Poston *et al.* (1993) found that the protein A gene didn't seem to be linked to adhesion properties of *S. aureus* to cells they tested. Hoefnagels-Schuermans *et al.* (1997) also found no connection between spreading behaviour and number of 24bp repeats in 43 MRSA strains, although in their isolates the number of repeats only ranged from nine to eleven. Any such link must therefore be considered to be highly speculative.

3.3.7 Further analysis

Further analysis of some of the data collected in this study is carried out elsewhere. The ward transmission data are considered further in chapter 8 and the consequences of patient heterogeneity and patient-carer contact patterns are examined in more detail in chapter 4.

Chapter 4

Extensions to the basic model: patient and contact pattern heterogeneity

4.1 Motivation

In previous chapters it was assumed that contact patterns between patients and carers were homogeneous; that each patient contact was equally likely to be met by each of the carers on the ward, and that all patients required contacts at the same rate.

The data described in chapter 3 contradict this assumption. The following sources of heterogeneity were identified:

- Some patients require more frequent contacts than others;
- On some wards nurses work in teams, where one team is more likely to contact one particular group of patients;
- On ICUs at any one time most of a given patient's contacts will be made by one particular nurse.

Other factors, such as antibiotic use and the type of required contacts may contribute to further heterogeneity and cause some patients to be more likely than others to acquire an organism when contacted by a transiently colonized carer. Similarly, carers may be more likely to become colonized on contact with certain colonized patients, again as a result of the types of contact and other host factors (Cookson *et al.*, 1989).

Such heterogeneity is important because it may impact on the dynamics of hospital infections, affecting the chance of an organism becoming established in a hospital, the endemic level given that it becomes established, and the chance of stochastic fade-outs occurring given this endemicity. Consequently, such heterogeneity may have implications for a number of patient management issues including: segregation of different classes of patients into different wards, or parts of wards; carer-to-patient ratios; nursing teams; and cohort nursing.

In this chapter the assumption of homogeneous mixing is relaxed, and structure is allowed in the contact patterns between patients and carers. Heterogeneity between the patients is also considered.

4.2 Method

The modelling framework of Hasibeder and Dye is adopted (Dye and Hasibeder, 1986; Hasibeder and Dye, 1988), and adapted so that it is applicable to the small populations under consideration. General results are presented, and this framework is then used to explore three different aspects of contact pattern heterogeneity: nursing teams (partial cohorting); "named-nurses" and ICU-type contact patterns, where each patient is assigned a single nurse who is responsible for a disproportionate amount of their care; and patient heterogeneity (some patients need more contacts than others; some patients are more susceptible to colonization than others). In each case threshold conditions for invasion are first considered and (where possible), determin-

istic endemic levels are calculated. Finally, stochastic effects are explored through simulation experiments using standard methods implemented in a C++ program available on request from the author.

4.2.1 Modelling framework

The basic host-vector framework of chapter 2 is retained, but each patient is now assumed to belong to one of g groups, and each carer to one of g' groups. The mixing patterns between the different groups are now determined by the parameters γ_{ij},where $1 \leq i \leq g$ and $1 \leq j \leq g'$. γ_{ij} represents the proportion of the contacts of patients in group i that are made by carers in group j. Clearly, $\sum_j \gamma_{ij} = 1$.

In the deterministic formulation, the system can then be described by the equations

$$\frac{dy_i}{dt} = \beta_i \left(\sum_j \gamma_{ij} \frac{y'_j}{n'_j} \right) x_i - \mu y_i + \sigma \mu n_i \tag{4.1}$$

$$\frac{dy'_j}{dt} = \left(\sum_i \beta'_i \gamma_{ij} y_i \right) \frac{x'_j}{n'_j} - \mu' y'_j \tag{4.2}$$

Here y_i and y'_j are the number of colonized patients in group i and colonized carers in group j respectively, and x_i and x'_j the number of uncolonized patients and carers in these groups. β_i represents the product of the rate contacts are made on patients in group i and the probability of transmission to uncolonized patients on contact with colonized carers. β'_j is the same, except that transmission is now to uncolonized carers from colonized patients. As before, σ gives the proportion of patients who are colonized on admission. μ is the patient discharge rate and μ' the carer handwashing rate. n_i and n'_j represent the number of patients and carers respectively in patient group i and carer group j. Since both of these are assumed to remain constant x_i and x'_j are given by $n_i - y_i$ and $n'_j - y'_j$, and the system is completely determined by the above equations.

In the case where $\sigma = 0$, these equations are analogous to those obtained by Dye and Hasibeder (1988) for modelling vector-borne disease transmission dynamics in a heterogeneous population. In this case, however, there is the important difference that it is assumed that the host (patient) contact rate doesn't change with vector (carer) density. Since these contacts are driven by patients' needs, this is a natural assumption, given that there are adequate staffing levels, and is supported by reports of nursing workload falling with decreases in the number of patients (Farrington *et al.*, 1998).

Another important distinction is that in this case a finite (and small) population is being considered, and y_i and y'_j take only integer values. In contrast, Dye and Hasibeder were considering large populations where the deterministic and continuous treatment is more readily justified.

When considering the basic reproduction number, R_0, in a stochastic and individual-based framework the formulation from the mean-field model may need to be modified. Expressions for approximations for R_0 for the current model are derived which are closely related to those obtained by Dye and Hasibeder for the deterministic model. Limitations of these approximations are considered in the discussion.

4.2.2 Sources of heterogeneity

In the next section deterministic and stochastic results are presented for three different scenarios:

- Nursing teams ($g = g' = 2$).

  Two nursing teams are considered, each with primary responsibility for one group of patients' care. Both groups of patients are assumed to require contacts at the same rate. This corresponds to the contact patterns observed in the observational study presented in chapter 3. The situation where patients in one of the groups are more susceptible to colonization is also considered.

- Patient heterogeneity $(g = 2; g' = 1)$.

  All nursing staff are assumed to have equal responsibility for treating all patients, but some patients are assumed to require more contacts than others. Again, the motivation comes from ward-based observations of contact patterns. The situation where patients requiring more contacts are more susceptible is also considered. This is also motivated by the observation in chapter 3 that patients taking antibiotics also had much higher contact rates.

- Assigned nurses $(g' = n')$.

  In this case a disproportionate amount of a given patient's care comes from one assigned nurse. Though no evidence was found for this from the observations on the medical/surgical ward presented in chapter 3, in some cases a patient's named nurse may tend to have a disproportionate role in their care (Dooley, 1999). Contact patterns on ICUs also follow this pattern, with each nurse typically assigned to one or two patients and contributing only occasionally to the care of other patients. In this case carer groups consist of only a single individual while patient groups may range from a few individuals on most wards to a single individual on an ICU.

4.3 Results

4.3.1 The basic reproduction ratio

Following Diekmann *et al.* (1990), the basic reproduction ratio for the model with non-homogeneous mixing and patient heterogeneity is considered to be the dominant eigen-value of the next-generation matrix for the linearized process. Rather than taking the approach given in the example of Diekmann *et al.*, the more natural approach of Macdonald (see Bailey, 1975b, and

references therein) is adopted, and the new cases amongst hosts (patients) are considered to constitute the next generation, rather than the transient colonization of vectors (carers).

The epidemic process is approximated by making two simplifying assumptions. Firstly it is assumed that the expected number of transiently colonized carers at any one time depends only on the number of colonized patients at that time. That is, transient effects can be ignored for the carer colonization process. This assumption is justified by the fact that carer colonization and loss of colonization occur on a much faster timescale than patient colonization: while a patient's length of stay is typically of the order of several days, the duration of transient colonization of carers will typically range from a few hours to minutes.

The second assumption is that all carers contacted by colonized patients are uncolonized, and all patients contacted by colonized carers are uncolonized, unless they are themselves the source of the transmission under consideration. In most situations this will be a reasonable assumption at the start of an outbreak. Observations have shown the prevalence of hand colonization with *S. aureus* amongst staff to be low. Limitations of this assumption are considered in the discussion

Because of these assumptions, only approximations to the basic reproduction number are obtained here. In practice, for most realistic parameter values, they represent upper bounds to the true values.

With these assumptions, if the patient is assumed to belong to group i, and the carer to group j, then from equations 4.1 and 4.2 a colonized patient will have a mean length of stay of $1/\mu$, and during this time each susceptible carer will become transiently colonized with this strain according to a Poisson process with intensity $\beta_i'\gamma_{ij}/n_j'$. Assuming that every carer contacted by the index patient is uncontaminated gives the upper bound for the mean number of carers colonized by this patient: $\beta_i'\gamma_{ij}/(\mu n_j')$.

Similarly, the rate a susceptible patient becomes colonized from a colonized carer is $\beta\gamma_{ij}/n'_j$. The mean number of patients colonized before the hand contamination is cleared will therefore be at most $\beta_i\gamma_{ij}/(\mu' n'_j)$.

Putting these two expressions together gives an expression for the mean number of secondary cases of patient colonization in patient group k caused by one colonized patient in group i. If $i = k$ this is:

$$\frac{\beta_i}{\mu} \sum_j \frac{\beta'_i \gamma_{ij}^2 (n_i - 1)}{\mu' n'_j} \tag{4.3}$$

and if $i \neq k$ it is given by

$$\frac{\beta_k}{\mu} \sum_j \frac{\beta'_i \gamma_{ij} \gamma_{kj}}{\mu' n'_j} \tag{4.4}$$

The expected number and distribution of new cases in the patient groups ($\mathbf{y_1}$) caused by initial cases ($\mathbf{y_0}$) is then given by $\mathbf{y_1} = W\mathbf{y_0}$, where W is the next-generation matrix.

The diagonal elements of w_{ii} of W are given by expression 4.3 above and the off-diagonal elements w_{ik} are given by expression 4.4.

If λ_i are the eigenvalues of W, and $\zeta = max(\lambda_i)$ then, if $\mathbf{w}_\zeta$ is the corresponding eigenvector $\mathbf{y_n}$ will approach $k\zeta^n \mathbf{w}_\zeta$ for some constant k. The condition for the number of colonized patients to increase, initially, is therefore $\zeta > 1$, and the initial course of the epidemic is determined by the dominant eigenvalue. W is a square non-negative matrix, and as long as it is also irreducible it can be shown that ζ must be real and non-negative (Hasibeder and Dye, 1988). If W is not irreducible the patients may be divided up into two or more groups of patients each having no chance of causing infections in other groups (however indirectly). In such cases the patients belong to two or more distinct populations and the ward may be divided into two or more subwards, each having an irreducible next generation matrix. These subwards are then independent and are the units to which the analysis should be applied.

4.3.2 Nursing teams

In this case there are two nursing teams and two groups of patients. It is assumed that contact patterns between these groups are symmetrical: patients in group 1 get the same proportion of contacts from carers in group 2 as patients in group 2 get from carers in group 1. Then $\gamma_{ii} = 1-k, i = 1, 2$, and $\gamma_{ij} = k, i \neq j$. Here $0 \leq k \leq 1$, and k represents the proportion of contacts that patients in one group get from one group of carers (so if $k = 0.5$ the mixing is homogeneous).

If both groups of patients and both groups of carers have the same sizes ($n_1 = n_2 = n/2$ and $n'_1 = n'_2 = n'/2$), and the same contact rates and susceptibilities to becoming colonized ($\beta_1 = \beta_2 = \beta$ and $\beta'_1 = \beta'_2 = \beta'$) , then the dominant eigenvalue, ζ, of the matrix W is given by

$$\zeta = \frac{\beta\beta'(2k^2 - 2k + 1)(n-2)}{\mu\mu' n'} + \sqrt{(4k^4 - 8k^3 + 4k^2)n^2 - (4k^2 - 4k + 2)^2(n-1)} \tag{4.5}$$

Figure 4.1 shows how this changes with k, and figure 4.3 presents corresponding simulation results.

If the $n_i - 1$ term in expression 4.3 is replaced with n_i, then all the terms in k in equation 4.5 cancel and the dominant eigenvalue is given by

$$\frac{\beta\beta' n_g}{2\mu\mu' n'_g}$$

which is the basic reproduction number for the continuous deterministic model. Thus the only effect of the team structure on the invasibility criterion is caused by the small reduction in the number of susceptibles becoming more important as there is more separation between the two groups of patients. Unless the number of patients in each group is very small, this effect is likely to be of little importance.

When the two patient groups have different properties, so that one is more susceptible to colonization than the other (as might, for example, happen when pre-operative and post-operative patients are separated on

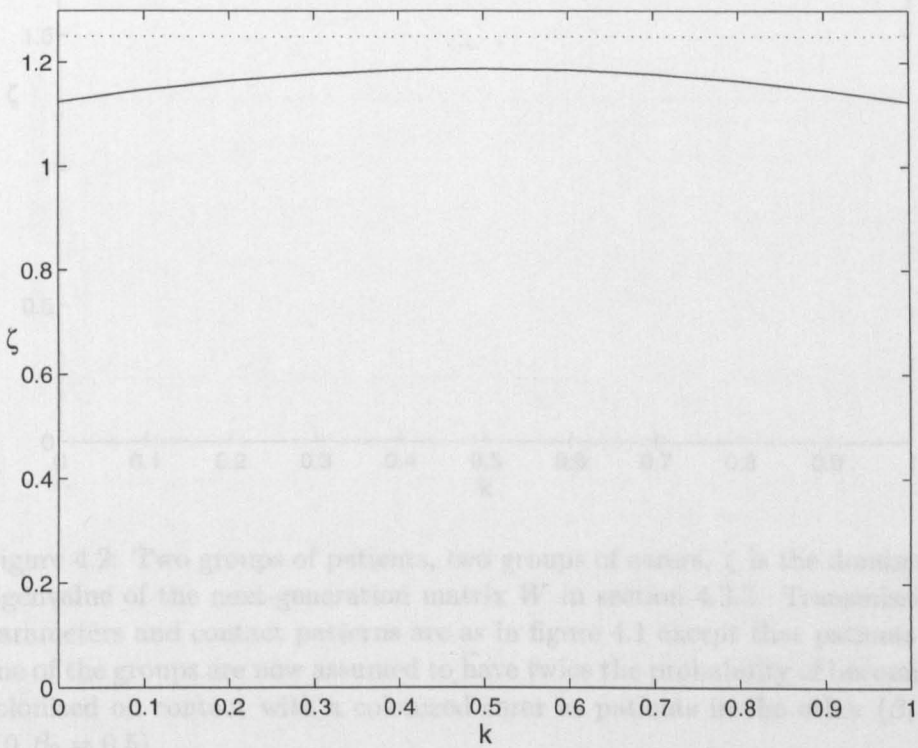

Figure 4.1: Two groups of patients, two groups of carers. ζ is the dominant eigenvalue of the next-generation matrix W defined in section 4.3.2 and represents the approximation to the basic reproduction number for this model (and is given by equation 4.5). k is the mixing parameter, and represents the proportion of the contacts made by carers of a single team on patients of a single group. Other parameters are: $n_1 = n_2 = 10; n_1' = n_2' = 2; \beta_1 = \beta_2 = 0.5; \beta_1' = \beta_2' = 1; \mu = 0.1; \mu' = 20; \sigma = 0$. The broken line shows the value of R_0 for the model, obtained by replacing the $n_i - 1$ terms with n_i in expression 4.3.

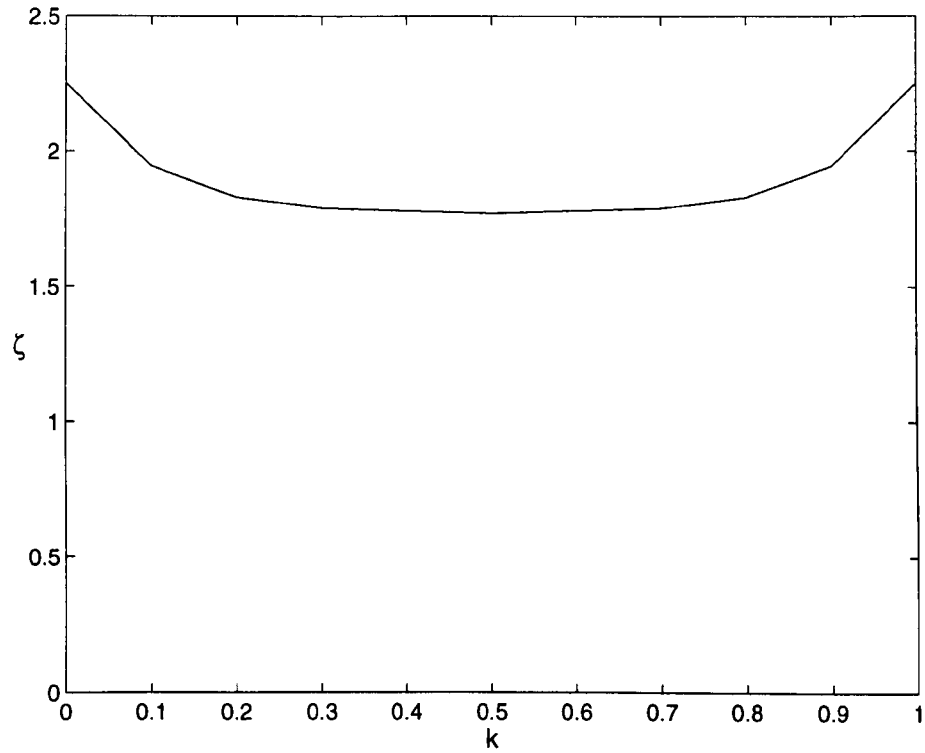

Figure 4.2: Two groups of patients, two groups of carers. ζ is the dominant eigenvalue of the next-generation matrix W in section 4.3.2. Transmission parameters and contact patterns are as in figure 4.1 except that patients in one of the groups are now assumed to have twice the probability of becoming colonized on contact with a colonized carer as patients in the other ($\beta_1 = 1.0, \beta_2 = 0.5$).

a ward), then the heterogeneity in contact patterns has the opposite effect (see figure 4.2): grouping the high risk patients together increases the ability of the pathogen to invade.

Prevalence

In general it is not possible to obtain an equilibrium solution to the deterministic equations for this 2×2 model, or for more complex models. In the symmetric case, however, where the parameters are the same within the patient and carer groups, and where the mixing matrix with elements γ_{ij} is also symmetric, then it is easy to show that the equilibrium solution doesn't

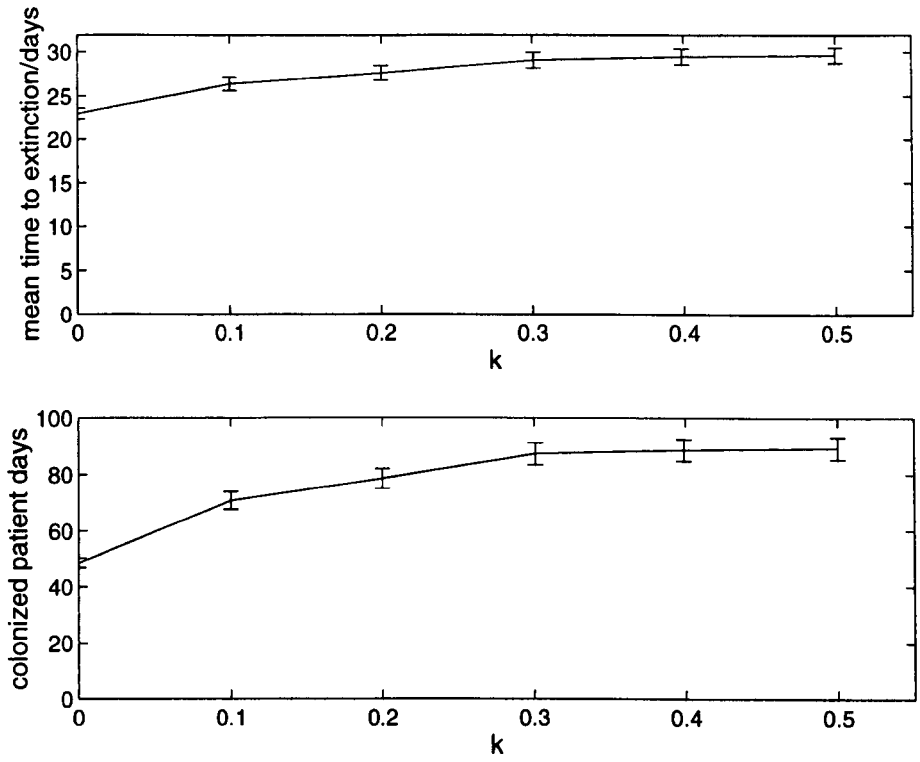

Figure 4.3: Simulation results from the stochastic model described in section 4.3.2 with two patient groups and two carer groups. Parameters and mixing patterns are the same as those listed in figure 4.1. Means are based on 10,000 simulation runs, and 95% confidence intervals for these means are shown.

change with k and is identical to that in the case of homogeneous mixing (this follows from the fact that for one value of k, the mixing patterns will in fact be homogeneous mixing). The same conclusion is reached for all such symmetric mixing patterns, where the ratio of the number of carers to the number of patients within each group doesn't change.

In the case where one of the patient groups is more susceptible to becoming colonized than the other, then in the deterministic model the prevalence in each group will vary as shown in figure 4.4, where the deterministic equilibrium has been determined numerically using Maple (Waterloo Software, 1996). As expected, the effect of increasing the separation between the groups is to increase the prevalence in the high risk group and decrease it in the low risk group. The mean of the quasi-stationary solution to the stochastic process would be expected to be close to these deterministic means.

4.3.3 Patient heterogeneity

In this scenario there are two groups of patients but only one group of carers. One of the patient groups receives a disproportionate amount of the total number of contacts. The total patient contact rate (and hence the work rate of the carers) is assumed to remain constant. In this case W is a 2×2 matrix. The dominant eigenvalue, ζ, is easily calculated, and the solid line in figure 4.5 shows how this changes as the contact rates in the two patient groups diverge. Increasing the proportion of contacts received by group A patients from 50% (homogeneous mixing) to 97.5% results in a substantial increase in ζ. However, the graph suggests that the effect is only likely to be important if one group of patients accounts for a very large proportion of the total contacts. Even when one group of patients receives three quarters of all contacts, the increase in ζ is fairly small. If patients in the group requiring more contacts also have a greater chance of becoming colonized on contact with a colonized carer then the effect of the heterogeneity becomes far more

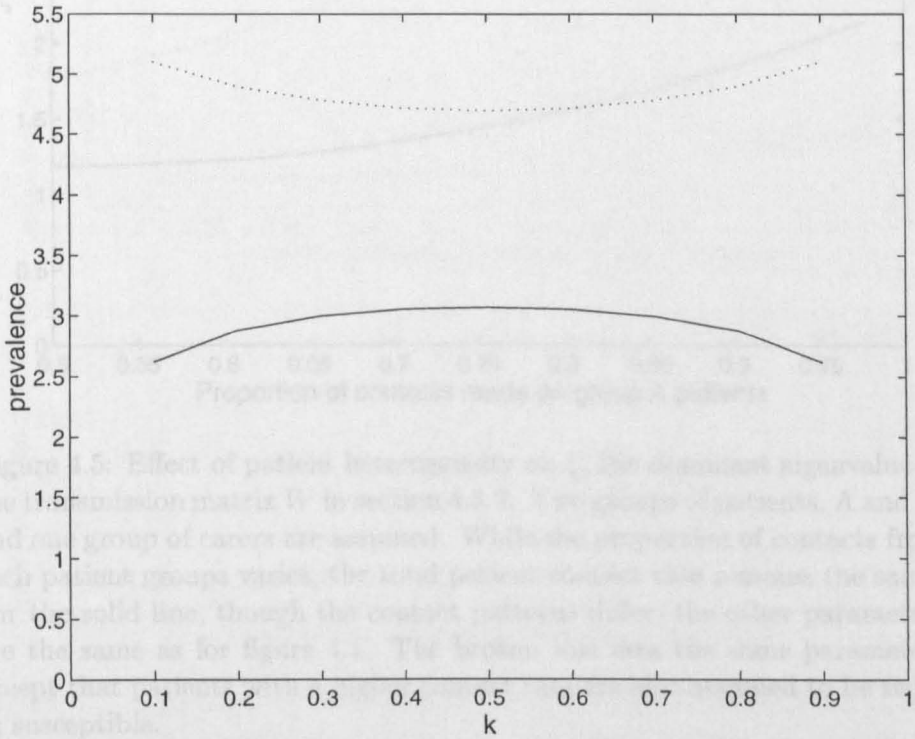

Figure 4.4: Prevalences from the deterministic model of two groups of patients, two groups of carers, where patients in one group (broken line) are twice as susceptible as patients in the other group (solid line). Parameters are the same as those used in figure 4.2.

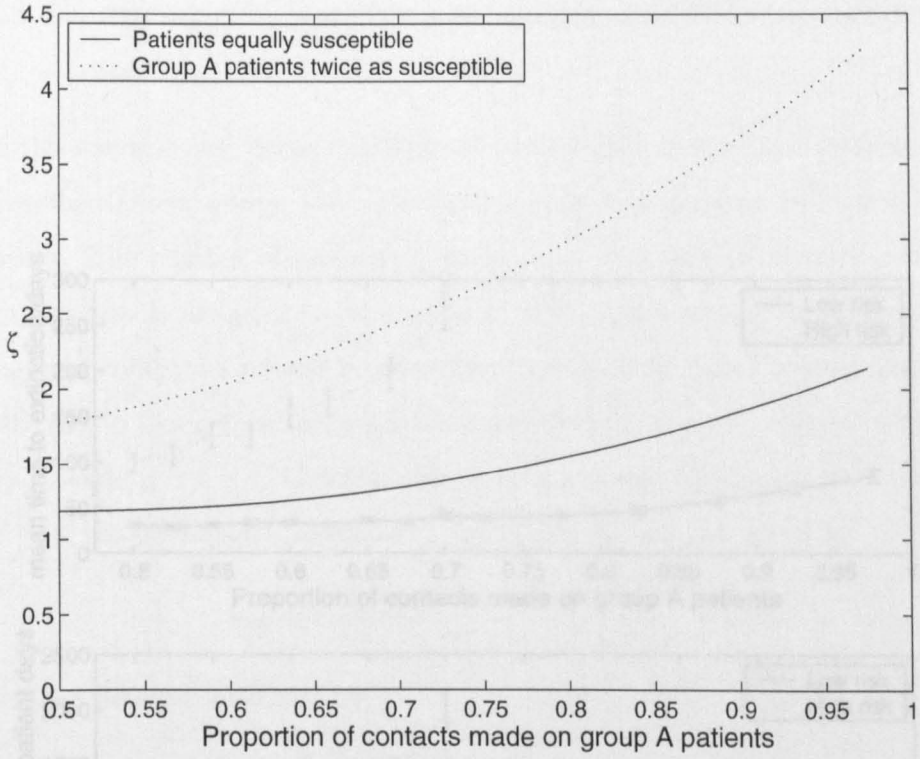

Figure 4.5: Effect of patient heterogeneity on ζ, the dominant eigenvalue of the transmission matrix W in section 4.3.3. Two groups of patients, A and B, and one group of carers are assumed. While the proportion of contacts from each patient groups varies, the total patient contact rate remains the same. For the solid line, though the contact patterns differ, the other parameters are the same as for figure 4.1. The broken line uses the same parameters except that patients with a higher contact rate are also assumed to be twice as susceptible.

important. This is shown in the broken line in figure 4.5, where group A patients are assumed to have twice the probability of becoming colonized on contact with a colonized carer. Figure 4.6 presents corresponding simulation results, and emphasises that such effects can greatly increase the ability of a pathogen to persist, particularly when higher contact rates in some patients are accompanied by a greater susceptibility to colonization.

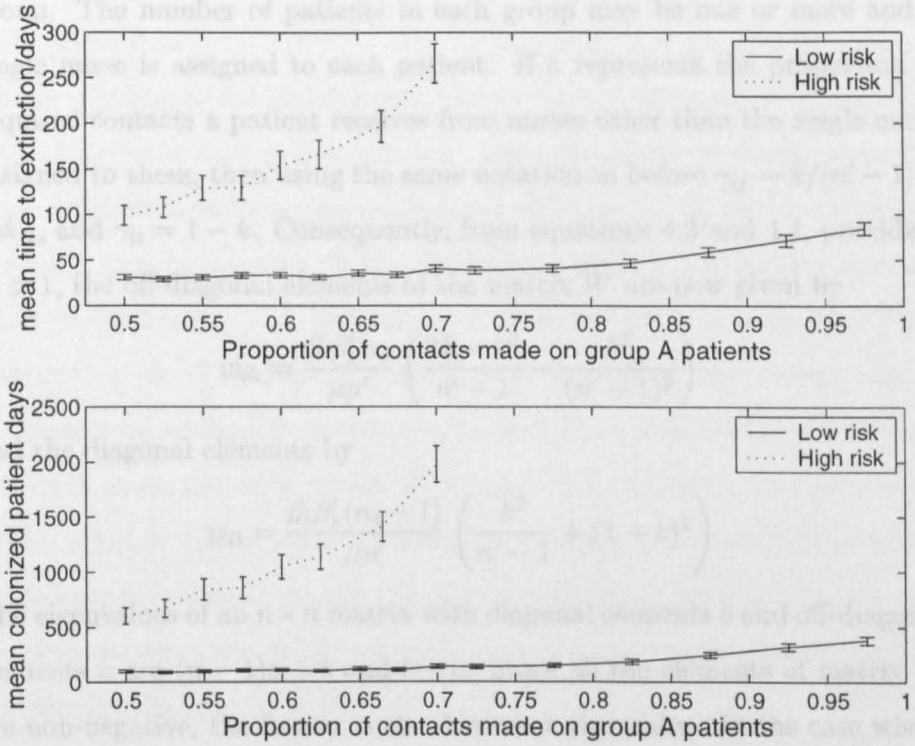

Figure 4.6: Simulation results from the stochastic models described in section 4.3.3 (and corresponding to the two models in figure 4.5). There are two patient groups, A and B, and one carer group. The graph illustrates the effect of heterogeneity in contact patterns alone, and heterogeneity in contact patterns and patient susceptibilities combined. For the solid line parameters not related to the contact pattern are the same as for figure 4.1. The broken line corresponds to the case where patients with a higher contact rate are assumed to be twice as susceptible. Each point is the mean of 1000 simulation runs, and 95% confidence intervals for these means are shown.

4.3.4 Assigned nurses ("named nurses" and ICU contact patterns).

In this scenario the contact pattern can be described in the same framework described above, except that now there is only a single carer in each carer group. The number of patients in each group may be one or more and a single nurse is assigned to each patient. If k represents the proportion of required contacts a patient receives from nurses other than the single nurse assigned to them, then using the same notation as before $\gamma_{ij} = k/(n'-1)$ if $i \neq j$, and $\gamma_{ii} = 1-k$. Consequently, from equations 4.3 and 4.4, providing $n' > 1$, the off-diagonal elements of the matrix W are now given by

$$w_{ik} = \frac{\beta_i \beta'_k n_k}{\mu\mu'} \left(\frac{2k-k^2}{n'-1} - \frac{k^2}{(n'-1)^2} \right)$$

and the diagonal elements by

$$w_{ii} = \frac{\beta_i \beta'_k (n_i - 1)}{\mu\mu'} \left(\frac{k^2}{n'-1} + (1-k)^2 \right)$$

The eigenvalues of an $n \times n$ matrix with diagonal elements b and off-diagonal elements a are $(n-1)a + b$ and $b - a$. Since all the elements of matrix W are non-negative, the former is the dominant eigenvalue. In the case where $\beta_i = \beta, \forall i$ and $\beta'_k = \beta', \forall k$, then substituting in the w_{ij} gives the dominant eigenvalue

$$\zeta = \frac{\beta\beta'}{\mu\mu'} \left[2k + n - 1 - \frac{n'k^2}{n'-1} \right]. \tag{4.6}$$

To be able to make comparisons between different staff/patient ratios it is necessary to express the above using the handwashing frequency f_{HW} (the probability of a handwash after each patient contact) rather than rate μ'. To do this μ' is replaced with $(f_{HW} cn)/n(1-f_{HW})$ in the above equation, where c is the rate that each patient makes contacts.

Figure 4.7 then shows how ζ changes with k for different values of n' in a 20 bed ward with a constant carer handwashing frequency. Simulation results are presented in figure 4.8 showing how such mixing patterns can

translate into reductions in the time to extinction of a pathogen following its introduction on a ward, and a reduction in the colonized patient days. Clearly the greatest sensitivity to n' occurs when $n' = n$ so that each patient may have a single nurse assigned to them. In the extreme case where each nurse contacts only one patient (so $k = 0$) clearly no transmission is possible, while when $k = (n' - 1)/n'$ ζ will be maximal, and the mixing patterns will be identical to the homogeneous mixing case. The figure illustrates that contact patterns such as these may be effective control strategies when the nurse to patient ratio is very high (such as in an ICU), but at the ratios normally seen in other wards it is unlikely to make a noticeable difference unless an extremely high proportion of patients' contacts come from their assigned carers. This has special relevance for staff-patient ratios: even if the carer handwashing frequency stays constant, decreasing the staff-patient ratio will cause the basic reproduction number to increase. The magnitude of this effect increases with the fidelity of carers to their assigned patients. In the case of homogeneous mixing ($k = (n' - 1)/n'$), ζ does not change with the staff-patient ratio, while for contact patterns typically seen in the ICU the effect is likely to be large.

4.4 Discussion

4.4.1 Patient heterogeneity

In the deterministic model of Dye and Hasibeder the heterogeneity in contact patterns could only increase the basic reproduction number. In this case it has been shown, both through individual-based approximations to the basic reproduction number in a multi-group setting and simulation results, that heterogeneity can also, in some circumstances, make it harder for a pathogen to become established in the population. The most extreme example of this is in the ICU setting, where non-homogeneous mixing can have

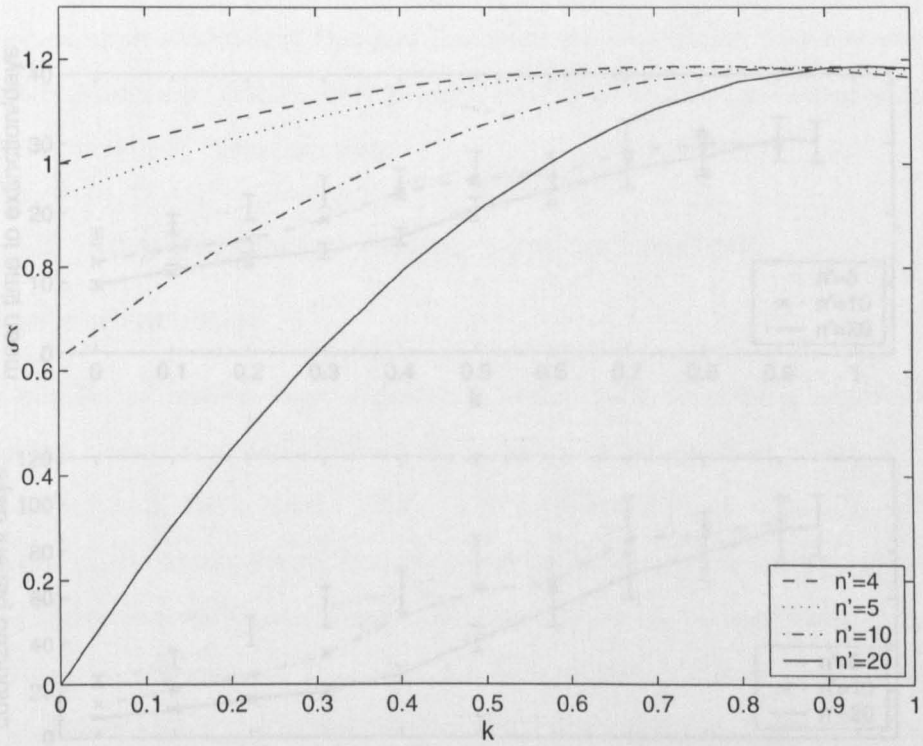

Figure 4.7: Assigned nurses and the basic reproduction number. Here ζ is the approximation to the basic reproduction number in the stochastic model described in section 4.3.4, where for each patient one nurse is responsible for a disproportionate number of their contacts. The mixing parameter k represents the proportion of each patient's contacts coming from nurses other than the nurse assigned to them. Parameters used were: $n = 20$; $\beta = 0.5; \beta' = 1; c = 10; \mu = 0.1; \sigma = 0$. c represents the patient contact rate. μ' is varied to keep the handwashing frequency, f_{HW}, constant and equal to 0.286.

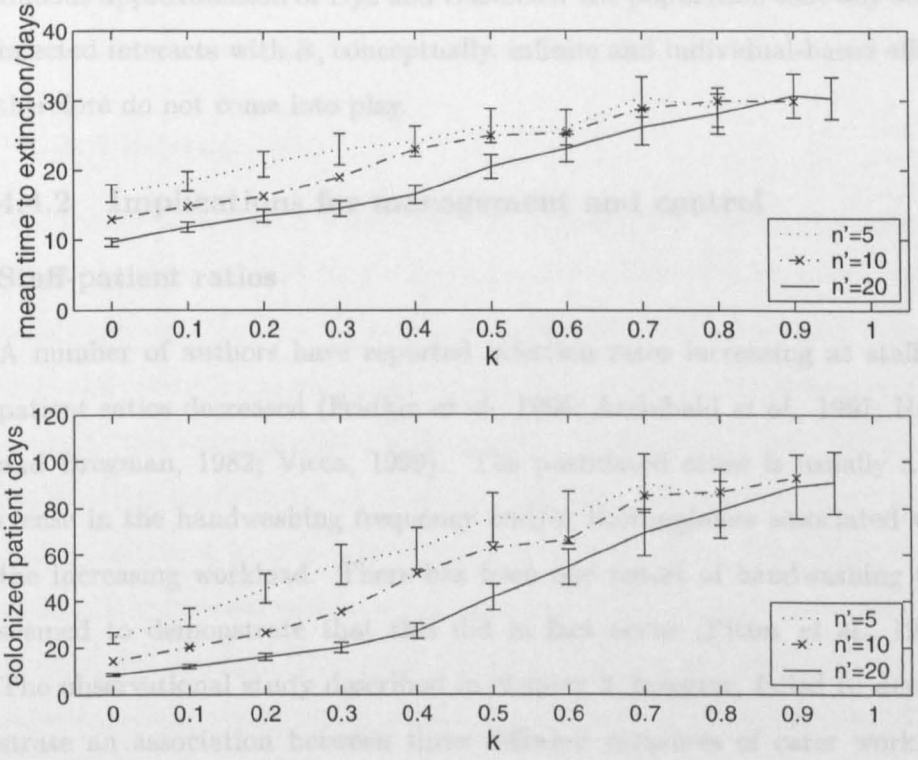

Figure 4.8: Simulation results from the stochastic model described in section 4.3.4, where a disproportionate amount of care for each patient is done by one nurse (k represents the proportion of a patient's contacts done by other nurses). Parameter values are the same as those used in figure 4.7. Means are based on 1000 simulation runs, and 95% confidence intervals for these means are shown.

a dramatic influence on the ability of a pathogen to invade and persist. The difference from the continuous deterministic result can only be accounted for by taking into account effects at the level of the individual; in particular the replacement of the n_i term in expression 4.3 with $n_i - 1$ due to the fact that the source if infection cannot, by definition, be susceptible. In the continuous approximation of Dye and Hasibeder the population that any single infected interacts with is, conceptually, infinite and individual-based effects therefore do not come into play.

4.4.2 Implications for management and control

Staff-patient ratios

A number of authors have reported infection rates increasing as staff-to-patient ratios decreased (Fridkin *et al.*, 1996; Archibald *et al.*, 1997; Haley and Bregman, 1982; Vicca, 1999). The postulated cause is usually a decrease in the handwashing frequency and/or thoroughness associated with the increasing workload. There has been one report of handwashing that seemed to demonstrate that this did in fact occur (Pittet *et al.*, 1999). The observational study described in chapter 3, however, failed to demonstrate an association between three different measures of carer workload and handwashing frequency (though no attempt to measure handwashing technique was made). The results of this chapter suggest that, whether or not decreased handwashing frequency and/or effectiveness does occur with increasing workload, there are likely to be other causes of the association between lower carer-to-patient ratios and higher infection rates. In particular, section 4.3.4 showed that the association would be expected to increase with departures from homogeneous mixing patterns. Thus, in a ward where patients and carers mixed homogeneously, changing carer-to-patient ratios could only affect the infection rate through reduced infection control measures or other indirect effects. For contact patterns typically seen in ICUs,

however, even if handwashing frequency and effectiveness didn't change, the infection rate would still be expected to show an inverse relationship with the carer-to-patient ratio. This suggests that in such situations increasing the staff-patient ratio may be an effective control measure. Indeed, closing of wards to new admissions has just this effect, and may, in addition to the important aspect of reducing the influx of susceptibles, account for some of the success of this method of controlling outbreaks (Farrington *et al.*, 1998).

Patient heterogeneity

The results from section 4.3.3 show that patient heterogeneity can have the effect of making it easier for the pathogen to invade even when carers behave homogeneously. For contact rate heterogeneity the effect was small, but dramatic increases in the persistence of the pathogen were seen when increased contact rates were associated with greater susceptibility. Results from chapter 3 suggest that this is indeed likely in practice. Patients taking antibiotics had contact rates almost twice as great as other patients. There may also be associations between transmissibility from patients to carers and contact frequency. For example, Burke and Corrigan (1961) found that patients with infected wounds were much greater dispersers than those without. These patients would also be expected to require more contacts. Though no data on the subject are presented here, it also seems likely that the same high-risk patients will tend to have longer stays. In this respect, with a relatively small number of patients responsible for a large amount of the transmission, the situation is analogous to the "core groups" of individuals considered important for the dynamics of sexually transmitted diseases (Hethcote and Yorke, 1984).

The results of section 4.3.3 then have implications for the management of a heterogeneous group of patients. For example, the separation of pre-operative and post-operative patients has been proposed as an infection con-

trol measure (Lidwell *et al.*, 1970; Shooter *et al.*, 1963). Acquisition rates of resistant strains have been found to be much higher amongst post-operative than pre-operative patients in the above studies. However simulation results suggest that the effect of grouping high risk patients together in this way is likely to increase the ability of a pathogen to persist. Fewer colonized patient days are seen when high and low risk patients are mixed. In this respect, the low risk patients offer some protection for the high risk patients, but at the expense of having greater risk of acquiring the organisms themselves. Overall, the total risk to patients is lower, but clearly ethical questions may arise in this situation.

4.4.3 Individual-based considerations

In section 4.3.1 it was suggested that the expression derived for R_0 was an upper bound to the true value. This is because, in considering the number of secondary cases arising from the primary case, it was assumed that each contact of the primary case was uncolonized. No account was taken of the reduction in susceptibles attributable to the primary case itself.

R_0 is normally defined as the mean number of secondary cases arising from a primary case in a completely susceptible population. In heterogeneous populations, rather than giving the mean number of secondary cases, R_0 gives the "typical" number of secondary cases; that is the mean number of secondary cases that is approached in the exponential phase of the epidemic, before non-linear effects due to the reduction of susceptibles become important (Diekmann *et al.*, 1990). When considering an individual-based model, however, where each individual is in contact with only a finite (and generally small) number of other individuals, the reduction in the number of susceptibles can be important even for the primary case. In the individual-based SIR model discussed by Keeling and Grenfell (2000) this is particularly important, as once contacts of the index case become infected they cannot

be re-infected. For the present model, this effect of susceptible contacts being "used up" applies both to the carer contacts of the index patient and to the susceptible patients contacted by carers. The effect may be expected to be less important than in the SIR model, however, as both patients and carers once colonized will not in general remain colonized for the rest of the stay of the index patients: carers decontaminate their hands, and patients are replaced by new susceptible patients. But overall, the effect will act to reduce the actual mean number of secondary cases below that predicted by the expression given in section 4.3.1.

To see how important this is likely to be for the R_0 estimates, the effects on the level of carer hand contamination and on the expected number of patients colonized by a carer are considered.

If $p_{ij}(t)$ represents the probability of a single carer in group j being colonized at time t as the result of a single colonized patient in group i initially colonized at $t = 0$ then

$$p_{ij}(t+\delta t) = p_{ij}(t)(1-\mu'\delta t) + (1-p_{ij}(t))\frac{\beta_i'\gamma_{ij}}{n_j'}\delta t + o(\delta t).$$

which gives

$$\frac{dp_{ij}(t)}{dt} = -p_{ij}(t)\mu' + (1-p_{ij}(t))\frac{\beta_i'\gamma_{ij}}{n_j'}$$

where $p_{ij}(0) = 0$. This has solution

$$p_{ij}(t) = \frac{\beta_i'\gamma_{ij}}{n_j'\mu' + \beta_i'\gamma_{ij}}(1 - \exp(-t[\mu' + \frac{\beta_i'\gamma_{ij}}{n_j'}])$$

so

$$\lim_{t\to\infty} p_{ij}(t) = \frac{\beta_i'\gamma_{ij}}{n_j'\mu' + \beta_i'\gamma_{ij}}$$

On many wards, the expected time before a carer becomes colonized as a result of contact with one colonized individual will be much larger than the expected persistence of hand contamination (assuming the handwashing

frequency, patient-carer contact rate, and patient-to-carer transmissibility values obtained from the study in chapter 3 are typical). Consequently

$$\mu' n'_j \gg \beta'_i \gamma_{ij}.$$

which implies that

$$p_{ij}(t) \approx \frac{\beta'_i \gamma_{ij}}{\mu' n'_j}$$

The one situation where this second assumption may be hardest to justify is in ICUs, where one carer repeatedly touches the same patient, and very high contact rates are seen. In this case, the approximation for R_0 may be a significant overestimate.

Considering the colonization of patients by carers, if a patient is colonized at a rate r by one colonized carer then they will be colonized with probability $r/(r+\mu')$ before the carer's colonization is cleared (as before μ' is the rate the carer loses the hand carriage). In the continuous version the r term doesn't appear in the denominator because an infinite susceptible population is being considered, and the rate any single patient is colonized is vanishingly small. For finite populations, when μ' is large compared to r, there will be little difference to the usual derivation of R_0 used in section 4.3.1.

Clearly, reconciling individual-based considerations within the setting of a heterogeneous population represents a significant challenge. Exact results represented by simple formulae will rarely be obtained. For this reason, approximations were used here. In populations as small as those considered here, which are both dominated by stochastic effects and for which non-linear effects are likely to be important from early in the course of the epidemic, the basic reproduction ratio cannot be as important as it is in large populations (though even in very large populations stochastic effects cannot be ignored, as the number of cases at the start of an epidemic will be small). Despite these caveats, the approximation to the basic reproduction ratio

does represent a readily computable quantity that provides an indication of an important aspect of the model's behaviour. As a tool to explore the effects of different model parameters it can also motivate an exploration of the parameter-space through simulation experiments which remain, for all but the simplest of models, the most reliable way of studying their behaviour.

Chapter 5

Antibiotic resistance: a review of the literature

The previous analysis is now extended by taking into account antibiotic use. This chapter starts by reviewing the literature relating antibiotic use to resistance, with particular emphasis on *S. aureus*. The aim here is to inform decisions about model choice, and where possible use published results to obtain parameter estimates. Subsequent chapters then present mathematical models (chapter 6), laboratory comparisons of growth rates of sensitive and resistant strains (chapter 7), and a preliminary analysis of data relating the acquisition of VRE to antibiotic consumption (chapter 8).

Recently there have been excellent reviews of the problem of bacterial antibiotic resistance, both from perspective of the UK (Standing Medical Advisory Committee, 1998) and globally (Baquero and Blàzquez, 1997). It is not the intention of this chapter to duplicate this work. However, section 5.1 presents essential background on drug resistant bacteria. Section 5.3 then goes on to critically review published attempts to relate consumption of antibiotics to acquisition of resistant organisms. This is considered at the community level, in hospital wards, and at the level of the individual. Section 5.4 then reviews studies of direct relevance to *S. aureus* and its

transmission in hospitals in some detail. Where possible reported results from these studies are used to arrive at provisional estimates for the new model parameters.

5.1 An overview of antibiotic resistance

Antibiotics have been the mainstay of treatment of bacterial infectious diseases for over 50 years. During this time the resistance profiles of both pathogenic bacteria and commensals forming part of the normal flora of the skin, intestinal tract, and upper respiratory tract have undergone dramatic changes. Bacterial strains isolated before the start of the antibiotic era have been found not to possess any acquired resistance determinants (Hughes and Datta, 1983). Thus, before the 1940s the vast majority of bacteria would have been susceptible to all antibiotics (except, of course, those to which they possess innate resistance). Penicillin was first used clinically in 1941(Abraham *et al.*, 1992). Levels of resistance to penicillin in *S. aureus* as high as 80% were already being reported in hospitals in 1958 (Ridley *et al.*, 1970). By the mid-1980s over 80% of *S. aureus* isolates from almost any part of the world were penicillin-resistant (O'Brien, 1986). Now only about 10% of *S. aureus* strains recovered from hospitals are sensitive to penicillin (Standing Medical Advisory Committee, 1998). A similar picture is found in the faecal flora (Levy, 1997). For example, in one study over 60% of individuals without a recent history of antibiotic use were found to have at least 10% of the *E. coli* sampled from their faecal flora resistant to one of seven antibiotics tested (Levy *et al.*, 1988).

Antibiotic resistance is not distributed evenly amongst bacterial strains, but tends to be clustered, so strains resistant to one antibiotic are more likely to also be resistant to other unrelated drugs (Goldstein and Acar, 1996; Livermore and Yuan, 1996). Such unrelated resistance is caused both through single plasmids encoding diverse mechanisms, and also through the

independent accumulation of distinct resistant determinants. Such multiple-resistance poses particularly severe therapeutic problems. This is especially true for Gram-positive organisms, for which no novel chemical class of antibiotics has been introduced in last 20 years (Cohen, 1992). For example, strains of VRE exist which are resistant to all available antibiotics, and recent strains of the more virulent *S. aureus* have been found with intermediate resistance to glycopeptides, previously the last remaining class of antibiotics to which they were all susceptible (CDC, 1997; Hiramatsu *et al.*, 1997b,a; Navarro Marin, 1996). Even when antibiotics are available to treat infections, resistance remains a serious problem owing to the consequent reliance on more toxic and expensive antibiotics such as vancomycin, and failure of empirical therapy.

When considering the spread of resistance in bacteria it is important to distinguish between the clonal spread of organisms, the spread of resistance genes, and the repeated emergence of resistance arising from mutations which are then selected for by antibiotic use. Most resistance genes, whether plasmid or chromosomally mediated, are mobile to some extent, and there are many accounts of such resistance determinants crossing species or even genus barriers (Roberts, 1997; Hall, 1997; Dowson *et al.*, 1990). However, the frequency of this varies enormously. Thus the *vanA* gene encoding for vancomycin resistance commonly found in enterococci is readily transferred to other strains (Chadwick *et al.*, 1997), and this may account for much of its spread in hospitals (Bingen *et al.*, 1991). In contrast, most MRSA transmission is clonal, though there are occasional reports of apparent horizontal transfer of the *mecA* gene (which confers methicillin-resistance) during outbreaks (Dominguez *et al.*, 1994). Only such transmission of pre-existing genes is considered in this and subsequent chapters. In some cases resistance repeatedly occurs during antibiotic therapy due to the selection of spontaneous mutations. Examples include quinolone resistance in *Enterobacter*

and carbapenems resistance in *Pseudomonas aeruginosa* (Standing Medical Advisory Committee, 1998). These processes have important differences, and care should be taken when constructing models and drawing conclusions to differentiate between them. For example, while multiple-antibiotic therapy may be a good strategy for preventing the emergence of resistance through mutations, it may be a poor strategy when resistance genes to the antibiotics are already common.

5.2 Antibiotic resistance in hospitals

In the UK hospital prescribing accounts for about 20% of all human antibiotic consumption and approximately 10% of total antibiotic use (Standing Medical Advisory Committee, 1998). However, for the transfer of existing resistant genes the concentration of antibiotic use is likely to be more important than the total volume, and the prevalence of resistance closely follows the gradient of the concentration of antibiotic usage (Levy, 1997). Thus, in ICUs, where audits have found that 40-50% of patients receive antibiotics in any 24 hour period, the problems are greatest, and resistant strains are often endemic. Resistance levels are less severe but still problematic on other hospital wards, where self-limiting outbreaks of resistant strains are more common than endemicity. Outside ICUs, hospital patients are about half as likely to receive antibiotics in any given day (Standing Medical Advisory Committee, 1998). Amongst out-patients resistance levels are lower than amongst in-patients (Monnet *et al.*, 1998), and outside hospitals a greater prevalence of resistance organisms is found in nursing homes and amongst intravenous drug users than in the population as a whole (Cox and Bowie, 1999; Muder *et al.*, 1991; Mulligan *et al.*, 1993). Both of these groups have higher than average levels of antibiotic consumption (Novick and Ness, 1984).

The picture is complicated, however, by the fact that there are likely

to be different rates of detection in the different groups. Typically, only resistant organisms causing disease will be detected. Also, as individuals frequently move between the different groups, incidence may be a more appropriate measure than prevalence of the effect when considering the effect of antibiotics in the different settings. However, estimating the incidence for a commensal is not easy, and the hospital infection literature is often unreliable, with patients who have positive swabs within a sufficiently short time after admission often being arbitrarily classified as community acquisitions. Thus, what appear to be community acquisitions may simply be nosocomial acquisitions occurring on earlier hospital stays.

Another difficulty in assessing the role of hospitals in the spread and maintenance of resistance is that much of the resistance seen in hospitals (and ICUs in particular) may only be due to selection of pre-existing strains, rather than nosocomial acquisition (Silvestri *et al.*, 1999). For organisms such as VRE, which may be hard to detect in the absence of antibiotic therapy, and for which much transmission may be accounted for by the mobility of the transposon carrying the resistance gene, assessing the importance of hospital transmission is particularly difficult (Chadwick *et al.*, 1997). This problem is considered further in chapter 8.

5.3 Relating consumption to resistance

5.3.1 In large populations

Resistance data at the national level typically consist of percentages of strains resistant to different antibiotics. Strains tested usually come from hospitals isolates, though some community isolates are also tested at reference laboratories. This represents only a small and selective sample of total bacterial flora. Different combinations of antibiotics and bacterial species are tested in different countries; different sampling procedures are used;

test methods and materials differ between regions; and different antibiotic-resistance breakpoints are used in different countries, so strains classified as being resistant in one location will be classified as sensitive in another (Baquero, 1990; Trzciński *et al.*, 2000). In light of these difficulties, it is unsurprising that an NIH task force on antibiotic resistance world-wide should report that "available data on global prevalence of resistance to antibacterial agents were barely adequate" (O'Brien and the Members of Task Force 2, 1987). They also claimed that resistance to older antibiotics had stabilized, but that new problems were being caused by the transfer of resistance genes to new species. Resistance to newer antimicrobial agents was considered to be increasing. Because of all these deficiencies in the data it is difficult to make meaningful quantitative comparisons between countries. Recent surveillance initiatives should mean that in the future better data become available (World Health Organization, 1997; Department of Health, 1998).

There are also difficulties in obtaining reliable data on antibiotic use, much of which is not publicly available, apparently for competitive reasons. Indeed, a parallel NIH task force, reporting on antibiotic use world-wide, described much of the data as "conjectural, anecdotal, or of questionable reliability" (Nananda and O'Connor, 1987).

Despite all these problems, there have been recent attempts to relate antibiotic consumption to resistance in large populations. However, even with good data there are many obstacles, and predicting the spread of antibiotic resistance remains a major challenge. Thus Baquero (1996) relates resistance of respiratory pathogens to drug-use, though points out that the analysis will be complicated by factors such as the initial level of resistance in the bacterial populations. High quality surveillance data from Finland and Iceland coupled with detailed data on antibiotic use have enabled resistance in the community to *Morexella catarrhalis* and *Streptococcus pneumoniae* respectively to be related to changes in antibiotic consumption using a simple

mathematical model (Austin *et al.*, 1999a). The fitted parameters suggest that the decay in resistance following reduced prescribing levels is likely to be slower than the rise in resistance (assuming antibiotics are prescribed at a level high enough to maintain a resistant population). There are, however, some problems with this analysis. In the Icelandic data, a rapid rise in penicillin-resistant pneumococci in children under seven was followed by a 13% reduction in antibiotic prescribing for this age group. A more gradual decrease in penicillin-resistant pneumococci followed. However, for children under five years of age the rate of acquisition of pneumococci from the family has been estimated to be about 25 times greater than that from the community (Auranen *et al.*, 2000). Consequently, much of the acquisition in these children is likely to be from individuals experiencing no change in levels of antibiotic consumption. These individuals would be expected to have higher levels of resistant pneumococci than they would have had if they had also experienced a reduction in antibiotic consumption. Furthermore, acquisition rates of pneumococci in young children differ by an order of magnitude from older age groups (Auranen *et al.*, 2000). Since the model takes no account of this important age-structuring, it is difficult to know how to meaningfully interpret the parameter estimates. Similar problems can be found with the analysis of the Finish data set.

For the above reasons, caution should be exercised in drawing inferences about transmission in the wider community and about long-term dynamics from these results. This example also serves to illustrate how difficult it can be to measure the effect of the antibiotic use on resistance, even with very good data. The Icelandic resistance data consists of only eight yearly prevalences, all of these with large confidence intervals. These were used to fit a model with four parameters. The authors' reluctance to introduce more parameters to the model—as would be required to account for the age structuring—is therefore understandable.

5.3.2 In small populations

There have been few attempts to relate the consumption of antibiotics to the prevalence of resistant bacteria in whole hospitals, and what evidence there is suggests that the relationship is not simple (Monnet *et al.*, 1998). Factors such as hospital size can complicate matters (Panlilio *et al.*, 1992) and casemix will affect the chance of patients becoming infected, and is therefore likely to introduce reporting biases (Glynn *et al.*, 1997).

At the hospital level, Westh and Rosdahl (1989) found that the level of erythromycin-resistance in *S. aureus* in nine large Danish hospitals was significantly associated with erythromycin consumption. In another study, Westh and co-workers (1998) compared occurrence of multiply-resistant *S. aureus* strains in 17 hospitals world-wide. They found that use of broad-spectrum antibiotics was more closely associated with the amount of resistance found than total antibiotic consumption. Monnet and co-workers (1998), however, compared eight US hospitals, and found high use of a particular antibiotic was not necessarily associated with resistance to it.

At the ward level, there are a number of reports of rapid changes in resistance levels following changes in antibiotic usage, and individual wards may have markedly different resistance levels to those found in the hospital as a whole (Ridley *et al.*, 1970). For example, Franco and co-workers report an intervention in an intensive care nursery (Franco *et al.*, 1973). Faced with high prevalence (> 10%) of kanamycin-resistance in enterobacteria, they substituted gentamycin for kanamycin. This led to a rapid decrease in kanamicin-resistance—accounted for by colonized patients being discharged, rather than patients losing resistant organisms—and a significant increase in gentamicin resistance in the intestinal flora. After reinstituting kanamycin use, there was a rapid rise in kanamycin resistance.

Conversely, Van der Zwet and co-workers (1999) describe how an outbreak of gentamicin-resistant *Klebsiella pneumoniae* in a neonatal intensive

care unit was apparently controlled by replacing gentamicin with amikacin. In a more extreme intervention, Price and Sleigh (1970) apparently controlled a serious outbreak of drug-resistant *Klebsiella aerogenes* by withdrawing *all* antibiotics. A number of other authors have reported control of outbreaks of resistant *S. aureus* strains by altering antibiotic prescribing policies (Barber *et al.*, 1960; Ridley *et al.*, 1970; Witte *et al.*, 1994). In chapter 8 a study in which ceftazidime was replaced by piperacillin/tazobactam on a ward with endemic VRE is discussed in detail. This change was associated with elimination of VRE from the ward after a period of several months (Bradley *et al.*, 1999).

However, the interpretation of reports such as these can be complicated by the importance of stochastic effects, and the fact that other interventions are often made simultaneously. The anecdotal nature of many of the reports also suggests that reporting biases may be important.

5.3.3 In individuals

Attempts to relate antibiotic consumption to resistance using formal statistical methods have mostly been inconclusive. For example, Asensio and co-workers (1996) used logistic regression to examine the factors affecting acquisition of MRSA. Although antibiotic therapy was strongly associated with acquisition of MRSA, the analysis failed to demonstrate it to be an independent risk factor once ward assignment, time at risk, age, coma and invasive procedures were taken into account. They suggested that previous reports could have overestimated the importance of antibiotic therapy "confounded by a prolonged period from admission to isolation of MRSA".

Hershow and co-workers (1992) also failed to demonstrate a significant association of antibiotic use with MRSA acquisition after accounting for length of hospital stay, though again, there was a very strong association between these variables. A meta-analysis of 20 studies relating VRE acqui-

sition to glycopeptide use reached a similar conclusion, finding only a small and non-significant association (Carmeli *et al.*, 1999).

A number of studies *have* found MRSA and VRE acquisition to be significantly associated with antibiotic consumption, but have not controlled for time from admission to MRSA acquisition (Crossley *et al.*, 1979a,b; Peacock *et al.*, 1980; Law and Gill, 1988; Carmeli *et al.*, 1999). Since length of stay and antibiotic use are so closely correlated, these studies could simply be reporting the finding that the longer one waits, the more likely something is to happen. As a result some commentators have even questioned whether antibiotic use does actually increase the chance of patients becoming colonized with multiply-resistant strains of bacteria (Thompson *et al.*, 1982). If it doesn't, one must ask why resistant strains persist in areas with high concentrations of antibiotic use, but are found less frequently outside these areas. Other possible explanations do exist. Firstly, it is possible that patients taking antibiotics have little additional risk of acquiring resistant organisms, but, if colonized, transmit them to other patients at a greater rate due to overgrowth of the organism. Antibiotic treatment may also prolong the carriage of resistant organisms, again leading to more transmission. In this case however, since lengths of stays are usually short compared to the timescales that individuals remain colonized with commensals such as *S. aureus*, this is unlikely to make much difference. The former mechanism, however, is intuitively reasonable. Unfortunately, there is little evidence to suggest how important a factor it might be.

Before rejecting the link between antibiotic use and the risk of acquiring resistant bacteria, there are, however, other studies that should be considered.

- Knight and White (1958) found that amongst patients taking tetracycline, tetracycline-sensitive strains were almost completely replaced by multiply-resistant "hospital" staphylococci within only seven to nine

days. Untreated patients showed a more gradual increase in resistant strains, rising from about 10 to 30% of all carriage strains over 30 days of hospitalization. Penicillin was found to cause similar behaviour, though at a slower rate.

- Bonten and co-workers (1998), using a Cox proportional hazards model, showed that antibiotic use was significantly associated with acquisition of VRE in an intensive care unit.

- Lidwell and co-workers (1971) used a multiple regression analysis to study the factors influencing the rate of nasal acquisition of *S. aureus* in two medical wards between 1965 and 1966. Use of antibiotics (apart from penicillin) was highly significantly associated with the acquisition rate of tetracycline-resistant strains (though not for other strains). Amongst *S. aureus* carriers, those who received no antibiotics had a significantly lower rate of acquisition of tetracycline-resistant strains.

- In chapter 8, using a model which allows the instantaneous acquisition rate to vary according to the antibiotics being taken by a patient at a given moment, simultaneous estimates are obtained for acquisition rates of VRE amongst patients in a haematology unit for six different classes of antibiotic treatment. In all cases, acquisition rates were greater when antibiotics were being taken.

All of these studies use different methods, but they have in common the fact that they are all making inferences about the effect of antibiotics on the *rate* of acquisition. Simple logistic regressions do not do this. Trivially, length of stay will be strongly associated with acquisition of strains[1]. Studies

[1]If patient discharge and strain acquisition can both be thought of as Poisson processes with respective rates μ and λ, then the mean length of stay for patients who don't become colonized will be $1/(\mu+\lambda)$, while for those who do become colonized it will be $1/(\mu+\lambda)+1/\mu$.

that conclude, on the basis of logistic regressions, that length of stay is a risk factor for colonization are really stating a tautology. The most plausible explanation for the failure of case-control studies to show an association between antibiotic use and acquisition of resistant strains is simply that antibiotic use and length of stay are so closely related. Logistic regression analysis is likely to be a poor tool for disentangling the two. Furthermore, the parameter of importance to the transmission dynamics is the rate of acquisition. Simply stating that antibiotic use is a risk factor tells us little about how the prevalence will change if antibiotic use changes.

The above studies (with the notable exceptions of those by Lidwell *et al.* and Bonten *et al.*) considered the problem only at the level of the individual. The analysis was restricted to what would be appropriate for a non-infectious disease. While this may be appropriate for many of the organisms responsible for nosocomial infections for which the main source is patients' endogenous flora, it is clearly not true of *S. aureus* and VRE, where person-to-person spread is important, and the risk to one patient depends on colonization status of others (Silvestri *et al.*, 1999). A number of studies investigating the transmission of drug-resistant *S. aureus* within hospital populations and presenting data at the population level have, however, been carried out, though not with MRSA. From the late 1950s to early 1970s there were several large studies where each patient in a ward was swabbed regularly, usually once or twice a week. These studies are considered in detail in the next section.

5.4 *Staphylococcus aureus*

The leading causes of hospital acquired infections are usually reported to be *S. aureus* and *E. coli* (Jarvis and Martone, 1992; NNIS, 1996; Central Public Health Laboratory, 1999). For *E. coli* a large proportion of these are due to endogenous flora, rather than the epidemic spread that is typical

for *S. aureus*, and MRSA in particular (Stamm *et al.*, 1981; Silvestri *et al.*, 1999). A recent report, based on data from 70 hospitals in England and Wales between 1997 and 1998, found that 46% of all surgical site infections were caused by staphylococci (about four times as many as were caused by coliforms, the next highest category). Of these staphylococci, 80% were *S. aureus*, and 67% of those were MRSA (Central Public Health Laboratory, 1999). Because *S. aureus* also has the potential to be highly virulent and is frequently resistant to multiple antibiotics, it represents probably the most troublesome nosocomial pathogen at the moment. For this reason, this organism is the primary focus of attention here.

5.4.1 Drug resistance in *Staphylococcus aureus*

MRSA was first reported in patients almost 40 years ago, shortly after the introduction of methicillin in 1959 (Jevons, 1961). Numbers increased throughout the 1960s in the UK, with increases both in the percentage of resistant isolates from individual hospitals, and in the number of hospitals yielding MRSA. However, most of the increase in the second half of the decade could be attributed to the former; that is, an increasing prevalence in hospitals where MRSA was already established (Parker and Hewitt, 1970). During this decade many other countries also reported significant MRSA problems (Casewell, 1986).

In contrast to penicillin-resistance in *S. aureus* however, the rise in methicillin-resistance has not been monotone (O'Brien and the Members of Task Force 2, 1987). During the early 1970s, MRSA incidence in many countries actually declined (Shanson, 1981), only to come back up in the second half of the 1970s with the emergence of the new—"modern"—MRSA strains with different resistance patterns and phage types to most of those from the previous decade. It was reported that these strains apparently had an increased transmissibility (Casewell, 1986). Not until the mid-1980s, however,

did strains with comparable potential for epidemic spread as the methicillin-sensitive, tetracycline-resistant, penicillinase-producing strains of the 1950s and 1960s emerge (Casewell and Hill, 1986).

The causes of these changes remain open to question. Speculation that epidemic strains may have a greater ability to survive on inanimate or animate surfaces through enhanced resistance to desiccation and skin fatty acids has not been supported by laboratory studies (Farrington *et al.*, 1992). It is possible that the changes are due to compensatory mutations ameliorating the deleterious pleiotropic effects of resistance genes. Chapter 7 considers this question. Much of the change, though, may simply be due to changing patterns of antibiotic resistance and antibiotic use. Thus, it has been pointed out that the decline in MRSA in the early 1970s coincided with the introduction of gentamicin, and later increases have coincided with the emergence of strains resistant to both methicillin and gentamicin (Shanson and Kensit, 1976). Tetracycline-resistance in *S. aureus* is now relatively uncommon in the UK. In the 1960s, however, it represented an extremely serious problem. This decrease in tetracycline-resistance followed a decrease in tetracycline use in the UK hospitals, which now have relatively low levels of usage (Standing Medical Advisory Committee, 1998). Indeed, the early MRSA strains were also resistant to tetracycline, whereas the most common MRSA strains now are usually sensitive to it (Shanson and Kensit, 1976; Cox *et al.*, 1995). In 1979 Ayliffe *et al.* reported that a progressive decline in the proportion of patients carrying *S. aureus* resistant to tetracycline, erythromycin, and kanamycin was associated with a reduction in the use of tetracycline (Ayliffe *et al.*, 1979). Much higher levels of tetracycline-resistance are now seen in countries with higher levels of tetracycline usage. Thus, in Poland, where tetracyclines are the second most commonly prescribed antibiotics in hospitals, MRSA strains are typically tetracycline-resistant (Trzciński *et al.*, 2000).

Similar patterns of spread of MRSA have been observed in many other countries, but it is interesting to compare these with the markedly different experience in Denmark. By the end of the 1960s over 40% of all *S. aureus* strains from blood cultures of hospital patients in Denmark, and 15% of all hospital strains were methicillin-resistant (Jessen *et al.*, 1969; Rosdahl and Knudsen, 1991). Most of these strains represented just a few closely related clones. By 1984 this figure had fallen to 0.2%, and it has stayed near that level since (Voss *et al.*, 1994), despite repeated challenges of MRSA strains introduced from patients acquiring them abroad. At the same time there was a fall in erythromycin resistance, decreasing from 10% of hospital isolates, to only 1-2% over a period of about 20 years (Westh *et al.*, 1989). The cause was a decline in numbers of the closely related strains. This started with a decline of those with the most resistance determinants, and was followed by a decrease in the resistance of all related types (Westh *et al.*, 1992). Rosdahl and Knudsen (1991) reported a correlation between decrease in streptomycin and tetracycline consumption and the decline in these MRSA strains, which were typically tetracycline and streptomycin resistant as well. They suggested that early detection and precautions taken when an MRSA strain is introduced has prevented MRSA from becoming established in Denmark .

A high rate of false positives was reported when strains from the 1980s were re-tested for methicillin-resistance. Strains from the 1960s and 1970s don't appear to have been re-tested for methicillin-resistance, so perhaps a little caution should be exercised in the interpretation of these data (Westh *et al.*, 1989).

In recent years hospitals in England and Wales have experienced rapid increases in the numbers of MRSA recovered from patients: in 1997 nearly 32% of *S. aureus* bloodstream infections were caused by MRSA, compared with half that figure in 1995, and less than 5% two years before that. Most

of this increase is attributable to three main epidemic strains: EMRSA-3, 15 and 16 (CDSC, 1998). This has been accompanied by simultaneous increases in resistance to other antibiotics such as erythromycin, gentamicin and ciprofloxacin.

In Europe levels of methicillin-resistance now range from below 0.5% of all hospital *S. aureus* strains in Sweden and Denmark, to about 30% in France and Spain, generally increasing in numbers from north to south (Voss *et al.*, 1994). In the USA, the percentage of MRSA in all hospital *S. aureus* isolates rose from 2.4% in 1975 to 29% in 1991, with significantly greater rates of increase in larger hospitals (Panlilio *et al.*, 1992).

5.4.2 Antibiotic use and *Staphylococcus aureus* carriage in individuals

Aly and co-workers (1970) studied the effects on the nasal flora in *S. aureus* carriers of taking the cephalosporin cephalexin orally. Figure 5.1 presents a schematic view of their results. They found a 10^5-fold reduction in *S. aureus* numbers, and complete loss of carriage in some. The greatest reduction for most bacterial species was seen three days after stopping antibiotic treatment. The reduction in *S. aureus* numbers was the most striking, falling from 38% to 0.6% of the total nasal flora. Two out of seven subjects completely lost *S. aureus* carriage and had not reacquired these organisms 60 days later (despite the fact that, in one case, they had formed 94% of the total nasal count pre-treatment). Similarly, Knight and White (1958) treated six nasal *S. aureus* carriers with erythromycin, and found nasal counts diminished from several thousand per swab to all negative cultures. After treatment stopped levels increased slowly, returning to pre-treatment levels after about three weeks. Similar results were obtained by Martin and White (1968) with gentamicin (applied topically) , and Shinefield *et al.* (1966) with oxacillin (applied topically and orally). In all cases, antibiotic

therapy suppressed *S. aureus* nasal carriage in adults to very low or undetectable levels, but most subjects were found to be still colonized with the same strain after treatment.

The effects of such treatment on the chances of an individual becoming colonized when challenged with a resistant organism have not been studied in detail. Intuitively, one would expect a resistant strain reaching the anterior nares of an individual undergoing antibiotic therapy to have a far greater chance of becoming established than an organism reaching an individual with an undisturbed flora. However, figure 5.1 also suggests that there might be a substantially greater chance of becoming colonized for the period after antibiotic therapy, when the original flora are recovering to their former levels.

Another effect of the reduced numbers that one might expect is a reduced rate of transmission to others from such a source. Indeed, Bøe and Soldberg found that the reduction in numbers of sensitive strains during antibiotic therapy is accompanied by a reduced shedding into the environment (Bøe, 1965, cited by Lidwell *et al.* (1966)). Similar results are reported by White and Smith (1964, cited in Aly *et al.* (1977)).

No similar studies have been reported for resistant strains. However, it is known that at the temperature of the anterior nares and other staphylococcal carriage sites, the entire population of MRSA will express its methicillin resistance. Since this confers resistance to all penicillins, a large selective advantage for such strains is likely (Parker and Hewitt, 1970; Casewell, 1986).

5.4.3 Interactions of staphylococcal colonization

It has been demonstrated in an extensive series of experiments that colonization with one strain of *S. aureus* (the resident strain) can inhibit the acquisition of another strain (the challenge strain) (Shinefield *et al.*, 1974; Eichenwald *et al.*, 1965). This was shown to occur both in infants (Shine-

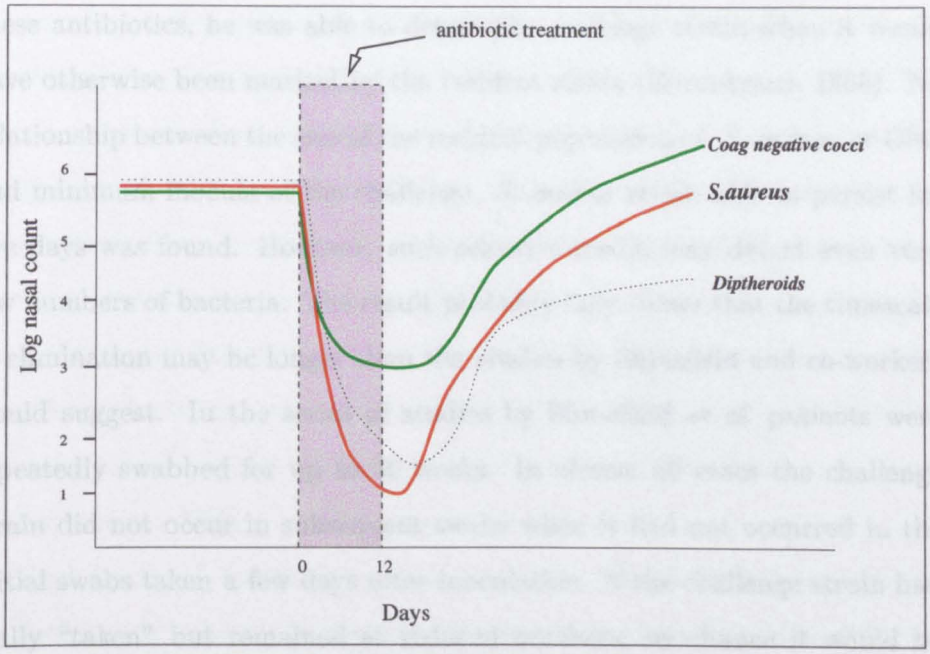

Figure 5.1: A schematic view of mean nasal bacterial counts before, during, and after antibiotic treatment in seven *S. aureus* carriers. Three of these also carried small numbers of gram-negative rods (not shown). Adapted from Aly *et al.* (1970).

field *et al.*, 1963) and adults (Boris *et al.*, 1964; Shinefield *et al.*, 1966). By deliberately inoculating individuals with a strain of low virulence, this effect has been successfully used to eradicate endemic virulent strains from hospital wards, and to treat families with recurrent staphylococcal disease (after first using antibiotics to clear the virulent strain) where, owing to recolonization with the virulent strain, treatment with antibiotics alone had failed (Eichenwald *et al.*, 1965; Boris *et al.*, 1968).

One objection to all of these studies is that the greater numbers of the resident strain compared to the challenge strain could mask the acquisition of the challenge strain; only a finite number of colonies can be selected for typing from any nasal swab. Ehrenkranz conducted similar experiments to those mentioned above, deliberately inoculating individuals with a known *S. aureus* strain (Ehrenkranz, 1966). By using a challenge strain resistant to tetracycline and streptomycin and culturing on selective agar containing

these antibiotics, he was able to detect the challenge strain when it would have otherwise been masked by the resident strain (Ehrenkranz, 1966). No relationship between the size of the resident population of *S. aureus* or CNS and minimum inocula of the challenge *S. aureus* strain able to persist for five days was found. However, such selective media may detect even very low numbers of bacteria. The result probably only shows that the timescale of elimination may be longer than the studies by Shinefield and co-workers would suggest. In the series of studies by Shinefield *et al.* patients were repeatedly swabbed for up to 46 weeks. In almost all cases the challenge strain did not occur in subsequent swabs when it had not occurred in the initial swabs taken a few days after inoculation. If the challenge strain had really "taken" but remained at reduced numbers, by chance it would be expected to have some subsequent positive swabs. Furthermore, if a strain is present in only very low numbers, its importance from an epidemiological perspective should be minor compared to the dominant strain.

Studies have shown that more than one mechanism is likely to be responsible for this "interference". *In vitro* studies by Bibel and co-workers (1983) provide strong evidence that competitive adherence is an important factor, and showed that the first bacteria to attach to a nasal epithelial cell are able to the reduce the ability of bacteria arriving later to attach . Studies by Ribble, however, showed that the filtrate of cultures of one *S. aureus* strain could inhibit the growth of another (Ribble, 1967). More recently it has been shown that one *S. aureus* strain may inhibit the expression of virulence factors and other extracellular proteins in another strain, rather than effect its growth (Guangyong *et al.*, 1997). Since such proteins may be related to the ability to attach to host cells, such behaviour provides another explanation for the observed competitive exclusion.

Other studies have shown that other bacterial species also interfere with colonization by *S. aureus.* Martin and White (1968) treated some nasal

S. aureus carriers with gentamicin (active against all staphylococci and diptheroids) and others with lysostaphin (which selectively lyses *S. aureus*). Amongst those who carried large numbers of CNS and diptheroids, those treated with lysostaphin had a much slower rate of reacquiring *S. aureus* than those treated with gentamicin. For patients who carried lower numbers, the acquisition rate changed little with treatment, but was higher than that in the first group. More recently it has been shown that implanting *Corynebacterium* sp. alone (with no prior antibiotic treatment) into the nares of *S. aureus* carriers can eliminate carriage in most individuals (Uehara *et al.*, 2000).

Such bacterial interference is also important for other bacteria, and may be particularly valuable for preventing infections amongst neonates (Sprunt and Leidy, 1988; Fuller and Gibson, 1998).

These results then suggest that the important aspects of the bactericidal and bacteriostatic behaviour of antibiotics are twofold. Firstly, there is the desirable effect of killing pathogens susceptible to the antibiotic. Secondly, there is the undesirable side-effect of suppressing the resident flora. This second effect makes it easier for patients to become colonized with new strains. If antibiotic concentration is sufficiently high, this will only lead to increased colonization by resistant strains. When the chemotherapy ends and antibiotic concentrations fall, all strains may have an increased chance of colonizing the host.

5.4.4 Studies on hospital wards

The most obvious approach to investigating the relationship between antibiotic use and the spread of resistant strains on a ward is to record detailed transmission data of all strains, by regularly swabbing all patients, and relate this to the changing levels of drug consumption. In chapter 8 just such a data set for VRE transmission is analysed. Unfortunately, few stud-

Reference	Year	Setting (ward type)	Duration	# patients	swabbing freq. swabs/week	Estimates possible
(Williams *et al.*, 1959)	1959	surgical 25 beds	8-9 months	602	1	acquisition rate of penicillin-resistant strains per source and its dependence on initial colonization status
(Burke and Corrigan, 1961)	1961	surgical	1.5 years	320	admission & discharge	limited use as no length of stay data
(Shooter *et al.*, 1963)	1963	surgical 22 beds	10 months	412	1	force of infection for penicillin and tetracycline resistant strains for pre- and post-operative patients
(Parker *et al.*, 1965)	1965	medical 20	15 months	446	2	acquisition rates per source for sensitive and resistant strains and their dependence on whether patient took antibiotics
(Selwyn, 1965)	1965	dermatological	2 years	352	1	force of infection
(Lidwell *et al.*, 1966)	1966	surgical 32–34	20 months	714	1	acquisition rates per source for sensitive and resistant strains and acquisition rates per known source
(Lidwell *et al.*, 1970)	1970	surgical 22 beds	39 months	unknown	1	acquisition rates per source for resistant and sensitive strains
(Lidwell *et al.*, 1971)	1971	medical Two 29-bed wards	2 years	3327	1	acquisition rates per source for resistant and sensitive strains and its dependence on whether patient took antibiotics
(Talon *et al.*, 1995)	1995	surgical ICU	6 months	157	1	force of infection for sensitive and resistant strains and its dependence on initial colonization status

Table 5.1: *S. aureus* transmission studies. Phage typing was used to identify cross-infection in all studies except the last, which used RFLPs.

ies have done this for *S. aureus* in the period since MRSA has become a major problem in hospitals, and none permit estimation of the parameters of interest. There were, however, a number of studies carried out in the 1960s and early 1970s which did this. The main concern then was caused by the tetracycline-resistant strains, which were also often resistant to many unrelated antibiotics. There are some problems in interpreting these data, though. Firstly, hospital populations have changed since then, and patients are likely to have more risk factors for infection with resistant *S. aureus* strains (Casewell, 1995). Also, the *S. aureus* strains have changed greatly as well. However, there is probably much that can be learned from studies of hospital staphylococci from the 1960s, and it has been argued that there is little reason why MRSA strains should differ (Casewell, 1986).

In many of these studies the main interest of the investigators was in assessing the role of the design of the ward or other interventions[2]. The main interest here, however, is in obtaining estimates for the transmission rates for sensitive and resistant strains, and their dependence on whether or not the patient has taken antibiotics, and whether or not the patient was initially colonized with *S. aureus*.

The largest and most relevant of these studies are listed in table 5.1. The earlier of the studies presented little or no formal analysis of their data, but did present their results in largely undigested format enabling estimates to be made as indicated below. Later studies tend to present only summary statistics. Unfortunately, the original records of the most useful of these studies do not survive, so the parameter estimates obtained below rely on making inferences, where possible, from the published data. Where

[2]None of the main studies that considered ward design were able to conclude with any confidence that the changes made significant differences (Shooter *et al.*, 1963; Lidwell *et al.*, 1970, 1971). One study by Lidwell and co-workers tentatively concluded that segregation of patients may have accounted for a low frequency of cross-infections (Lidwell *et al.*, 1966).

possible, maximum likelihood estimates are made, and confidence limits obtained using the likelihood ratio test. In most cases these estimates are fairly crude and depend on a number of assumptions that may be difficult to justify; confidence intervals are therefore likely to underestimate the degree of uncertainty. Nonetheless, they do serve as an aid to model construction, and give an idea of the area of parameter space in the model likely to be worth exploring. They may also be useful in the construction of informative priors in a Bayesian analysis.

Williams and co-workers, (1959) This study was designed to assess the importance of self-infection by *S. aureus* carriers for the aetiology of septic lesions. Nasal swabs were taken from patients on admission, and then at weekly intervals. Figure 5.2 and table 5.2 present the data of interest here; the carriage frequencies of penicillin-resistant and sensitive strains.

Lengths of patient stays can be estimated from the reported number of patients still present at given numbers of weeks post-admission (shown in figure 5.2). These show excellent agreement with the negative exponential distribution, with a maximum likelihood estimate of the mean length of stay (and 95% CI) of 14.9 days (13.8,16.2). Table 5.2 shows data on colonization with penicillin-resistant strains classified by initial colonization status. Departure from independence of the two classifications is significant at the 5% level (p=0.043, G-test 1 df), with patients initially colonized with sensitive strains less likely to acquire resistant strains: odds ratio (and 95% confidence interval) 0.575 (0.332, 0.997). No significant differences were found between initial colonization status and whether or not the patient took antibiotics. There are no reason for believing that nasal carriers of penicillin-sensitive strains would tend to have longer stays, and there are no significant differences in antibiotic consumption amongst non-carriers and carriers. The most plausible explanation for the discrepancy is bacterial interference, as

described in section 5.4.3.

It is possible that resistant strains acquired by patients already colonized with sensitive strains may have gone undetected due to being masked by the sensitive strains. However, if a selective medium had been used for selecting the penicillin-resistant strains this would be unlikely to be the cause. The authors do not state how penicillin-resistance was detected.

The authors also reported patient antibiotic use in relation to acquisition of drug-resistant staphylococci, and this appeared to be a significant risk factor (odds ratio = 1.87, 95% CI = (1.169, 2.996)). However, from the data presented it is not possible to determine how much of this difference is accounted for by the antibiotic and how much by the increased length of stay associated with antibiotic use. As a result, little useful information about the effect of antibiotics can be found here.

Despite having typed the organisms, no data on acquisition rates per source are presented. To estimate these the prevalences of the sensitive and resistant strains on the ward are needed. A crude estimate of these can be made. Since the distribution of lengths of stays is known to be negative exponential, patient-discharge can be thought of as a Poisson process. The distribution of lengths of stays so far for the patients on the ward at any one time should therefore also be negative exponential (though the distribution of total lengths of stays for patients on the ward at any one time will be $\Gamma(2,1)$; long-stay patients will be over-represented). Data presented in figure 5.2 provide estimates for $p_i(t)$, the probability that a patient who has been on the ward for time, t, will be colonized with a strain i. Thus, assuming the probabilities of being colonized do not change after 11 weeks for the very few patients who stay that long, the mean prevalence for strain i can be estimated to be:

$$\sum_{t=0,1,2\ldots} \Pr(l = t) p_i(t) \tag{5.1}$$

where $\Pr(l = t)$ is the probability that the length of stay so far, l, for any given patient is t weeks. For these data this gives mean prevalences of 24% for the penicillin-resistant strain, and 23% for the penicillin-sensitive strain.

Of the 353 patients not initially colonized with resistant strains who had two or more nasal swabs, there were 98 apparent acquisitions. If patient colonization can be assumed to be a Poisson process with rate λ (the force of infection), and patient discharge is also a Poisson process with rate μ, then the probability of any one patient becoming colonized is $p = \lambda/(\lambda+\mu)$. The binomial distribution with parameter p gives the likelihood of the observed data. The maximum likelihood estimate for λ is then $98\mu/(353-98)$. Using the earlier estimate for μ (1/14.89) , the rate susceptible patients become colonized is estimated to be 0.026 /day, with a 95% confidence interval of (0.020,0.032). This gives a mean time before becoming colonized of between about 30 and 50 days. Since this was in a 25 bed ward, where about 24% of patients were colonized with penicillin-resistant strains, there would on average be six patient sources. Ignoring other possible sources (i.e. long-term colonized carers) this gives a mean acquisition rate of 4.30 per 1000 patient days, per source, and a 95% confidence interval of (3.39, 5.4). No data for the number of acquisitions of sensitive strains are given.

One problem with these data is that putative sources of the acquired strains were not identified, and some of the apparent acquisitions may therefore be caused by initial false-negative swabs before later detection of the organism. If patients were already harbouring small numbers of penicillin-resistant staphylococci these may have been preferentially selected by antibiotic therapy, increasing the chance of detection. Colonized carers were also not considered as sources. For both of these reasons the transmission rate per source arrived at here may be an overestimate.

week 1 carrier state	acquired penicillin-resistant *S. aureus*	didn't acquire penicillin-resistant *S. aureus*
no *S. aureus*	77	173
penicillin-sensitive *S. aureus*	21	82

Table 5.2: Number of patients carrying penicillin-resistant *S. aureus* strains by initial nasal colonization status. Data from Williams *et al.* (1959).

Shooter and co-workers, (1963) Rates of acquisition of tetracycline-resistant strains were 0.01 per patient day of exposure for penicillin-resistant strains, and 0.005 for tetracycline-resistant strains. Insufficient data are presented to estimate acquisition rates per source.

Parker and co-workers, (1965) The main purpose of this study was to investigate the effect of the physical subdivision of wards on the transmission of *S. aureus*. Nasal swabs were taken from patients in a 20 bed ward on admission and then twice weekly. The data presented allow for estimates of the acquisition rates per source for drug-sensitive *S. aureus* strains (S strain), strains resistant to penicillin only (P), and strains resistant to two or more antibiotics, including penicillin (M). Estimates of the affect of antibiotic use on acquisition rates for these strains can be made from the published data.

The number of acquisitions of each strain are presented and broken down by antibiotic status of patients in table 5.3. . If λ is the per-patient colonization rate (the force of infection), then the probability of a patient becoming colonized in an interval Δ_t is $p = 1 - e^{-\lambda\Delta_t}$. The log likelihood of there being a acquisitions from n patient-intervals (all of length Δ_t) is then (from the binomial distribution) $a\ln(p)+(n-a)\ln(1-p)+c$, where c is a constant. Differentiating with respect to λ and equating to zero gives the maximum likelihood estimate for λ, $-\ln((n-a)/n)/\Delta_t$.

To estimate the acquisition rates per source for different strains listed in

Strain type	Patient taking antibiotics	# acquisitions in half week from admission/number swabbed 1	2	3	4	Acquisition rate /1000 patient days [first swab ignored]	95% CI	Acquisition rate /patient source /1000 patient days	95% CI
S	No	6/256	5/210	3/130	2/79	6.85[6.90]	(4.02,10.78)	2.48 [2.50]	(1.46,3.90)
S	Yes	3/190	2/175	3/150	1/109	4.15[3.98]	(1.99,7.48)	1.50[1.44]	(0.72,2.71)
P	No	9/256	9/210	3/130	0/79	9.03 [8.30]	(5.70,13.46)	2.55[2.34]	(1.61,3.80)
P	Yes	8/190	0/175	3/150	1/109	5.55 [2.64]	(2.97,9.30)	1.57[0.75]	(0.84,2.63)
M	No	2/256	1/210	0/130	0/79	1.27[0.68]	(0.32,3.30)	1.20[0.64]	(0.30,3.11)
M	Yes	4/190	3/175	3/150	2/109	5.55[5.32]	(2.97,9.30)	5.24[5.02]	(2.80,8.78)

Table 5.3: Numbers of acquisitions and estimated acquisition rates of *S. aureus* strains sensitive to all antibiotics(S), resistant to penicillin (P), and multiply-resistant (M). Acquisition rates are calculated as described in the text. Because some of the apparent acquisitions may be due to false negative admission swabs (taken prior to the first weekly swab), rates calculated ignoring apparent acquisitions between the first and second swab are also shown. If such false-negatives are important, this rate should be lower than the rate based on all the data. Data from Parker *et al.* (1965). Note that acquisition rates presented in table IV of this paper are incorrect and should be multiplied by 2.

table 5.3 the number of sources for each strain must be estimated. Using the same calculations that were used for Williams' study described above, mean prevalences of different strains in patients can be estimated to be 1.06, 3.54 and 2.76 for the M, P and S strains respectively. Note that since only patient sources are considered these are likely to be overestimates. Of 86 apparent acquisitions, a possible source was found for only 28 by phage typing, and for 12 of these a member of staff was the only possible source found. Furthermore, if the frequencies of the different strain types are different in patients and carers, then the relative values for acquisition rates of different strains will also be unreliable. Unfortunately, no data are given on the strains recovered from carers.

If apparent acquisitions due to initial false-negative swabs are important, then a disproportionate number of acquisitions should appear in swabs taken shortly after admission. To compensate for this, in table 5.3 acquisition rates have also been estimated by ignoring what appeared to be acquisitions based on the first post-admission swab. The large discrepancy between acquisition rates for P strains amongst patients taking antibiotics suggests that many of these acquisitions are likely to be spurious. There is some evidence that the same may be true for acquisitions of P strains amongst patients not taking antibiotics. There is also a large discrepancy in estimates for the acquisition rate of M strains amongst patients not taking antibiotics, but since there were only three acquisitions in this group, the difference does not necessarily indicate spurious acquisitions.

No significant differences in total rates of nasal acquisition of *S. aureus* strains were found between those taking antibiotics and those not, but those who received antibiotics acquired multiply-resistant strains between four and eight (if the first-weekly swab is ignored) times faster than those who didn't. Antibiotic use was associated with a reduction in the acquisition rate of both S and P strains by about half, and the acquisition rate of M

Strain	Acquisition rate per 1000 patient days per patient source	per patient or staff source
S	0.017	0.039
P	0.11	0.083
T	0.63	0.384

Table 5.4: Acquisitions rates per source for: (i) antibiotic sensitive *S. aureus* strains (S); (ii) penicillin-resistant strains; (iii) tetracycline-resistant strains. Acquisition rates per patient source (calculated by the authors) are a weighted mean from rates per source in same room and per source in other rooms, where weights are the number of sources of each (table 4 in the paper). Data from Lidwell *et al.* (1966).

strains per source amongst patients not taking antibiotics was found to be substantially lower than for S or P strains. This could be due to lower numbers of carers carrying these strains, or indicative of a fitness cost of resistance. However, the confidence intervals are large, and any evidence of a fitness cost is weak at best.

The assumption made above that each patient is equally susceptible is unrealistic, as colonization with one strain will hinder the acquisition of another. Unfortunately, the authors do not say in how many cases a patient carrying one strain subsequently acquired another, and prevalences of colonization with different strains after different lengths of stay are likely to depend on prior antibiotic therapy. Because of this, there is no obvious way to adjust the rates in table 5.3. However, acquisition rates per source for all patients can be adjusted, if the data illustrated in figure 5.2 is used to estimate the proportion of patients swabbed on each date carrying each strain. If it is then assumed that carriage of one strain provides total protection against acquisition of another, then the estimates for acquisition rates per source (ignoring apparent acquisitions detected on the first post-admission swab) are 2.60, 2.03 and 3.79 per source per 1000 patient days, for the S, P and M strains respectively.

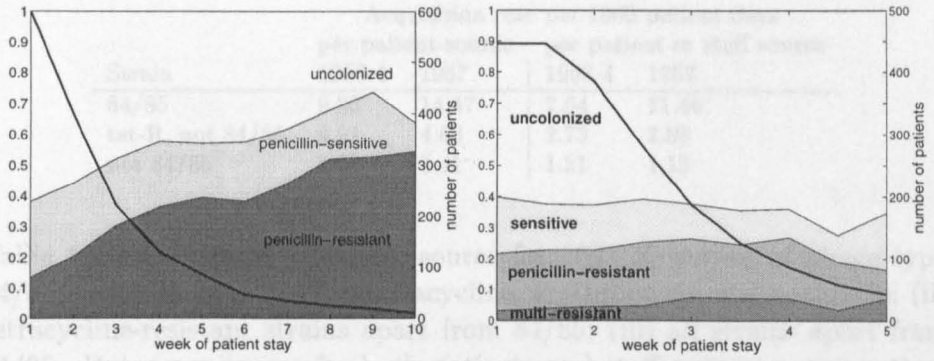

Figure 5.2: Carriage of penicillin-sensitive and resistant strains of *S. aureus* by time spent on the ward. In B, multi-resistant strains are all resistant to penicillin and at least one other antibiotic (91% to tetracycline, and 53% to streptomycin).Data from Williams *et al.* (1959)(A) and Parker *et al.* (1965)(B).

Lidwell and co-workers, (1966) This paper describes an investigation into whether the subdivision of a ward was effective at preventing staphylococcal cross-infection in a 30 bed surgical ward. Patients were swabbed on admission, and at weekly intervals. 71% of the patients received an antibiotic (63% took penicillin, 14% streptomycin and 7% tetracycline. Other antibiotics were taken by less than 10% of patients). Sources of infecting or colonizing strains were identified, where possible, by phage typing.

A reduction in the *S. aureus* carriage rate was observed. In the first two weeks of patient stay carriage levels fell from 38% to 25%, largely due to loss of carriage of sensitive strains. This loss continued, though at a slower rate, throughout patients' stays. Just over 5% of patients carried tetracycline-resistant strains on admission, and the frequency of carriage of these strains increased to no more than 9% with increased length of stay.

The total apparent acquisition rate was estimated to be 34.4 per 1000 patient weeks, or 13.3 per 1000 patient weeks for multiply-resistant staphylococci. The authors considered about 25% of these acquisitions to be spurious (most of these were sensitive strains). Estimates of acquisition rates

	Acquisition rate per 1000 patient days			
	per patient source		per patient or staff source	
Strain	1963-4	1967	1963-4	1967
84/85	9.03	14.47	7.64	11.46
tet-R, not 84/85	4.91	4.63	2.73	2.98
not 84/85	4.05	5.41	1.21	1.13

Table 5.5: Acquisition rates per source for: (i) *S. aureus* of phage type 84/85, resistant to penicillin, tetracycline, erythromycin and neomycin; (ii) tetracycline-resistant strains apart from 84/85; (iii) all strains apart from 84/85. Rates per source for both patients and staff sources count patient and staff carriers equally, and are taken directly from (Lidwell *et al.*, 1970), while rates per patient source are derived from data therein and assume that the only sources for all acquisitions are patients.

per source were obtained by the authors and are shown in table 5.4

Lidwell and co-workers, (1970) The aim of this study, which was conducted in two distinct phases, was to examine the effect of physically separating pre-operative and post-operative patients, although the same staff attended all patients. Carriers of tetracycline resistant strains of *S. aureus* were put in side-rooms or isolation cubicles when possible. A single multi-resistant *S. aureus* strain (type 84/85) became established on the ward during the study, which was acquired by over 20% of patients who stayed for 5-6 weeks.

Though the raw data are not presented in this paper, and no information is given about patient antibiotic use, the average numbers of carriers of different strains present are given, as are the acquisition rates. Acquisition rates per 1000 patient days per patient source can be calculated from these (table 5.5).

On the basis of the typeable strains, the authors estimated the proportion of acquisitions that were spurious (due to false negative swabs) to be about 16%.

Strain	Acquisition rate per 1000 patient days	
	per patient source	per patient or staff source
Any	6.41	2.78
Tetracycline-resistant	12.05	5.20

Table 5.6: Nasal acquisition rates per source for *S. aureus*. Data from Lidwell *et al.* (1971).

Lidwell and co-workers, 1971 This study of two 29-bed medical wards was intended to examine the effect of nursing patients in 4-bed rooms, instead of an open-plan "Nightingale" ward.

The total nasal carriage frequency of *S. aureus* increased with time spent in the ward, as did carriage of multiply-resistant and to a lesser extent penicillin-resistant strains. The frequency of carriage of strains sensitive to all antibiotics decreased slightly with the length of stay.

Of 257 apparent acquisitions, the authors considered about 20% to be spurious, based on a consideration of phage types and antibiograms.

The authors calculated acquisition rates per patient source and per patient or staff source (excluding those considered spurious). These are shown in table 5.6.

The authors also presented a multiple-regression analysis to determine the factors influencing the rate of nasal acquisition of *S. aureus*. Apart from patient age (and after accounting for a lower acquisition rate for patients during their first week in the ward), the only significant explanatory variables for the acquisition of tetracycline-resistant strains were antibiotic use: patients who weren't carrying *S. aureus* strains who had received antibiotics (apart from penicillin) acquired strains at a rate of 20 acquisitions per 1000 patient-days faster than those who received only penicillin; and *S. aureus* carriers who received no antibiotics acquired tetracycline resistance significantly more slowly (27 acquisitions/1000 patient-days) than carriers who received antibiotics.

5.4.5 Other studies

Little useful data can be gleaned from the other major studies listed. Shooter and co-workers (1963) report colonization rates with tetracycline-resistant staphylococci ranging from 6 per 1000 days patient days in a post-operative division of a ward, to 2.8 in the pre-operative side, but no figures are given to allow a colonization rate per source to be estimated. Selwyn (1965) presents insufficient data to enable estimates of acquisition rates to be made. In the study by Burke and Corrigan (1961) patients were swabbed on admission and discharge, but the lengths of patient stays were not reported, so no estimates of acquisition rates are possible. Interpretation of the results of this study is further confused by the extraordinarily high level of *S. aureus* carriage amongst patients entering the ward (79%). This suggests either an extremely unusual patient population or unreliable laboratory results.

In one of the few recent studies of this kind applicable to MRSA, Talon and co-workers (1995) took nasal, surgical wound, and tracheal swabs from patients on admission and then at weekly intervals. The aim of this study was to examine the effect of mupirocin treatment on acquisition, but during the first four months no interventions were made. During this period 31 out of 99 untreated patients acquired nasal *S. aureus*. Most of these were caused by MRSA strains of three distinct RFLP types, while MSSA strains caused only sporadic cases. However, no length of stay data are given and the size of the ward is not specified. Estimates of acquisition rates are not possible without further information.

5.5 Discussion

Parameter estimates obtained from these studies are summarized in table 5.7. This table also presents estimates of the R_0 value for different wards and strains based on the estimated per-source colonization rate. For

an endemic infection, R_0 can usually be estimated by the reciprocal of the proportion of susceptibles at the endemic equilibrium (Anderson and May, 1991). In this case, however, this formula will not hold because of the importance of the constant admissions of colonized patients to the ward. Instead, in table 5.7 it is estimated as the product of colonization rate per-source, mean length of patient stay, and the number of susceptibles given that all patients except the index case are uncolonized. Although these are crude estimates, they do give an illustration of the range of values found on different wards. These can be related to observed transmission patterns on the wards.

Thus, in the study by Williams and co-workers (1959), frequency of carriage of resistant strains increased rapidly with length of stay. A strikingly different pattern was seen in the study by Parker and co-workers, with only a gradual increase in the frequency of resistance with length of stay (figure 5.2). This difference occurred despite the fact that similar numbers of patients received antibiotics in both studies (45-50% and 43% respectively), and only slightly higher rates of acquisition per source were found for penicillin-resistant strains in the former study as were found for multiply-resistant strains in the latter. The fact that the estimated R_0 in the former study is about twice the value of that for multiply-resistant strains in the latter, however, reflects the markedly different dynamics. In addition to the slightly higher acquisition rate per source, the longer-length of stay, and the larger ward size account for the increased value. The high immigration rate of patients colonized with penicillin-resistant in the former study also has an important influence on the ward dynamics.

The very low transmission rates reported in the study by Lidwell and co-workers (1966) for drug-sensitive and penicillin-resistant strains can perhaps be explained in part by the very high levels of antibiotic use (most patients took antibiotics prophylactically). The transmission rate for the

tetracycline-resistant strains was considerably higher, but still low compared to other studies. It is not clear why this should be so.

The results from the 1963 and 1967 studies by Lidwell and co-workers (1970) are particularly interesting, as they provide evidence that different multiply-resistant strains may have large differences in their transmissibilities . Thus, the endemic strain on the ward appeared to be between three and four times more transmissible than other tetracycline-resistant strains, and was reported by the authors to be dispersed by carriers far more profusely than other strains. This strain had a wide range of antibiotic resistance, but it is not clear if this was the cause of its enhanced transmissibility, or whether there were larger fitness costs associated with the other resistant phenotypes, or if other factors accounted for the difference.

5.6 Summary

Antibiotic treatment causes the suppression of drug-sensitive strains, leaving carriage sites free for colonization with new strains, and drug-resistant strains in particular. There is evidence that carriers of sensitive strains are colonized at a slower rate than non-carriers.

Although it has been argued that antibiotic therapy in non-carriers does not increase the acquisition rate of staphylococci, there is strong evidence to contradict this. However, while in almost all cases the percentage of carriage *S. aureus* strains that are resistant has been to found to increase with the length of stay of patients on wards where significant numbers of antibiotics are used, the overall carriage frequency of *S. aureus* has been found to both increase and decrease with time (Lidwell *et al.*, 1966, 1970). Increases may be attributed to both antimicrobial therapy increasing the chances of non-carriers acquiring strains, and simply due to increased contact rates found in hospital wards; hospital staff in contact with patients typically also have higher carriage rates than members of the general public (Casewell and

Ref.	strain	acquisition rate /source /day	LOS (days)	ward size	R_0
(Williams *et al.*, 1959)	penicillin-resistant	0.0043	14.9	25	1.54
(Parker *et al.*, 1965)	sensitive	0.0025 (no antibiotics)			
		0.0015 (antibiotics)			
		0.0026† (all patients)	11.5	20	0.57
	penicillin-resistant	0.0026 (no antibiotics)			
		0.0016 (antibiotics)			
		0.0020† (all patients)	11.5	20	0.44
	multi-resistant	0.0012 (no antibiotics)			
		0.0052 (antibiotics)			
		0.0038† (all patients)	11.5	20	0.83
(Lidwell *et al.*, 1966)	sensitive	0.000017	15*	33	0.0082
	penicillin-resistant	0.00011	15*	33	0.0528
	tetracycline-resistant	0.00063	15*	33	0.30
(Lidwell *et al.*, 1970)	84/85 (1963)	0.0076	15*	22	2.41
	84/85 (1967)	0.011	15*	22	3.61
	other tet-resistant (1963)	0.0027	15*	22	0.86
	other tet-resistant (1967)	0.0030	15*	22	0.94
(Lidwell *et al.*, 1971)	all strains	0.0028	15*	29	1.17
	tetracyline-resistant	0.0052	15*	29	2.18

Table 5.7: R_0 estimates from the reviewed studies. R_0 is calculated as the product of the patient length of stay, acquisition rate per source, and [ward size -1].

* indicates data not available, so value given is a guess.

In the study by Parker and co-workers (1965) acquisition rates obtained depend on whether patients have taking antibiotics or not. 43% of patients did take antibiotics, but the numbers on the ward at any one time who had taken antibiotics are unknown.

† These estimates of total acquisition rates are based on the assumptions that one strain provides complete protection against the acquisition of another, and that all apparent acquisitions detected in the first post-admission swab are in fact spurious.

Strain 84/85 in (Lidwell *et al.*, 1970) was resistant to penicillin, tetracycline, erythromycin and neomycin.

Hill, 1986). Decreases may be attributable to antibiotic therapy suppressing sensitive flora.

Chapter 6

Antibiotic resistance models

6.1 Introduction

In this chapter the basic model of chapter 2 is adapted to allow for two strains: antibiotic-resistant and antibiotic-sensitive. The impact of changing antibiotic prescribing rates and the duration of antibiotic courses is investigated, again within the context of a single ward. As these changes to the model entail an increase in model complexity, to simplify the analysis the host-vector framework is dropped, and patients and carers are considered to mix homogeneously. Again, the model is constructed with an organism which usually behaves as a commensal (such as *S. aureus*) in mind. Thus, antibiotic treatment is assumed to be unrelated to the presence or absence of the organism.

6.1.1 Previous models

Models are beginning to shed some light on emergence and spread of drug-resistance resistance (Levin *et al.*, 1999). A menagerie of mathematical models simulating the spread and evolution of drug-resistance has been developed and applied in diverse settings. These include anthelmintic resistance (Barnes *et al.*, 1995), fungicide resistance (Gubbins and Gilligan, 1999), vi-

ral (Frost and McLean, 1994), as well as bacterial resistance (Blower *et al.*, 1996).

To date the emphasis has been on community transmission and selection, and deterministic approximations to stochastic systems. Most of this work has also concentrated on drug-resistance occurring in the organisms which the drug use is intended to control, and successful treatment is assumed to completely eradicate the pathogen. In contrast, most of the antibiotic pressure on bacteria capable of colonizing humans will affect bacteria behaving as commensals, and the effect of treatment is usually to temporarily suppress their numbers. Consequently, much of the work on drug-resistance is not directly relevant to the problem of drug-resistance in bacteria responsible for most nosocomial infections.

Much of the modelling of relevance to nosocomial infections has concentrated on the competitive interactions between antibiotic resistant and sensitive strains. Krus and Rvachev (1971) first presented a very simple deterministic model of the spread of resistant and sensitive strains in an isolated population. This work was extended by Massad and co-workers (1993) who presented a model intended to be more appropriate to a hospital ward, but which nonetheless still ignored stochastic effects and considered an isolated population. As well as cross-infection, this model allowed for direct emergence of resistant organisms through mutations and plasmid transfer of resistance factors.

Austin and co-workers (1997) considered a model of the competitive interactions of sensitive and resistant strains of commensal bacteria. The spread of resistance was explicitly related to antibiotic consumption, where antibiotic treatment was assumed to be independent of the colonization status of the host. Again, however, the main focus was on community spread and equilibrium conditions. Also, individuals were assumed to be initially uncolonized, antibiotic use was assumed to eradicate sensitive strains, and

stochastic effects were ignored.

Bonhoeffer and co-workers (1997) used a deterministic model to explore different antibiotic treatment protocols, and concluded that cycling antibiotics could never be an effective strategy. While this conclusion may be justified for transmission in the community, the model is not applicable to hospital populations. Their conclusion relies on the property of the model that withdrawal of antibiotics leads to a slow decline of resistant strains, while reinstating their use results in a rapid increase in their numbers. In a community setting this conclusion seems reasonable. On a hospital ward, however, patient turnover is rapid, stochastic effects are crucial and fade-outs of resistant strains not only would be expected to happen, but have in fact been repeatedly observed to occur after changes in antibiotic policy (see chapter 5). It is also far from certain that reinstating the use of the antibiotic should lead to a rapid rise of resistant strains. This will depend on their immigration rate into the ward, which in turn may be reduced by screening of incoming patients. In the same paper the authors also considered treatment with a combination of antibiotics to be, in most cases, optimal. Again, it is important to stress the limitations of the model. Only one species of bacteria was considered, and this was treated as if it were an obligate pathogen. There was no consideration of the additional selection pressure exerted by the extra antibiotic use this could lead to on the normal flora of patients. For most of the bacteria important for nosocomial infections, however, such incidental chemotherapy will represent the vast majority of their exposure to antibiotics. Furthermore, in most cases such treatment is likely to be insufficient to eradicate the commensals, but will provide strong selection pressure for resistance to the antibiotics. In other words, such multiple-antibiotic therapy may in fact exacerbate the resistance problem.

Austin and co-workers (1999) considered a similar deterministic model.

Antibiotics were assumed not to affect transmission, but to act only by reducing the persistence of sensitive strains, and to select for resistant mutants (or pre-existing resistant strains). Patients were assumed to be uncolonized on admission. As the authors make clear, these assumptions would be appropriate for an upper respiratory tract infection, for example, rather than an organism that usually behaves as a commensal such as MRSA or VRE. Multiple resistance and combination antibiotic therapies were considered. The authors reach only the tentative conclusions that both rotation of antibiotics and combination therapy may be effective.

In another study Austin and co-workers (1999b) used prevalence data on an ICU to fit a deterministic host-vector model of a similar form to that used in chapter 2 . Simulations of the stochastic process were then used to obtain confidence intervals. However, no attempt to estimate the effect of antibiotic therapy on transmission was made. In this model colonization with VRE was assumed to lead to a reduced discharge rate. No justification of this assumption is given in the text, though it was reported that VRE-positive patients had a mean stay of 10.9 days, and VRE-negatives only 7.0 days. But if patients are assumed to become colonized at some constant rate λ and discharged at a rate μ, then the expected length of stay of those who become colonized will be $1/\mu$ greater. On this basis, it might be thought that lengths of stays of VRE-positives are actually shorter than those of VRE-negatives. More plausibly, the high proportion of patients who were colonized on admission, the timing of antibiotic therapy, and the incubation period before VRE becomes detectable may all act to bring the means closer together.

Levin and colleagues (1997) considered a simple stochastic model of commensal sensitive and resistant bacteria interacting only by transmission to and from an assumed environmental reservoir, and not directly with each other. In this model there is no threshold effect and any level of treatment

retains some resistant organisms. This is a consequence of two assumptions. First, all hosts are assumed to carry some resistant bacteria provided that there are some in the environment, though this number may be very low. Second, the numbers of resistant and sensitive organisms in the environment are treated as a ratio, and fadeout is not a possibility. Their main conclusions were that even very low fitness costs for resistant bacteria would cause a big decline in numbers in the absence of antibiotics within a decade or less, but that if resistant bacteria were still present, then reinstating the use of antibiotics would be likely to lead to a rapid rise in resistance. For the reasons mentioned above this model is not directly applicable to hospital populations, but may perhaps be best thought of as a representation of bacteria normally found in the faecal flora in large populations.

Sébille and her colleagues have modelled the spread of an antibiotic resistant pathogen in an intensive care unit (ICU), initially in a deterministic framework (Sébille *et al.*, 1997), but also using a stochastic individual-based approach (Sébille and Valleron, 1997). Their model again considered the interactions between sensitive and resistant strains and simultaneously assumed vector-borne transmission by carers. Handwashing was accounted for by considering that staff had a pre-set probability of washing their hands before patient contacts. Different immigration rates of sensitive and resistant strains were allowed for, and antibiotic use was considered to be independent of the initial colonization status of patients. Their model also allowed for direct patient-to-patient transmission, as well as staff-to-staff transmission. Antibiotic treatment was modelled by assuming that each course of treatment had some probability of eliminating strains sensitive to the drug, rather than increasing the chance of acquisition. This model was used by the authors to explore the effects of three different levels of handwashing compliance (including compliance that varied according to the number of colonized patients) on a 10-bed ICU with 30 carers. In the stochastic treat-

ment, the model output presented is limited, and intended to be illustrative of of the model's potentialities, rather than representative of the full range of behaviour possible. This approach, however, is the closest to the modelling framework used here, and seems to capture the essential aspects of the dynamics of most nosocomial infections, though is somewhat more elaborate than the models developed here, for which different models are used to address antibiotic selection for resistant strains and the effect of carer handwashing behaviour.

Recently Lipsitch and co-workers (2000) also presented a model using a similar framework, though without the carers and presented only in a deterministic formulation. Like Sébille's model, this had the important improvement on other models that individuals were allowed to be colonized on admission. Antibiotic use was assumed to be independent of colonization status, so this model is appropriate for the most common causes of hospital infections. It was assumed that antibiotic use completely cleared carriage of sensitive strains. The authors showed how elimination of the sensitive strains had the result that, at the individual level, an antibiotic would be positively associate with carriage of bacteria resistant to other antibiotics (and not the current one), though negatively associated at the population level. In this model, if the resistant strain could be maintained, the authors found the counterintuitive result that changes in the transmission rate (common to both resistant and sensitive strains) affected only the prevalence of the resistant strain.

Others models have also explicitly looked at within-host dynamics of drug-resistant bacteria as a function of antibiotic treatment (Austin *et al.*, 1997; Steward *et al.*, 1998), ignoring between-host transmission.

6.2 Model for drug-resistant *Staphylococcus aureus*: methods

From the review in chapter 5, it appears that acquisition of antibiotic-resistant *S. aureus* in hospital wards can be attributed to the suppression of the sensitive bacterial flora and its replacement with resistant organisms acquired from other patients, and to a lesser extent from hospital staff. For MRSA, staff carriage is usually found to be rare and its main importance is likely to be the occasional introduction (or re-introduction) of MRSA into the patient population. For this reason colonized HCWs are not considered as a source for the acquisition of organisms by patients. Below a simple model that incorporates this behaviour is presented.

6.2.1 Two strain model: sensitive and resistant strains

To reduce model complexity no explicit assumptions about transmission routes are made here, and HCWs are thus omitted from the model. This is in accordance with the philosophy that a model should be as simple as possible, but sufficiently complex to answer the questions of interest.

The same assumptions regarding lengths of patient stays and homogeneity are made as made in chapter 2. The following additional assumptions are also made to account for the interactions of antibiotic-resistant and sensitive *S. aureus* strains with each other and with the antibiotic:

1. Two strains are considered: one resistant and one sensitive to antibiotics. Patients have some probabilities, σ and σ', of already carrying these when admitted to the ward. Only one strain can be carried at a time.

2. When challenged by a resistant strain, patients taking antibiotics are assumed to have an increased risk of becoming colonized compared to

those not taking antibiotics. For uncolonized patients the ratio of the probabilities of becoming colonized is a, while for patients colonized with sensitive strains it is a_s.

3. Patients who are already colonized with one strain are assumed to have a reduced probability of becoming colonized when challenged with another strain compared to uncolonized patients. The ratios of probabilities in the absence of antibiotics are: s for resistant strain challenges on patients colonized with sensitive strains, and r for sensitive strain challenges on patients colonized with resistant strains.

4. Patients colonized with sensitive strains taking antibiotics are assumed to lose carriage of those sensitive strains when taking antibiotics at a rate μ_{yA}.

5. All patients colonized with resistant strains are assumed to contribute equally to the chance of other patients becoming colonized with these strains. For sensitive strains, however, only those patients not taking antibiotics are assumed to contribute. This assumption is made because of the very low levels of sensitive strains found in carriers taking antibiotics (fig 5.1).

6. Patients are assumed to be prescribed antibiotics at a rate ϕ, independently of colonization status. The duration of antibiotic use is assumed to follow a negative exponential distribution with mean L.

7. The cost of resistance (if it exists) is manifest by a reduced colonization probability for patients not taking antibiotics when challenged with a resistant strain, compared to the probability of colonization when challenged with a sensitive strain. This is reflected in the transmission parameters for the sensitive and resistance strains, β_S and β_R. So $\beta_R \leq \beta_S$.

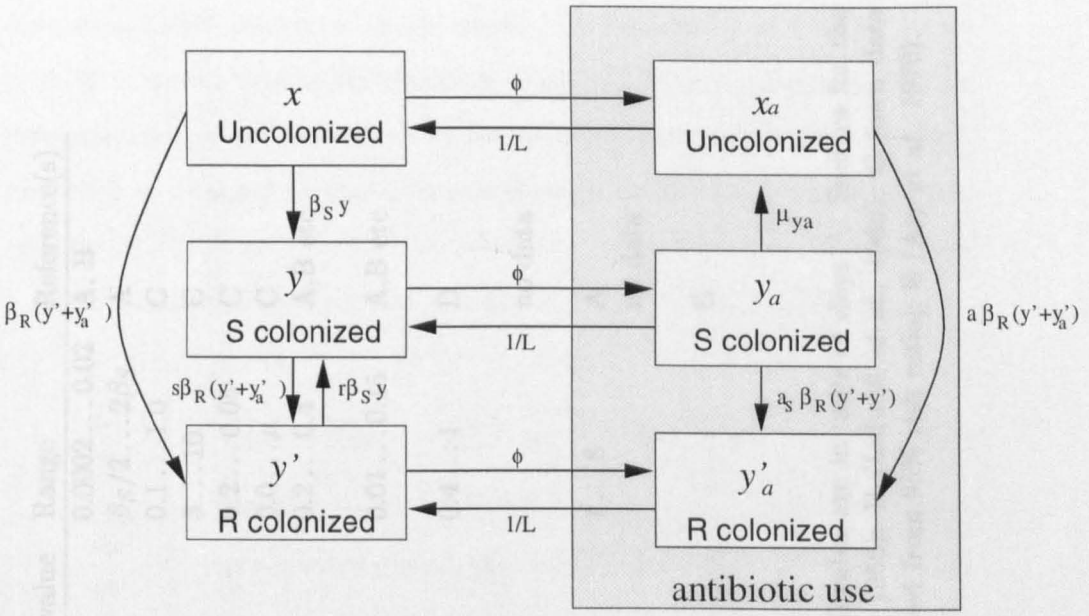

Figure 6.1: Flow diagram of the model for resistant strains (y' and y'_a) and antibiotic-sensitive strains (y and y_a), illustrating *per capita* rates of flow from each compartment. Admission and discharge rates are not shown, but we assume that patients not taking antibiotics are discharged at a rate μ, and those taking antibiotics at a (generally lower) rate μ_a. All discharges are matched by admissions. A fraction σ of these are assumed to be initially colonized with the sensitive strain, and go into the compartment y, and a fraction σ' go into the compartment y' (i.e. carry the resistant strain). The rest, $(1-\sigma-\sigma')$, go into the uncolonized compartment x. It is assumed that none are initially taking antibiotics.

8. In the absence of antibiotics, the rate at which strains are lost from patients is assumed to be very small compared to the rate at which patients are discharged from the ward, and can therefore be ignored.

Parameters are summarized in table 6.1 and the model is illustrated schematically in figure 6.1.

Deterministic formulation

The model described above is formulated in stochastic terms, and the stochastic version remains the primary interest. However, it can be instructive to first consider the properties of the deterministic analogue of the model. In

Parameter	Meaning	Default value	Range	Reference(s)
β_S	colonization rate with sensitive strains	0.001	0.0002...0.02	A, B
β_R	colonization rate with resistant strains	β_S	$\beta_S/2 \ldots 2\beta_S$	A
ϕ	antibiotic prescribing rate	0.25	0.1...1.0	C
L	mean length of antibiotic treatment	5	3...10	C
μ	discharge rate	0.1	0.2...0.05	C
μ_a	discharge rate during antibiotic treatment	0.02	$0.0 \ldots \mu$	C
σ	proportion of new admissions colonized with sensitive strains	0.3	0.2...0.4	A,B etc
σ'	proportion of new admissions colonized with resistant strains	0.05	0.01...0.15	A,B etc
s	reduction in β_R if colonized with sensitive strain	0.667	0.4...1	D
r	reduction in β_S if colonized with resistant strain	s		no data
a	multiplier for β_R if taking antibiotics	6	1...8	A
a_S	as a, but for patients colonized with sensitive strains	a		no data
μ_{ya}	rate of loss of sensitive strains during antibiotic therapy	0.03		E

Table 6.1: Parameters and their default values for the two strain model. All rates are in units of days^{-1}. Sources for the default parameter values are given. Reference codes are: A (Parker *et al.*, 1965); B (Lidwell *et al.*, 1966); C (own data collected during study described in chapter 3); D (Williams *et al.*, 1959)[derived from 95% risk ratio]; E (Aly *et al.*, 1970).

discussing this formulation of the model, the convention of talking about it *as if* it were a stochastic system is adopted. Strictly speaking, for the deterministic system *individuals* or *probabilities* have no meaning. The terminology is retained to enable connections to be drawn to the stochastic system.

The model can be expressed as follows:

$$\begin{aligned}
\frac{dx}{dt} &= -\beta_S xy - \beta_R x(y' + y'_a) - \phi x + (1/L)x_a - \mu x && (6.1)\\
&\quad + (\mu n_0 + \mu_a n_a)(1 - \sigma - \sigma') \\
\frac{dy}{dt} &= \beta_S xy + r\beta_S y'y - s\beta_R y(y' + y'_a) - \phi y + (1/L)y_a - \mu y && (6.2)\\
&\quad + (\mu n + \mu_a n_a)\sigma \\
\frac{dy'}{dt} &= \beta_R x(y' + y'_a) + s\beta_R y(y' + y'_a) - r\beta_S y'y - \phi y' + (1/L)y'_a && (6.3)\\
&\quad - \mu y' + (\mu n_0 + \mu_a n_a)\sigma' \\
\frac{dx_a}{dt} &= \mu_{ya} y_a - a\beta_R x_a(y' + y'_a) + \phi x - (1/L)x_a - \mu_a x_a && (6.4)\\
\frac{dy_a}{dt} &= -\mu_{ya} y_a - a_s\beta_R y_a(y' + y'_a) + \phi y - (1/L)y_a - \mu_a y_a && (6.5)\\
\frac{dy'_a}{dt} &= a_s\beta_R y_a(y' + y'_a) + a\beta_R x_a(y' + y'_a) + \phi y' - (1/L)y'_a && (6.6)\\
&\quad - \mu_a y'_a && (6.7)
\end{aligned}$$

where $n_a = x_a + y_a + y'_a$ and $n_0 = x + y + y'$. Since $n_0 + n_a$ is assumed to be a constant (n), only five of the above equations are independent.

For notational convenience, the dependence of the transmission rate on n is not shown, and has been absorbed into the constants β. When considering the effects of changing n, therefore, it should be borne in mind that the βs will also need to change.

Stochastic framework

The stochastic version of the deterministic model described by equations 6.2–6.7 is derived in the same manner as described in chapter 2 . There are

now six compartments, and since the population size is assumed to remain constant, the state of the systems is uniquely determined by the numbers in any five of these. There are now 28 possible transitions between these compartments. These are listed in table 6.2.

The behaviour of this model was explored with simulation studies, using a program written in C++ (available on request from the author). Experiments investigating the invasion of resistant strains were performed by introducing one patient colonized with a resistant strain into a ward at equilibrium with regard to the sensitive strain and antibiotic use. This condition was satisfied by running the simulation for a (simulated) period of 500 days before introducing the resistant strain. Persistence of resistant strains was investigated by introducing resistant strains and sampling only from those simulation runs where the resistant strain was present after a sufficiently long "burn-in" period.

Apart from σ', which was set to 0 in these experiments, and ϕ which was typically varied, the default parameters from table 6.1 were used unless stated otherwise.

6.3 Results

6.3.1 Deterministic results for the two strain model

In this model antibiotic treatment is independent of the colonization status of the patients, and the proportion of patients taking antibiotics at any one time, p_a, is given by

$$p_a = \frac{\phi}{\mu_a + \phi + 1/L} \tag{6.8}$$

So, in the absence of any bacterial strains, the equilibrium values of x and x_a (denoted $x_0^\star$ and $x_{a,0}^\star$)are given by

$$x_0^\star = n(1 - p_a) \tag{6.9}$$

Transition	Description	Rate
$y \to y+1, x \to x-1$	transmission (sensitive strain)	$\beta_S x y \Delta t$
$y \to y+1, y' \to y'-1$	transmission (sensitive strain)	$r\beta_S y' y \Delta t$
$y' \to y'+1, x \to x-1$	transmission (resistant strain)	$\beta_R x(y'+y'_A)\Delta t$
$y' \to y'+1, y \to y-1$	transmission (resistant strain)	$s\beta_R y(y'+y'_A)\Delta t$
$y'_a \to y'_a+1, x_a \to x_a-1$	transmission (resistant strain)	$a_S\beta_R x_a(y'+y'_A)\Delta t$
$y'_a \to y'_a+1, y_a \to y_a-1$	transmission (resistant strain)	$a_S\beta_R y_a(y'+y'_A)\Delta t$
$y_a \to y_a-1, x_a \to x_a+1$	loss of sensitive strain	$\mu_{ya} y_a \Delta t$
$x_a \to x_a+1, x \to x-1$	start antibiotics	$\phi x \Delta t$
$y_a \to y_a+1, y \to y-1$	start antibiotics	$\phi y \Delta t$
$y'_a \to y'_a+1, y' \to y'-1$	start antibiotics	$\phi y \Delta t$
$x_a \to x_a-1, x \to x+1$	stop antibiotics	$(x_a/L)\Delta t$
$y_a \to y_a-1, y \to y+1$	stop antibiotics	$(y_a/L)\Delta t$
$y'_a \to y'_a-1, y' \to y'+1$	stop antibiotics	$(y'_a/L)\Delta t$
$x \to x-1, y \to y+1$	discharge	$\mu x \sigma \Delta t$
$x \to x-1, y' \to y'+1$	discharge	$\mu x \sigma' \Delta t$
$x_a \to x_a-1, x \to x+1$	discharge	$\mu_a x_a(1-\sigma-\sigma')\Delta t$
$x_a \to x_a-1, y \to y+1$	discharge	$\mu_a x_a \sigma \Delta t$
$x_a \to x_a-1, y' \to y'+1$	discharge	$\mu_a x_a \sigma' \Delta t$
$y \to y-1, x \to x+1$	discharge	$\mu y(1-\sigma-\sigma')\Delta t$
$y \to y-1, y' \to y'+1$	discharge	$\mu y \sigma' \Delta t$
$y_a \to y_a-1, x \to x+1$	discharge	$\mu_a y_a(1-\sigma-\sigma')\Delta t$
$y_a \to y_a-1, y \to y+1$	discharge	$\mu_a y_a \sigma \Delta t$
$y_a \to y_a-1, y' \to y'+1$	discharge	$\mu_a y_a \sigma' \Delta t$
$y' \to y'-1, x \to x+1$	discharge	$\mu y'(1-\sigma-\sigma')\Delta t$
$y' \to y'-1, y \to y+1$	discharge	$\mu y' \sigma \Delta t$
$y'_a \to y'_a-1, x \to x+1$	discharge	$\mu_a y'_a(1-\sigma-\sigma')\Delta t$
$y'_a \to y'_a-1, y \to y+1$	discharge	$\mu_a y'_a \sigma \Delta t$
$y'_a \to y'_a-1, y' \to y'+1$	discharge	$\mu_a y'_a \sigma' \Delta t$

Table 6.2: Stochastic transitions in the two strain model. All other transitions occur at $o(\Delta t)$.

$$x^{\star}_{a,0} = np_a. \tag{6.10}$$

$$\tag{6.11}$$

In the absence of constant immigration for sensitive or resistant strains ($\sigma = \sigma' = 0$), a sensitive strain will only be able to become established on the ward given a single index case if its basic reproduction ratio, $R_{y|0^\star}$ is greater than 1. Here, $R_{y|0^\star}$ represents the number of secondary cases caused by the index case when no other strains are present on the ward and x and x_a are at their equilibrium values.

Intuitively, this can be calculated as the product of the time the index case spends on the ward, T, and the rate, C, at which other patients are colonized by this index case. Since sensitive strains are assumed to only contribute to the spread of the organism when their hosts are not taking antibiotics, only the mean time spent in compartment y is needed. Let T_0 represent this quantity, then

$$T_0 = \frac{1}{\phi + \mu} + p_r T_0 \tag{6.12}$$

where p_r is the probability of moving from y to y_a and returning to y, and is given by

$$p_r = (\frac{\phi}{\phi + \mu})(\frac{1/L}{1/L + \mu_a + \mu_{ya}})$$

which gives

$$T_0 = \frac{1 + L\mu_a + L\mu_{ya}}{\mu + (\phi + \mu)(L\mu_a + L\mu_{ya})} \tag{6.13}$$

During this time susceptibles become colonized at a rate $\beta_S x^{\star}_0$. Combining the two gives

$$R_{y|0^\star} = \frac{\beta_S(1/L + \mu_a + \mu_{ya})}{[\mu/L + (\phi + \mu)(\mu_a + \mu_{ya})]} \frac{n(\mu_a + 1/L)}{(\mu_a + \phi + 1/L)} \tag{6.14}$$

So $R_{y|0^\star}$ is a decreasing function of the prescribing rate ϕ, and as $\phi \to \infty$, $R_{y|0^\star} \to 0$. For a sufficiently high value of ϕ a sensitive strain will not be

able to invade. Three effects are responsible for this: firstly, patients are assumed not to be susceptible to strains sensitive to antibiotics they are taking; secondly, antibiotic treatment is assumed to clear carriage of sensitive strains at a rate μ_{ya}; thirdly, patients colonized with sensitive strains are assumed not to cause any transmission during antibiotic therapy due to the suppression of the resident organisms.

In a similar manner, the corresponding basic reproduction ratio for a resistant strain, $R_{y'|0^\star}$, can be shown to be

$$R_{y'|0^\star} = \frac{\beta_R(\phi + 1/L + \mu_a)}{(\phi\mu_a + \mu/L + \mu\mu_a)} \frac{n(\mu_a + 1/L + a\phi)}{(\mu_a + \phi + 1/L)} \tag{6.15}$$

Differentiating this with respect to ϕ shows that this is an increasing function of ϕ provided that $\mu_a \leq \mu$ and $a \geq 1$. When these conditions hold (as they usually will), increasing ϕ will increase the chance of a resistant strain invading.

Two distinct effects are responsible for this increase: firstly if $a > 1$ patients taking antibiotics will become colonized faster than those who aren't; secondly, if $\mu_a < \mu$, patients taking antibiotics spend longer on the ward, providing more opportunity for a resistant strain to spread. If $\mu_a = \mu$ only the first effect occurs, and the increase of $R_{y'|0^\star}$ with p_a is linear, as shown in figure 6.2.

In a hospital setting, for *S. aureus*, the situation described here with $\sigma = 0$ is generally not realistic. In most wards a significant proportion of admissions will be carrying sensitive strains (though neonatal wards represent an important exception).

If $R_{y|0^\star} > 1$, then, at the equilibrium, the numbers of susceptibles are reduced to a number $x_y^\star$ such that each patient colonized with a sensitive strain causes exactly one more case. Hence

$$R_{y|0^\star} \frac{x_y^\star}{x_0^\star} = 1 \tag{6.16}$$

where $x_0^\star$ is given by 6.10.

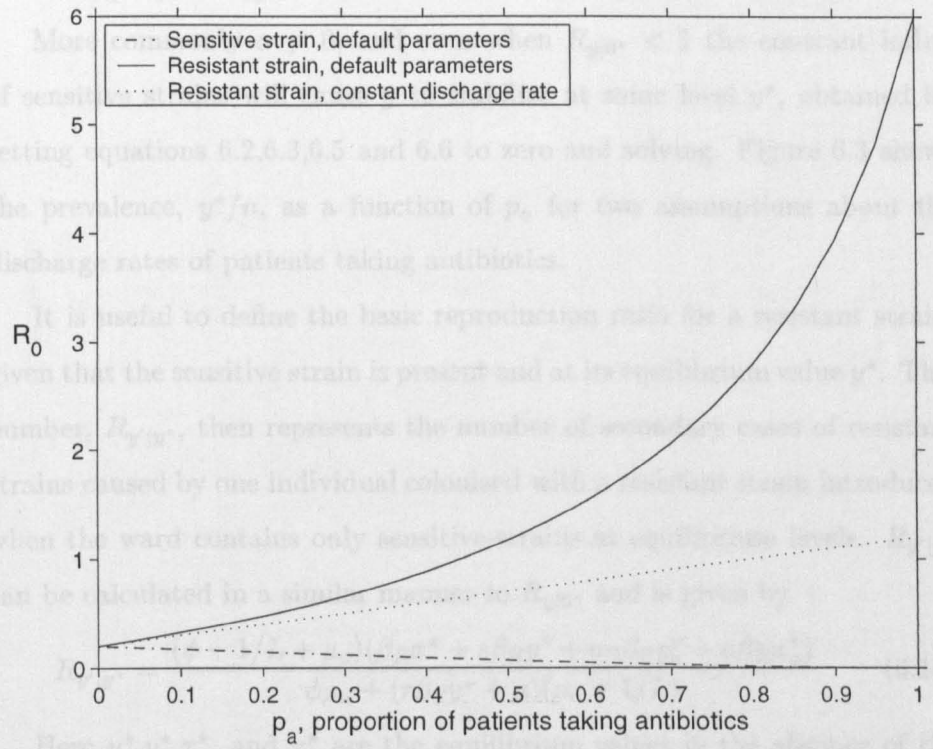

Figure 6.2: $R_{y|0^*}$ (dash-dotted line) and $R_{y'|0^*}$ (solid line) for default parameter values. Also shown (dotted line) is $R_{y'|0^*}$ assuming $\mu_a = \mu = 0.1$ (i.e. constant patient discharge rate). For the default parameters $p_a = 0.53$.

This gives

$$x^\star = \frac{\mu(\phi+\mu)(\mu_a+\mu_{ya})}{(L\mu_{ya}+L\mu_a+1)}\frac{1}{\beta_S} \tag{6.17}$$

Again, $\sigma = \sigma' = 0$ is assumed.

Then, from (6.8), $y^\star = (1-p_a)n - x^\star$, and from (6.6)

$$y_a^\star = \frac{\phi y^\star}{\mu_{ya}+\mu+1/L}$$

and $x_a^\star = p_a n - y_a^\star$.

More commonly $\sigma \neq 0$, and even when $R_{y|0^\star} < 1$ the constant influx of sensitive strains will cause y to stabilize at some level $y^\star$, obtained by setting equations 6.2,6.3,6.5 and 6.6 to zero and solving. Figure 6.3 shows the prevalence, $y^\star/n$, as a function of p_a for two assumptions about the discharge rates of patients taking antibiotics.

It is useful to define the basic reproduction ratio for a resistant strain, given that the sensitive strain is present and at its equilibrium value $y^\star$. This number, $R_{y'|y^\star}$, then represents the number of secondary cases of resistant strains caused by one individual colonized with a resistant strain introduced when the ward contains only sensitive-strains at equilibrium levels. $R_{y'|y^\star}$ can be calculated in a similar manner to $R_{y|0^\star}$ and is given by

$$R_{y'|y^\star} = \frac{(\phi+1/L+\mu_a)(\beta_R x^\star + s\beta_R y^\star + a_S\beta_R y_a^\star + a\beta_R x_a^\star)}{\phi\mu_a + (r\beta_S y^\star+\mu)(\mu_a+1/L)} \tag{6.18}$$

Here $y^\star$,$y_a^\star$,$x^\star$, and $x_a^\star$ are the equilibrium values in the absence of the resistant strain, as given above.

Figure 6.4 shows the relative contribution of the different compartments to new cases caused by the index case. For the range of parameter values considered here, at the beginning of an outbreak most of the new cases are likely to come from patients undergoing antibiotic therapy. Since antibiotic treatment is assumed to be independent of colonization status, this suggests that in an outbreak situation, where the resistant strain would not persist in the absence of antibiotics, the influx of sensitive strains may not be important in determining the chance of an outbreak. Figure 6.5 emphasizes

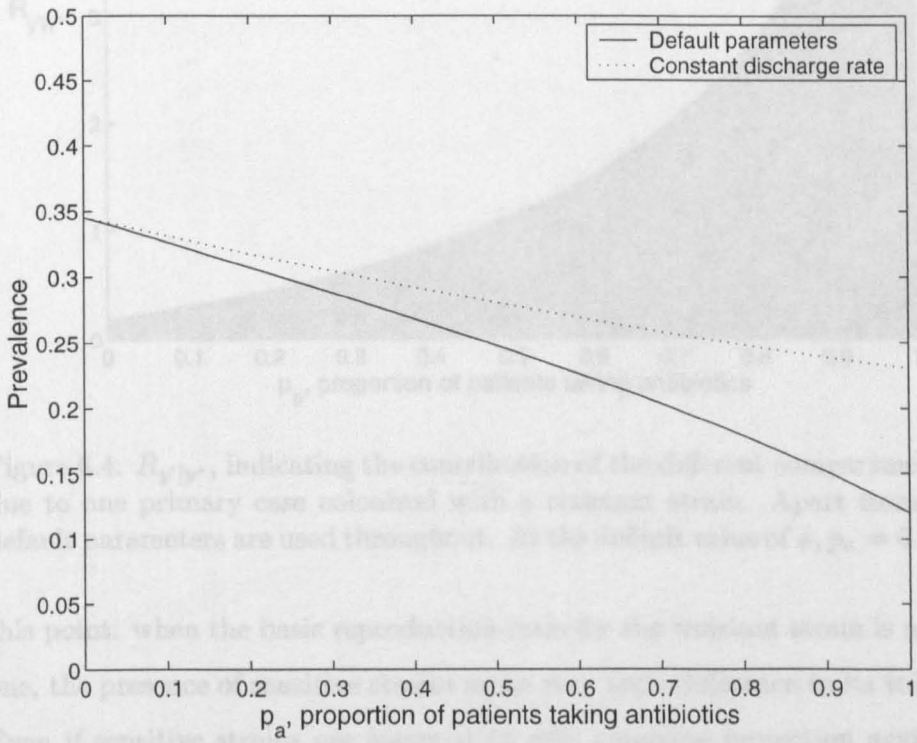

Figure 6.3: Equilibrium prevalences for sensitive strains ($y^\star/n$) with immigration ($\sigma = 0.3$). Solid line uses default parameter values, except for ϕ. The dotted line assumes $\mu_a = \mu = 0.1$ (i.e. constant patient discharge rate), but otherwise uses the same parameters. For the default parameters $p_a = 0.53$. In both cases, $R_{y|0^\star} \leq 0.2$.

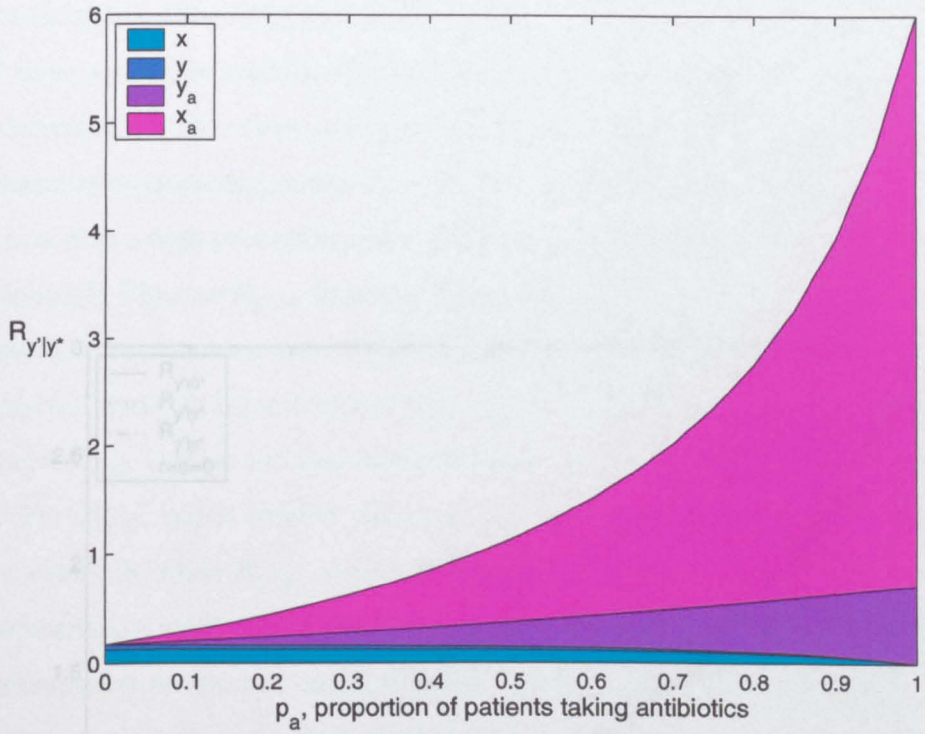

Figure 6.4: $R_{y'|y^\star}$, indicating the contribution of the different compartments due to one primary case colonized with a resistant strain. Apart from ϕ, default parameters are used throughout. At the default value of ϕ, $p_a = 0.53$.

this point: when the basic reproduction ratio for the resistant strain is near one, the presence of sensitive strains make very little difference to its value. Even if sensitive strains are assumed to offer complete protection against acquiring resistant strains for patients not taking antibiotics ($s = 0$), $R_{y'|y^\star}$ is not substantially reduced.

6.3.2 Stochastic results for the two strain model

Figure 6.6 illustrates the model's behaviour following the introduction of one patient colonized with a resistant-strain into a ward at equilibrium with regard to antibiotic use and the sensitive strain. The proportion of patients taking antibiotics at any one time (p_a) is increased from 0 to 0.8 by varying ϕ, the prescribing rate. For low values of p_a, when $R_{y'|y\star}$ is much smaller than

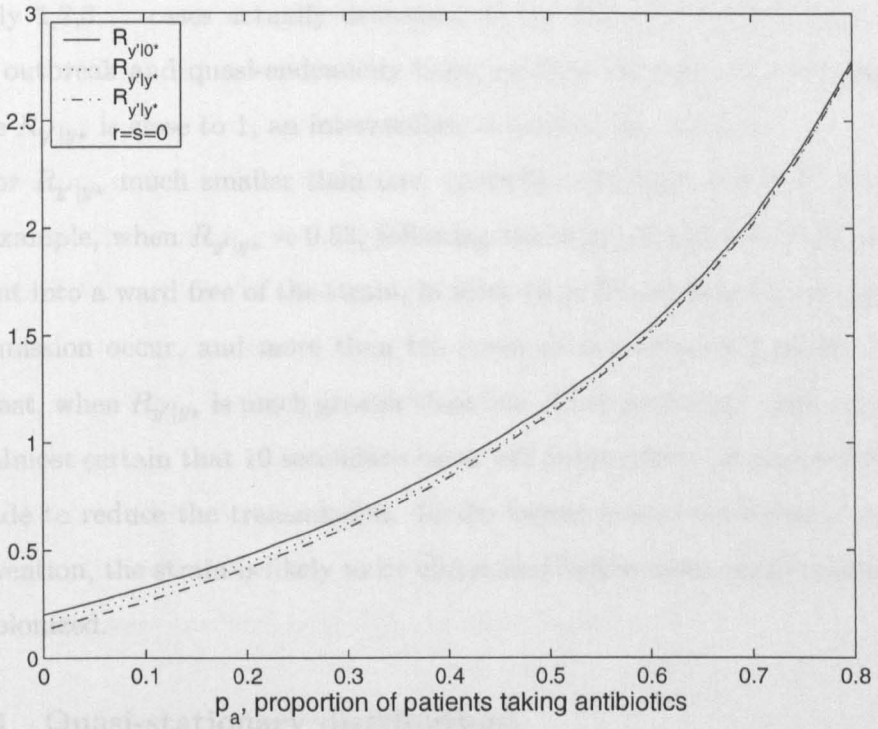

Figure 6.5: Dependency of reproduction numbers on antibiotic use in the two strain model. Solid line: $R_{y'|0^\star}$. Dotted line: $R_{y'|y^\star}$. Dot-dashed line: $R_{y'|y^\star}$ with $r = s = 0$, so in the absence of antibiotics one strain cannot replace another. Otherwise, apart from ϕ, default parameters are used throughout. At the default value of ϕ, $p_a = 0.53$.

one (less than about 0.5), though there are sporadic outbreaks, the frequency of these decreases rapidly with outbreak size. In contrast, when $R_{y'|y\star}$ is substantially higher than one, although there is still a high probability of no transmission occurring, when there are just one or two secondary cases there is now also a high probability that many more patients will subsequently be colonized. Thus as $R_{y'|y\star}$ increases above one, the frequency of there being exactly 1,2,3 ... cases actually decreases, as the chances of there being a large outbreak and quasi-endemicity being reached increase. In the region where $R_{y'|y\star}$ is close to 1, an intermediate behaviour is observed.

For $R_{y'|y\star}$ much smaller than one, sporadic outbreaks will still occur. For example, when $R_{y'|y\star} = 0.53$, following the admission of one colonized patient into a ward free of the strain, in more than 7% of cases five or more transmission occur, and more than ten occur in one admission in 50. In contrast, when $R_{y'|y\star}$ is much greater than one, if five secondary cases occur it is almost certain that 10 secondary cases will occur unless an intervention is made to reduce the transmission. In the former case, even without any intervention, the strain is likely to be eliminated before many more patients are colonized.

6.3.3 Quasi-stationary distributions

Figure 6.7 shows how the mean number of patients colonized with a resistant strain changes with time, following the introduction of one colonized case on day 0.

If a long time has elapsed since the introduction of the resistant strain, then it can be assumed that, if it is still present on the ward, the distribution of the number of patients colonized with it will be well-described by the quasi-stationary distribution, that is the stationary distribution conditional on non-extinction of the epidemic. Figure 6.8 shows the approximate quasi-stationary distributions obtained from the simulation runs, by conditioning

on non-extinction, and sampling either 75 or 150 days after the introduction of the resistant strain.

For comparison, also shown on figure 6.7 are the corresponding quasi-stationary distributions for the stochastic SIS epidemic model with basic reproduction ratio, R_0, set equal to $R_{y'|y\star}$. These distributions were obtained as the left eigenvalues of the matrix obtained by deleting the first row and column from the matrix A, where A is taken from the Kolmogorov forward equations for the SIS model: $p' = pA$. Here $p(t)$ is the row vector of state probabilities, where the ith component gives the probability of there being i infecteds at time t (Nåsell, 1999).

Clearly, they show excellent agreement with the simulation results. This suggests that despite the additional complexity, the quasi-stationary behaviour of the current model behaves essentially like the stochastic SIS model, but governed by $R_{y'|y\star}$ instead of R_0.

Adopting Nåsell's terminology for the SIS model, the behaviour of the model in three distinct regions is considered: $R_{y'|y\star}$ distinctly greater than one; $R_{y'|y\star}$ distinctly less than 1; and $R_{y'|y\star}$ in a transitional region between the two, and near the threshold value of one (Nåsell, 1999).

- $R_{y'|y\star}$ distinctly less than one

  In this case the quasi-stationary distribution is approximated by a geometric distribution. Following the introduction of one colonized case carrying the resistant strain into a ward previously free of this strain, the mean number of patients colonized with this strain decreases monotonically to zero.

- $R_{y'|y\star} \approx 1$

  The quasi-stationary distribution is approximated by a truncated normal distribution. The mean number of colonized patients stays close to one at first, before decreasing slowly due to fadeouts.

- $R_{y'|y\star}$ distinctly greater than one

  The quasi-stationary distribution is approximated by a normal distribution. Although in a large number of cases fadeout will occur shortly after the introduction of the index case, there is a rapid increase in the mean number of colonized patients; this reaches a plateau at a quasiendemic level close to the deterministic endemic level. Since fadeout is certain this must eventually decrease to zero. However, the rate at which the mean decreases itself decreases with $R_{y'|y\star}$; for large values fadeouts are very rare, and the mean remains almost constant with time.

6.3.4 Extinction times and intensity

Figure 6.9 shows times to extinction from the introduction of one colonized patient, and its dependency on $R_{y'|y^\star}$, shown on a logarithmic scale. $R_{y'|y^\star}$ was changed by changing the prescribing rate ϕ.

When the default parameters are used the mean time to extinction increases faster than exponentially with $R_{y'|y^\star}$. The mean increases much faster than the median since it is dominated by the very long persistence in a small proportion of simulation runs. The mean time to extinction is also shown as a function of p_a, the proportion of patients taking antibiotics at any one time. When patients taking antibiotics are discharged at a slower rate, the time to extinction becomes very sensitive to this number; this results from the dependency of $R_{y'|y^\star}$ on p_a, as shown in figure 6.2. When the discharge rate for patients taking antibiotics is the same as that for other patients $R_{y'|y^\star}$ increases only linearly with p_a, and the time to extinction increases only exponentially with both. In contrast to the quasi-stationary distributions, times to extinction do not depend solely on $R_{y'|y^\star}$. For the same $R_{y'|y^\star}$ values, longer extinction times are seen when the discharge rate of patients taking antibiotics is lower. This shouldn't be surprising; in the

SIS model, the expected time to extinction is found to be inversely proportional to the removal rate of infecteds (the recovery rate, or discharge rate in the case of hospitals wards) (Nåsell, 1999).

Figure 6.10 shows similar graphs for the colonized patient days (intensity) following the introduction of one patient colonized with a resistant strain. It shows a similar relationship, but the greater rate of increase with $R_{y'|y^\star}$ when patients taking antibiotics have a reduced discharge rate is more apparent.

Figure 6.11 shows how colonized patient days, the mean time to extinction, and the quasi-stationary distribution are related. Colonized patient days can be closely approximated by the product of the mean time to extinction and the quasi-stationary mean (for the SIS model), particularly for larger values of $R_{y'|y^\star}$ when most of the colonized patient days will come from outbreaks that persist for a long time, and will therefore be closer to the quasi-stationary distribution.

Times to extinction from the quasi-stationary distribution are shown in figure 6.12. As for the stochastic SIS model, these have a negative exponential distribution, regardless of the value of $R_{y'|y^\star}$.

Figure 6.12 shows times to extinction following the withdrawal of antibiotics. In all cases this has the effect of reducing the value of $R_{y'|y^\star}$ to 0.17. For values of $R_{y'|y^\star}$ distinctly less than one a similar distribution of times to fadeout is seen. Here, the mean time to extinction is reduced from 102 when $R_{y'|y^\star} = 0.53$ to 48 days. For values of $R_{y'|y^\star}$ distinctly greater than one the distribution of times to extinction from the quasi-stationary appears to approach gamma distribution as $R_{y'|y^\star}$ increases. In this case, the mean time to extinction was reduced from 7860 days for $R_{y'|y^\star} = 2.36$ to 160 days. Only in this case ($R_{y'|y^\star}$ distinctly greater than one) is there likely to be a good chance that results of an intervention are statistically significant at conventionally accepted levels. In this case, there is 5% chance

that a fadeout in the number of resistant strains will occur within 400 days without an intervention, but in almost all cases fadeout will have occurred by this time when the indicated intervention is made.

When $R_{y'|y^*}$ is close to the threshold value the times to extinction following the reduction in $R_{y'|y^*}$ have a bimodal distribution. In this case the mean extinction time was reduced from 198 days when $R_{y'|y^*} = 1.14$ to 103 days.

6.4 Discussion

The value of any model depends on the validity of its assumptions, and (as in most biological models) many of this model's assumptions may be questioned. Of particular interest are the assumptions made regarding antibiotic usage. Estimates of durations of courses and prescribing rates were based on data collected in the observational study described in chapter 3. However, a far larger set of data on antibiotic use was collected in a recent study by Bradley *et al.* (1999), and this is presented in figure 8.8 in chapter 8. Some qualitative aspects are accurately captured by the model, others aren't. Clearly many patients do go through repeated courses of antibiotics, and patients' longer stays are associated with antibiotic use. The time before a patient takes antibiotics however has a negative exponential distribution in the model, but a bimodal distribution in the data in figure 8.8, with a large proportion of patients taking antibiotics within 2 days of being admitted, and the other peak occurring about 12 days later. Duration of one course of antibiotics is (surprisingly) reasonably approximated by the negative exponential distribution in these data, but again there is in fact a bimodal distribution, with the main peaks at 1 day and a smaller peak at 5 to 7 days. For the study described in chapter 3 the negative exponential distribution also seems like a reasonable approximation. In both sets of data (as in the model) distributions of durations of antibiotic treatments

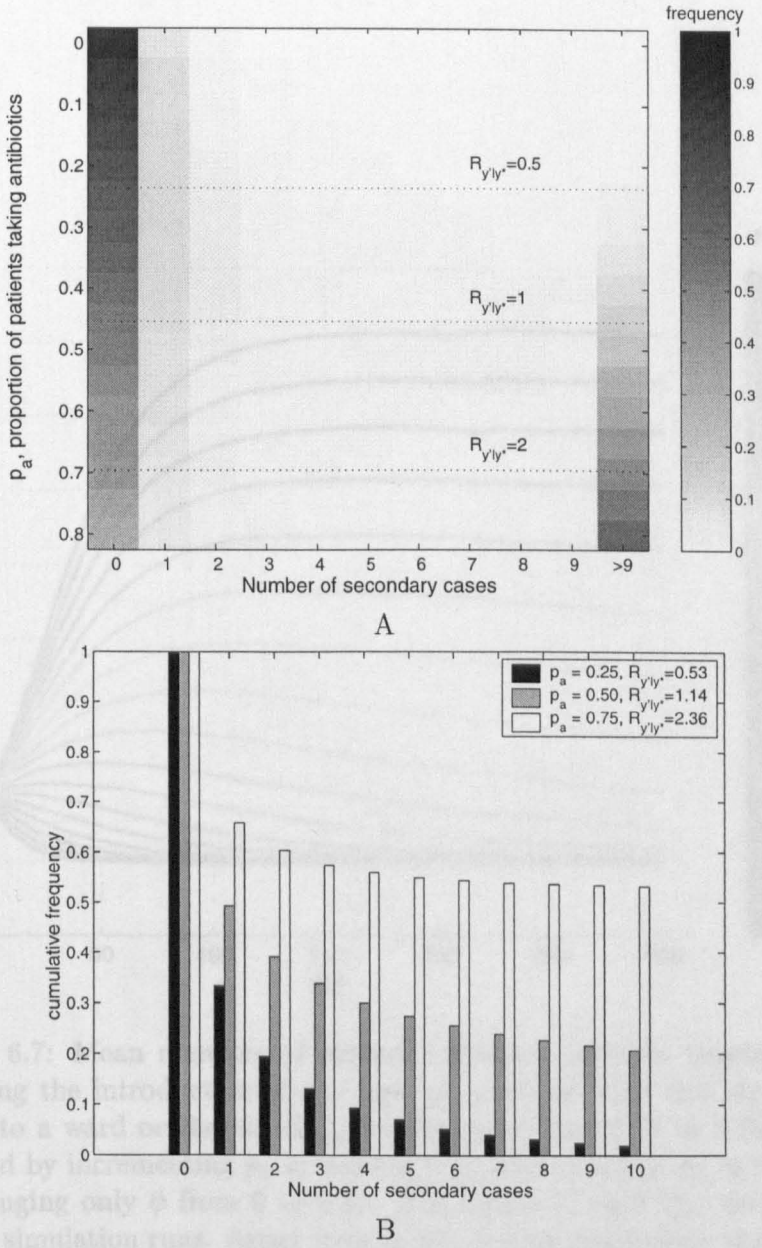

Figure 6.6: A: Frequencies of $0, 1, 2 \ldots$ secondary cases of the resistant strain, following the introduction of one patient colonized with this strain introduced to a ward at equilibrium. B: cumulative frequencies for three $R_{y'|y^\star}$ values. Each bar indicates the probability of the indicated number of secondary cases or more. For each value of p_a the results are based on 10,000 simulation runs. Apart from ϕ, the default parameters were used.

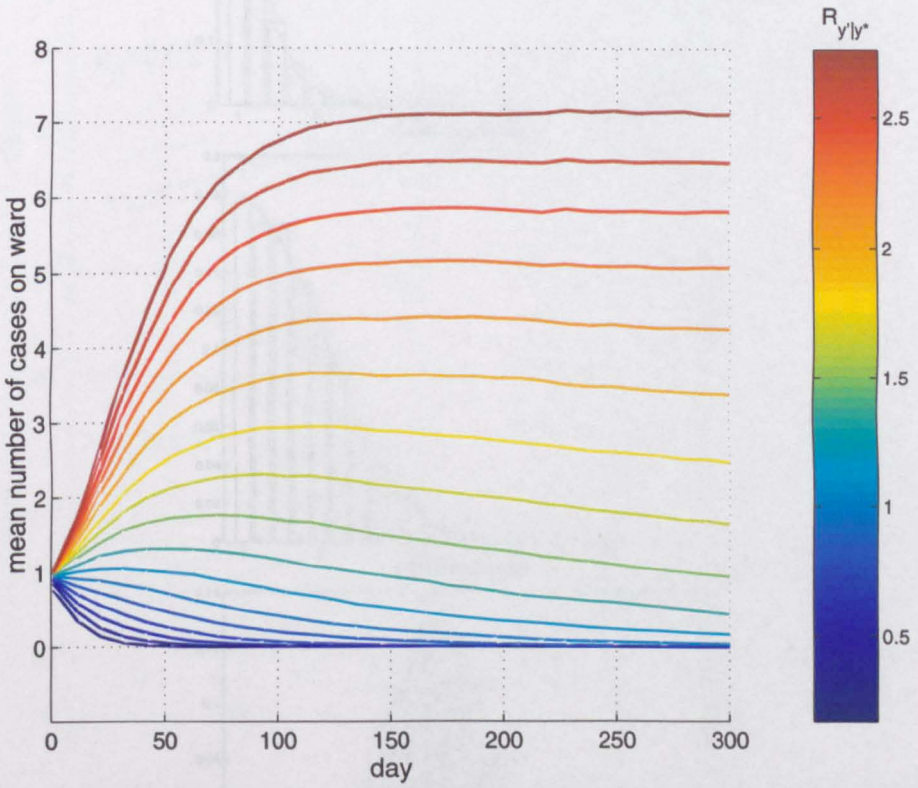

Figure 6.7: Mean numbers of patients colonized with the resistant strain following the introduction of one patient colonized with this strain introduced to a ward on day 0. $R_{y'|y^\star}$ values range from 0.17 to 2.76, and are changed by incrementing p_a in units of 0.05 from 0 to 0.8. p_a in turn is set by changing only ϕ from 0 to 0.88. The means of each line are based on 10,000 simulation runs. Apart from ϕ, the default parameters were used.

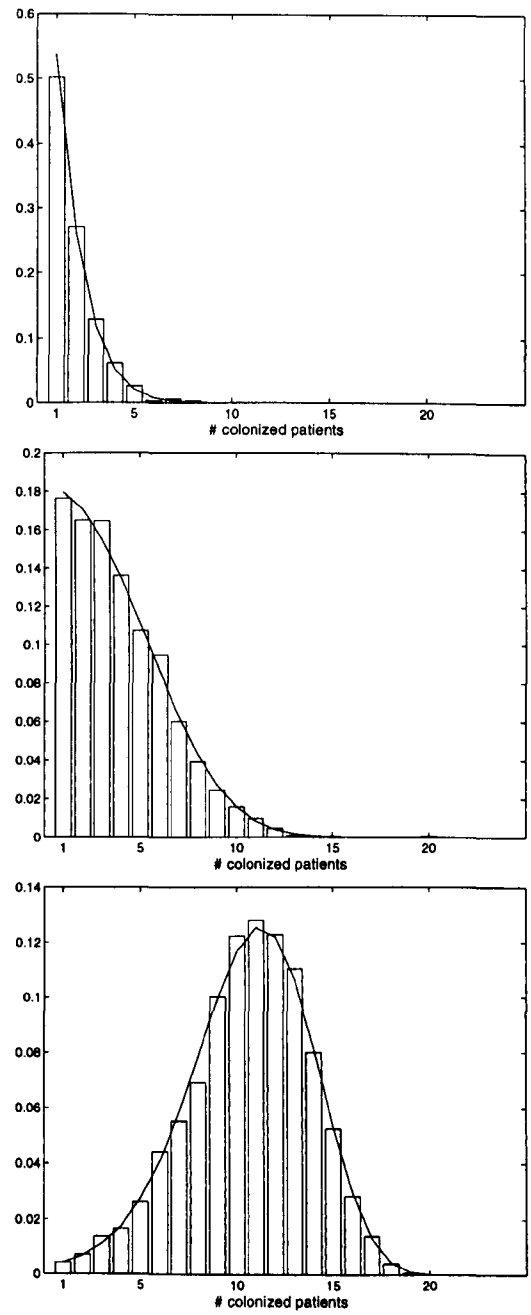

Figure 6.8: Quasi-stationary distributions of numbers of colonized patients. Bars show results from 10,000 simulation runs, and the solid line the distribution from the corresponding SIS. Parameter values were: top, $R_{y'|y^\star} = 0.528$ ($p_a = 0.25$); centre, $R_{y'|y^\star} = 1.135$ ($p_a = 0.5$); bottom, $R_{y'|y^\star} = 2.356$ ($p_a = 0.75$). For the last case simulation results are taken 150 days after the introduction of the index case (by which time resistant strain fade out had occurred in over 48% of the runs). For the first two cases, samples are based on the 3.4% and 21.7% of runs not reaching extinction 75 days after the introduction of the resistant strain. Apart from ϕ, the default parameters were used.

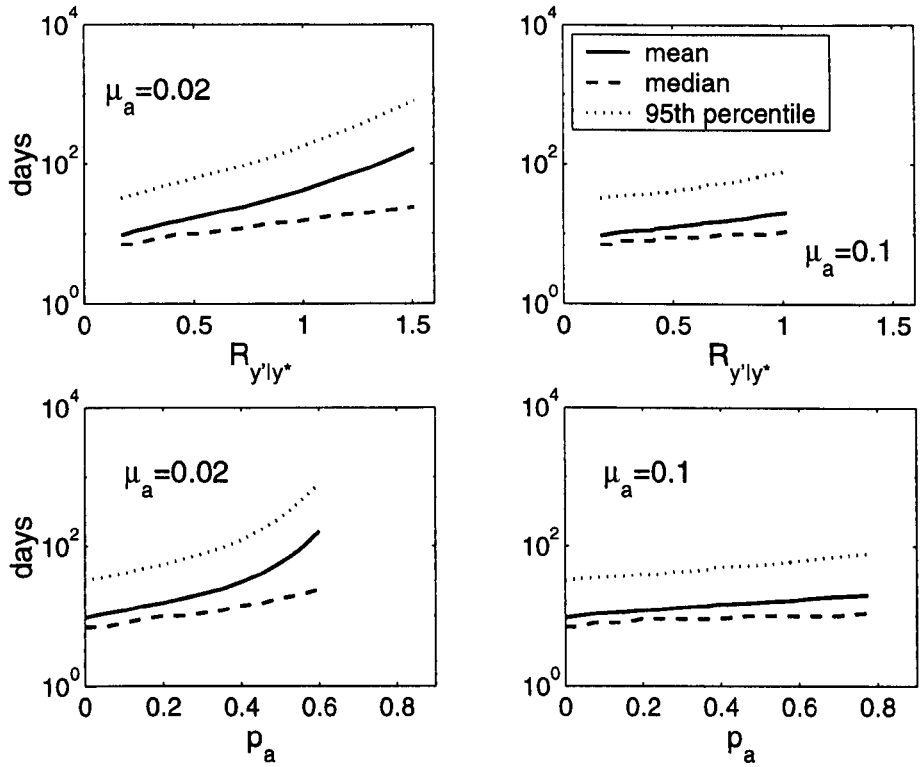

Figure 6.9: Means, medians, and 95th percentiles of the times to extinction following the introduction of one patient colonized with a resistant strain into a ward at equilibrium. In the top row persistence is plotted against $R_{y'|y^*}$, and in the bottom row against p_a. These were varied by varying ϕ between 0 and 0.88. The left column shows the results using the default value for μ_a, and the right using $\mu_a = \mu$. For each value of ϕ, 10,000 simulation runs were made. Confidence intervals for the means are too small to be distinguished on this scale and are not shown.

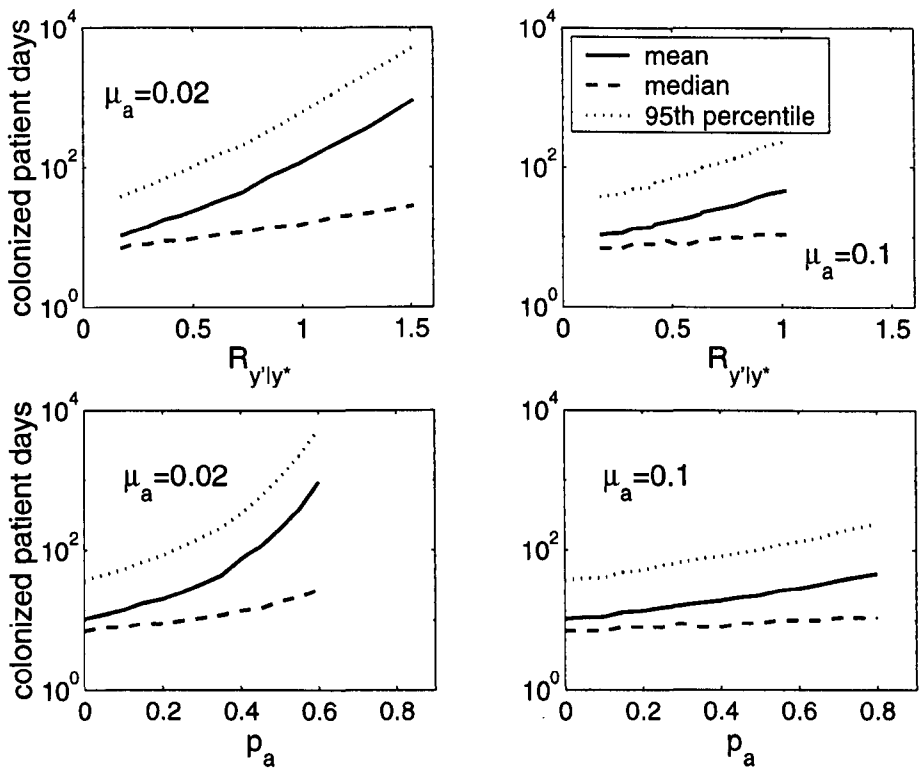

Figure 6.10: Means, medians, and 95th percentiles for the colonized patient days (intensity) following the introduction of one patient colonized with a resistant strain into a ward at equilibrium. Parameter values and simulation details are identical to those described in figure 6.9. Confidence intervals for the means are too small to be distinguished on this scale and are not shown.

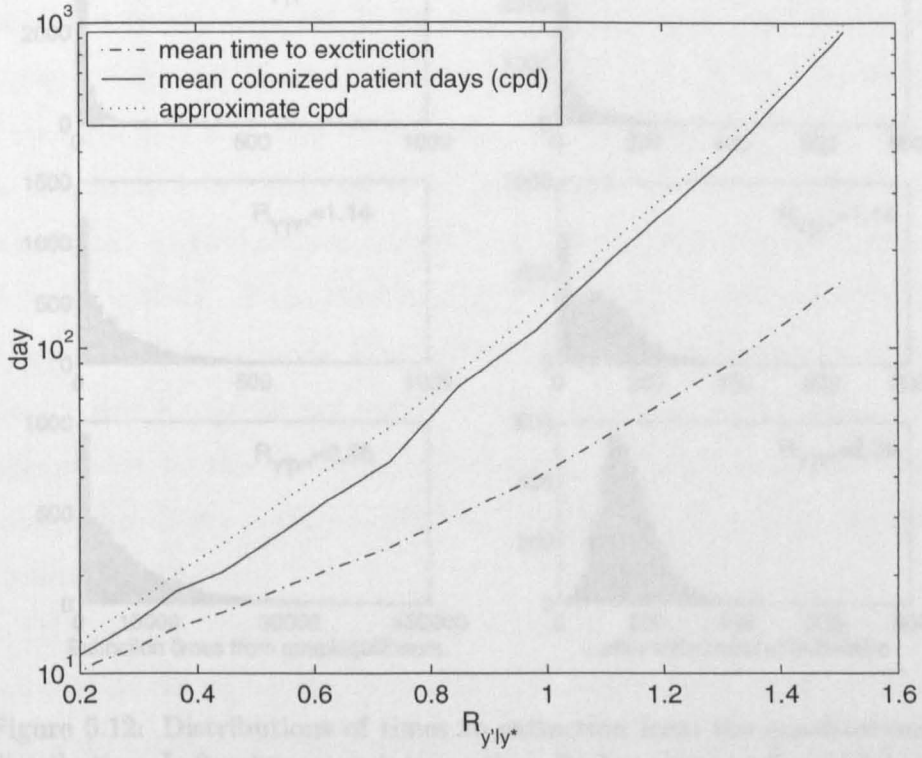

Figure 6.11: The mean colonized patient days are approximated by the product of the mean time to extinction and the mean of the quasistationary distribution (see section 6.3.3, here calculated for the SIS model with R_0 set equal to $R_{y'|y^*}$). Simulation details are as described in figure 6.9 for $\mu_a = 0.02$.

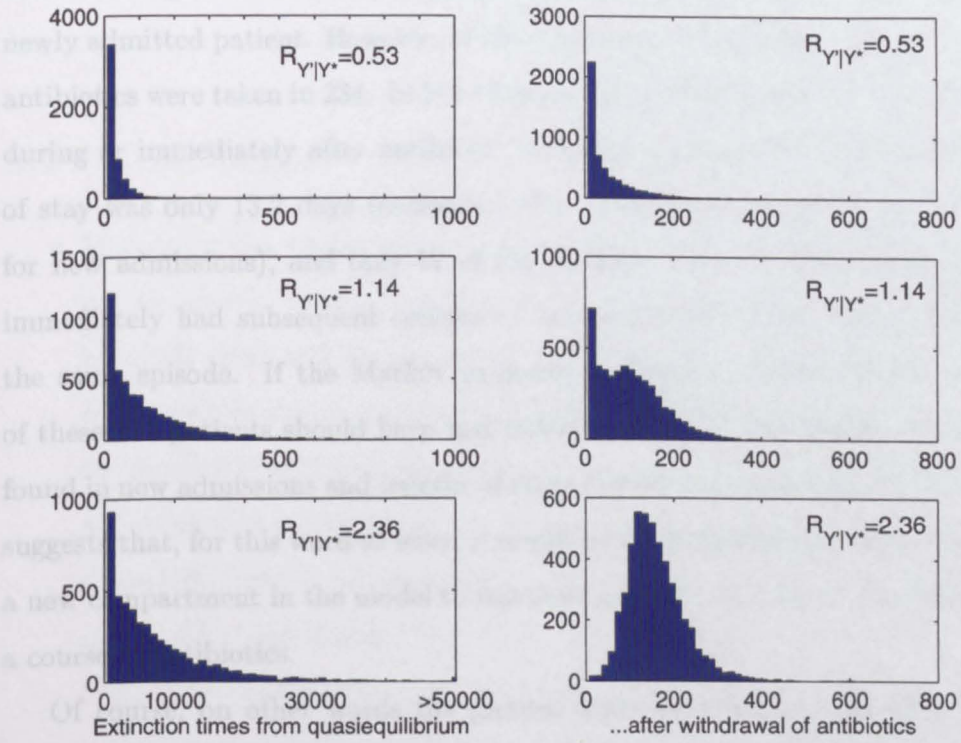

Figure 6.12: Distributions of times to extinction from the quasistationary distribution. Left column: no intervention. Right column: after withdrawal of antibiotic therapy. Parameter values for the three $R_{y'|y^*}$ values are identical to those described in figure 6.7. Each histogram is based on 5000 extinctions. So that the quasistationary distributions were (approximately) reached, the simulations were run for a "burn-in" period of 75 days (first two rows), or 150 days (third row), and times to extinction were recorded for those runs that had already reached extinction by that time. Withdrawal of antibiotics was simulated by setting ϕ to 0 after the burn-in period.

have long tails, and a large number of very short durations of antibiotic use. Of course, other wards may have very different patterns of antibiotic usage.

Another difference is that the model has the Markov property. Thus, in the model a patient who has just finished one course of antibiotics and not been discharged is as likely to undergo another course or be discharged as a newly admitted patient. However, of the 290 patients episodes in figure 8.8, antibiotics were taken in 234. In 211 of these the patients weren't discharged during or immediately after antibiotic treatment; subsequent mean length of stay was only 13.2 days (compared with a mean length of stay of 25.2 for new admissions), and only 57 of the patients who weren't discharged immediately had subsequent courses of antibiotics at a later date during the same episode. If the Markov property did hold a similar proportion of these 211 patients should have had further courses of antibiotics as that found in new admissions and lengths of stays should have been similar. This suggests that, for this ward at least, it might be more appropriate to include a new compartment in the model to represent patients who have completed a course of antibiotics.

Of course, on other wards the picture could be different, and such a modification carries the cost of further increasing model complexity. However, since a disproportionate amount of colonization should occur amongst patients who have taken antibiotics, length of stay on the ward following antibiotic therapy is likely to be an important factor for the persistence of resistant strains on the ward.

Other models have been of a similar form to that used here, but have simplified the analysis by using only three compartments: one for uncolonized patients; and one for those colonized with each of the two strains (for example Austin and Anderson, 1999; Lipsitch *et al.*, 2000). Antibiotics are then assumed to act for a fixed proportion of the time for each patient class. This has the advantage that the system reduces to two independent

equations and solutions are readily found. It has the disadvantage that correlations between antibiotic use and colonization status are lost, and these can have important effects on the dynamics of the system. In situations where a large proportion of each individual's time is spent taking antibiotics, as is likely on many hospital wards, the effects will be particularly important.

6.5 Conclusions

- Models for the spread of antibiotic resistance in the community will not be appropriate for hospitals. The important difference is that in hospitals there will be a constant influx of new hosts colonized only with sensitive strains, and these will rapidly replace resistant strains in the absence of antibiotic pressure. Furthermore, the small populations will mean that stochastic effects are important and fadeouts cannot be neglected. Here, it has been shown that changes in antibiotic policies can lead to large changes in the prevalence of resistant strains. Moreover, these changes can occur on a fast timescale, and rapidly lead to extinction of resistant strains, contrary to predictions of deterministic models. Previous arguments based on deterministic model results that claim that cycling of antibiotics will never be a good strategy cannot be applied to hospital wards. Antibiotic policies suggested by models that consider only the effect of antibiotics on obligate pathogens may be more likely to select for resistance in commensals, and therefore exacerbate the problem. Great care is therefore needed in the interpretation of models, and should be exercised in indicating their range of applicability.

- The model is very sensitive to the persistence of patients undergoing antibiotic therapy. Thus, if such patients have reduced discharge rates,

big increases in the basic reproduction number for the resistant strains can occur. This is because most of the patients who become colonized, at least in the early stages of an outbreak, are those who have taken antibiotics. For the same reason, length of stay following antibiotic therapy will also have a large impact on transmission; the longer it is, the greater the basic reproduction number. The assumptions made here about length of stay following antibiotic treatment were mathematically convenient, but probably unrealistic on most wards. More realistic models that aim to make quantitative predictions should pay particular attention to this.

- Colonization with sensitive strains may significantly reduce the chance of an individual becoming colonized with resistant *S. aureus* strains in the absence of antibiotics. When antibiotics are taken, however, the numbers of sensitive strains are reduced to such low numbers that such interference is unlikely to be important. Because of this, the fact that most transmission is likely to be associated with antibiotic therapy, and the relatively low numbers of patients colonized with sensitive *S. aureus* strains, models that ignore sensitive strains may be adequate for many purposes. Nonetheless, as shown by Lipsitch and co-workers (2000), consideration of sensitive strains and the role they play in preventing colonization with other strains can explain apparent paradoxes in colonization patterns. It is also likely that patients who have taken antibiotics are likely to be more susceptible to colonization with sensitive strains (as well as with resistant strains) due to the reduced numbers of the rest of the flora.

- All the models mentioned in this chapter consider only different strains of the same species of bacteria. Thus patients who don't carry these are considered to be the same as patients who have had their sensitive

flora suppressed or removed through antibiotic therapy. For *S. aureus* however, experiments have shown that other bacteria, such as CNS, can also reduce the risk of acquiring new *S. aureus* strains. When the antibiotics also suppress these (see figure 5.1), it may be more appropriate to model treated patients in a separate class. This would also allow lengths of stays following antibiotic treatment to be modelled explicitly.

- A major conclusion of this work is that not only the prevalence of antibiotic therapy on a ward are important, but also the timing. The effect of reduced discharge rates for patients taking antibiotics not only increases the basic reproduction number for the resistant strain, $R_{y'|y^*}$, thus increasing the chance of transmission, and the quasi-endemic level, it also increases the persistence of such a strain on the ward, over and above the increase that can be attributed to the change in $R_{y'|y^*}$. Consequently, an effective strategy to reduce resistant strains would be to discharge patients who have taken or are taking antibiotics promptly when this is possible.

- Because of stochastic effects, in many circumstances it can be difficult to interpret the results of interventions that result in the elimination of a resistant strain from a ward, as fadeout may have occurred by chance. Simulation results of extinction times from quasiequilibrium both with no intervention, and with an intervention to reduce the basic reproduction number can aid in the interpretation of these results, as well as in the design of studies. In particular, they suggest that unless the basic reproduction number is significantly higher than one and the effect of the intervention is large, then the variability of extinction times will be so large that, for most possible results of effective interventions, one would have little confidence that the results didn't

occur by chance.

- One consequence of this model, and all models like these, is that the amount of resistance will have a non-linear relationship with the amount of antibiotic consumption. Thus, given the choice, it may be a better strategy to have two wards with equal levels of antibiotic use rather than one with twice the level and one with none at all. This is an immediate consequence of the threshold effect. If both wards prescribe antibiotics below the threshold where long-term persistence can occur, only sporadic outbreaks in each will be possible. Doubling these levels, however, may enable long-term persistence of the resistant strains, resulting in many more patients becoming colonized.

- Despite its moderate complexity, the model explored here has fairly simple behaviour. It can usefully be considered to be an extension of the simple SIS model. Indeed, the quasistationary distributions are essentially the same as those from the corresponding SIS model. Times to extinction from the quasistationary distribution, however, now depend on parameters relating to antibiotic consumption as well as the "recovery rate".

- The model presented here has a large number of parameters. In this case, only simplified versions of this model were considered where some of these were set to zero or chosen to equal each other, reducing the parameter space. Exploring the full parameter space would be a difficult task and would be getting ahead of available data (though chapter 8 shows how many of the unknown parameters may be estimated). One of the major goals of this work has been to show that much of the model's behaviour can be characterized by derived quantities. In this case it was shown that some of the most important aspects of the model's behaviour can be understood by consideration of the quasis-

tationary distributions, and the mean time to extinction. The form of the quasistationary distribution in turn can be approximated using the basic reproduction ratio for the resistant strain, and through this the effect of different parameters can be determined. Simulation studies showed that the former was approximated well by that for the simpler SIS model, but with the R_0 replaced by $R_{y'|y^*}$. Such approximations may be important for further developments, including determining optimal antibiotic policies and investigating the interactions of a number of wards.

Chapter 7

A comparison of growth kinetics between methicillin-resistant and methicillin sensitive *S. aureus* strains

This chapter describes a study comparing growth kinetics between collections of MRSA and MSSA strains. The intention is to examine whether there are obvious fitness costs associated with the methicillin-resistance.

7.1 Background: the fitness cost of resistance

Most mutations that make significant changes to the physiology of a cell would be expected to be deleterious (Maynard Smith, 1989). Mutations conferring resistance to antibiotics should be no different in the absence of antibiotics. *In vitro* studies have borne out this intuition, with maladaptive

pleiotropic effects accompanying mutations conferring resistance to viruses in *Escherichia coli* (Lenski, 1988), rifampicin in *Bacillus subtilis* (Cohan *et al.*, 1994) and streptomycin resistance in *E. coli* (Schrag and Perrot, 1996). These studies showed that this initial "cost" of resistance is subsequently reduced through one or more compensatory mutations.

Significant variations in the initial costs are seen, caused in part by the resistance genes themselves, but mostly by variations in the genetic background. Increasing the antibiotic use should increase the cost that a resistant organism is capable of supporting in the absence of antibiotics while still persisting. Consequently, hospitals, with their exceptionally high concentration of antibiotic usage, might by expected to play a disproportionate role in the selection of resistance: even resistance genes that carry a high fitness cost in the absence of antibiotics may persist for long enough to enable the reduction in the cost to occur.

Generally, very little is known about what makes one bacterial clone a better competitor than another. Most laboratory experiments aimed at investigating fitness and fitness costs have relied on *in vitro* competition experiments between strains (when two or more strains are grown together) or simply on studies of growth kinetics (see for example Lenski *et al.*, 1998). For *S. aureus*, interpretation of competition experiments may be complicated by the fact that different groups of strains are able to inhibit production of extracellular proteins in other groups (Guangyong *et al.*, 1997). Competitive fitness may therefore not be hierarchical, but instead depend on prevalences of other strains in the environment.

To avoid these complications, growth kinetics of *S. aureus* strains grown in isolation were studied. For a commensal such as *S. aureus* the two important components of fitness are (i) ability to spread to new hosts (ii) ability to persist on hosts (in hospital populations this can have an important affect on the admission rate of colonized patients; many patients are readmitted

at varying times after discharge). In general, trade-offs may be expected between these two factors, but higher growth rates may be expected to be associated with both factors, and represent an obvious starting point when looking for any costs associated with resistance.

7.2 Growth rate studies on MRSA and MSSA

7.2.1 Method

Bacterial strains

S. aureus strains used in this study are listed in table 7.1.

All the London strains in this table correspond to those described in chapter 3. The molecular typing results in that chapter were used to choose strains that were epidemiologically unrelated. The Polish strains in table 7.1 have also been typed previously using a variety of molecular and phenotypic methods (Trzciński *et al.*, 1997). Again, strains chosen for inclusion were shown by the typing results to be epidemiologically unrelated. The remaining strains listed came from a variety of sources and had only been typed using antibiograms. On the basis of these results, and by considering the location and date of recovery, strains were selected that were thought unlikely to represent single clones. MSSA strains isolated from adult population have been shown to come from a large number of distinct clones (Kobayashi *et al.*, 1995). For those strains coming from neither London nor Poland, it was therefore assumed that two MSSA strains isolated from patients in the same hospital were unlikely to be related, unless there was information to the contrary. When choosing between two epidemiologically related strains to include in the analysis, the choice was always made randomly (by coin flipping).

Some additional growth rate measurements were performed to test whether observed growth rates were consistent at three levels: between different runs

using the same strains (tested with repeated runs of strains B28, B29, and reference strain ATCC25923); between different isolates of the same strain obtained from the same patients on different occasions (strains B2 and B29); and between different isolates of the same strain (as defined by typing results in chapter 3) obtained from different patients where there appeared to have been transmission (strains B10 and B13, and strains B28 and B29).

Culturing of bacterial strains

All strains were inoculated on to brain heart infusion (BHI) agar plates and incubated overnight at 37°C. Cells from one colony of each strain were suspended in phosphate buffered saline and diluted to an optical density at 600nm (OD_{600}) of $0.1 \pm 15\%$ using a microplate reader (EMS Reader MF, Labsystems, Finland). Once at the correct OD, $1\mu l$ of each suspension was added to 200 μl of Tryptic Soy Broth (TSB, Difco laboratories, Detroit) in each of three wells on the 96-well plate. In addition to the strains listed in table 7.1 the type strain (ATCC25923) was added to three wells of each plate as a control.

Growth conditions and measurements

Using the microplate reader the cells were incubated at 37°C, shaking every 10 minutes. OD_{600} readings were taken from each well every 10 minutes over a period of 10 hours.

7.2.2 Statistical methods

Exponential phase growth rates for each well were taken to be the maximum growth rates observed over any 90 minute period. The growth rates were estimated by taking the gradient of the least squares line through the $ln(OD_{600})$ readings. The lag period was then estimated as the time from

Strain ID	Isolated from	Location	Year	Resistance Pattern
MRSA strains				
B28	Hospital patient	London	1998	C,E,M,P
K1678	Hospital patient	Poland	1990-1992	D,M,P,T,To
M38	Hospital	Warwick	1999	C,E,M,P
M19	Hospital patient	Poland	1990-1992	Ch,Cl, D, E,G, K, L,M,P,Sp,St,T,To
M15	Hospital patient	Poland	1990-1992	D,M,Mu,P, To,T
M22	Hospital patient	Poland	1990-1992	D,G, K,M, Mi, P,St,T,To
M61	Hospital	Warwick	1999	C,M,P
M141	Hospital	Bulgaria	1996	Ch,D,E, G,K,M,P,T,To
M152	Hospital	Bulgaria	1998	D, Mh,P,T
M138	Hospital	Bulgaria	1996	G,P, K, M, To
M146	Hospital	Bulgaria	1997	Cl,E, G,K,L,M,P,To
M120	Hospital	Bulgaria	1995	M,P,St
M129	Hospital	Bulgaria	1995	E, G,K,N,Mh,P,St,to
M112	Hospital	Bulgaria	1994	G,P, K, M, To
M128	Hospital	Bulgaria	1995	E,G, K, Mh,P,St,To
M93	Hospital	Ipswich	1999	E, G,K,M,P,To
M95	Hospital	Ipswich	1999	E,G,K, Mh,P,Su,To
M110	Hospital	Bulgaria	1994	Ch,G,K,M,St,To
M88	Hospital	Rugby	1999	C,E,M,P
MSSA strains				
B10	Hospital patient	London	1998	P
B22A	Hospital staff	London	1998	P
B30A	Hospital staff	London	1998	P
B20A	Hospital staff	London	1998	P
B35	Hospital staff	London	1998	P,k
B3A	Hospital patient	London	1998	P
B7	Hospital patient	London	1998	
M49	Hospital	Warwick	1999	P
M8	Community	Coventry	1998	D,T
M57	Hospital	Warwick	1999	
M43	Hospital	Warwick	1999	C,P
M94	Hospital	Ipswich	1999	st
M3	Community	Coventry	1998	P
M87	Hospital	Rugby	1999	cl,D, f,T
M1	Community	Coventry	1998	D,P,T
B16	Hospital patient	London	1998	
B21	Hospital patient	London	1998	P
M36	Hospital	Warwick	1999	
M75	Hospital	Rugby	1999	

Table 7.1: Strains used in the growth rate experiment. Antibiotic resistance codes: A, amikacin; C, ciprofloxacin ; Ch, Chloramphenicol; Cl,clindamycin; D, doxycycline; E, erythromycin; F, fusidic acid;G, gentamicin, K kanamycin;L, lincomycin; M, methicillin (homogeneous resistance; Mh, methicillin (heterogeneous resistance); Mi, minocycine;Mu, mupirocin; N, neomcyin;P, penicillin, R, rifampin, Sp, Spiramcyin; St, streptomycin;Su, sulphamethoxazole; T, tetracycline; To, tobramycin. Lower case indicates intermediate resistance.

starting incubation until the start of the 90 minute period with the maximum growth rate.

A two-level nested analysis of variance (mixed model) was then used to study the differences in growth rates and lag periods between the groups of MRSA and MSSA strains, and between the individual strains (Sokal and Rohlf, 1994).

In addition to the MRSA/MSSA comparison (the purpose of the experiment) a number of other exploratory comparisons between different subgroups of strains were performed. Such additional comparisons were performed between tetracycline-resistant and tetracycline-sensitive strains, and between antibiotic sensitive strains (or those resistant to penicillin only) and multiply-resistant strains (classified as those with four or more distinct resistance mechanisms). Mean growth rates and lag periods were compared using two-tailed t-tests. Growth rate parameters were also examined for linear trends with the time since isolation, and with the number of distinct antibiotic resistance mechanisms identified.

As consistency checks, comparisons of growth rate parameters between pairs of isolates were made using two-tailed t-tests.

7.2.3 Results

Mean growth rates and lag periods are listed in table 7.2. In all cases the straight lines fitted to the $ln(OD_{600})$ values over 90 minute periods showed an excellent fit to the data, with all coefficients of determination (R^2 values) for the lines of maximum slope exceeding 0.996. This indicates that growth rates were measured over periods of exponential growth.

Tables 7.3 and 7.4 summarize the analyses of variance for the growth rate data and the lag period data. Though a slightly higher mean growth rate was found in the MRSA strains, the data provide no evidence to reject the null hypothesis that MRSA and MSSA strains have the same growth rates.

MRSA strains			MSSA strains		
Strain	mean growth rate(SD)	mean lag(SD)	Strain	mean growth rate (SD)	mean lag (SD)
B28	0.093(0.002)	6.7(0.6)	B10	0.078(0.004)	6.7(0.6)
K1678	0.092(0.002)	17.7(1.5)	B16	0.055(0.003)	17.7(1.5)
M110	0.085(0.004)	7.7(1.2)	B20A	0.081(0.002)	7.7(1.2)
M112	0.089(0.003)	13.7(0.6)	B21	0.096(0.003)	13.7(0.6)
M120	0.090(0.001)	7.7(1.5)	B22A	0.078(0.003)	7.7(1.5)
M128	0.084(0.003)	12.3(2.9)	B30A	0.090(0.003)	12.3(2.9)
M129	0.088(0.002)	10.0(2.0)	B35	0.078(0.001)	10.0(2.0)
M138	0.087(0.007)	11.3(0.6)	B3A	0.089(0.003)	11.3(0.6)
M141	0.091(0.007)	9.3(0.6)	B7	0.076(0.003)	9.3(0.6)
M146	0.090(0.004)	18.3(5.9)	M1	0.068(0.003)	18.3(5.9)
M152	0.091(0.006)	17.3(1.5)	M3	0.104(0.004)	17.3(1.5)
M19	0.070(0.005)	12.0(0.0)	M36	0.066(0.001)	12.0(0.0)
M22	0.053(0.002)	7.3(0.6)	M43	0.070(0.002)	7.3(0.6)
M38	0.090(0.005)	17.3(4.7)	M49	0.067(0.007)	17.3(4.7)
M61	0.089(0.003)	10.7(0.6)	M57	0.108(0.005)	10.7(0.6)
M88	0.094(0.003)	20.7(0.6)	M75	0.071(0.002)	20.7(0.6)
M93	0.056(0.004)	13.3(1.2)	M8	0.089(0.002)	13.3(1.2)
M95	0.079(0.002)	14.3(0.6)	M87	0.063(0.003)	14.3(0.6)
M15	0.091(0.005)	20.3(0.6)	M94	0.084(0.001)	20.3(0.6)
MRSA mean:	0.084	15.9	MSSA mean:	0.079	13.1

Table 7.2: Mean growth rates and lag periods of MRSA and MSSA strains, based on OD_{600} readings from cell cultures in three wells for each strain. Lag periods were estimated as described in the text, and are expressed as multiples of 10 minutes. Growth rates give the maximum gradient of the $ln(OD_{600})$ readings plotted against time (expressed in units of ten minutes). Assuming OD_{600} is proportional to the number of cells present, then the doubling period (in minutes) is given by $10 \times ln2$ (or 6.93) times the reciprocal of the growth rate.

Source of variation	df	SS	MS	F_s	P-value
MRSA/MSSA	1	0.000688	0.000688	1.365	0.25
Among strains	36	0.0182	0.000504	36.276	3.63×10^{-35}
Within strains (between wells)	76	0.00106	1.39×10^{-5}		

Table 7.3: ANOVA table for MRSA and MSSA growth rates. The within strain variance (1.39×10^{-5}) represents only 7.7% of the total, while the estimated among strain variance (0.00016) accounts for 90.5% of the total.

Source of variation	df	SS	MS	F_s	P-value
MRSA/MSSA	1	233.06	233.06	4.45	0.042
Among strains	36	1886.07	52.39	12.09	1.67×10^{-19}
Within strains (between wells)	76	329.33	4.33		

Table 7.4: ANOVA table for MRSA and MSSA lag periods. The within strain (between well) variance (4.33) represents 18.4% of the total, while the estimated among strain variance (16.019) accounts for 68.1% of the total. The difference between the estimated lag periods of MRSA and MSSA strains, though small, is just significant at the 5% level.

Clearly, there is considerable variation in growth rates between the strains, which accounts for over 90% of the total variance. Minimal difference is seen between wells with the same strain.

The estimated lag periods of the MRSA strains were, on average, almost 20 minutes longer than those of the MSSA strains. Again, there was large variation between the strains, and more variation between the individual wells containing the same strains than was seen for the growth rates. Though the MRSA/MSSA classification explained a relatively small proportion of the differences in lags compared to the strain classification, the difference was just significant at the 5% level.

The results of the consistency checks found that for all three strains where repeated runs were made, there was no evidence to reject the null hypothesis that growth rates were consistent between runs (for all three pairwise comparisons $P > 0.1$). Similarly, there was no evidence for any difference in growth rates between isolates B2 and B29, which typed identically in chapter 3, and had been obtained from the same patient on different occasions ($P = 0.68$). Comparing growth rates between strains typing identically but isolated from different patients also gave similar results ($P = 0.41$ for isolates B28 and B29, and $P = 0.094$ for B10 and B13). Overall, though large differences in growth rates were found between different strains, there

was no reason to reject the hypothesis that isolates that were directly epidemiologically related had the same growth rates.

No significant trends were found between growth rates or lag periods and time since isolation ($P = 0.83$ and $P = 0.87$ respectively). Similarly no significant linear relationship was detected between growth rates or lag periods and the number of resistance mechanisms ($P = 0.32$ and $P = 0.87$).

Tetracycline-resistant strains had, on average, slightly lower growth rates than tetracycline-sensitive strains, but again the difference was non-significant ($P = 0.16$) as was the difference in lags between the two groups of strains ($P = 0.92$). There were, however, only a few strains resistant to tetracycline.

Comparing isolates with four or more distinct resistance mechanisms with those with zero or one, the less resistant strains did have slightly higher mean growth rates (0.082 compared to 0.079), but this difference was far from being significant ($P = 0.60$). There was a more pronounced difference in the mean lag period between the multiply-resistant strains and the sensitive strains (13.59 compared to 16.27), though again this was not significant ($p = 0.13$).

7.2.4 Discussion

The main conclusion from the growth rate experiments is that there is considerable heterogeneity in growth rate parameters between different strains, and these parameters seem to be stable properties of the strains. There was no evidence to suggest that MRSA strains had different mean growth rates to MRSA strains, although there was some evidence for a small difference in the lag periods associated with these groups of strains, with the MRSA strains having, on average, slightly greater lags (though many MRSA strains had shorter lag periods than many MSSA strains). Other factors, such as years since isolated and number resistance mechanisms present, did not help explain any of the heterogeneity between the strains.

Although these differences in lags were significant at the 5% level, the variation between the strains accounts for much more of the overall variation than the small differences between the MRSA and MSSA groups. Only tentative conclusions about differences in the length of the lag phase between MRSA and MSSA should be drawn. The experimental protocol was designed more to measure the maximum growth rate rather than the duration of the lag phase. Consequently, no attempt was made to standardize the growth phase of the cells used to inoculate the growth medium in the microplate wells at the start of the experiment, and estimates of the lag may therefore be biased by the initial status of the cells (there are, however, no reasons for thinking that there should be systematic differences between MRSA and MSSA strains, both of which were treated identically once recovered from the frozen samples). Since MRSA and MSSA strains are not, however, generally treated identically from isolation, it is possible that systematic differences may arise as a result of their history of subculturing. Also, the strains examined are not by any means a random sample of all MRSA and MSSA strains, with many of them coming from just a few locations. A different selection of strains may have produced very different results.

Perhaps the one reason for attaching any weight to these comparisons is that the one other study which attempted to compare the *in vitro* growth rates and lag periods of MRSA and MSSA reached a similar conclusion (Mizobuchi *et al.*, 1994): no differences were found in growth rates in exponential phase, but the growth of MRSA was much slower in the lag phase.

Parameter estimation

The growth curves sometimes showed irregularities in the later growth stages. Because of this they were not analysed by fitting the full range of data to standard forms for growth curves as these irregularities would have led to a reduction in the accuracy with which the maximal growth rates and the

lag period were measured. Growth rates were estimated using only data from the 90 minute period where the maximum growth rate occurred in each well. This period was chosen to be long enough to prevent stochastic fluctuations and/or measurement errors from dominating, but short enough so that growth was accurately described by an exponential curve over the period. In fact, all of the final fitted straight lines to the log-transformed data had coefficients of determination (r^2 values) greater than 0.996. Inspection of the fitted lines indicated that the lines with maximal slopes gave better estimates of the exponential growth rates than did lines with maximal r^2 values, though in most cases identical, or near-identical, results were obtained.

The estimation of the lag period is somewhat arbitrary, and may lead slower growing strains to have apparently higher lags, since there will be a greater region over which growth is exponential and to which a straight line segment may be fitted to the data. Although there was a small negative association between growth rate and lag period (product-moment correlation coefficient $r = -0.18$) this was not statistically significant ($P = 0.26$).

The cost of resistance

Clearly *in vitro* studies such as this can at best be a poor substitute for *in vivo* studies in human hosts, and the growth rate is but a single component of a strain's fitness.

However, experiments like this are easy to carry out. As Mizobuchi *et al.* (1994) found, there was no evidence of fitness cost of methicillin-resistance manifesting itself in a reduced growth rate, though there was some evidence for an increased lag period.

Chapter 8

Fitting models to data

8.1 Introduction

This chapter deals with the problem of how to fit simple transmission models, such as that described in chapter 2, to hospital data using Markov Chain Monte Carlo (MCMC) methods.

The hospital data are assumed to come from studies where all patients are swabbed, with the possible exception of a number of "non-consenters", where it is assumed that non-consent occurs at random. The data then consist of a series of patient swabs indicating the presence or absence of a given pathogen, and in some cases also a strain type.

Where data consist only of clinical isolates for pathogens normally behaving as commensals (such as MRSA and VRE) more complex models, accounting for both transmission and for progression from colonization to infection, are required. The methods presented here are therefore not appropriate for such data-sets. Modified MCMC-based methods, however, are likely to be valuable tools for the analysis of such data. For community-acquired infections they have already been used to estimate incidence rates for subclinical *Haemophilus influenzae* type b (Hib) infection in the community, using only clinical infection data (Auranen, 2000).

In the past little formal analysis has been carried out on data arising from such transmission studies of nosocomial pathogens. Typically, only summary statistics are presented. In the best cases authors have made meticulous attempts to estimate acquisition rates per source (for example Lidwell *et al.*, 1966, 1971). Sometimes these acquisition rates have been related to the antibiotic consumption of patients acquiring the strains. In doing this authors are forced to develop sets of *ad hoc* rules for determining the times of acquisition (Lidwell *et al.*, 1966), and for assigning the acquisition of strains to specific sources. For example, for acquisitions having more than one possible source, Lidwell *et al.* (1971) assumes the same distribution of sources as for acquisitions where the source can be unambiguously located. In contrast, Jernigan *et al.* (1996) used a panel of independent observers who considered temporal and spatial factors along with staff contact patterns to assess the "most likely" sources. Sometimes the process can seem even more arbitrary. Shooter *et al.* (1963), for example, assumed the true source was always the nearest.

Earlier studies also had the disadvantage that they depended on phage typing. This technique is unable to type many strains and is not as effective at grouping outbreak-related strains as DNA-based techniques (Tenover *et al.*, 1994) . However, even if one can be almost certain of the identity of strains colonizing two or more patients, these can only ever be observed at a finite number of time points, and there will inevitably be uncertainty in the underlying process being observed. Further uncertainty is likely to arise due varying sensitivities of sampling and culturing methods.

Two attempts have been made to fit hospital data to models. Firstly Austin and co-workers (1999b) used prevalence data on an ICU to fit a deterministic host-vector model of a similar form to that used in chapter 2. Simulations of the stochastic process were then used to obtain confidence intervals. No attempt to estimate the effect of antibiotic therapy on trans-

mission was made.

In another study, Bonten and co-workers (1998) cultured rectal swabs collected daily from patients on an ICU, and used a Cox proportional hazards model to relate the acquisition rate of VRE to factors including antibiotic use, and what they termed the "colonization pressure". By this they meant the prevalence of VRE. An unfortunate consequence of their choice of model is that the hazard for acquiring VRE increases exponentially with the prevalence. Epidemiologically, this is meaningless. Nonetheless, the "colonization pressure" still appeared to be the most important variable affecting VRE acquisition, with a weaker association with use of third-generation cephalosporins.

One possible approach to dealing with such data would be to analyse the number of transmissions on each day using a generalized linear model to investigate the dependence of the number of transmission on factors such as the number of colonized patients, antibiotic use (see Becker, 1989, chap. 6). For infections where acquisition is clearly indicated by a show of symptoms this can be a powerful approach. For asymptomatic infections, however, arbitrary decisions again have to be made about the true acquisition times unless swabbing is sufficiently frequent to remove this uncertainty. Also, since patient swabs are usually taken weekly in this type of study, and in this time interval a high turnover of patients would be expected on most wards, the analysis of the number of acquisitions in any given time period will necessarily be complicated by the arrival of new, potentially colonized patients. There is also the problem of there being some patients for whom no swabs are obtained, which further complicates this method.

Recently, however, methods for fitting data to stochastic epidemic models with missing data have been developed (Auranen *et al.*, 2000; O'Neill and Roberts, 1999). Loosely speaking, these approaches work by using Monte Carlo methods to integrate over all possibilities for the unobserved process

that are consistent with the data. In this chapter, this approach is adapted to fitting simple single ward stochastic transmission models to data.

In subsection 8.2.1 an approach suitable for a single strain is developed. Simulated data is used to test the approach in section 8.2.3, and also to illustrate the effects of different study designs on parameter estimates.

In section 8.3.1 the method is extended to allow for the transmission of multiple strains on a ward, and different assumptions regarding the immigration of strains. In section 8.3.3 this is then used to estimate a single transmission parameter for the *S. aureus* transmission data collected in the study described in chapter 3.

Finally, section 8.4 shows how this approach can easily be extended to cope with more complex models. This is illustrated with a preliminary analysis of a large VRE data-set in section 8.4.2, where acquisition of VRE is related to antibiotic consumption.

8.2 Single strain model

8.2.1 Methods

A Bayesian data augmentation approach is used, where the unobserved times of acquiring the organism (the latent event times, which constitute the augmented data) are included in the set of model unknowns, with posterior distributions to be derived (see, for example Green, 1995). Each accepted set of values for the augmented data thus constitutes one possible realisation of the epidemic process, and the acquisition times are parameters to be estimated. Because the number of acquisitions is itself an unknown the dimension of the model changes, and an MCMC sampling algorithm with reversible jump extensions is required to explore the joint posterior distribution of all model unknowns (augmented data and model parameters) (Green, 1995).

The overall model is hierarchical in nature, with three levels: the observational model, the transmission model, and the prior model. The observation model determines the likelihood of the observed data (the patient swabs) for a given realisation of the epidemic process, and the transmission model the likelihood of the realisation given the model parameters.

Because swab and patient-presence data are typically available on a daily basis, colonization statuses are assumed to remain constant during any one day.

The following notation is used: **D** represents the observed data, which consists of the set of swab results $S = \{s_{ij}\}$, and the timing of patient stays, $\{w_{ij}\}$. Here s_{ij} is the result of the swab taken on day j for patient i, and is equal to one if the result is positive, and zero otherwise. w_{ij} is equal to 1 if patient i is present on the ward on day j, and zero otherwise (it is assumed that patients are not readmitted). The time in days is represented by t, where $0 \leq t \leq T$. The augmented data **A** consist of the set $\{c_{ij}\}$, where c_{ij} represents the assumed (though generally unknown) colonization status of patient i on day j, and is equal to 1 if the patient is colonized and present on the ward on that day, and zero otherwise. From this and $\{w_{ij}\}$ the latent event times (colonization times) $\{\tau\}$, the number of colonized and susceptible patients at time t, $y(t)$ and $x(t)$, and the colonization statuses of each patient on admission $\{a_i\}$ can be derived in an obvious manner. For example, the set of transmission days $\{\tau\}$ gives the (unknown) days at which uncolonized patients become colonized (so $\tau_i \in \{\tau\}$ iff $\tau_i = \min\{\tau : c_{i,\tau} = 1\}$ and $w_{i,\tau_i - 1} = 1$). The admission colonization status for patient i is given by $a_i = c_{it}$, where $t = \min\{t : w_{it} = 1\}$.

Transmission model

Patient stays are assumed to be perfectly observed. Only transmissions and introductions of the organism need to be considered.

It is assumed that there is a single strain with which patients may become colonized. Once colonized, patients are assumed to stay colonized for the rest of their stays on the ward. The mass action model for transmission is assumed, so the instantaneous rate at which any uncolonized patient becomes colonized is $\lambda y(t)$, for some constant rate λ. This model is effectively the same as that presented in chapter 2 with the detection rate γ set to 0, and without explicit consideration of the vectors (inclusion of vectors into the model does not greatly affect the dynamics as the timescale at which they become colonized and "recover" is short compared to that for patients). Each patient is assumed to have some probability, σ, of already carrying the strain when admitted to the ward.

The transmission model defines the likelihood of a given set of values for the augmented data given the model parameters. This is given by

$$L = \exp\left(-\lambda \int_0^T y(t)x(t)dt\right) \prod_{t\in\{\tau\}} \lambda y(t_-) \tag{8.1}$$

where t_- is the time just before t. The likelihood of the set of admission colonization statuses, $\{a_i\}$, is just

$$\prod_i \sigma^{a_i}(1-\sigma)^{1-a_i}$$

Since in practice event times are only resolved at the level of days, t_- is taken as $t-1$. The total log likelihood of the data is then given by

$$l = -\lambda \sum_{t=0\ldots T} y(t)x(t) + \lambda \sum_{t\in\{\tau\}} y(t_-) + \sum_i a_i \ln\sigma + (1-a_i)\ln(1-\sigma). \tag{8.2}$$

Observation model

Patients' colonization statuses are assumed to be determined by a number of arbitrarily timed swabs. There is assumed to be some probability, ξ, of recovering the organism from a swab taken from a colonized patient.

For each patient i, for each day of their stay j, observed colonization status is given by b_{ij}. Here b_{ij} is arbitrarily taken to be 1 if the patient is

known to be colonized on that day, 0 if the patient is known to be uncolonized (only possible if $\xi = 1$), and -1 if the colonization status is unknown.

The observation model defines the probability of the observed data (the swab results) $\mathbf{D}$, for a given set of values in the augmented data. This is 0 if $\exists i, j$ such that $s_{i,j} = 1$ and $c_{ij} = 0$, since false positives are assumed not to occur. Otherwise it is given by

$$\prod_{ij \ (c_{ij}=1, \ s_{ij} \in S)} \xi^{s_{ij}} (1-\xi)^{1-s_{ij}}.$$

In the case where $\xi = 1$, so there is no uncertainty in the swab data, this is simply equal to one if the observations are consistent with the augmented data, and zero otherwise.

Posterior inference

Conditionally on the realization of the latent process, $\mathbf{A}$, and on ξ, the observed data $\mathbf{D}$ is independent of the model parameters $\{\lambda, \sigma\}$. The joint posterior density of λ, σ, ξ, $\mathbf{A}$ and $\mathbf{D}$ is thus given as the product of observation model, the transmission model and the prior model:

$$\begin{aligned} p(\mathbf{D}, \mathbf{A}, \lambda, \sigma, \xi) &= p(\mathbf{D}, \mathbf{A} | \lambda, \sigma, \xi) p(\lambda, \sigma, \xi) && (8.3) \\ &= p(\mathbf{D} | \mathbf{A}, \xi) p(\mathbf{A} | \lambda, \sigma) p(\lambda, \sigma, \xi) && (8.4) \end{aligned}$$

The joint posterior density of λ, σ, ξ and $\mathbf{A}$ is then explored numerically using an MCMC sampling approach outlined below.

Implementation

For simplicity, only the special case where the probability of colonization on admission, σ, is known is treated here. In practice σ is easy to estimate from admission swabs and is not the primary interest. Similarly, a swab sensitivity of 100% is assumed for the moment, so $\xi = 1$. Although in practice it is clearly not possible to achieve such a sensitivity, it is also not

particularly useful to consider an individual colonized if they harbour only a few cells of the infectious organism, since then the risk of infecting others will be negligible. Effectively, therefore, a positive swab is treated as the *definition* of colonization with the organism.

Metropolis sampling was used (see, for example Gamerman, 1997, and references therein). A proposal for a new value for λ was chosen by drawing a proposal for $\ln\lambda$ from the proposal distribution $N(\ln\lambda_c, \sigma_p^2)$ (the log transformation allows the proposal to be drawn from the whole real line). Here λ_c is the current value of λ, and σ_p^2 is the proposal variance, which acts as a tuning constant: too large and too few moves are accepted; too small, and the new values are too close to the old ones for the Markov chain to mix well. If $\ln\lambda^\star$ is the proposed value, then this value is accepted into the sample with probability

$$\alpha(\lambda_c, \lambda^\star) = min\left[1, \frac{p(\mathbf{A}|\lambda^\star)p(\lambda^\star)\lambda^\star}{p(\mathbf{A}|\lambda_c)p(\lambda_c)\lambda_c}\right]$$

Here $p(\mathbf{A}|\lambda)$ is the probability of the augmented data for given λ, $p(\lambda)$ is the prior density for λ, and the final λ term in both denominator and numerator of the acceptance ratio is needed since the proposal is for $\ln\lambda$ rather than λ. These terms are the Jacobians for this change of parameterization.

If the new value is not accepted the old value, λ_c, is retained in the sample.

Updates to the augmented data Let $\mathbf{A}^\star$ represent the proposed augmented data, and $\mathbf{A_c}$ the current augmented data.

A proposal $\mathbf{A}^\star$ is accepted as the new state of the augmented data with probability

$$\alpha(\mathbf{A_c}, \mathbf{A}^\star) = min(1, A)$$

where

$$A = \left[\frac{p(\mathbf{D}|\mathbf{A}^\star)p(\mathbf{A}^\star|\lambda)}{p(\mathbf{D}|\mathbf{A_c})p(\mathbf{A_c}|\lambda)}\right]\Pi_p \tag{8.5}$$

In this case, since it is assumed that there are no false negative swabs, $p(\mathbf{D}|\mathbf{A}^\star)$ is equal to one if the augmented data are consistent with observed data (the swab results), and zero otherwise. Assuming that initially $\mathbf{A_c}$ is consistent with $\mathbf{D}$ then $p(\mathbf{D}|\mathbf{A_c})$ must always be equal to one, since only consistent data can be accepted. Π_p is the proposal ratio: the ratio of the density of a proposal for a move from $\mathbf{A}^\star$ to $\mathbf{A_c}$, $q(\mathbf{A}^\star \rightarrow \mathbf{A_c})$, to the density of the proposal for the reverse move, $q(\mathbf{A_c} \rightarrow \mathbf{A}^\star)$.

To see that see that the Markov chain defined above has the required stationary distribution (i.e. equation 8.3) it is sufficient to show that the detailed balance equations are satisfied, and that the chain is irreducible.

For the updates to the model parameters, this is standard (see, for example Gamerman, 1997). For the updates to the augmented data, first assume without loss of generality that $A < 1$, and consider a move to another feasible state, $\mathbf{A}^\star$, so that $p(\mathbf{D}|\mathbf{A}^\star) = 1$. Then, from equation 8.5, and writing $p(\mathbf{A_c} \rightarrow \mathbf{A}^\star)$ as the probability of updating the augmented data from $\mathbf{A_c}$ to $\mathbf{A}^\star$:

$$p(\mathbf{A_c}|\lambda)p(\mathbf{A_c} \rightarrow \mathbf{A}^\star) = q(\mathbf{A_c} \rightarrow \mathbf{A}^\star)p(\mathbf{A}^\star|\lambda)\Pi_p$$

where

$$\Pi_p = \frac{q(\mathbf{A}^\star \rightarrow \mathbf{A_c})}{q(\mathbf{A_c} \rightarrow \mathbf{A}^\star)}$$

so

$$p(\mathbf{A_c}|\lambda)p(\mathbf{A_c} \rightarrow \mathbf{A}^\star) = p(\mathbf{A}^\star|\lambda)q(\mathbf{A}^\star \rightarrow \mathbf{A_c})$$

Since $A < 1$, the acceptance ratio for the reverse move is one, and the right hand side is equal to $p(\mathbf{A}^\star|\lambda)p(\mathbf{A}^\star \rightarrow \mathbf{A_c})$, showing that detailed balance is satisfied. Irreducibility is also required to show that the chain has the desired stationary distribution. However, providing that all moves that update the augmented data are reversible, with the acceptance ratio defined above, new updating moves can be added until this condition is satisfied. Similar considerations are required in the choice of updating moves as are

needed when choosing the proposal variance: if the moves are too small the chain will have a high autocorrelation, but if too large, few of them may be accepted, again resulting in poor mixing.

Three sorts of changes to the augmented data are used here. When updating the augmented data one of these types was chosen at random. In the first move type only event times are updated. Thus for patients who become colonized in the current set of augmented data, but for whom there is uncertainty as to the exact colonization date, the day on which they are assumed to acquire the organisms may change. This move is carried out on all such patients simultaneously. Suppose that when updating the acquisition time for patient i, there are n_i possible days on which the strain may have been acquired. Because of the assumption that once colonized a patient remains colonized for the duration of their stay, these n_i days are necessarily contiguous. One of these n_i days is selected uniformly, and in $\mathbf{A}^\star$ patient i is made colonized from the selected day to discharge. This move doesn't change the dimension of the augmented data and hence $\Pi_p = 1$.

The second type of move updates the actual transmission events. This is needed since any of the patients whose final colonization status is unknown may have become colonized following their final negative swab. For this move, only one patient is updated at a time, so a single transmission event is changed. A decision is first made whether to add or remove a transmission event.

When adding a transmission event a patient i is selected uniformly from m_c possible patients in the current augmented data whose final colonization status is unknown, but who don't become colonized in $\mathbf{A_c}$. One of the n_i days during which their colonization status is unknown is then selected at random, and in the proposed augmented data $\mathbf{A}^\star$ they are made to be colonized from this day until discharge.

When removing a transmission event a patient j is selected. To be a

candidate for transmission event removal j must currently be assumed to be colonized in $\mathbf{A_c}$, but have no positive swabs (so in $\mathbf{D}$ the final colonization status is unknown). $\mathbf{A}^\star$ is then updated by making the patient uncolonized during each day of their stay.

The proposal ratio for adding a transmission event for patient i is then given by

$$\Pi_p = \frac{m_c n_i}{m_c' + 1}$$

where m_c' is the number of candidate patients for removal in $\mathbf{A_c}$. When removing a transmission event the proposal ratio is

$$\Pi_p = \frac{m_c'}{(m_c + 1)}$$

All patients who are swabbed are assumed to have an admission swab, so there is no uncertainty as to whether or not they are colonized on admission. However, for patients for whom no swabs are taken it is not known whether they were colonized on admission or not. Since the augmented data must integrate over all the possibilities for the unobserved process consistent with the observed data, a third move type is required which changes the admission colonization status of these unobserved patients. This move chooses an unswabbed patient at random who in $\mathbf{A_c}$ is assumed to be uncolonized throughout their whole stay. This patient is then made colonized throughout their stay in the proposed augmented data, $\mathbf{A}^\star$. The reverse move simply chooses one such unswabbed patient who is assumed colonized on admission in $\mathbf{A_c}$ and makes them uncolonized. The proposal ratio for the forward move is given by

$$\Pi_p = \frac{u_c}{v_c + 1}$$

where u_c is the number of unswabbed patients who are currently assumed to never become colonized in $\mathbf{A_c}$, and v_c is the number of unswabbed patients currently assumed to be colonized on admission. The proposal ratio for the

reverse move is given by:

$$\Pi_p = \frac{v_c}{u_c + 1}.$$

Irreducibility can easily be shown in this case (though when considering the multi-strain model in section 8.3 it is more difficult to ensure). Aperiodicity follows from irreducibility, since some states will clearly have a periodicity of one.

The algorithm was implemented in a C++ program (available on request from the author). Convergence was assessed primarily through the use of the Raftery-Lewis diagnostic (Rafter and Lewis, 1992), as implemented in CODA (Best *et al.*, 1995). A sufficient number of samples were recorded from a single chain to estimate the 2.5th percentile (± 0.005) with a probability of attaining this accuracy of 0.95, as calculated with the Raftery-Lewis diagnostic. An initial "burn-in" period was used where all samples were discarded. The length of this burn-in was taken to be at least that recommended by the Raftery-Lewis diagnostic.

8.2.2 Test data

Simulated data were generated from a single-strain model of a 20 bed hospital ward with mean length of stay of 10 days and $\lambda = 0.00263$ (giving a value of R_0 of 0.5.), where 20% of patients were assumed to be colonized on admission.

Two sets of data were generated corresponding to 30 and 500 days of surveillance. In both cases the MCMC algorithm was used to estimate λ under four different scenarios (assuming that σ was known). First, full data were assumed. This corresponds to swabbing each patient every day of their stay. Second, all patients were assumed to be swabbed on admission and then at weekly intervals. The third and fourth scenarios were identical to the second, except that 10% and 50% of patients respectively were assumed to be non-consenters, and provide no swabs. Non-consenters were chosen

at random, and the observed colonization status, b_{ij} for patient i on day j was coded either as 0 for uncolonized, 1 for colonized, and -1 for unknown colonization status. The rules for assigning these values are based on the transmission model and the assumption that $\xi = 1$. If there is any negative swab for patient i on day j, then for all $j' < j, b_{ij'} = 0$. Similarly, a positive swab on day j means that for all $j' > j, b_{ij'} = 1$. If there are positive swabs for patient i on days j_1 and j_2 $(j_1 < j_2)$ and $j_1 \leq j \leq j_2$ then $b_{ij} = 1$. However, if there is a positive swab on day j_2, and the previous swab was negative and taken on day j_1, then for $j_1 < j < j_2$, $b_{ij} = -1$. Patients for whom no swabs are taken will clearly have unknown colonization status on each day.

8.2.3 Single strain model results

Figure 8.1 shows the posterior densities obtained for the eight sets of simulated data using the single strain model.

8.3 Multiple strain models

Real data from a single hospital ward often includes typing information that shows that two or more strains of the same organism were in fact circulating among the patients. The section describes the extensions that need to be made to the model to account for this, and for the introduction of new strains. In this section it is assumed that all the circulating strains have an identical transmission rate, λ, although modifications to allow different strains to have different transmission rates are straightforward.

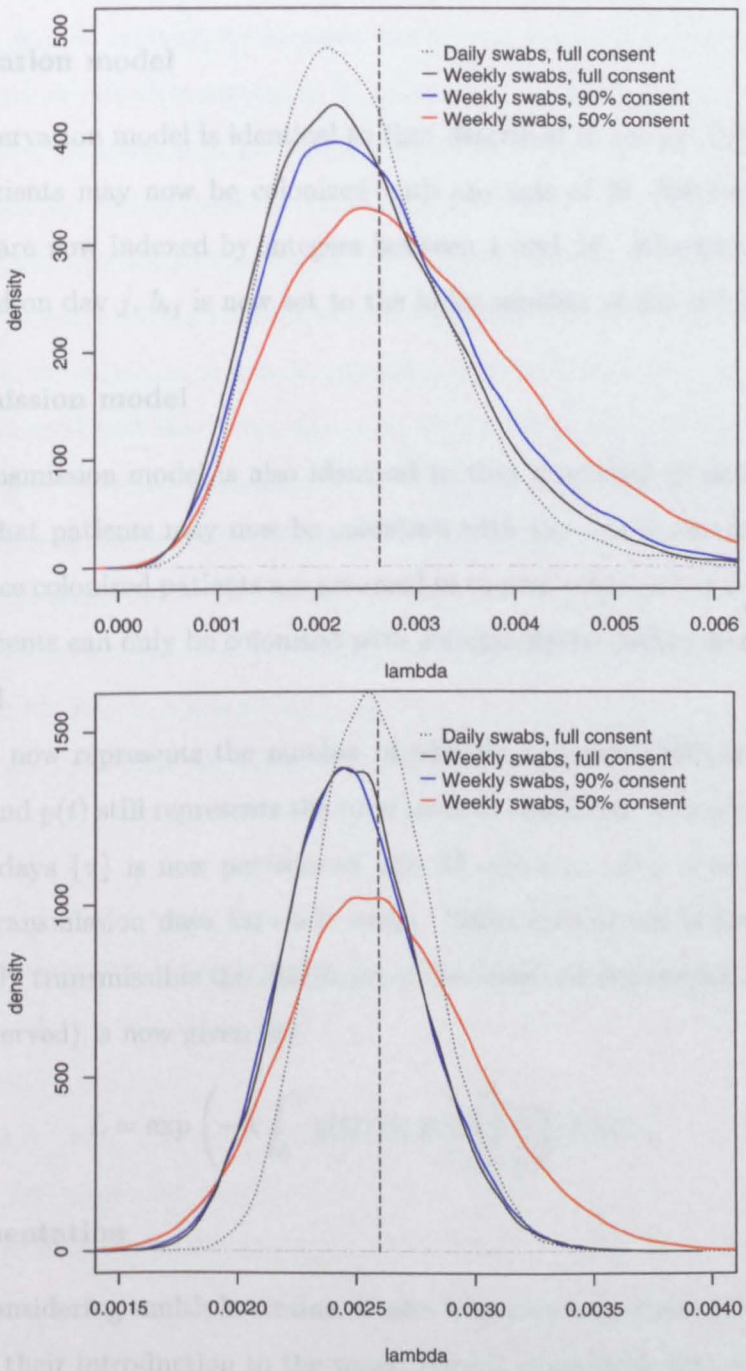

Figure 8.1: Posterior densities obtained from simulated transmission data corresponding to one 20 bed ward over 30 days (top), and 500 days (bottom). Priors in all four cases were $\Gamma(0.001, 0.001)$. The vertical dashed line shows the value of λ used when generating the data (0.00263).

8.3.1 Methods

Observation model

The observation model is identical to that described in section 8.2.1 except that patients may now be colonized with any one of M different strains. Strains are now indexed by integers between 1 and M. When patient i is colonized on day j, b_{ij} is now set to the index number of the strain.

Transmission model

The transmission model is also identical to that described in section 8.2.1 except that patients may now be colonized with any one of the M strains. Since once colonized patients are assumed to remain colonized, it is assumed that patients can only be colonized with a single strain during their stay on the ward.

$y_r(t)$ now represents the number of patients colonized with strain r at time t, and $y(t)$ still represents the total number colonized. The set of transmission days $\{\tau\}$ is now partitioned into M sets $U_1, ..., U_M$ corresponding to the transmission days for each strain. Since each strain is assumed to be equally transmissible the likelihood of the observed transmission data (if fully observed) is now given by:

$$L = \exp\left(-\lambda \int_0^T y(t)x(t)dt\right) \prod_{k=1}^{M} \prod_{t \in U_k} \lambda y_k(t_-) \tag{8.6}$$

Implementation

When considering multiple strains, it also becomes important to explicitly consider their introduction to the ward. Here it is assumed that the typing methodology used has sufficiently high resolution, and that there are enough distinct strains, so that each unique strain type identified on the ward is introduced by exactly one patient (although more than one patient may, of course, be colonized with that strain at the beginning of the observation

period). The assumption that admission swabs are taken for all swabbed patients is now relaxed, so in some cases there may be uncertainty as to which patient introduces a strain. Consequently, a new update move for the augmented data needs to be introduced. This move first chooses a strain at random for which there is uncertainty as to the true introducer, then selects uniformly one of the feasible introducers of the strain from amongst (i) those patients who are known to carry the strain at some point, but whose admission colonization status is unknown, and (ii) those patients who are never swabbed and also never colonized in the current augmented data. In $\mathbf{A}^\star$ the newly selected introducer is colonized from their admission with the selected strain. Before a decision is made whether to accept this move a not, all the acquisition times are updated as described previously. The purpose of this last move is to increase the number of problems for which the algorithm is irreducible and to allow a simpler form for the proposal ratio.

The proposal ratio for this move, which is its own reverse, is

$$\Pi_p = \frac{1 + n_i}{1 + n_j}$$

when both patients i and j are known to become colonized n_i and n_j are the number of days during which patients i and j have unknown colonization status. If i and/or j are unswabbed patients, then the numerator/denominator is replaced by one.

As well being able to introduce strains which are seen on the ward, patients for whom no swabs are taken may also introduce strains which never appear in swabs. To account for this, the move that changes patients' colonization status on admission is retained with the same proposal ratio, but only for patients who have no swabs at all. Since introductions for all strains observed on the ward are catered for by the previous move, this move now makes patients colonized on admission with a strain $M + 1$. This appears only in the augmented data, and represents all unobserved strains. This strain may also be acquired by other patients in the augmented data.

Although several patients may introduce strain $M+1$ in the augmented data, conceptually these should be thought of as separate introductions of different unobserved strains. The likelihood calculation for the augmented data will still be valid, though the sampled augmented data need to be interpreted with care: the number of acquisitions of this strain in the augmented data should be interpreted as the number of acquisitions of any unobserved strain, rather than any particular strain with one introducer. For an organism such as *S. aureus* which a high proportion of patients might be expected to carry on admission and for which interference/bioexclusion is important, failing to take this into account would result in an overestimate of the number of susceptible patients.

In general the above moves are not sufficient to ensure irreducibility of the chain, as figure 8.2 demonstrates. Similar deadlock situations to that shown in the figure can occur if there are two candidate patients who may each be the introducers of one of two strains. Once one scenario is chosen, a deadlock situation is reached where other possible scenarios cannot be reached. Further moves would need to be introduced to resolve such deadlocks or the assumption that one patient can only carry one strain would need to be relaxed. However, for many data, the above rules will be sufficient to ensure irreducibility. For example, if there are no gaps in the observed chain of transmission of any one strain, and either the introducers of each strain are known, or the candidate introducers of one strain are not also candidate introducers for another strain, then such deadlocks will be avoided. In the latent process, patients of unknown colonization status can be made colonized with any strain consistent with the data, and all feasible latent processes will be explored. Attention here is restricted to such data where these moves do ensure irreducibility. For the more general case, additional moves need to be added. However, choosing moves that ensure the chain is irreducible and mixes well may not always be an easy

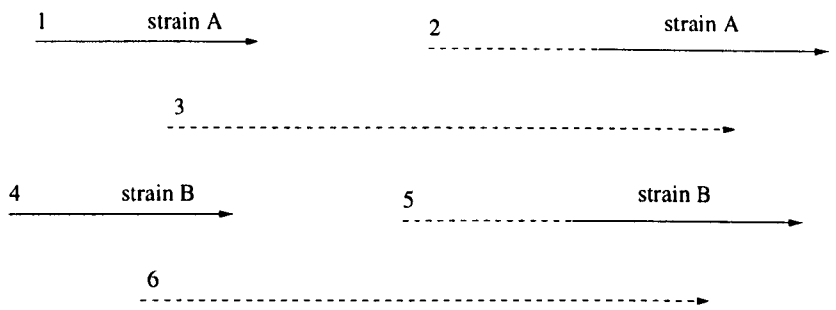

Figure 8.2: One example where "deadlock" could occur with the set of moves in section 8.3.1. Patients are indicated by the lines, and discharges by arrowheads. Solid lines indicate periods when patients are known to be colonized, and dashed lines periods when their colonization status is unknown. Assuming no other patients are present, since only one patient can introduce each strain, to be consistent either patient 3 or patient 6 must be colonized with strain A in the augmented data. Suppose patient 3 is colonized with A in the current augmented data, so patient 6 must be colonized with strain B. With the existing updating moves, there is no way to now move to the situation where patient 3 is colonized with strain B, and patient 6 with strain A.

task.

8.3.2 Application to *Staphylococcus aureus* data

Figure 3.6 shows the transmission data collected in the study described in chapter 3, with strain types indicated by numbers. Solid coloured lines indicate periods when patients were known to be colonized or not colonized with *S. aureus*. Strains are indicated by the circled numbers. Green lines indicate periods where patients are deduced to be uncolonized on the basis of the swab data. Black lines indicate periods of unknown colonization status. For one patient (in bed 12), a series of positive swabs was followed by a large number of negative swabs, with no positive swabs for the remainder of the study. This observation apparently refutes the model. However, since the model should be thought of as a tool rather than a conjecture, rather than changing it for a less wieldy one, the data are changed instead.

Thus this patient is split into two. From the beginning of the study to the mid-point of the period of unknown colonization status (indicated by the dashed pink line), the patient is assumed to be colonized. From this point onwards, until discharge, the patient is treated as a new patient, assumed to be uncolonized[1]. Similarly, another patient (in bed 1), had positive swabs for two different strain types, the second of which was identical to one isolated from another patient. This contradicts the assumption that patients can only be colonized with one strain at a time. To cope with this, only the acquired strain was considered.

8.3.3 *Staphylococcus aureus* results

Figure 8.3 shows the smoothed posterior density for λ when a diffuse prior ($\Gamma(0.001, 0.001)$) is assumed. This gives a mean colonization rate of 0.00145 per colonized patient per day, with a 95% credible interval of $[0.0002, 0.0040]$. The estimate for R_0 is then given as product of λ, the mean length of stay, and the number of susceptible patients. From data obtained for all patients stays in 1997, mean length of stay on the ward was 10.01 days CI: [9.49,10.87]. The ward has 15 beds, with nearly 100% bed occupancy. Assuming that all 14 patients are susceptible, and using the mean length of stay gives an R_0 value of 0.203 with 95% CI $[0.028, 0.56]$.

The informative prior $\Gamma(2, 0.0015)$ was then used, based on estimates from chapter 5. In these studies λ only once took a value exceeding 0.01, and had a mode in the region of 0.003. Figure 8.4 shows the smoothed posterior density obtained using this prior. The data modify the prior only slightly, by increasing the confidence that λ takes a value below about 0.01, but also reducing the support for very low values. The mean (and 95% CI) for the posterior are now 0.00437 $[0.0011, 0.0077]$. This corresponds to

[1] An alternative approach would be to simply allow the swab sensitivity to be less than 100%

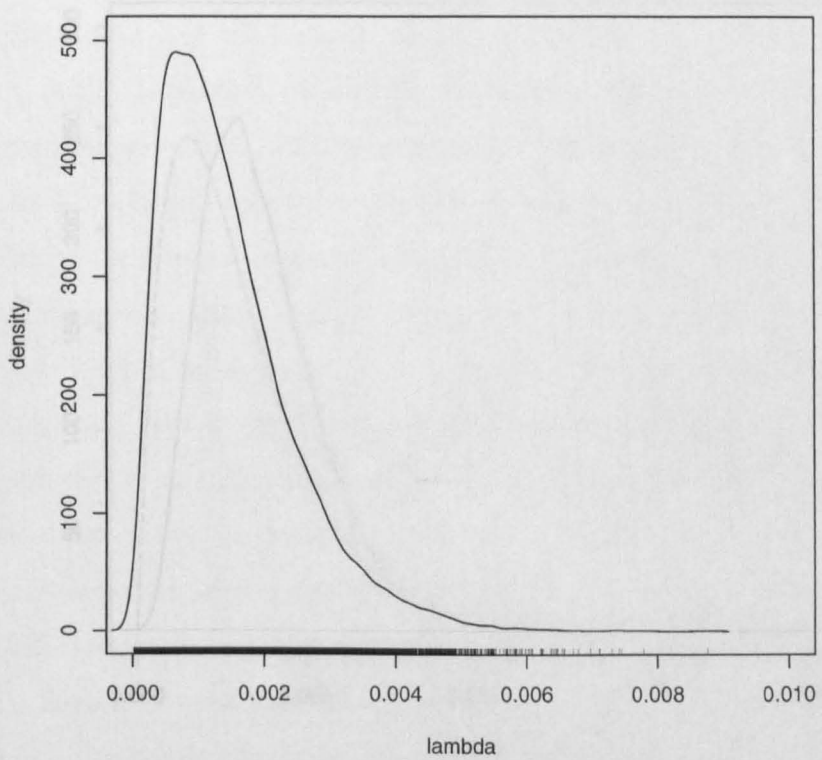

Figure 8.3: Posterior density for transmission parameter λ for the *S. aureus* transmission data. The diffuse prior $\Gamma(0.001, 0.001)$ was used, and 25,000 iterations were made, with a thinning of 25 (i.e. storing every 25th value).

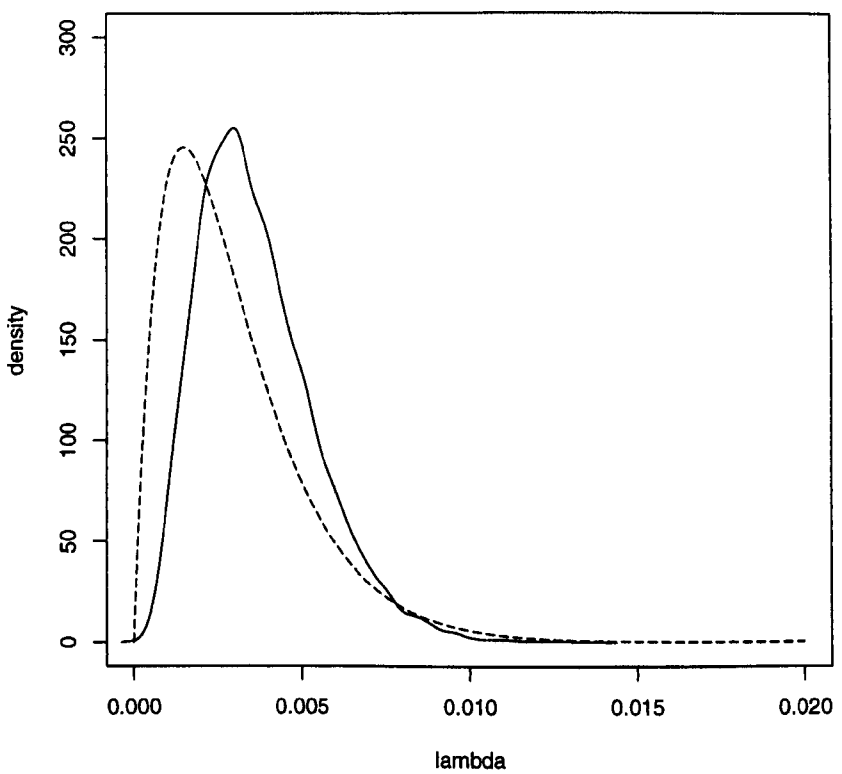

Figure 8.4: Smoothed posterior density (solid line) for transmission parameter λ for the *S. aureus* transmission data. The broken line shows the prior, $\Gamma(2, 0.0015)$. 20,000 iterations were made, with a thinning of 25.

a mean R_0 value of 0.51, with 95% CI $[0.15, 1.08]$. This suggests there is still a high degree of confidence that R_0 is below the critical value, but also suggests that there is non-negligible chance that it lies in the transitional region between which quasiendemicity and rapid fadeouts occur.

8.4 Application to VRE data

In section 8.2.1 and 8.3.1 above, simulated transmission data, and data from a month long study on a single ward were used to estimate a single transmission parameter. With minor modification the algorithms could have been used to also estimate the proportion of patients who were colonized on admission. In general, however, interest lies in determining what factors affect transmission rates, and by how much. For the spread of antibiotic-resistant bacteria in hospitals, for example, the most important questions are: how much does antibiotic use affect the transmission of bacteria between patients, and what difference do infection control activities make? Below, simple extensions to the model are made to allow such estimates to be made, and preliminary estimates are made for the effect of six different classes of antibiotic treatment on the acquisition of strains of VRE, based on a subset of data from a 16 month study in a haematology unit.

It is a preliminary analysis because the data are preliminary, based on initial typing of a single isolate from each patient.

8.4.1 VRE study description

Data concerning the transmission of VRE were obtained from the study described in detail by Bradley *et al.* (1999). In brief, this was a prospective three-phase sequential study where colonization with VRE was established by rectal swabs from consenting patients (90-95% of all patients) on a three ward haematology unit. In the first phase (four months) ceftazidime (a third

generation cephalosporin) was used as the first-line treatment for febrile neutropenic episodes; in the second phase (8 months) piperacillin/tazobactam was used instead; and in the third phase (4 months) ceftazidime was used as the first line antibiotic again. Bradley and co-workers also collected extensive data on patient antibiotic use for all consenting patients, recording antibiotics used on most days of each patient's stay. Frequencies of different combinations of antibiotics used are summarized in table 8.4.1, and table 8.4.1 shows total antibiotic use during the whole study. Glycopeptide use did not change significantly throughout the study, while ceftazidime use was close to zero in phase two, and piperacillin/tazobactam use close to zero in phases one and three. In the second and third phases there was also an intensive education programme to improve hygiene on the wards. A survival analysis showed that acquisition rates of VRE decreased significantly in phase two, and then returned in phase three to a value only slightly lower than that seen in phase one (Bradley *et al.*, 1999).

Preliminary PFGE typing has since been carried out on the isolates, and the analysis here is made on the results.

Data description and modelling assumptions

The data analysed here come only from the largest ward under study, which had 18 beds. Figure 8.6 shows colonization statuses for each patient on each day they were on the ward, based on the assumption that patients could both lose and acquire strains, and that there were no false negative swabs. The figure shows that once a positive VRE swab was obtained, the patient typically remained colonized for the rest of their stay. When subsequent negative swabs were obtained, later swabs were often positive again. Because of this, the assumption that once a patient was colonized with VRE they remained colonized for the rest of their stay was considered adequate. Figure 8.7 shows the data based on this assumption, including available in-

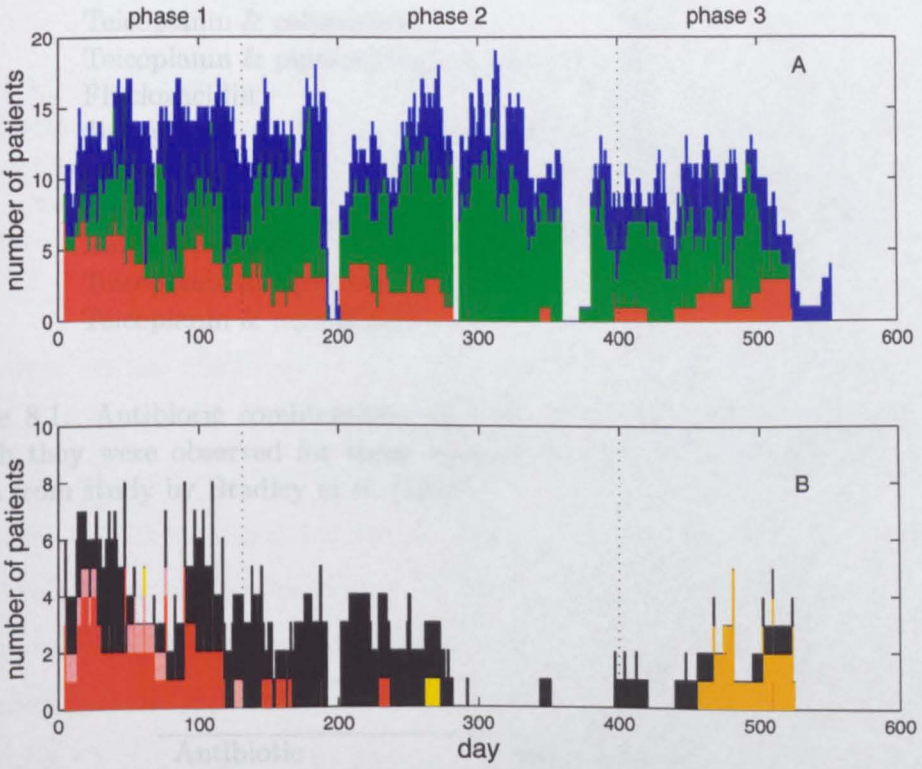

Figure 8.5: VRE transmission on a haematology ward. Data from study by Bradley *et al.* (1999) with preliminary PFGE typing results. **A**. See text for study details. Number of patients who are colonized (red), uncolonized (green) and of unknown colonization status (blue). **B** Colonized patients classified by the PFGE strain type of the patient's first positive isolate. Black–colonized with unique strain; red–strain 1; orange–strain 2; pink–strain 3; yellow–strain 5.

Antibiotics	patient days
None	2810
Piperacillin/tazobactam	298
Ciprofloxacin	225
Ceftazidime	210
Teicoplanin & ceftazidime	183
Teicoplanin & piperacillin/tazobactam	176
Flucloxacillin	167
Teicoplanin	104
Metronidazole	73
Erythromycin	69
Imi/Merpoenem	62
Teicoplanin & ciprofloxacin	56
Teicoplanin & ciprofloxacin	56

Table 8.1: Antibiotic combinations, and the number of patient days for which they were observed for those used for more than 50 patient days. Data from study by Bradley *et al.* (1999)

Antibiotic	patient days
Teicoplanin	1091
Ceftazidime	807
Piperacillin/tazobactam	760
ciprofloxacin	610
Metronidazole	550
Flucloxacillin	347
Gentamicin	326
Erythromycin	250

Table 8.2: Antibiotic used, and the number of patient days for which they were observed for those used for 250 patient days or more. Data from study by Bradley *et al.* (1999)

Class	Antibiotics	Patient days
A	None	2810
B	Ceftazidime + glycopeptide	418
C	Ceftazidime without glycopeptide	371
D	Piperacillin/tazobactam + glycopeptide	449
E	Piperacillin/tazobactam without glycopeptide	396
F	Glycopeptide (without ceftazidime & pip./tazo.)	523
G	Other antibiotics	1046

Table 8.3: The seven mutually exclusive antibiotic classes, and the number of patient days for which they were observed. Except for A, other unlisted antibiotics were also used simultaneously for some of the patient days in each class. Teicoplanin accounted for most of the glycopeptide use, but 155 patient days of vancomycin use were also observed. Data from study by Bradley *et al.* (1999)

formation on the PFGE type of the strains recovered from each patient, which in most cases is just the PFGE type of the first positive isolate. The rules used here to assign the colonization status for each patient are the same as those used for the simulated data as outlined in section 8.2.2. Figure 8.5 uses this information to show how total numbers colonized with each strain changed during the three phases of the study. A large proportion of the colonized patients gave isolates with unique PFGE types (indicated in black in figures 8.5 and 8.7). These present important difficulties of interpretation. While strains with identical PFGE types can be assumed to share a common source, strains of unique types may represent either pre-existing flora, endogenous sensitive flora that has acquired the transposon containing the vancomycin resistance gene as a result of transmission from other patients, or it may be due to the transmission of strains which are present but undetected in other patients (Chadwick *et al.*, 1997). The effect of antibiotic therapy may be both to change the chance of a patient acquiring the transposon or particular strain, and also to change the chance of detecting such a strain if it is present.

The fact that apparent acquisitions of strains of unique type continues

in phase two, but decreases as the number of colonized patients decreases suggests that selection of pre-existing resistant flora as a result of antibiotic use is unlikely to be the sole explanation. If this were the case a much faster response to the change in antibiotic therapy would be expected. The observed data are probably accounted for both by transmission and selection of pre-existing flora. While it would be possible to fit a model that allowed for both processes, this has not been done yet and the analysis presented here ignores the possibility of transmission of these strains, and they are treated as if they were all attributable to pre-existing flora. For the other strains, the multi-strain transmission model is first used to compare transmission rates in three periods. This is then modified to allow the transmission to also depend on the antibiotics being taken by the susceptible patients. Other possible transmission models are considered in the discussion.

Transmission model with antibiotic-dependent susceptibilities

The transmission model is identical to that described in section 8.3.1, except that the instantaneous acquisition rate is assumed to depend on the antibiotics which a susceptible patient is currently taking. There are too many possible combinations of antibiotics to treat each one individually (270 different combinations were used, if a distinction is made between oral and intravenous administration) and synergistic effects are likely, so there is no obvious way to combine the effects of two or more antibiotics taken together. Instead, combinations of antibiotics are assigned to classes. For example, in the current analysis seven different classes are considered (table 8.3).

The likelihood of the observed transmission data (if fully observed) is now given by:

$$L = \exp\left(-\sum_d \lambda_d \int_0^T y(t)x_d(t)dt\right) \prod_{k=1}^{S} \prod_{r=1}^{Q} \lambda_r y_k(t_{r-}) \qquad (8.7)$$

where λ_d is now the rate at which susceptible patients in antibiotic class

d become colonized due to one colonized patient, $x_d(t)$ is the number of patients in antibiotic class d at time t. There are assumed to be Q transmissions, and λ_r is the transmission rate corresponding to the antibiotic class of the patient becoming colonized in the rth transmission .

Immigration assumptions

In order to completely specify the likelihood it is necessary to specify the probability of each patient being already colonized when admitted to the ward. The approach described in section 8.3 might seem to be appropriate here. For the strains which colonize several patients, exactly one patient should be required to introduce the strain. Patients who have no swabs may also be assumed to have some probability of introducing other strains of unique PFGE type to the ward. This probability then becomes another parameter of the model. This approach would work well for the third part of the study, where a strain previously not seen on the ward is introduced, and spreads rapidly to other patients. However, in the first part of the study several patients were already colonized with the predominant ward strain when admitted. This is almost certainly due to those patients acquiring the strains during previous episodes on the unit. Out of 283 patients, 125 had two or more episodes on the ward during the study. There were 91 episodes where patients who were colonized with VRE on their previous episode were readmitted, and in 53 of these they were again found to be colonized; in 21 cases no swabs were taken; and in the remaining 17 episodes only negative swabs were recorded. Because of this constant recycling of patients through the ward, the assumptions of section 8.3 are no longer valid. A full model for the data should specify the probability of a patient being colonized with a given strain on admission to the ward, given their colonization status when leaving the ward during the previous episode and the time interval between the two. The most obvious assumptions for such a model would be to assume

this probability was very small if the patient was uncolonized at the end of the last stay, and decreased exponentially at some rate ζ if the patient was colonized at the end of the previous episode. ζ is then another parameter to be estimated from the data, though one about which there is extensive prior information.

The preliminary analysis presented here ignores these complications, and treats each patient episode as a new patient. Previous patient episodes on the unit are, however, accounted for in the following way: patients who are found to be colonized during the current episode, and who have no negative swabs before the first positive swabs for this episode are assumed to have been already colonized on admission if they were known to be colonized at the end of their previous episode. Also, patients with strains of unique types were assumed to introduce these to the ward.

Once these episodes have been accounted for, there were four remaining episodes where it is possible that the patient was colonized on admission with strain 1 (the predominant strain during phase 1). These episodes are dealt with on a case-by-case basis.

- Episode 59: The patient was found to be colonized on the first swab taken, which was on the eighth day of stay. This patient was not a previous in-patient on the unit, and was therefore assumed to acquire the strain during this episode.

- Episode 140: The patient was found to be colonized on the first swab taken, which was on the third day of stay. At the end of their previous stay on the ward their colonization status was unknown, but many other patients were colonized with strain 1 on the ward at this time. This patient was therefore assumed to be colonized on admission.

- Episodes 124 and 283: Both patients were found to be colonized with strain 1 on their previous episodes, but no swabs were taken for the

above-mentioned episodes. These episodes were treated in the same way as patients who had no previous exposure to the ward, and it is assumed that they are not colonized on admission with strain 1.

Previously colonized readmitted patients with no swabs or only negative swabs were treated in an identical manner to patients with no previous episodes.

There is assumed to be some constant probability that patients are admitted with strains of unique type. This probability is taken from the 222 admissions to the ward whose colonization status on admission could be deduced as described above. Of these 28 (13%), were assumed to be colonized with VRE on the basis of the assumptions outlined above.

To make the data consistent with the model two further assumptions are required. First, the patient who becomes colonized during episode 325 must be assumed to be colonized on admission (the same patient was previously on the ward, and left with unknown colonization status). This is because there are no possible patient sources on the ward at this time.

Finally, there is one patient episode that is inconsistent with the model: the patient in episode 703 acquired strain 1 during their stay. This patient was not a previous in-patient on the unit, so this cannot be an apparent acquisition due only to a false negative swab. Not only were there no other patients who harboured this strain present on the ward prior to this acquisition, there were no other patients of unknown colonization status who could feasibly have been colonized with this strain. In fact there were no patients of unknown colonization status during the period when the strain could have been acquired. This acquisition therefore cannot be accounted for by this simple model. To account for it the model would have to modified to allow for cross-infection from other wards (presumably through a carer acting as a vector), direct transmission from a colonized carer, or colonization resulting from a reservoir of VRE in the form of environmental contamination on the

ward. For the purposes of fitting this model however, the episode is simply divided into two. During the first one, lasting from the admission date to the midpoint of dates where the patient had unknown colonization status, the patient is assumed to be uncolonized. During the second one, the patient is assumed to be colonized "on admission".

Simulation details

In all cases diffuse priors, $\Gamma(0.001, 0.001)$, were used for all parameters. In the first run colonization rates for the three phases were allowed to vary independently. In the second run, colonization rates in phases one and three of the study were constrained to be equal, but the rate in the second phase was allowed to vary independently. Finally, colonization rates were allowed to vary for individual patients according the antibiotics they were taking at the time, where antibiotic treatment was deemed to fall into one of seven classes (see table 8.3).

8.4.2 VRE results

Figure 8.9 shows posterior densities when the colonization rate λ was allowed to vary according the phase of the study. Phases one and three clearly seem to have markedly higher values than phase two. There is little evidence that phase one and three have different transmission rates, despite the fact that in phase three there was an intensive education programme aimed at improving hygiene on the ward. The survival analysis, which ignored the effect of there being different numbers of susceptibles (Bradley *et al.*, 1999) showed a slightly lower rate of acquisition in phase three than in phase one. The opposite conclusion here arises because of the different numbers of colonized patients initially present in phase one and three, and the different immigration rates of colonized patients. Both of these were greater in phase one than in phase three. Because estimates for phase one and three were so

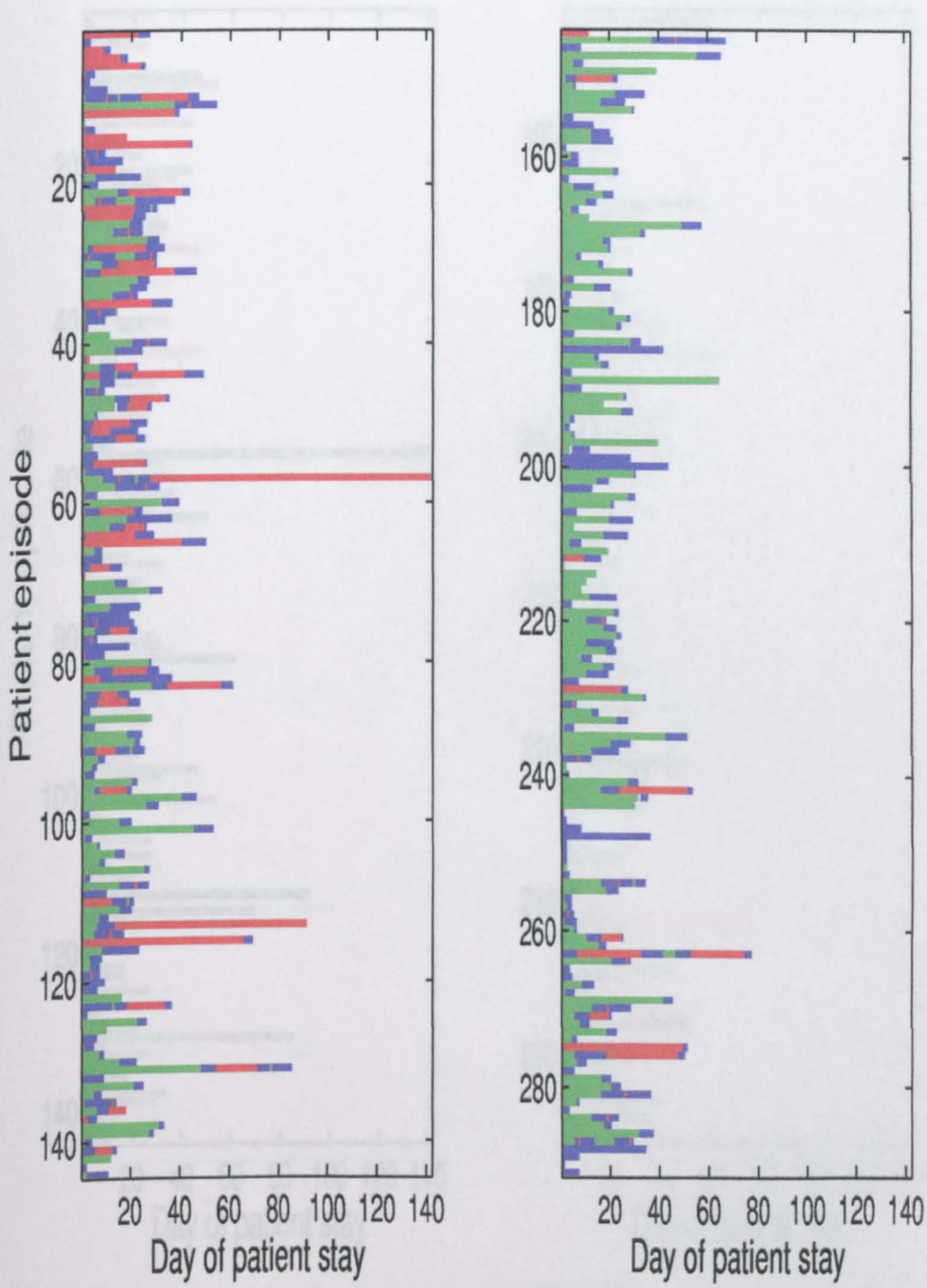

Figure 8.6: VRE colonization statuses on the ward assuming patients can lose as well as acquire VRE during their stays. Key: red–colonized; green–uncolonized; blue–unknown. Where patients spent time on other wards during their episode, they have been split into two episodes in the figure.

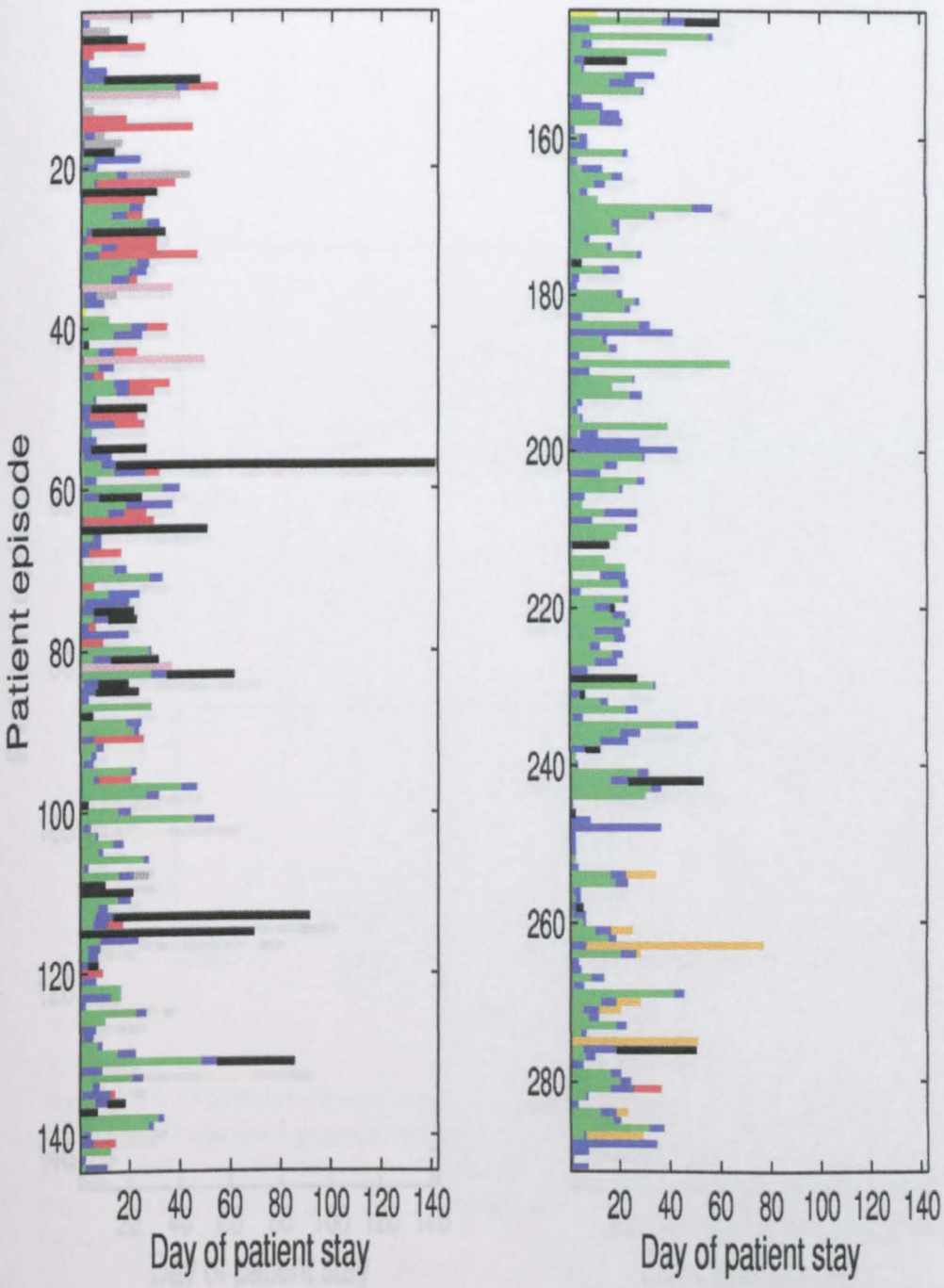

Figure 8.7: VRE colonization statuses assuming that patients who acquire VRE remain colonized for the rest of their stays. Key: green–uncolonized; blue–unknown; black–colonized, unique strain; grey–colonized, unknown strain. Other colours indicate strains as determined by the PFGE typing: red– strain 1; orange–strain2; pink–strain 3; yellow–strain 5. Patients spending time on other wards during their episodes have been split into two episodes in the figure.

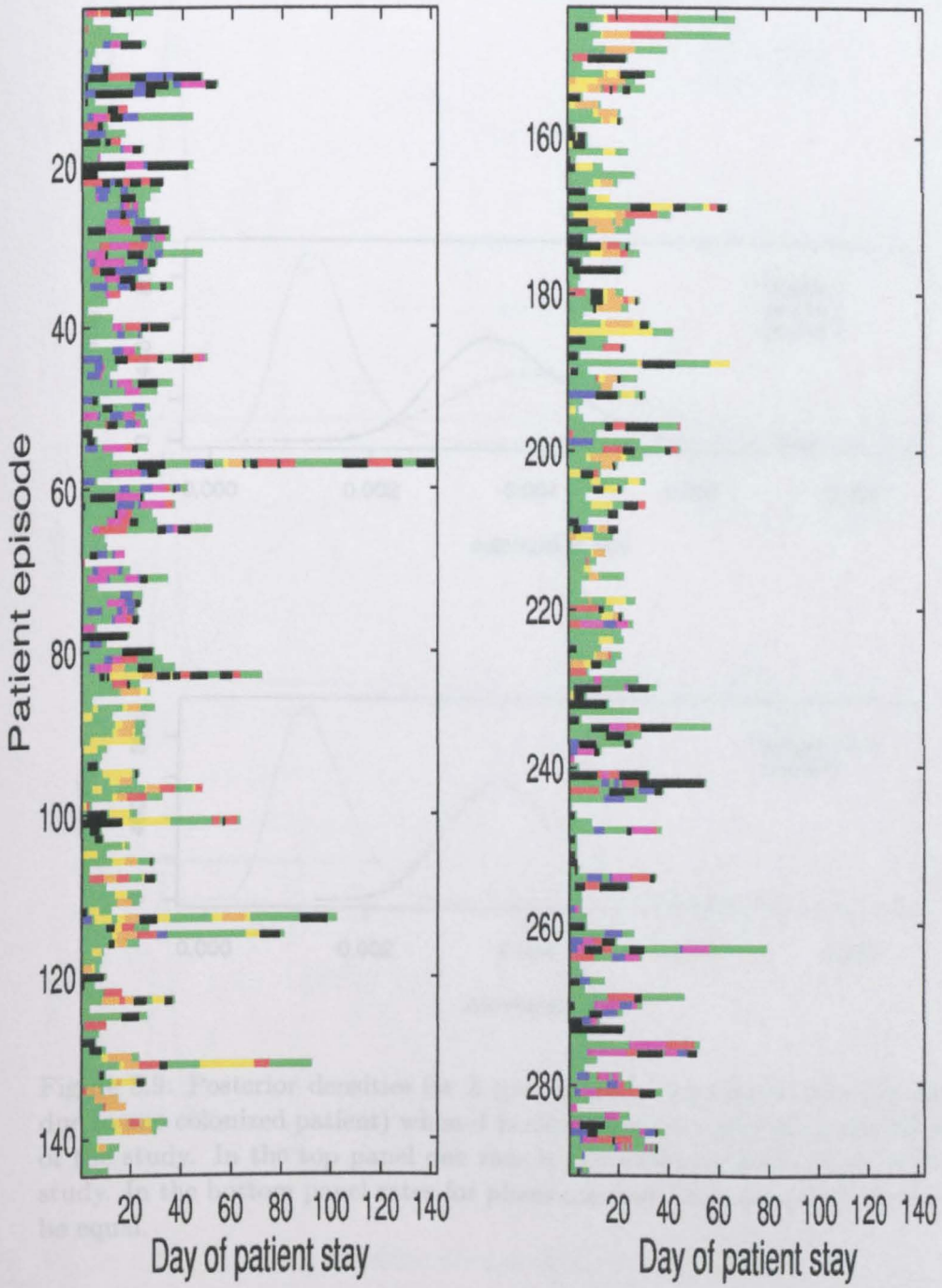

Figure 8.8: Antibiotic courses of patients. Key: green—no antibiotics; blue—ceftazidime, no glycopeptide; purple—ceftazidime & glycopeptide; yellow—piperacillin/tazobactam, no glycopeptide; orange—piperacillin/tazobactam & glycopeptide; red– glycopeptide, no ceftazidime, no pip./tazo.; black—other antibiotic(s).

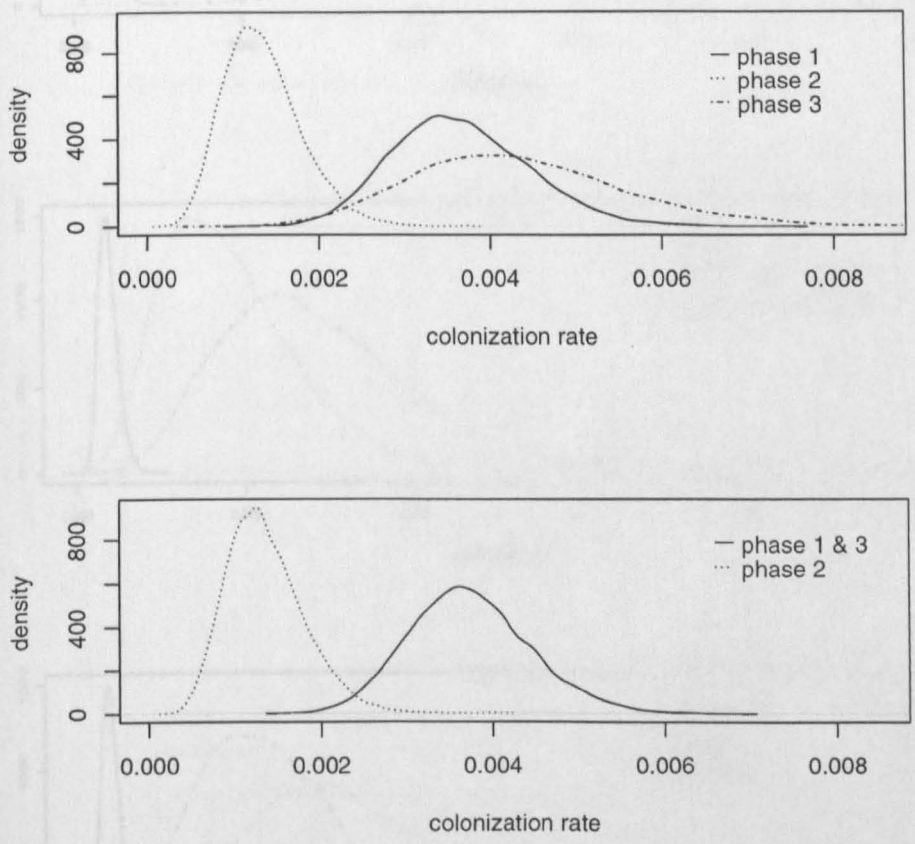

Figure 8.9: Posterior densities for λ (per-patient colonization rate per day due to one colonized patient) when it is allowed to vary according the phase of the study. In the top panel one rate is estimated for each phase of the study. In the bottom panel rates for phase one and three are constrained to be equal.

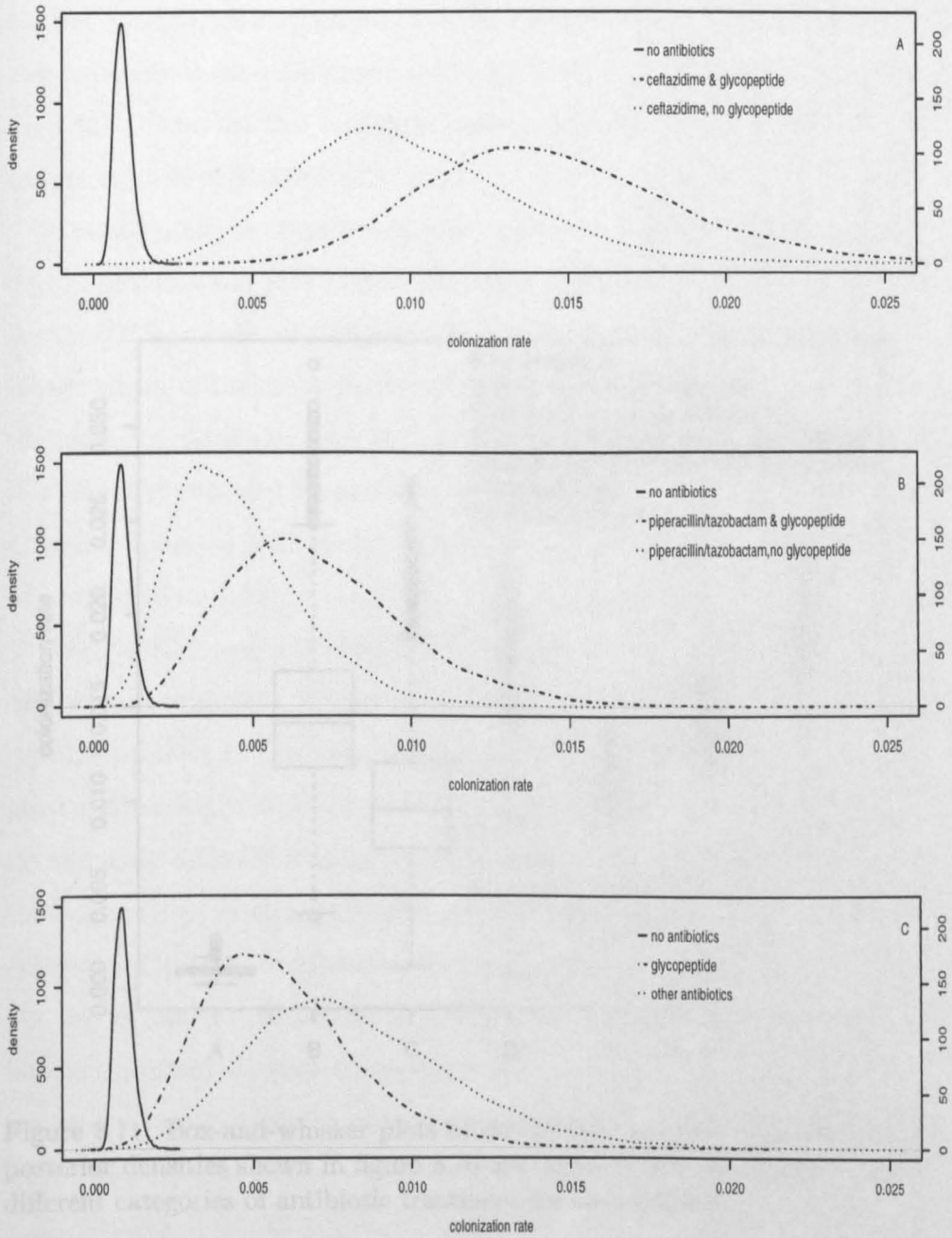

Figure 8.10: Posterior densities for the colonization rates, λ_d. This rate is assumed to vary with antibiotic treatment, and seven categories of therapy are considered: no antibiotics (panels A,B & C), ceftazidime and a glycopeptide (A); ceftazidime and no glycopeptide (A); piperacillin/tazobactam and a glycopeptide (B);piperacillin/tazobactam with no glycopeptide (B); glycopeptide without both piperacillin/tazobactam and ceftazidime (C); and other antibiotics (C). In all categories some patients may have been taking additional antibiotics, apart from those named above. Densities for the solid line (no antibiotics) are shown on the left axis, those for the broken lines on the right.

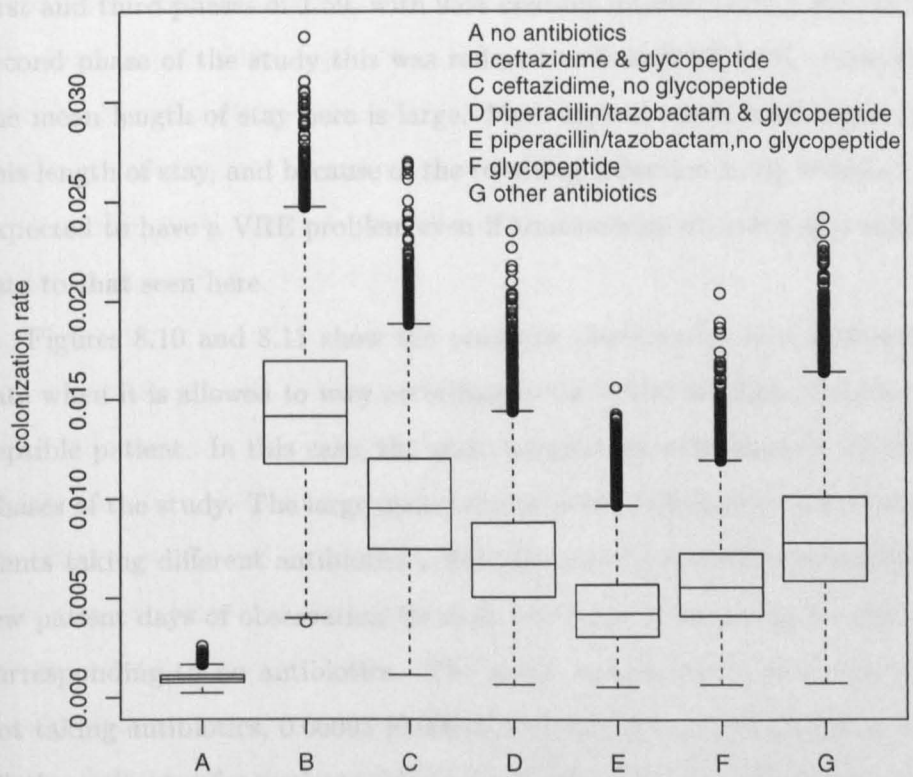

Figure 8.11: Box-and-whisker plots of the MCMC samples from which the posterior densities shown in figure 8.10 are derived, corresponding to seven different categories of antibiotic treatment for susceptibles.

similar, a single colonization rate was then specified for them. The results of this parameterization are shown in the lower panel of figure 8.9. The mean (and 95%CI) for the first and third phase is 0.0037 [0.0025, 0.0042], and for the second 0.0014 [0.00062, 0.0024]

The mean length of patient stay during the study on this ward was 25.2 days. Since this was an 18 bed ward, this gives a mean value of R_0 for the first and third phases of 1.59, with 95% credible interval [1.07, 1.80]. In the second phase of the study this was reduced to 0.60 [0.27, 1.01]. Note that the mean length of stay here is large. More typical wards have about half this length of stay, and because of the resulting reduction in R_0 wouldn't be expected to have a VRE problem even if transmission occurred at a similar rate to that seen here.

Figures 8.10 and 8.11 show the posterior densities for the colonization rate when it is allowed to vary according to the antibiotic class of each susceptible patient. In this case, the same parameters were used for all three phases of the study. The large uncertainties in the colonization rates for patients taking different antibiotics reflect the fact that there were relatively few patient days of observation for each antibiotic class, except for the one corresponding to no antibiotics. The mean colonization rate for patients not taking antibiotics, 0.00093 [0.00046, 0.0016], is clearly much lower than all the estimates for patients taking antibiotics. The largest of these corresponds to joint ceftazidime and glycopeptide use, (0.0145,[0.0078, 0.023]), with a slightly lower value when the glycopeptide was not used simultaneously (0.0099, [0.0043, 0.017]). Piperacillin/tazobactam use without a glycopeptide gave the lowest acquisition rate (0.0046, [0.0015, 0.0092]), which was increased slightly when glycopeptides were used simultaneously (0.0071, [0.0027, 0.014]. Glycopeptide use with ceftazidime and piperacillin/tazobactam gave a lower rate (0.0058[0.0021, 0.011]) than use of other antibiotics (0.0081, [0.003, 0.015]).

8.5 Discussion

8.5.1 Simulated data

The results suggest that relatively little information is gained by taking daily instead of weekly swabs. In practice, the situation may be complicated by the detection of very short-term carriage, when a newly arrived strain is present in enough numbers to be detected, but not to become established (Cookson *et al.*, 1989). This phenomenon was not included in the model used for generating the data. With real data, more frequent swabs would have a greater chance of correctly identifying such carriage as transient. With weekly swabs, for each swab there is a large chance that a patient will be discharged before a second swab can be made to determine if the carriage does persist.

A surprising result is that even with very low levels of patient consent good estimates can be obtained. It is not clear, however, if this will still be the case in a situation where there are multiple strains or when there are large uncertainties attached to initial colonization status. Low consent rates can also introduce significant bias if the assumption that non-consent occurs at random doesn't hold.

Given these caveats, however, the results do show when planning such studies different designs are likely to have radically different efficiencies.

For example, taking daily swabs from all patients for 30 days requires nearly as many swabs as taking weekly swabs from 50% of patients over 500 days, but provides much less information about the transmission rate. Such studies are time-consuming and expensive. In large part this is due to laboratory work needed to type the isolates obtained. Also, because of the large stochastic fluctuations it can be difficult to distinguish even quite large effects from chance. Analysis of simulated data may therefore help in the design of such studies. However, because of the size of the stochastic fluc-

tuations associated with such hospital epidemics, even large well-designed studies may be unable to show effects significant at conventionally accepted α values. Adopting a Bayesian approach, and taking prior information from previous studies into account, could provide a more productive and rational framework for analysing such studies.

The simulation studies suggest that the best approach might be to take fewer swabs from each patient, but extend the study over a longer period of time or several wards. If transient carriage is not important, taking only admission and discharge swabs from patients is likely to be sufficient.

Most studies have used weekly swabs, but Parker *et al.* (1965) tried taking twice-weekly swabs. The main difference they found was that increased transmission was found this way, as more transient colonization was included. For VRE data some studies have used daily swabs (Bonten *et al.*, 1998). Though very time-consuming, expensive, and tiresome for the patient, this would remove most of the uncertainty with the data (provided swabs were obtained from each patient) and analysis using a generalized linear model might be a more productive approach.

8.5.2 *Staphylococcus aureus* data

The large credible interval obtained in the estimates of the transmission rate reflects the short duration of the study and low consent rate. However, the estimate is consistent with the pattern of MRSA transmission seen on the ward, which is characterized by sporadic cases of cross-infection. When such "clusters" are spotted by infection control staff it is likely that an intervention will be made. This was seen during the study described in chapter 3 when alcohol handscrub was placed at the end of every bed. However no subsequent increase in handwashing frequency was observed when this happened. It is likely that on a ward such as this outbreaks will be self-limiting, provided reasonable levels of hygiene are maintained.

The Bayesian approach provides a natural way to incorporate information from previous studies by specifying an informative prior. In many cases this is necessarily a subjective process, but by subjecting data to a "community of priors" the degree to which previous beliefs are modified by the new data can be examined.

In this case, a prior was elicited from previous studies, most of which were conducted more than 30 years ago. Consequently, the relevance of such estimates for contemporary wards and strains is questionable.

Such limited data can only justify fitting the simplest of models. In practice, though, this model has severe limitations. Firstly, since the population will never (or almost never) be completely susceptible, R_0 is of relevance only as an upper bound. In chapter 6 an effective R_0 value for resistant strains was introduced, where the effect of patients colonized with sensitive strains was accounted for. However, when all the strains behave identically, as assumed here, then if the ward can be assumed to be observed at equilibrium, the mean number of secondary cases arising from each case can at most be one. Long-term stability of any one strain will not be possible (in the model), due to the constant influx of new strains with identical behaviour to those present.

To account for observed patterns of behaviour where a single strain becomes endemic to a ward requires a model that differentiates between the strains. Amongst the most important differences are likely to be the antibiotic-resistance patterns. Antibiotic data were collected during this study, but there are too few transmissions for it to be usefully applied. In particular, there was no observed transmission of MRSA during the study period.

Note that when assessing the effect of different numbers of beds on the transmission dynamics, it will not be valid to simply substitute the number of beds in the above calculation. The reason for this is that as the size of

the ward increases, λ would be expected to decrease.

Again, as mentioned above, the assumption that once a patient is colonized they remain colonized is contradicted by data. In the present case, colonization of a patient was deliberately eliminated. Transient carriage by patients may also be important. However, if the former is rare and can be treated on case-by-case basis, and the latter is unimportant for transmission, then this may still be a useful working assumption. The assumption that patients are not simultaneously colonized with two or more strains is also contradicted by the data. The fact that carriage of multiple strains has been found to be rare in other studies (for example Shinefield *et al.*, 1974), suggests that this too may still be a convenient, if incorrect, assumption. In the current study, swabs from multiple sites of each patient were taken, and all colonies of differing morphologies were cultivated, but only two patients were identified as carrying more than one strain.

8.5.3 VRE data

These are the most interesting results, and represent the first attempt to fit data relating antibiotic use to the spread of resistance to a dynamic model. Uncertainties are still large but may be reduced when full typing data becomes available, and all three wards are combined.

8.5.4 The effect of antibiotics

Carmeli and co-workers conducted a meta-analysis of 20 studies relating VRE acquisition to antecedent vancomycin usage (Carmeli *et al.*, 1999). They found that studies that used patients harbouring vancomycin-susceptible enterococci as controls found a stronger association between use of the antibiotic and VRE acquisition when compared to those studies that used patients not acquiring VRE as controls. They also found, when analysing these latter studies, if only those studies that adjusted for length of stay

were included, only a weak and non-significant association was found with vancomycin treatment and VRE acquisition. This seems to contradict conventional view that the emergence of VRE "mainly results from the extensive use of glycopeptides" (Mouthon and Mainardi, 1996).

Again, however, there is the problem that drug use is likely to be highly correlated with length of stay, so this may obscure the effect of antibiotic use. In chapter 5 it was argued with reference to *S. aureus* acquisition that simple logistic regressions are likely to be of limited value when investigating the effect of antibiotic use because of this association. In the VRE data considered in this chapter, for example, figure 8.8 shows that all but one of the patients who stayed longer than 20 days received antibiotics. The appropriate tool is survival analysis, but with asymptomatic infections there will usually be large uncertainties regarding the true acquisition times. This can limit the applicability of such techniques. The methods in the present chapter overcome these problems by allowing for all possible acquisition times in the unobserved data, and simultaneously making inferences about model parameters and the unobserved process. In all six of the antibiotic classes, the patient acquisition rate was found to be well above that for patients not taking antibiotics. Some of this increase, however, may simply be due to the fact that patients taking antibiotics are contacted by carers almost twice as frequently as those who aren't (see chapter 3).

Another study overcame the problems inherent in routine hospital data by giving healthy volunteers oral glycopeptides. Using non-selective media, VRE could be found in 14 out of 22 subjects after three weeks of glycopeptide treatment, while none of the pre-treatment screens were positive (Van der Auwerea *et al.*, 1996). When using a selective medium containing vancomycin VRE could only be recovered from 11 out of 40 healthy untreated volunteers. This study clearly suggests that glycopeptides do select for VRE, at least when orally administered.

The study by Carmeli *et al.* (1999) also suggests that resident antibiotic-sensitive enterococci may play a important role in inhibiting acquisition of VRE in the absence of antibiotic treatment. Vancomycin might be expected to select for enterococci by killing resident susceptible enterococci, leaving a vacant niche for the VRE. This could explain why there was a stronger association between vancomycin use and VRE acquisition when controls were those colonized with sensitive enterococci. Cephalosporins, however, are not active against enterococci, and might be expected to select for the resident enterococcal population, inhibiting acquisition of VRE. The results obtained in this chapter suggest that this is not the case, with the highest acquisition rates being found for patients taking cephalosporins. This may be because the effect of the cephalosporin depends on the response time of the endogenous flora to the antibiotic pressure. During treatment with a cephalosporin, endogenous enterococci should initially increase in numbers. If competition between strains is principally through competition for attachment sites as suggested for *S. aureus* (Bibel *et al.*, 1983), rather than direct, then during this period any incoming enterococci should also be able to increase in numbers. This period of growth in the enterococcal population may also provide excellent conditions for the spread of the transposon containing the resistance gene. The lowest acquisition rates were found amongst patients taking piperacillin/tazobactam and glycopeptides. This is also a little paradoxical, as both drugs should be active against sensitive but not resistant strains, and therefore might be expected to select for VRE. It should be noted, though, that acquisition rates for patients taking these drugs were still much higher than the corresponding rate for patients taking no antibiotics. In other words, it appears that they do increase the risk of acquiring VRE, but not as much as cephalosporins do. It may be the case, as was found with the interference experiments with *S. aureus* described in section 5.4.3, that the interference caused by other bacteria species making up

the faecal flora is at least as important as that attributable to enterococci themselves.

8.5.5 Model selection

The approach taken here provides a framework for fitting a whole family of epidemiologically meaningful models. In the present case this was illustrated by fitting a simple transmission model where the rate that patients become colonized depends on the number of colonized patients on the ward at that time (the mass action assumption). Clearly many other assumptions are possible and many interesting questions can be framed in terms of choosing between competing models.

For example, the effect of antibiotics may change the chance of a patient becoming colonized, may change the chance of a colonized patient colonizing other patients, or the effect could be some combinations of the two. Patients are treated with many different combinations of antibiotics, and there are many ways that these could be accounted for in a model. The mass action assumption that each new colonized individual provides a constant additional risk should also be examined. If, for example, the acquisition rate were to remain constant providing there was at least one colonized patient on the ward, different conclusions might be drawn about the transmission process.

Most importantly, the strains of unique PFGE types need to be considered, and the colonization status of a patient on admission to the ward needs to be related to their colonization status on departure in their previous episode. A fuller treatment should also make allowance for incubation periods. The delayed effect of antibiotic therapy due to suppression of endogenous flora may also be important. Other factors that may be worth considering include: heterogeneities in the transmissibilities of different strains; and the effect on transmission of diarrhoea in colonized patients.

The Bayesian approach used here provides an attractive framework for doing this. When full typing information becomes available further work will need to concentrate on the problems of model selection and assessment.

Clearance of VRE carriage

It was assumed when looking at the VRE data that once a patient became colonized they remained colonized for the rest of that episode. Other studies have shown low clearance rates of VRE carriage. Unsurprisingly, clearance rates appear to be closely related to the number of organisms present. Green *et al.* (1991) found that 7 out of 21 subjects with more than 10^7 CFU/ml of VRE in their stool specimens had persistent colonization, while 0 out of 4 of those with less than 10^4 CFU/mls had persistent colonization. If antibiotic therapy increases the numbers of resistant organisms given that they are already present, then an important aspect of antibiotic therapy may be to increase persistence in colonized hosts. In most hospital wards, inclusion of this effect may not be important, since length of hospital stay is typically short compared to the rate of loss of carraige. However, when considering transmission in long-term care settings, and looking at the problem at a hospital level where many of the patient episodes are readmissions, the effect could be important.

A high rate of "loss" of VRE was also found in the study by Van der Auwerea *et al.* (1996). One week after the last dose of glycopeptide the authors found that amongst patients given a low dose of teicoplanin 78% of samples were VRE negative. 64% had been positive after three weeks of glycopeptide treatment. Note, however, that unlike the studies by Bradley *et al.* (1999) and Green *et al.* (1991), in this case there was no enrichment step, and specimens were not inoculated directly onto glycopeptide-containing agar, so a lower sensitivity for the detection of VRE would be expected in this case. Both of these latter studies have much lower rates of loss.

Admission of colonized patients

A particularly interesting aspect of these data is the importance of readmissions of colonized patients. Patients were frequently readmitted soon after they were discharged from the ward. The effect of this is to decrease the rate of loss of strains from the ward. In phase one of the study, in those patients for whom initial colonization status on admission could be established, 14 out of 53 were colonized. In phase two the corresponding figure was 12 out of 129, but this decreased from 10 out of 59 in the first four months of phase two, to only 1 out of 70 in the second four months. In phase three only 2 out of 40 were colonized on admission. This suggests that the assumption of a constant probability that new patients are colonized with unique VRE strains on admission cannot be justified. A full model that allows for acquisition of the transposon and relates patients' colonization statuses to those of previous episodes would be needed to account for this.

As well as decreasing the rate at which VRE was lost from the ward, the same effect could explain why hospitals see gradually increasing levels of resistant organisms following their introduction. Since a large number of patient episodes are likely to be readmissions, a gradual increase in the proportion of patients colonized on admission would be expected. The rate of this increase would depend on the proportion of patient episodes that are readmissions, the time interval between readmissions, the rate of loss of resistant organisms in the absence of antibiotics, and also transmission in the community if this is important.

Environmental contamination

The role of environmental contamination in the transmission of VRE remains contentious, and it has proved difficult to disentangle animate and inanimate reservoirs (Weber and Rutala, 1997). Environmental contamination is frequently found in the rooms of colonized patients, particularly

those with diarrhoea, but high levels of hand contamination of healthcare workers are also reported (Bonilla *et al.*, 1997). To demonstrate that environmental contamination is important for the transmission of VRE requires doing more than showing that organisms can be found surviving on fomites; it also requires showing that transmission is reduced by reducing the level of environmental contamination. Since colonized patients are almost certainly also an important reservoir, the reduction is likely to be partial at best, and because of stochastic effects and associated large uncertainties, evidence from any single study is likely to be inconclusive.

If there is a high level of contamination of fomites, it seems highly likely that there will be at least *some* transmission attributable to this reservoir. This could be demonstrated by observing acquisition of a strain by a patient which no current patients on the ward harboured. In practice, however, it is difficult to exclude all other patients as potential reservoirs, especially as there may also be HCW-borne transmission from contacts with colonized patients on other wards. HCWs would also need to be excluded as potential sources.

More important, though, is an assessment of the importance of this reservoir compared with the reservoir of colonized patients. Highly detailed studies such as the one described above may help to answer this question by considering, for example, whether patients have an increased risk of acquiring strains carried by previous occupants of their rooms or beds. Such analyses, however, would inevitably be complicated by the fact that if such an association were found alternative explanations could be equally plausible. Most importantly, neighbouring patients would be expected to have a greater chance of being colonized with a strain harboured by a departed patient. Such spatial correlations may arise solely from carer contact patterns, rather than environmental contamination or airborne spread.

A more conclusive demonstration of the importance of such environ-

mental contamination would depend on interventional studies. Studies that compare both carer and patient hand contamination after contact with colonized patients and surfaces should also be valuable. Without such studies, the role of environmental contamination must remain largely a matter of speculation.

Chapter 9

Conclusions

This chapter summarises the main conclusions from the previous chapters, their implications for hospital infection control, and considers directions for future work.

9.1 An overview of the main results

9.1.1 Chance

Simulation results from chapter 2 suggest that chance (stochastic) effects are likely to be extremely important for hospital epidemics, particularly for ward-level outbreaks. Huge variation in the course of outbreaks occurs even when the underlying processes are in every way identical. For many scenarios it was found that almost any sensible measure of the scale of a ward's problem is likely to be highly unpredictable, with similar wards having widely differing outcomes.

Such stochastic effects are almost never considered in outbreak reports, but a consideration of them may profoundly modify any interpretation of observed outcomes.

The large reporting biases likely in this literature, together with the huge heterogeneity in source data that theory predicts, suggest that such

retrospective reports may contribute little to our knowledge, other than the information that what was observed is possible. Attempts at the study of hospital epidemics should consider whether effects usually attributed to interventions may not equally well have occurred by chance.

9.1.2 Length of stay and detection rate

Model results in chapter 2 showed that decreasing the lengths of patients' stays could result in both increases and decreases in the total colonized patient days. This was a consequence of two opposing processes: shorter lengths of stays result in more introductions of the pathogen into the ward, but less opportunity for its spread. Which effect dominates depends on the transmissibility of the pathogen (or the susceptibility of the patient population) and the prevalence of colonization amongst new admissions. For a highly transmissible strain, decreasing length of stay should decrease colonized patient days. For a strain of low transmissibility the effect of more introductions can dominate. In both cases total colonized patient days increase linearly (or almost linearly) as the proportion of patients colonized on admission increases.

Assuming that known carriers of the pathogen are removed, isolated, or cleared, increasing the detection rate of the pathogen is equivalent to decreasing the length of stay for carriers. Consequently, for a highly transmissible strain increasing the detection rate can results in big reductions in colonized patient days, while only small effects are seen when transmissibility is lower.

Decreasing the proportion of patients bringing the pathogen into the ward (by admission screening, for example) is likely to be a good strategy in all settings. Increased surveillance should be more effective in settings (or with strains) where there is a lot of transmission.

9.1.3 Handwashing

Simulation results from chapter 2 suggest (if hand-borne transmission is as important as is widely believed) that the spread of a pathogen will be highly sensitive to handwashing frequency. For parameter values chosen to be representative of a general medical ward, effective handwashing frequencies as low 30 or 40% were found sufficient to eliminate almost all patient colonization, leaving only sporadic and self-limiting outbreaks.

Handwashing frequencies in this range are in fact commonly observed, as was the case in the observational study described in chapter 3. Handwashing frequency was found to vary greatly with contact type, with handwashes least likely for simple touches, and most likely for invasive or semi-invasive procedures. Also in agreement with other studies (for example Pittet *et al.*, 1999) was the observation that doctors washed their hands far less frequently than other carers, even after adjusting for the contact type. Surprisingly, once differences in carer type had been accounted for, little heterogeneity in the handwashing behaviour of carers was seen. Heterogeneity between observation periods was, however, significant, but did not seem to be linked to ward activity levels, staffing levels, or the presence of senior staff.

The implications for control are that, away from wards with highly susceptible patients (such as ICUs, burns units, and neonatal units), fairly modest and achievable handwashing frequencies may be sufficient to prevent sustained outbreaks. Lower grade (types 1 and 2) contacts were found to be far more frequent than high grade (type 3) contacts and far less likely to be followed by handwashes, but are not necessarily associated with lower risk of transmission of organisms to carers' hands (Wong, 2000). Efforts at increasing compliance might therefore usefully be directed at emphasising the importance of handwashing after these simpler contacts, as well as at improving the consistently poor performance of doctors.

9.1.4 Contact patterns: ward teams, named-nurses, patient-carer ratios

The common assumption in epidemic models of homogeneous mixing (that each individual is equally likely to contact every other individual) is rarely likely to be found in practice, and the consequences of departures from this assumption should be considered. In the observational study of the surgical/medical ward, only the nurse team structure (two nurse teams, each with primary responsibility for one side of the ward) was found to be an important source of structure in the contact patterns. Results from chapter 4 suggest that this team structure has the potential to reduce the burden of infection in a ward, but the effect is small and only likely to become evident at levels of separation between the two halves of the ward far greater than those observed.

On the ICU, however, 60% of each patient's contacts came from the single nurse assigned to them at that time. Chapter 4 results show that—as has been suggested elsewhere (Austin *et al.*, 1999b)—this structuring should be a highly effective control measure, becoming more so as the fidelity of a carer to their patient increases. Interestingly, the results are sensitive to the carer-patient ratio, with the colonized patient days and time to extinction both increasing as staffing levels decrease. No such dependency is seen when there is only homogeneous mixing. Reports that link decreased carer-patient ratios with higher infection rates usually attribute the association to lower handwashing rates among busier staff. These results show that the structure of contact patterns can also be important. They predict that on wards such as ICUs, where staffing patterns similar to these are seen, infection rates should go up when staffing levels go down, even when handwashing frequency is unchanged. In fact, in chapter 3 no link between three different measures of nursing workload and handwashing frequency could be found. Others, however, have found such a link (Pittet *et al.*, 1999),

using the number of handwashing opportunities as a proxy for staff workload. Measuring ward-activity is not simple, though. For example, type 3 contacts are likely to take much longer than simple touches, so when many carers are performing these invasive procedures a crude count of the number of handwashing opportunities would falsely suggest a period of low activity. Because these contacts are likely to be associated with higher handwashing frequencies, a spurious association between low ward activity and high handwashing frequency could arise.

Increased staffing levels in high risk areas and contact patterns typical of an ICU may therefore be an effective control measure, whether or not handwashing frequency does go down as workload goes up. Indeed, one of the reasons for the effectiveness of closing wards to new admissions may be just this change in carer-patient ratios.

9.1.5 Contact pattern heterogeneity: the importance of high risk patients

Large heterogeneities in patient-carer contact rates were seen, with significant variation between observation periods and between patients. Simulation results in chapter 4 suggest that such heterogeneity in contact rates would result in modest increases in transmission. However, antibiotic use and having had an operation in the last 24 hours were both strongly linked with higher contact rates; each was associated with nearly a doubling of the rate. Both of these factors might themselves be expected to be independent risks for becoming colonized or infected. When higher patient contact rates are associated with greater susceptibility, the simulation results show that the ability of the pathogen to persist is greatly enhanced. A small group of high-risk patients may account for much of the spread and persistence.

Theoretical consideration suggest that as a control measure, separating such high risk patients from low risk patients is likely to exacerbate the

problem, and the best option may be to mix such patients together. If—as seems likely—100% handwashing compliance cannot be achieved, a more focussed approach that emphasises hand decontamination before and after contacts with high risk patients may be an effective control strategy.

9.1.6 Transmission rates

The *S. aureus* transmission data collected as described in chapter 3 and analysed further in chapter 3 provided only limited information on transmission patterns. No cases of MRSA cross-infection were seen, despite there being three MRSA colonized patients on the ward for most of the time. In contrast, two cases of MSSA cross-infection were observed. It is possible that MSSA cross-infection is more frequent than has been previously thought (see, for example Casewell and Hill, 1986), but simply less likely to be detected and less visible because of the multitude of strains and the fact that a single strain is unlikely to become endemic[1]. Analysed together, the data suggested that the *S. aureus* transmission rate observed in the medical/surgical ward was unlikely to reach the critical level where long-term persistence becomes a possibility. This is consistent with the observation that the patterns of transmission in such wards are usually characterized by sporadic clusters of cases. During such outbreaks interventions—such as encouraging staff to wash their hands more—may be made to reduce transmission. While these may indeed limit the spread, it seems likely—from these limited data—that most such outbreaks would rapidly fade out on their own.

Considerations such as these again suggest that a targeted approach to infection control, that concentrates on preventing endemicity in high risk areas rather than fighting many sporadic outbreaks elsewhere, may pay div-

[1]Neonatal units are an exception. Babies are highly susceptible to colonization, and MSSA strains sometimes do become endemic.

idends.

9.1.7 Antibiotics and the spread of resistance

Chapter 5 reviewed studies relating transmission of resistant bacteria to antibiotic use. Surprisingly, those studies that used logistic regressions to relate the risk of acquiring resistant organisms such as MRSA or VRE to prior antibiotic use tended to find only weak associations, or no association at all once length of stay had been accounted for. Because length of stay and antibiotic use will usually be closely linked, the many studies that do not adjust for the former cannot be considered to contribute useful information. A variety of methods, however, were used in other studies to make inferences about the *rate* of acquisition. These all found the rates to be greatly increased in patients taking antibiotics. It was argued that the high levels of antibiotic use and its close association with length of stay mean that the power of logistic regressions to detect any extra risk due to antibiotic use is likely to be very low, even if that extra risk is large. Further analysis of such data, and of simulation experiments, may be able to confirm this suspicion. Studies that report no link may not only be counter-intuitive, but also highly misleading if naively interpreted as providing evidence of no effect, rather than no evidence of an effect. The link between antibiotic use and the spread of resistant organisms may not be simple or always easy to detect, but the rarity of resistance genes before widespread antibiotic use, the fact that resistant bacteria are consistently found in greater numbers as antibiotic use increases, estimates relating acquisition rates to antibiotic use, and basic theoretical considerations all support the obvious conclusion: that antibiotic use is indeed amongst the most important determinants for the spread of antibiotic-resistant bacteria.

Previous models relating antibiotic use and the transmission of resistant organisms were critically reviewed in chapter 6. It was argued that claims

that the cycling of antibiotics could never be an effective strategy were based on models inappropriate to hospital populations, and the conclusions unjustified. In particular, chance effects and patient turnover are important. A stochastic model of the spread of resistant bacteria in a hospital ward was presented, and simulation experiments showed that the elimination of an endemic resistant strain following withdrawal of antibiotics (or a change in antibiotic policy) could indeed be rapid. Away from endemic settings, however, any effect of a change in policy would be far harder to detect because of the large variability in periods required for elimination of the strain from the ward. In common with other models, a threshold effect was predicted, whereby long-term persistence does not occur below a certain level of antibiotic use, but becomes a possibility (though by no means a certainty) above that level.

The model proved to be very sensitive to the assumptions regarding discharge rates for patients undergoing antibiotic therapy, suggesting that, where it is possible, rapid discharge of patients undergoing or having recently undergone antibiotic therapy may be effective at controlling the spread of resistant organisms.

If transmission rates are enhanced by antibiotics as much as the data suggest, reducing antibiotic use—and particularly the density of antibiotic use—would be expected to make a large impact, particularly if it could be reduced below the critical level where long-term persistence is no longer possible. Again, a more focussed attitude to infection control that concentrated on preventing transmission to patients taking antibiotics might also be effective.

9.1.8 The fitness cost of resistance

Both theory and experiment suggest that a mutation conferring antibiotic-resistance to a bacterium is likely to be associated with a fitness cost in the

absence of the antibiotic. Subsequent secondary mutations may then reduce this cost. Such a fitness cost could reduce a strain's ability to persist on host or colonize new hosts, and consequently hinder its ability to spread in hospitals and the community.

In an attempt to see if there was any such cost associated with methicillin-resistance in *S. aureus* a comparison of growth kinetics was made between MRSA and MSSA strains. No differences in maximal (exponential) growth rates were found, though there was a suggestion of an increased lag period in MRSA strains. Both MRSA and MSSA strains, however, exhibited great variability in both aspects of their growth curves.

This experiment should not be considered as evidence for the lack of a fitness cost. To date, however, there is little evidence that any such cost does exist, although research in this area has been minimal.

9.1.9 Fitting models to data

Chapter 8 showed how model parameters could be estimated from detailed ward-level data using a Markov chain Monte Carlo (MCMC) approach. Such MCMC methods are computationally expensive and have only recently been applied to epidemic models, but have great potential for fitting stochastic models to data, particularly when the epidemic process is only partially observed. In one of the examples it was shown how the approach could be used to make inferences about the effects of different antibiotics, and combinations of antibiotics, on the rate of acquisition of VRE strains amongst patients in a haematology unit. The results contradicted a previous meta-analysis that suggested that glycopeptide use did not select for VRE, though provided support for the view that cephalosporin use was an important additional risk factor for VRE acquisition.

9.2 Directions for future work

Patterns of patient movement

The work presented in this thesis has concentrated on transmission within single hospital wards. Clearly, other levels need to be considered. In particular, the transfer of patients and movement of carers between wards will be important for the dynamics within a hospital as a whole. Data are readily available for these movements, and models of the likely consequences of these movements need to be developed and explored.

Patterns of patient readmission are likely to be particularly important for long-term dynamics. If a colonized or infected patient colonizes on average 0.5 other patients during a stay in a ward free of the pathogen, the pathogen will not become established in the hospital from a single episode. However, if each such patient has three episodes before being cleared of carriage, then an average of 1.5 patients will become infected in a susceptible population. A chain reaction is then possible, and a long-term increase in prevalence may occur in the hospital. If such readmissions are indeed important for the long-term dynamics, then a number of possible control measures immediately suggest themselves.

Antibiotic policies

As suggested above, cycling of antibiotics has *not* been shown to be ineffective. Whether or not it works should depend on the time taken to eliminate a resistant strain when antibiotic use is changed, and the rate at which new resistant strains come into a ward. If the first rate is high and the second low, then cycling antibiotics could be an effective policy. Again, modelling work should shed light on this question. Stochastic optimization techniques may also allow optimal antibiotic policies to be identified. For this to be feasible it may be necessary to work with approximations of the epidemic

process suggested by the results of chapter 6. Multiply-resistant strains represent the other major problem, and their emergence and spread must be a major consideration when assessing all policies involving the use of more than one antibiotic.

Transmissibility

Transmissibility is amongst the most important of model parameters, yet very little is known about its determinants, and no attempts have been made to measure the transmission rate for different strains in different settings.

The methods of chapter 8 show how such transmission rates could be measured, but there is also a need for further laboratory investigations comparable to those of Farrington *et al.* (1992).

Further studies on the growth kinetics of *S. aureus* strains will also be of interest. As well as competition experiments, it would be interesting to compare kinetics of early MRSA strains to the more recent epidemic variety. In this way any change in the fitness cost of resistance could be explored.

Repeating these experiments at temperatures slightly below 37^oC, closer to the normal temperatures of the usual colonization sites of *S. aureus*, may also be worthwhile.

Fittings models to data

The analysis of the VRE data in chapter 8 represents just the beginning of what is possible with these data and methods. A lot of further work is required. In particular, the areas of model assessment and selection need attention, as a number of different models may be appropriate. Fitting different models should allow an evaluation of the consistency of the data with different assumptions regarding the underlying processes. For example, it is not clear how to interpret VRE strains of unique types. They may have been present in low numbers before antibiotic use and selected by treatment,

or they may represent strains that have acquired the resistance-conferring transposon by cross-infection from other patients.

It should be possible to apply the same methods to many other hospital infection problems. For example, formal assessments of the effect of side-room isolation on transmission of MRSA could be made, without the need for *ad hoc* assumptions.

Assessing interventions

Ward-level experiments are essential for the assessment of any suggested intervention, ideally with a cluster-randomized design. Mathematical models are important tools for proposing interventions and assessing the likely costs and benefits, and stochastic models allow an exploration of the expected variation in these costs and benefits. Models may not only help choose which interventions should be tested in controlled trials, they can also help in the design of the trials themselves.

Bibliography

Abraham, E., Chain, E., Fletcher, C., Florey, H., Gardner, A., Heatley, N., and Jennings, M. (1992). Further observations on penicillin. 1941. *Eur J Clin Pharmacol*, **42**, 3–9.

Aly, R., Maibach, H., Strauss, W., and Shinefield, H. (1970). Effects of a systemic antibiotic on nasal bacterial ecology in man. *Appl Microbiol*, **20**, 240–244.

Aly, R., Shinefield, H., Strauss, W., and Maiback, H. (1977). Bacterial adherence to nasal mucosal cells. *Infect Immun*, **17**, 546–549.

Anderson, R. and May, R. (1991). *Infectious diseases of humans: dynamics and control.* Oxford University Press, Oxford.

Archibald, L., Manning, M., Bell, L., Banerjee, S., and Jarvis, W. (1997). Patient density, nurse-to-patient ratio and nosocomial infection risk in a pediatric cardiac intensive care unit. *Pediatr Infect Dis J*, **16**, 1045–1048.

Aron, J. and May, R. (1982). *The population dynamics of infectious diseases: theory and applications*, chapter The population dynamics of malaria, pages 139–179. Chapman and Hall, London.

Asensio, A., Guerrero, A., Quereda, C., Lizán, M., and Martinez-Ferrer, M. (1996). Colonization and infection with methicillin-resistant *Staphylococcus aureus*: associated factors and eradication. *Infect Control Hosp Epidemiol*, **17**, 20–28.

Auranen, K. (2000). Backcalculating the age-specific incidence of recurrent subclinical *Haemophilus influenzae* type b infection. *Stat Med*, **19**, 281–296.

Auranen, K., Arjas, E., Leino, T., and Takala, A. (2000). Transmission of pneumococcal carriage in families: a latent Markov process model for binary longitudinal data. *Submitted to J Am Stat Assoc.*

Austin, D. and Anderson, R. (1999). Studies of antibiotic resistance within the patient, hospitals and the community using simple mathematical models. *Proc R Soc Lond B*, **354**, 721–738.

Austin, D., Kakehashi, M., and Anderson, R. (1997). The transmission dynamics of antibiotic-resistant bacteria: the relationship between resistance in commensal organisms and antibiotic consumption. *Proc R Soc Lond B*, **264**, 1629–1638.

Austin, D., Kristinsson, K., and Anderson, R. (1999a). The relationship between the volume of antimicrobial consumption in human communities and frequency of resistance. *Proc Natl Acad Sci*, **96**, 1152–1156.

Austin, D., Bonten, M., Slaughter, S., Weinstein, R., and Anderson, R. (1999b). Vancomycin-resistant enterococci in intensive care hospital settings: persistence, dynamics and impact of infection control measures. *Proc Natl Acad Sci*, **96**, 6908–6913.

Ayliffe, G., Babb, J., Bridges, K., Lilly, H., Lowbury, E., Varney, J., and Wilkins, M. (1975). Comparison of two methods for assessing the removal of total organisms and pathogens from the skin. *J Hyg(Lond)*, **75**, 259–274.

Ayliffe, G., Babb, J., Taylor, L., and Wise, R. (1979). A unit for source and protective isolation in a general hospital. *BMJ*, **2**, 461–465.

Bailey, N. (1975a). *The Biomathematics of Malaria.* Charles Griffin, London.

Bailey, N. (1975b). *The Mathematical Theory of Infectious Diseases.* Charles Griffin, London.

Baquero, F. (1990). European standards for antibiotic susceptibility testing: towards a theoretical consensus. *Eur J Clin Microbiol Inf Dis*, **9**, 492–495.

Baquero, F. (1996). Trends in antibiotic resistance of respiratory pathogens: an analysis and commentary on a collaborative surveillance study. *J Antimicrob Chemother*, **38**(Suppl. A), 117–132.

Baquero, F. and Blàzquez, J. (1997). Evolution of antibiotic resistance. *Trends in Ecology and Evolution*, **12**(12), 482–487.

Barber, M., Dutton, A. C., Beard, M., Elmes, P., and Williams, R. (1960). Reversal of antibiotic resistance in hospital staphylococcal infections. *BMJ*, **1**, 11–17.

Barnes, E., Dobson, R., and Barger, I. (1995). Worm control and anthelmintic resistance: adventures with a model. *Parasitol Today*, **11**, 56–63.

Bauer, T., Ofner, E., Just, H., and Daschner, F. (1990). An epidemiologicial study assessing the relative importance of airborne and direct contact

transmission of microorganisms in a medical intensive care unit. *J Hosp Infect*, **15**, 301–309.

Becker, N. (1989). *Analysis of Infectious Disease Data.* Monographs on statistics and applied probability. Chapman and Hall, London.

Best, N., Cowles, M., and Vines, S. (1995). *CODA Convergence Diagnosis and Output Analysis software for Gibbs Sampler output: Version 0.3.*

Bibel, D., Aly, R., Bayles, C., Strauss, W., Shinefield, H., and Maibach, H. (1983). Competitive adherence as a mechanism of bacterial interference. *Can J Microbiol*, **29**, 700–703.

Bingen, E., Denamur, E., Lambert-Zechovsky, N., and Elion, J. (1991). Evidence for the genetic unrelatedness of nosocomial vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. *J Clin Microbiol*, **29**, 1888–1892.

Blower, S., Small, P., and Hopewell, P. (1996). Control strategies for tuberculosis epidemics: new models for old problems. *Science*, **273**, 497–500.

Bøe, A.J.and Solberg, C. (1965). Behandling av nasale stafylokokkbaerere med fucidin. *Tidsskr norske Laegeforen*, **85**, 527.

Bonhoeffer, S., Lipsitch, M., and Levin, B. (1997). Evaluating treatment protocols to prevent antibiotic resistance. *Proc Natl Acad Sci*, **94**, 12106–1211.

Bonilla, H., Zervos, M., Lyons, M., Bradley, S., Hedderwick, S., Ramsey, M. A., Paul, L., and Kauffman, C. (1997). Colonization with vancomycin-resistant *Enterococcus faecium*: comparison with an acute-care hospital. *Infect Control Hosp Epidemiol*, **18**, 333–339.

Bonten, M. J., Slaughter, S., Ambergen, A., Hayden, M., Voorhis, J. v., Nathan, C., and Weinstein, R. (1998). The role of "colonization pressure" in the spread of vancomycin-resistant enterococci. *Arch Intern Med*, **158**, 1127–1132.

Boris, M., Sellers Jr, T., Eichenwald, H., Ribble, J., and Shinefield, H. (1964). Bacterial interference. Protection of adults against nasal *Staphylococcus aureus* infection after colonization with a heterologous *S. aureus* strain. *Am J Dis Child*, **108**, 252–261.

Boris, M., Shinefield, H., Romano, P., McCarthy, D., and Florman, A. (1968). Bacterial interference. Protection against recurrent intrafamilial staphylococcal disease. *Am J Dis Child*, **115**, 521–529.

Borland (1990). *Turbo Pascal.* Borland International Inc., Scotts Valley, CA, 6.0 edition.

Boyce, J., Potter-Bynoe, G., Chenevert, C., and King, T. (1997). Environmental contamination due to methicillin-resistant *Staphylococcus aureus*: possible infection control implications. *Infect Control Hosp Epidemiol*, **18**, 622–627.

Bradley, S., Wilson, A., Allen, M., Sher, H., Goldstone, A., and Scott, G. (1999). The control of hyperendemic glycopeptide-resistant *Enterococcus* spp. on a haematology unit by controlling antibiotic usage. *J Antimicrob Chemother*, **43**, 261–266.

Brown, J., Froese-Fretz, A., Luckey, D., and Todd, J. (1996). High rate of hand contamination and low rate of hand washing before infant contact in a neonatal intensive care unit. *Paediatr Infect Dis J*, **15**, 908–911.

Burke, J. and Corrigan, M. (1961). Staphylococcal epidemiology on a surgical ward. *New Eng J Med*, **264**, 321–326.

Carabin, H., Gyorkos, T., Soto, J., Joseph, L., Payment, J., and Collet, J. (1999). Effectiveness of a training program in reducing infections in toddlers attending day care centres. *Epidemiology*, **10**, 219–227.

Carmeli, Y., Samore, M., and Huskins, C. (1999). The association between antecedent vancomycin treatment and hospital-acquired vancomycin-resistant enterococci: a meta-analysis. *Arch Intern Med*, **159**, 2461–2468.

Carter, K. and Carter, B. (1994). *Childbed fever: a scientific biography of Ignaz Semmelweis*. Greenwood Press, London.

Casewell, M. (1995). New threats to the control of methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect*, **30 (S)**, 465–471.

Casewell, M. and Hill, R. (1986). The carrier state: methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother*, **18 (suppl A)**, s1–s12.

Casewell, M. W. (1986). Epidemiology and control of the 'modern' methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect*, **7 (suppl A)**, S1–S11.

Casewell, M. W. and Phillips, I. (1977). Hands as route of transmission for klebsiella species. *BMJ*, **2**, 1315–1317.

CDC (1992). Public health focus: surveillance, prevention and control of nosocomial infections. *MMWR Morb Mortal Wkly Rep*, **41**, 783–787.

CDC (1997). Reduced susceptibility of *Staphylococcus aureus* to vancomycin—Japan. *MMWR Morb Mortal Wkly Rep*, **46**, 624–626.

CDSC (1998). Methicillin resistant *Staphylococcus aureus*. *Communicable Disease Report CDR Weekly*, **8**(42), 369–372.

Central Public Health Laboratory (1999). Surgical site infection. Analysis of a year's surveillance in English hospitals. Technical report, PHLS.

Chadwick, P., Fox, A., and Woodford, N. (1997). Molecular epidemiology of glycopeptide-resistant *Enterococcus faecium* on a renal unit. *Epidemiol Infect*, **119**, 159–166.

Coello, R., Glenister, H., Fereres, J., Bartlett, C., Leigh, D., Sedgwick, J., and Cooke, M. (1993). The cost of infection in surgical patients. *J Hosp Infect*, **25**, 239–250.

Cohan, F., King, E., and Zawadzki, P. (1994). Amelioration of the deleterious pleiotropic effects of an adaptive mutation in *Bacillus subtilis*. *Evolution*, **48**, 81–95.

Cohen, M. (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science*, **257**, 1050–1055.

Cookson, B., Peters, B., Webster, M., Phillips, I., Rahman, M., and Noble, W. (1989). Staff carriage of epidemic methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol*, **27**, 1471–1476.

Cooper, B. (1996). *A model for the spread of methicillin-resistant Staphylococcus aureus within a hospital ward.* Master's thesis, University of Warwick, UK.

Cooper, B., Medley, G., and Scott, G. (1999). Preliminary analysis of the transmission dynamics of nosocomial infections: stochastic and management effects. *J Hosp Infect*, **43**, 131–147.

Coovadia, Y., Bhana, R., Johnson, A., Haffejee, I., and Marples, R. (1989). A laboratory-confirmed outbreak of rifampicin-methicillin-resistant *Staphylococcus aureus* (RMRSA) in a newborn nursery. *J Hosp Infect*, **14**, 303–312.

Courvalin, P. and Soussy, C.-J. (1996). Report of the comité de l'antibiogramme de la Societé Francaise de Microbiologie. *Clin Microbiol Inf*, **2 (suppl 1)**, S11–S25.

Cox, D. and Miller, H. (1965). *The Theory of Stochastic Processes.* Chapman and Hall, London.

Cox, R. and Bowie, P. (1999). Methicillin-resistant *Staphylococcus aureus* colonization in nursing home residents: a prevalence study in northamptonshire. *J Hosp Infect*, **43**, 115–122.

Cox, R., Conquest, C., and Mallaghan, C. amd Marples, R. (1995). A major outbreak of methicillin-resistant *Staphylococcus aureus* caused by a new phage-type (EMRSA-16). *J Hosp Infect*, **29**, 87–106.

Crossley, K., Loesch, D., Landesman, B., Mead, K., Chern, M., and Strate, R. (1979a). An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides, i: clinical studies. *J Infect Dis*, **139**, 273–279.

Crossley, K., Landesman, B., and Zaske, D. (1979b). An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides, ii: epidemiological studies. *J Infect Dis*, **139**, 280–287.

Cytel Software Corporation (1997). *StatXact 3.1.* Cambridge, MA.

Department of Health (1998). Government response the house of lords science & technology committee seventh report - resistance to antibiotics. Technical report, The Stationery Office, London.

Diekmann, O., Heesterbeek, J., and Metz, J. (1990). On the definition and computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. *J Math Biol*, **28**, 365–382.

Dominguez, M., de Lencastre, H., Linares, J., and Tomasz, A. (1994). Spread and maintenance of a dominant methicillin-resistant *Staphylcoccus aureus* (MRSA) clone during an outbreak of MRSA disease in a Spanish hospital. *J Clin Microbiol*, **32**, 2081–2087.

Dooley, F. (1999). The named nurse in practice. *Nursing Standard*, **13**(34), 33–38.

Dowson, C., Hutchinson, A., Woodford, N., Johnson, A., George, R., and Spratt, B. (1990). Penicillin-resistant viridans streptococci have obtained altered penicillin-binding protein genes from penicillin-resistant strains of *Streptococcus pneumoniae. Proc Natl Acad Sci*, **87**, 5858–5862.

Draper, D. and Browne, J. (2000). A comparison of Bayesian and likelihood methods for fitting multilevel models. *Submitted to J R Stat Soc B.*

Dye, C. and Hasibeder, G. (1986). Population dynamics of mosquito-borne disease: effects of flies which bite some people more frequently than others. *Trans R Soc Trop Med Hyg*, **80**, 69–77.

Edmunds, W., O'Callaghan, C., and Nokes, D. (1997). Who mixes with whom? A method to determine the contact patterns of adults that may lead to the spread of airborne infections. *Proc R Soc Lond B*, **264**, 949–957.

Ehrenkranz, N. (1966). Nasal rejection of experimentally inoculated *Staphylococcus aureus*: evidence for an immune reaction in man. *J Immunol*, **96**, 509–517.

Eichenwald, H., Shinefield, H., Boris, M., and Ribble, J. (1965). "Bacterial interference" and staphylococcic colonization in infants and adults. *Ann N Y Acad Sci*, **128**, 365–380.

Farrington, M., Brenwald, N., Haines, D., and Walpole, E. (1992). Resistance to desiccation and skin fatty acids in outbreak strains of methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect*, **36**, 56–60.

Farrington, M., Redpath, C., Trundle, C., Coomber, S., and Brown, N. (1998). Winning the battle but losing the war: methicillin-resistant *Staphylococcus aureus* (MRSA) infection at a teaching hospital. *QJM*, **91**, 539–548.

Foster, W. and Hutt, M. (1960). Experimental staphylococcal infections in man. *The Lancet*, **i**, 1373–1376.

Franco, J., Eitzman, D., and Baer, H. (1973). Antibiotic usage and microbial resistance in an intensive care nursery. *Am J Dis Child*, **126**, 318–321.

Frénay, H., Theelen, J., Schouls, L., Vandenbroucke-Grauls, C., Verhoef, J., van Leeuwen, W., and Mooi, F. (1994). Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. *J Clin Microbiol*, **32**(3), 846–7.

Fridkin, S., Pear, S., Williamson, T., Galgiani, J., and Jarvis, W. (1996). The role of understaffing in central venous catheter-related bloodstream infections. *Infect Control Hosp Epidemiol*, **17**, 150–158.

Friedman, M. and Friedland, G. (2000). *Medicine's ten greatest discoveries*. Yale Nota Bene, New Haven.

Frost, S. and McLean, A. (1994). Quasispecies dynamics and the emergence of drug resistance during zidovudine therapy of HIV infection. *AIDS*, **8**, 323–332.

Fuller, R. and Gibson, G. (1998). Probiotics and prebiotics: microflora management for improved gut health. *Clin Microbiol Infect*, **4**, 477–480.

Gamerman, D. (1997). *Markov chain Monte Carlo : stochastic simulation for Bayesian inference*. Chapman and Hall, London.

Glynn, A., Ward, V., Wilson, J., Charlett, A., Cookson, B., Taylor, L., and Cole, N. (1997). Hospital-acquired infection: surveillance, policies, and practice. Technical report, PHLS, London.

Goh, S., Byrne, S., Zhang, J., and Chow, A. (1992). Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms. *J Clin Microbiol*, **30**(7), 1642–5.

Goldstein, F. and Acar, J. (1996). Antimicrobial resistance among lower respiratory tract isolates of *Streptococcus pneumoniae*: results of a 1992-93 western Europe and USA collaborative surveillance study. the Alexander project collaborative group. *J Antimicrob Chemother*, **38 (Suppl.A.)**, 71–84.

Goldstein, H. (1995). *Multilevel Statistical Models.* Edward Arnold, London.

Goldstein, H. and Rasbash, J. (1996). Improved approximations for multilvel models with binary responses. *J R Stat Soc A*, **159**, 505–513.

Goldstein, H., Rasbash, J., Plewis, I., and Draper, D. (1998). *A user's guide to MLwiN.* Insitute of Education, London.

Gould, D. (1994). Nurses' hand decontamination practice: results of a local study. *J Hosp Infect*, **28**, 15–30.

Green, M., Barbadora, K., and Michaels, M. (1991). Recovery of vancomycin-resistant gram-positive cocci from pediatric liver transplant patients. *J Clin Microbiol*, **29**(11), 2503–2506.

Green, P. (1995). Reversible jump Markov chain Monte Carlo computation and bayesian model determination. *Biometrika*, **82**, 711–732.

Guangyong, J., Beavis, R., and Novick, R. (1997). Bacterial interference caused by autoinducing peptide variants. *Science*, **276**, 2027–2030.

Gubbins, S. and Gilligan, C. (1999). Invasion thresholds for fungicide resistance: deterministic and stochastic analyses. *Proc R Soc Lond B*, **266**, 2539–2549.

Haley, R. and Bregman, D. (1982). The role of under-staffing and overcrowding in recurrent outbreaks of staphylococcal infections in neonatal special-care units. *J Inf Dis*, **145**, 875–883.

Hall, R. (1997). Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria. In *Antibiotic resistance: origins, evolution, selection and spread.*, pages 192–205. Ciba Foundation Symposium 207, Wiley, Chichester.

Hasibeder, G. and Dye, C. (1988). Population dynamics of mosquito-borne disease: persistence in a completely heterogeneous environment. *Theor Popul Biol*, **33**, 31–53.

Hershow, R., Khayr, W., and Smith, N. (1992). A comparison of clinical virulence of nosocomially acquired methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* infections in a university hospital. *Infect Control Hosp Epidemiol*, **13**, 587–593.

Hethcote, W. and Yorke, J. (1984). *Gonorrhea transmission dynamics and control.* Number 56 in Lecture notes in biomathematics. Springer-Verlag, Berlin.

Hiramatsu, K. (1998). The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. *Am J Med*, **104(5A)**, 7S–10S.

Hiramatsu, K., Aritaka, N., Hanaki, H., Kawasaki, S., Hosoda, Y., Hori, S., Fukuchi, Y., and Kobayashi, I. (1997a). Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet*, **350**, 1670–1673.

Hiramatsu, K., Hanaki, H., Ino, T., Yabuta, K., Oguri, T., and Tenover (1997b). Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother*, **40**, 135–136.

Hoefnagels-Schuermans, A., Peetermans, W., Struelens, M., Van Lierde, S., and Van Eldere, J. (1997). Clonal analysis and identification of epidemic methicillin-resistant *Staphylococcus aureus* by antibiotyping and determination of protein A gene and coagulase gene polymorphisms. *J Clin Microbiol*, **35**, 2514–2520.

Hox, J. (1995). *Applied Multilevel Analysis.* TT-Publikaties, Amsterdam.

Hughes, V. and Datta, N. (1983). Conjugative plasmids in bacteria of the 'pre-antibiotic' era. *Nature*, **302**, 725–726.

Isham, V. and Medley, G., editors (1996). *Models for infectious human diseases: their structure and relation to data.* Cambridge University Press.

Jarvis, W. and Martone, W. (1992). Predominant pathogens in hospital infections. *J Antimicrob Chemother*, **29, (suppl A)**, 19–24.

Jernigan, J., Titus, M., Groschel, D., Getchell-White, S., and Farr, B. (1996). Effectiveness of contact isolation during a hospital outbreak of methicillin-resistant *Staphylococcus aureus*. *Am J Epidemiol*, **143**, 496–504.

Jessen, O., Rosendal, K., Bulow, P., Faber, V., and Eriksen, K. (1969). Changing staphylococci and staphylococcal infections. a ten-year study of bacteria and cases of bacteremia. *New Eng J Med*, **281**, 627–637.

Jevons, M. (1961). 'Celbenin'-resistant Staphylococci. *BMJ*, **i**, 124–125.

Keeling, M. and Grenfell, B. (2000). Individual-based perspectives on R_0. *J Theor Biol*, **203**, 51–61.

Kjølen, H. and Andersen, B. (1992). Handwashing and disinfection of heavily contaminated hands—effective or ineffective? *J Hosp Infect*, **21**, 61–71.

Knight, V. and White, A. (1958). The effect of antimicrobial drugs on the staphylococcal flora of hospital patients. *Ann Intern Med*, **49**, 536–543.

Knittle, M., Eitzman, D., and Baer, H. (1975). Role of hand contamination of personnel in the epidemiology of gram-negative nosocomial infections. *J Pediatr*, **86**, 433–437.

Kobayashi, N., Taniguchi, K., Kojima, K., Urasawa, S., Uehara, N., Omizu, Y., Kishi, Y., Yagihashi, A., and Kurokawa, I. (1995). Analysis of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* by a molecular typing method based on coagulase gene polymorphisms. *Epidemiol Infect*, **115**, 419–426.

Krus, V. and Rvachev, L. (1971). The mathematical theory of epidemics: a study of the evolution of resistance in microorganisms. *Adv Appl Prob*, **3**, 206–208.

Larson, E. (1988). A causal link between handwashing and risk of infection? Examination of the evidence. *Infect Control Hosp Epidemiol*, **9**, 28–36.

Larson, E. and Kretzer, E. (1995). Compliance with handwashing and barrier precautions. *J Hosp Infect*, **30(S)**, 88–106.

Law, M. and Gill, O. (1988). Hospital-acquired infection with methicillin-resistant and methicillin-sensitive staphylococcis. *Epidmiol Infect*, **101**, 623–629.

Lenski, R. (1988). Experimental studies of pleiotropy and epistasis in *Escherichia coli*. 2. Compensation for maladaptive effects associated with resistance to virus-T4. *Evolution*, **42**, 425–432.

Lenski, R. (1997). The cost of antibiotic resistance—from the perspective of a bacterium. In *Antibiotic resistance: origins, evolution, selection and spread*, pages 131–151, Chichester. (Ciba Foundation Symposium 207), Wiley.

Lenski, R., Mongold, J., Sniegowski, P., Travisano, M., Vasi, F., Gerrish, P., and Schmidt, T. (1998). Evolution of competitive fitness in experimental populations of *E. coli*: what makes one genotype a better competitor than another? *Antonie Van Leeuwenhoek*, **73**, 35–47.

Lessing, M., Jordens, J., and Bowler, I. (1996). When should healthcare workers be screened for methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect*, **34**, 205–210.

Levin, B., Lipsitch, M., Perrot, V., Schrag, S., Antia, R., Simonsen, L., Moore Walker, N., and Stewart, F. (1997). The population genetics of antibiotic resistance. *Clin Infect Dis*, **24 (Suppl 1)**, S9–16.

Levin, B., Lipsitch, M., and Bonhoeffer, S. (1999). Population biology, evolution, and infectious disease: convergence and synthesis. *Science*, **283**, 806–809.

Levy, S. (1997). Antibiotic resistance: an ecological imbalance. In *Antibiotic resistance: origins, evolution, selection and spread*, pages 1–14. Ciba Foundation Symposium 207, Wiley, Chichester.

Levy, S., Marshall, B., Schluederberg, S., Rowse, D., and Davis, J. (1988). High frequency of antimicrobial resistance in human fecal flora. *Antimicrob Agents Chemother*, **32**, 1801–1806.

Lidwell, O., Polakoff, S., Jevons, M., and Parker, M. (1966). Staphylococcal infection in thoracic surgery: experience in a subdivided ward. *J Hyg (Lond)*, **64**, 321–337.

Lidwell, O., Polakoff, S., Davies, J., and Hewitt, J. (1970). Nasal acquisition of *Staphylococcus aureus* in a subdivided and mechanically ventilated ward: endemic prevalence of a single staphylococcal strain. *J Hyg (Lond)*, **68**, 417–433.

Lidwell, O., Davies, J., Payne, R., Newman, P., and Williams, R. (1971). Nasal acquisition of *Staphylococcus aureus* in partly divided wards. *J Hyg (Lond)*, **69**, 113–123.

Lingnau, W. and Allerberger, F. (1994). Control of an outbreak of methicillin-resistant *Staphylococcus aureus* by hygienic measures in a general intensive care unit. *Infection*, **22 S(2)**, 135–139.

Lipsitch, M., Bergstrom, C., and Levin, B. (2000). The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions. *Proc Natl Acad Sci*, **97**, 1938–1943.

Livermore, D. and Yuan, M. (1996). Antibiotic resistance and production of extended-spectrum beta-lactamases amongst klebsiella spp. from intensive care units in Europe. *J Antimicrob Chemother*, **38**, 409–424.

Martin, R. and White, A. (1968). The reacquisition of staphylococci by treated carriers: a demonstration of bacterial interference. *J. Lab & Clin Med*, **71**, 791–797.

Massad, E., Lundberg, S., and Hyun, M. Y. (1993). Modeling and simulating the evolution of resistance against antibiotics. *Int J Biomed Comput*, **33**, 65–81.

Massanari, R. (1997). The calculus of transmission. *Infect Control Hosp Epidemiol*, **18**, 81–83.

Maynard Smith, J. (1989). *Evolutionary Genetics.* Oxford University Press, Oxford.

Mazel, D. and Davies, J. (1998). *Resolving the antibiotic paradox: progress in understanding drug resistance and development of new antibiotics*, chapter Antibiotic resistance: the big picture, pages 1–6. Kluwer Academic, New York.

McGowan Jr., J. E. (1981). Environmental factors in nosocomial infection - a selective focus. *Rev Infect Dis*, **3**, 76–769.

Mehtar, S. (1995). Infection control programmes—are they cost-effective? *J Hosp Infect*, **30(S)**, 26–34.

Mizobuchi, S., Minami, J., Jin, F., Matsushita, O., and Okabe, A. (1994). Comparison of the virulence of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *Microbiol Immunol*, **38**, 599–605.

Monnet, D., Archibald, L., Phillips, L., Tenover, F., McGowan, J. J., and Gaynes, R. (1998). Antimicrobial use and resistance in eight US hospitals: complexities of analysis and modeling. *Infect Control Hosp Epidemiol*, **19**, 388–394.

Mortimer, E., Wolinsky, F., Gonzaga, A., and Rammelkamp, C. (1966). Role of airborne transmission in staphylococcal infections. *BMJ*, **1**, 39–322.

Mouthon, L. and Mainardi, J.-L. (1996). Vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*: the gram-positive challenges. *Curr Opin Infect Dis*, **9**, 256–260.

Muder, R., Brennen, C., Wagener, M., Vickers, R., Rihs, J., Hancock, G., Yee, Y., Miller, J., and Yu, V, L. (1991). Methicillin-resistant staphylococcal colonization and infection in a long-term care facility. *Ann Intern Med*, **114**, 107–112.

Mulligan, M., Ribner, B., Standiford, H., John, J., Korvick, J., Kauffman, C., and Yu, V. (1993). Methicillin-resistant *Staphylococcus aureus:* a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. *Am J Med*, **94**, 313–328.

Murakami, K., Minamide, W., Wada, K., E., N., Teraoka, H., and Watanabe, S. (1991). Identification of methicillin resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol*, **20**, 2240–2244.

Murchan, S., Trzciński, K., Skoczyńska, A., Van Leeuwen, W., Van Belkum, A., Pietuszko, S., Gadomski, T., and Hryniewicz, W. (1998). Spread of old and new clones of epidemic methicillin-resistant *Staphylococcus aureus* in Poland. *Clin Microbiol Infect*, **4**, 481–90.

Nananda, F. C. and O'Connor, R. (1987). Estimating worldwide current antibiotic usage: report of task force 1. *Rev Infect Dis*, **9**(s3), s232–s243.

Nåsell, I. (1991). On the quasi-stationary solution of the Ross malaria model. *Math Biosciences*, **107**, 187–207.

Nåsell, I. (1999). On the quasi-stationary distribution of the stochastic logistic epidemic. *Math Biosciences*, **156**, 21–40.

Navarro Marin, J. (1996). The first infection due to vancomycin-resistant methicillin-resistant *Staphylococcus aureus*? *Int J Antimicrob Agents*, **7**, 293–294.

NCCLS (1997). *Performance standards for antimicrobial disk susceptibility tests; Approved standard M02-A6.* NCCLS, Villanova Pa, 6th edition.

Nicholson, T., Milne, R., and Stein, K. (1997). Development and evaluation committee report no. 76. screening staff for methicillin-resistant *Staphylococcus aureus.* Technical report, Wessex Institute for Health Research and Development.

NNIS (1996). National nosocomial infections surveillance report, data summary from October 1986-April 1996, issued May 1996. *Am J Infect Control*, **24**, 380–388.

Novick, D. and Ness, G. (1984). Abuse of antibiotics by abusers of parenteral heroin or cocaine. *South Med J*, **77**, 302–3.

O'Brien, T. (1986). Resistance to antibiotics at medical centers in different parts of the world. *J Antimicrob Chemother*, **18 (suppl C)**, 243–253.

O'Brien, T. and the Members of Task Force 2 (1987). Resistance of bacteria to antibacterial agents: report of task force 2. *Rev Infect Dis*, **9**(S3), S244–S260.

Ojajärvi (1980). Effectiveness of hand washing and disinfection methods in removing transient bacteria after patient nursing. *J Hyg (Lond)*, **85**, 193–203.

O'Neill, P. and Roberts, G. (1999). Bayesian inference for partially observed stochastic epidemics. *J R Stat Soc A*, **162**, 121–129.

Panlilio, A., Culver, D., Gaynes, R., Benerjee, S., Henderson, T., Tolson, J., and Martone, W. (1992). Methicillin-resistant *Staphylococcus aureus* in US hospitals, 1975-1991. *Infect Control Hosp Epidemiol*, **13**, 582–586.

Parker, M. and Hewitt, J. (1970). Methicillin resistance in *Staphylococcus aureus*. *Lancet*, **1(7651)**, 800–804.

Parker, M., M., J., Emond, R., and Machacek, K. (1965). Acquisition of *Staphylococcus aureus* by patients in cubicles. *BMJ*, **1**, 1101–1105.

Peacock, J., Marsik, F., and Wenzel, R. (1980). Methicillin-resistant *Staphylococcus aureus*: introduction and spread with a hospital. *Ann Intern Med*, **93**, 526–532.

Pittet, D., Mourouga, P., and Perneger, T. (1999). Compliance with handwashing in a teaching hospital. *Ann Intern Med*, **130**, 126–130.

Plowman, R., Graves, N., Griffin, M., Roberts, J., Swan, A., Cookson, B., and Taylor, L. (1999). The socio-economic burder of hospital-acquired infection. Technical report, PHLS, London.

Poston, S., Glancey, G., Wyatt, J., Hogan, T., and Foster, T. (1993). Co-elimination of *mec* and *spa* genes in *Staphylococcus aureus* and the effect of *agr* and protein A production on bacterial adherence to cell monolayers. *J Med Microbiol*, **39**, 422–428.

Pratt, R., Pellowe, C., Loveday, H., and Robinson, N. (2000a). EPIC phase 1: The development of national evidence-based guidelines for preventing hospital-acquired infections in England: standard principles. Technical report, Thames Valley University, London.

Pratt, R., Pellowe, C., Loveday, H., and Robinson, N. e. (2000b). National evidence-based guidelines for preventing hospital-acquired infections (final draft). Technical report, Thames Valley University, London.

Press, W., Flannery, B., Teukolsky, S., and Vetterling, W. (1989). *Numerical Recipes in Pascal*. Cambridge University Press, Cambridge.

Price, D. and Sleigh, J. (1970). Control of infection due to *Klebsiella aerogenes* in a neurosurgical unit by withdrawal of all antibiotics. *Lancet*, **2(7685)**, 1213–1215.

Rafter, A. and Lewis, S. (1992). How many iterations in the Gibbs sampler? *Bayesian Statistics*, **4**, 763–773.

Renshaw, E. (1991). *Modelling biological populations in space and time*. Cambridge University Press, Cambridge.

Reybrouck, G. (1983). Role of the hands in the spread of nosocomial infections. *J Hosp Infect*, **4**, 103–110.

Ribble, J. (1967). A mechanism of bacterial interference *in vitro*. *J Immunol*, **98**, 716–723.

Ridley, M., Barrie, D., Lynn, R., and Stead, K. (1970). Antibiotic resistant *Staphylococcus aureus* and hospital antibiotic policies. *Lancet*, **1(7640)**, 230–233.

Riley, T. and Rouse, I. (1995). Methicillin-resistant *Staphylococcus aureus* in Western Australia, 1983-1992. *J Hosp Infect*, **29**, 117–188.

Ringertz, S., Olsson-Liljequist, B., Kahlmeter, G., and Kronvall, G. (1997). Antimicrobial susceptibility testing in Sweden. ii. species-related zone diameter breakpoints to avoid interpretive errors and guard against unrecognized evolution of resistance. *Scand J Infect Dis*, **Suppl 105**, 8–12.

Roberts, M. (1997). Genetic mobility and distribution of tetracycline resistance determinants. In *Antibiotic resistance: origins, evolution, selection and spread.*, pages 206–222. Ciba Foundation Symposium 207, Wiley, Chichester.

Rosdahl, V. and Knudsen, A. (1991). The decline on methicillin resistance among Danish *Staphylococcus aureus* strains. *Infect Control Hosp Epidemiol*, **12**, 83–88.

Rotter, M. (1997). 150 years of hand disinfection—Semmelweis' heritage. *Am J Epidemiol*, **22**, 332–39.

Samore, M., Venkataraman, L., DeGirolami, P., Arbeit, R., and Karchmer, A. (1996). Clinical and molecular epidemiology of sporadic and clustered cases of nosocomial *Clostridium difficile* diarrhea. *Am J Med*, **100**(1), 32–40.

Sanderson, P. and Weissler, S. (1992). Recovery of coliforms from the hands of nurses and patients: activities leading to contamination. *J Hosp Infect*, **21**, 85–93.

Schrag, S. and Perrot, V. (1996). Reducing antibiotic resistance. *Nature*, **381**, 120–121.

Sébille, V. and Valleron, A. (1997). A computer simulation model for the spread of nosocomial infections caused by multidrug-resistant pathogens. *Comput Biomed Res*, **30**, 307–322.

Sébille, V., Chevret, S., and Alain-Jacques, V. (1997). Modeling the spread of resistant nosocomial pathogens in an intensive-care unit. *Infect Control Hosp Epidemiol*, **18**, 84–92.

Selwyn, S. (1965). The mechanism of prevention of cross-infection in dermatological wards. *J Hyg (Cambridge)*, **63**, 59–71.

Shanson, D. (1981). Antibiotic-resistant *Staphylococcus aureus*. *J Hosp Infect*, **2**, 11–36.

Shanson, D. and Kensit, J. (1976). Outbreak of hospital infection with a strain of *Staphylococcus aureus* resistant to gentamicin and methicillin. *Lancet*, pages 1347–1348.

Shinefield, H., Ribble, J., Boris, M., and Eichenwald, H. (1963). Bacterial interference: its effect on nursery-acquired infection with *Staphylococcus aureus*. i. preliminary observations on artificial colonization in newborns. *Am J Dis Child*, **105**, 646–654.

Shinefield, H., Wilsey, J., Ribble, J., Boris, M., Eichenwald, H., and Dittmar, C. (1966). Interactions of staphylococcal colonization. *Am J Dis Child*, **111**, 11–21.

Shinefield, H., Ribble, J., Boris, M., Eichenwald, H., Aly, R., and Maibach, H. (1974). Bacterial interference between strains of *Staphylococcus aureus*. *Ann N Y Acad Sci*, **236**, 444–455.

Shooter, R., Thom, B., Dunkerley, D., Taylor, G., Parker, M. T., John, M., and Richards, I. (1963). Pre-operative segregation of patients in a surgical ward. *BMJ*, **1**, 1567–1569.

Silvestri, L., Monti Bragadin, C., Milanese, M., Gregori, D., Consales, C., Gullo, A., and van Saene, H. (1999). Are most ICU infections really nosocomial? A prospective observational cohort study in mechanically ventilated patients. *J Hosp Infect*, **42**, 125–133.

Sokal, R. and Rohlf, F. (1994). *Biometry*. W H Freeman, New York.

Spiegelhalter, D., Thomas, A., and Best, N. (1999). *WinBUGS Version 1.2 User Manual*. MRC Biostatistics Unit, Cambridge, England.

Sprunt, K. and Leidy, G. (1988). The use of bacterial interference to prevent infection. *Can J Microbiol*, **34**(3), 332–338.

Stamm, W., Weinstein, R., and Dixon, R. (1981). Comparison of endemic and epidemic nosocomial infections. *Am J Med*, **70**, 393–7.

Standing Medical Advisory Committee, sub-group on Antimicrobial Resistance (1998). The path of least resistance. Technical report, Department of Health, (UK), http://www.doh.gov.uk/smacful.htm.

Starr, J., Rogers, T., and Impallomeni, M. (1997). Hospital-acquired *Clostridium difficile* diarrhoea and herd immunity. *Lancet*, **349**, 426–428.

Steward, F., Antia, R., Levin, B., Lipsitch, M., and Mittler, J. (1998). The population genetics of antibiotic resistance ii: analytical theory for sustained populations of bacteria in a community of hosts. *Theor Popul Biol*, **53**, 152–165.

Talon, D., Rouget, C., Cailleaux, V., Bailly, P., Thouverez, M., Barale, F., and Michel-Briand, Y. (1995). Nasal carriage of *Staphylococcus aureus* and cross-contamination in a surgical intensive care unit: efficacy of mupirocin ointment. *J Hosp Infect*, **30**, 39–49.

Tenover, F., Arbeit, R., Archer, G., Biddle, J., Byrne, S., Goering, R., Hancock, G., Hebert, G., Hill, B., and Hollis, R. e. a. (1994). Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J Clin Microbiol*, **32**, 407–415.

The Hospital Infection Working Group of the DoH and PHLS (1995). Hospital infection control. Guidance on the control of infections in hospitals. Technical report, DoH/PHLS, London.

Thompson, R., Cabezudo, I., Wenzel, R., and Mandell, G. (1982). Epidemiology of nosocomial infections casued by methicillin-resistant *Staphylococcus aureus*. *Ann Intern Med*, **97**, 309–317.

Trzciński, K., van Leeuwen, W., van Belkum, A., Grzesiowski, P., Kluytmans, J., Sijmos, M., Verbrugh, H., Witte, W., and Hryniewicz, W. (1997). Two clones of methicillin-resistant *Staphylococcus aureus* in poland. *Clinical Microbiology and Infection*, **3**, 198–207.

Trzciński, K., Cooper, B., Hryniewicz, W., and Dowson, C. (2000). Expression of resistance to tetracyclines in methicillin-resistant *Staphylococcus aureus* strains. *J Antimicrob Chemother*, **45**, 763–770.

Uehara, Y., Nakama, H., Agematsu, K., Uchida, M., Kawakami, Y., Abdul Fattah, A., and Maruchi, N. (2000). Bacterial interference among nasal inhabitants: eradication of *Staphylococcus aureus* from nasal cavities by artificial implantation of corynebacterium sp. *J Hosp Infect*, **44**, 127–133.

Uhlén, M., Guss, B., Nilsson, B., Gatenbeck, S., Philipson, L., and Lindberg, M. (1984). Complete sequence of the staphylococcal gene encoding protein A. *J Biol Chem*, **259**, 1695–1702.

Ukoumunne, O., Gulliford, M., Chinn, S., Sterne, J., Burney, P., and Donner, A. (1999). Methods in health service research. Evaluation of health interventions at area and organisation level. *BMJ*, **319**, 376–379.

van Belkum, A., Riewerts Eriksen, N., Sijmons, M., van Leeuwen, W., van den Bergh, M., Kluytmans, J., Espersen, F., and Verbrugh, H. (1997). Coagulase and protein A polymorphisms do not contribute to persistence of nasal colonization by *S. aureus*,. *J Med Microbiol*, **46**, 222–232.

Van der Auwerea, P., Pensart, N., Korten, V., Murray, B., and Leclerq, R. (1996). Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly glycopeptide-resistant enterococci. *J Inf Dis*, **173**, 1129–1136.

van der Zwet, W., Parlevliet, G., Savelkoul, P., Stoof, J., Kaiser, A., Koelman, J., and Vandenbroucke-Grauls, C. (1999). Nosocomial outbreak of gentamicin-resistant *Klebsiella pneumoniae* in a neonatal intensive care unit controlled by a change in antibiotic policy. *J Hosp Infect*, **42**, 295–302.

Vicca, A. (1999). Nursing staff workload as a determinant of methicillin-resistant *Staphylococcus aureus* spread in an adult intensive therapy unit. *J Hosp Infect*, **43**, 109–113.

Voss, A., Milatovic, C., Wallrauch-Schwarz, C., Rosdahl, V., and Braveny, I. (1994). Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Inf Dis*, **13**, 50–55.

Waterloo Software (1996). *Maple 5*. Waterloo, Canada.

Weber, D. and Rutala, W. (1997). Role of environmental contamination in the transmission of vancomycin-resistant enterococci. *Infect Control Hosp Epidemiol*, **18**, 306–309.

Westh, H. and Rosdahl, V. (1989). Erythromycin resistance in Danish *Staphylococcus aureus* hospital strains with emphasis on erythromycin consumption. *APMIS*, **97**, 1121–1124.

Westh, H., Jensen, B., Rosdahl, V., and Prag, J. (1989). Development of erythromycin-resistance in *Staphylococcus aureus* as a consequence of high erythromycin consumption. *J Hosp Infect*, **14**, 107–115.

Westh, H., Jarlov, J., Kjersem, H., and Rosdahl, V. (1992). The disappearance of multiresistant *Staphylococcus aureus* in Denmark: changes in strains of the 83a complex between 1969 and 1989. *Clin Infect Dis*, **14**, 1196–1194.

Westh, H., Elsberg, C., and Rosdahl, V. (1998). Antibiotic consumption and level of resistance in *Staphylococcus aureus* in 15 hospitals world wide. *J Hosp Infect*, **40**, P5.1.4.

White, A. and Smith, J. (1964). Nasal reservoir as the source of extranasal staphylococci. *Antimicrob Agents Chemother*, pages 679–683.

Williams, R. (1963). Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriological Reviews*, **27**, 56–71.

Williams, R., Jevons, M. P., Shooter, R., Hunter, C., Girling, J., Griffiths, J., and Taylor, G. (1959). Nasal staphylococci and sepsis in hospital patients. *BMJ*, **ii**, 658–662.

Witte, W., Braulke, C., D., H., and Cuny, C. (1994). Analysis of nosocomial outbreaks with multiply and methicillin resistant *Staphylococcus aureus* (MRSA) in Germany: impplications for hospital hygiene. *Infection*, **22**(Suppl 2), s128–s134.

Wolinsky, E., Lipsitz, P., Mortimer, E., and Rammelkamp, C. (1960). Acquisition of staphylococci by newborns: direct versus indirect transmission. *Lancet*, **2**, 620–622.

Wong, E. (2000). The epidemiology of contact transmission: beyond Semmelweis. *Infect Control Hosp Epidemiol*, **21**, 77–79.

Working Party Report (1998). Revised guidelines for the control of methicillin-resistant *Staphylococcus aureus* infections in hospitals. *J Hosp Infect*, **39**, 253–290.

World Health Organization (1997). Resistance to antimicrobial agents. *Weekly Epidemiological Record*, **72**, 333–340.

Zimmerman, T. (1996). Personal area networks: near-field intrabody communication. *IBM Systems Journal*, **35**, 609–617.

Appendix A

Data capture forms for observational study

Form CR1. Contact Recording Form

Date:.....Start time:..... Stop time:..... Beds being observed:.....

Time	Bed No.	Carer No.	Event detail (0 = observed ; G = guessed)	Hand decontam. B	Hand decontam. A	Contact Type[2]	Gloves ✓ or × if used	Apron ✓ or × if used	Finger swab id (if done)

[2] Contact types: 1 = simmple touch; 2 = extensive touch; 3 = invasive/semi-invasive touch..

Form C1. Shift Details

Date:.....

First shift	Name	ID	Assigned to which beds/patients	Team	Date of last swab
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					

Second shift	Name	ID	Assigned to which beds/patients	Team	Date of last swab
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					

Night shift	Name	ID	Assigned to which beds/patients	Team	Date of last swab
1.					
2.					
3.					

Form P1. Patient Details

The following info will be recorded when each patient enters the ward, or at the start of the study for patients already present.

Surname: .....

First name: : .....

Hospital number : .....

Initial bed number: .....

Named nurse:.....

Consultant.....

Sex: .....

Date of birth:.....

Reason for hospitalisation: .....

Date admitted to ward: .....

Time admitted to ward: .....

Date of expected discharge from ward.....

Where patient admitted to ward from (e.g. another ward, nursing home, home):

.....

If transferred from another ward

Name of previous wards: .....

Date admitted to this hospital: ... .

Dates of last period of hospitalisation. From..... To: .....

Was there a hospital-acquired infection during last hospitalisation? (YES/NO).....
If YES, give details .....

Total number of days in hospital in last 12 months:.....

Consent for study obtained (YES/NO) .....

Today's date:.....

Notes:

Form P2. Daily Patient Details

PatientID: .....

Intended Discharge date:..... Reason for delay (if any):.....

Actual Discharge date:..... .....

Start date of information collection.....

Wound site: 1). 2). 3). 4).

Wound status:[1]:

Date							
Wound 1							
Wound 2							
Wound 3							
Wound 4							

Wound swabs (wound numbers and dates):

Operation date:.....What was done:.....

Lines

Type	Start date	Stop date	Notes

Observations :

[1] Wound status codes: A = wound clean and healing; B = suspicion of infection; C= Definite Infection.; D=Dressings not to be removed while patient on ward; E = healed.

Daily Patient Details ...continued PatientID: .....

Regular drugs including fluids and pumps

Drugs being taken	1.	2.	3.	4.
Route				
Start date				
Stop date				

Drugs being taken	5.	6.	7.	8.
Route				
Start date				
Stop date				

Durgs "as required" or once only

Changes to above data or extra information