Comparison of Epidemiological Markers Used in the Investigation of an Outbreak of Methicillin-Resistant *Staphylococcus aureus* Infections

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An outbreak of nosocomial infections was caused by a single strain of methicillin-resistant (MR) *Staphylococcus aureus*. This strain was followed as it was transmitted from the index case to 17 patients, 3 hospital personnel, and 12 items in the hospital environment. The MR *S. aureus* strain was traced by using four specific epidemiological markers: antibiogram, phage type, production of aminoglycoside-inactivating enzymes, and plasmid pattern. These markers were assessed for their reliability in differentiating the epidemic *S. aureus* strain from resident nonepidemic strains and for the ease and rapidity with which they determined differences. The epidemic strain was resistant to beta-lactam antibiotics, gentamicin, erythromycin, clindamycin, and rifampin. Resistance to rifampin was the only unique marker in the antibiogram which distinguished the epidemic strain from the indigenous strains, and it was the easiest marker to use for screening isolates from culture surveys. Phage typing was poorly reproducible and did not yield results rapidly enough to be useful for ongoing epidemiology. The epidemic strain produced a unique aminoglycoside-inactivating enzyme (3'-phosphotransferase) which distinguished it from indigenous gentamicin-resistant staphylococci, but this marker was not easily identified, nor was identification helpful during the course of the investigation. Plasmid pattern analysis was rapidly performed (in less than 24 h), allowed many isolates to be examined at a time, was stable and reproducible, and yielded a unique fingerprint which distinguished the epidemic strain from all indigenous isolates. Plasmid pattern analysis is a promising epidemiological tool for MR *S. aureus* outbreaks in which epidemic strains lack unique antibiotic resistance markers.

The incidence of nosocomial infections caused by methicillin-resistant (MR) *Staphylococcus aureus* strains is increasing dramatically in hospitals in the United States (5). MR *S. aureus* isolates have been shown to be fully virulent, causing staphylococcal endocarditis and septicemia at a frequency similar to that of methicillin-sensitive *S. aureus* isolates (16; E. Lewis and L. D. Saravolatz, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 366, 1982). Furthermore, these isolates are usually resistant to multiple additional antibiotics, including cephalosporins, aminoglycosides, macrolides, and lincosamides (2, 8, 10, 16). The antibiotic resistance of *S. aureus* strains allows increased colonization of patients in areas of high antibiotic use and impedes effective antibiotic therapy for infections. Therefore, it is important to be able to document the spread of an epidemic MR

S. aureus isolate so that effective control measures can be rapidly instituted.

The first MR *S. aureus* isolate identified at the Medical College of Virginia Hospitals was introduced by a patient who was transferred from a Florida hospital where several such isolates were endemic. Before this patient was identified as being infected, he had contact with medical personnel and other patients. Therefore, an epidemiological investigation was instituted to trace contacts and document environmental contamination. In the process of tracing the epidemic isolate, many surveillance cultures were obtained from patients, personnel, and the environment. The separation of the epidemic isolate from other, resident *S. aureus* isolates required that specific markers for this isolate be identified. In this study, we compared the traditional epidemiological markers, antibiograms and phage typing, with newer markers, amino-

glycoside-inactivating enzyme analysis and plasmid pattern fingerprinting, for their ability to identify MR *S. aureus* during the investigation of the outbreak.

MATERIALS AND METHODS

Collection of cultures. Isolates analyzed for specific markers were obtained from the wounds, blood, nares, and secretions of infected patients; the nares and wounds of asymptomatic contacts; and the hospital environment. Culture swabs were rolled on the surface of S110 agar (BBL Microbiology Systems, Cockeysville, Md.) containing 20 μg of methicillin per ml and incubated at 37°C for 72 h. Colonies growing on S110 methicillin agar were picked and tested for coagulase production. As confirmation of their resistance to methicillin, some colonies growing on this agar were inoculated into drug-free broth and grown overnight at 37°C; 0.1 ml of the broth was then replated on agar containing 20 μg of methicillin per ml. The presence of subpopulations on this agar after 72 h of incubation at 37°C confirmed the methicillin resistance of the colonies.

Antibiotic sensitivity. The sensitivity of all isolates to antibiotics other than methicillin was determined by standard agar dilution techniques on Mueller-Hinton agar (9).

Phage typing. Phage typing was performed by Gary Hancock at the Centers for Disease Control, Atlanta, Ga., and by Harry Dalton in the Medical College of Virginia Clinical Microbiology Laboratory. Phages were tested at the Medical College of Virginia only at the standard (1/100) dilution. The Centers for Disease Control used both the standard and a 1/10 dilution.

Aminoglycoside-inactivating enzyme analysis. Selected gentamicin-resistant *S. aureus* isolates were lysed and tested for the production of aminoglycoside-inactivating enzymes by David Bobey at Bristol Laboratories, Syracuse, N.Y. Lysis was done with lysostaphin in Tris buffer by the method of Scott et al. (15). The assay was a variation of the nitrocellulose-binding assay described by Haas and Dowding (4).

Plasmid pattern determination. Staphylococcal isolates were lysed and their DNA was visualized by the following scheme: *S. aureus* isolates were grown overnight on agar, and the growth from one-fourth of an agar plate was transferred with a swab to 10 ml of a solution containing 0.1 M NaCl and 0.05 M EDTA at pH 6.9. Bacteria were washed twice and resuspended in 1 ml of this salt solution to which lysostaphin (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 70 μg/ml. After 30 min of incubation at 37°C, the solution of lysed bacteria was spun at high speed (40,000 × g) for 45 min, and the cleared supernatant was removed and treated with 10 μg of pancreatic RNase A (Sigma) per ml for 1 h at 37°C, followed by treatment with 10 μg of proteinase K (Sigma) per ml for 1 h at 37°C. A 40-μl portion of the cleared lysate was then electrophoresed at 80 V (35 mA) for 3 h on an 11.5-cm vertical slab gel through 0.7% agarose in Tris-borate buffer as previously described (1). The gel was washed, stained with ethidium bromide, transilluminated with UV light, and photographed. This lysis procedure produced a lysate with abundant plasmid DNA and very little chromosomal DNA. Plasmid size was determined by comparing the gel migration distance of unknown plasmids with that of plasmids of known molecular size.

RESULTS

Antibiotic sensitivity. MR *S. aureus* organisms were recovered from 17 patients (8 infected, 9 colonized), 3 nurses, and 12 items in the environment, including charts, telephones, and stethoscopes. No MR *S. aureus* organisms were recovered from air samples. Detailed results of the epidemiological investigation will be presented in a separate report.

The epidemic MR *S. aureus* isolate was found to be resistant to the following antibiotics: nafcillin, cefazolin, gentamicin, amikacin, clindamycin, chloramphenicol, tetracycline, erythromycin, and rifampin. Resistance to methicillin, gentamicin, rifampin, and clindamycin was used as the characteristic antibiotic resistance markers for the epidemic isolate.

During the investigation of the spread of the epidemic MR *S. aureus* isolate, surveillance cultures uncovered a variety of antibiotic resistance patterns. Some *S. aureus* isolates were sensitive to all antibiotics; some were resistant to gentamicin and clindamycin, but sensitive to methicillin and rifampin; and one, found at the affiliated McGuire Veterans Administration Medical Center, was resistant to methicillin, gentamicin, and clindamycin but sensitive to rifampin. All of these isolates were used as controls for evaluating the other epidemiological markers considered below.

Phage typing. A comparison of all markers for both the epidemic MR *S. aureus* isolate and the controls is shown in Table 1. Although the epidemic isolate was typed with phage 85, a reaction was only obtained at a 1/10 phage dilution after two previous typing attempts had classified the isolates as nontypable. Three of the nonepidemic *S. aureus* isolates were nontypable even at the 1/10 dilution, including one of the MR *S. aureus* isolates recovered at the Veterans Administration Medical Center.

Aminoglycoside-inactivating enzymes. The epidemic MR *S. aureus* isolate contained aminoglycoside-inactivating enzymes with substrate profiles different from those of the resident gentamicin-resistant, methicillin-sensitive *S. aureus* isolates (Table 1). Furthermore, virtually all of the resident gentamicin-resistant *S. aureus* isolates at our hospital have this resistance encoded on a self-transmissible plasmid (G. Archer and J. L. Johnston; Program Abstr. Intersci. Conf. Antimicrob. Agents Intersci. Chemother. 22nd, Miami Beach, Fla. abstr. no. 366, 1982), but gentamicin resistance could not be transferred from the epidemic MR *S. aureus* isolate to a suitable recipient by the filter mating techniques described by Schaberg et al. (14).

TABLE 1. Markers for epidemic MR and nonepidemic *S. aureus* isolates

Isolate	Phage type	Antibiogram[a]	Aminoglycoside-inactivating enzyme[b]	Plasmids (megadaltons)
Epidemic (W-1)	85	M^r G^r R^r CL^r	3'-APH (I and II), 6'-AAC (I)	34, 1.75, 1.5
Nonepidemic				
S-1	77, 84	M^r G^r R^s CL^r	ND^c	34
W-2	Nontypable	M^r G^s R^s CL^r	ND	None
985	96	M^s G^s R^s CL^r	ND	20
962	Nontypable	M^s G^s R^s CL^s	ND	25
605	Nontypable	M^s G^s R^s CL^s	ND	1.65
866	52, 52A, 79, 80, 6, 42E, 47, 53, 54, 75, 85	M^s G^r R^s CL^r	2"-APH, 6'-AAC (I)	37, 1.65
265	Nontypable	M^s G^r R^s CL^r	2"-APH, 6'-AAC (I), 4'-ANT (II)	34, 31

[a] M, Methicillin; G, gentamicin; R, rifampin; CL, clindamycin.

[b] APH, Phosphotransferase; AAC, acetyltransferase; ANT, adenyltransferase. Roman numerals indicate different isozymic forms of the enzyme.

[c] ND, Not done.

Plasmid pattern analysis. All of the epidemic MR *S. aureus* cells contained three plasmids, approximately 34, 1.8, and 1.5 megadaltons in molecular size. None of the other isolates examined had this profile, which readily identified the epidemic isolate (Table 1 and Fig. 1). Attempts to transfer gentamicin (see above), clindamycin, erythromycin, or chloramphenicol resistance to a suitable recipient from the epidemic MR *S. aureus* isolate by filter mating or mixed culture (6) were unsuccessful. With the lysis method outlined above, cells obtained in the morning could be completely processed (lysis, electrophoresis, and photography) by late afternoon.

DISCUSSION

The epidemic MR *S. aureus* isolate responsible for the outbreak in the Medical College of Virginia Hospital had a unique antibiogram which made its identification relatively easy; it was resistant to rifampin. Rifampin resistance was a stable chromosomal marker not seen in any other staphylococcal isolate cultured from patients, contacts, or the hospital environment. However, a unique antibiotic resistance marker such as rifampin resistance has not been found in the majority of MR *S. aureus* isolates associated with outbreaks in other hospitals (2, 8, 10, 16). The reliability of the antibiogram in identifying a particular nosocomial staphylococcal isolate associated with an outbreak can be questioned for several reasons. First, methicillin resistance is a phenotypic trait which may be difficult to detect by standard antibiotic susceptibility testing methods. Its expression may vary with inoculum size, incubation temperature, medium composition, and exposure to beta-lactam antibiotics (12). Second, staphylococci resistant to multiple antibiotics, including methicillin and gentamicin, may be present in the hospital be-

fore the introduction of an epidemic strain. These resident resistant staphylococci may then be discovered during culture surveys performed as part of an outbreak investigation. A different methicillin- and gentamicin-resistant *S. aureus* strain which was colonizing patients at the affili-
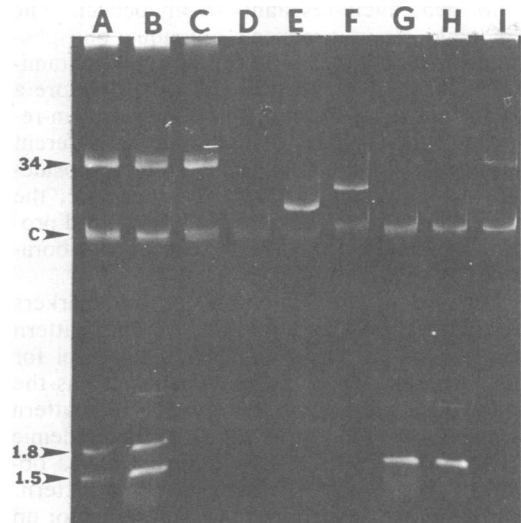

FIG. 1. Plasmid patterns of the epidemic MR and control *S. aureus* strains. The bands represent covalently closed circular and chromosomal DNA. Lanes A and B, Epidemic MR *S. aureus* strain; an isolate from the index case (lane A) (strain W-1 in Table 1), and a positive culture from a colonized hospital staff contact (lane B). Lanes C through I show the nonepidemic isolates listed in Table 1: lane C, S-1; lane D, W-2; lane E, 985; lane F, 962; lane G, 605; lane H, 866; and lane I, 265. The numbers to the left are the sizes (in megadaltons) of the plasmids in the epidemic MR *S. aureus* strain; C indicates chromosomal DNA.

ated Veterans Administration Hospital was found during our outbreak investigation. It was not detected earlier because it caused no infections and was associated with no outbreaks at that hospital. However, had resistance to rifampin not been a specific marker for the epidemic strain, the similar antibiogram of this resistant staphylococcal strain and of the epidemic strain may have confounded the outbreak investigation by raising the possibility that the outbreak had spread to a second hospital.

Phage typing was not particularly helpful in marking the epidemic strain in this outbreak. The epidemic strain was susceptible to lysis by only one phage and then only at 10 times the routine test dilution. This result was only obtained when the isolates were sent to the Centers for Disease Control after variable typing results had been reported by local laboratories for several weeks. The inability of MR *S. aureus* isolates to be typed by phage has been reported in other outbreaks (13).

The determination of the production of specific proteins, such as catalases, has been used to identify and mark specific staphylococcal isolates (11). Since the epidemic MR *S. aureus* isolate was resistant to gentamicin, we sought to identify specific aminoglycoside-inactivating enzymes which might distinguish it from the resident gentamicin-resistant staphylococci. The epidemic strain produced a unique phosphotransferase that was not seen in other gentamicin-resistant staphylococci and was therefore a useful marker. However, many gentamicin-resistant staphylococci of the same or different species may produce the same aminoglycoside-inactivating enzymes (3, 7). Furthermore, the detection of these enzymes is a specialized procedure not readily available to clinical laboratories.

Because of the limitations of the markers discussed above, we found that plasmid pattern analysis was a useful epidemiological tool for fingerprinting MR *S. aureus* strains. It has the following advantages. First, the plasmid pattern is stable. We found that all 32 of the epidemic (rifampin-resistant) MR *S. aureus* isolates obtained over 7 months had an identical pattern. Furthermore, the pattern remained stable for up to 1 year of storage at −70°C (G. Archer, unpublished data). Second, it can be used to differentiate isolates with similar antibiograms. The two rifampin-sensitive MR *S. aureus* strains (Table 1, strains S-1 and W-2) discovered during culture surveys at the Veterans Administration Hospital were differentiated from each other and the epidemic strain by their dissimilar plasmid patterns (Fig. 1). Finally, the lysis, electrophoresis, and photography procedures involved in plasmid pattern analysis can be performed rapid-

ly and inexpensively by unspecialized personnel. Multiple isolates can be screened in less time than is required to determine a minimum inhibitory concentration or a phage type. However, there are limitations to the procedure. Someone with some understanding of DNA electrophoresis and microbial genetics must be available to interpret the gels. For instance, two patterns may be very similar but not identical, differing by only a single plasmid. The loss or gain of a single plasmid may be associated with the loss or gain of antibiotic resistance; this can only be determined by curing or plasmid transfer studies. The acquisition by an epidemic MR *S. aureus* strain of a chloramphenicol resistance plasmid was shown by Locksley, et al. (8). Other problems, such as the existence of a single plasmid in different molecular forms, may have to be interpreted as well. Furthermore, isolates with no or only one plasmid will not have distinct patterns on gels and cannot be reliably distinguished from similar isolates.

In our study of a discrete MR *S. aureus* outbreak involving a single strain newly introduced into the hospital, plasmid pattern analysis quickly and reliably fingerprinted the epidemic isolate. It was superior to both phage typing and the antibiogram of routine antistaphylococcal antibiotics as a marker. It should be even more useful for epidemiological investigations of complex MR *S. aureus* outbreaks involving multiple strains in different hospitals.

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