Spontaneous peritonitis and bacteremia caused by *Streptococcus equinus*: Value of 16S rRNA gene sequencing analysis

John Dotis¹, Nikoleta Printza¹, Stella Stabouli¹*, Efthymia Petinaki² and Fotios Papachristou¹

¹1st Department of Pediatrics, Aristotle University, Hippokratario Hospital, Greece
²Department of Microbiology, University Hospital of Larissa, Greece

Abstract

*Streptococcus equinus* constitutes an uncommon Gram-positive cocci that can cause infections in humans rarely. We have recently encountered a case of *S. equinus* peritonitis, established by 16S rRNA gene sequence analysis, in a peritoneal dialysis (PD) patient which, in our knowledge, is the first reported case in a PD child complicated with bacteremia.

Introduction

*Streptococcus equinus* is Gram-positive cocci, catalase negative, which has seldom been reported to cause infections in humans [1]. Even rarer are the peritonitis cases presented in the bibliography in previous decades [2,3]. We have recently encountered a case of *S. equinus* peritonitis in a peritoneal dialysis (PD) patient that, in our knowledge, is the first reported case in a PD child complicated with bacteremia.

Case report

A 9-year old boy who had end stage renal disease, due to infantile-type polycystic kidney disease, was treated with continuous ambulatory PD from the age of 10-months old. He was admitted to the hospital with cloudy effluent, fever (39°C), vomiting and diarrhea. Empirically treatment with intraperitoneal (i.p.) vancomycin (30 mg/L) plus ceftazidime (125 mg/L) was initiated in addition to intravenous (i.v.) cefuroxime (50 mg/kg). The PD fluid obtained, was slightly opalescent, and was sent immediately to the microbiological laboratory for further analysis. In parallel, blood cultures were also obtained. The PD fluid analysis revealed the presence of 650 cells/µL with 85% neutrophils, while, Gram stain was negative for microorganism. PD fluid was injected into both aerobic and anaerobic blood culture bottles which were processed by automated BACTEC 9120 System (Becton Dickinson). Furthermore, 20 ml of the PD fluid was collected in rubber sealed pyrogen-free tubes for direct detection of bacterial DNA (Endo Tube ET; Chromogenix AB, Vienna, Austria). A broad-range 16S rRNA PCR with subsequent DNA sequencing, detecting bacterial DNA directly in clinical specimens of patients with serious infections is applied on a routine basis. The isolation and the detection of bacterial DNA in the PD fluid were performed as previously reported [4]. The PCR amplicons (520 bp) were sequenced in both directions by the same primer set: 5’-GACGAACGCTGCGGCGTGCCTA-3’ and 5’-CGCTCGTTGCGGGACTTAAACG-3’ were used. Analysis of the sequences results demonstrated 100% identity of the microorganism with *Streptococcus equinus* (Gen Bank accession No KJ803948.1) and was available one day after admission (Figure 1).

Three days after admission and two days after the molecular

Specifically, primers 5’-GACGAACGCTGCGGCGTGCCTA-3’ and 5’-CGCTCGTTGCGGGACTTAAACC-3’ were used. Analysis of the sequences results demonstrated 100% identity of the microorganism with *Streptococcus equinus* (Gen Bank accession No KJ803948.1) and was available one day after admission (Figure 1).

Three days after admission and two days after the molecular

Lane 1, ladder 10.000bp; Lane 2, our *Streptococcus equinus* isolate; Lane 3, positive control; Lane 4, negative control

**Figure 1.** Detection of the *Streptococcus equinus* isolate with DNA sequencing initial PCR fragment on agarose gel.

**Correspondence to:** Stella Stabouli, MD, PhD, 1st Department of Pediatrics, Hippokratario Hospital, Konstantinoupoleos 49, 546 42 Thessaloniki, Greece, Tel:0030 2310892466, Fax: 0030 2310992784, E-mail: sstaboul@auth.gr

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discussion

streptococcus equinus is a lancefield group d streptococcus which belongs to streptococcus bovis/equinus-complex and the taxonomy of this complex has undergone several changes during the last decade. specifically by 16s rRNA gene sequence analysis, was divided into S. equinus, Streptococcus bovis, Streptococcus galloyticus subsp. galloyticus, streptococcus infantarius subsp. coli, Streptococcus infantarius subsp. infantarius, streptococcus pasteurianus, streptococcus galloyticus subsp. macedonius, streptococcus alactolyticus and streptococcus lutetiensis [5,6].

Published cases of human infections due to S. equinus are rare with only two peritonitis cases presented till now. In the first case, a patient with cirrhosis developed spontaneous bacterial peritonitis plus bacteremia and was treated with aztreonam [2]. The second case was in a 63-year-old man with end-stage renal disease of unknown origin treated with CAPD. The isolate was identified as a S. equinus by an API 32 (bioMérieux, Lyon, France) automated identification system and the patient was treated with i.p. cefalotin for a total of 14 days [3]. However, 16s rRNA gene sequence analysis was not available for both cases.

The scarcity of reports concerning S. equinus infections may be due to low pathogenicity, but it also may be due to difficulties in culturing and identifying the bacteria. If it is available a 16s rRNA gene sequence analysis is useful to establish certain biotypes and subspecies when a S. equinus is isolated from a culture, and although S. equinus is rare as a cause of human bacteremia, it is very easy to be treated even in severe cases of both bacteremia and peritonitis such in our case.

references