Invasive neonatal GBS infections from an area-based surveillance study in Italy

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Abstract

During an area-based study, 75 group B streptococcus (GBS) strains isolated both from early-onset disease (EOD, 37 strains) and from late-onset disease (LOD, 38 strains) were analysed for serotype, pulsed field gel electrophoresis (PFGE) and multilocus sequence typing profiles, protein markers and antibiotic resistance. Serotype III, possessing the *rib* gene, was the most frequent (54 strains, 72%) and responsible for 89.5% and 54% of LOD and EOD, respectively. Forty-six serotype III strains belonged to the same PFGE type and clonal complex 17, already described as an over-represented clone in neonatal invasive GBS infections. Other serotypes were Ia (9.3%), II (6.7%), Ib (5.3%), V (5.3%) and IV (1.3%). Seventeen PFGE groups were identified comprising strains with related sequence types; conversely, strains displaying the same sequence type could belong to different PFGE groups. When both neonate and maternal strains from vaginorectal swabs and/or milk were available (eight cases), they were indistinguishable. Resistance to erythromycin (12%) was associated with a constitutive resistance to clindamycin in five cases (four carrying the *erm*(B) gene and one both the *erm*(B) and *mef*(E) genes) and with an inducible clindamycin resistance in two cases (one possessing the *erm*(A) gene, the other the *erm*(T) gene). Two isolates displayed the M phenotype (*mef*(E) gene). All strains but five were resistant to tetracycline, mostly mediated by the *tet*(M) gene (97.1%). The study underlined the importance of an active surveillance system for the elucidation of a GBS population structure causing neonatal infections and allowed the detection of rare antibiotic resistance determinants [*erm*(T)].

Keywords: Alpha-like protein family, antibiotic resistance, group B streptococcus, MLST, molecular epidemiology, neonatal infection, PFGE, *S. agalactiae*, serotype

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Introduction

Group B streptococcus (GBS) is the leading cause of infectious diseases among newborns. Invasive neonatal GBS infections can present either as an early-onset disease (EOD) occurring within the first week (generally within the first day) and resulting in sepsis and/or pneumonia or as a late-onset disease (LOD) occurring between 1 week and 3 months of life and accounting for most meningitis cases and deaths [1].

Since 2002, CDC recommendations for the prevention of invasive GBS disease in newborns opted for the universal prenatal culture-based screening for vaginal and rectal GBS Centre for Disease Control colonization of pregnant women at 35–37 weeks of gestation, with intrapartum antibiotic prophylaxis (penicillin/ampicillin or macrolide in the case of serious penicillin allergy) for those with positive cultures [2]. Nevertheless, the observed decline in the incidence of GBS disease since the adoption of the prevention strategies related mostly to EOD, with only a slight reduction in LOD [3].

A total of ten different GBS capsular polysaccharide antigenes (Ia, Ib, II–IX) have been described so far; prevalence studies indicated that EOD is associated with Ia, Ib, II, III and V serotypes whereas LOD is associated primarily with type III [4–8]. Other serotypes have been reported mainly in colonization studies, with a predominance of serotypes VI and VIII in Japan and serotype IV in the United Arab Emirates [9,10].

Besides the capsule type, the Alpha-like protein (Alp) family, which comprises the surface-localized protein antigens Alpha-C, Alp1 to 3, and Rib/R4, is commonly used as a GBS protein marker in epidemiological studies [11].

The analysis of serotypes, protein markers, antibiotic resistance profiles and clonal relationships of invasive neonatal GBS strains from an area-based multicentre study is reported here. This is the first time this has been investigated in our country.

Materials and Methods

Bacterial strains and serotyping

From February 2005 to June 2008, 75 non-redundant GBS strains were isolated from blood and/or cerebrospinal fluid of infected newborns and infants. In one EOD and seven LOD cases, neonatal strains were accompanied by isolates from the maternal vaginorectal swabs collected at the onset of the neonatal disease symptoms and, in four LOD cases, from the maternal milk. In total, 87 GBS strains were analysed.

Bacterial strains were plated on defibrinated sheep blood agar plates and incubated at 37°C in 5% CO_2. Identification was confirmed by the Dryspot Streptococcal Grouping Kit (Oxoid). Serotyping was performed by using both a latex agglutination test (Statens Serum Institut) and a multiplex PCR assay [12].

Alpha-like protein (Alp) family

Surface protein markers were inferred by using a multiplex-PCR for the direct identification of the Alp protein genes [13].

Pulsed field gel electrophoresis (PFGE) analysis

Total DNA was extracted as previously described [14] and digested with 40 U of *Sma*I. Four isolates, all serotype III – *rib*, were not DNA-digested by the *Sma*I enzyme but they could be resolved by using the *Xma*I restriction enzyme, a *Sma*I isoschizomer. Interpretation of PFGE results and assignation to PFGE group were performed according to the previously reported criteria [14]. In particular, isolates with indistinguishable profiles were assigned to the same PFGE type and subtype; isolates with similar profiles (differing by up to five bands) were considered possibly related and assigned to different subtypes within the same PFGE type [15]. Isolates with more than five bands of difference were considered unrelated and were identified as different PFGE types.

Multilocus sequence typing (MLST) analysis

A subset of 32 isolates from each PFGE type and possessing different serotype/surface protein combinations were further genotyped by MLST (http://pubmlst.org/sagalactiae/). Sequence types (STs) were assigned to one of the previously described clonal complexes (CCs) included in the GBS MLST database if they shared five or more alleles from the most frequent ST in that CC.

Erythromycin, clindamycin and tetracycline resistance determinants

Resistance to erythromycin and clindamycin was assessed phenotypically by both Etest (Biomériela Italia, Milan, Italy) for determination of MIC values and the Kirby–Bauer double-disk diffusion method to assign the constitutive (CR), inducible (IR) and M resistant phenotype [16]. The presence of the macrolide resistance genes *erm*(A) (subclass *erm*(TR)), *erm*(B) and *mef* was investigated in a multiplex PCR, as already described [14]. The *mef* amplicon was sequenced for the identification of the *mef* class. PCR conditions and primer sequences used for amplification of the *erm*(T) gene were as described [17] and the amplicon was then sequenced to confirm its identity. Tetracycline resistance was determined both phenotypically by E-test and genotypically by studying the occurrence of the resistance genes *tet*(M) and *tet*(O) [18].

Statistical analysis

Fisher's exact test was used to evaluate the differences in distribution of isolates. Two-sided p values <0.05 were considered statistically significant (SPSS 17 for Windows).

Results

Clinical features

Among the 37 EOD cases, the most common manifestation was sepsis (59.4%), followed by septic shock (18.9%) and bacteraemia (13.5%). Sepsis was defined as GBS bacteraemia in the presence of clinical signs and symptoms consistent with a systemic inflammatory response. A septic shock was defined as a sepsis with tachycardia and signs of decreased perfusion. GBS bacteraemia was confirmed by a blood culture obtained from a peripheral or umbilical vessel (if <6 h after catheter insertion) [19].