Predominant *Staphylococcus aureus* Isolated from Antibiotic-Associated Diarrhea Is Clinically Relevant and Produces Enterotoxin A and the Bicomponent Toxin LukE-LukD

ALAIN GRAVET,1 MURIELLE RONDEAU,2 COLETTE HARF-MONTEIL,1 FABIENNE GRUNENBERGER,3 HENRI MONTEIL,1 JEAN-MICHEL SCHEFTEL,1 AND GILLES PREVOST1*

UPRES EA-1318, Institut de Bactériologie de la Faculté de Médecine (Université Louis Pasteur-Hôpitaux Universitaires de Strasbourg),1 and Service de Médecine Interne, Clinique Médicale A,2 and Service de Médecine Interne, Hôpital de Hautepierre,3 Hôpitaux Universitaires de Strasbourg, F-67000 Strasbourg, France

Received 28 May 1999/Returned for modification 7 June 1999/Accepted 16 August 1999

* Corresponding author. Mailing address: UPRES EA-1318, Institut de Bactériologie de la Faculté de Médecine (Université Louis Pasteur-Hôpitaux Universitaires de Strasbourg), 3 rue Koeberlé, F-67000 Strasbourg, France. Phone: (33) 3 88 21 19 70. Fax: (33) 3 88 25 11 13. E-mail: gilles.prevost@medecine.u-strasbg.fr.

From 1955 to 1970, *Staphylococcus aureus* was suspected as a cause of antibiotic-associated diarrhea (AAD) (30, 36). Investigations established a frequent relation between pseudomembranous colitis and administration of tetracycline with the isolation at high yields of *S. aureus* from stools (21, 32, 36). Altemeier et al. (2) studied patients with *S. aureus*-associated enteritis; the majority of the patients had undergone gastrointestinal surgery. One-third of the patients had undergone no operation before development of diarrhea. *S. aureus* was the only species isolated from stools on culture and was resistant to tetracycline. Hinton et al. (17) emphasized the emergence of staphylococci in stools as important pathogens responsible for AAD for three reasons: first, the close time relation between the onset of fulminating diarrhea and the occurrence of a heavy intestinal staphylococcal flora; second, the time relation between the defecescence from the disease and the decrease in abundance of staphylococci in stools after an appropriate antibiotic therapy; and third, the relation between the intestinal lesion and the location of the organism in patients who died (17, 30). Therefore, isolation of *S. aureus* as the predominant organism in the fecal flora should lead to treatment. *S. aureus* strains previously incriminated in enterocolitis (36) were reported (6) to be secondary enterotoxin A (SEA) producers.

In the 1970s, pseudomembranous enterocolitis was observed in patients treated with clindamycin and other antibiotics (4, 14). Isolation and identification of *Clostridium difficile*, which produces an enterotoxin (toxin A) and a cytotoxin (toxin B), favored the hypothesis that this bacterium was the cause of pseudomembranous colitis associated with AAD (3, 4, 14). The role of *S. aureus* was neglected and became controversial. However, methicillin-resistant and gentamicin-resistant *S. aureus* strains were recognized as the causes of outbreaks of enteritis in hospitalized patients who were treated with extended-spectrum antibiotics (25, 33) and in whom mild to fatal illnesses were observed.

The aim of the work described here was to study the clinical significance of *S. aureus* strains isolated for 2 years as the predominant bacteria from stools in a routine laboratory and to study the toxins production in comparison to those produced by isolates from other sites of infection. As LukE-LukD seemed to be frequently present in strains isolated from stools (15), the members of this bicomponent leucotoxin family and the staphylococcal enterotoxins were typed.

MATERIALS AND METHODS

Clinical data. (i) Group A patients. *S. aureus* was isolated as the only isolate or as the predominant isolate in cultures of stools of 60 patients whose hospital charts were reviewed. A standardized questionnaire was completed and included age, sex, hospital unit, reasons for admission, medical history, presence and duration of clinical diarrhea, additional symptoms, and a list of other samples that had been collected. Receipt of antimicrobial treatment or a gastrointestinal tract decontamination procedure within 1 month before the occurrence of diarrhea was recorded. The therapeutic outcomes in terms of the evolution of diarrhea and death as a direct consequence of this complaint were recorded.

Clinical diarrhea was defined as three or more liquid or soft stools a day for at least 2 days. Resolution of diarrhea was defined as normal stool frequency and normal consistency for at least 3 consecutive days.

(ii) Group B patients. Stools from 57 different patients who originated from two medical care units and who had received antibiotics for at least 5 days without diarrhea were systematically processed from March to June 1998. Samples were inoculated onto a 5% (vol/vol) sheep blood agar plate and onto Chapman-Stone medium for selective isolation of staphylococci (Difco, Detroit,
Mich.). The presence and relative abundance (on blood agar) of \( S. aureus \) isolates were recorded.

(iii) Random isolates. The toxin production study included 146 consecutive strains that were collected during two periods (July 1996 and March 1998) and that originated from different sites of infection, including 11 from blood, 38 from skin, 51 from the throat, mouth, and nose, 18 from the urinary tract, 23 from abcesses and infected materials, and 5 from stools.

Bacteriologic analyses. (i) \( S. aureus \) isolates. All stool samples referred by clinicians from patients hospitalized in Strasbourg University Hospital, Strasbourg, France, between July 1996 and June 1998 were evaluated. Stools and other samples (urinary tract, slough, vagina, and nose) were inoculated onto 5% sheep blood agar plates, and the plates were incubated for 24 h at 37°C. \( S. aureus \) was identified after plating stools on Mueller-Hinton agar with 2% (wt/vol) NaCl at 37°C for 24 h of incubation with a disk of oxacillin (5 μg; Sanofi-Diagnostic Pasteur, Marne la Coquette, France), according to the standards of the French Committee for Antibiotic (34).

Tests for detection of other pathogens, such as \( S. aureus \), \( E. coli \), \( Yersinia \), and \( P. aeruginosa \), were done by conventional methods (1, 7, 27).

(ii) \( C. difficile \) isolates. \( C. difficile \) was identified after plating stools on George-selective agar medium (15) containing cefoxitin (10 μg/ml) and cefoxime (250 μg/ml). Colonies suspected as being \( C. difficile \) were embedded in agarose plugs as described previously (30). After lysis of the plugs for 18 h at 50°C, the plugs were washed and stored at 4°C in 10 mM Tris-HCl–1 mM EDTA (pH 8.0). Macrorestriction of the DNA was accomplished with \( SmaI \) (New England Biolabs, Beverly, Mass.). Plugs (agarose plugs, 2.5 by 5.0 by 1 mm) were equilibrated in 300 μl of restriction enzyme buffer for 30 min at 0°C. They were incubated for 4 h at room temperature in 60 μl with 10 U of enzyme. Electrophoresis was performed at 12°C with a Bio-Rad Genelink system in a 1% agarose gel. Electrophoresis (TAFE) system with a 100 mM in 0.5% TAE buffer system (20× TAE buffer system buffer at 0.2 M Tris, 10 mM free acid EDTA, and 87 mM CH₃COOH [pH 8.2]) in a 1% (wt/vol) agarose gel. Electrophoresis of 5 μl DNA fragments began with 2-s pulses for 1 h, followed by 14-s pulses for 1 h, 12-s pulses for 1.5 h, 10-s pulses for 2.5 h, 8-s pulses for 3 h, and 6-s pulses for 6 h. The bacteriophage lambda PFG Marker (New England Biolabs) was used as a molecular marker. The gels were stained with ethidium bromide and were photographed under UV transillumination. Pulsootypes were compared and were classified in a dendrogram by using the Dice coefficient and the unweighted pair group method with arithmetic mean cluster-

Pulsed-field gel electrophoresis (PFGE). \( S. aureus \) strains at midexponential growth in 25 ml of TY (BioTryptase, 1.6% [wt/vol]; yeast extract, 1% [wt/vol]; NaCl, 0.5% [wt/vol]) were embedded in agarose plugs as described previously (31). After lysis of the plugs for 18 h at 50°C, the plugs were washed and stored at 4°C in 10 mM Tris-HCl–1 mM EDTA (pH 8.0). Macrorestriction of the DNA was accomplished with \( SmaI \) (New England Biolabs, Beverly, Mass.). Plugs (agarose plugs, 2.5 by 5.0 by 1 mm) were equilibrated in 300 μl of restriction enzyme buffer for 30 min at 0°C. They were incubated for 4 h at room temperature in 60 μl with 10 U of enzyme. Electrophoresis was performed at 12°C with a Bio-Rad Genelink system in a 1% agarose gel. Electrophoresis (TAFE) system with a 100 mM in 0.5% TAE buffer system (20× TAE buffer system buffer at 0.2 M Tris, 10 mM free acid EDTA, and 87 mM CH₃COOH [pH 8.2]) in a 1% (wt/vol) agarose gel. Electrophoresis of 5 μl DNA fragments began with 2-s pulses for 1 h, followed by 14-s pulses for 1 h, 12-s pulses for 1.5 h, 10-s pulses for 2.5 h, 8-s pulses for 3 h, and 6-s pulses for 6 h. The bacteriophage lambda PFG Marker (New England Biolabs) was used as a molecular marker. The gels were stained with ethidium bromide and were photographed under UV transillumination. Pulsootypes were compared and were classified in a dendrogram by using the Dice coefficient and the unweighted pair group method with arithmetic mean clustering provided by Molecular Analyst (version 1.3) and Fingerprinting (version 1.12) software (Bio-Rad, Ivry sur Seine, France).

Toxin determinations. (i) Enterotoxins A, B, C, and D and Tst-1. \( S. aureus \) strains were detected as recommended by the manufacturer (Oxoid) by detection of enterotoxins A, B, C, and D, and toxin shock syndrome toxin 1 (TSS-1) by semi-quantitative tests with reversed passive latex agglutination (RPLA) toxin detection kits (SET-RPLA and TST-RPLA [Oxoid], respectively). These tests were reported to be sensitive and specific, although large amounts of enterotoxin E seemed to be detected as low levels of enterotoxin A (8).

(ii) Enterotoxin E. The total DNAs of the isolates were digested by EcoRI restriction of total DNA. After agarose gel electrophoresis of the restricted DNAs, \( endE \) was probed by Southern blotting (35) with \( 5^\prime \)-terminal labelled specific oligonucleotide, position 636–5′-TTCGAGGCTCAAGTAAAGTTT–3′ position 660) (11).

(iii) Bicomponent leucotoxins and exfoliative toxins. Components of different leucotoxins (Panton-Valentine leucocidin, LukE-LukD, LukM-LukF–PV, gamma-hemolysin, and exfoliative toxins A and B) were detected by radial gel immunodiffusion with component-specific rabbit polyclonal and affinity-purified antibodies prepared as described previously (25) and tested after 18 h of growth in YCP (yeast extract, Casamino Acids, pyruvate) medium (15).

RESULTS

Clinical results. (i) Group A patients. During the study period, a total of 60 patients were enrolled in group A. Forty-one patients (68.3%) were admitted to medical units, 10 (16.7%) were in surgical units, and 9 (15%) were in pediatric wards. The mean age was 60.0 ± 29.0 years (age range, 1 month to 95 years). The male/female ratio was 1.2:1. One hundred fourteen \( S. aureus \) isolates from these patients were evaluated: 81 were from stools, 28 were from blood, and 5 were from other samples (urinary tract, slough, vagina, and nose). During the same period, stools of 3,437 different patients were examined. Culture and/or toxin assays for \( C. difficile \) were positive for 460 patients. For 247 of these patients, a diagnosis of \( C. difficile \)-associated AAD was made. The distribution of the \( C. difficile \) isolates according to age (mean age, 63.7 ± 24.3 years; age range, 3 months to 99 years) and medical units was similar to that for \( S. aureus \) isolates.

Among the 60 patients in group A, 6 had no clinical diarrhea. The mean age of these patients was 1.8 ± 3.3 years (age range, 1 month to 9 years). All were hospitalized in pediatric units and presented no more than two watery stools. These patients had normal body temperatures, but two presented with moderate elevations in C-reactive protein levels.

Fifty-four (90%) patients had clinical diarrhea, from most of whom it was acute and watery. The patients had a mean of 4.1 liquid or soft stools per day (range, 3 to 10) liquid or soft stools. The mean age of the patients was 66.5 ± 22.6 years (age range, 1 month to 95 years). Only 3 of 54 patients did not receive antibiotics in the month before the onset of diarrhea. For the first of the latter patients without a specific medical background, \( Campylobacter jejuni \) was found in the stools in association with \( S. aureus \), which was predominant. For the second patient, a 3-month-old, an enteropathogenic \( Escherichia coli \) strain was found. The third patient was a premature infant who presented with an elevation in the C-reactive protein level and from whom a methicillin-susceptible \( S. aureus \) strain was isolated. A diagnosis of colitis was made, and the patient was treated with intravenous vancomycin. \( C. difficile \) was isolated from the stools of four patients. The tests for enterotoxin A and cytotoxin B were also positive. Patients were treated with metronidazole, and the diarrhea disappeared rapidly, but the tests for \( C. difficile \) remained positive. After a 3-week remission, the diarrhea reappeared, and both \( C. difficile \) and \( S. aureus \) were still isolated from the stools. The test for toxin B was positive, but at a lower level. For three of these patients, oral treatment with vancomycin, 500 mg every 8 h, was prescribed, and the diarrhea disappeared within 3 days but the stools remained positive for \( C. difficile \) and cytotoxin B only. For the fourth patient, the remission of diarrhea was longer (2 months); he was then treated with metronidazole but soon died.

The 47 remaining patients presented with diarrhea, and no enteropathogens other than \( S. aureus \) were isolated as the predominant isolate in culture or were associated with AAD. The mean age of these patients was 68.6 ± 18.2 years. Table 1 summarizes the different antibiotics prescribed for these 47 patients. Diarrhea began an average of 11.7 ± 5.4 days (range, 4 to 30 days) after administration of the first antibiotic dose. For 30 of 47 (63.8%) patients, combination therapy with two or three antibiotics was administered. Thirty-four of the 47 (72.3%) patients received fluoroquinolones: 11 as monotherapy and 23 in combination with other agents.

The patients with AAD were then distinguished according to their underlying diseases. Twenty-nine patients had not undergone abdominal surgery and had no digestive disease. These
patients were admitted for cardiac pulmonary decompensa-
tion, cerebrovascular accident, or general deterioration. All of 
these patients presented or had presented with an infection: 
pneumonia, urinary tract infection, or an infection of undeter-
determined origin. These infections were assumed to be the 
reason for hospitalization or were considered nosocomial infections. 
These patients were hospitalized for $22.2 \pm 33.9$ days (range, 0 
to 170 days) before the onset of diarrhea. Eighteen other 
patients had a particular underlying disease with digestive 
manifestations: one had pancreatitis, one had cirrhosis, three 
had digestive carcinoma with recent surgery, four had digestive 
carcinoma without recent surgery, one had multiple endocrine 
neoplasia, three had surgery for malformation, one had diver-
ticulosis, two had liver graft, one had bone marrow graft, and 
one had graft-versus-host disease (digestive form). Among the 
latter group of 18 patients, 7 patients had received an immu-
nosuppressive therapy before the onset of diarrhea: 4 had 
received chemotherapy for neoplastic disease and three had 
received chemotheraphy for grafts. Two patients, one with mul-
tiple neoplasia and one with graft-versus-host disease, had 
frequent watery stools, but the stools became diarrheic with 
isolation of $S. aureus$. These 18 patients were hospitalized for 
$15.9 \pm 10.1$ days (range, 0 to 37 days) before the onset of 
diarrhea.

The patients had normal body temperature just before the 
beginning of the diarrhea. The clinical manifestations of diar-
rhea in patients were a rise in body temperature for 28 of 47 
(59.6%) patients and a rise in biological parameters of infec-
tion for 26 of 47 (55.2%) patients. Finally, 11 (23.4%) patients 
did not present any general or biological signs of infection 
except the diarrhea.

**Treatment and evolution of disease in group A patients.** 
The outcomes of the diarrhea for the 47 patients with AAD were 
considered according to the antimicrobial treatment. For eight 
patients, oral vancomycin was applied. For six of these pa-
tients, the first dose of glycopeptide was administered 5.1 days 
after the beginning of diarrhea, which stopped within 4.5 days (range, 2 to 7 days). The patients had lower signs of infection; 5 of the 10 patients 
had no rise in body temperature. Diarrhea disappeared from all patients in this group, with a rapid improvement in their 
general condition.

For the remaining 10 patients, including the patient with 
blood contamination, the previous antimicrobial treatment was 
replaced by another drug but not vancomycin. The diarrhea 
stopped for two patients. Eight patients died, and the diarrhea 
was an important factor in the changes in their health.

**Group B patients.** The mean age of the 57 group B patients 
was 75.8 ± 11.2 years. They received antibiotics for at least 5 
days before stool sampling. Two patients had digestive surgery, 
and one patient had amputation surgery. Seven patients had 
digestive diseases but had not undergone surgery. Twenty pa-

### TABLE 1. Antimicrobial therapy administered to 47 patients within the month before the onset of diarrhea$^a$

<table>
<thead>
<tr>
<th>Antibiotic(s)</th>
<th>Group A ($n = 47$)</th>
<th>Group B ($n = 57$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monotherapy</td>
<td>Therapy in association with other antibiotic(s)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Cephalosporins + carbapenem</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>β-Lactams + β-lactamase inhibitor</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Macrolides</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Others$^a$</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

---

$^a$ Group A: pristinamycin, 1 patient, trimethoprim-sulfamethoxazole, 1 patient. Group B: trimethoprim-sulfamethoxazole, 2 patients; rifampin, 1 patient; nitrofuran-
toin, 1 patient.
patients had complicated diabetes. All 57 patients presented with infectious diseases, which affected, in order of frequency, the lungs, urinary and digestive tracts, and skin. Therefore, the medical background for the group B patients was similar to that for the group A patients. Table 1 summarizes the antibiotics prescribed. Eighteen (31.6%) patients received amoxicillin and 22 (38.6%) received fluoroquinolones. Colonies of Staphylococcus aureus were found on blood agar plates and on Chapman medium plates for two patients. For one of these patients, an Enterococcus sp. was predominant (90% of the total CFU), while for the second patient, S. aureus seemed to be present in amounts equal to the amounts of Enterococcus. For three patients, 1 to 10 CFU of S. aureus was isolated only on Chapman medium. For the latter three patients and the 54 remaining patients, the predominant isolated floras were an Enterococcus sp., gram-negative rods, and yeast and coagulase-negative Staphylococcus.

Biological results. (i) Antimicrobial susceptibilities of S. aureus strains from group A patients. Isolates from 58 patients (97%) were resistant to amoxicillin and 55 (92%) were methicillin-resistant S. aureus (MRSA), but all were susceptible to vancomycin (100%). They were susceptible to trimethoprim-sulfamethoxazole (58 of 60; 97%), nitrofurantoin (58 of 60; 97%), rifampin (49 of 60; 82%), and chloramphenicol (53 of 60; 88%). Susceptibility to the other antibiotics was variable: 59 (82%) were resistant to ciprofloxacin, 48 (80%) were resistant to kanamycin, 48 (80%) were resistant to tobramycin, 15 (25%) were resistant to gentamicin, 45 (75%) were resistant to neomycin, and 48 (80%) were resistant to amikacin.

(ii) Differentiation of the S. aureus strains by DNA pulsedtyping. The total DNAs of all 114 S. aureus isolates from the patients were analyzed by SmaI macrorestriction and PFGE. Figure 1 provides a schematic representation of the 27 different pulsortypes, 26 of which were for isolates from stools. The number of isolates corresponding to each pulsortype was noted. Taking into consideration the profiles obtained for three different isolates retested on a simple or different gel, standard deviations of ±5% were recorded for various DNA fragments. For example, PFGE patterns 3, 4, and 5 differed only in the locations of single DNA fragments of 180, 197, and 165 kb, respectively. PFGE profiles 10 and 13, which differed only in the locations of single DNA fragments of 450 and 420 kb, respectively, were also considered to be similar, even though the other fragments were similarly separated. PFGE fingerprints 20 and 21 also differed because of an additional frag-
ment of 420 kb in pattern 21. The pulsotypes were distributed in 15 clusters, with the cutoff for a cluster being 80% similarity.

Considering the distribution of isolates from the 60 patients into 27 PFGE fingerprints, it appeared that isolates of pulsotypes 13, 10, 16, and 17 were the most frequently encountered (10, 7, 7, and 7 isolates from different patients, respectively) (Fig. 1). In all cases, when \textit{S. aureus} was isolated several times from a given patient, all related stool isolates had the same pattern (Fig. 2). For one patient, with a clinical history of diarrhea for 3 months, the three isolates from stools and isolates originating from the nose and the urinary tract were pulsotype 16, whereas isolates from a slough and the vagina were pulsotype 17. For each patient with septicemia, isolates from stools and blood cultures were of the same pulsotype (Fig. 2). PFGE profiles 14, 21, and 22 were observed for isolates from single patients whose stools and blood were sampled. The isolate from one positive blood culture considered to be contaminated with \textit{S. aureus} and those from stools of a single patient were of pulsotypes 24 and 10, respectively.

(iii) Toxin production. The results of toxin production are summarized in Table 2. The newly discovered bicomponent leucotoxin LukE-LukD was produced by 93.6% of the 47 representative isolates from patients with \textit{S. aureus}-associated AAD, 76.9% of the 13 \textit{S. aureus} isolates not associated with AAD, and 33.6% of the 146 random isolates. SEA enterotoxin A was produced by 85.1, 46.1, and 54.1% of the three groups of isolates cited above, respectively. Both groups of toxins were produced by 80.9, 22.6, and 30.8% of the isolates in the same groups, respectively.

Among the six isolates from young patients without diarrhea in whom \textit{S. aureus} was the predominant organism, three produced LukE-LukD, two produced SEA, and two produced staphylococcal enterotoxin D but no isolate produced both SEA and LukE-LukD. The isolate from the young patient

<table>
<thead>
<tr>
<th>Disease or organism</th>
<th>Total no. of isolates</th>
<th>Staphylococcal enterotoxin</th>
<th>No. (%) of patients positive for each toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-associated AAD</td>
<td>47</td>
<td>A 40 (85.1) B 0 C 0 D 0 E 0</td>
<td>TSST-1 0 PVL 0 ET 0 LukE-LukD 44 (93.6) SEA + LukE-LukD 38 (80.9)</td>
</tr>
<tr>
<td>SA-nonassociated AAD</td>
<td>13</td>
<td>A 6 B 0 C 0 D 1 E 0</td>
<td></td>
</tr>
<tr>
<td>Enterocolitis with SA</td>
<td>1</td>
<td>A 0 B 0 C 0 D 2 E 0</td>
<td></td>
</tr>
<tr>
<td>AAD caused by SA + CD</td>
<td>4</td>
<td>A 3 (75) B 0 C 0 D 0 E 0</td>
<td></td>
</tr>
<tr>
<td>SA + other pathogens</td>
<td>2</td>
<td>A 1 B 0 C 0 D 1 E 0</td>
<td></td>
</tr>
<tr>
<td>Predominance of SA in patients without AAD</td>
<td>6</td>
<td>A 2 (33.3) B 0 C 0 D 2 (33.3) E 0</td>
<td></td>
</tr>
<tr>
<td>No predominance of SA in patients without AAD</td>
<td>5</td>
<td>A 5 (100) B 0 C 0 D 0 E 0</td>
<td></td>
</tr>
<tr>
<td>Random SA isolates</td>
<td>146</td>
<td>A 79 (54.1) B 6 (4.1) C 8 (5.5) D 10 (6.8) E ND</td>
<td>TSST-1 15 (10.3) PVL 2 (1.4) ET 2 (1.4) LukE-LukD 49 (33.6) SEA + LukE-LukD 33 (22.6)</td>
</tr>
</tbody>
</table>

*Abbreviations: SA, \textit{S. aureus}; CD, \textit{C. difficile}; PVL, Panton-Valentine leucocidin; ET, enterotoxin; ND, not determined.*
presenting with enterocolitis with diarrhea produced LukE-LukD but none of the staphylococcal enterotoxins.

All 47 isolates were negative for production of enterotoxins B, C, D, E, TSST-1, and Panton-Valentine leucocidin. Only one isolate produced the exfoliative toxin A. Among the 146 random S. aureus isolates, 6 isolates produced enterotoxin B, 8 produced enterotoxin C, 10 produced enterotoxin D, 15 produced TSST-1, 2 produced Panton-Valentine leucocidin, and 2 produced one of the exfoliative toxins. The gamma-hemolysin was produced by all the isolates studied; LukM-LukF-PV (15) was never produced. Of the five isolates from patients who did not have diarrhea and who received antibiotics for at least 5 days, all produced SEA; only two isolates produced LukE-LukD.

**DISCUSSION**

With the identification of *C. difficile* and its toxins, the etiologic role of *S. aureus* in AAD became controversial (4, 14). The arguments developed were (i) the increase in the carriage rate of *S. aureus* following admission to the hospital (29), (ii) the lack of clear evidence of the association of *S. aureus* with pseudomembranous colitis, (iii) culture conditions more favorable to the growth of *S. aureus* (5), and (iv) the efficacy of vancomycin, which became apparent in 1959, against both *S. aureus* and *C. difficile*, although *C. difficile* was not identified at that time (5). However, there is some evidence against the etiologic role of *S. aureus* and *C. difficile* as well. As opposed to *S. aureus*, a satisfactory animal model of colitis evidenced the role of *C. difficile* in these infections (9). The frequency with which each bacterium was associated with AAD was not assessed. The rate of lack of recovery of *C. difficile* despite detection of toxin in stools is generally estimated to be no more than 5%. Recent studies indicate that when *S. aureus* is the predominant isolate in a culture it is a possible cause of AAD (20, 25, 33).

Over 2 years, 60 patients for whom *S. aureus* was isolated from stool samples as the predominant or only isolate were considered. Fifty-four patients (mean age, 66.5 years) had diarrhea, whereas the others were young infants without diarrhea. For 6 of the 54 patients, the role of *S. aureus* could not be clearly established. One young patient with diarrhea from whom *S. aureus* was recovered did not receive antibiotics. Finally, 47 patients were assumed to have *S. aureus* associated AAD. The frequency of *S. aureus*-associated AAD was a fifth of that of *C. difficile*-associated AAD and was therefore significant. Most patients with *S. aureus*-associated AAD presented with signs of infection, whereas this was more seldom the case for those with *C. difficile*-caused AAD (4, 14).

To fulfill the second Koch’s postulate, stools from 57 patients (group B) who did not have diarrhea after at least 5 days of antimicrobial treatment in two medical units also affected by AAD and who were examined over 4 months during the 2 years of the study. It appeared that an *Enterococcus* sp. was the predominant organism, and *S. aureus* was isolated from only five patients and was obtained on a selective culture medium (Chapman medium) for three of the patients. The frequency of *S. aureus* isolation from stools differed for group A and B patients, although their medical backgrounds were similar. However, previous antimicrobial therapy also differed for the two groups, since fluoroquinolones alone or in combination with another antibiotic were mostly administered to group A patients (*P* < 0.001), whereas amoxicillin was more frequently administered to group B patients (*P* < 0.02). The reason why fluoroquinolones may select for MRSA over bacteria like *Enterococcus* spp. remains unexplained. The rate of carriage of *S. aureus* increases with the length of hospitalization (38). However, the predominance of *S. aureus* in stools is probably an essential condition for induction of the disease, as is the case for *C. difficile*. The pathologic yields of abundance of *S. aureus* and *C. difficile* in stools remain to be defined.

Discontinuation of antibiotics seemed to be a good choice for resolution of the *S. aureus*-associated AAD when patients presented with few clinical signs of infection. Otherwise, oral vancomycin applied rapidly after the beginning of AAD was satisfactory. Intravenous vancomycin was more or less efficient, in accordance with previous data (20, 25, 33). For every patient in the present study, *S. aureus* was no longer isolated after relapse of diarrhea consecutive to a dedicated treatment.

For the six young patients, the rate of occurrence of *S. aureus* in stools was generally frequent, especially for patients fed nasogastrically (4, 16), and the significance of these isolates rarely seemed to be pathogenic. The absence of diarrhea may be due to the absence of the association of SEA and LukE-LukD or, as for the *C. difficile* toxins (4), to the non-susceptibility of young children. In such patients, cases of diarrhea due to *S. aureus* were reported in a previous study (16).

The frequency of 91% MRSA strains among the isolates from 60 group A patients (97% for the isolates from the 47 patients with AAD) with *S. aureus* in stools differed from the 40% frequency of MRSA found in the same hospital (22). The *S. aureus* strains originating from stools in this study appeared to be polymorphic when analyzed by PFGE, since 26 different pulsortypes were distinguished, although 15 clusters were evidenced, which favors a nonepidemic origin. However, pulotype 13 corresponded to pulsortype 1 found a few years ago for nosocomial MRSA strains in the same hospital (31). Eleven patients were infected with such an isolate. Therefore, strains associated with AAD cannot be easily defined as being responsible for hospital-acquired infections or being of an endogenous origin. In fact, little is known about (i) the presence at very low yields and frequency of *S. aureus* in the gastrointestinal flora, (ii) the selective pressure of feeding on this ecology for the selection of resistant strains, (iii) the possibility of several types of strains in the fecal flora, and (iv) the more or less rapid growth of contaminating MRSA in antibiotic-treated gastrointestinal fluids. The spread of *S. aureus* strains from patients with AAD must be considered as a possible and important source of nosocomial infections that require specific hygienic measures in hospitals. Moreover, PFGE analysis revealed pulsortypes which never differed for isolates from a single patient, including those from patients with bacteremia in association with AAD. These data strongly suggest that *S. aureus*-associated AAD may constitute an entry to septicemia.

The production of LukE-LukD and SEA was significantly different between the *S. aureus* strains associated with AAD and the group of random isolates (*P* < 0.001; *χ*² = 51.36 and 14.45, respectively). The 44 representative isolates positive for LukE-LukD among the *S. aureus* isolates associated with AAD were polymorphic, with 18 different pulsortypes distributed in 10 clusters. Most of these isolates were MRSA (97%), but only 24 of the 49 random isolates that produced LukE-LukD were MRSA. Production of this toxin was not linked to methicillin resistance.

The frequency of production of SEA in the random group of isolates was comparable to the results in the literature, despite variability in this frequency (10, 24, 26). The general occurrence of TSST-1 production by *S. aureus* isolates from clinical samples appeared to be quite frequent (10 to 12%), but data were not significant for isolates originating from feces (18). A comparable frequency was observed for random isolates in this study, but because of the absence of such isolates from stools,
the frequency cannot be assessed. The production of both SEA and LukE-LukD was significantly different among the 47 isolates associated with AAD and the random group of isolates (P < 0.001; \( \chi^2 = 51.87 \)). The frequencies of the two toxins were not significantly different from the observed frequencies of association (Z test, P < 0.05; Z = 0.122 and 1.063). There is no genetic link between the two toxins.

In four other patients, the presence of S. aureus and diarrhea disappeared after oral vancomycin treatment, while C. difficile and cytotoxin B were still detected. The patients concerned were treated with metronidazole, with relapses in 3 to 6 weeks. When the diarrhea appeared again, S. aureus and C. difficile and its toxins were still isolated, although at lower yields than in the first event. Three of these patients were then treated with oral vancomycin. The diarrhea disappeared and S. aureus was no longer isolated, whereas they remained positive for C. difficile and its toxins. The last patient was treated with metronidazole, and the outcome was fatal, with diarrhea as an important factor. These four S. aureus isolates produced LukE-LukD, and three produced SEA. A recurrent C. difficile-associated diarrhea (12) and a complication of diarrhea caused by S. aureus were also envisaged. The particular profile of toxin production may be an argument favoring a staphylococcal etiology.

In conclusion, S. aureus infection in association with AAD exists and must be taken into consideration, especially for relatively older patients, even though it is less frequent than AAD caused by C. difficile. Rapid and specific care is recommended for such patients and to prevent the spread of MRSA. Production of both SEA and LukE-LukD is associated with these isolates. An attempt is being made to develop experimental models.

ACKNOWLEDGMENTS

We are grateful to the staff of Strasbourg University Hospital for cooperation in the collection of clinical data. Thanks are given to N. Boord for reading of the English. We greatly appreciate the technical assistance of B. Muller and D. Keller and the help of O. Meunier and J.-M. Rousée for the computer analysis of the PGFE fingerprints.

This work was supported by grant EA1318 from “Direction de la Recherche et des Etudes Doctorales.”

REFERENCES


