Microbial Pathogenesis 99 (2016) 119–122

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

Characterization of virulence factors, antimicrobial resistance pattern and clonal complexes of group B streptococci isolated from neonates

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ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form
16 July 2016
Accepted 18 August 2016
Available online 20 August 2016

Keywords:
GBS
CC19
Neonates
Resistance
Virulence gene

ABSTRACT

Between January and December 2013, swab samples were taken for the throat and external ear canals of 1037 newborns for screening of Group B Streptococcus (GBS or *S. agalactiae*). Isolates were analyzed form Multilocus sequence typing (MLST), capsular type, virulence genes and antibiotic susceptibility. The MLST analysis of 19 GBS isolates showed 8 sequence types (STs). Overall the most common STs were ST19 and ST28. Other STs were ST1, ST4, ST8, ST12, ST335 and ST734 (a new ST). The most common clonal complexes (CCs) were CC19 (68.4%) and CC10 (21%). The *scpB*, *hlyB* and *bca* virulence genes were detected in all ST_S, while the *bac* gene was predominant in ST12 with capsular type (CT) Ib. The IS*1548* and the *rib* genes were particularly prevalent in CTIII and were detected in isolates belong to ST19, ST335 and ST734 and were grouped in CC19. All isolates were susceptible to penicillin, vancomycin, linezolid and quinupristin-dalfopristin. Resistance to tetracycline was observed in all 19 (100%) strains and was correlated with presence of the *tetM* gene except for one isolate with ST12. All the ST8 and ST12 isolates were resistant to macrolide carrying two resistance genes; the *ermTR* and the *ermB*, respectively. The results of this study showed that the CC19 was a major clone in the neonatal intensive care unit (NICU) of Imam Khomeini hospital which can cause severe infections in susceptible neonates (particularly in premature infants). As a result, an intensive infection control policy is needed to prevent the spread of this clone.

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1. Introduction

Group B Streptococcus (GBS or *S. agalactiae*) is known as a leading cause of neonatal sepsis and meningitis [1,2]. It is estimated that 10–30% of pregnant women are rectovaginal colonized by GBS and maternal carriage is the major source of neonatal infections [3]. In the absence of any intervention, between 1 and 2% of neonates born from GBS-colonized mothers develop severe diseases [3]. Neonatal disease results from vertical transmission from mothers to neonates during childbirth or via horizontal transfer by nursery personnel [4]. GBS infections are separated into Early-Onset Disease (EOD) occurring in newborns 0–6 days of age and Late-Onset Disease (LOD) occurring in newborns 7–90 days of age [4]. Sepsis and pneumonia are more common in EOD, while meningitis and bacteremia are more common in LOD [3,4]. In order to understand the population structure of GBS, a variety of techniques have been developed among which multilocus sequence typing (MLST) was introduced as the standard approach for typing [5]. MLST of GBS isolates from different countries have shown that only limited numbers of clonal complexes (CCs) including CC1, CC10, CC17, CC19 and CC23 were associated with colonizing or invasive isolates [6,7]. Among these CCs, CC17 is a hypervirulent clone, mostly associated with invasive disease in neonate, whereas CC19 causes invasive diseases among either neonates or adults [8,9]. However, more recent studies have shown that CC1, CC19 and CC23 were responsible for a high proportion of colonizing isolates [5,10]. Several studies have revealed that CC17 is relatively a homogenous group of capsular type (CT) III isolates and other CCs are heterogeneous groups which express different CTs [11,12]. The polysaccharide capsule is the most important virulence factor and, based on the differences in the structure of surface polysaccharides, 10 CTs (Ia, Ib, II–IX) have been described [13,14]. Moreover, GBS has several

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http://dx.doi.org/10.1016/j.micpath.2016.08.016
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mobile genetic elements (MGEs) in the genome, among which GBSi1 and IS*1548* can serve as genetic markers for lineages CC17 and CC19, respectively [15]. There are several reports from different areas of Iran on the prevalence of GBS among pregnant women [16,17]; however, to our knowledge no data exist on the molecular characteristics of GBS strains isolated from neonates. The goal of current study was to determine phenotypic and genotypic characteristics of GBS isolated from neonates in Tehran, Iran.

2. Methods

2.1. Patients sampling

Between January and December 2013, swab samples were taken from the throat and external ear canals of 1037 newborns that did not develop EOD or LOD, and were hospitalized in the neonatal intensive care unit (NICU) of Imam Khomeini hospital, Tehran-Iran due to various clinical conditions. Sample processing was performed according to the recommendations of the Centers of Disease Control and Prevention (CDC) guidelines [3].

2.2. Bacterial strains

Briefly, after sampling, swabs were inserted directly into Todd-Hewitt broth with 8 μg/ml gentamicin and 15 μg/ml nalidixic acid and were transferred to the microbiology laboratory. After overnight incubation at 35 °C, the incubated broth was then sub cultured on 5% sheep blood agar (SBA) for 18–24 h at 35 °C with 5% CO2. Presumptive GBS colonies on the SBA plate were identified to the species level by a combination of standard tests [13]. To confirm the identity of isolate as GBS, the *dltS* gene was amplified by polymerase chain reaction (PCR) [13].

2.3. Ethical approval

The study was approved by the Ethics Committee of Tehran University of Medical Sciences.

2.4. Antimicrobial susceptibility testing

Antibiotic susceptibilities were determined against clindamycin (2 μg), vancomycin (30 μg), erythromycin (15 μg), linezolid (30 μg), penicillin (10 units), quinupristin-dalfopristin (synercid; 15 μg) and tetracycline (30 μg) using disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Antibiotic discs were purchased from Mast Company, UK.

2.5. DNA extraction

For DNA isolation, the bacterial DNA extraction kit (Gene All Exgene™ Cell SV) was used according to the manufacturer's protocol (Gene ALL, Seoul, Korea). All DNA preparations were stored at 4 °C until they were used.

2.6. Detection of antimicrobial resistance, virulence and capsular genes

The genes encoding resistance to the macrolides and lincosamides (*ermA*, *ermB*, *ermC*, *ermTR*, *mefA* and *linB*), tetracyclines (*tetM*, *tetL*, *tetK* and *tetO*), virulence factors (*bca*, *bac*, *rib*, *hylB* and *scpB*) and mobile genetic elements (IS*1548* and GBSi1) were investigated by PCR as described previously and validated using the control strains [13]. The identification of CTs (Ia, Ib, II–VIII) of all GBS isolates were carried out by multiplex PCR assay as previously described [13].

2.7. MLST

MLST was carried out as described by Jones et al. [19]. Briefly, 7 housekeeping genes (*adhP*, *atr*, *glcK*, *glnA*, *pheS*, *sdhA*, *tkt*) were amplified by PCR and were sequenced in both directions by Macrogen Inc. (Korea). The unique sequence of each gene was then uploaded to the GBS database at http://pubmlst.org/*sagalactiae*/ to provide a unique allele number, and the combination of the allele numbers of the seven loci was given a sequence type (ST). New allelic and ST numbers were confirmed by the MLST website curator. The eBURST v3 software (http://eburst.mlst.net/) was applied used to determine the relationships between isolates and isolates grouping to group isolates into CCs based on five out of seven shared alleles, otherwise, an ST was considered as singleton.

3. Results

Of the 1037 studied neonates, 19 (2%) were found to be colonized with GBS. The phenotypic and genotypic characteristics of GBS isolates are shown in Table 1. The MLST analysis of 19 GBS isolates showed 8 STs. Overall the most common STs were ST19 (34.6%; 6/19), followed by ST28 (21%; 4/19), ST335 (10.5%; 2/19), ST8 (10.5%; 2/19) and ST12 (10.5%; 2/19). Other STs, including ST1, ST4, and ST734 (a new ST) were represented by a single isolate. Using eBURST, the STs were grouped in three CCs and one singleton. The most common CC was CC19 (68.4%, 13/19). The most common CT was type III, found in 52.6% of isolates, followed by type II (31.6%), Ib (10.5%), and V (5.2%). No strains of CTIa, IV, VI–VIII were identified. All isolates harbored the *scpB*, *hlyB*, and *bca* virulence genes, whereas the *rib*, IS*1548* and *bac* genes were detected in 73.7%, 47.4% and 10.5% of the isolates, respectively. The GBSi1 was not detected. The *scpB*, *hlyB* and *bca* virulence genes were detected in ST_S, while the *bac* gene was predominant in ST12 with CTIb. The IS*1548* and the *rib* genes were particularly prevalent in CTIII and were detected in isolates belong to ST19, ST335 and ST734 and were grouped in CC19.

All isolates were susceptible to penicillin, vancomycin, linezolid and quinupristin-dalfopristin. Resistance to erythromycin and clindamycin were found in 5 (26.3%) and 6 (31.6%) isolates respectively. Considering the CC and antibiotic susceptibility, the CC10 revealed the highest resistance rate to erythromycin and clindamycin. Resistance to tetracycline was observed in all 19 (100%) strains and was correlated with presence of the *tetM* gene except for one isolate with ST12. All the ST8 and ST12 isolates were resistant to macrolide carrying two resistance genes; the *ermTR* and the *ermB*, respectively. Of 19 studied isolates, 14 isolates with 6 various STs showed one resistant genotype while the other 5 isolates, consisted of 4 STs, revealed two resistant genotypes (Table 1). Regarding tetracycline and macrolide resistant genes, 18 (98%) had *tetM*, 4 (21%) and 2 (10.5%) had *ermTR* and *ermB* gene respectively. None of the isolates were positive for *ermA, ermC, mefA, linB, tetL, tetK* and *tetO* in the PCR assay.

4. Discussion

Our investigation revealed that the most prevalent STs in the studied neonatal in intensive care units were ST-19, ST-28, ST-335, ST-12 and ST-8. The majority of STs recognized in this study have also been reported previously as common STs for strains isolated from neonatal and/or adult infections [9,11,20]; however, despite the small number of strains, we found one new ST (ST-734) that was present in the MLST database. Associations between CT and STs have been previously reported by many authors [9,11,19]. The number of strains examined in our study was too small to confirm the association between CTs and STs. However, our results suggest

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