Case Report

Human bloodstream infection caused by *Staphylococcus pettenkoferi*

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Staphylococcus pettenkoferi is a recently isolated human pathogen with only a few reported cases of infection. We report a case of bloodstream infection caused by *S. pettenkoferi* in a patient with pulmonary tuberculosis.

Introduction

Coagulase-negative staphylococci (CoNS) are among the most common organisms causing nosocomial bacteraemia worldwide (Pfaller *et al.*, 1999). *Staphylococcus pettenkoferi* is a novel member of the CoNS. It was first isolated from blood culture from a 25-year-old patient with extrapulmonary tuberculosis and from a wound of 76-year-old patient with leukaemia, cancer and insulin-dependent diabetes mellitus in 2002 (Trülsch *et al.*, 2002). The same study group recently reported three more isolates of *S. pettenkoferi* from different patients (Trülsch *et al.*, 2007). Osteomyelitis caused by this organism in a diabetic patient was also reported recently (Loiez *et al.*, 2007), but to our knowledge there are no more reported cases to date. Here we describe a case of bloodstream infection caused by *S. pettenkoferi*, which was identified using 16S rRNA sequencing.

Case report

A 76-year-old male was admitted to Seoul National University Bundang Hospital for the treatment of recurring pulmonary tuberculosis which seemed to be multidrug-resistant. Sudden skin eruptions developed, and he was suspected to have Stevens–Johnson syndrome. A central venous catheter was inserted due to the poor venous access. During the management of the tuberculosis and Stevens–Johnson syndrome, he developed a sustained unexplained fever and unidentified CoNS were grown in two blood culture bottles from different lumens of a central venous

catheter using different single-use vacutainers. Subculture onto blood agar plates produced glistening, yellow-pigmented colonies 1–2 mm in diameter, with no haemolysis. As the isolates were negative for coagulase and positive for catalase, they were initially concluded to be CoNS. With the MicroScan WalkAway Pos Combo Panel (Dade Behring), the isolates were positive for urease, nitrate reduction, growth on bacitracin, micrococcus screen, glycosidase, β -lactamase and growth in 6.5% NaCl, and identified as *Staphylococcus hominis* subsp. *hominis* (92%) or *Staphylococcus auricularis* (99%). The micrococcus screen test is used to differentiate micrococci (negative) and staphylococci (positive) by identification of growth on 0.05 μ g bacitracin ml⁻¹. With the VITEK 2 Gram Positive Identification system (bioMérieux), the isolates were weakly positive for urease, acid production from D-mannitol and O/129 resistance, and positive for arginine dihydrolase 1, pyrrolidonylarylamidase, growth in 6.5% NaCl and optochin resistance. The O/129 resistance test is used to differentiate certain species that can grow in the presence of the vibriostatic compound O/129. The isolates were identified as *S. auricularis* (70%), *Staphylococcus capitis* (50%) or *Staphylococcus warneri* (50%). With the API STAPH V4.1 kit (bioMérieux), the isolates were positive for acid production from glucose, D-fructose and sucrose, nitrate reduction and alkaline phosphatase, and negative for urease, arginine dihydrolase and acetoin production (numerical profile 6006010). The isolates were susceptible to novobiocin and identified as *S. capitis* (61.5%) or *Kocuria varians/rosea* (27.8%). By an antimicrobial susceptibility test using MicroScan WalkAway, the isolates were susceptible to chloramphenicol, gentamicin, trimethoprim/sulfamethoxazole, tetracycline, teicoplanin and vancomycin, and resistant to

Abbreviation: CoNS, coagulase-negative staphylococci.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the *S. pettenkoferi* isolate is FJ222447.

oxacillin, penicillin, rifampicin, clindamycin, ciprofloxacin and erythromycin. The resistance to oxacillin was confirmed by PCR of the *mecA* gene.

As the biochemical identification systems produced indeterminate and different results, we proceeded to the sequencing of the 16S rRNA gene using the MicroSeq Microbial Identification System (Applied Biosystems) and obtained a consensus sequence of 495 bp. The system reported the organism to be *Staphylococcus caprae* (99.36 %), *Staphylococcus hyicus* (96.94 %) or *Staphylococcus cohnii* (97.08 %). We further analysed the 16S rRNA gene sequence using three different primer sets and obtained a sequence of 1533 bp. The analysed sequence data were submitted to GenBank (strain SNUBH406; accession no. FJ222447) and found to have the highest similarity to the sequence of *S. pettenkoferi* or '*Staphylococcus pseudolugdunensis*'. The 16S rRNA gene sequences of the two species were not included in the MicroSeq database. All of the sequenced nucleotides except for one mismatched nucleotide were in agreement with the 16S rRNA sequence of the recent isolates of *S. pettenkoferi*. A phylogenetic tree based on the 16S rRNA gene sequences of the isolate and 45 organisms with highest similarity to the isolate was drawn using the neighbour-joining method (Fig. 1). It showed a subline closely related to the previously reported *S. pettenkoferi* or '*S. pseudolugdunensis*' cases. As it was negative for ornithine decarboxylase, we concluded that our organism was *S. pettenkoferi* as previously reported (Tang *et al.*, 2008). The isolates from two blood culture bottles showed the same biochemical and genetic characteristics so they were thought to be the same strain.

After the patient was treated with parenteral vancomycin 2 g every 24 h for 1 week, no organisms were grown from blood and the patient is currently under treatment for pulmonary tuberculosis.

Discussion

The importance of CoNS as opportunistic pathogens has increased recently (Huebner & Goldmann, 1999). *S. pettenkoferi* is a novobiocin-susceptible member of the CoNS and may also be regarded as the cause of opportunistic infection. To the best of our knowledge, this is the first case of pathogenic infection caused by the organism in Asia.

As the isolate is a novel species of CoNS, it could not be identified by any of the three biochemical test kits used in our study. It would be helpful if phenotypic characteristics from previous reports and our case were included in the identification algorithm of those kits in the future. Our study shows that sequencing of the 16S rRNA gene is a useful tool for the identification of unknown isolates. Sequencing of the *rpoB* gene is accepted as another approach for *Staphylococcus* species identification (Drancourt & Raoult, 2002; Mellmann *et al.*, 2006). In

Fig. 1. Unrooted neighbour-joining phylogenetic tree based on the 16S rRNA gene sequence of the isolate and that of 45 organisms with highest similarity after a BLAST search. Bootstrap values are given at branching points. The scale bar represents 1 nucleotide substitution per 200 nucleotides.

the analysis of the sequence data, NCBI GenBank was superior to the commercial database as it contained the most up-to-date sequences. Though verified on evidence and useful in routine identification in clinical laboratories (Lau *et al.*, 2006; Woo *et al.*, 2003), the commercial

database might be unsuitable for identification of novel sequences (Fontana *et al.*, 2005).

S. pettenkoferi has been shown to be present in the indoor environment by direct analysis of the 16S rRNA gene from settled dust samples (Rintala *et al.*, 2008). Our isolates were found to have more than 99% similarity with these samples from indoor dust (accession no. AM695858). Thus *S. pettenkoferi* may commonly exist in the environment and serve as an opportunistic pathogen. The possibility of contamination from the environment had to be considered in our case. However, as the isolates from the two blood culture bottles had the same 16S rRNA gene sequence and the patient had unexplained fever which was controlled after use of appropriate antibiotics, we concluded that the isolate served as a pathogen. Although not always considered necessary, the identification of CoNS to species level using molecular techniques might differentiate pathogens from contaminants (Kim *et al.*, 2000). A careful approach for an unidentified isolate is required for relevant support to the clinical decision.

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