Isolation of *Staphylococcus caprae* from Blood Cultures of a Neonate with Congenital Heart Disease

During the past two decades, coagulase-negative staphylococci have been increasingly recognized as important nosocomial pathogens, and many new species and subspecies have been identified (1). *Staphylococcus caprae*, which was described as a new species in 1983, was initially isolated from goat milk (2). Since then, numerous human isolates and several cases of human infection have been reported (3).

We describe here the isolation of *Staphylococcus caprae* from the blood culture of a neonate with severe cardiac malformations. Species identification was achieved by conventional biochemical methods and DNA sequencing of the 16S rRNA gene. To the best of our knowledge, this is the first time that the 16S rRNA nucleotide sequence of this species has been published.

A 2-day-old neonate was transferred to the neonatal intensive care unit of the University Hospital of the Technical University Aachen, Germany, with tachypnea and cyanosis. On admission, blood cultures were obtained and antibiotic therapy with cefotiam was initiated. Increasing tachypnea and cardiac decompensation necessitated mechanical ventilation; in addition, prostaglandin E_2 and dobutamin were given. Diagnosis by right heart catheterization revealed congenital heart disease with interrupted aortic arch (type B), ventricular septal defect, atrial septal defect, and patent ductus arteriosus.

After one day of incubation, coagulase-negative staphylococci (CNS) were isolated from the blood culture (Bact Alert, Paedi Bact bottle, USA) drawn on admission. Antibiotic susceptibility testing by disk diffusion according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (4) showed the strain to be resistant to penicillin and ampicillin and susceptible to oxacillin, cefotiam, erythromycin, ofloxacin, and vancomycin. Species identification by the API ID Staph system (bioMérieux, France) yielded *Staphylococcus caprae* (code 363132000). In contrast to the type strain (DSM 20608), our clinical isolate failed to produce acid from lactose, a reaction that is positive for the majority of *Staphylococcus caprae* strains. Since misidentification and poor identification of *Staphylococcus caprae* using the API Staph system have been described (3) and isolation of *Staphylococcus caprae* from sterile body sites has been reported in only three cases (3), the results of the biochemical identification were confirmed by DNA sequencing.

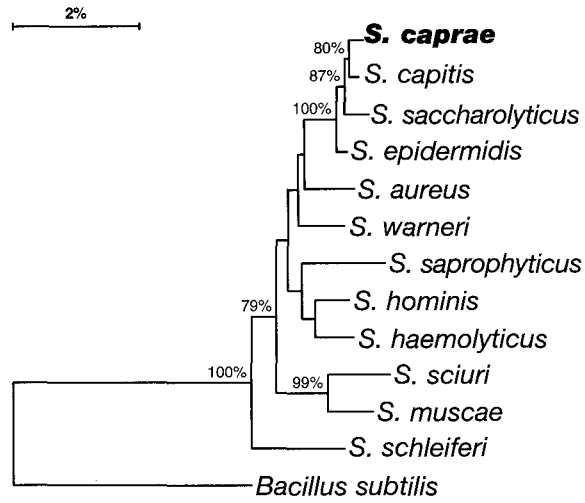

Figure 1: Rooted phylogenetic tree inferred by analysis of the 16S rRNA genes of staphylococci using the neighbor-joining method, showing *Staphylococcus caprae* as a paraphyletic group of *Staphylococcus capitis*. Sequence data were obtained from the SSU rRNA database at the Department of Biochemistry, University of Antwerp, Belgium. Bootstrap values above 75% are displayed.

To allow species identification of the clinical isolate, the 16S rRNA gene of both the clinical isolate and the type strain was sequenced. 1.47 kb of DNA representing > 95% of the entire gene was amplified by polymerase chain reaction (PCR) employing primers to highly conserved nucleotide regions, a method published previously by Hiraishi (5). The PCR product was purified using the Qiaquick PCR purification kit (Qiagen, Germany) according to the manufacturer's instructions and sequenced on an Abi 373 automated DNA sequencer. The DNA sequence of the type strain, which has been isolated from goats milk, was identical to that of the clinical isolate (EMBL accession number Y12593). Using this sequence and published 16S rRNA sequences of the genus *Staphylococcus*, a phylogenetic analysis was performed on the basis of 1470 alignable positions by neighbor-joining method and bootstrap analysis with 500 resampled data sets. Methods and programs used for this analysis have been described previously in detail (6). In the resulting phylogenetic tree (Figure 1), *Staphylococcus caprae* clustered together with *Staphylococcus capitis* and *Staphylococcus epidermidis* as a monophyletic group.

The patient received antibiotic therapy with cefotiam for 14 days. On day 12 the aortic arch was reconnected and the atrial septal defect, the ventricular septal defect, and the patent ductus arteriosus were closed. Mechanical ventilation was discontinued on day 7 postoperatively, and the patient was discharged from the neonatal intensive care unit two weeks after cardiac surgery.

With the availability of automated DNA-sequencing and a rapidly growing database, the analysis of 16S rRNA genes has become one of the most powerful taxonomic tools for phylogenetic analysis and species identification. The nucleotide sequences of the 16S rRNA gene of the human isolate and the *Staphylococcus caprae* type strain (DSM 20608) were identical, supporting the preliminary species identification obtained by biochemical methods. The phylogenetic placement of *Staphylococcus caprae* is in accordance with the results of DNA-DNA hybridization studies (7). In contrast to the results of hybridization, the 16S rRNA nucleotide sequence revealed no difference between strains of human versus those of animal origin.

Coagulase-negative staphylococci are regarded as important opportunistic pathogens in the neonatal intensive care unit. Diagnosis of bacteremia in neonates has been based on the isolation of a single morphologic type of CNS from one or more blood cultures (8), since the frequency of skin contamination by CNS appears to be much less than that in adults (8) and the number of blood cultures is restricted by the limited blood volume of neonates. Very few cases of infections caused by *Staphylococcus caprae* have been reported. Isolation from blood cultures has been described in only three cases (3): an elderly woman with catheter-associated sepsis, a middle-aged man with endocarditis, and a neonate with aortic coarctation and sepsis. Together with the case described here, three of four isolations of *Staphylococcus caprae* from blood cultures were associated with cardiac malformations or endocarditis. These numbers are clearly too small for statistical analysis, but the potential association with cardiac disease warrants further studies of *Staphylococcus caprae* and its role in human infections.

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Two Fatal Cases of *Veillonella* Bacteremia

Veillonella spp. are anaerobic, nonmotile, nonsporulating gram-negative cocci that form part of normal flora in the upper respiratory, lower gastrointestinal, and female genital tracts. They often appear as a component of polymicrobial cultures (1) and infrequently grow from blood cultures (2, 3). The pathogenic role of these anaerobes has not been established thus far (1). We report here two fatal cases of *Veillonella parvula* bacteremia in which pure *Veillonella parvula* was isolated in