Pediatric Bacteremia Due to \textit{Staphylococcus warneri}: Microbiological, Epidemiological, and Clinical Features

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Between 1991 and 1995, an apparent high rate of \textit{Staphylococcus warneri} bacteremias at the Royal Children’s Hospital, Melbourne, Victoria, Australia, raised the possibility of a virulent nosocomial strain. In a retrospective review of 30 \textit{S. warneri} bacteremias in children, organisms were viable and verified in 22 episodes, 12 representing significant bacteremias. Of these 12 episodes, 2 pairs shared chromosomal DNA pulsed-field gel electrophoresis patterns in unconnected patients, dispelling concerns about a single virulent strain.

\textit{Staphylococcus warneri} was first described in 1975 by Kloos and Schleifer (12). It has been reported to cause bacteremia (8, 9), infective endocarditis (6, 9, 24, 25), cerebrospinal fluid shunt infection (23), subdural empyema (20), vertebral osteomyelitis (3, 4, 10, 25), and urinary tract infection (14). The distribution of coagulase-negative staphylococci isolated from blood at the Royal Children’s Hospital comprises 80% \textit{S. epidermidis} and 10% \textit{S. warneri}, with \textit{S. capitis} and \textit{S. hemolyticus} making up almost all of the remaining isolates. Reports from other institutions have described \textit{S. warneri} as the third to fifth most common coagulase-negative staphylococcus species from blood (5, 8, 16) and foreign body infections (21). The only previously published series of bacteremias due to \textit{S. warneri} described 27 patient episodes of positive blood cultures at Long Island Jewish Medical Center in New York, N.Y., between 1984 and 1989 (9). The authors identified 14 true bacteremias (5 of which were in children): 8 were central venous catheter (CVC) related, 5 were of an unknown source, and 1 was native valve endocarditis.

The Royal Children’s Hospital is the major tertiary referral pediatric hospital in Melbourne, Victoria, Australia, a city with a population of 3.5 million. Clinical services include oncology, bone marrow transplant, and neonatal and pediatric intensive care units. From January 1991 to April 1995, 98,672 inpatients were treated, and bacteria were cultured from 2,163 of the 30,505 blood culture samples received by the bacteriology laboratory. All blood culture isolates are stored at the hospital. In 30 patients, the organism was identified as \textit{S. warneri}. The following clinical records of these patients were reviewed, and the following clinical information was obtained: age, sex, underlying condition, reason for hospitalization, CVC presence and duration in situ, presence of other indwelling devices, number of total and positive blood cultures drawn, presence of fever, leukocyte count, isolate antibiotic susceptibility pattern, and outcome of infection. Because CVC tip isolates from this period were not stored or speciated, these results were not available.

Definitions. Significant \textit{S. warneri} bacteremia was defined as fulfilling the following criteria. (i) There were at least two positive blood cultures drawn on separate occasions, and either (ii) there was no other cause found for fever and/or clinical deterioration or (iii) there was isolation of \textit{S. warneri} from a normally sterile body site concomitantly with blood cultures positive for \textit{S. warneri}.

Fever was defined as a temperature of 38.5°C or more on two consecutive occasions. Nosocomial bacteremia was defined as onset of symptoms of new sepsis and first positive blood culture occurring more than 48 h after admission. Patients were identified from laboratory blood culture records. Initial identification was based upon coagulase-negative staphylococci shown to be negative for trehalose reduction and desferrioxamine susceptibility (15) and then were identified as \textit{S. warneri} by API Staph-Ident (bioMerieux Vitel, Inc., Hazelwood, Mo.). Patients were included if these organisms were subsequently viable and identified as \textit{S. warneri} according to the methods described by Kloos and Schleifer (13). From January 1991 to May 1994, adult and pediatric nonradiometric Bactec 660 blood culture bottles (Becton-Dickinson) were used. Subsequently, PediBact and BacTalert culture bottles (BacTalert Microbial Detection Systems; Organon Teknika, Belgium) were used.

Methicillin MICs were determined with Mueller-Hinton agar incubated for 24 h at 35°C in ambient air by the E-Test method (AB Biodisk, Sweden) (17). Testing of sensitivity to penicillin, methicillin, erythromycin, trimethoprim, chloramphenicol, and vancomycin was also performed according to National Committee for Clinical Laboratory Standards guidelines for agar dilution susceptibility tests (18).

PFGE. Whole-cell DNA was prepared for pulsed-field gel electrophoresis (PFGE) by a modification of the method described by Smith and Cantor (22), according to the manufacturer’s instructions (2). DNA digestion was performed overnight at 27°C with 30 U of \textit{SmaI} (Boehringer) in 30 μl of restriction buffer. Quarter blocks were loaded into wells (5 by 1 mm) in a 1.0% agarose gel and electrophoresed at 12°C for 10 h with a pulse time of 5 s, 10 h at a pulse time of 20 s, and then for 10 h at a pulse time of 80 s at 170 V (100 mA) in dilute Tris-borate EDTA (0.0445 M Tris base, 0.0445 M boric acid, 0.001 M EDTA). Molecular markers (λ DNA [PFGE]; Pharmacia) were included in each run. The same procedure was performed with undigested DNA to identify extrachromosomal DNA on a separate gel.

Twenty-two patient episodes of positive \textit{S. warneri} blood cultures were identified. Of these, 12 fulfilled the previously stated criteria of significant bacteremia; the remaining 10 were
TABLE 1. Clinical and laboratory data from children with S. warneri bacteremia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Significant infection</th>
<th>Nosocomial CVC No. of samples blood culture positive/total drawn</th>
<th>Age (mo)</th>
<th>Fever</th>
<th>Immunosuppressed</th>
<th>Antibiogram</th>
<th>MIC (mg/ml)</th>
<th>Treatment</th>
<th>Underlying condition(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Y</td>
<td>4/7</td>
<td>1.5</td>
<td>Y</td>
<td>Y</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>4/7</td>
<td>1.5</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>B</td>
<td>Y</td>
<td>4/7</td>
<td>0.5</td>
<td>N</td>
<td>N</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>4/7</td>
<td>16</td>
<td>Flucloxacillin, vancomycin</td>
</tr>
<tr>
<td>C</td>
<td>Y</td>
<td>3/3</td>
<td>1</td>
<td>Y</td>
<td>Y</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>3/3</td>
<td>1</td>
<td>Flucloxacillin</td>
</tr>
<tr>
<td>D</td>
<td>Y</td>
<td>2/5</td>
<td>59</td>
<td>Y</td>
<td>Y</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>2/5</td>
<td>3.25</td>
<td>Vancomycin, clindamycin</td>
</tr>
<tr>
<td>E</td>
<td>Y</td>
<td>5/8</td>
<td>2.5</td>
<td>Y</td>
<td>N</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>5/8</td>
<td>12</td>
<td>Piperacillin, vancomycin</td>
</tr>
<tr>
<td>F</td>
<td>Y</td>
<td>4/8</td>
<td>125</td>
<td>Y</td>
<td>Y</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>4/8</td>
<td>256</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>G</td>
<td>Y</td>
<td>2/2</td>
<td>0.4</td>
<td>Y</td>
<td>N</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>2/2</td>
<td>1.5</td>
<td>Flucloxacillin</td>
</tr>
<tr>
<td>H</td>
<td>Y</td>
<td>2/5</td>
<td>39</td>
<td>Y</td>
<td>Y</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>2/5</td>
<td>256</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>I</td>
<td>Y</td>
<td>5/8</td>
<td>2.5</td>
<td>Y</td>
<td>N</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>5/8</td>
<td>12</td>
<td>Piperacillin, vancomycin</td>
</tr>
<tr>
<td>J</td>
<td>Y</td>
<td>4/8</td>
<td>125</td>
<td>Y</td>
<td>Y</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>4/8</td>
<td>256</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>V</td>
<td>N</td>
<td>4/4</td>
<td>115</td>
<td>N</td>
<td>Y</td>
<td>Pen Met Tri Ery Chl Van</td>
<td>4/4</td>
<td>256</td>
<td>Vancomycin</td>
</tr>
</tbody>
</table>

a Y, yes; N, no; Pen, penicillin; Met, methicillin; Tri, trimethoprim; Ery, erythromycin; Chl, chloramphenicol; Van, vancomycin.
considered contaminants. In 8 of the 30 episodes originally reported as \textit{S. warneri}, the organism could not be regrown (seven patients) or was not confirmed as \textit{S. warneri} on regrowth (one patient). The isolate from this patient was identified as \textit{Staphylococcus cohnii}. The original API Staph-Ident profile code was not kept for this organism, and there was a profile identifying it as \textit{Staphylococcus xylosis} on repeat. \textit{S. warneri} is distinguished from \textit{S. cohnii} by the ability to reduce sucrose in Kloos and Schleifer’s simplified scheme (13). This isolate has been sent to the Microbiological Diagnostic Unit, University of Melbourne, for further characterization. In a prospective study compared to reference procedures, API Staph-Ident had questionable accuracy in identifying \textit{S. warneri}, with only 25 of 39 \textit{S. warneri} isolates correctly identified and with another 40 isolates incorrectly identified as \textit{S. warneri} (19). Other studies have yielded varied results, with API Staph-Ident identifying 1 of 8 and 17 of 17 \textit{S. warneri} isolates correctly (1, 7). In the original identification, the specificity of API Staph-Ident in identifying \textit{S. warneri} in our isolates was good, with only 1 of 23 incorrectly identified as \textit{S. warneri}.

Table 1 summarizes the data from the 22 patients with \textit{S. warneri} bacteremia. Subsequent patient discussion is restricted to the 12 patients with significant bacteremia. Age ranged from 13 days to 15 years, with a mean age of 6.3 years. There were seven males and five females. In the eight patients with documented CVC insertion dates, the average duration in situ was 8.7 months (range, 3 days to 33 months). The two patients without a CVC both had indwelling peripheral intravenous lines, and one also had an intra-arterial line in situ. Six patients had undergone CVC removal during treatment for the bacteremia. Five patients were immunocompromised (four with hematological malignancies, one with primary immunodeficiency), and there was an additional 2-week-old patient extremely premature after birth at 25 weeks gestation. Of the 11 patients who had blood leukocyte counts performed, two had neutrophil counts below 500 per mm\(^3\). Eight patients were febrile. Of the two patients with non-nosocomial bacteremia, one became febrile and recorded a subsequent positive blood culture following a CVC flush at home. The remaining patient had a CVC in situ for over 2 years and became febrile 2 days prior to admission.

There were no deaths attributable to \textit{S. warneri} bacteremia. Eleven patients improved with treatment. Bacteremia did not recur in these patients following cessation of antibiotics. The other bacteremic patient died from disseminated aspergillosis in the setting of progressive varicella and natural killer cell deficiency. All patients received courses of intravenous flucloxacinil, vancomycin, or clindamycin.

The 12 children had 62 blood cultures taken during sepsis episodes. Thirty-nine (62\%) were positive—12 peripherally drawn, 11 drawn from CVCs, and 16 drawn from unspecified sites. Twenty-nine became positive on day 1, 9 became positive on day 2, and 1 became positive on day 3. Six of the 12 strains were methicillin resistant. One strain demonstrated markedly higher (than the other 11) levels of methicillin resistance for which the MIC was >256 \text{mg}/\text{ml}. PFGE of chromosomal DNA, shown in Fig. 1, demonstrated related band patterns in 20 of the 22 strains. The remaining two strains had patterns markedly different from those of other local isolates of \textit{S. warneri} (G and R). These isolates have also been sent for characterization. There was significant variation between the patterns of isolates from different patients but close similarity between isolates from the same patient. One pair differed by two chromosomal bands (N and O), and 2 pairs differed by one extrachromosomal band (E and F and J and K), as shown in Fig. 2. No patients within these pairs shared wards or treating medical units.

This study supports \textit{S. warneri} as a cause of clinically significant pediatric bacteremia. At our institution, it is the second most common coagulase-negative staphylococcus species isolated from blood after \textit{S. epidermidis}. Like other coagulase-negative staphylococcus species, it is almost exclusively a nosocomial blood pathogen, with 10 of our 12 cases of infection fulfilling our criteria for nosocomial acquisition and another temporally related to CVC manipulation at home. Intravascular devices (10 CVCs, 2 peripheral intravenous cannulae, and one peripheral intra-arterial line) were present in all our patients. These devices have previously been associated with coagulase-negative bacteremia (11, 16) and \textit{S. warneri} infection (9). There was no evidence of a single virulent nosocomial strain. Chromosomal DNA analysis by PFGE demonstrated the heterogeneity of our isolates, with six temporally and geographically separate patients sharing three similar patterns. Two of the 22 isolates had PFGE band patterns markedly different from those of the other 20. These results support the role of PFGE in coagulase-negative strain differentiation as an epidemiological tool.

![FIG. 1. PFGE of chromosomal DNA following digestion by Smal of 22 patient strains of \textit{S. warneri}. Lanes: Std, λ DNA molecular marker; A to V, patient strains as per Table 1. Lanes were aligned with GelCompar 4.0 (Applied Maths, Kortrijk, Belgium) after the gel had been scanned by HP DeskScan II, version 2.3 (Hewlett-Packard).](http://jcm.asm.org/on/August_14_2017_by_guest)

![FIG. 2. PFGE of undigested DNA of 22 patient strains of \textit{S. warneri} showing extrachromosomal DNA band patterns. Lanes: Std, λ DNA molecular marker; A to V, patient strains as per Table 1. Lanes were aligned with GelCompar 4.0 (Applied Maths) after the gel had been scanned by HP DeskScan II, version 2.3 (Hewlett-Packard).](http://jcm.asm.org/on/August_14_2017_by_guest)
REFERENCES


