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Original Article

Estimating the burden of invasive Group B Streptococcal disease in young infants in southern mainland China: an observational study

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Abstract: Objectives: To estimate the incidence, case fatality ratio and serotypes associated with early-onset (EOD) and late-onset (LOD) invasive GBS disease in infants in southern mainland China. Methods: During the six-month study period, infants aged ≤ 90 days with culture-confirmed GBS disease born in the study hospitals or elsewhere, but presenting to a study hospital, were enrolled. GBS-positive cultures were genotyped, serotyped and sequence typed. The incidence rate was calculated for infants born in the study hospitals, and case fatality ratio and causative serotypes identified for all enrolled GBS cases. Results: Ten cases were enrolled: 2 EOD cases born in the study hospitals and 8 LOD cases born elsewhere. Incidence rate was 0.28 (95% confidence interval: 0.08-1.03, n = 2/7061 successfully followed-up consenting subjects); no cases resulted in fatality. In the 8 GBS isolates available for typing, 4 serotypes (Ia, Ib, III and V) and 5 multi-locus sequence types (1, 10, 12, 17 and 23) were identified. Conclusions: This is the first study specifically investigating the incidence of GBS invasive disease in infants in southern mainland China. Incidence and case fatality were low but further research is needed in larger, more diverse cohorts to estimate disease burden for the broader Chinese population.

Keywords: Group B Streptococcus, China, infants, incidence, case fatality, serotype

Introduction

Since the 1970s, *Streptococcus agalactiae*, or Group B Streptococcus (GBS), has been recognised as one of the major causes of neonatal sepsis and meningitis worldwide [1]. The majority of neonatal GBS disease occurs within the first week of life (early-onset disease; EOD), following vertical transmission from the colonised mother during delivery [2]. The introduction of routine screening for recto-vaginal colonisation in late pregnancy (35-37 weeks) and intra-partum antibiotic prophylaxis (IAP) administration at delivery has significantly reduced the incidence of EOD in countries where it has been implemented, such as the USA [3, 4]. However, there has been no impact on GBS disease in pre-term infants or on the incidence of disease after the first week of life (late-onset disease; LOD), which is related to both maternal and nosocomial transmission [5, 6]. To assess the impact of potential interventions in a country, such as different screening methods or future vaccines, a good understanding of the epidemiology and incidence of neonatal GBS disease in that country is vital.

Incidence rates of neonatal GBS disease vary globally, from an estimated mean incidence of 0.02 per 1000 live births in South East Asia to 1.97 per 1000 live births in South Africa and 2.60 per 1000 live births in Slovakia [7]. Reported case fatality ratios range from 1%-33% depending on the study population, and neurological sequelae are common, with up to 19% of survivors suffering severe impairment [8, 9]. There is some variation in serotype distribution globally. However, serotype III is the

dominant serotype in all regions, accounting for nearly half of all neonatal GBS cases worldwide, with serotype Ia being responsible for a further 23% of cases [7].

Despite being well documented in western countries, there is a lack of data on GBS incidence, case fatality, and serotype distribution data in certain regions, such as Asia [10, 11]. In China, there is no universal culture-based screening campaign as the incidence of neonatal GBS disease is thought to be very low, in line with the values reported by the few studies from other parts of Asia [12, 13]. However, GBS has been identified as one of the major causative agents of neonatal invasive disease in China [14-17], and neonatal mortality rates due to GBS infection as high as 20% have been reported in Asia [12, 18, 19]. This study aimed to assess the incidence rate, case fatality ratio and serotypes of early- and late-onset GBS disease in infants ≤ 90 days of age in southern mainland China.

Methods

Study design

This prospective observational study was conducted at two hospitals in Guangzhou and Changsha, China, between September 2013 and September 2014. Both hospitals are situated in major cities in southern mainland China. The hospital in Guangzhou is a large urban maternity and children's hospital which has approximately 16,000 births per year and a catchment area with a population of around five million people. The Changsha hospital, one of two major hospitals in the city with paediatric and maternity facilities, has approximately 7,000 births per year and a catchment of approximately 3.6 million people. The study was conducted in accordance with the principles of Good Pharmacoepidemiological Practice and the Declaration of Helsinki. The protocol was reviewed and approved by the Medical Ethical Committees of Guangzhou Women and Children's Medical Center and of Changsha Hospital for Maternal and Child Health prior to commencement of the study.

Subjects

Infants were enrolled into the study if their parents/legal guardians provided written informed consent and the infants met the inclusion criteria of being ≤ 90 days of age and having a positive GBS culture. Infants born outside the study hospitals but presenting to the study hospital who had positive GBS culture were also invite to enrol in the study if they met the inclusion criteria. Only cases born in the study hospitals were included for the estimation of incidence rate, however all enrolled cases were included for assessment of case fatality ratio and causative serotypes (see *Analysis* section below).

All infants born in the study hospitals during the six month study period were included in the "study hospital birth cohort". In order to increase case ascertainment, written informed consent for a telephone call 90 days after birth was requested from parents of all infants in the study hospital birth cohort who had not developed GBS disease prior to discharge, in order to identify any cases of GBS disease during this 90 day period that had not been presented to the study hospitals. Additionally, parents of babies who had been diagnosed with GBS and did not die in the study hospital were contacted at 90 days by phone to confirm the disease outcome.

Group B Streptococcus case identification was according to routine diagnostic standards in study hospitals: for any infant showing clinical signs or symptoms of sepsis, such as body temperature changes, breathing problems, bowel movement changes, reduced movements etc. (suspected GBS cases), blood and/or cerebrospinal fluid (CSF) samples were taken. The presence of GBS in samples was determined within routine internal hospital laboratory services using the BD BACTEC™ 9210 Culture System and API 20STREP (Guangzhou) and BD BACTEC™ 9120 Blood Culture System and CAMP test (Changsha).

Enrolment into the study occurred following culture confirmation of GBS at a study hospital. Following informed consent and enrolment, demographics and clinical case details were collected for each infant. Data included whether intrapatrum antibiotic prophylaxis (IAP) were used during delivery, date of birth, place of birth, district of residence, gestational age at birth (estimated by date of last menstruation), birth weight, gender, date of admission for GBS case, date of onset of illness, and GBS culture results.

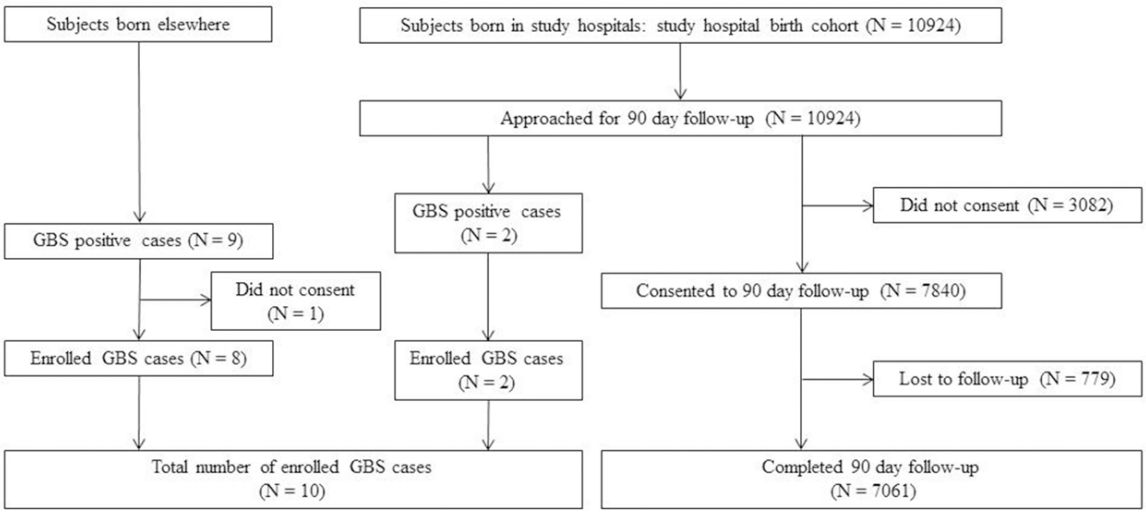

Figure 1. Flowchart of subject disposition and participation in the study.

GBS strain analysis

Isolates were stored and shipped for analysis to the Novartis central laboratory in Siena, Italy. GBS isolates were grown at 37°C in 5% CO_2 in Todd Hewitt Broth (Difco Laboratories) or in trypticase soy agar supplemented with 5% sheep blood. Serotyping was performed using Strep-B-Latex® rapid latex agglutination test kit (Statens Serum Institute, Hillerød, Denmark), according to the manufacturer's instructions. The capsular genotype was determined using a previously described multiplex PCR assay [20]. Multi-locus sequence typing (MLST) was performed by sequencing the internal fragments of seven house-keeping genes (adhP, pheS, atr, glnA, sdhA, glcK and tkt). Alleles and sequence types (ST) were determined using the S. agalactiae MLST website (http://pubmlst.org/sagalactiae/).

Study period and expected sample size

The study enrolment took place over a six month time period, plus a 90 day follow-up period. Assuming 20,000 live births during this period, and an incidence of 0.5 cases per 1000 live births, the expected number of cases was 10 and the 95% confidence intervals around the estimated incidence rates would be 0.24-0.92. As the actual GBS incidence rate in China is unknown, the number of cases could have varied from < 10 to > 30, based on the range of global GBS incidence estimates [7].

Bias

Selection bias and loss to follow up were seen as potential limitations for this study. To minimise the risk of infants with severe illness presenting to a non-study hospital, parents were informed of the study and the importance of returning to the study hospital prior to discharge post-delivery. The telephone call at 90 days aimed to increase case ascertainment. To maximise the number of infants with 90 day follow-up information, hospital staff were instructed to make at least three attempts to contact parents who had given consent over three consecutive days. In addition, to increase sensitivity of case detection, sites routinely collected and analysed culture specimens prior to antibiotic administration, as recommended by CDC [21].

Analysis

No formal statistical hypotheses were tested in this study. The incidence rate per 1000 live births was defined as the total number of confirmed GBS cases born in the study hospitals divided by the number of infants in the study hospital birth cohort who was successfully followed up at 90 days, all multiplied by 1000. Incidence rates were also calculated using the total study hospital birth cohort as a denominator, for sensitivity analysis. Case fatality ratio was calculated as the number of cases resulting in death divided by the number of enrolled

Table 1. Demographics of enrolled subjects by early-(EOD) and late-onset (LOD) disease. Percentages of the total number of cases are given in parenthesis

	EOD	LOD	Total
Number of subjects enrolled	2	8	10
Sex			
Male	1 (50%)	6 (75%)	7 (70%)
Female	1 (50%)	2 (25%)	3 (30%)
Median (range) age at admission, d	0.5 (0-1.0)	28 (8-75)	19 (1-75)
Median (range) birth weight, kg	3.2 (3.0-3.3)	3.5 (2.7-3.6)	3.3 (2.7-3.6)
Birth weight ≥ 2.5 kg	2 (100%)	7 (88%)	9 (90%)
Unknown birth weight	0 (0%)	1 (12%)	1 (10%)
Median (range) gestational age, weeks	37.5 (37.0-38.0)	39.0 (36.0-40.0)	38.0 (36.0-40.0)
Premature (34-36 weeks)	0 (0%)	2 (25%)	2 (20%)
Term (≥ 37 weeks)	2 (100%)	5 (63%)	7 (70%)
Unknown age	0 (0%)	1 (12%)	1 (10%)
IAP administration to mother			
Yes	0 (0%)	1 (13%)	1 (10%)
No	2 (100%)	4 (50%)	6 (60%)
Unknown	0 (0%)	3 (37%)	3 (30%)
Median (range) days hospitalised	24 (12-36)	27 (1-85)	27 (1-85)
Outcome at discharge			
Recovered	1 (50%)	2 (25%)	3 (30%)
Recovered with sequelae	1 (50%)	6 (75%)	7 (70%)

cases. For both the incidence rate and case fatality ratio, 95% confidence intervals (CI) were calculated using the Wilson interval method.

Results

In total, 10,924 infants were born in the two study hospitals during the six-month enrolment period. Of these, 7,840 (72%) parents/legal guardians provided written consent for the Day 90 follow-up telephone call, and 7,061 of the consenting parents/legal guardians (90%) were successfully contacted (**Figure 1**). The main reasons for not providing consent for participation were "do not want to be interrupted", "worried about the potential risk associated with participation in any study" and "no reason given". The 10% of parents/legal guardians who consented but could not be contacted did not answer the follow-up telephone calls despite three attempts.

Two infants born at the study hospitals (one at each site) had culture-confirmed GBS and were enrolled in the study. Eight additional subjects were enrolled as they were confirmed as GBS-positive at a study hospital but had been born elsewhere. An additional subject born elsewhere was confirmed GBS-positive but was not enrolled in the study as no consent was obtained.

Demographics of enrolled subjects

The two subjects born in the study hospitals were diagnosed with EOD, whereas all eight subjects born in other locations had LOD. Of the EOD cases, one infant was male and one was female, and both suffered disease onset within one day of birth (**Table 1**). Both of the infants were ≥ 37 weeks gestational age at delivery and weighed 2.5 kg or above. Neither of the mothers of the infants received IAP. One infant fully recovered and the other suffered sequelae during the study period.

The majority of the LOD cases were male (6 out of 8) and 63% (n = 5) were ≥ 37 weeks gestational age at delivery. Two infants were born prematurely, and the data for the other infant were not available. One mother received IAP prior to delivery; four infants were born to mothers who did not receive IAP and the IAP status of the other three mothers was unknown. The median age of GBS onset was 28.5 days (range: 8-75 days). Three-quarters of the infants with

Table 2. Serotype, genotype, multi locus sequence type (MLST) alleles and sequence type (ST) for each of the isolates from culture-confirmed GBS cases

Case no.	Born in study hospital?	Sample source	Onset of Disease	Serotype (Latex)	Genotype (PCR)	MLST alleles							Sequence type (ST)
						adhP	pheS	atr	glnA	sdhA	glcK	tkt	
1	Yes	blood	EOD	Ia	Ia	5	4	6	3	2	1	3	23
2	No	CSF	LOD	V	V	1	1	2	1	1	2	2	1
3	No	CSF	LOD	III	III	2	1	1	2	1	1	1	17
4	No	Blood	LOD	Ib	Ib	10	1	4	1	3	3	2	12
5	Yes	Blood	EOD	Ib	Ib	10	1	4	1	3	3	2	12
6	No	Blood	LOD	III	III	2	1	1	2	1	1	$1^{286}G\text{-}^{286}A$	NEW (17-like)
7	No	CSF	LOD	Ib	Ib	9	1	4	1	3	3	2	10
8	No	blood	LOD	Ia	Ia	5	4	6	3	2	1	3	23

Abbreviations: CSF = cerebrospinal fluid, EOD = early-onset disease (0 to < 7 days), LOD = late-onset disease (7-90 days).

LOD suffered sequelae, and the other two infants recovered without any sequelae recorded within the study period.

Incidence rate, case fatality ratio and serotypes of isolated strains

Based on the infants who were followed up at 90 days after birth, the estimated incidence rate was 0.28 per 1000 live births (2/7061 subjects), with a 95% CI of 0.08-1.03 per 1000 live births. Individual incidence rates for the study hospitals were 0.31 (95% CI: 0.05-1.75, 1/3230 subjects) and 0.26 (95% CI: 0.05-1.48, 1/3831 subjects) per 1000 live births for the Changsha and Guangzhou hospitals, respectively. The incidence rate calculated using the entire study hospital birth cohort as denominator (n = 10924) was 0.18 (95% CI: 0.02-0.67) per 1000 live births. Analysis of separate EOD and LOD incidence was not performed as there were only EOD cases reported at the study hospitals. As there were no GBS-positive subjects who died in the study, the case fatality ratio was 0.

Eight of the GBS-positive samples were available for serotyping/genotyping; samples from the other two cases were not retained and thus could not be serotyped. For all analysed strains, serotyping and genotyping were concordant. The two EOD cases (study hospital birth cohort) were identified as serotypes Ia and Ib, while serotypes Ia, Ib, III and V were found in LOD strains from cases born outside the hospitals (**Table 2**). MLST allele sequences and Sequence Types were identified for all eight samples, and all strains except one belonged to previously identified Sequence Types present in the GBS MLST database (**Table 2**).

Discussion

This is the first study estimating the incidence and case fatality ratio of GBS invasive disease in infants ≤ 90 days of age in southern mainland China. To date, there have been very few studies on neonatal GBS disease carried out in Asia, and the incidence rate estimates have been quite variable. For example, in a recent study of a 400,000 infant cohort in Japan, the incidence rate of EOD and LOD were estimated to be 0.08 and 0.10 per 1000 live births, respectively [12]. Similarly, a study in Thailand estimated incidence rates to be relatively low (0.10-0.27 per 1000 live births for EOD, and 0.05 per 1000 live births for LOD) [13]. In contrast, a much higher incidence rate was found in studies in Taiwan (1 per 1000 live births) and Hong Kong (1.13 per 1000 live births) [22, 23]. The variability in reported incidence between studies may reflect population differences, differences in access to care, clinical sepsis case identification, or protocols for specimen collection and microbiological methods in some of these countries [10, 11]. In addition, differences in caesarean section rates, antibiotic usage, serotype distribution and invasiveness, or uncertainty in incidence rate estimates may all contribute to variation in reported incidence rates [7, 11, 24].

The incidence rate in the current study (0.28 per 1000 live births) may suffer from under-ascertainment bias as 28% of parents did not consent for the active follow-up call, meaning that some GBS cases may have been missed if these parents presented a sick infant to a non-study hospital, (e.g. at another hospital with paediatric facilities, which may have been the case in Changsha where there are other tertia-

ry facilities available, but less was likely in Guangzhou as the study hospital was the main tertiary facility for the city) or moved away from the area.

In China, it has been reported that a high percentage of women give birth by caesarean section (27-55%) [24, 25]. Although the percentage of caesarean sections was not recorded in this study, antibiotics were administered to 38% of women who received a caesarean section in study hospitals during the study period, which may have reduced the incidence of GBS invasive disease in the study population. Alternatively, as maternal colonisation is the main risk factor for infant invasive GBS disease, there may be a lower GBS colonisation than is observed in Europe and the US, where incidence is higher [2]. The reported colonisation rates for pregnant women in Europe and the US are 20-30% [26, 27]. In contrast, two studies of pregnant women in Beijing reported colonisation rate of 6.5% and 7% [14, 28], and a previous study of multiple sites in China found that 19% were colonised [29]. As colonisation was not assessed in the current study, future research is required to gain a reliable estimate of the influence of maternal colonisation on infant GBS invasive disease incidence in China.

Similarly, the case fatality ratio was much lower than expected from other studies. Worldwide, the mean case fatality ratio for EOD and LOD is estimated to be 10% [7]. Reports from other Asian countries estimate case fatality to be 2.5% to 40%, depending on the health care setting [12, 13, 22]. The two study hospitals included in the present study have previously reported low GBS fatality ratios, with the Guangzhou hospital reporting a case fatality ratio of 2.8% for GBS from 2011-2014 (Guangzhou Women and Children's Medical Center-unpublished data). All the GBS-positive infants enrolled in this study were born at a minimum of 36 weeks gestation and all were normal weight, which may have increased the likelihood of survival of the disease. In addition, the specialist paediatric care and timely use of high dose, wide spectrum antibiotics may have also reduced the case fatality ratio for the current study, and therefore this may not be representative of China as a whole. Finally, the small sample size adds greater uncertainty to the current case fatality ratio estimates; given that the mean case fatality ratio worldwide has been estimated as 9.6%, it is not unexpected that there were no deaths out of the 10 cases in this study [7].

EOD is generally considered to be more prevalent than LOD [3, 30], however there was a much higher proportion of LOD cases (80%) than EOD cases in our study. It is possible that some EOD cases may have been misclassified if parents first presented their sick infant to a non-study hospital for treatment, and later transferred to the study hospitals. However, as EOD frequently presents within the first 48 hours of life, it is likely that these cases would have been identified and treated in the hospital in which the infant was born prior to discharge, and only LOD cases would present to the study hospitals post-discharge. As most of the infants with LOD in this study were more than two weeks old when diagnosed, it is unlikely that these were misclassified EOD cases. Some other studies in Asia have also reported a higher proportion of LOD cases (65% of cases in Japan [12], 80% in Korea [18]), although this was not the case for other studies in Taiwan, Thailand and Hong Kong [13, 22, 23]. In order to interpret these differences, a better understanding of the differences in health care settings in these countries (e.g. antibiotic usage and caesarean section rate) is needed.

Due to the low number of cases in this study, it would be inappropriate to extrapolate any findings about the distribution of serotypes causing GBS from this study to a population level. The four serotypes isolated are known to be four of the five predominant disease-causing serotypes, which account for nearly 90% of infant invasive GBS disease worldwide [7], although the frequency of Ib cases was higher than that reported in other studies, particularly for LOD. Additionally, the identified sequence types were similar to those associated with neonatal GBS disease in other studies, although we found only two LOD cases associated with serotype III ST-17, which causes the majority of LOD in developed countries [31-34]. There were also no cases of the rarer serotypes reported (e.g. serotype VI), in contrast to recent studies in the Philippines, Thailand and Japan [35, 36].

Further research is needed to accurately assess the incidence and case fatality for GBS disease in infants across the whole of China, incorporating more diverse cohorts of the pop-

ulation. In addition to a larger, more widespread survey, mechanisms would need to be implemented to closely monitor potential limiting factors, such as antibiotic administration and loss to follow-up, which could influence the results. A larger cohort would also allow for more reliable measures of serotype distribution and assessment of the overall burden of GBS in this part of China.

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Disclosure of conflict of interest

IM, CDR, TS, MC, WJJ and GGZ were all permanent employees of Novartis group of companies at the time of the study. Following the acquisition of Novartis non-influenza vaccines business by GlaxoSmithKline Plc on 2nd March 2015, IM, CDR, MC, WJJ and GGZ are now employees of the GSK group of companies. IM holds stock options in Novartis and GSK; MC has shares in GSK. All other authors have no conflicts of interest to declare.

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