International Journal of Infectious Diseases 37 (2015) 115–118

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Serotype distribution, antimicrobial resistance, and molecular characterization of invasive group B Streptococcus isolates recovered from Chinese neonates

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ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form 19 June 2015
Accepted 25 June 2015

Keywords:
Group B Streptococcus isolates
Serotype distribution
Microbial drug resistance
Newborn infant

SUMMARY

Background: Group B Streptococcus (GBS) is an important neonatal pathogen associated with high morbidity and mortality in developed countries. However, data describing neonatal GBS disease in developing countries, particularly in Asia, are largely incomplete. The aim of this study was to determine the serotype distribution, antimicrobial resistance, and molecular characteristics of invasive GBS isolates recovered from Chinese neonates.

Methods: From 2008 to 2013, 40 GBS isolates were recovered from infected neonates less than 3 months of age. All isolates were identified with the CAMP test and commercially available techniques. Serotyping was performed by latex agglutination. Antibiotic susceptibility was tested with Etest strips and the disk diffusion method. Multilocus sequence typing and erythromycin resistance gene detection (*erm*B and *mef*A) were performed by PCR.

Results: Four serotypes were identified. Serotype III (85%) was the most prevalent, followed by Ia (7.5%), Ib (5%), and V (2.5%). All isolates were sensitive to penicillin, ceftriaxone, and levofloxacin. However, resistance to erythromycin (92.5%), clindamycin (87.5%), and tetracycline (100%) was observed. Among erythromycin-resistant isolates, 73.0% carried the *erm*B gene alone, 5.4% carried the *mef*A gene alone, and 21.6% expressed both *erm*B and *mef*A genes. A total of seven sequence types (STs) were identified; the most prevalent was ST17, accounting for 80% of all isolates. Further, serotype III isolates contained ST17 (94.2%), ST19 (2.9%), and ST650 (2.9%).

Conclusion: Serotype distribution, antimicrobial susceptibility, and sequence type characterization in Asia and in other global regions may contribute to improve the prevention and treatment of neonatal GBS infections.

Key points

Group B Streptococcus isolates recovered from neonates at two Chinese hospitals were predominantly serotypes III and Ia.

All isolates were sensitive to penicillin; however, a large proportion exhibited multidrug resistance to erythromycin, clindamycin, and tetracycline.

1. Introduction

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is a Gram-positive bacterium that is a leading causative pathogen of severe and invasive neonatal infections such as meningitis and sepsis, which are often associated with high morbidity and mortality. In neonates, a GBS infection occurs as one of two distinct types. Early-onset disease (EOD) is characterized by the onset of symptoms during the first week of life (0–6 days), while late-onset disease (LOD) is characterized by the onset of symptoms up to 3 months of age (7–89 days).[1] An invasive GBS

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http://dx.doi.org/10.1016/j.ijid.2015.06.019

infection is defined as GBS isolated from a normally sterile site such as blood or cerebrospinal fluid (CSF), or, less commonly, joint, pleural, or pericardial fluid.[2]

Serotype distribution is critical to understanding the epidemiology of GBS infections. Currently, a total of 10 distinct GBS serotypes (Ia, Ib, and II–IX) have been characterized according to the capsular polysaccharide (CPS), one of the major known virulence factors underlying invasive GBS disease.[3] The most common serotypes, Ia, Ib, II, III, and V, account for more than 85% of serotypes in global regions that have reported serotype data, including the Americas (96%), Europe (93%), and the Western Pacific (89%).[4] A substantial proportion of EOD isolates and the majority of LOD isolates are serotype III; this serotype is largely responsible for invasive GBS disease.[5]

Recently, the incidence of neonatal GBS EOD in Australia, New Zealand, and the USA has decreased putatively because of the implementation of intrapartum antibiotic prophylaxis (IAP).[6,7] In developing countries such as China, however, neonatal GBS disease remains problematic and has not been addressed adequately. Only a few cases have been reported.

Widespread IAP may be disadvantageous to both mother and child as it can cause anaphylaxis and/or contribute to the development of antibiotic-resistant strains. To address these concerns, vaccination of pregnant women remains an attractive prospect for the prevention and treatment of GBS infections. To provide a basis for the application of effective clinical antimicrobial agents and the development of a GBS vaccine, the serotype distribution, antibiotic susceptibility profiles, and molecular features of invasive GBS isolates recovered from a cohort of neonates in mainland China were examined.

2. Methods

2.1. Bacterial strains and serotype identification

Forty non-redundant, invasive GBS isolates collected from 2008 to 2013 were analyzed in this study. These included 36 isolates recovered from neonates at Shenzhen Children's Hospital (Shenzhen, China) and four isolates recovered from neonates at Beijing Obstetrics and Gynecology Hospital (Beijing, China). Bacteria were cultured on sheep blood plates and confirmed by CAMP test and a commercially available streptococcal grouping kit according to the methods described by Wang et al.[8] Serotype identification was performed with a latex agglutination kit (Statens Serum Institute, Denmark) as described previously.[9] This study protocol was in accordance with the ethical standards of the responsible regional committee on human experimentation and the Declaration of Helsinki of 1975 (revised in 1983). It was approved by the ethics committees at Shenzhen Children's Hospital and Beijing Obstetrics and Gynecology Hospital. Informed written consent was obtained from the care providers of all neonates enrolled in this study.

2.2. Antimicrobial susceptibility tests

The minimum inhibitory concentrations (MICs) of penicillin, ceftriaxone, erythromycin, clindamycin, clarithromycin, levofloxacin, and azithromycin were determined using Etest strips (AB Biodisk, Sweden). Susceptibilities to telithromycin and tetracycline were determined by disk diffusion method (Oxoid). The breakpoints adopted were in accordance with the 2012 criteria set by the Clinical and Laboratory Standards Institute (CLSI). The breakpoint for telithromycin was determined by comparison with the quality control culture *Streptococcus pneumoniae* ATCC 49619. GBS isolates were considered multidrug-resistant if they were not susceptible to three or more classes of antimicrobials tested in this study.

All antibiotic susceptibility data were analyzed using WHONET 5.6 software, as recommended by the World Health Organization (WHO).

2.3. Detection of erythromycin resistance genes

Chromosomal DNA was extracted from overnight cultures of GBS isolates grown on sheep blood agar plates containing 5% defibrinated sheep blood using a DNA Mini Kit (SBS Genetech, China). PCR was used to detect the erythromycin resistance genes *erm*B and *mef*A in all erythromycin-resistant GBS isolates using primers and reaction conditions described previously.[10] A total of 5 μl each PCR product was analyzed by electrophoresis on a 1.5% agarose gel, stained with gold view (SBS, Beijing, China), and visualized by UV transillumination.

2.4. Multilocus sequence typing (MLST)

MLST analyses were based on the amplification and sequencing of internal fragments within seven housekeeping genes (*adh*P, *phe*S, *atr*, *gln*A, *sdh*A, *glc*K, and *tkt*). Primer pairs corresponding to these genes were designed as described previously.[11] Sequencing results were submitted to the GBS MLST website (http://pubmlst.org/sagalactiae/) to allow for allelic assignment and to determine sequence type (ST).

3. Results

The collection for this study comprised 40 GBS isolates recovered from 40 different neonatal patients with invasive infections. Of these, 10 neonates were diagnosed with EOD and were symptomatic within the first week after birth (0–6 days), while 30 neonates were diagnosed with LOD and were symptomatic between 7 and 89 days after birth. The majority of these GBS infection diagnoses were sepsis, pneumonia, and meningitis.

3.1. Serotype distribution

A total of four serotypes were identified in this study. Overall, the predominant serotype was III, accounting for 85% of all isolates, followed by Ia (7.5%), Ib (5%), and V (2.5%). A significantly greater proportion of serotype III isolates was observed in neonates with LOD (94%) when compared to those with EOD (60%). The other identified serotypes (Ia, Ib, and V) were more likely to cause EOD. Three different serotypes were identified in the 36 isolates recovered at Shenzhen Children's Hospital: 33 isolates were serotype III, two isolates were serotype Ib, and one isolate was serotype Ia. The distribution of isolates recovered from Beijing Obstetrics and Gynecology Hospital was as follows: two isolates were serotype Ia, one isolate was serotype III, and one isolate was serotype V.

3.2. Antimicrobial susceptibility

The antimicrobial susceptibility and MIC results for nine antimicrobial agents tested against all 40 GBS isolates are presented in Table 1. All isolates were susceptible to penicillin, ceftriaxone, levofloxacin, and telithromycin; and all were resistant to tetracycline. In contrast, 95%, 90%, 97.5%, and 100% of the 40 GBS isolates were non-susceptible to erythromycin, clindamycin, clarithromycin, and azithromycin, respectively. Approximately 90% (36/40) were multidrug-resistant, and these isolates exhibited co-resistance to erythromycin, clindamycin, and tetracycline.

Table 1
Antimicrobial susceptibility and MICs of group B Streptococcus isolates

Antimicrobials	Susceptibility			MIC[a]		
	S (%)	I (%)	R (%)	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Range (mg/l)
Penicillin	100	0	0	0.064	0.094	0.032–0.125
Ceftriaxone	100	0	0	0.125	0.19	0.047–0.5
Erythromycin	5	2.5	92.5	≥256	≥256	0.125–≥256
Clindamycin	10	2.5	87.5	64	≥256	0.094–≥256
Clarithromycin	2.5	5	92.5	≥256	≥256	0.25–≥256
Levofloxacin	100	0	0	1	1.5	0.5–2
Azithromycin	0	2.5	97.5	≥256	≥256	1–≥256
Telithromycin	100%	0	0	-[b]	-[b]	-[b]
Tetracycline	0	0	100	-[b]	-[b]	-[b]

MIC, minimum inhibitory concentration; S, susceptible; I, intermediate; R, resistant.
[a] MIC_{50}, minimum inhibitory concentration at which 50% of isolates were inhibited; MIC_{90}, minimum inhibitory concentration at which 90% of isolates were inhibited; MIC range, range of minimum inhibitory concentration.
[b] No data available for the disk diffusion test.

3.3. Detection of macrolide resistance genes

A total of 37 isolates were resistant to erythromycin. Of these, 27 (73.0%) harbored the *erm*B gene alone, two (5.4%) isolates harbored the *mef*A gene alone, and eight (21.6%) isolates harbored both *erm*B and *mef*A.

3.4. MLST

MLST analyses revealed seven different STs among the 40 GBS isolates. ST17 was the predominant ST; this accounted for 80% of all isolates. ST650 represents a new ST identified in this study. Relationships between STs and GBS serotypes of the 40 isolates characterized in this study are presented in Table 2. A correlation between serotype III and ST17 was evident, as ST17 accounted for 94.1% of all serotype III isolates. The other two serotype III isolates belonged to ST19 and ST650.

4. Discussion

Reported data on invasive neonatal GBS infections in Asia are sparse. To address this knowledge gap, a study that examined serotype distribution, antimicrobial resistance, and molecular characterization of invasive GBS isolates recovered from neonatal patients at two different hospitals situated in mainland China was performed.

In this study, it was found that neonatal GBS infections were most commonly caused by serotypes III and Ia, and this finding concurs with a previous report from the USA.[12] However, the data differ from the results of a previous study comprising 1500 pregnant Chinese women that was performed in the 1990s, wherein the reported serotype distribution was 36% serotype II, 26% serotype III, and 18% serotype Ia, and neonates born from this cohort were colonized with a serotype distribution of 13.5% Ia, 9.5% II, 11.5% III, and 57% NT (non-typeable).[13–16] Additionally, it was found in the present study that serotype III and Ia isolates were responsible for 60% and 10% of EOD, as well as 94% and 3% of LOD, respectively. These results differ from those of a previous report from France in which serotypes III and Ia were recovered in 54% and 24% of EOD, and 82% and 7% of LOD, respectively.[17] It is hypothesized that serotypes responsible for GBS infection differ globally, and that these serotypes may vary over time. Therefore, active investigation of serotype distribution in China and Asia, as well as other global regions, should precede vaccine development.

Table 2
Relationships between sequence type and serotype in group B Streptococcus isolates

Sequence type	Serotype				
	III (%)	Ia (%)	Ib (%)	V (%)	Total (%)
ST17	32	0	0	0	32 (80)
ST23	0	2	0	0	2 (5)
ST12	0	0	2	0	2 (5)
ST24	0	1	0	0	1 (2.5)
ST19	1	0	0	0	1 (2.5)
ST1	0	0	0	1	1 (2.5)
ST650	1	0	0	0	1 (2.5)
Total (%)	34 (85)	3 (7.5)	2 (5)	1 (2.5)	40 (100)

ST, sequence type.

A retrospective study was performed of 200 children with fatal neonatal pneumonia who died between 1953 and 2004, with 34 fatal neonatal cases without any infectious disease serving as a control group. Paraffin-embedded lung tissues were collected for total genomic DNA extraction, and PCR and Southern blot analyses were used for GBS detection and molecular serotyping. It was demonstrated that the positive GBS rate of the pneumonia group was significantly higher than that of the control group (PCR: 26% vs. 3% ($p < 0.01$); Southern blot: 65% vs. 18% ($p < 0.01$)). The positive rate in neonates aged younger than 7 days was significantly higher than that in neonates aged older than 7 days in the pneumonia group. The isolates of 22 GBS-positive neonates were serotypeable. Of these, seven were identified as serotype Ia, six isolates were serotype III, five were serotype II, and one was serotype Ib.[18] These results suggest that GBS is an important pathogen in fatal neonatal pneumonia in China, particularly in EOD. No further data on this phenomenon appear to have been reported.

In this study, all isolates were susceptible to penicillin and ceftriaxone. These data suggest that penicillin should be the first choice for antibiotic prophylaxis and treatment of GBS infections, as recommended previously.[19] Notably, the MIC of penicillin in 50% of strains was ≥0.094 mg/l, which is higher than the maximum MIC (≤0.06 mg/l) reported in a previous study by this group.[20] GBS isolates with reduced penicillin susceptibility have also been reported in Japan[21] and Hong Kong.[22] Further, all GBS isolates tested in this study were sensitive to levofloxacin; however, fluoroquinolone-resistant GBS clinical isolates, mainly belonging to serotype III/ST19, have been reported previously.[23] The incidence of erythromycin resistance was 92.5%. This is higher when compared to Taiwan (58.3%) and Western countries, with reported resistance ranging from 11.5% to 32%.[24–27] Last, the non-susceptible rate to clindamycin reported here is higher than that in Korea (54.0%)[28] and the USA (38.4%).[29]

MLST allowed for the comparison of genetic profiles of isolates recovered from various geographic areas. In the present study, ST17 was most prevalent – as high as 80.0%. Interestingly, it has been suggested previously that ST17 isolates have unique characteristics independent of *cps* type that contribute to more invasive disease.[30]

In summary, the data from this study provide important epidemiological information on invasive GBS isolates recovered from neonates in China. The most prevalent serotype III /ST17 clone exhibited increased resistance to clindamycin and erythromycin. This study contributes to the basic knowledge required for successful GBS vaccine development suited for disease prevention and treatment in China, as well as the implementation of effective clinical antimicrobials.

Acknowledgements

The authors sincerely thank all staff who took part in this study, in particular co-workers at Shenzhen Children's Hospital, for their valuable assistance in collecting the GBS isolates.

Funding: This work was supported by the Research Funds of Profession Quota Budget administered by the Beijing Municipal Science and Technology Commission (2015-bjsekyjs-3).

Ethical approval: The study protocol was in accordance with the ethical standards of the responsible regional committee on human experimentation and the Declaration of Helsinki of 1975 (revised in 1983). It was approved by the ethics committees of the two hospitals, Ethics Committee of Beijing Obstetrics and Genecology Hospital and Ethics Committee of Shenzhen Children's Hospital.

Conflict of interest: No conflict of interest for all authors.

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