Diagnostic Microbiology and Infectious Disease 92 (2018) 56–61

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Characterization of group B *Streptococcus* isolated from sterile and non-sterile specimens in China

Shuping Nie [a,b], Xuedong Lu [a], Zhengjiang Jin [c], Juyi Gao [d], Dongli Ma [e], Jikui Deng [f], Xianglin Wu [g], Yan-Wei Hu [b], Lei Zheng [b], Qian Wang [b,*]

[a] Department of Laboratory Medicine, The Eighth Affiliated Hospital of Sun Yat-Sen University, Shenzhen, China
[b] Laboratory Medicine Centre, Nanfang Hospital, Southern Medical University, Guangzhou, China
[c] Department of Laboratory Medicine, Hubei Provincial Maternity & Child Healthcare Hospital,Wuhan, China
[d] Department of Laboratory Medicine, The university of Hong Kong-Shenzhen Hospital, Shenzhen, China
[e] Department of Laboratory Medicine, Shenzhen Children's Hospital, Shenzhen, China
[f] Department of Infectious Disease, Shenzhen Children's Hospital, Shenzhen, China
[g] Department of Laboratory Medicine, Shenzhen Guangming New District People's Hospital, Shenzhen, China

ARTICLE INFO

Article history:
Received 22 September 2017
Received in revised form 29 April 2018
Accepted 30 April 2018
Available online 16 May 2018

Keywords:
Group B *Streptococcus*
Multilocus sequence typing
Serotyping
Clonal complex
Virulence factor

ABSTRACT

Group B *streptococcus* (GBS) is a leading cause of invasive neonatal infections and has increasingly been associated with invasive diseases in non-pregnant adults. We collected 113 GBS isolates recovered from sterile and non-sterile specimens from seven tertiary hospitals in China between October 2014 and September 2016. Medical records were retrospectively reviewed and the sequence types, serotypes, virulence, and antimicrobial resistance profiles of the isolates were characterized and correlated. Significantly higher C-reactive protein and procalcitonin levels and absolute neutrophil counts were observed in patients with invasive infections than in those with non-invasive infections ($P < 0.05$). The 113 isolates were grouped into 24 sequence types, 5 clonal complexes, and 6 serotypes. multivariate analysis revealed that clonal complex 17 isolates characterized by serotype iii, the surface protein gene *rib*, and the pilus island pi-2b were independently correlated with invasive infection (or: 6.79; 95% ci: 2.31–19.94, $P < 0.001$). These results suggest alternative molecular biomarkers for diagnosis and prognosis of GBS infections.

1. Introduction

Group B *Streptococcus* (GBS or *Streptococcus agalactiae*) is a primary cause of neonatal sepsis and meningitis and is increasingly being associated with invasive infections in men and non-pregnant women (Edmond et al., 2012; Phares et al., 2008; Skoff et al., 2009). Continuous surveillance and epidemiological studies provide a basis for prevention and treatment of clinical GBS infections. Molecular epidemiological studies utilizing multilocus sequence typing (MLST) have the advantages of high discriminatory power and data portability (Manning et al., 2009).

Although GBS isolates with reduced susceptibility to penicillin have been reported, penicillin remains the first-line agent for the prevention and treatment of GBS infections (Kimura et al., 2011; Longtin et al., 2011). Clindamycin and erythromycin are recommended alternatives, but increased resistance to these agents is raising concerns (Garland et al., 2011; Lu et al., 2014). Macrolide resistance in GBS is primarily mediated by 23S rRNA methylation via the *ermB* and *ermA* genes or by active drug efflux pumps encoded by the *mefA/E* genes. GBS resistance to tetracycline is also common and is frequently associated with the *tetM* and *tetO* genes (Culebras et al., 2002). Importantly, tetracycline and macrolide resistance genes are often found on the same mobile genetic elements(Varaldo et al., 2009).

The polysaccharide capsule is one of the most important GBS virulence factors. The capsular serotype contributes to disease severity and is a major focus of vaccine development. Ten GBS capsular serotypes have been identified, including Ia, Ib, and II–IX, and vaccinations against five serotypes (Ia, Ib, II, III, and V) are currently in development (Melin and Efstratiou, 2013). In addition to the capsule, GBS possesses a pilus-like structure that mediates adhesion and permits host cell invasion (Lauer et al., 2005). Three pilus island (PI) alleles (PI-1, PI-2a, and PI-2b) have been described and their proteins are being explored as vaccine candidates (Margarit et al., 2009). Proteins belonging to the α-like surface protein (Alp) family also play an important role in GBS pathogenesis and are potential vaccine candidates (Lindahl et al., 2005). Alp family members are encoded by *alpha-C* (*bca*), *epsilon* (*eps*), *alp2/3*, *rib*, and *alp4* genes and are located on pathogenicity island IV. β protein, another virulence factor encoded by the *bac* gene, combines with α protein to form the C antigen (Lindahl et al., 2005). In

* Corresponding author. Tel.: +86-20-61641888; fax:+86-20-61641888.
E-mail address: 793499131@qq.com (Q. Wang).

https://doi.org/10.1016/j.diagmicrobio.2018.04.014
0732-8893/

addition, numerous other virulence factors, including C5a peptidase (ScpB), β-hemolysin/cytolysin (CylE), hyaluronidase (HylB), and laminin-binding protein (Lmb), are also involved in host adhesion and invasion as well as in immune system evasion (Rajagopal, 2009).

This study was conducted to characterize the sequence type (ST), serotype, virulence, and antimicrobial resistance of GBS isolates and to compare the clinical features of invasive and non-invasive GBS isolates. These data may provide a useful basis for the prevention of invasive GBS infections.

2. Materials and methods

2.1. Patients and clinical variables

This was a retrospective study. Medical records were retrospectively reviewed for all non-pregnant inpatients that were admitted between October 2014 and September 2016 to the following seven tertiary hospitals: Nanfang Hospital (Guangzhou, China), The Eighth Affiliated Hospital of Sun Yat-sen University (Shenzhen, China), Shenzhen Children's Hospital (Shenzhen, China), Hubei Provincial Maternity and Child Healthcare Hospital (Wuhan, China), University of Hong Kong-Shenzhen Hospital (Shenzhen, China), Shenzhen People's Hospital (Shenzhen, China), and Shenzhen Guangming New District People's Hospital (Shenzhen, China). This study was approved by the Institutional Review Board of each study center and informed consent was waived due to the retrospective nature of this study.

Inclusion criteria were as follows: (1) non-pregnant patients with a positive culture of GBS and a discharge diagnosis of GBS infection, and (2) pathogen cultures, C-reactive protein (CRP), procalcitonin (PCT), and complete blood count measurements were performed at admission before the use of antimicrobials. The following data were retrieved from patients' medical records: demographic characteristics, clinical presentations, and laboratory findings, including total white blood cell counts (WBC), absolute neutrophil counts (ANC), CRP, and PCT. Patient characteristics are summarized in Table 1.

We defined invasive GBS infection as the laboratory isolation of GBS from a normally sterile site, such as blood, cerebrospinal fluid, peritoneal fluid, or synovial fluid, accompanied by any signs of clinical disease, such as sepsis, pneumonia, or meningitis (Edmond et al., 2012). Non-invasive infection was defined as a positive culture from a non-sterile site, such as urine, tracheal and gastric aspirates, wound and skin ulcers, or male genital tract discharge from non-sepsis/meningitis patients.

2.2. Bacterial isolates

A total of 113 GBS isolates recovered from sterile sites (blood, CSF, synovial fluid and peritoneal fluid) (45.1%) and non-sterile sites (urine, sputum, gastric aspirates, wound secretions and male genital tract discharges) (54.9%) were collected for this study. All isolates were confirmed to be GBS using Gram staining, CAMP testing, and the Vitek-2 Compact Microbiology System (bioMérieux, Marcy-l'Étoile, France).

2.2.1. Molecular typing, capsule serotyping, and virulence-associated gene detection

MLST was conducted by sequence analysis of seven reference genes as previously described (Jones et al., 2003). The *Streptococcus agalactiae* MLST online database (http://pubmlst.org/sagalactiae/) was used to assign allele numbers and STs. The sequences of the seven loci obtained were concatenated and used to build a phylogenetic tree using the MEGA6 program with the unweighted pair group method with arithmetic mean (UPGMA) method (Manning et al., 2009). STs that grouped together with >80% bootstrap support were considered part of the same GBS clonal complex (CC). STs that were not part of a CC were classified as singletons. The Simpson index of diversity (SID) was calculated for GBS isolates recovered from sterile and non-sterile sites as previously described (Grundmann et al., 2001). GBS capsular serotypes (Ia, Ib, and II-IX) were determined using multiplex PCR as previously described (Imperi et al., 2010). Isolates that failed to type were considered non-typeable. PCR assays were performed to identify the PI-1, PI-2a, or PI-2b genes using primer pairs as previously described (Springman et al., 2014). The presence of Alp genes (*bca*, *eps*, *rib*, *alp*2/3, and *alp*4) and five other virulence genes (*bac*, *lmb*, *hylB*, *cylE*, and *scpB*) was confirmed with PCR using previously described primers (Creti et al., 2004; Park et al., 2012). Virulence genes were detected by direct evaluation of amplicon sizes.

Table 1
Characteristics of patients with group B *Streptococcus* isolates recovered from sterile and non-sterile sites.

	Sterile	Non-sterile	*P* value[a]
City sites, n (%)			<0.001
Shenzhen	47 (92.2)	36 (58.1)	
Guangzhou and Wuhan	4 (7.8)	26 (41.9)	
Gender, n (%)			NS
Female	28 (54.9)	34 (54.8)	
Male	23 (44.2)	28 (45.2)	
Age, n (%)			<0.001
<7 days	12 (23.5)	24 (38.7)	
7−89 days	23 (45.1)	4 (6.5)	
+90 days	16 (31.4)	34 (54.8)	
Collection site (n)	Blood (41), Cerebrospinal fluid (4), Synovial fluid (3), Peritoneal fluid (3)	Urine (24), Sputum (21), Gastric aspirates (8), Wound secretions (5), Male genital tract discharges (4)	
Manifestation (n)	Sepsis (37), Septic arthritis (3), Pneumonia (4), Meningitis (4), Peritonitis (3)	UTI (24), Pneumonia (6), Bronchitis (4), Neonatal asphyxia (5), Premature infant (6), NRDS (8), GTI (4), Other[b] (5)	
Initial laboratory findings, median (IQR)			
WBC, 10^9/L	14.41 (9.90−18.26)	10.03 (6.77−16.44)	NS
ANC, 10^9/L	9.28 (4.68−13.61)	5.79 (3.40−11.48)	0.027
hs-CRP, mg/L	29 (8.00−61.10)	4 (1.00−22.4)	<0.001
PCT, ng/mL	11.6 (3.94−35.00)	0.12 (0.05−0.78)	<0.001
Other pathogens	*Candida albicans* (1), *Pseudomonas aeruginosa* (1)	*Klebsiella pneumoniae* (3), *Escherichia coli* (2), *Stenotrophomonas maltophilia* (1), *Staphylococcus epidermidis* (1), *Staphylococcus aureus* (1), *Haemophilus influenzae* (1), *C. albicans* (1)	NS

ANC = absolute neutrophil counts; GTI = genital tract infection; hs-CRP = high-sensitivity C-reactive protein; IQR = interquartile range; NRDS = neonatal respiratory distress syndrome; NS = not significant; PCT = procalcitonin; UTI = urinary tract infection; WBC = white blood cell count.

[a] *P* values <0.05 were considered statistically significant.

[b] Aural fistula infection and skin ulcer.