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Bacterial Load and Molecular Markers Associated with Early-Onset Group B *Streptococcus.*

A Systematic Review and Meta-Analysis

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Background: The natural history of neonatal group B *Streptococcus* (GBS) is poorly understood. Little is known about the bacterial factors influencing the transmission of GBS from mother to neonate, or the development of invasive early-onset GBS disease (EOGBS) in colonized neonates. We reviewed whether bacterial load and molecular markers are associated with GBS vertical transmission and progression to EOGBS.

Methods: We searched Medline, Embase, Cochrane and Web of Science from inception to 10th October 2016 for observational studies in English. We also hand-searched reference lists of relevant publications and experts cross-checked included studies. Two reviewers independently screened studies, extracted data and appraised the quality of included studies using the QUIPS tool. We conducted random-effects meta-analyses where possible and narratively synthesized the evidence in text and tables.

Results: Seventeen studies were included from 1,107 records retrieved from electronic databases and publication references. Meta-analyses of three studies showed that neonates colonized by serotype III had a higher risk of developing EOGBS than serotype Ia (pooled risk ratio [RR] = 1.51, 95% confidence interval [CI] 1.12 to 2.03) and serotype II (RR = 1.95, 95% CI 1.10 to 3.45). Eleven studies showed that in heavily colonized mothers 2 to 3 times more neonates were colonized, and in heavily colonized neonates up to 15 times more neonates had EOGBS, compared to light colonization. Most evidence was pre-2000 and at risk of bias.

Conclusion: Acknowledging the difficulty of natural history studies, well-controlled studies are needed to assess the predictive value of pathogen subtype and heavy load; they may be useful for better-targeted prevention.

Keywords: *Streptococcus agalactiae*, bacterial load, molecular markers, systematic review, transmission

Introduction

Group B *Streptococcus* (GBS) is the leading cause of morbidity and mortality from neonatal sepsis.[1] Early-onset GBS disease (EOGBS, first six days of life) has a global estimated incidence of 0.4 per 1000 live births and a case fatality rate of 12.1%, although this incidence is likely to be an underestimate.[2] A precondition for EOGBS is maternal GBS colonization of the gastrointestinal and/or genitourinary tract. A meta-analysis found that GBS colonizes approximately 17.9% of women globally, from 11.1% in Southeast Asia to 22.4% in Americas.[3] If a woman has GBS vaginal colonization during labor, there is approximately a 36% chance that GBS might be transmitted to her neonate.[4] Without treatment, most neonates colonized with GBS will be asymptomatic, but a small proportion (around 1%) will have EOGBS.[5]

The natural history of GBS disease is poorly understood. There is a paucity of data on the pathogen-specific factors influencing the transmission of GBS colonization from mother to neonate or the development of invasive EOGBS in colonized neonates. Of 10 GBS polysaccharide capsule types, serotype Ia, Ib, II, III and V are more commonly responsible for EOGBS.[5-7] A number of virulence factors, such as clonal complexes and surface proteins have also been proposed in laboratory and clinico-epidemiologic studies,[8-10] and maternal bacterial load has been associated with increased neonatal colonization and sepsis.[11]

Data on the GBS characteristics that increase the risk of neonatal colonization and EOGBS may have important implications for targeting intrapartum antibiotic prophylaxis (IAP) prevention to only those women at most risk of having a baby with EOGBS. This may reduce exposure to the potential harms associated with IAP, such as antimicrobial resistance and Gram negative infections as a result of selection pressure and mutations of the organisms causing infection.[12, 13] Therefore, we systematically reviewed the evidence on the bacterial load and bacterial molecular

markers associated with GBS vertical transmission, and progression from neonatal GBS colonization to EOGBS.

Materials and Methods

This systematic review is reported according to recommendations from the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) 2015 statement.[14] The protocol is registered at the International Prospective Register of Systematic Reviews (PROSPERO): CRD42016037196.

Search strategy and selection criteria

We conducted electronic searches in MEDLINE, MEDLINE In-Process & Other Non-Indexed Citations, EMBASE, Cochrane Library: Cochrane Database of Systematic Reviews, CENTRAL, DARE and HTA databases and Science Citation Index Expanded from inception to 10th October 2016. The search combined both text words and MeSH terms for GBS, neonate/pregnancy and bacterial load/molecular markers, and was limited to English and humans (Supplemental Digital Content 1, http://links.lww.com/INF/D106). We hand-searched reference lists of included studies and relevant systematic reviews and experts cross-checked included studies.

Two reviewers independently screened titles, abstracts and full texts of all identified records. Any disagreements were resolved by discussion, with involvement of a third reviewer if necessary. We included cohort or case-control studies that evaluated the association between bacterial load or any individual molecular marker with the transition of GBS from (a) maternal colonization in the third trimester to neonatal colonization or EOGBS (as defined by authors, ideally confirmed by culture from a sterile site; fewer than seven days), (b) maternal colonization in labor to neonatal colonization or EOGBS or (c) neonatal colonization to EOGBS. We excluded studies in which more than 10% participants were pregnant women before the third

trimester [for study objective (a) above] or neonates who had late-onset GBS. However, we included any studies where the data for mothers in the third trimester or for neonates less than seven days of age could be separated from the other participants regardless of the percentage of total participants that met the exclusion criteria. We also excluded studies in which participants received an intervention that would interfere with GBS transmission, such as IAP treatment or elective caesarean section delivery as well as any studies that were conducted in the context of IAP treatments. Finally, we excluded case reports, case series, abstracts, reviews, editorials, letters, books, consensus statements and opinions.

Data extraction and quality assessment

Two reviewers independently extracted relevant data on an *a priori* defined and piloted extraction sheet. Data included study settings, participants, bacterial factors, outcomes and results. Two authors independently appraised the risk of bias of included studies using the Quality in Prognosis Studies (QUIPS) tool, judging six risk of bias domains as low, moderate or high.[15] Any disagreements were resolved by discussion, with involvement of a third reviewer if necessary.

Data synthesis

All analyses were conducted in Stata 14 (Stata Corp, College Station, Texas). Where data permitted, we calculated odds ratios (ORs) for case-control studies and risk ratios (RRs) for all other designs, along with 95% confidence intervals (CIs). We only conducted meta-analyses on the serotypes associated with progression from neonatal GBS colonization to EOGBS because of heterogeneity in the studies on the remaining factors. We used a random effects model due to anticipated between-study differences.[16] As only raw numbers and proportions were reported in the studies and summary measures such as RRs were not, we calculated the RRs and 95% CIs for

each study and pooled them using STATA command *metan*. Heterogeneity was assessed using forest plots, the chi-squared test for heterogeneity with a 10% level of statistical significance and the I^2 statistic where a value of less than 50% represents low to moderate heterogeneity.[17] Comparisons were only made for serotypes included in at least two studies. For the remaining studies, we conducted narrative syntheses and displayed results in tables and texts.

Results

Our search identified 1,107 unique records, of which 17 articles were included in the synthesis (see Figure 1 and supplementary material, http://links.lww.com/INF/D106).[7, 18-33] Study designs, bacterial factors, populations and definitions of GBS colonization and EOGBS differed between studies (see Table 1). Most studies were cohort, with two case-control studies[21, 31] and one secondary analysis of a control group in a randomized controlled trial.[28] Nine studies were on vertical transmission of GBS colonisation,[18, 20, 23, 25-30] five on maternal colonization to EOGBS[20, 26-28, 30] and eight on neonatal GBS colonization to EOGBS.[7, 19, 21, 22, 24, 31-33] Thirteen studies were conducted before 1990,[19, 20, 22-29, 31-33] two during the 1990s[21, 30] and two after 2000[7, 18] possibly as a result of the widespread use of IAP inhibiting natural history studies. Six studies investigated the association of serotype,[7, 18, 19, 21, 31, 32] 11 investigated bacterial load[20, 22-30, 33] and one investigated C-protein antigen.[21]

Risk of bias

Figure 2 shows the methodological quality of included studies. Risk of bias was considered high in two or more QUIPS domains in 10 of 17 studies (59%), and in one domain in 4 of 17 studies (24%). No study was judged as low risk of bias in all six domains. The study confounding domain had the highest risk of bias, as important potential confounders such as gestational age at birth, birth weight, intrapartum fever and prolonged rupture of membranes were not accounted

for in 76% of study designs (13/17, high risk).[7, 18, 20-26, 28, 29, 32, 33] The remaining four accounted for some, but not all, relevant confounders (moderate risk).[19, 27, 30, 31] In the study participation domain, nine studies (53%)[18-23, 25, 26, 33] were at high risk and the remaining eight were at moderate risk of selection bias[7, 24, 27-32] as baseline characteristics were not adequately described and/or recruitment methods were not fully stated.

Serotypes

Information on serotypes associated with GBS transmission from mother to neonate was available from one study. Al-Sweih et al. (2005)[18] found that mothers colonized with serotypes V (13/27, 48%) and Ia (5/11, 45%) on vaginal-anorectal swabs were more likely to transmit GBS than mothers colonized with Ib (1/3, 33%), III (11/33, 33%), serotypes not typeable (7/22, 32%), and the remaining serotypes.

Information on serotypes associated with progression from GBS neonatal colonization to EOGBS was available in five studies.[7, 19, 21, 31, 32] Meta-analyses could only be performed on three studies,[7, 21, 32] as the required data were not available in the others. Of the omitted studies, Baker et al. (1973)[19] reported that serotype III was more frequently present in EOGBS cases (56%) than in asymptomatic colonization (36%). However, in this study, the number of participants in the asymptomatic GBS colonization group was inconsistently reported, therefore, the number of participants with each serotype could not be calculated. Similarly, Baker et al. (1974)[31] inconsistently reported the number of individuals with GBS sepsis, so the numbers could not be calculated from this study either.

The pooled RRs from the meta-analyses for EOGBS in neonates colonized by comparisons of GBS serotypes are shown in Figure 3. Neonates colonized by serotype III had a higher risk of developing EOGBS than neonates colonized by serotype Ia (pooled RR = 1.51, 95% CI 1.12 to

2.03, three studies, 439 neonates). Among 261 neonates colonized by serotype III, 98 (37.5%) developed EOGBS compared with 45 of 178 (25.3%) colonized by serotype Ia. Similarly, neonates colonized by serotype III were twice as likely to have developed EOGBS than neonates colonized by serotype II (pooled RR = 1.95, 95% CI 1.10 to 3.45, three studies, 355 neonates). Among 261 neonates colonized by serotype III, 98 (37.5%) developed EOGBS compared with 19 of 94 (20.2%) colonized by serotype II. The forest plots for each comparison are presented in Supplementary Figure 1, http://links.lww.com/INF/D106. For the statistically significant serotype III comparisons, the forest plots show that the data from Madzivhandila et al. (2011)[7] may have had considerable influence on the results.

Bacterial load

Eleven studies investigated bacterial load, and although they defined load differently, studies agreed that heavy maternal load was more strongly associated with GBS transmission, and heavy neonatal load more strongly associated with EOGBS, compared with light load (see Table 2).[20, 22-30, 33] Three studies reported the number of colonized sites.[22, 25, 33] Hoogkamp-Korstanje et al. (1982)[25] found that women colonized in two or more sites compared to one site only were two and a half times more likely to have a neonate colonized with GBS (91% versus 36%, RR calculated from percentages = 2.53, 95% CI 1.93 to 3.31). Sites swabbed included throat, nose, vagina, cervix, rectum and midstream urine in labor. Similarly, two studies found up to a 15 times higher risk of EOGBS in neonates with three to four colonized sites as compared to one to two colonized sites (see Table 2 for results).[22, 33] Sites reported in these studies were external ear canal, umbilicus, oropharynx and rectum within an hour of birth,[22] and external canal, umbilicus, throat and anus within one to two hours of birth.[33]

Three studies reported the number of colony counts on a plate.[23-25] Hoogkamp-Korstanje et al. (1982)[25] found that heavy maternal colonization (>50 colonies, 87% transmission rate) in labor was associated with GBS transmission more often than light (<10 colonies, 30% transmission rate) or moderate colonization (10-50 colonies, 50% transmission rate). Gerards et al. (1985)[24] combined the number of sites with the number of colony counts, finding that neonates colonized in three or more sites with >50 colonies (heavy, 4/8, 50% transmission rate) were more likely to have EOGBS than neonates with fewer than three sites with >50 colonies, three or more sites with <10 or 10-50 colonies (moderate, 15/35, 42.9% transmission rate) or fewer than three sites with <10 or 10-50 colonies (light, 2/44, 4.5% transmission rate). Sites swabbed were nose, throat, external auditory meatus, eyes, umbilicus, skin and rectum immediately after admission to NICU. Easmon et al. (1985)[23] also reported the number of colonies in mothers' vaginas and rectum, however, conclusions could not be drawn as data labelling in their report was unclear. Three studies investigated colony-forming units (cfu) of GBS finding that the risk of vertical GBS transmission and EOGBS increases with cfu of GBS.[26, 29, 30] Jones et al. (1994)[26] found a statistically significant linear correlation ($p<0.001$) between the cfu of GBS in mothers' vaginas during delivery and neonates' rectums at birth, but a poor correlation between cfu of GBS in infant umbilical or nasopharyngeal culture with that found in the mother's vagina. They also found that mothers' swabs had to contain at least 10^2 GBS before their neonate's swab yielded a positive result, and that neonates colonized with $\geq 10^5$ GBS per rectal swab were delivered by mothers colonized with $\geq 3 \times 10^4$ GBS per vaginal swab. Three infants developed EOGBS; two had blood culture positive sepsis and one had a positive rectal culture with respiratory distress. All three infants had mothers who were heavily colonized with GBS during delivery (7.70×10^6, 6.62×10^7, 2.5×10^6). However, only two of the infants were heavily colonized (7.02×10^5,

$5.25x10^6$); one infant with blood culture positive sepsis was lightly colonized ($<10^1$). Authors noted that this infant might have been cleaned before culture.[26] Sensini et al. (1997)[30] found that mothers with $\geq 10^6$ cfu/GBS ml at the time of delivery were more likely to transmit GBS to their neonates than mothers with 10^2 to 10^6 cfu/GBS ml (74/148 [50%] versus 34/112 [30%] RR = 1.65, 95% CI 1.19 to 2.28). One neonate developed EOGBS whose mother had light colonization. Persson et al. (1986)[29] investigated cfu/GBS ml in the mothers' urine during delivery finding that those with $\geq 10^4$ cfu/GBS ml were six times more likely to transmit GBS to their neonates compared to mothers with $<10^4$ cfu/GBS ml (6/9 [67%] versus 6/55 [11%] RR = 6.11, 95% CI 2.52 to 14.81).

Morales et al. (1986, 1987)[27, 28] investigated bacterial load in mothers by a rapid slide coagglutination test and found that mothers with heavy colonization in labor (GBS antigens detectable within five hours) were twice as likely to transmit GBS to their term neonates (24/30 [80%] versus 35/98 [36%] RR = 2.24, 95% CI 1.63 to 3.09), and three times more likely to transmit GBS to their pre-term neonates (8/11 [73%] versus 9/37 [24%] RR = 2.99, 95% CI 1.52 to 5.87) than mothers with light colonization (agglutination negative at five hours but positive at 20 hours). They found three cases of term GBS sepsis, all in heavily colonized mothers, and pre-term GBS sepsis that was four times more likely in heavily compared to lightly colonized mothers (7/11 [64%] versus 6/37 [16%] RR = 3.92, 95% CI 1.66 to 9.25). Finally, Boyer et al. (1983)[20] found that neonatal colonization was 3.29 times more likely in heavily colonized mothers (intrapartum vaginal culture positive on direct plate as well as selective culture) compared to light (intrapartum vaginal culture negative but postpartum rectal or vaginal culture positive) or moderate colonization (intrapartum vaginal culture positive on selective culture) during labor (69/107 [64%] versus 20/102 [20%] RR = 3.29, 95% CI 2.17 to 4.99). Of the

women who transmitted GBS to their infants, heavily colonized women were more likely to have neonates colonized at multiple sites (55%) compared to moderate or light colonization (30%, p=0.04).[20] Sites included throat, umbilicus, rectum, external ear and nasogastric aspirate. Four neonates developed EOGBS, all in heavily colonized mothers.[20]

C-protein antigen

Chun et al. (1991)[21] examined whether asymptomatic GBS and EOGBS strains reacted to C-protein antiserum and four antigens – α, β, γ, δ. They found that GBS isolates in 87% (41/47) of neonates with EOGBS and 73% (54/74) of asymptomatically colonized individuals reacted to C-protein antiserum; this difference was not statistically significant. When comparing the distribution of the four C protein-associated antigens, antigen δ was expressed more often in isolates from neonates with EOGBS (12/41, 29%) than in asymptomatic neonates (10/54, 19%). The remaining antigens were present less often in EOGBS (α = 28/41, 68%, β= 7/41, 17%, and γ= 15/41, 36.5%) than in healthy neonates (α = 44/54, 81%, β= 15/54, 28%, and γ= 20/54, 37%). Summary measures were not calculated as more than one antigen can be expressed in one strain.

Discussion

This is the first systematic review investigating bacterial load and molecular markers associated with GBS vertical transmission, or progression from neonatal coloniation to EOGBS. Our findings suggest that the epidemiology and natural history of neonatal GBS has not been extensively researched. Only two bacterial markers have been investigated in addition to bacterial load, and most of the evidence is published before 2000 and at high risk of bias. While IAP can reduce EOGBS morbidity,[34] there are potential harms associated with it,[35] and in current prevention approaches some mothers and their neonates may be unnecessarily treated and exposed to these potential harms. For example, in a screening program, up to 30% of GBS

positive pregnant women may become negative by birth,[24] and only 1% of GBS positive women in labor will have a baby with EOGBS;[5] all of these women and their neonates would be unnecessarily treated and potentially exposed to the harms. Similarly, approximately 5% of GBS negative pregnant women may become positive by birth and would not be treated. Bacterial factors could provide innovative opportunities for more efficient prevention, allowing patients with the hypervirulent strains of GBS to be targeted, avoiding unnecessary exposure to IAP and reducing potential under-treatment. Bacterial load is the most promising of the factors, as irrespective of how it was defined and measured, heavier load was consistently associated with GBS transmission and EOGBS. Women colonized with heavy GBS load had approximately two to three times higher risk of having a neonate colonized with GBS compared to mothers with light load. Heavier GBS load in neonates was also consistently associated with EOGBS. The pooled comparison of serotypes in GBS colonized neonates showed that the risk of EOGBS disease was highest for neonates colonized with serotype III.

Previous literature shows that serotype III, along with Ia, Ib, II and V, is one of the most frequently identified invasive neonatal serotypes.[2, 5-7] Our review showed that compared to Ia and II, serotype III is more often associated with invasive EOGBS. Contrary to expectations, we found no evidence of a difference between non-typeable and other serotypes. When comparing colonized mothers to EOGBS cases, Fabrini et al. (2016), for example, found no cases of EOGBS in neonates with a non-typeable serotype compared with 8% of colonized mothers who had a non-typeable serotype.[36] We may not have found this difference in our review as there were only 23 neonates colonized with a non-typeable serotype in the meta-analysis. Within serotype III, a study excluded from this review due to the context of IAP found that ST-17 was the most common sequence type amongst invasive serotype III strains.[37] Laboratory experiments

have demonstrated that a determinant of this hypervirulence is a ST-17 specific surface protein, which promotes attachment to intestinal and meningeal cells.[38] ST-17 is also more likely to invade decidual cells than colonizing strains.[39]

The finding that heavy bacterial load is consistently associated with GBS vertical transmission and EOGBS is in line with evidence that women with GBS bacteriuria (a surrogate for heavy maternal colonization) have a higher risk of delivering neonates who develop EOGBS.[40, 41] There is also more recent evidence (excluded as the study was conducted in the context of IAP) showing that heavy neonatal colonization as defined by the number of sites is more strongly associated with EOGBS than light load (25/1000 versus 4/1000 respectively, $p<0.001$).[42] In contrast, evidence on the association directly between maternal load and EOGBS was slightly unclear, possibly due to the small numbers of EOGBS in such studies. We were only able to perform analyses on one study, where pre-term EOGBS was almost four times more likely in infants with heavily colonized mothers.[27]

Several limitations of the evidence should be considered. The risk of bias across the evidence was high or moderate, especially regarding confounding variables and study participation domains. Furthermore, we calculated the point and interval estimates (RRs, ORs and 95% CIs) reported in this review using unadjusted statistical analyses which did not control for potential confounders. Therefore, the identified relationships could be partially or entirely due to confounding factors. Majority of the evidence is also published before 2000 and may not be applicable to today's context. For example, the association of serotypes with invasive disease may be influenced by circulating strains or clones rather than serotype alone, and these associations may change over time. To fully understand the mechanisms of virulent GBS types, and to confirm that bacterial load is independently associated with EOGBS, larger and better-

controlled studies are required. We acknowledge that such a study may no longer be feasible as IAP is now the recommended treatment. However, it may be possible to conduct a prospective cohort study in contexts where IAP prevention is not adopted, for example, in countries in Africa or Asia. Alternatively, it might be possible to conduct a retrospective cohort study on culture positive mothers who did not end up being treated in screening programs across countries. Clinical studies are required to confirm findings on other virulence factors indicated from laboratory studies,[8, 9] as they are not yet available.

There are also some limitations of our review. Studies in which participants were given IAP were excluded, as IAP would interfere with the natural history of GBS transmission and progression to EOGBS. This may have resulted in the exclusion of more recent studies, as it may be less feasible to conduct studies on untreated women only. As such, it may be worth systematically reviewing whether serotype, bacterial load and other factors predict risk of transmission and EOGBS in the presence of IAP. As non-English studies were also excluded, prognostic studies in other languages may have been missed.

Conclusions

While IAP treatment can reduce EOGBS morbidity,[34] the persistence of EOGBS combined with the potential harms from IAP stress the need for better targeted prevention and therapy. Bacterial load, serotype, sequence type and the more specific isolate characterization feasible with the advent of genome sequencing, could potentially be involved in guiding future prevention interventions. There is good evidence to further investigate serotype, and particularly bacterial load, in better quality studies. Beyond these factors, greater insights into the mechanisms which underlie the natural history of GBS vertical transmission and EOGBS are essential for the development of new interventions to prevent EOGBS.

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Figure legends

Figure 1: Flow diagram of study selection

See supplementary information for list of excluded studies with reasons

Figure 2: Risk of bias across included studies, according to the QUIPS tool[15]

Figure 3. Pooled relative risk of EOGBS by colonising GBS serotypes in neonates

Comparisons should be read from right to left. The pooled estimate is located at the intersection of the row-defining serotype and column-defining serotype. A RR value greater than 1 means higher risk of early-onset group B *Streptococcus* disease (EOGBS) in neonates colonised by the row-defining serotype. For example, neonates colonised by serotype III had a higher risk of developing EOGBS than neonates colonised by serotype Ia (pooled RR = 1.51, 95% CI 1.12 to 2.03). RRs for comparisons in the opposing direction can be established by using reciprocals. Significant results are in bold and underlined.

Table 1: Characteristics of included studies

Study ID Country	**Study design**	**Risk factors**	**GBS natural history pathway**	**Participants and GBS culture method**	**Outcome, definition and measurement**
Al-Sweih 2005[18] Kuwait	Prospective cohort study	Serotype	Maternal colonisation to neonatal colonisation	124 women colonised with GBS on vaginal-anorectal swabs in labour (selective culture)	74 neonates colonised with GBS on external ear canal and umbilicus swabs at unspecified time (selective culture)
Baker 1973[19] USA	Prospective cohort study	Serotype	Neonatal colonisation to EOGBS disease	66 neonates colonised with GBS on throat and umbilical or external auditory canal swabs at mean age of 13.8 hours (selective culture)	12/13 neonates with bacteriologically confirmed EOGBS disease ≤10 days (all infants developed symptoms in the first five days of life)

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
Baker 1974[31] USA	Case-control study	Serotype	Neonatal colonisation to EOGBS disease	53 neonates colonised with GBS on throat, umbilical cord or ear swabs at <3 days	Unknown number of neonates with EOGBS sepsis or pneumonia: clinical symptoms and pre-mortem blood cultures or post-mortem heart and lung cultures in neonates with pneumonia 15 neonates with EOGBS meningitis: CSF culture ≤5 days
Chun 1991[21] USA	Case-control study	Serotype, Reaction to c-protein	Neonatal colonisation to EOGBS disease	121 neonates colonised with GBS at birth on nasopharynx,	47 neonates with EOGBS sepsis: Blood and CSF culture <7 days

Study ID Country	**Study design**	**Risk factors**	**GBS natural history pathway**	**Participants and GBS culture method**	**Outcome, definition and measurement**
		and c-protein ß antigen gene		throat, umbilicus or rectum swabs	
Embil 1987[32] Canada	Prospective cohort study	Serotype	Neonatal colonisation to EOGBS disease	55 strains from 54 neonates colonised with GBS on rectal swabs within 1 hour of birth (selective culture)	12 neonates with symptomatic EOGBS <3 days
Madzivhandila 2011[7] South Africa	Prospective cohort study	Serotype	Neonatal colonisation to EOGBS disease	525 neonatal isolates colonised with GBS on ears, nose and umbilicus swabs shortly after birth (standard	136 neonates with EOGBS: Blood and CSF culture <7 days

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
				culture)	
Hoogkamp 1982[25] Netherlands	Prospective cohort study	Bacterial load - number of positive sites, number of colony counts per plate	Maternal colonisation to neonatal colonisation	46 women colonised with GBS on throat, nose, vagina, cervix, rectum and midstream urine swabs in labour (selective culture)	Unknown number of neonates colonised with GBS on skin, throat, external ears and umbilicus swabs at <6 hours of birth (selective swab)
Dillon 1987[22] USA	Prospective cohort study	Bacterial load - number of positive sites	Neonatal colonisation to EOGBS	1448 neonates colonised with GBS on external ear canal, umbilicus, oropharynx and rectum swabs within 1 hour of birth (selective culture)	24 neonates with EOGBS: Symptoms and blood, CSF, urine and other clinical specimens <3 days

Study ID Country	**Study design**	**Risk factors**	**GBS natural history pathway**	**Participants and GBS culture method**	**Outcome, definition and measurement**
Pass 1979[33] USA	Prospective cohort study	Bacterial load - number of positive sites	Neonatal colonisation to EOGBS	290 neonates colonised with GBS on external canal, umbilicus, throat and anus swabs 1-2 hours after birth (selective culture)	8 neonates with EOGBS: Blood and CSF culture
Easmon 1985[23] England	Prospective cohort study	Bacterial load - number of colony counts per plate	Maternal colonisation to neonatal colonisation	140 women colonised with GBS on vaginal swabs in labour (selective and standard culture)	38 neonates colonised with GBS on rectum, umbilicus, ear and external nares swabs within 24 hours of birth and/or on discharge from hospital (selective culture)

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
				141 women colonised with GBS on rectal swabs in labour (selective and standard culture)	39 neonates colonised with GBS on rectum, umbilicus, ear and external nares swabs within 24 hours of birth and/or on discharge from hospital (selective culture)
Gerards 1985[24] Netherlands	Prospective cohort study	Bacterial load - number of colony counts per plate	Neonatal colonisation to EOGBS	68 neonates colonised with GBS on nose, throat, external auditory meatus, eyes, umbilicus, skin and rectum swabs immediately	21 neonates with EOGBS: Sepsis symptoms with GBS cultured from normally sterile culture <7 days

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
				after admission to NICU (selective culture)	
			Neonatal colonisation to probable GBS	66 neonates colonised with GBS on nose, throat, external auditory meatus, eyes, umbilicus, skin and rectum swabs immediately after admission to NICU (selective culture)	19 probable sepsis: Symptoms with nose, throat, external auditory meatus, eyes, umbilicus, skin and rectum swans but no culture from sterile site
Jones 1984[26] USA	Prospective cohort study	Bacterial load - Colony-	Maternal colonisation to neonatal	130 women colonised with GBS on vaginal	61 neonates colonised with GBS on rectum,

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
		forming units (cfu) per ml	colonisation	swabs at labour (selective culture)	nasopharynx, and umbilicus swabs at birth (selective culture)
			Maternal colonisation to EOGBS		2 neonates with EOGBS: blood culture positive 1 neonate with probable EOGBS: symptoms and surface culture positive but not blood culture positive
Persson 1986[29] Sweden	Secondary analysis combined with a prospective cohort	Bacterial load - Colony-forming units (cfu) per ml	Maternal colonisation to neonatal colonisation	64 women colonised with GBS on urine swab in labour (selective culture)	12 neonates colonised with GBS on rectal swabs <5 days (selective culture)

Study ID Country	**Study design**	**Risk factors**	**GBS natural history pathway**	**Participants and GBS culture method**	**Outcome, definition and measurement**
	study				
Sensini 1997[30] Italy	Prospective cohort study	Bacterial load - Colony-forming units (cfu) per ml	Maternal colonisation to neonatal colonisation	260 women colonised with GBS on lower vaginal swabs in labour (selective culture)	108 neonates colonised with GBS on auricular, pharyngeal and gastric aspirate swabs before first bath (selective culture)
		Bacterial load - Colony-forming units (cfu) per ml	Maternal colonisation to EOGBS		1 neonate with EOGBS sepsis: Blood culture and sepsis symptoms <24 hours

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
Boyer 1983[20] USA	Prospective cohort study	Bacterial load - other	Maternal colonisation to neonatal colonisation	207 women colonised with GBS on vaginal swabs in labour who gave birth to 209 neonates (selective culture)	89 neonates colonised with GBS on throat, umbilicus, rectum, external ear and nasogastric aspirate swabs in the delivery room
			Maternal colonisation to EOGBS		4 neonates with EOGBS (definition not stated)
Morales 1986 USA[28]	Untreated control group of RCT	Bacterial load - other	Maternal colonisation to neonatal colonisation	128 women colonised with GBS at labour identified by a rapid slide coagglutination test on selective vaginal culture	59 term neonates colonised with GBS on oropharynx and the skin swabs at delivery and a urine latex-agglutination

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
			Maternal colonisation to EOGBS		3 term neonates with GBS sepsis: Positive body fluid
Morales 1987[27] USA	Prospective cohort study	Bacterial load - other	Maternal colonisation to neonatal colonisation	48 women colonised with GBS in labour identified by latex agglutination on selective vaginal culture	17 pre-term neonates colonised with GBS detected by urine latex-agglutination after delivery
			Maternal colonisation to EOGBS		13 pre-term neonates with GBS sepsis: Blood, CSF, or urine culture, and oropharynx cultures with radiographic and clinical signs of

Study ID Country	Study design	Risk factors	GBS natural history pathway	Participants and GBS culture method	Outcome, definition and measurement
					infection

CSF cerebrospinal fluid, EOGBS early-onset GBS disease, GBS group B *Streptococcus*

Table 2: Statistical findings of the association between bacterial load and neonatal GBS

Study ID Country	Outcome	Bacterial load definition	Number/ % without outcome	Number/ % with outcome	Summary measure (95% CI)	Covariates adjusted for
Number of positive sites						
Hoogkamp 1982[25] Netherlands	Neonatal colonisation	Maternal colonisation Light: 1 site Heavy ≥2 sites	 64% 9%	 36% 91%	*RR of heavy: 2.53 (1.93-3.31) (calculated from %)*	None
Dillon 1987[22] USA	EOGBS	Neonatal colonisation Light: 1-2 sites Heavy: 3-4 sites	 1041 383	 4 20	*RR of heavy: 12.97 (4.46- 37.70)*	None
Pass 1979[33]	EOGBS	Neonatal colonisation			*RR of heavy: 15.31*	None

Study ID **Country**	**Outcome**	**Bacterial load definition**	**Number/ % without outcome**	**Number/ % with outcome**	**Summary measure (95% CI)**	**Covariates adjusted for**
USA		Light: 1-2 sites Heavy: 3-4 sites	198 84	1 7	*(1.91- 122.60)*	
Number of colony counts per plate						
Hoogkamp 1982[25] Netherlands	Neonatal colonisation	Maternal colonisation Light: <10 colonies Moderate: 10-50 colonies Heavy: >50 colonies	 70% 50% 13%	 30% 50% 87%	Not calculated for heavy versus light/moderate as no raw numbers	None
Gerards 1985[24] Netherlands	EOGBS – culture proven	Neonatal colonisation Light: <3 sites positive that were <10 or 10-50 colonies per plate	38	2	Moderate and heavy versus light: p<0.0005	None

Study ID Country	Outcome	Bacterial load definition	Number/ % without outcome	Number/ % with outcome	Summary measure (95% CI)	Covariates adjusted for
		Moderate: <3 sites positive that were >50 colonies per plate OR ≥3 sites positive that were <10-50 colonies per plate	9	15		
		Heavy: ≥3 sites positive that were >50 colonies per plate	0	4		
	Probable sepsis (no confirmatory culture from	Neonatal colonisation			*RR of heavy versus light and moderate: 3.13 (2.06- 4.76)*	None
		Light: as above	38	4		
		Moderate: as above	9	11		
		Heavy: as above	0	4		

Study ID Country	Outcome	Bacterial load definition	Number/ % without outcome	Number/ % with outcome	Summary measure (95% CI)	Covariates adjusted for
	a sterile site)					
Colony-forming units (cfu) per ml						
Jones 1984[26] USA	Neonatal colonisation	Continuous variable of maternal GBS colonisation from 10^2 to 10^8 colony counts	See text	See text	Correlation between cfu/GBS ml in mothers' vagina and neonates' rectum: p<0.001	None
Persson 1986[29]	Neonatal colonisation	Maternal colonisation Light colonisation: $<10^4$ cfu/ml in	49	6	*RR of heavy: 6.11 (2.52-14.81)*	None

Study ID Country	**Outcome**	**Bacterial load definition**	**Number/ % without outcome**	**Number/ % with outcome**	**Summary measure (95% CI)**	**Covariates adjusted for**
Sweden		urine Heavy colonisation: $\geq 10^4$ cfu/ml in urine	3	6		
Sensini 1997[30] Italy	Neonatal colonisation	Maternal colonisation Light: 10^2-10^5 cfu/ml Heavy: 10^6 or greater	78 74	34 74	*RR of heavy: 1.65 (1.19- 2.28)*	None
	EOGBS	Maternal colonisation Light: as above Heavy: as above	111 148	1 0	Not applicable	Not applicable
Other						

Study ID Country	Outcome	Bacterial load definition	Number/ % without outcome	Number/ % with outcome	Summary measure (95% CI)	Covariates adjusted for
Boyer 1983[20] USA	Neonatal colonisation	Maternal colonisation Light: Negative intrapartum vaginal culture but positive postpartum rectal/vaginal culture Moderate: Positive intrapartum vaginal culture on selective broth enrichment only Heavy: Positive intrapartum vaginal culture on direct plate as well as enrichment	 47* 35 38	 10* 10 69	*RR of heavy versus light and moderate: 3.29 (2.17- 4.99)*	None

Study ID **Country**	**Outcome**	**Bacterial load definition**	**Number/ % without outcome**	**Number/ % with outcome**	**Summary measure (95% CI)**	**Covariates adjusted for**
	EOGBS	Maternal colonisation Light: as above Moderate: as above Heavy: as above	 57* 45 103	 0 0 4	Not applicable	Not applicable
Morales 1986[28] USA	Neonatal colonisation	Maternal colonisation Light colonisation: Agglutination with GBS antigens was negative at 5 hours but positive at 20 hours Heavy colonisation: Agglutination with GBS antigens was detectable within 5	 63	 35	*RR of heavy: 2.24 (1.63- 3.09)*	None

Study ID Country	Outcome	Bacterial load definition	Number/ % without outcome	Number/ % with outcome	Summary measure (95% CI)	Covariates adjusted for
		hours	6	24		
	GBS sepsis	Maternal colonisation Light colonisation: as above Heavy colonisation: as above	 98 27	 0 3	Not applicable	Not applicable
Morales 1987[27] USA	Neonatal colonisation	Maternal colonisation Light colonisation: Positive latex agglutination identification at 20 hours but not at 5 hours Heavy colonisation: Positive latex agglutination identification at 5 hours	 28 3	 9 8	*RR of heavy: 2.99 (1.52-5.87)*	None

Study ID Country	Outcome	Bacterial load definition	Number/ % without outcome	Number/ % with outcome	Summary measure (95% CI)	Covariates adjusted for
	GBS sepsis	Maternal colonisation Light colonisation: as above Heavy colonisation: as above	 31 4	 6 7	*RR of heavy: 3.92 (1.66-9.25)*	None

Cfu colony forming units, CI confidence interval, GBS group B *Streptococcus*, EOGBS early-onset GBS, RR risk ratio

*Two extra births: 57 infants from 55 mothers

Numbers in italics were calculated by authors

Figure 1

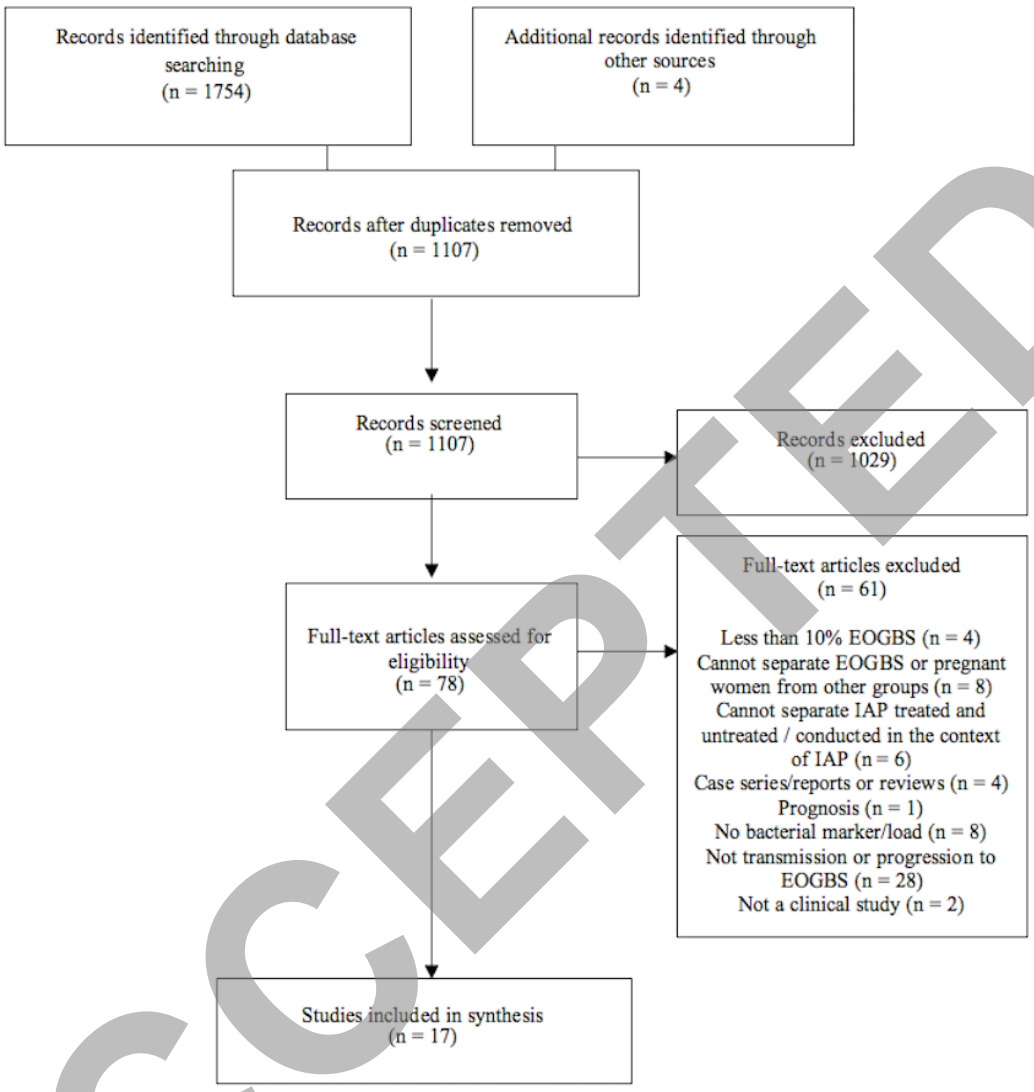

Figure 2

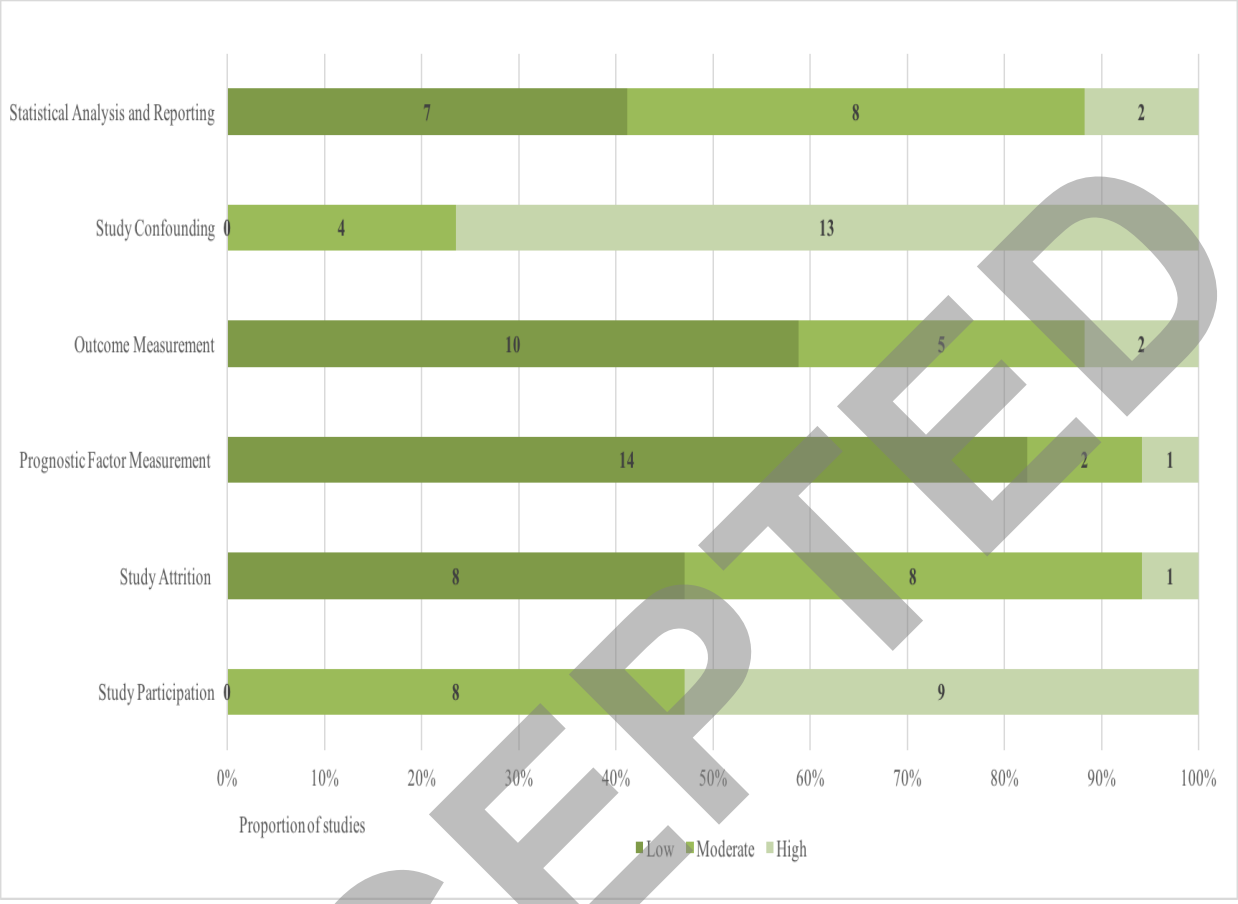

Figure 3