Original Paper

Neonatology

Neonatology 2015;108:157–163
DOI: 10.1159/000434716

Received: March 19, 2015
Accepted after revision: June 1, 2015
Published online: July 11, 2015

Maternal Colonization with Group B *Streptococcus* Is Associated with an Increased Rate of Infants Transferred to the Neonatal Intensive Care Unit

Anne Karin Brigtsen[a, b] Anne Flem Jacobsen[a, c] Lumnije Dedi[d]
Kjetil K. Melby[a, d] Drude Fugelseth[a, b] Andrew Whitelaw[a]

[a]Institute of Clinical Medicine, University of Oslo, and Departments of [b]Neonatal Intensive Care, [c]Obstetrics and Gynaecology and [d]Microbiology, Oslo University Hospital Ullevaal, Oslo, Norway

Key Words
Group B *Streptococcus* · Newborn infant · Maternal colonization · Intrapartum infection · Neonatal intensive care

Abstract
Background: *Streptococcus agalactiae* (group B *Streptococcus*, GBS) is the most common cause of early neonatal infection, but restricting the diagnosis to culture-positive infants may underestimate the burden of GBS disease. Our objective was to determine whether maternal GBS colonization was associated with an increased risk of transfer of term infants to the neonatal intensive care unit (NICU) and, if so, to estimate the incidence of probable early-onset GBS disease. ***Methods:*** We conducted a prospective cohort study of 1,694 term infants whose mothers had vaginal-rectal swabs collected at delivery. Data collected on each mother and infant included demographics, clinical findings and laboratory investigations. The medical staff were unaware of the maternal GBS colonization status. ***Results:*** A total of 26% of the mothers were colonized. Infants born to colonized mothers did not differ from infants born to non-colonized mothers with respect to birth weight or Apgar score. Altogether, 30 (1.8%) of the term infants were transferred to the NICU. Only 1 infant born to a colonized mother had culture-positive early-onset GBS disease. Infants born to colonized mothers were more than 3 times as likely to be transferred to the NICU compared to infants of non-colonized mothers (3.6 vs. 1.1%; OR 3.4, 95% CI 1.6–6.9, p = 0.001); 5 infants of colonized mothers had probable GBS disease with tachypnoea and raised C-reactive protein (3.0/1,000 live term births). ***Conclusions:*** Maternal GBS colonization is associated with increased risk of transfer to the NICU in term infants. The burden of neonatal GBS disease may be greater than indicated by the number of culture-positive cases.

Introduction

Streptococcus agalactiae (group B *Streptococcus*, GBS) remains a leading cause of serious neonatal infection [1, 2]. Maternal vaginal-rectal colonization is considered the main route for neonatal infection. GBS colonizes the vagina or rectum in 20–30% of women giving birth and a vertical transmission rate of about 50% among infants born to colonized women has been reported [3]. The incidence of culture-proven, neonatal GBS infection varies between 0.3 and 0.7 cases per 1,000 live births in most Western countries [4]. However, regional differences exist and the disease burden is higher in many non-Western countries [5]. A case fatality rate of 4–10% in Europe has been reported, which is quite similar to mortality rates in the USA [4, 6]. Long-term outcomes are mainly reported for survivors of GBS meningitis and neurological impair-

1661–7800/15/1083–0157$39.50/0

E-Mail karger@karger.com
www.karger.com/neo

Anne Karin Brigtsen, MD
Institute of Clinical Medicine, University of Oslo
Kirkeveien 166
NO–0450 Oslo (Norway)
E-Mail a.k.brigtsen@medisin.uio.no

ment has been observed in approximately half of these children [7]. The continued threat from GBS has prompted work on developing vaccines for pregnant women. The introduction of a GBS vaccination program requires an accurate knowledge of the full burden of disease associated with GBS.

The diagnosis of invasive GBS disease is based on the isolation of GBS from normally sterile sites, e.g. blood or cerebrospinal fluid. However, GBS can give rise to clinical disease in the absence of positive cultures. This can occur if the volume of the blood for culture is low, if the concentration of organism in blood or cerebrospinal fluid is low and, especially, if the mother has received antibiotic therapy prior to delivery. The term 'probable early-onset GBS disease' has been used to describe this condition, and seems to be more common than culture-positive GBS, but there is very limited information on the scale and clinical implications of this problem [8, 9]. There is no uniform definition of probable early-onset disease. Luck et al. [8] defined probable early-onset GBS disease as follows: positive deep ear swab in a baby with clinical pneumonia, meningitis or sepsis (fever >38°C on one occasion or >37.5°C on two occasions an hour apart or two or more incidences of poor perfusion, respiratory distress, thrombocytopenia, leucopenia $<5 \times 10^9$/l, persisting glucose imbalance, abdominal distension, bilious aspirates, or blood in stool) [8, 10]. Carbonell-Estrany et al. [9] used GBS colonization in the mother, amniotic fluid, placenta or neonatal ear swabs, clinical signs, and at least one laboratory abnormality consistent with infection. These two different definitions share the same essentials, i.e. neonatal clinical and laboratory findings consistent with infection and documented GBS colonization.

To gain more insight into the epidemiology and potential adverse consequences of maternal GBS colonization, we wanted to determine whether infants born to mothers colonized with GBS are at increased risk of being transferred to the neonatal intensive care unit (NICU) compared to infants born to non-colonized mothers and, if so, whether transfer is due to probable early-onset GBS disease. Admission of preterm infants was not considered a useful outcome because most infants below 36 weeks are admitted to the NICU automatically because of immaturity, e.g. the need for feeding and temperature maintenance, regardless of whether they are ill or not. Conversely, term infants are routinely cared for by their mothers unless there is a specific dysfunction requiring investigation and management. Thus, admission of infants of 37 or more weeks was considered a proxy unspecific indication of illness.

Methods

Study Population

The Oslo GBS Study is a prospective cohort study of pregnant women admitted to the delivery department at Oslo University Hospital Ullevaal. The hospital serves a population of approximately 600,000 in the metropolitan Oslo area. From June 2009 to September 2011, 16,000 pregnant women were invited to participate at the time of routine ultrasound screening in the second trimester. A total of 4,450 women consented to participate in the study and 1,739 women had one vaginal-rectal sample obtained. Of these, 1,682 women gave birth at term. Sample size calculation was based on the following assumptions: an alpha (risk of type I error) of 5%, a power (risk of type II error) of 80% and a prevalence of 25% maternal GBS colonization. With these assumptions, detecting a difference in NICU admission of 1 versus 4% would require a total sample of 976 and a difference of 1 versus 3% would require a total sample of 1,820 [11]. We originally considered 1,820 as a recruitment target but decided to proceed with analysis on the 1,682 actually recruited.

The Regional Committees for Medical and Health Research Ethics approved the study and the biobank is registered in the Norwegian Biobank Registry. Written informed consent was obtained from the participants.

Data Collection

Combined vaginal-rectal swabs were obtained during the vaginal examination at the onset of labour and prior to antibiotic administration. The swabs were collected and processed in accordance with the CDC recommendations [12]. The members of the hospital staff taking care of the women and infants participating in the study were blinded to the results of the GBS cultures. In Norway, a risk-based rather than a screening-based approach is applied to prevent early-onset GBS disease and intrapartum antibiotic prophylaxis (IAP) was administered to women with any of the following risk factors: having previously given birth to an infant with invasive GBS disease, intrapartum fever >38°C, GBS bacteriuria during pregnancy, premature rupture of membranes ≥18 h, and chorioamnionitis.

Demographic, epidemiological and clinical information was obtained from clinical records, the Medical Birth Registry of Norway and the Norwegian Surveillance System of Communicable Diseases. The Medical Birth Registry of Norway prospectively records perinatal data on all births in Norway based on compulsory notification. The clinical records of every infant transferred to the NICU were examined in detail.

Our definition of probable early-onset GBS infection contained the same essentials as Luck et al. [8] and Carbonell-Estrany et al. [9], i.e. neonatal clinical and laboratory findings consistent with infection and documented GBS colonization. Our requirements were as follows: maternal GBS colonization, clinical signs before 72 h of age (e.g. respiratory rate >60/min, temperature >38°C, heart rate >160 beats/min, lethargy, irritability, or vomiting) and raised C-reactive protein (CRP >10 mg/l) [9, 13].

Statistical Analysis

Baseline characteristics are reported as counts and percentages for categorical variables. Normally distributed continuous variables are reported as means and standard deviations. For skewed distributions, we report medians and interquartile ranges. Con-

cerning continuous variables, differences between groups were assessed by the Student t test or the Mann-Whitney U test. Differences in categorical characteristics between groups were compared by χ^2 or Fisher's exact tests, as appropriate. Logistic regression analysis was used to estimate the risk associated with maternal colonization. Risk estimates are presented as odds ratios (OR) with 95% CI. A two-sided p value <0.05 was considered to indicate statistical significance.

Results

Baseline Characteristics of the Mothers and Infants

From the total of 1,694 infants born at term to 1,682 mothers with GBS samples cultured, 1,255 infants were born to non-colonized mothers and 439 infants to colonized mothers. Table 1 shows the characteristics of the colonized and non-colonized mothers and infants. The baseline characteristics did not differ significantly between groups, except that significantly more boys than girls were born to colonized mothers and the gestational age was slightly shorter, i.e. on average 1 day less in the infants born to colonized mothers.

Outcomes

Of the 1,682 women giving birth at term, 437 (26.0%) were colonized with GBS. A total of 16 out of 439 infants born to colonized women were transferred to the NICU, whereas only 14 of 1,255 infants born to non-colonized women were transferred. Thus, infants born to colonized mothers were more than 3 times as likely to be transferred to the NICU compared to infants born to non-colonized mothers (3.6 vs. 1.1%; OR 3.4, 95% CI 1.6–6.9, p = 0.001). Excluding 1 infant transferred to the NICU who had culture-proven early-onset GBS disease, the risk of transfer was marginally attenuated and remained 3 times higher for infants born to colonized compared to non-colonized mothers (3.4 vs. 1.1%; OR 3.1, 95% CI 1.5–6.6, p = 0.002).

The characteristics of the 30 term infants admitted to the NICU are shown in table 2. Gestational age, birth weight and Apgar scores at 1 and 5 min did not differ statistically between the infants born to colonized and non-colonized women. There were no differences between the colonized and non-colonized women due to interventions like the use of forceps, vacuum extraction or Caesarean section. However, emergency Caesarean section was performed in 6 of the 16 colonized women and in only 1 of the 14 non-colonized women (p = 0.09). In addition, 5 (31.3%) infants were born to colonized women receiving antibiotics during labour, whereas antibiotics where not given to any of the non-colonized women (p = 0.045).

Table 1. Characteristics of mothers and mature infants, according to maternal GBS colonization status

	Non-colonized women (n = 1,255)	Colonized women (n = 439)	p value
Maternal age, years	32.2±4.1	32.0±3.9	0.41
Nulliparous, n	816 (65.2)	283 (64.8)	0.86
Birth weight, g	3,567±463	3,541±455	0.32
Gestational age, days	283±8	282±9	0.003
Male, n	613 (48.8)	241 (50.4)	0.03
Apgar score at 1 min	9 (9–9)	9 (9–9)	0.09
Apgar score at 5 min	9 (9–9)	10 (9–10)	0.91
Apgar score ≤5 at 5 min	6 (0.5)	3 (0.7)	0.70
PROM	177 (14.2)	62 (14.3)	0.95
Intervention	348 (27.7)	124 (28.2)	0.84

Values represent means ± SD, medians (with interquartile ranges) or counts (with percentages), as appropriate. Intervention: the use of forceps, vacuum extraction or Caesarean section. PROM = Prolonged rupture of membranes (≥18 hours).

Table 2. Characteristics of infants transferred to the NICU

	Non-colonized women (n = 14)	Colonized women (n = 16)	p value
Maternal age, years	32.2±4.2	31.1±3.8	0.48
Nulliparous, n	10 (71.4)	13 (81.3)	0.53
Birth weight, g	3,536±544	3,461±374	0.67
Gestational age, days	284±10	278±11	0.15
Male, n	8 (57.1)	9 (56.3)	0.96
Apgar score at 1 min	6 (4–9)	7 (5–9)	0.97
Apgar score at 5 min	8 (6–9)	8 (6–10)	0.90
Apgar score ≤5 at 5 min	3 (21.4)	2 (12.5)	0.64
PROM	2 (14.3)	2 (12.5)	1.00
Intervention	9 (64.3)	13 (81.3)	0.30
Emergency Caesarean section	1 (7.1)	6 (37.5)	0.09
IAP, complete	0 (0)	2 (12.5)	0.49
IAP, any	0 (0)	5 (31.3)	0.045

Values represent means ± SD, medians (with interquartile ranges) or counts (with percentages), as appropriate. Intervention: the use of forceps, vacuum extraction or Caesarean section. IAP, complete: antibiotics administered ≥4 h before delivery. IAP, any: complete or incomplete intrapartum antibiotic prophylaxis.

Only 2 of the 7 colonized women with risk factors for giving birth to an infant with GBS disease received complete IAP with antibiotics at least 4 h before delivery [12].

In table 3, clinical and laboratory findings are summarized for each infant admitted to the NICU. Of the 16 in-

Neonatology 2015;108:157–163
DOI: 10.1159/000434716

Table 3. Infants transferred to the NICU

Patient	Maternal GBS cultures	Maternal GBS risk factors	Reason for admission to NICU	Onset of symptoms, h	IAP	Infant GBS cultures	AB infant	O_2 delivery	O_2 NICU	Highest CRP (mg/l)	Leucocyte count	Serotype	Severity of disease	Outcome
1	+	–	tachypnoea	<1	–	n.a.	–	+	+	<0.5	normal	II	mild	no complications
2	+	PROM	asphyxia	<1	>4 h	n.a.	–	–	–	1	normal	Ia	mild	no complications
3	+	–	*tachypnoea*	*<1*	–	*n.a.*	–	+	+	*15*	*normal*	*V*	*mild*	*no complications*
4	+	fever	asphyxia, hypotension	<1	<4 h	BC–	3	–	–	2	normal	V	moderate	no complications
5	+	fever	asphyxia, hypotension	<1	<4 h	n.a.	3	–	–	2	normal	V	moderate	no complications
6	+	–	tachypnoea	<1	–	n.a.	–	–	–	7	normal	II	mild	no complications
7	+	–	subgaleal haemorrhage	<1	–	n.a.	–	–	–	9	normal	III	moderate	no complications
8	+	*UTI*	*respiratory distress*	*<1*	*>4 h*	*BC–*	*9*	+	–	*77*	*32.9*	II	*moderate*	*no complications*
9	+	–	rhesus-immunization	<1	–	n.a.	–	–	–	<1	normal	VI	moderate	no complications
10	+	*fever*	*respiratory distress*	*<1*	*<4 h*	*BC–/CSF–*	*10*	+	+	*89*	*normal*	*NA*	*severe*	no *complications*
11	+	–	HSV	<1	–	BC–/CSF–	5	–	–	6	normal	NA	moderate	HSV not confirmed no complications
12	+	–	asphyxia	<1	–	n.a.	–	–	–	NA	normal	III	mild	no complications
13	+	–	*respiratory distress*	*<1*	–	*BC–*	*5*	+	+	*25*	*normal*	*V*	*moderate*	*no complications*
14	+	–	CLP	<1	–	n.a.	–	–	–	10	4.2	V	moderate	CLP, otherwise no complications
15	+	*PROM*	*tachypnoea*	*21*	–	*BC–*	*7*	–	–	*95*	*normal*	*IV*	*moderate*	*no complications*
16[a]	+	–	tachypnoea	47	–	BC–/CSF+	14	–	–	66	normal	III	severe	normal development at 2 years of age
17	–	–	tachypnoea	1.5	–	n.a.	–	–	–	3	normal		mild	no complications
18	–	–	tachypnoea	26	–	BC–	8	–	–	90	normal		moderate	no complications
19	–	–	asphyxia	<1	–	BC–	5	+	–	6	normal		moderate	no complications
20	–	–	tachypnoea	<1	–	n.a.	–	+	–	6	normal		mild	pneumothorax no complications
21	–	PROM	tachypnoea	<1	–	n.a.	–	+	–	21	normal		mild	no complications
22	–	–	respiratory distress	<1	–	n.a.	–	+	–	5	normal		moderate focal seizures	normal development at 2 years of age
23	–	–	meconium aspiration, tachypnoea	<1	–	BC–	12	+	+	106	5.0		severe	no complications
24	–	–	polycythaemia	13	–	n.a.	–	–	–	<1	normal		mild	no complications
25	–	–	asphyxia	<1	–	n.a.	–	+	+	<1	33.7		mild	no complications
26	–	–	tachypnoea	<1	–	n.a.	–	+	–	<1	normal		mild	no complications
27	–	–	tachypnoea	<1	–	n.a.	–	–	–	7	normal		mild	no complications
28	–	–	tachypnoea	<1	–	n.a.	–	+	–	2	normal		mild	no complications
29	–	PROM	asphyxia, tachypnoea	<1	–	BC–	6	+	–	30	normal		moderate	no complications
30	–	–	asphyxia, tachypnoea	<1	–	BC–/CSF–	7	+	–	79	normal		severe	normal exam at 4 months of age

Italic type: infants with probable early-onset GBS disease. IAP: ≥4 h before delivery = complete prophylaxis, <4 h = incomplete prophylaxis. AB infant: number of days antibiotics were administered to the infant. Leucocyte count: normal = 5–30 × 10^9/l. Severity of disease: mild = not treated with antibiotics; moderate = treated with antibiotics or requiring other pharmacological treatment; severe = requiring the use of mechanical ventilation and/or inotropes and/or diagnosed with meningitis. PROM = Prolonged rupture of membranes (≥18 h); UTI = GBS urinary tract infection; HSV = maternal primary genital herpes simplex virus infection; CLP = cleft lip and palate; n.a. = not applicable; BC = blood culture; CSF = cerebrospinal fluid. [a] Infant with GBS meningitis.

fants from colonized women admitted to the NICU, 7 presented with tachypnoea, including 5 with probable early-onset GBS disease with raised CRP values, giving an incidence of 3 per 1,000 total live births at term. Of these, the mothers of 2 infants had received IAP. Only 1 infant had culture-positive early-onset GBS disease. This was an infant with GBS meningitis (0.6/1,000 total live births at term). Of the term infants born to non-colonized mothers, 5 (3.0/1,000 live births) had tachypnoea and raised CRP but none of these infants had positive cultures and none of their mothers had received intrapartum antibiotic therapy. There were no deaths among the infants transferred to the NICU.

Among the 16 infants transferred to the NICU, information about the maternal colonizing GBS serotype was available for 14 infants. The rate of colonization with serotype V was higher in mothers of transferred infants (5 of 14, 35.7%) than in mothers whose infants were not transferred (68 of 402, 16.9%; $p = 0.07$). Serotypes II and III colonized 3 mothers each, whereas 1 mother each was colonized by serotypes Ia, IV and VI.

Discussion

In this large-scale, prospective cohort study from Norway, we found that term infants of mothers colonized with GBS were more than 3 times as likely to require admission to the NICU as infants of non-colonized mothers. The increased risk of transfer to the NICU was not altered after the exclusion of a single patient with invasive GBS disease. A third of the transferred infants met the criteria for probable early-onset GBS disease. A higher proportion of the infants transferred to the NICU born to colonized mothers had respiratory distress and raised CRP compared to the transferred infants born to non-colonized mothers. These infants may have had genuine bacterial invasion even though the blood culture was negative due to low bacterial concentrations, inadequate volume of blood obtained for culture or maternal antimicrobial treatment prior to delivery [14, 15]. In some cases, placentally transferred maternal antibodies may have inhibited the growth of bacteria in neonatal blood but the bacteria may still have given rise to clinically significant inflammation.

We speculate that the increased transfer rate to the NICU of infants born to GBS-colonized mothers may have been caused by an immune response in the foetus and infant without the invasion of bacteria. GBS induces an inflammatory reaction which triggers the release of various proinflammatory cytokines. Several cytokines, inflammatory markers and prothrombotic factors such as thromboxanes, tumour necrosis factor-α, interleukin 6 (IL-6), IL-8 and IL-12 are involved in the host response to the microbial presentation [3, 16–20]. Respiratory distress is a prominent sign of GBS infection and it is likely that such proinflammatory activity in the amniotic fluid can access the fetal airways and lungs, thus inducing symptomatic disease in infants even when the bacterial exposure has not been proven by culture [3, 21]. Another biologically plausible explanation for our findings is pulmonary hypertension induced by phospholipids released by GBS (alive or dead). This mechanism has been demonstrated in lambs [18], although Stroustrup et al. [22] did not confirm a link between intrapartum exposure to GBS and self-limiting respiratory distress.

Only a few studies have previously examined the incidence of probable early-onset GBS disease and to our knowledge no previous study has specifically investigated the association between GBS colonization intrapartum and the risk of transfer to the NICU. In the study by Luck et al. [8] from a UK tertiary referral centre, the infants included underwent a sepsis screen before 72 h of life and received antibiotic treatment. In their population, the incidence of probable early-onset GBS was 2.5 per 1,000 live births and 1.1 per 1,000 had culture-positive GBS. Our findings suggest that the burden of probable early-onset GBS disease is at least as high in our unselected population as that previously reported from a tertiary referral centre.

Carbonell-Estrany et al. [9], in a Spanish population with universal GBS screening, found the incidence of culture-proven and probable early-onset GBS disease to be 0.39 and 0.47 per 1,000 live births, respectively. Their results diverge from our estimate of 3.0 per 1,000 of probable early-onset disease. The difference may be due to population dissimilarities such as differences in the use of antibiotics in the population. Scandinavian countries have a more restrictive antibiotic use policy than Southern European countries [23, 24] and as antibiotics can reduce the load of maternal GBS colonization, the infant exposure to this important risk factor for infant GBS disease may be decreased [25]. In Norway, a risk-based policy for the prevention of neonatal GBS disease is applied and fewer women receive IAP than in Spain. Thus, more infants in Spain have been exposed to antibiotics during birth, which may potentially reduce the risk of early-onset and probably early-onset GBS disease. Most infants with probable early-onset GBS disease in the Spanish study

had received either complete or incomplete IAP, whereas a smaller proportion of infants with probable early-onset GBS disease in our study received IAP – 84 versus 40%, respectively. Further, their more strict criteria for white cell count and CRP have probably also contributed to the lower incidence rate.

In Norway, serotype III has so far been the predominant serotype in early-onset GBS disease, in accordance with reports from other countries [26–28]. We observed that serotype V was the major maternal colonizing serotype of infants transferred to the NICU. In a recently published study from the same population, we found serotype III to be the major maternal colonizing strain followed by serotype V, constituting 25 and 17% of the isolates, respectively [29]. This indicates that serotype V may be more virulent than the other serotypes, although this requires confirmation in other studies.

Strengths of our study include the prospective inclusion of patients from an unselected population and the blinded design, which kept the maternal colonization status unknown to the hospital staff. Limitations of our study include the selection of consenting women, who may not be fully representative of the whole population of pregnant women, and the fact that the midwives were not able to obtain vaginal-rectal samples from all the women participating in the study. This was mainly due to a high workload in the delivery ward during the study period and is thus unlikely to have introduced a systematic bias.

Conclusion

Mature infants born to colonized mothers are at substantially increased risk of transfer to the NICU. A considerable proportion of the transferred infants born to colonized mothers had findings suggesting probable early-onset GBS infection. Thus, our findings provide further evidence that maternal colonization with GBS produces a considerably greater burden of neonatal disease than generally recognized and gives further support for vaccine development.

Acknowledgements

We wish to thank everyone involved in enrolment, sampling, culturing, and serotyping of the GBS strains at the Department of Obstetrics and Gynaecology and the Department of Microbiology at Oslo University Hospital Ullevaal. We are grateful to Cathrine Nygaard for her excellent technical assistance. This work was partially supported by funds from the Norwegian SIDS and Stillbirth Society, the Eckbo Foundation and the Renée and Bredo Grimsgaard Foundation. The sponsors were not involved in the design and execution of the study.

References

1 Stoll BJ, Hansen NI, Sanchez PJ, Faix RG, Poindexter BB, Van Meurs KP, Bizzarro MJ, Goldberg RN, Frantz ID 3rd, Hale EC, Shankaran S, Kennedy K, Carlo WA, Watterberg KL, Bell EF, Walsh MC, Schibler K, Laptook AR, Shane AL, Schrag SJ, Das A, Higgins RD: Early onset neonatal sepsis: the burden of group B Streptococcal and *E. coli* disease continues. Pediatrics 2011;127:817–826.

2 Okike IO, Ribeiro S, Ramsay ME, Heath PT, Sharland M, Ladhani SN: Trends in bacterial, mycobacterial, and fungal meningitis in England and Wales 2004–11: an observational study. Lancet Infect Dis 2014;14:301–307.

3 Remington J, Klein J, Wilson C, Nizet V, Maldonado Y: Infectious Diseases of the Fetus and Newborn Infant, ed 7. Philadelphia, Elsevier-Saunders, 2011.

4 Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Cousens S, Heath PT: Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet 2012;379:547–556.

5 Afshar B, Broughton K, Creti R, Decheva A, Hufnagel M, Kriz P, Lambertsen L, Lovgren M, Melin P, Orefici G, Poyart C, Radtke A, Rodriguez-Granger J, Sorensen UB, Telford J, Valinsky L, Zachariadou L, Efstratiou A: International external quality assurance for laboratory identification and typing of *Streptococcus agalactiae* (Group B streptococci). J Clin Microbiol 2011;49:1475–1482.

6 Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, Craig AS, Schaffner W, Zansky SM, Gershman K, Stefonek KR, Albanese BA, Zell ER, Schuchat A, Schrag SJ: Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. JAMA 2008;299:2056–2065.

7 Libster R, Edwards KM, Levent F, Edwards MS, Rench MA, Castagnini LA, Cooper T, Sparks RC, Baker CJ, Shah PE: Long-term outcomes of group B streptococcal meningitis. Pediatrics 2012;130:e8–e15.

8 Luck S, Torny M, d'Agapeyeff K, Pitt A, Heath P, Breathnach A, Russell AB: Estimated early-onset group B streptococcal neonatal disease. Lancet 2003;361:1953–1954.

9 Carbonell-Estrany X, Figueras-Aloy J, Salcedo-Abizanda S, de la Rosa-Fraile M: Probable early-onset group B streptococcal neonatal sepsis: a serious clinical condition related to intrauterine infection. Arch Dis Child Fetal Neonatal Ed 2008;93:F85–F89.

10 Bedford Russell AR, Breathnach A, Sender P: Confirmed group B streptococcus infection: the tip of the iceberg. Arch Dis Child Fetal Neonatal Ed 2001;84:F140.

11 UCSF Clinical and Translational Science Institute: Sample Size Calculators. San Francisco, CTSI, 2015.

12 Verani JR, McGee L, Schrag SJ: Prevention of perinatal group B streptococcal disease – revised guidelines from CDC, 2010. MMWR Recomm Rep 2010;59:1–36.

13 Hofer N, Zacharias E, Muller W, Resch B: An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. Neonatology 2012;102:25–36.

14 Connell TG, Rele M, Cowley D, Buttery JP, Curtis N: How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. Pediatrics 2007;119:891–896.

15 Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP: Volume of blood required to detect common neonatal pathogens. J Pediatr 1996;129:275–278.
16 Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD: Early-onset neonatal sepsis. Clin Microbiol Rev 2014;27:21–47.
17 Cohen J: The immunopathogenesis of sepsis. Nature 2002;420:885–891.
18 Curtis J, Kim G, Wehr NB, Levine RL: Group B streptococcal phospholipid causes pulmonary hypertension. Proc Natl Acad Sci U S A 2003;100:5087–5090.
19 Mohamed MA, Cunningham-Rundles S, Dean CR, Hammad TA, Nesin M: Levels of pro-inflammatory cytokines produced from cord blood in vitro are pathogen dependent and increased in comparison to adult controls. Cytokine 2007;39:171–177.
20 Berner R, Welter P, Brandis M: Cytokine expression of cord and adult blood mononuclear cells in response to *Streptococcus agalactiae*. Pediatr Res 2002;51:304–309.
21 Doran KS, Chang JC, Benoit VM, Eckmann L, Nizet V: Group B streptococcal β-hemolysin/cytolysin promotes invasion of human lung epithelial cells and the release of interleukin-8. J Infect Dis 2002;185:196–203.
22 Stroustrup A, Weintraub AS, Cadet CT, Perez R, DeLorenzo E, Holzman IR: Group B *Streptococcus* exposure and self-limited respiratory distress in late preterm and term neonates. Neonatology 2013;104:210–215.
23 Goossens H, Ferech M, Vander Stichele R, Elseviers M: Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 2005;365:579–587.
24 Malo S, Bjerrum L, Feja C, Lallana MJ, Abad JM, Rabanaque-Hernandez MJ: The quality of outpatient antimicrobial prescribing: a comparison between two areas of northern and southern Europe. Eur J Clin Pharmacol 2014;70:347–353.
25 Ancona RJ, Ferrieri P, Williams PP: Maternal factors that enhance the acquisition of group B streptococci by newborn infants. J Med Microbiol 1980;13:273–280.
26 Bergseng H, Rygg M, Bevanger L, Bergh K: Invasive group B streptococcus (GBS) disease in Norway 1996–2006. Eur J Clin Microbiol Infect Dis 2008;27:1193–1199.
27 Imperi M, Gherardi G, Berardi A, Baldassarri L, Pataracchia M, Dicuonzo G, Orefici G, Creti R: Invasive neonatal GBS infections from an area-based surveillance study in Italy. Clin Microbiol Infect 2011;17:1834–1839.
28 Persson E, Berg S, Trollfors B, Larsson P, Ek E, Backhaus E, Claesson BE, Jonsson L, Radberg G, Ripa T, Johansson S: Serotypes and clinical manifestations of invasive group B streptococcal infections in western Sweden 1998–2001. Clin Microbiol Infect 2004;10:791–796.
29 Brigtsen AK, Dedi L, Melby KK, Holberg-Petersen M, Radtke A, Lyng RV, Andresen LL, Jacobsen AF, Fugelseth D, Whitelaw A: Comparison of PCR and serotyping of Group B *Streptococcus* in pregnant women: the Oslo GBS-study. J Microbiol Methods 2015;108:31–35.