JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1988, p. 2395–2401
0095-1137/88/112395-07$02.00/0

Vol. 26, No. 11

Immunoblots, Antimicrobial Resistance, and Bacteriophage Typing of Oxacillin-Resistant *Staphylococcus aureus*

MAURY E. MULLIGAN,[1,2]* RICHARD Y. Y. KWOK,[3] DIANE M. CITRON,[1] JOSEPH F. JOHN, JR.,[4] AND PETER B. SMITH[5]

Medical[1] and Research[3] Services, West Los Angeles Veterans Administration Medical Center, Los Angeles, California 90073; UCLA School of Medicine, Los Angeles, California 90024[2]; Department of Medicine, Medical University of South Carolina, Charleston, South Carolina 29425[4]; and Hospital Infections Program, Centers for Disease Control, Atlanta, Georgia 30333[5]

Received 12 April 1988/Accepted 26 July 1988

An immunoblotting system was developed for typing of oxacillin-resistant *Staphylococcus aureus*. Clinical isolates recovered during a 40-month period at a single institution were evaluated with this typing scheme. Results were compared with susceptibility patterns and with bacteriophage typing results for 100 clinical isolates and with plasmid fingerprints for 14 isolates. Immunoblotting was found to be a useful method with good reproducibility that distinguished seven major groups of patient isolates that were clinically and epidemiologically related. Susceptibility patterns showed specific correlations with other typing results but were inferior to immunoblotting and phage typing for differentiating major groups of organisms. Plasmid profiles failed to distinguish two major groups that were readily identified by immunoblots and phage typing. There was evidence of increasing antimicrobial resistance of endemic hospital strains. Immunoblotting correlated well with phage typing, offered an alternative method for typing isolates that could not be typed by phage typing, and was superior to susceptibility testing and plasmid profiles for distinguishing different groups of oxacillin-resistant *S. aureus* at our institution.

Staphylococcus aureus resistant to the antistaphylococcal penicillins such as methicillin and oxacillin has become an increasingly common pathogen (2, 17). Some institutions have experienced outbreaks caused by a distinctive strain, whereas in other settings multiple strains of resistant *S. aureus* have been recognized (1–5, 7, 10–13, 15). Various typing systems including bacteriophage typing, antibiograms, plasmid profiles, electrophoresis of radiolabeled cellular proteins, and restriction endonuclease patterns as well as the presence of unique markers such as aminoglycoside-inactivating enzymes have been used as epidemiologic tools (1, 12, 15). There has been interest in evaluating new methods for typing oxacillin-resistant *S. aureus* because the standard method, phage typing, cannot be used to type all isolates (1, 13) and may give variable results with repeated testing of isolates (12). In response to an increased frequency at our institution of strains of oxacillin-resistant *S. aureus* with several antimicrobial susceptibility patterns, we evaluated a new typing method using immunoblots and compared results with phage typing, susceptibility patterns, and plasmid profiles.

MATERIALS AND METHODS

Selection of isolates. Strains of oxacillin-resistant *S. aureus* were recovered from clinical specimens of 211 patients at the West Los Angeles Veterans Administration Medical Center from February 1984 to June 1987. A total of 100 isolates from 62 of the patients were selected for study. There were single isolates from 40 patients and multiple isolates from 22 patients. Patients were selected to represent clusters of cases. Multiple isolates from individual patients were selected to compare organisms recovered from different body sites and, in selected cases, so that isolates associated with recurrent or prolonged colonization (up to 20 months duration) could be examined. The majority of patients were identified because oxacillin-resistant *S. aureus* was recovered from a clinical specimen submitted by the primary physician. Colonized patients were occasionally identified because of hospital policy that required that nasal cultures be obtained from roommates of individuals found to have oxacillin-resistant *S. aureus*. If the organism was recovered from any site, patients also had cultures taken from the nares, perineum, and open skin lesions. All culture-positive sites were sampled repeatedly during hospitalization. For 56 patients, acquisition of the organism was judged to be nosocomial because of prior hospitalization at the institution; six other patients had been outpatients or had been transferred from other institutions. In addition to the clinical isolates, a test isolate of oxacillin-resistant *S. aureus* from the College of American Pathologists was examined.

All isolates were catalase and tube-coagulase positive, and the MIC of oxacillin for the isolates was >16.0 μg/ml as determined by broth microdilution with Mueller-Hinton broth supplemented with calcium, magnesium, and 2% NaCl (8). The identity of 20 representative isolates was confirmed to be *S. aureus* by the Staph-Ident kit (Analytab Products, Sherwood Medical, Plainview, N.Y.). Isolates were collected from the clinical laboratory and stored in skim milk at −40°C until further study.

Antimicrobial susceptibility testing. Susceptibilities to antimicrobial agents other than oxacillin were determined by disk diffusion as described by the National Committee for Clinical Laboratory Standards protocol M2-A3 (9) for amikacin, chloramphenicol, clindamycin, erythromycin, gentamicin, novobiocin, penicillin, sulfisoxazole, tetracycline, trimethoprim-sulfamethoxazole, tobramycin, and vancomycin (Difco Laboratories, Detroit, Mich.) as well as rifampin (BBL Microbiology Systems, Cockeysville, Md.). To provide single breakpoints to facilitate classification, we labeled isolates resistant if, by National Committee for Clinical

* Corresponding author.

Laboratory Standards criteria, they would be considered resistant or intermediate. Because no standard guidelines were available for interpreting susceptibility of *S. aureus* to rifampin by disk diffusion, a zone diameter of <23 mm (which correlated with an MIC of >2 μg/ml) was selected to define resistance. For 30 isolates, microdilution MICs were also determined to confirm the reliability of disk diffusion test results.

Bacteriophage typing. Bacteriophage typing (14) was performed with the international set of phages at standard and 100-fold concentrations. Phages used included group I (29, 52, 52A, 79, 80), group II (3A, 3C, 55, 71), group III (6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85), and miscellaneous phages (81, 94, 95, and 96). Phage typing was performed twice for 40 isolates to evaluate reproducibility of results. For 33 of these repeated tests, the isolates were typed in a single batch.

Plasmid profiles. Plasmid DNA was isolated by the method of Archer and Mayhall (1) except that *S. aureus* strains were grown on sheep blood agar overnight. After lysostaphin was added to a suspension of organisms in salt solution, the mixture was incubated for at least 1 h at 37°C. The cleared lysates were electrophoresed in 0.7% agarose at 50 mA for approximately 3 h. Plasmid size was determined by comparing the distance travelled by the unknown plasmids with that of plasmids of known molecular size (6). The gel was stained with ethidium bromide and transilluminated with UV light and photographed with a Polaroid camera.

Immunoblotting. EDTA extracts were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Brain heart infusion broth (15 ml; BBL Microbiology Systems) was inoculated with two to three colonies of *S. aureus* from blood agar and incubated at 35°C for 24 h. Cells were washed in 10 ml of 0.15 M phosphate-buffered saline (PBS; pH 7.4) twice. EDTA (70 mM, 100 μl) was added to each tube of washed cells, and the cells were then incubated at 45°C for 30 min and centrifuged at 1,500 × *g* for 15 min. The supernatant (EDTA extract) was then stored at −20°C until further study. A 50-μl portion of each EDTA extract was mixed with 12 μl of sample buffer (containing 0.5 ml of glycerol, 0.25 ml of 20% sodium dodecyl sulfate, 0.05 ml of beta-mercaptoethanol, and 0.05 ml of 0.1% bromphenol blue per ml) and heated at 65°C for 15 min. Polyacrylamide gel electrophoresis was performed as described previously (19) with 12.5% running and 5% stacking gels at 20 mA for the stacking gel and 40 mA for the running gel for a total of 6 h. Immunoblotting was based on the method of Tsang et al. (16) for enzyme-linked immunoelectrotransfer blotting. Transfer onto a nitrocellulose membrane was performed in a Trans Blot chamber (Bio-Rad Laboratories, Richmond, Calif.) containing 0.025 M Tris, 0.193 M glycine, and 20% methanol (vol/vol) (pH 8.35) at 4°C for 16 h at 10 V. The nitrocellulose was washed four times with 0.15 M PBS-Tween 20 (0.3%) with shaking at 40°C.

Batches of pooled human sera were used as the source of antibody; each pool was from approximately 50 patients (who were not evaluated for prior exposure to oxacillin-resistant *S. aureus*). Three different batches were collected over the course of 1 year. The different pools of sera were tested in parallel to determine whether results were similar and reproducible. The sera were from specimens submitted for clinical testing and had been refrigerated at 4°C for 1 week. They were subsequently stored in aliquots at −40°C until use. Thawed sera were diluted 1:50 in PBS-Tween 20 and incubated with shaking at room temperature for 1 h with the nitrocellulose. After four washings with PBS-Tween 20 for 1 h, the nitrocellulose was incubated for 1 h at 25°C in peroxidase-conjugated goat anti-human immunoglobulin G (Sigma Chemical Co., St. Louis, Mo.). The conjugate was diluted (1:1,000) in PBS-Tween 20 immediately before use. After washing, the nitrocellulose was reacted with a mixture of 50 mg of 3,3′-diaminobenzidine (Sigma) and 0.1 ml of 3% hydrogen peroxide in 100 ml of PBS. To estimate molecular weights, prestained standards were run with blots.

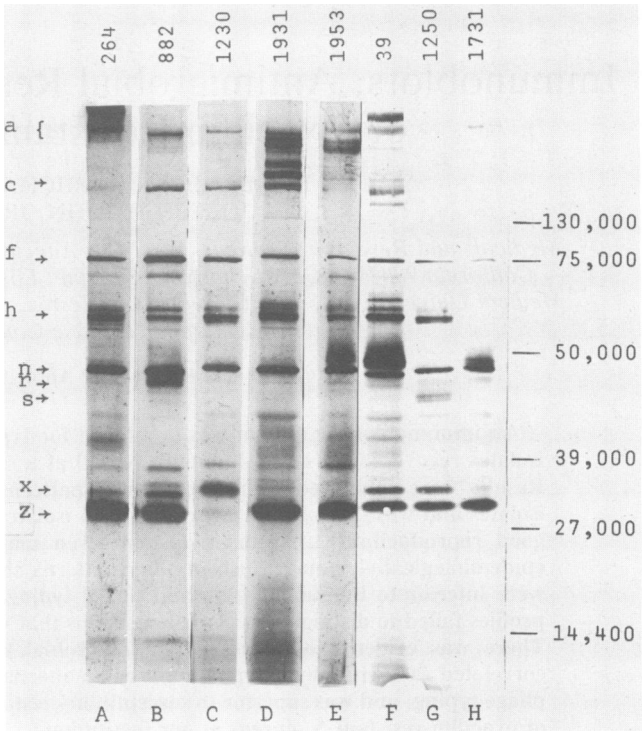

FIG. 1. Immunoblots of eight types of oxacillin-resistant *S. aureus*. Isolate numbers are listed above lanes. Immunoblot types are listed below lanes. Molecular weights are indicated in the right margin, and major bands are indicated in the left margin.

To determine reproducibility of results, we subjected 55 isolates to repeated susceptibility testing and 31 isolates to repeated immunoblotting on at least two occasions, the isolates having been stored in skim milk at −40°C in the interval. In addition, to evaluate the possible variation in immunoblot patterns for a single strain of *S. aureus*, we also evaluated by immunoblotting 22 isolates recovered from five different body sites of a single patient during a 9-week period.

RESULTS

Immunoblots for representative isolates are presented in Fig. 1 and 2; isolates are identified by laboratory numbers above the lanes and immunoblot types below the lanes. Figure 3 is a schematic representation of the major common bands and the distinctive patterns used to distinguish the immunoblot types. For initial analysis, bands were labeled a through z. Ultimately, the designation a was used to indicate a complex group of bands at a very high molecular weight range (above 130,000) and b was used to designate a double band just below the a complex (Fig. 3). There were common bands that we designated c, f, h complex, and n. Every isolate had one of two prominent bands in the low-molecular-weight range (approximately 27,000); these bands were designated x and z. Other distinctive bands were inconstant,

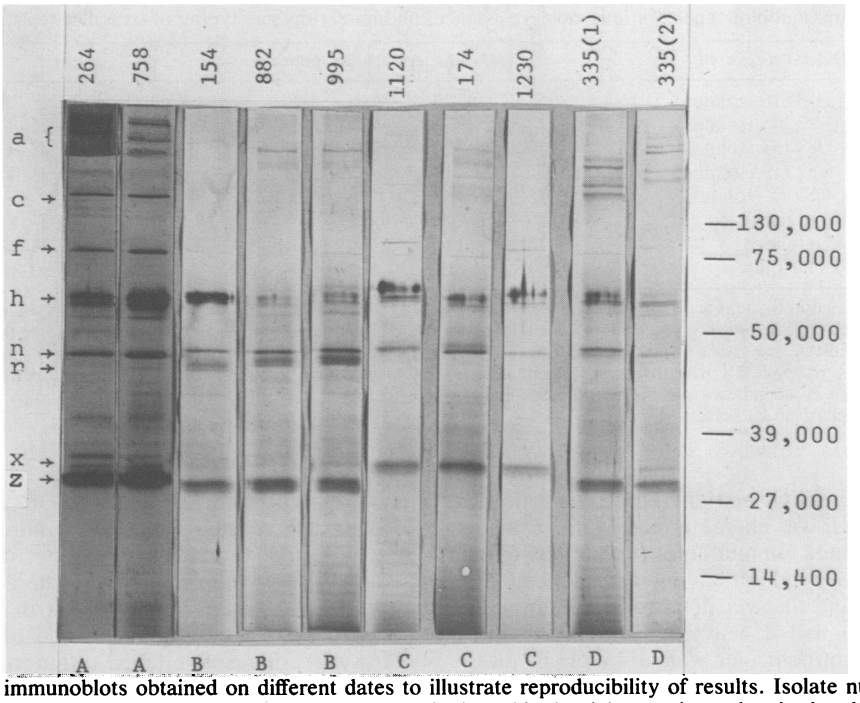

FIG. 2. Composite of immunoblots obtained on different dates to illustrate reproducibility of results. Isolate numbers are listed above lanes. Immunoblot types are listed below lanes. Molecular weights are indicated in the right margin, and major bands are indicated in the left margin.

seen on some preparations but not on others. In addition, there were bands that were reproducible but not useful for typing. Photography improved the resolution of bands.

Figure 1 illustrates the eight immunoblot types on a single run; the blots depicted in Fig. 2 are from different runs with different batches of pooled sera to illustrate the reproducibility of the method. Immunoblot type A isolates were unique in having a distinctive, very high molecular weight complex of bands, the a complex, above the 130,000-molecular-weight range. The r band, a distinctive band below the common n band, characterized immunoblot type B isolates. Immunoblot type C isolates had x bands at approximately 30,000 molecular weight, whereas all other isolates had z bands at approximately 27,000 molecular weight. Immunoblot type D isolates resembled the immunoblot type C pattern except for having z bands instead of x bands. In addition, immunoblot type D isolates had a high-molecular-weight complex just below the b complex. However, this complex was not consistently distinct and was not needed for typing.

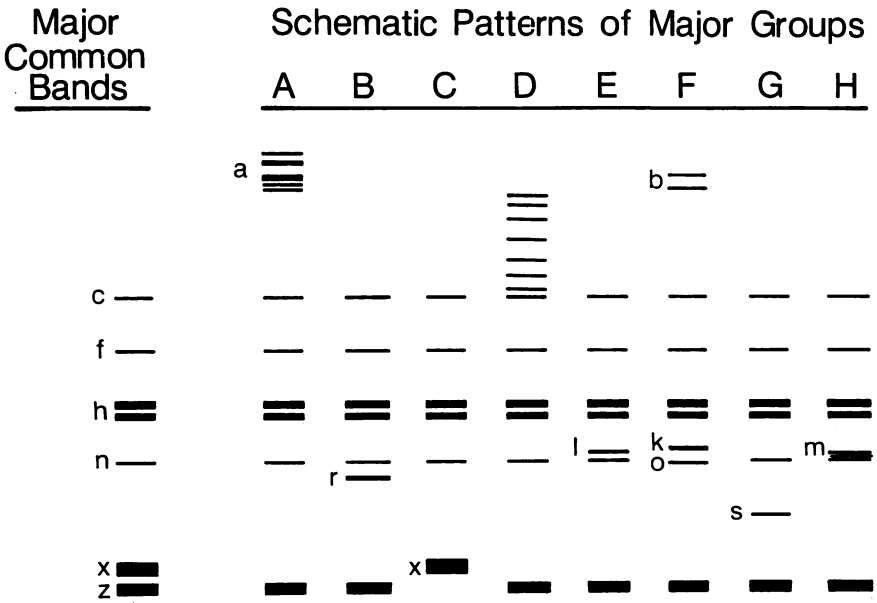

FIG. 3. Schematic presentation of common bands and patterns of major groups of oxacillin-resistant *S. aureus* identified by immunoblotting.

TABLE 1. Immunoblot types, antimicrobial resistance, and bacteriophage typing of oxacillin-resistant *S. aureus*

Blot type[a]	Date(s) recovered	Bacteriophage typing[b]	Resistance to[c]:
A	3/85–11/85 (6 isolates)	53 (77, 83A)	C, E, TC, N, R, TS, S (G, T, A)
B	3/84–3/87 (35 isolates)	47, 54 (29, 52, 52A, 80, 81, 6, 95, 75)	C, E, T, TC (G, A, R, TS, S)
C	8/84–5/87 (33 isolates)	54, 75 (42E, 47, 77, 83A, 81, 95, 6)	C, E, T (G, A, R, Ch)
D	2/84–6/87 (19 isolates)	Not typable	C, E, T (G, A, TC, R, TS)
E	5/86, 6/87 (5 isolates)	75 (47, 54, 85, 81)	T (C, E, G, T, A, TS, Ch)
F	4/84 (1 isolate)	Not typable	C, E, G, T, S
G	8/86 (1 isolate)	Not typable	C, E, G, T, TC
H	CAP[d] (1 isolate)	84	TC, Ch

[a] Criteria for types are defined in the text and illustrated in Fig. 1 through 3.
[b] All isolates were susceptible to the phage(s) listed first. Susceptibility to phages listed in parentheses was present for some but not all isolates.
[c] Letters in this column indicate resistance to antimicrobial agents: C, clindamycin; E, erythromycin; G, gentamicin; T, tobramycin; A, amikacin; TC, tetracycline; N, novobiocin; R, rifampin; TS, trimethoprim-sulfamethoxazole; S, sulfisoxazole; Ch, chloramphenicol. All isolates were resistant to the drugs listed first. Resistance to drugs listed in parentheses was present for some but not all isolates.
[d] Isolate from College of American Pathologists.

Immunoblot types E, F, G, and H (Fig. 1) were uncommon. Immunoblot type E was characterized by an l band just above the common n band. Immunoblot type F was unique in lacking the common n band but having instead a band that we designated the o band that was located just below the n region. This type also had a heavy k band above the n location and a very high molecular weight double band, the b complex. Immunoblot type G was characterized by an s band below the common n band. Immunoblot type H was characterized by an m complex that blended into the common n band.

Immunoblot patterns were identical when different batches of pooled sera were compared (Fig. 2). However, of a total of 183 blots obtained for 101 organisms, 5 (2.7%) results were discrepant, even when the same batch of sera was used. Reproducibility was variable for the eight different types. Results were always reproducible in the 88 tests for isolates in immunoblot types C, D, F, G, and H. However, 1 of 25 tests for type A isolates (4%), 2 of 63 tests for type B isolates (3%), and 2 of 7 tests for E isolates (28%) were discrepant. The discrepancies resulted when there was failure to visualize a distinctive band that characterized a type. This seemed to be the result of freezing and thawing of EDTA extracts prior to repeat testing and was minimized by preparation of fresh extracts. Experience with the technique also allowed recognition of poor quality blots with bands too faint for analysis. Antigenic reactivity of the pooled sera that had been stored at −40°C remained stable throughout the study. Individual batches of sera were stored for longer than 1 year. Immunoblots were not altered by storage of the isolates at −40°C but were, as previously noted, affected by repeated freezing and thawing of EDTA extracts.

Table 1 presents the eight different immunoblot types with their bacteriophage typing results and patterns of resistance to antimicrobial agents. There was good correlation between immunoblots and phage typing, given the inherent variability of phage typing results when testing is done on different occasions and when weak reactions must be taken into account. For presentation in Table 1, phages that reacted with all isolates in a blot type are indicated first and additional phages that reacted with some but not all isolates are listed in parentheses. Immunoblot type A isolates were the only isolates susceptible to phage 53. All 35 of the immunoblot B isolates were susceptible at standard concentrations to group III phages 47 and 54. On initial testing, 30 of the 35 immunoblot type B isolates were susceptible to phage 6. However, when phage typing was performed for 33 of the immunoblot B isolates in a single batch, all these isolates reacted with phage 6. Of the immunoblot type B isolates, 18 reacted with one or more of group I phages 29, 52, 52A, and 80, whereas 17 did not. The immunoblot type B isolates might therefore be considered to include two phage groups: one group that reacted with one or more of group I phages 29, 52, 52A, and 80, and one group that did not. However, one isolate failed to react with group I phages on the first testing but did so when testing was repeated. Two other isolates from the same patient reacted with group I phages on all tests. Although it is possible that there were two different but closely related phage groups among the immunoblot type B isolates (one susceptible to group I phages and one not), the apparent differences may simply reflect variability in phage typing results.

All immunoblot C isolates were susceptible to phages 54 and 75. Of the 33 immunoblot C isolates, 31 also reacted with phage 83A, and 29 of the isolates reacted with phage 77. Only one immunoblot type C isolate reacted with phage 6, and none reacted with group I phages 29, 52, 52A, and 80.

Although all isolates of an immunoblot type did not have identical phage typing results, the two systems had very good correlation; the differences in phage typing appeared to be due to the known variations obtained with different runs and to the difficulties of interpreting weak reactions.

Immunoblot types D, F, and G were isolates not typable with phages. There were 19 type D isolates, but only one each of types F and G. Of the five type E isolates, four were from a single patient. The type E isolates all reacted with phage 75 either at standard or 100-fold concentration.

Of the 40 isolates subjected to repeat phage typing, 12 (30%) had discrepant results if a discrepancy is defined as a difference of more than two phage reactions based on initial reports that did not list all weak reactions. However, for 27 isolates that had duplicate testing within the same year, detailed review of phage typing results (that included noting all weak reactions) identified a significant discrepancy in 5 of 27 (18.5%) tests.

Isolates came from a variety of sites: lower extremity, 27; nares, 24; sputum, 19; perineum, 8; pressure sore, 7; surgical wound, 6; blood, 3; eye, 3; urine, 2; and mouth, 1. In three cases, isolates obtained from the same patient were distinctly different (by immunoblotting, phage typing, and susceptibility testing), suggesting the presence of two different strains. In all other cases, isolates from the same patient had identical immunoblots and susceptibilities. The 22 isolates obtained from a single patient during a 9-week period also had identical blots. There were variations in phage typing of isolates from the same patient, but in most cases these were

TABLE 2. Increased antimicrobial resistance of oxacillin-resistant *S. aureus*

Isolate	No. susceptible or resistant/no. tested (%)
Type B	
Susceptible to trimethoprim-sulfamethoxazole before 12/86	25/25 (100)
Resistant to trimethoprim-sulfamethoxazole after 12/86	11/12 (92)
Type C	
Susceptible to gentamicin before 12/85	11/11 (100)
Resistant to gentamicin after 12/85	14/22 (64)
Type D	
Susceptible to gentamicin before 3/87	13/13 (100)
Resistant to gentamicin after 3/87	6/6 (100)

minor. However, two isolates from one patient differed by three phage reactions and two isolates from another patient differed by four phage reactions. When these isolates were tested in a single batch, there were no longer significant discrepancies.

Immunoblot patterns correlated closely with specific types of antimicrobial resistance. All immunoblot type A isolates demonstrated resistance to many classes of agents including novobiocin, rifampin, and trimethoprim-sulfamethoxazole. These isolates were, in fact, the only novobiocin-resistant isolates tested (zone diameter was ≤16 mm for all but one isolate, which had a zone diameter of 18 mm; MICs were ≥4 μg/ml for all isolates). Another consistent correlation was that all type A and B isolates were resistant to tetracycline, whereas all type C isolates lacked resistance to this agent. Except in the case of the multiply resistant type A isolates, susceptibility testing could not be used to differentiate major groups; for example, the majority of C and D isolates were resistant only to erythromycin, clindamycin, and the aminoglycosides and thus were not distinguishable.

It appeared that isolates of the same immunoblot and phage type became increasingly resistant during the study period (Table 2). Type B isolates were found to be resistant to trimethoprim-sulfamethoxazole only after December 1986. The more resistant isolates could not be distinguished by immunoblotting or by bacteriophage typing. Increased resistance to gentamicin was also noted for immunoblot types C and D.

Results of susceptibility testing were reproducible within expected limitations of the method and were not affected by storage. The most difficult interpretation was for amikacin susceptibility, which varied such that 22% of isolates with repeated testing moved between intermediate and susceptible classifications. However, this change in classification never reflected a change in zone diameter greater than 5 mm and usually was within 1 to 2 mm.

Fourteen isolates from the four major immunoblot groups (A to D) were examined for plasmids. There were three distinctive plasmid fingerprints for the isolates studied. The three type A isolates contained one large plasmid band and three small bands (smaller than the chromosomal DNA band). The five type B isolates had five major bands, four of which were smaller than the chromosomal band, with a conspicuous grouping between 2 and 5 megadaltons. The type B isolates that might be considered to constitute two phage groups (based on the presence or absence of susceptibility to group I phages) had identical plasmid fingerprints. All of the three type C and three type D isolates had a single large plasmid band in the same size range as the large plasmid bands seen with types A and B. Therefore, these two groups could not be clearly differentiated by plasmid fingerprints. It was difficult to distinguish between closed circular and open circular plasmid DNA in these preparations, but the fingerprints were consistent among the types. Because types C and D could not be differentiated by plasmid fingerprints, analyses of plasmid contents were not performed for the remaining isolates in this study.

Three patients had organisms recovered that were of different types (based on immunoblots and phage typing). For one patient who had two different types of oxacillin-resistant *S. aureus* recovered, complete clinical records were not available. A second patient had an immunoblot type D isolate first detected in April 1984 and received multiple courses of antimicrobial agents designed to eradicate his colonization. Typing of a later isolate that was recovered in May 1985 showed it to be an immunoblot type C isolate. There was no difference in susceptibility patterns, so that acquisition of the new strain would not have been detected by susceptibility typing. A third patient was originally colonized with an immunoblot B strain. He then had transient acquisition of a second, more resistant immunoblot type A strain while receiving antimicrobial therapy for colonization. Subsequently, he was persistently colonized with the more susceptible type B strain.

The immunoblot types of isolates recovered throughout the study period are illustrated in Fig. 4. The four patients with isolates of the uncommon immunoblot types E, F, and G did not acquire the organism at our institution. Two were referred from nursing home facilities and two had been outpatients immediately prior to detection of oxacillin-resistant *S. aureus*. Of the two other patients with acquisition of oxacillin-resistant *S. aureus* that could not be related to prior hospitalization at our institution, one had an immunoblot type A isolate and one had an immunoblot type C isolate. This latter patient was the first at our hospital to have an immunoblot type C isolate recovered and may have been responsible for introducing this strain to the institution. This isolate could not have been distinguished from the earlier type D isolates by susceptibility testing or plasmid profiles, although it was clearly identified by immunoblotting and phage typing.

Type A isolates were recovered only from a cluster of cases in 1985 (Fig. 4). Type D isolates were common in early 1984 and again in an outbreak in 1987. In contrast, immunoblot type B and C isolates appeared sporadically and were the predominant strains accounting for nosocomial acquisition of oxacillin-resistant *S. aureus* during the period of study. Because the 1987 outbreak involved multiple strains, typing was useful for identifying unrelated clusters of cases and judging the effectiveness of infection control measures.

DISCUSSION

As isolates of oxacillin-resistant *S. aureus* have become more common in the United States, there has been increased interest in determining optimal methods for management of patients who are either infected or colonized. Reliable typing systems are necessary for epidemiologic studies. In addition, they may help to evaluate the efficacy of regimens used to treat patients who are carriers of *S. aureus* because the evaluation of such regimens is often complicated by the occurrence of recolonization or relapse (20).

We found immunoblotting to be a typing system that distinguished major groups of organisms that were clinically and epidemiologically related. Immunoblot typing results

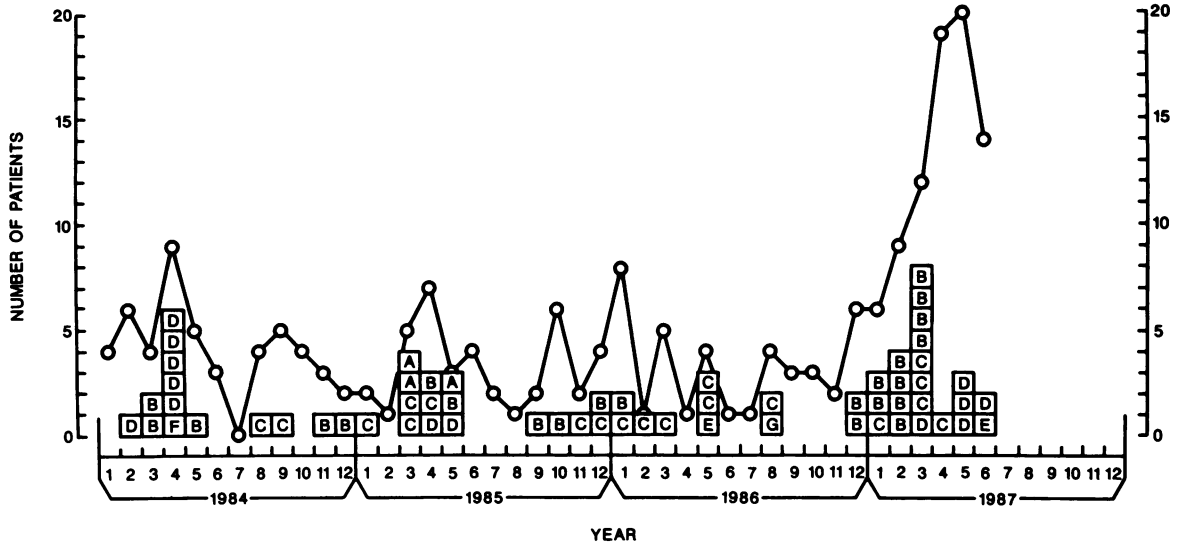

FIG. 4. Numbers of patients with oxacillin-resistant *S. aureus* identified each month from January 1984 to June 1987 and immunoblot types for isolates tested.

corresponded to bacteriophage typing results, susceptibility patterns, and plasmid fingerprints but offered some advantages over each of these other systems. Nonetheless, additional study to compare immunoblots with plasmid fingerprints for a larger number of isolates would be of value. A typing scheme based on restriction endonuclease digests of plasmid DNA has been described and appears to be superior to the use of simple plasmid fingerprints (12); immunoblot typing should be compared with this and other new methods to evaluate the utility and technical difficulties of these various methods.

It is important to note that, for immunoblotting, the clonal variation within staphylococcal populations and the frequency of different patterns are not defined. Our finding that multiple isolates from the same patients (including 22 isolates recovered from five body sites of one patient during 9 weeks) had identical immunoblots provides preliminary evidence that there is not marked clonal variation, but detailed population analyses would be required to define fully the degree of variation that may exist. Similarly, although the utility of immunoblotting as compared with other typing methods was demonstrated in this study of isolates from a single institution, the frequency of different immunoblot patterns occurring in the species *S. aureus* cannot be predicted. The stability of immunoblot patterns and their frequencies in natural populations would need to be determined before immunoblot typing could be used alone as a comprehensive typing system.

The technical difficulty of immunoblotting appears to be comparable to that of obtaining plasmid fingerprints or determining bacteriophage susceptibilities. For economic reasons only, isolates should be batched for immunoblotting; 30 isolates can be tested in a single run. Immunoblotting appears to be superior to phage typing in three respects: results appear to be less variable and to require less expertise for interpretation; isolates not susceptible to phages can be typed; and there is no need to batch isolates to get optimal results. However, it would be important to determine the number of immunoblot patterns among strains of *S. aureus* that are not susceptible to standard bacteriophages.

We did not attempt to characterize the staphylococcal antigens or the human antibodies that were reacting in our immunoblot system. We did find, however, that immunologic activities of both were stable when isolates and sera were stored at −40°C.

Because our antibody source consisted of different batches of pooled sera from patients who were not evaluated for previous exposure to oxacillin-resistant *S. aureus*, we assume that results would be comparable if pooled sera from patients in other geographic locations were used. It has been reported that the sera from patients with systematic staphylococcal infections frequently contain high levels of antibody to staphylococcal cell wall peptidoglycans and that these antibodies cross-react even with other species of gram-positive bacteria (18); the high frequency of *S. aureus* as a pathogen may account for the antistaphylococcal antibody in pooled human sera. However, evaluation of our immunoblotting method with different sources of sera would be necessary to confirm the assumption that pooled sera would invariably contain sufficient antibody.

Although we used immunoblotting to distinguish major groups of organisms, it is clear that additional subgroups could be identified. Many of our so-called prominent bands were in reality composites of multiple fine bands. By altering the electrophoresis time, it was possible to increase resolution of certain areas of the blots and to bring out differences that could not be appreciated by the original technique. Further refinement of the methodology to identify subgroups may be of value. The technique would also seem to warrant evaluation for typing of *S. aureus* not resistant to oxacillin as well as coagulase-negative staphylococci.

An unexpected finding in our study was the apparent acquisition over time of resistance of trimethoprim-sulfamethoxazole and gentamicin by endemic strains of oxacillin-resistant *S. aureus*. This possibility of nosocomial acquisition of resistance that could be related to use of antimicrobial agents raises a concern about the wisdom of using antimicrobial agents to treat patients colonized with oxacillin-resistant *S. aureus*. Trimethoprim-sulfamethoxazole and gentamicin are commonly used in our institution, but we did not investigate the possibility that use of these drugs had increased prior to the observance of increased resistance. We also did not determine the previous antimicrobial therapy of patients who acquired the more resistant strains.

Our results suggest that typing is helpful in differentiating relapse from recolonization. One of our patients acquired a new type after therapy for colonization, consistent with exposure to a new strain rather than failure of therapy. Another patient had only transient acquisition of a different type during therapy but subsequent persistent colonization with his original strain, indicating that antimicrobial therapy had failed to eradicate the original colonizing strain. Typing of isolates from patients who have positive cultures after therapy for colonization may help to evaluate the efficacy of such regimens.

We found immunoblotting to be a useful epidemiologic tool at our institution that was comparable or superior to the other typing methods evaluated. It would be of value to examine isolates from other geographic regions, especially from regions with a high frequency of isolates that are not susceptible to bacteriophages, to compare immunoblot typing results with restriction endonuclease digests of plasmid DNA, and to determine the clonal variation within staphylococcal populations and the frequency of different patterns of immunoblots.

ACKNOWLEDGMENTS

This work was supported in part by Veterans Administration research funds.

We thank the medical technologists and the infection control nurses of the West Los Angeles Veterans Administration Medical Center, the medical media staff for artwork and photography, and Kimi Ishii for manuscript preparation.

LITERATURE CITED

1. **Archer, G. I., and C. G. Mayhall.** 1983. Comparison of epidemiological markers used in the investigation of an outbreak of methicillin-resistant *Staphylococcus aureus* infection. J. Clin. Microbiol. **18**:395–399.
2. **Boyce, J. M.** 1980. Methicillin-resistant *Staphylococcus aureus* infections: a growing infection control problem? Infect. Control **1**:335–337.
3. **Boyce, J. M., M. Landry, T. R. Deetz, and H. DuPont.** 1981. Epidemiologic studies of an outbreak of nosocomial methicillin-resistant *Staphylococcus aureus* infections. Infect. Control **2**: 110–116.
4. **Crossley, K., B. Landesman, and D. Laske.** 1979. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. J. Infect. Dis. **138**:280–287.
5. **Gilbert, G. L., V. Asche, A. S. Hewstone, and J. L. Mathiesen.** 1982. Methicillin-resistant *Staphylococcus aureus* in neonatal nurseries. Med. J. Aust. **i**:455–458.
6. **John, J. F., and J. A. Twitty.** 1986. Plasmids as epidemiologic markers in nosocomial gram-negative bacilli: experience at a university hospital and review of the literature. Rev. Infect. Dis. **8**:747–756.
7. **Locksley, R. M., M. L. Cohen, T. C. Quinn, L. S. Tompkins, J. M. Coyle, J. M. Kirihara, and G. W. Counts.** 1982. Multiply antibiotic-resistant *Staphylococcus aureus*: introduction, transmission, and evolution of nosocomial infection. Ann. Intern. Med. **97**:317–324.
8. **National Committee for Clinical Laboratory Standards.** 1985. Tentative standard M7-A. Standard methods for dilution antimicrobial tests for bacteria which grow aerobically. Approved standard. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **National Committee for Clinical Laboratory Standards.** 1984. Performance standards for antimicrobial disk susceptibility tests, 3rd ed. Approved standard M2-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Peacock, J. E., F. J. Marsik, and R. P. Wenzel.** 1980. Methicillin-resistant *Staphylococcus aureus*: introduction and spread within a hospital. Ann. Intern. Med. **93**:526–532.
11. **Peacock, J. E., D. R. Moorman, R. P. Wenzel, and G. L. Mandell.** 1981. Methicillin-resistant *Staphylococcus aureus*: microbiological characteristics, antimicrobial susceptibilities, and assessment of virulence of an epidemic strain. J. Infect. Dis. **144**:575–582.
12. **Rhinehart, E., D. M. Shlaes, T. F. Keys, J. Serkey, B. Kirkley, C. Kim, C. A. Currie-McCumber, and G. Hall.** 1987. Nosocomial clonal dissemination of methicillin-resistant *Staphylococcus aureus*. Arch. Intern. Med. **147**:521–524.
13. **Saravolatz, L. D., N. Markowitz, L. Arking, A. Pohlok, and E. Fisher.** 1975. Methicillin-resistant *Staphylococcus aureus*: epidemiological observation during a community-acquired outbreak. Ann. Intern. Med. **131**:367–374.
14. **Smith, P. B.** 1972. Bacteriophage typing of *Staphylococcus aureus*, p. 431–441. *In* J. O. Cohen (ed.), The staphylococci. John Wiley & Sons, Inc., New York.
15. **Stephenson, J. R., S. J. Crook, and S. Tabaqchali.** 1986. New method for typing *Staphylococcus aureus* resistant to methicillin based on sulfur-35 methionine labelled proteins: its application in an outbreak. Br. Med. J. **293**:281–283.
16. **Tsang, V. C. W., J. M. Peratta, and A. R. Simons.** 1983. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. Methods Enzymol. **92**:377–391.
17. **Wenzel, R. P.** 1979. The emergence of methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:440–442.
18. **Wergeland, H. I., and C. Endresen.** 1987. Antibodies to various bacterial cell wall peptidoglycans in human and rabbit sera. J. Clin. Microbiol. **25**:540–545.
19. **Wexler, H., M. E. Mulligan, and S. M. Finegold.** 1984. Polyacrylamide gel electrophoresis patterns produced by *Clostridium difficile*. Rev. Infect. Dis. **6**:S229–S234.
20. **Yu, V. L., A. Goetz, M. Wagener, P. B. Smith, J. D. Ribs, J. Hanchett, and J. J. Zuravleff.** 1986. *Staphylococcus aureus* nasal carriage and infection in patients on hemodialysis. N. Engl. J. Med. **315**:91–96.