Phage Pattern-Specific Oxacillin-Resistant and Borderline Oxacillin-Resistant *Staphylococcus aureus* in U.S. Hospitals: Epidemiological Significance

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For a 13-year period (1978 through 1990), oxacillin-resistant (MIC, >4 $\mu\text{g/ml}$) *Staphylococcus aureus* (ORSA) strains were collected from Clinical Center (National Institutes of Health) patients and patients from five other U.S. hospitals. From Clinical Center patients, 251 of 253 isolates (99%) were bacteriophage typed as phage group III. Five other hospitals contributed 203 ORSA strains, of which 188 (93%) were group III. The group III ORSA strains predominantly included a characteristic core pattern of phages, 7/47/53/54/75/77. For the low-level (borderline) oxacillin-resistant strains (MIC, 2 to 4 $\mu\text{g/ml}$), amoxicillin-clavulanic acid combination (Augmentin) testing disclosed 62 hyper- β -lactamase producers, of which 59 (95%) were of a separate, distinct *S. aureus* strain, with the phage pattern 92/94/96/292/D-11 (group V). Thus, ORSA and hyper- β -lactamase producing *S. aureus* are distinct epidemic strains.

The first *Staphylococcus aureus* strains shown resistant to methicillin were not from methicillin-treated patients. These strains possessed natural resistance to this drug (1, 2, 10, 14). Of 5,440 *S. aureus* strains collected from England before methicillin usage, 3 were methicillin resistant (10). All three had a group III phage pattern, 7/47/53/54/75/77. Barber reported in 1964 (2) on natural methicillin-resistant *S. aureus* among strains collected from hospitals in England, France, and Denmark. Knox (14) and Sutherland and Rolinson (22) described the characteristics of methicillin-resistant *S. aureus*. These also had the same general phage pattern, 7/47/53/54/75/77. Pattern length variation for a given group III strain was and is common (18). About 1968, a marked increase of clinically significant methicillin-resistant *S. aureus* isolates was noted in England (19). Methicillin-resistant *S. aureus* infections in the United States increased dramatically in the late 1970s and reached epidemic proportions in the early 1980s. This increase was noted in four large hospital centers, affiliated with medical schools (8). Boston City Hospital had no methicillin-resistant strains until 1967 (3), but in the succeeding 12 months, 22 strains were isolated from 18 patients. The present study uncovered additional evidence that the group III strain(s) described in the first 1960s reports continues into the 1990s as a nosocomial pathogen. Oxacillin resistant *S. aureus* (ORSA) strains characteristically possess a distinct orange-yellow pigmentation, have much higher catalase production than non-ORSA strains, and have increased resistance to Tween 80 and mercuric chloride (20, 22). Borderline oxacillin-resistant *S. aureus* strains (6, 16) have been shown in this study to be hyper- β -lactamase-producing *S. aureus* (HBLPSA) strains (17) whose resistance is greatly neutralized in the presence of clavulanic acid. HBLPSA strains from a number of geographical areas in the United States were phage typed.

ORSA. From outside sources, 39 isolates were received from University Hospital of Jacksonville, Fla.; 108 isolates

were received from Veterans Administration (VA) Hospital, Sioux Falls, S. Dak., courtesy of Mary Jo Jaqua; 5 isolates were received from Walter Reed Army Medical Center, courtesy of Alan Cross; 7 isolates were received from VA Hospital, Loma Linda, Calif., courtesy of Harry Elston; and 44 isolates were received from the University of Chicago Hospital, courtesy of Mary Fran Smaron. Clinical Center, National Institutes of Health, contributed 253 isolates.

HBLPSA. Five isolates were received from the Centers for Disease Control, courtesy of Robert C. Cooksey. All ORSA or HBLPSA isolates submitted were retested in the National Institutes of Health laboratory to confirm the category of oxacillin resistance. Sixty-two HBLPSA isolates (one isolate per separate patient per year) were recovered in the Clinical Center in 4 years (1987 through 1990).

All isolates were tested with oxacillin, and so they will be referred to as ORSA in this study. A standardized suspension of morphologically similar colonies was inoculated into calcium- and magnesium-supplemented Mueller-Hinton broth to achieve a final concentration of 5×10^5 CFU/ml. The test procedure recommended by the manufacturer was followed in preparing Sensititre custom air-dried microtiter antibiotic-susceptibility test panels (Sensititre Microbiology Systems, Radiometer America Inc., Westlake, Ohio), which included oxacillin plus 2% NaCl and amoxicillin-clavulanic acid. The agar diffusion test used a 1- μg oxacillin disk. Test panels were incubated at 35°C in 5% CO₂ for 18 to 24 h. Results were read visually. In addition, all of the ORSA isolates were resistant by MIC determination to a concentration of 32 μg of amoxicillin and 16 μg of clavulanic acid per ml, and the HBLPSA isolates were susceptible at a concentration of 4 μg of amoxicillin and 2 μg of clavulanic acid per ml.

Phage typing. A total of 29 bacteriophages at 100 routine test doses were used: 3B, 3C, 6, 7, 29, 42B, 42E, 44A, 47, 52, 52A, 53, 54, 55, 71, 75, 77, 80, 81, 83, 86, 92, 94, 95, 96, 99, 187, 292, and D-11. Group III phages within the set included 6, 7, 42B, 42E, 47, 53, 54, 75, 77, and 83. Group V phages included 92, 94, 96, 292, and D-11. A lysis pattern of one or

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TABLE 1. ORSA in the Clinical Center, by year

Year	No. of ORSA isolates			Phage typed at one/patient/yr
	Group III ^a	Not phage typed	Total	
1978	1	1	2	1
1979	14	9	22	2
1980	2	1	3	1
1981	18	12	30	6
1982	25	8	33	9
1983	30	27	57	8
1984	12	21	33	5
1985	10	19	29	5
1986	30	28	58	8
1987	46	19	65	15
1988	50	25	75	12
1989	4	1	5	3
1990	9	0	9	7
Total	251	171	422	82

^a One patient was infected with two ORSA strains in groups other than group III. This patient had one ORSA strain with the phage pattern 3B/3C/55/71 and one ORSA strain with the phage pattern 29/52/80. Therefore, of the 84 phage-typed ORSA isolates (one/patient/year), 82 (98%) were group III.

more phages of a single group places the tested *S. aureus* strain within that group. Given the large number of group III phages (i.e., 10), a firm strain differentiation within the group phages requires a matching pattern, usually within two phages in length, and core or common phages of the same numbers. Core phages for the studied strain were 7/47/53/54/75/77.

To minimize the risk of phage mutation during propagation and to ensure the same concentration of viral particles for succeeding typing runs, phage lots were lyophilized in aliquots (25). After postlyophilization titering, the phages, sealed in glass vials under vacuum, were stored at -20°C, at which the titers remained stable for at least 18 years (23). Sufficient vials of each phage were stored to supply 15 years of phage typing, performed once a month. Tryptic soy broth of the same lot number was used throughout this study for growing the bacterial strains before typing. For agar, SeaKem agarose of the same lot number was used throughout, combined with tryptic soy broth at 1% wt/vol. Anhydrous calcium chloride (147 mg/liter) was added. Eagle's vitamin mix (100×) was added at 1%. A bacteriophage applicator (24) was used. Other facets of the technique were previously published (25). Square petri plates (100 by 100 mm) were used, each plate receiving 40 ml of molten agar.

Clinical Center ORSA strains (Table 1) had predominantly a group III lytic pattern. One patient carried three different phage-patterned ORSA strains, one of which, the group III strain, was recovered from blood over a 2-week period. One ORSA was of group I and one was of group II. The possibility of resistance transfer between this patient's three separate ORSA strains exists. Many patients carried the group III epidemic ORSA for a number of years and were infected repeatedly with their endogenous strain.

Among Clinical Center HBLPSA strains, 59 of the 62 (95%) borderline oxacillin-resistant strains (HBLPSA strains) had the group V phage pattern 92/94/96/292/D-11. The remaining three strains had phage patterns 42E/44A/47, 47, and 3B/3C/55. (Of the 62 strains, 6 were collected in 1987, 16 were collected in 1988, 24 were collected in 1989, and 16 were collected in 1990.) For four isolates, the oxacillin MIC was >4 µg/ml, but these isolates were susceptible to amox-

acillin-clavulanic acid. Their phage pattern, 92/94/96/292/D-11, also indicated that these strains were HBLPSA, and they were included in that group. Of the five HBLPSA isolates from the Centers for Disease Control, three were group V, and two were nontypeable.

Of 89 Clinical Center patients, 7 carried both the ORSA and the HBLPSA strains.

Of the 253 ORSA strains (National Institutes of Health), 251 (99%) were group III. The University Hospital, Jacksonville, strains were all (39 of 39) group III. The VA Hospital, Sioux Falls, ORSA strains were 94% group III (102 of 108), with six strains (5%) being nontypeable. For the ORSA strains from University of Chicago Hospital, 36 of 44 (82%) were group III, with 8 (18%) being nontypeable. VA Hospital, Loma Linda, had six of seven strains (87%) that were group III, with one (14%) that was nontypeable, and five of five (100%) ORSA strains from Walter Reed Hospital were group III. Combined ORSA strains from the six hospitals were 96% group III (439 of 456).

Although bacteriophage typing of *S. aureus* strains is the most sensitive tool available for epidemiological study, many phage-typing programs around the world have become less useful because of viral mutation during continuous phage propagations. Reactions from phage sets at separate laboratories vary so much, particularly for the older group III phages, that it is difficult to evaluate data from different institutions. In this laboratory, phage lyophilized in aliquots and stored in vacuo at -20°C permitted use of the same phage propagation lot for about 15 years of monthly typing (23). Most propagations in our laboratory require only one passage.

In 1961, Barber (1) reported phage patterns for the first three methicillin-resistant *S. aureus* strains found in England. The patterns were 53/54/75/77, 7/47/53/75/77, and 7/47/53/54/75/77. The ORSA strains reported here have the same general patterns as those reported by Barber. It is reasonable to assume that present-day ORSA strains are derived from the worldwide spread of closely related epidemic ORSA strains. This is supported by numerous reports from around the world of similar group III-patterned ORSA strains (3-9, 11, 13, 15, 16, 18-20).

Other strains have arisen, some with group I phage patterns (20), represented by phage pattern 29/52/80. Group II methicillin-resistant *S. aureus* phage patterns are seen less often and are represented by selections from phages 3A/3B/3C/55/71 (12).

During a detailed 1981 study of three epidemic ORSA strains at the University of Virginia, Peacock et al. (20) recorded the phage pattern 6/47/54/75/83A and a MIC of >128 µg/ml (methicillin) for one strain. The MIC for the second strain was 2.0 µg/ml, and the phage pattern was 96; the MIC for the third strain was 2.0 µg/ml and the phage pattern was 94/96. The latter two strains were probably HBLPSA strains. This established that yet another U.S. hospital has had the pandemic, specific-phage-patterned ORSA and HBLPSA strains introduced. From the evidence presented in this paper, it seems likely that these two distinct strains represent the predominant ORSA strain in many U.S. hospitals and the predominant HBLPSA strain (92/94/96/292/D-11). Most phage typing sets do not include all phages of this lytic group of phages. Schaeffer et al. (21) used only phage 92. However, susceptibility to even one phage of the group places the *S. aureus* strain within this closely related group of phages. Thus, the strains reported as methicillin-resistant phage 92 strains by Schaeffer may have been borderline resistant or HBLPSA strains. The level of resis-

tance was not given. If so, since Schaeffer studied strains from a number of New York City hospitals, the HBLPSA strain is also widely disseminated in the New York City area.

It is apparent that the group III and group V phage patterns constitute positive markers for the predominant worldwide ORSA strains and in the United States, at least, for HBLPSA strains.

ORSA strains were present in very small numbers before the advent of methicillin or oxacillin. These antibiotics paved the way for an increase in ORSA strains by eliminating susceptible *S. aureus*. The general increase and subsequent decrease in the incidence of ORSA infections in most areas of the world, with notable exceptions in individual hospitals and localized areas, follows the epidemiologic characteristics of previous *S. aureus* pandemics. Among these are the postantibiotic sloughing enteritis of the 1950s attributed to phage group III *S. aureus* and the devastating pandemic of generalized infections in the 1950s and early 1960s caused by the phage group I *S. aureus*, phage pattern 80/81 (9). Transmission of the *S. aureus* phage core pattern 7/47/53/54/75/77 around the world is similar to that of *S. aureus* phage pattern 80/81, which originated in 1954 in Australia. *S. aureus* 80/81, of course, was penicillin resistant and left an unparalleled path of serious infections, with mortality exceeding that of any strain on record. Perhaps if the few ORSA strains had not been present at the advent of methicillin and oxacillin therapy, no significant resistance to these drugs would have been encountered over the years.

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