Case Report

Neonatal bacterial meningitis caused by *Streptococcus gallolyticus* subsp. *pasteurianus*

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This report describes a case of neonatal bacteraemia and meningitis due to *Streptococcus gallolyticus* subsp. *pasteurianus*. Based on the identification kit results, this species may have been reported as *Streptococcus bovis* or *S. bovis* biotype II. The accurate identification of this organism is mandatory for evaluating the aetiology of neonatal meningitis.

Case report

A 5-day-old female was admitted to Fukuoka Red Cross Hospital. She was born at term, weighing 3192 g, and the culture of a maternal prenatal vaginal swab was negative for group B streptococcus. The labour was uneventful, without premature rupture of the membrane. The patient had a fever of 38.4°C on the fourth day after birth. The results of blood examination revealed that there were 3600 leukocytes ml⁻¹ and that the C-reactive protein level was less than 1 mg l⁻¹. The fever persisted the next day, and the patient was then admitted to the hospital. The patient's anterior fontanel bulged slightly, and her overall activity was poor. A sepsis work-up and lumbar puncture were performed. The results of the blood examination were as follows: 13 900 leukocytes ml⁻¹; 12.8 g haemoglobin dl⁻¹; 279 000 platelets ml⁻¹; and 65 mg C-reactive protein l⁻¹. The cerebrospinal fluid was cloudy with 12 971 leukocytes ml⁻¹ (12 800 polymorphonuclear cells ml⁻¹ and 171 mononuclear cells ml⁻¹). The cerebrospinal glucose level was 21 mg dl⁻¹ and the protein level was 3.32 g dl⁻¹. No antigens for *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, *Neisseria meningitidis* group A, B, or C, or *E. coli* K1 were detected (Slidex-Meningite-Kit5; bioMérieux) in the cerebrospinal fluid. Treatment with cefotaxime (200 mg·kg⁻¹ per day), panipenem–betamipron (120 mg·kg⁻¹ per day) and intravenous γ -globulin (300 mg·kg⁻¹ per day for 2 days) was started.

Cultures of both the cerebrospinal fluid and blood showed Gram-positive cocci, which were initially reported as *Streptococcus* species (non-enterococcus). The cerebrospinal fluid isolate was susceptible to penicillin G (MIC 0.06 μ g ml⁻¹), cefotaxime (MIC 0.06 μ g ml⁻¹) and imipenem (MIC $\leq$ 0.008 μ g ml⁻¹). Panipenem–betamipron treatment was

discontinued, and treatment with cefotaxime alone continued for 14 days. Culture of the cerebrospinal fluid was negative on day 3 of hospitalization. Non-contrast head computed tomography scans, which were obtained on day 11 of hospitalization, revealed no intracranial haemorrhage or subdural abscess. The patient was discharged without sequelae.

The isolate possessed Lancefield's D antigen (Streptex; Remel). According to an API 20 Strep test (bioMérieux), the isolate was identified as *S. bovis* biotype II/2. Because the *S. bovis* complex has been recently reclassified (Schlegel *et al.*, 2003; and see below), we tested for gallate hydrolysis (tannase) activity of the isolate according to the reference by Osawa *et al.* (1995). Various biochemical activities of the isolate are described in Table 1 with comparison to those of *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus* and *S. gallolyticus* subsp. *macedonicus*. The biochemical characteristics of the isolate coincided well with those of *S. gallolyticus* subsp. *pasteurianus*. A positive result was obtained with the recently developed PCR test for detecting *sodA* of *S. gallolyticus* (data not shown) (Sasaki *et al.*, 2004). The 5' side of the isolate's 16S rRNA gene sequence revealed 99.4% (354/356 bp) and 100% (356/356 bp) homology with those of *S. gallolyticus* subsp. *gallolyticus* (ATCC 43143) and *S. gallolyticus* subsp. *pasteurianus* (ATCC 43144), respectively. From the results of these biochemical and molecular tests, the isolate was identified as *S. gallolyticus* subsp. *pasteurianus*.

Discussion

S. gallolyticus subsp. *pasteurianus* belongs to the group D streptococci, and was previously recognized as *S. bovis*

Table 1. Biochemical characteristics of the isolate from this case and the three subspecies of *S. gallolyticus*

The characteristics of three subspecies of *S. gallolyticus* refer to a reference by Schlegel *et al.* (2003).

Characteristic	Our isolate	<i>S. gallolyticus</i> subsp. <i>gallolyticus</i>	<i>S. gallolyticus</i> subsp. <i>pasteurianus</i>	<i>S. gallolyticus</i> subsp. <i>macedonicus</i>
Hydrolysis of:				
Aesculin	+	+	+	–
Gallate (tannase activity)	–	+	–	–
Production of:				
β -Glucosidase	+	+	+	–
β -Glucuronidase	–	–	+	–
α -Galactosidase	+	+	v	v
β -Galactosidase	+	–	+	+
Acidification of:				
Starch	–	+	–	+
Glycogen	–	+	–	–
Inulin	–	+	–	–
Lactose	+	+	+	+
Mannitol	–	+	–	–
Raffinose	+	+	v	–
Trehalose	+	+	+	–

+, ≥ 80 % activity compared to positive control reaction; –, ≤ 20 % activity compared to positive control reaction; v, 21–79 % activity compared to positive control reaction.

biotype II/2. *S. bovis* is delineated into two biotypes according to their ability (biotype I) or inability (biotype II) to ferment mannitol (Facklam, 1972; Parker & Ball, 1976). *S. bovis* (biotype II) is further divided into biotypes II/1 and II/2 on the basis of phenotypic testing with the Rapid Strep system (bioMérieux) (Coykendall & Gustafson, 1985). It has been well documented that *S. bovis* (biotype I) is associated with colonic neoplasia and bacterial endocarditis in adults (Ruoff *et al.*, 1989; Herrero *et al.*, 2002). In contrast, *S. bovis* (biotype II) is associated with invasive infection in neonates and infants (Grant *et al.*, 2000; Cheung *et al.*, 2000; Gavin *et al.*, 2003; Nagai *et al.*, 2008), as well as adult bacteraemia both in Western and Eastern countries (Clarridge *et al.*, 2001; Lee *et al.*, 2003). Among the reported cases of neonatal invasive infection due to *S. bovis* (Gerber *et al.*, 2006; Nagai *et al.*, 2008), *S. bovis* biotype II/2 was described in two cases (Gavin *et al.*, 2003; Nagai *et al.*, 2008). No reports have described the aetiological organism of invasive infections as *S. gallolyticus* subsp. *pasteurianus*.

The taxonomic status of the *S. bovis* group has been evolving in the last few decades. Farrow *et al.* (1984) demonstrated that the *S. bovis*/*Streptococcus equinus* complex comprised six DNA groups. It was shown that *S. bovis* biotype II/2 belonged to the DNA group 2 of Farrow's classification (Schlegel *et al.*, 2003; Poyart *et al.*, 2002). According to the biochemical characteristics, the members in this DNA group have been reclassified and renamed *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus* or *S. gallolyticus* subsp. *macedonicus* (Schlegel *et al.*, 2003). These subspecies have similar 16S rRNA gene sequences and cannot be discriminated from

each other solely by 16S rRNA gene sequence (Clarridge *et al.*, 2001; Schlegel *et al.*, 2003). Instead, the aesculin- and gallate-hydrolysis activity measurement works for identifying these subspecies, though the latter is not included in the identification kit (Table 1) (Osawa & Sasaki, 2004). According to the new classification, *S. bovis* biotype I and *S. bovis* biotype II/2 correspond to *S. gallolyticus* subsp. *gallolyticus* and *S. gallolyticus* subsp. *pasteurianus*, respectively (Schlegel *et al.*, 2003).

The isolate from our case was susceptible to penicillin G, cefotaxime and panipenem, and resistant to erythromycin and minocycline. *Enterococcus* spp. have phenotypic characteristics similar to those of *S. gallolyticus* subsp. *pasteurianus*, i.e. they are non-haemolytic, positive for Lancefield's D antigen and positive for aesculin hydrolysis. Penicillin G is considered to be an efficient treatment for neonatal infections caused by *S. gallolyticus* subsp. *pasteurianus*, while vancomycin and/or aminoglycosides may be considered for the treatment of neonatal infections caused by *Enterococcus*. Thus, the accurate identification of the isolate is crucial for selecting appropriate antibiotic therapy.

This report describes a case of neonatal bacterial meningitis due to *S. gallolyticus* subsp. *pasteurianus*. The importance of this organism as a causative agent of invasive infection in neonates should be emphasized.

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