Epidemiological Markers for Epidemic Strain and Carrier Isolates in an Outbreak of Nosocomial Oxacillin-Resistant *Staphylococcus aureus*

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Received 8 January 1990/Accepted 26 March 1990

An outbreak of nosocomial infections occurring in a postoperative intensive care unit was caused by a single strain of oxacillin-resistant *Staphylococcus aureus*. Six patients were infected, or colonized, by this strain, which was traced by using the following four epidemiological markers: antibiogram, bacteriophage type, capsular polysaccharide type, and esterase electrophoretic type. This strain was compared with *S. aureus* isolates obtained from the noses of 13 carriers from a group of 42 staff members. A good correlation in terms of phenotypic markers was found between the epidemic strain and a strain isolated from one carrier. Both exhibited the same pattern of multiple resistance as well as the same phage type, 77, capsular polysaccharide type, 5, and esterase electrophoretic type, 6. In contrast, an oxacillin-resistant strain, isolated from another carrier, differed from the epidemic strain by susceptibility to rifampin and by susceptibility to four additional bacteriophages. The other 11 strains isolated from carriers were susceptible to oxacillin and exhibited widely different phenotypes. These results confirm the interest of using several epidemiological markers to trace the spread of epidemic *S. aureus* strains and to delineate the carrier strains.

Nosocomial infections caused by oxacillin-resistant (Oxa*) *Staphylococcus aureus* lead to an increasing problem in intensive care units, where virulent bacteria can spread from infected patients to the medical staff as well as to other patients with severe underlying conditions (25, 30). These strains have gradually become resistant to multiple additional antibiotics, such as aminoglycosides, macrolides, lincomamides, and fluoroquinolones (1, 23, 24), and were encouraged by the extensive use of antibiotics in intensive care units (3, 14, 27).

Various epidemiological markers, such as antibiotic susceptibility pattern and bacteriophage type, have been used for studies of outbreaks (3, 4, 6, 14, 27). Strains of *S. aureus* have been identified by capsular type (2, 19), and studies have shown the predominance of capsular types 5 and 8 among clinical isolates both in the United States (2) and in Europe (8, 19, 29). Electrophoretic analysis of cellular extracts, followed by staining for esterase activity, is a powerful tool for intraspecies delineation of bacteria (18, 28) and has been used for the study of methicillin-susceptible and methicillin-resistant strains of *S. aureus* (9, 10). This method can also provide extensive data for epidemiological investigations (11, 20).

We present here the results of the study of an outbreak of *S. aureus* in the Hôtel-Dieu hospital (Paris, France). In addition to antibiogram and phage type, capsular type and esterase electrophoretic type of the strains isolated from both patients and carriers were investigated. The overall comparison between these markers allowed the differentiation of the epidemic strain and the various other isolates of *S. aureus* that were found.

**MATERIALS AND METHODS**

**Background.** The Hôtel-Dieu hospital is a 575-bed university-affiliated hospital in the center of Paris and includes an 8-bed surgical intensive care unit. Strains resistant to multiple antibiotics identified in the microbiology laboratory are recorded daily by geographic origin, and the physicians in charge of the patient are immediately informed. The other patients in the unit are then examined for infection or colonization. An infection control nurse is responsible for surveillance, intervention, and control measures. No Oxa* strain had been recorded in the hospital during the 7 weeks prior to the transfer of patient 1 from another hospital.

**Isolation of strains.** Patient 1 was admitted to the surgical intensive care unit of Hôtel-Dieu hospital for a traumatic hemothorax infected with a multiple-antibiotic-resistant strain of *S. aureus*. Despite surgical investigations, chest tube drainage, and treatment with vancomycin, the same multiple-antibiotic-resistant strain was isolated from both the empyema and the intravenous catheter. The patient developed acute endocarditis and died 10 days after admission. Despite standard precautions, transmission of *S. aureus* to five patients of the same ward (patients 2 through 6) occurred during the subsequent 6 weeks (Fig. 1). Three patients developed both pneumonia and bacteremia, while the remaining were colonized only, as shown by cultures of their wounds or drainage tubes. Three isolates of the same strain were found during this period in two other wards but did not lead to severe infections.

To avoid further transmission of the organism, precautions were reinforced and a search for the strain was made among the hospital staff members. Swabs were taken from the anterior nares of 42 physicians, nurses, and members of the house staff who had contact with patients in the intensive care unit. The samples were plated on 5% sheep blood agar and incubated for 18 h at 37°C. Of these 42 subjects, 13 had

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PATIENTS

| No. 1 |  |
| No. 2 |  |
| No. 3 |  |
| No. 4 |  |
| No. 5 |  |
| No. 6 |  |

FIG. 1. Temporal relationship between the six patients in the surgical intensive care unit. Patients 3, 4, and 6 died as the result of both S. aureus infection and severe underlying disease. Symbols: - - - - - - hospitalization in the unit; ■ presence of the epidemic strain in specimens.

cultures positive for S. aureus. Species identification was made on the basis of morphology of colonies, Gram stain, positive catalase test, and positive tube coagulase test.

A total of 31 strains, including 10 from the Hôtel-Dieu hospital, with capsular polysaccharide type 5 of a total of 97 Oxa⁺ S. aureus strains from 406 isolates unrelated to this outbreak (15) were analyzed as control strains.

**Antimicrobial susceptibility testing.** Susceptibility to antimicrobial agents was determined by the standard disk diffusion method on Mueller-Hinton agar plates (Diagnostics Pasteur, Marnes la Coquette, France). Inhibition of growth was interpreted according to standard recommendations (13). The disks contained 6 μg of penicillin G, 5 μg of oxacillin, 10 IU of streptomycin, 30 IU of kanamycin, 10 IU of gentamicin, 10 μg of tobramycin, 30 μg of amikacin, 30 μg of netilmicin, 30 IU of tetracycline, 30 μg of chloramphenicol, 15 IU of erythromycin, 15 μg of lincomycin, 15 μg of pristinamycin, 200 μg of sulfadiazine, 5 μg of trimethoprim, 10 μg of fusidic acid, 30 μg of vancomycin, 30 μg of rifampin, 5 μg of pefloxacin, or 50 μg of fosfomycin.

**Phage typing.** The international set of 23 phage types (group I: 29, 52, 52A, 79, and 80; group II: 3A, 3C, 55, and 71; group III: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85; group V: 94 and 96; and miscellaneous: 81 and 95) was applied by using standard methods (7, 31). An additional experimental phage, 54A, was also tested. Isolates were typed both at the routine test dilution and at a 100-fold-higher concentration. Only reactions showing major lysis were considered.

**Capsular polysaccharide typing.** Type 5 and type 8 capsular polysaccharides were detected in bacteria grown on Columbia agar slants (Difco Laboratories, Detroit, Mich.). The bacteria were suspended in phosphate-buffered saline and autoclaved at 121°C for 1 h. After centrifugation for 10 min at 10,000 × g, the polysaccharides were detected in the supernatant by inhibition enzyme-linked immunosorbent assay (8), by using purified capsular antigen preparations (16, 17) and the corresponding monoclonal antibodies. Strains lacking both type 5 and type 8 capsular polysaccharides were designated as nontypeable.

**Esterase electrophoretic typing.** The electrophoretic mobility pattern of esterases was investigated as previously described (9). Briefly, esterases from supernatant fluids of lysostaphin-treated staphylococcal cultures were analyzed by polyacrylamide-agarose gel electrophoresis. They were characterized by their activities toward five synthetic substrates (α- and β-naphthyl acetates, α- and β-naphthyl butyrates, and indoxyl acetate), by their resistance to diisopropyl fluorophosphate, and by their heat inactivation characteristics.

**RESULTS**

**Antimicrobial susceptibility testing.** S. aureus isolates from all six patients were found to be highly resistant to oxacillin, since growth was detected to the disk edges. They were also resistant to penicillin G, amingglycosides, tetracycline, erythromycin, lincomycin, sulfonamides, rifampin, and pefloxacin but were found susceptible to chloramphenicol, pristinamycin, trimethoprim, fusidic acid, vancomycin, and fosfomycin (Table 1).

### TABLE 1. Epidemiological markers of S. aureus isolates from carriers and patients

<table>
<thead>
<tr>
<th>Origin of isolates</th>
<th>Antiogram*</th>
<th>Phage type</th>
<th>Capsular type</th>
<th>Esterase type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>P° O° A° T° E° L° S° R° F°</td>
<td>NT*</td>
<td>8</td>
<td>16</td>
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<tr>
<td>2</td>
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</tr>
<tr>
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<td>P°</td>
<td>96</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>P°</td>
<td>3C, 55, 77</td>
<td>8</td>
<td>10a</td>
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<tr>
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<td>8</td>
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<tr>
<td>12</td>
<td>P° OR PO A° T° E° L° S° R° F°</td>
<td>6, 54, 75, 77, 85</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>P° OR PO A° T° E° L° S° R° F°</td>
<td>77</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Patients 1 to 6</td>
<td>P° OR PO A° T° E° L° S° R° F°</td>
<td>77</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

* Markers for carrier strain 1 are indicated; for other strains, only different results are indicated. ---, Similarity with the antibiotic markers of strain 1. P, Penicillin G; O, oxacillin; A, amingglycosides (streptomycin, kanamycin, gentamicin, tobramycin, amikacin, and netilmicin); T, tetracycline; E, erythromycin; L, lincomycin; S, sulfadiazine; R, rifampin; F, fluoroquinolone (pentaloxacin). All the strains were identically susceptible to the following other antibiotic markers tested: trimethoprim, chloramphenicol, pristinamycin, fusidic acid, fosfomycin, and vancomycin.

* NT, Nontypeable.
S. aureus isolates from two carriers were found to be resistant to oxacillin. The isolate from carrier 13 exhibited the same antibiogram pattern as the epidemic strain. The isolate from carrier 12 differed by its susceptibility to rifampin (Table 1). Carrier 12 was the surgeon who operated on patients 4 and 6. After 10 days of vacation, his nasal cultures were found negative for S. aureus. Carrier 13 was a nursing assistant involved with patient care throughout the ward; he was treated with intranasal bacitracin ointment four times a day for 10 days and was cleared of nasal carriage when he returned to work in the hospital 2 weeks later.

The remaining 11 carrier isolates were found susceptible both to oxacillin and to multiple other antibiotics. Four different antimicrobial susceptibility patterns (Table 1) were exhibited.

**Phage typing.** The isolates from the six patients and from carrier 13 were phage type 77 (Table 1). The isolate from carrier 12 was lysed by phage 77 and by four additional group III phages (phages 6, 54, 75, and 85). Six other isolates had five different phage types, and five isolates were nontypeable, even at 100-fold the routine test dilution (Table 1).

**Capsular typing.** The isolates from the six patients were capsular type 5. The isolates from carriers 12 and 13 were also type 5 (Table 1). Among the other carriers, four isolates were type 5, five were type 8, and two were nontypeable (Table 1).

**Esterase electrophoretic typing.** All isolates from the six patients were esterase type 6. The isolates from carriers 12 and 13 were also esterase type 6 (Table 1). Seven different other esterase types were observed among the isolates from the remaining carriers (Table 1).

**Control strains.** Only 7 of 31 control isolates were the same capsular type, 5, esterase type, 6, and phage type, 77, as the epidemic strain. One of these strains was from the Hôtel-Dieu hospital, three were from one other hospital, and the remaining ones were of different origins.

**DISCUSSION**

The admission of a patient infected with a multiple-antibiotic-resistant S. aureus strain to an intensive care unit resulted in a nosocomial outbreak. The use of several markers showed a full correlation between the results for the isolates from the six patients and those from 1 of the 13 carriers. The isolate from another carrier differed only by its susceptibility to rifampin and by the phage lysis characteristics. The other carriers harbored oxacillin-susceptible strains, which exhibited striking phenotypic diversity.

The choice of markers for epidemic investigations depends on a concerted strategy, taking into account speed, cost, and local facilities. In our study, the epidemic strain was easily traced, since it was multiply resistant to antimicrobial agents, as shown by a routine test, i.e., antibiogram. However, these results required confirmation by other methods, which characterized the strain by its phage type, 77, its capsular type, 5, and its esterase type, 6.

The correlation between oxacillin resistance and susceptibility to lysis by the group III phages, such as phage 77 (21), is well known. Resistance to oxacillin was initially observed in S. aureus strains belonging to phage group III (5) and remained preponderant among the closely related strains which react primarily with phages of groups I and III (12, 22, 26).

Previous clinical surveys indicated that capsular types 5 and 8 account for approximately 70% of S. aureus isolates (19) and 90% of invasive strains responsible for bacteremias (8). The predominance of capsular type 5 among Oxa' S. aureus strains has already been described (15). Furthermore, capsular type 5 Oxa' S. aureus strains isolated in France have been recently characterized by their esterase type 6 (10, 11) activity. In the control strains, unrelated to this outbreak, we found the association between phage type 77 and esterase type 6 in less than 25% of capsular type 5 Oxa' strains.

By showing susceptibility to rifampin and susceptibility to additional phages, antibiogram and phage typing were equally reliable in delineating the strain of carrier 12 from the outbreak strain, although both were Oxa' and capsular type 5 and esterase type 6. Archer and Mayhall (3), studying an outbreak, also observed that resistance to rifampin was the unique marker in the antibiogram which allowed delineation of the epidemic from a resistant strain. Since rifampin resistance was a stable chromosomal marker, the antibiogram was the easiest tool with which to screen isolates from culture surveys. In our study, phage typing confirmed that the strain from carrier 12 differed from the epidemic strain, as already suggested by the antimicrobial susceptibility patterns.

For oxacillin-susceptible strains, the delineation was best achieved when antibiotic susceptibility pattern and phage typing were combined with capsular typing and esterase typing. These isolates exhibited various phenotypes, and the overall results of typing methods showed them to belong to at least nine different populations.

The full concordance, in terms of all markers, between the isolates from patients and that from one carrier is in contrast with the diversity of results obtained from the other isolates. This confirms the interest of a typing system using several markers for epidemic investigations. These markers allow the responsible strain to be traced and managed in order to prevent further spread of the infection.

**ACKNOWLEDGMENTS**

We thank P. Goulet for helpful discussion and constant support; Catherine Devine, Alain Boutonnier, Annick Fenneteau, Colette Gaillard, and Nicole Hautier for excellent technical assistance; and Isabelle Babilaëre for typing the manuscript. We are also grateful to William T. Stringfellow for critical review of the manuscript.

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