Bacterial Load and Molecular Markers Associated With Early-onset Group B *Streptococcus*

A Systematic Review and Meta-analysis

Farah Seedat, PhD, Colin Stewart Brown, FRCPath,† Chris Stinton, PhD,* Jacoby Patterson, MD,* Julia Geppert, PhD,* Karoline Freeman, MSc,* Bee Tan, FRCS,* Samantha Ann Johnson, MA,* Hannah Fraser, BSc,* Olalekan A. Uthman, PhD,* Esther R. Robinson, BMBCh DPhil,‡ Noel Denis McCarthy, MB Dphil,* Aileen Clarke, MD,* and Sian Taylor-Phillips, PhD**

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 October 10, 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 Quality in Prognosis Studies tool. We conducted random-effects meta-analyses where possible and narratively synthesized the evidence in text and tables.

Results: Seventeen studies were included from 1107 records retrieved from electronic databases and publication references. Meta-analyses of 3 studies showed that neonates colonized by serotype III had a higher risk of developing EOGBS than serotype Ia (pooled risk ratio: 1.51, 95% confidence interval: 1.12–2.03) and serotype II (risk ratio: 1.95, 95% confidence interval: 1.10–3.45). Eleven studies showed that in heavily colonized mothers, 2–3 times more neonates were colonized, and in heavily colonized neonates, up to 15 times more neonates had EOGBS, compared with light colonization. Most evidence was published before 2000 and was at risk of bias.

Conclusions: 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.

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

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From the *Division of Health Sciences, Warwick Medical School, University of Warwick, Gibbet Hill Campus, Coventry, United Kingdom; †Bacteria Reference Department, National Infection Service, Public Health England, London, England; and ‡Field Service, National Infection Service, Public Health England, Seaton House, Nottingham.

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Address for correspondence: Sian Taylor-Phillips, PhD, Division of Health Sciences, University of Warwick Medical School, Gibbet Hill Campus, Coventry CV4 7AL, United Kingdom. E-mail: S.Taylor-Phillips@warwick.ac.uk.

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Group B *Streptococcus* (GBS) is a leading cause of morbidity and mortality from neonatal sepsis.[1] Early-onset GBS disease (EOGBS, first 6 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 2015 statement.[14] The protocol is registered at the International Prospective Register of Systematic Reviews: CRD42016037196.

Search Strategy and Selection Criteria

We conducted electronic searches in MEDLINE, MEDLINE In-Process and 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 October 10, 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, 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 (1) 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 7 days), (2) maternal colonization in labor to neonatal colonization or EOGBS or (3) neonatal colonization to EOGBS. We excluded studies in which more than 10% participants were pregnant women before the third trimester [for study objective (1) 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 7 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 tool, judging 6 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, TX). Where data permitted, we calculated odds ratios 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 colonisation to EOGBS because of heterogeneity in the studies on the remaining factors. We used a random effects model because of 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 χ^2 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 2 studies. For the remaining studies, we conducted narrative syntheses and displayed results in tables and text.

RESULTS

Our search identified 1107 unique records, of which 17 articles were included in the synthesis (see Fig. 1 and Supplemental Digital Content, http://links.lww.com/INF/D106).[7,18–33] Study designs, bacterial factors, populations and definitions of GBS colonization and EOGBS differed between studies (Table 1). Most studies were cohort, with 2 case–control studies[21,31] and 1 subgroup analysis of a control group in a randomized controlled trial.[28] Nine studies were on vertical transmission of GBS colonization,[18,20,23,25–30] 5 on maternal colonization to EOGBS[20,26–28,30] and 8 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] 2 during the 1990s[21,30] and 2 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 1 investigated C-protein antigen.[21]

Risk of Bias

Figure 2 shows the methodologic quality of included studies. Risk of bias was considered high in 2 or more Quality in Prognosis Studies domains in 10 of 17 studies (59%), and in 1 domain in 4 of 17 studies (24%). No study was judged as low risk of bias in all 6 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 4 accounted for some, but not all, relevant confounders (moderate risk).[19,27,30,31] In the study participation domain, 9 studies (53%)[18–23,25,26,33] were at high risk and the remaining 8 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 1 study. Al-Sweih et al[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 5 studies.[7,19,21,31,32] Meta-analyses could only be performed on 3 studies,[7,21,32] as the required data were not available in the others. Of the omitted studies, Baker and Barrett[19] reported that serotype III was more frequently present in EOGBS cases (56%) than in asymptomatic colonisation (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 and Barrett[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 EOGBS than neonates colonized by serotype Ia (pooled RR: 1.51, 95% CI: 1.12–2.03, 3 studies, 439 neonates). Among 261 neonates colonized by serotype III, 98 (37.5%) developed EOGBS compared with 45 of 178 (25.3%) neonates colonized by serotype Ia. Similarly, neonates colonized by serotype III were twice as likely to have EOGBS than neonates colonized by serotype II (pooled RR: 1.95, 95% CI: 1.10–3.45, 3 studies, 355 neonates). Among 261 neonates colonized by serotype III, 98 (37.5%) developed EOGBS compared with 19 of 94 (20.2%) neonates colonized by serotype II. The forest plots for each comparison are presented in Supplemental Digital Content 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[7] may have had considerable influence on the results.

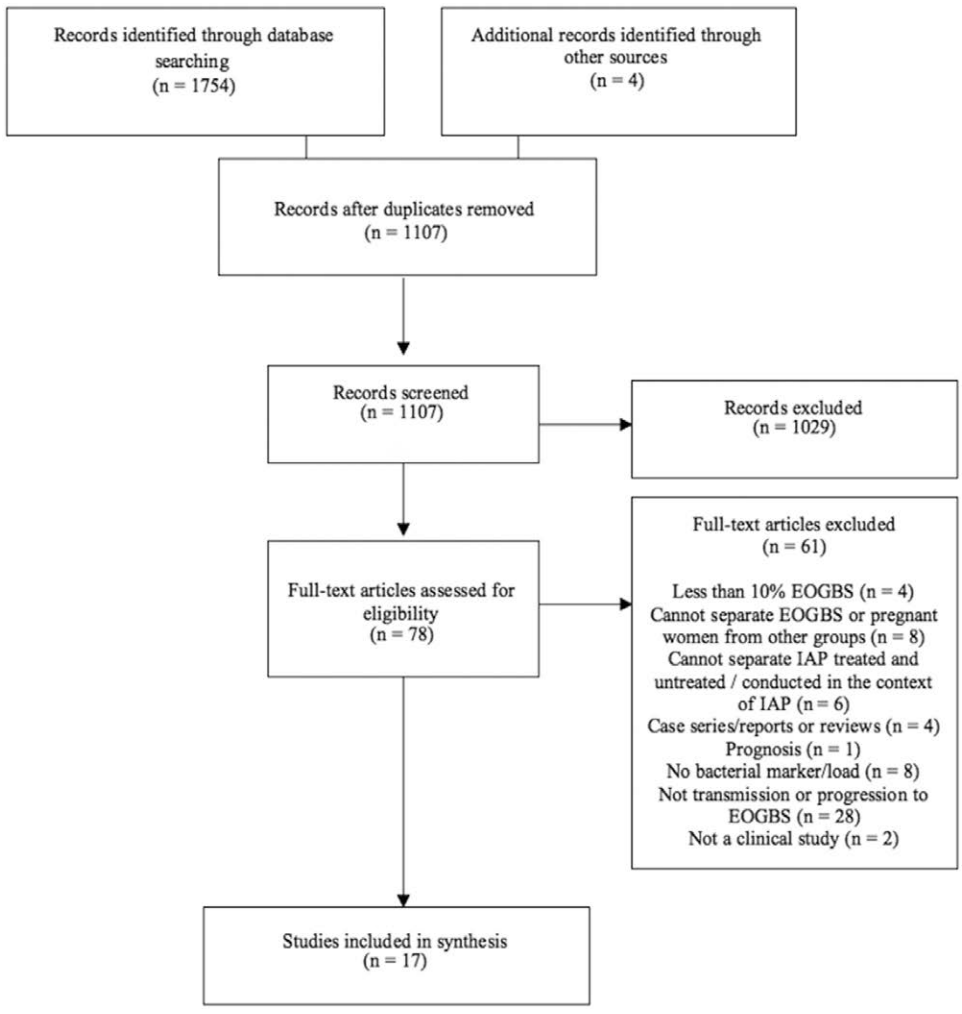

FIGURE 1. Flow diagram of study selection. See Supplemental Digital Content (http://links.lww.com/INF/D106) for the list of excluded full-text articles with reasons.

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 (Table 2).[20,22–30,33] Three studies reported the number of colonized sites.[22,25,33] Hoogkamp-Korstanje et al[25] found that women colonized in 2 or more sites compared with 1 site only were two and a half times more likely to have a neonate colonized with GBS (91% vs. 36%, RR calculated from percentages: 2.53, 95% CI: 1.93–3.31). Sites swabbed included throat, nose, vagina, cervix, rectum and midstream urine in labor. Similarly, 2 studies found up to a 15 times higher risk of EOGBS in neonates with 3 to 4 colonized sites compared with 1 to 2 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 1–2 hours of birth.[33]

Three studies reported the number of colony counts on a plate.[23–25] Hoogkamp-Korstanje et al[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[24] combined the number of sites with the number of colony counts, finding that neonates colonized in 3 or more sites with >50 colonies (heavy, 4/8, 50% transmission rate) were more likely to have EOGBS than neonates with fewer than 3 sites with >50 colonies, 3 or more sites with <10 or 10–50 colonies (moderate, 15/35, 42.9% transmission rate) or fewer than 3 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 neonatal intensive care unit. Easmon et al[23] also reported the number of colonies in mothers' vaginas and rectum; however, conclusions could not be drawn as data labeling 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[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; 2 had blood culture positive sepsis and 1 had a positive rectal culture with respiratory distress. All 3 infants had mothers who

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 et al (2005)[18] Kuwait	Prospective cohort study	Serotype	Maternal colonisation to neonatal colonisation	124 women colonized with GBS on vaginal-anorectal swabs in labor (selective culture)	74 neonates colonized with GBS on external ear canal and umbilicus swabs at unspecified time (selective culture)
Baker and Barrett (1973)[19] United States	Prospective cohort study	Serotype	Neonatal colonisation to EOGBS disease	66 neonates colonized with GBS on throat and umbilical or external auditory canal swabs at mean age of 13.8 h (selective culture)	12/13 neonates with bacteriologically confirmed EOGBS disease ≤10 d (all infants developed symptoms in the first 5 d of life)
Baker and Barrett (1974)[31] United States	Case–control study	Serotype	Neonatal colonisation to EOGBS disease	53 neonates colonized with GBS on throat, umbilical cord or ear swabs at <3 d	Unknown number of neonates with EOGBS sepsis or pneumonia: clinical symptoms and premortem blood cultures or postmortem heart and lung cultures in neonates with pneumonia 15 neonates with EOGBS meningitis: CSF culture ≤5 d
Chun et al (1991)[21] United States	Case–control study	Serotype, Reaction to c-protein and c-protein ß antigen gene	Neonatal colonisation to EOGBS disease	121 neonates colonized with GBS at birth on nasopharynx, throat, umbilicus or rectum swabs	47 neonates with EOGBS sepsis: Blood and CSF culture <7 d
Embil et al (1987)[32] Canada	Prospective cohort study	Serotype	Neonatal colonisation to EOGBS disease	55 strains from 54 neonates colonized with GBS on rectal swabs within 1 h of birth (selective culture)	12 neonates with symptomatic EOGBS <3 d
Madzivhandila et al (2011)[7] South Africa	Prospective cohort study	Serotype	Neonatal colonisation to EOGBS disease	525 neonatal isolates colonized with GBS on ears, nose and umbilicus swabs shortly after birth (standard culture)	136 neonates with EOGBS: Blood and CSF culture <7 d
Hoogkamp-Korstanje et al (1982)[25] The Netherlands	Prospective cohort study	Bacterial load: number of positive sites, number of colony counts per plate	Maternal colonisation to neonatal colonisation	46 women colonized with GBS on throat, nose, vagina, cervix, rectum and midstream urine swabs in labor (selective culture)	Unknown number of neonates colonized with GBS on skin, throat, external ears and umbilicus swabs at <6 h of birth (selective swab)
Dillon et al (1987)[22] United States	Prospective cohort study	Bacterial load: number of positive sites	Neonatal colonisation to EOGBS	1448 neonates colonized with GBS on external ear canal, umbilicus, oropharynx and rectum swabs within 1 h of birth (selective culture)	24 neonates with EOGBS: Symptoms and blood, CSF, urine and other clinical specimens <3 d
Pass et al (1979)[33] United States	Prospective cohort study	Bacterial load: number of positive sites	Neonatal colonisation to EOGBS	290 neonates colonized with GBS on external canal, umbilicus, throat and anus swabs 1–2 h after birth (selective culture)	8 neonates with EOGBS: blood and CSF culture
Easmon et al (1985)[23] England	Prospective cohort study	Bacterial load: number of colony counts per plate	Maternal colonisation to neonatal colonisation	140 women colonized with GBS on vaginal swabs in labor (selective and standard culture)	38 neonates colonized with GBS on rectum, umbilicus, ear and external nares swabs within 24 h of birth and/or on discharge from hospital (selective culture)
				141 women colonized with GBS on rectal swabs in labor (selective and standard culture)	39 neonates colonized with GBS on rectum, umbilicus, ear and external nares swabs within 24 h of birth and/or on discharge from hospital (selective culture)
Gerards et al (1985)[24] The Netherlands	Prospective cohort study	Bacterial load: number of colony counts per plate	Neonatal colonisation to EOGBS	68 neonates colonized with GBS on nose, throat, external auditory meatus, eyes, umbilicus, skin and rectum swabs immediately after admission to NICU (selective culture)	21 neonates with EOGBS: sepsis symptoms with GBS cultured from normally sterile culture <7 d
			Neonatal colonisation to probable GBS	66 neonates colonized 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 et al (1984)[26] United States	Prospective cohort study	Bacterial load: CFU per milliliter	Maternal colonisation to neonatal colonisation	130 women colonized with GBS on vaginal swabs at labor (selective culture)	61 neonates colonized with GBS on rectum, 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

(*Continued*)

TABLE 1. (*Continued.*)

Study ID Country	Study Design	Risk Factors	GBS Natural History Pathway	Participants and GBS Culture Method	Outcome, Definition and Measurement
Persson et al (1986)[29] Sweden	Secondary analysis combined with a prospective cohort study	Bacterial load: CFUs per milliliter	Maternal colonisation to neonatal colonisation	64 women colonized with GBS on urine swab in labor (selective culture)	12 neonates colonized with GBS on rectal swabs <5 d (selective culture)
Sensini et al (1997)[30] Italy	Prospective cohort study	Bacterial load: CFUs per milliliter	Maternal colonisation to neonatal colonisation	260 women colonized with GBS on lower vaginal swabs in labor (selective culture)	108 neonates colonized with GBS on auricular, pharyngeal and gastric aspirate swabs before first bath (selective culture)
		Bacterial load: CFUs per milliliter	Maternal colonisation to EOGBS		1 neonate with EOGBS sepsis: blood culture and sepsis symptoms <24 h
Boyer et al (1983)[20] United States	Prospective cohort study	Bacterial load: other	Maternal colonisation to neonatal colonisation	207 women colonized with GBS on vaginal swabs in labor who gave birth to 209 neonates (selective culture)	89 neonates colonized 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 et al (1986)[28] United States	Untreated control group of RCT	Bacterial load: other	Maternal colonisation to neonatal colonisation	128 women colonized with GBS at labor identified by a rapid slide coagglutination test on selective vaginal culture	59 term neonates colonized with GBS on oropharynx and the skin swabs at delivery and a urine latex-agglutination
			Maternal colonisation to EOGBS		3 term neonates with GBS sepsis: positive body fluid
Morales and Lim (1987)[27] United States	Prospective cohort study	Bacterial load: other	Maternal colonisation to neonatal colonisation	48 women colonized with GBS in labor identified by latex agglutination on selective vaginal culture	17 preterm neonates colonized with GBS detected by urine latex-agglutination after delivery
			Maternal colonisation to EOGBS		13 preterm neonates with GBS sepsis: blood, CSF or urine culture, and oropharynx cultures with radiographic and clinical signs of infection

CSF indicates cerebrospinal fluid; NICU, neonatal intensive care unit; RCT, randomized controlled trial.

were heavily colonized with GBS during delivery (7.70×10^6, 6.62×10^7, 2.5×10^6). However, only 2 of the infants were heavily colonized (7.02×10^5, 5.25×10^6); 1 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[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 10^2–10^6 CFU/GBS mL [74/148 (50%) vs. 34/112 (30%) RR: 1.65, 95% CI: 1.19–2.28]. One neonate developed EOGBS whose mother had light colonization. Persson et al[29] investigated CFU/GBS mL in the mothers' urine during delivery finding that those with $\geq 10^4$ CFU/GBS mL were 6 times more likely to transmit GBS to their neonates compared with mothers with $<10^4$ CFU/GBS mL [6/9 (67%) vs. 6/55 (11%) RR: 6.11, 95% CI: 2.52–14.81].

Morales and Lim[27] and Morales et al[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 5 hours) were twice as likely to transmit GBS to their term neonates [24/30 (80%) vs. 35/98 (36%) RR: 2.24, 95% CI: 1.63–3.09], and 3 times more likely to transmit GBS to their preterm neonates [8/11 (73%) vs. 9/37 (24%) RR: 2.99, 95% CI: 1.52–5.87) than mothers with light colonization (agglutination negative at 5 hours but positive at 20 hours). They found 3 cases of term GBS sepsis, all in heavily colonized mothers, and preterm GBS sepsis that was 4 times more likely in heavily compared with lightly colonized mothers [7/11 (64%) vs. 6/37 (16%) RR: 3.92, 95% CI: 1.66–9.25]. Finally, Boyer et al[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 with 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%) vs. 20/102 (20%) RR: 3.29, 95% CI: 2.17–4.99]. Of the women who transmitted GBS to their infants, heavily colonized women were more likely to have neonates colonized at multiple sites (55%) compared with 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[21] examined whether asymptomatic GBS and EOGBS strains reacted to C-protein antiserum and 4 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 4 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 1 antigen can be expressed in 1 strain.

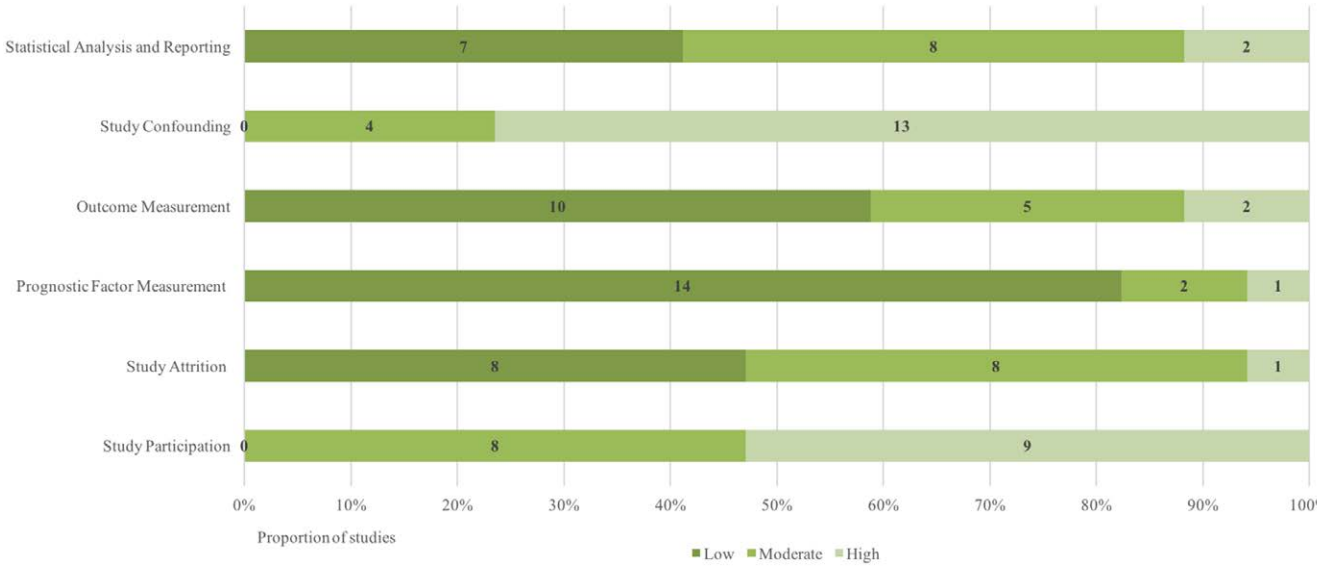

FIGURE 2. Risk of bias across included studies, according to the Quality in Prognosis Studies tool.[15]

Serotype Ia				
0.96 (0.59 to 1.58)	**Serotype Ib**			
0.76 (0.47 to 1.23)	0.82 (0.47 to 1.44)	**Serotype II**		
1.51 (1.12 to 2.03)	1.48 (0.94 to 2.35)	**1.95 (1.10 to 3.45)**	**Serotype III**	
0.67 (0.26 to 1.72)	0.77 (0.27 to 2.20)	0.82 (0.31 to 2.18)	0.45 (0.19 to 1.10)	**Nontypeable**

Serotype | Pooled association (Risk Ratio [95% Confidence Interval]

FIGURE 3. Pooled relative risk of EOGBS by colonizing 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 EOGBS disease in neonates colonized by the row-defining serotype. For example, neonates colonized by serotype III had a higher risk of developing EOGBS than neonates colonized by serotype Ia (pooled RR: 1.51, 95% CI: 1.12–2.03). RRs for comparisons in the opposing direction can be established by using reciprocals. Significant results are in bold and underlined.

DISCUSSION

This is the first systematic review investigating bacterial load and molecular markers associated with GBS vertical transmission, or progression from neonatal colonization to EOGBS. Our findings suggest that the epidemiology and natural history of neonatal GBS have not been extensively researched. Only 2 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,[36] 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 undertreatment. 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 2–3 times higher risk of having a neonate colonized with GBS compared with 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 with Ia and

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-Korstanjeet al (1982)[25]	Neonatal colonisation	Maternal colonization			*RR of heavy: 2.53 (1.93–3.31) (calculated from %)*	None
The Netherlands		Light: 1 site	64%	36%		
		Heavy ≥2 sites	9%	91%		
Dillon et al (1987)[22]	EOGBS	Neonatal colonization			*RR of heavy: 12.97 (4.46–37.70)*	None
United States		Light: 1–2 sites	1041	4		
		Heavy: 3–4 sites	383	20		
Pass et al (1979)[33]	EOGBS	Neonatal colonization			*RR of heavy: 15.31 (1.91–122.60)*	None
United States		Light: 1–2 sites	198	1		
		Heavy: 3–4 sites	84	7		
Number of colony counts per plate						
Hoogkamp-Korstanjeet al (1982)[25]	Neonatal colonisation	Maternal colonisation			Not calculated for heavy vs. light/moderate as no raw numbers	None
The Netherlands						
		Light: <10 colonies	70%	30%		
		Moderate: 10–50 colonies	50%	50%		
		Heavy: >50 colonies	13%	87%		
Gerards et al (1985)[24]	EOGBS: culture proven	Neonatal colonisation			Moderate and heavy vs. light: $P < 0.0005$	None
The Netherlands						
		Light: <3 sites positive that were <10 or 10–50 colonies per plate	38	2		
		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 a sterile site)	Neonatal colonisation			*RR of heavy vs. 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		
CFU per milliliter						
Jones et al (1984)[26]	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
United States						
Persson et al (1986)[29]	Neonatal colonisation	Maternal colonisation			*RR of heavy: 6.11 (2.52–14.81)*	None
Sweden		Light colonisation: $<10^4$ CFU/mL in urine	49	6		
		Heavy colonisation: $\geq 10^4$ CFU/mL in urine	3	6		
Sensini et al (1997)[30]	Neonatal colonisation	Maternal colonisation			*RR of heavy: 1.65 (1.19–2.28)*	None
Italy		Light: 10^2–10^5 CFU/mL	78	34		
		Heavy: 10^6 or greater	74	74		
	EOGBS	Maternal colonisation			Not applicable	Not applicable
		Light: as above	111	1		
		Heavy: as above	148	0		
Other						
Boyer et al (1983)[20]	Neonatal colonisation	Maternal colonisation			*RR of heavy vs. light and moderate: 3.29 (2.17–4.99)*	None
United States		Light: Negative intrapartum vaginal culture but positive postpartum rectal/vaginal culture	47*	10*		
		Moderate: Positive intrapartum vaginal culture on selective broth enrichment only	35	10		
		Heavy: Positive intrapartum vaginal culture on direct plate and enrichment	38	69		
	EOGBS	Maternal colonisation			Not applicable	Not applicable
		Light: as above	57*	0		
		Moderate: as above	45	0		
		Heavy: as above	107	4		

(*Continued*)

TABLE 2. (*Continued.*)

Study ID Country	Outcome	Bacterial Load Definition	Number/% Without Outcome	Number/% With Outcome	Summary Measure (95% CI)	Covariates Adjusted for
Morales et al (1986)[28] United States	Neonatal colonization	Maternal colonisation			*RR of heavy: 2.24 (1.63–3.09)*	None
		Light colonisation: Agglutination with GBS antigens was negative at 5 h but positive at 20 h	63	35		
		Heavy colonisation: Agglutination with GBS antigens was detectable within 5 h	6	24		
	GBS sepsis	Maternal colonisation			Not applicable	Not applicable
		Light colonisation: as above	98	0		
		Heavy colonisation: as above	27	3		
Morales and Lim (1987)[27] United States	Neonatal colonisation	Maternal colonization			*RR of heavy: 2.99 (1.52–5.87)*	None
		Light colonisation: Positive latex agglutination identification at 20 h but not at 5 h	28	9		
		Heavy colonisation: Positive latex agglutination identification at 5 h	3	8		
	GBS sepsis	Maternal colonisation			*RR of heavy: 3.92 (1.66–9.25)*	None
		Light colonisation: as above	31	6		
		Heavy colonisation: as above	4	7		

Numbers in italics were calculated by authors.
*2 extra births: 57 infants from 55 mothers.

II, serotype III is more often associated with invasive EOGBS. Contrary to expectations, we found no evidence of a difference between nontypeable and other serotypes. When comparing colonized mothers to EOGBS cases, Fabbrini et al[37], for example, found no cases of EOGBS in neonates with a nontypeable serotype compared with 8% of colonized mothers who had a nontypeable serotype. We may not have found this difference in our review as there were only 23 neonates colonized with a nontypeable serotype in the meta-analysis. Within serotype III, a study excluded from this review because of the context of IAP, found that ST-17 was the most common sequence type among invasive serotype III strains.[10] 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 vs. 4/1000, respectively, $P < 0.001$).[42] In contrast, evidence on the association directly between maternal load and EOGBS was slightly unclear, possibly because of the small numbers of EOGBS in such studies. We were only able to perform analyses on 1 study, where preterm EOGBS was almost 4 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, odds ratios and 95% CIs) reported in this review using unadjusted statistical analyses that did not control for potential confounders. Therefore, the identified relationships could be partially or entirely because of 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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