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Comparison of Traditional and Molecular Methods of Typing Isolates of *Staphylococcus aureus*

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Fifty-nine *Staphylococcus aureus* isolates and 1 isolate of *Staphylococcus intermedius* were typed by investigators at eight institutions by using either antibiograms, bacteriophage typing, biotyping, immunoblotting, insertion sequence typing with IS*257/431*, multilocus enzyme electrophoresis, restriction analysis of plasmid DNA, pulsed-field or field inversion gel electrophoresis, restriction analysis of PCR-amplified coagulase gene sequences, restriction fragment length polymorphism typing by using four staphylococcal genes as probes, or ribotyping. Isolates from four well-characterized outbreaks ($n = 29$) and a collection of organisms from two nursing homes were mixed with epidemiologically unrelated stock strains from the Centers for Disease Control and Prevention. Several isolates were included multiple times either within or between the sets of isolates to analyze the reproducibilities of the typing systems. Overall, the DNA-based techniques and immunoblotting were most effective in grouping outbreak-related strains, recognizing 27 to 29 of the 29 outbreak-related strains; however, they also tended to include 3 to 8 epidemiologically unrelated isolates in the same strain type. Restriction fragment length polymorphism methods with *mec* gene-associated loci were less useful than other techniques for typing oxacillin-susceptible isolates. Phage typing, plasmid DNA restriction analysis, and antibiogram analysis, the techniques most readily available to clinical laboratories, identified 23 to 26 of 29 outbreak-related isolates and assigned 0 to 6 unrelated isolates to outbreak strain types. No single technique was clearly superior to the others; however, biotyping, because it produced so many subtypes, did not effectively group outbreak-related strains of *S. aureus*.

Staphylococcus aureus continues to be a major cause of both nosocomial and community-acquired infections (9, 24, 43). Consequently, microbiologists are frequently asked to determine the relatedness of staphylococcal isolates collected during the investigation of an outbreak or as part of an ongoing surveillance system. While there are many different methods for typing *S. aureus*, not all methods divide groups of strains in a similar fashion (2, 8, 17, 20, 29, 30, 35–37, 42). Unfortunately, few studies have evaluated a broad sample of isolates or directly compared multiple techniques. Nonetheless, data from several studies suggest that phenotypic markers, such as biotypes or antimicrobial susceptibility patterns, are more likely to change over time than are the results of techniques, such as pulsed-field gel electrophoresis (PFGE) or multilocus enzyme electrophoresis (MLEE) (2, 6, 12, 13, 30, 37, 38, 42, 46).

Maslow et al. (26) have characterized typing systems using five criteria: typeability, reproducibility, discriminatory power, ease of interpretation, and ease of use. Typeability refers to the ability of the test to provide an unambiguous result for each isolate examined; nontypeable isolates are those that produce a null or ambiguous result. Reproducibility refers to the ability of a technique to produce the same result when a strain is tested repeatedly. Discriminatory power defines the ability of the test to discriminate between unrelated isolates. This discrimination is important, because some typing systems tend to group organisms into a few broad groups, while others divide collections of isolates into many small clusters, often subdividing groups of isolates that are tightly linked by epidemiologic data (6, 8, 40). Ease of interpretation and use are also key issues for many techniques. The greater the expertise that is required to discern differences between strains, the less likely the technique is to be readily accepted by clinical microbiologists who generally lack such expertise.

Typing of methicillin-resistant strains of *S. aureus* has proven to be particularly difficult because most strains are derived from relatively few clones (5, 6, 10, 22, 37, 40, 46). The present study was organized to determine the strengths and weaknesses of 12 currently available typing systems, ranging from a simple antibiogram method to a more tech-

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nically demanding MLEE method, for discriminating among methicillin-susceptible and methicillin-resistant isolates.

MATERIALS AND METHODS

Bacterial strains. Fifty-nine isolates of *S. aureus*, including isolates from four well-documented outbreaks and one pseudo-outbreak, were included in the study. One isolate of *Staphylococcus intermedius* was also included as a control to determine whether the various techniques could discriminate this organism, which can give a positive slide coagulase test and has colonies that may resemble *S. aureus*, from true *S. aureus* isolates. The identification of all isolates used in the study was confirmed by using standard biochemical methods (21).

Description of the three sets of isolates. The isolates were divided into three sets to facilitate analysis. Set A contained a cluster of nine *S. aureus* isolates from two nursing homes that were originally thought to represent dissemination of a single strain. All were found to be of group III by bacteriophage typing. However, additional epidemiologic investigations could not establish a link among the patients. Thus, the group of isolates is referred to as the isolates involved in a pseudo-outbreak. The isolates SA-01 and SA-02 (Table 1) were also included in set A as SA-09 and SA-15, respectively. The remaining nine isolates in set A included *S. aureus* ATCC 12600 (American Type Culture Collection, Rockville, Md.) (Table 1, SA-04) and seven unrelated isolates of *S. aureus* from the strain collection of the Centers for Disease Control and Prevention (CDC) from seven different states. Among these isolates were three strains (SA-12, SA-18, SA-20) that were bacteriophage type 47/54/75/77/83A and that were collected from three different states during 3 different years. The final isolate in the set (SA-16) was *S. intermedius* ATCC 49052.

Set B contained strains from outbreaks I and II, eight epidemiologically unrelated isolates, and *S. aureus* ATCC 12600 (SB-07). Outbreak I represents dissemination of a methicillin-resistant strain of *S. aureus* in the Iowa Veterans Affairs Medical Center (34). The outbreak cluster comprised seven isolates obtained from patients during June through August 1985; all infections met the National Nosocomial Infections Surveillance Study definitions (11). The outbreak isolates were originally defined on the basis of an epidemiologic investigation in the hospital and the plasmid restriction profiles of the isolates. Six additional epidemiologically unrelated isolates from the same hospital that were collected after the epidemic period were included as controls. Two of these isolates (SB-01, SB-16) were obtained from patients who had no obvious epidemiologic link to outbreak patients, although they were admitted to the same surgical service in the hospital, but after the outbreak period. These isolates had the same base plasmid restriction profiles as the outbreak strain but showed additional bands that represented acquisition of a new, low-molecular-size plasmid. The other four isolates were epidemiologically unrelated.

Outbreak II isolates were from the CDC collection and represented an outbreak of a methicillin-susceptible strain of *S. aureus* related to a contaminated anesthetic (7). Four isolates were in this cluster; three (SB-02, SB-04, SB-06) were from the same patient, and the fourth (SB-11) was from a different patient. The isolates were originally classified by bacteriophage typing as being part of the outbreak. Two additional isolates of the same bacteriophage type as those in outbreak II, but unrelated to the cluster, were provided as controls by Barbara Robinson, Michigan State Department of Health, East Lansing.

Set C contained isolates from outbreaks III and IV, an unrelated control strain of *S. aureus* that originally had a bacteriophage type similar to those of the isolates in outbreak IV, although it was nonreactive on repeat testing (SC-08), and *S. aureus* ATCC 12600 (SC-03). Outbreak III contained 10 isolates of a methicillin-resistant strain of *S. aureus* from an outbreak at the Sepulveda Veterans Affairs Medical Center, Sepulveda, Calif. (15). The isolates were obtained from cultures of hip wound, sputum, nose, or axilla samples from eight patients identified by infection control criteria as being part of the outbreak. One of the isolates was repeated within the set (SC-17, SC-20), and two samples for culture were taken from the same patient on the same day but from different sites (SC-14, SC-15). All isolates were collected within a 2-month period and were initially classified by immunoblotting as being outbreak related. Outbreak IV isolates were from another anesthetic-related outbreak of methicillin-susceptible *S. aureus* (7). The eight outbreak-related isolates, all from cultures of blood or wound specimens from separate patients, were provided by the Texas State Health Department and were originally classified by bacteriophage typing as being outbreak related.

Antibiograms. Antibiograms were determined by disk diffusion by using the following antimicrobial agent-containing disks: amoxicillin-clavulanate (20/10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), minocycline (30 μg), oxacillin (1 μg), penicillin (10 U), rifampin (5 μg), tetracycline (30 μg), and trospectomycin (30 μg). Plates were inoculated and zone sizes were interpreted as described by the National Committee for Clinical Laboratory Standards (32). The mean zone diameter was determined for each set of 20 organisms, and all zone diameters within ±2 mm of the mean were arbitrarily considered identical. Zone sizes of >2 mm from the mean were considered indicative of a different strain. Strains were given different letter designations if two or more of the antimicrobial agents tested had zone diameters of >2 mm from the mean for that drug. Strains that differed by a single antimicrobial agent were numbered as subtypes (e.g., A1). Oxacillin MICs were determined by the broth microdilution method with Mueller-Hinton agar (Becton Dickinson Microbiology Systems) as described previously (31).

Bacteriophage typing. Bacteriophage typing was performed as described previously by using the international bacteriophage typing set (4, 20) at the routine test dilution and 100× the routine test dilution. A plus sign indicates the presence of additional strong phage reactions. Phage types that differed by the presence or absence of one phage were considered related. Differences by the presence or absence of two or more phages were considered to be unrelated strains.

Biotyping. Biotyping was performed by using the system described by Hébert et al. (18) for typing coagulase-negative staphylococci. Tests included the Staph-Ident system (bioMérieux-Vitek, St. Louis, Mo.), adherence to glass tubes, and synergistic hemolysis by using a β-hemolysin-producing strain of *S. intermedius* (18). Codes represent a combination of biochemical (Staph-Ident) type codes (capital letters) and physiological test results (subtypes; numbers and lowercase letters).

RFLP typing using variable gene probes. Restriction fragment length polymorphism (RFLP) typing is a Southern blot method based on the restriction fragment banding patterns of the chromosomal DNAs produced by hybridization with

TABLE 1. Staphylococcal strain typing results by 12 methods[a]

Strain	Outbr	Ox	Phage type	Antibiogram	Biotype	Plasmid	*Hind*/Ribo	*Cla*/Ribo	IS type	RFLP type	PCR	PFGE	FIGE	Immuno	MLEE
SA-16	NO	S	NR	I	INTER	NP	D	e	NH	NH:NH:NH:NH	0.0	I	VII	K	F
SA-04	NO	S	6/47/54/75	B	A-2b	B	F	i	NH	NH:X:4:NH	2.1	E	IV	D	E
SA-12	NO	R	47/54/75/77/83A	G	A-3b	NP	B	b	C	I:A:1:NH	9.0	J	IC2	A	A5
SA-18	NO	R	47/54/75/77/83A	J	A-3b	I	B	b	C	I:A:1:NH	9.0	J	IC3	A2	A3
SA-20	NO	R	47/54/75/77/83A	K	A-3b	J	B	b	C	I:A:1:NH	9.0	J	IC1	A1	A1
SA-06	NO	I	NR	C	A-3b	C	A	a	B	II:NH:1:a	9.0	C	III	A4	A4
SA-07	NO	S	53/+	D	H-4	D	B	c	NH	NH:NH:1:NH	9.0	B	V	C	A2
SA-08	NO	R	54/75/77/81	E	I-2b	E	E	d	D	I:NH:6:NH	7.0	G	IIA	E1	D1
SA-11	NO	R	NR	F	A-2b	E	G	d	G	II:NH:6:NH	7.0	F	IIB	E2	D2
SA-01	NH1	R	54/77	A1	A-1b	A	A	a.1	A	I:A:5:a	9.0	K.1	IB	A1	A1
SA-09	NH1	R	54/77	A	A-1b	NP	A	a.1	A	I:A:5:a	9.0	K.2	IB	A1	A1
SA-03	NH1	R	47/54/75/77	A2	A-3b	NP	A	a	C	I:A:1:NH	9.0	A	IA	A	A1
SA-13	NH1	R	54/77	A3	A-1b	G	A	a	A	I:A:1:a	9.0	A	IA	A3	A2
SA-14	NH1	S	54/75/77	H	B-1b	H	C	i	NH	NH:NH:1:NH	9.0	H	VI	E3	C
SA-19	NH1	R	54/77	A4	G-1b	A	A	a.1	A	I:A:1:a	9.0	K.3	IB	A1	A1
SA-17	NH2	R	54/75/77	A	C-3b	A	A	a	A	I:A:1:a	9.0	A	IA	A	A1
SA-02	NH2	R	75/77	A	A-3b	A	A	a	A1	I:A:1:b	9.0	A	IA	A	A1
SA-15	NH2	R	77	A	A-3b	A	A	a	A1	I:A:1:a	9.0	A	IA	A1	A5
SA-05	NH2	R	77	A	A-3b	A	A	a	A	I:A:1:a	9.0	A	IA	A	A1
SA-10	NH2	R	77	A	A-3b	A	A	a	A	I:A:1:a	9.0	D	ID	A1	B
SB-07	NO	S	6/47/54/75	C	A-2b	D	C	i	NH	NH:X:4:NH	2.1	D	IIB3	D′	B3
SB-03	I	R	75/+	A	C-4	C	A	a	E	I:A:1:a	9.0	A	IA	A6	A1
SB-05	I	R	75/+	A	A-4	C	A	a	E	I:A:1:a	9.0	A	IA	A6	A1
SB-10	I	R	75/+	A	A-4	C	A	a	E	I:A:1:a	9.0	A	IA	A6	A1
SB-12	I	R	75/+	A	C-4	C	A	a	E	I:A:1:a	9.0	A.1	IA	A6	A1
SB-15	I	R	75/77/83A	A	C-4	C	A	a	E	I:A:1:a	9.0	A	IA	A6	A1
SB-19	I	R	75/+	A	A-4	C	A	a	E	I:A:1:a	9.0	A	IA	A5	A1
SB-20	I	R	75/+	A	A-4	C	A	a	E	I:A:1:a	9.0	A	IA	A5	A1
SB-01	NO	R	75/77	A	A-4	A	A	a	E	I:Y:1:a	9.0	A.1	IB1	A5	A1
SB-16	NO	R	75/77/83A	A	A-4	A	A	a	E	I:Y:1:a	9.0	A.1	IB1	A5	A1
SB-18	NO	R	75/+	A	C-4	J	A	a	E1	I:A:1:a	9.0	A	IA	A7	A1
SB-17	NO	I	96	E	B-3b	I	F	j	NH	NH:NH:1:NH	6.0	E	IV	G	A2
SB-14	NO	R	47/54/75/77/83A	A1	A-3b	H	E	a	D	I:A:1:NH	9.0	A.2	IB2	A5	A3
SB-08	NO	S	95	B1	C-4	E	D	d.1	NH	NH:NH:1:NH	2.0	F	III	E5	C
SB-02	II	S	3A/55	B	B-1b	B	B	b	NH	NH:NH:7:NH	6.0	B	IIA	D1	B1
SB-04	II	S	3A/55	B	D-1b	B	B	b	NH	NH:NH:7:NH	6.0	B	IIA	D1	B1
SB-06	II	S	3A/55	B	B-1b	B	B	b	NH	NH:NH:7:NH	6.0	B	IIA	D1	B1
SB-11	II	S	3A/55	B	B-3b	G	B1	b	NH	NH:NH:7:NH	14.0	C	IIB2	D2	B1
SB-09	NO	S	3A	D	D-3b	F	B	b	NH	NH:Z:7:NH	6.0	B	IIA	D1	B1
SB-13	NO	S	3A	B2	D-3b	G	B	b	NH	NH:NH:7:NH	6.0	B.1	IIB1	E6	B2
SC-03	NO	S	6/47/54/75	C	A-2b	C	A	i	NH	NH:NH:4:NH	2.1	C	III	D	B
SC-01	III	R	75	A	A-1b	A	A	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-04	III	R	75	A	A-1b	D	A	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-05	III	R	NR	A1	A-1b	D	A	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-09	III	R	75	A	A-1b	D	A	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-11	III	R	75	E	A-1b	NP	A	b	NH	I:A:4:NH	10.0	A	IB	F	A1
SC-12	III	R	75	A2	A-1b	A	A	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-14	III	R	75	A2	B-2b	A	A	b	F	I:A:4:a	10.0	A	IA	F	A2
SC-15	III	R	75	A	A-1b	D	B2	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-17	III	R	75	A	A-1b	A	A	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-20	III	R	75	A	A-1b	D	A	b	F	I:A:4:a	10.0	A	IA	F	A1
SC-08	NO	S	NR	B	B-3a	E	B1	g	NH	NH:NH:1:NH	2.0	B.1	II	E7	A3
SC-02	IV	S	52/52A/80/47/54/83A/84/95	B	E-1b	B	B	g	NH	NH:NH:1:NH	2.0	B	II	E7	C1
SC-06	IV	S	95	B	J-1b	B	B	g	NH	NH:NH:1:NH	2.0	B	II	E7	C1
SC-07	IV	S	95	D	I-1a	B	B	g	NH	NH:NH:1:NH	2.0	B	II	E7	C1
SC-10	IV	S	52A/79/80/47/54/75/77/83A/95	B	I-2a	B	B	g	NH	NH:NH:1:NH	2.0	B	II	E7	C1
SC-13	IV	S	95	B1	I-1b	B	B	g	NH	NH:NH:1:NH	2.0	B	II	E7	C1
SC-16	IV	S	95	B1	I-1b	B	A	g	NH	NH:NH:1:NH	2.0	B	II	H	D1
SC-18	IV	S	95	F	I-3b	B	B	g	NH	NH:NH:1:NH	2.0	B	II	E7	C1
SC-19	IV	S	95	B1	D-1a	B	B	g	NH	NH:NH:1:NH	2.0	B	II	E7	D2

[a] Outb, outbreak; NO, not in epidemiologically related cluster; YES, strain in epidemiologically related cluster; I to IV, outbreak number; Ox, oxacillin susceptibility test results; S, susceptible; R, resistant; INTER, *S. intermedius* biotype; Plasmid, plasmid restriction profile; NP, no plasmids; *Hind*/Ribo, ribotyping result with *Hind*III; *Cla*/Ribo, ribotyping result with *Cla*I; IS, insertion sequence; NH, no hybridization; PCR, coagulase gene PCR typing; PFGE, pulsed-field gel electrophoresis; FIGE, field inversion gel electrophoresis; Immuno, immunoblot typing; MLEE, multilocus enzyme electrophoresis.

four unique DNA probes (23). Whole-cell DNA from each strain was extracted as described previously (22) and digested with *Cla*I, and the fragments were separated on a 1.0% agarose gel. The DNA was transferred to Nytran or nitrocellulose filters (Schleicher & Schuell, Keene, N.H.) and was then hybridized sequentially with four probes that targeted the following genes or transposons: *mec*, Tn*554*, *agr*, and *aph*(2″)-*aac*(6′). Control strains of methicillin-resistant *S. aureus* were run on each gel. The *mec*-specific probe patterns were designated I to V, the Tn*554*-specific probe patterns were designated A to Y, the staphylococcal accessory gene regulator probe (*agr*) produced patterns designated 1 to 7, and the *aph*(2″)-*aac*(6′) aminoglycoside resistance gene probe produced two different patterns (a and b).

IS probe typing. Insertion sequence (IS) typing is based on the restriction polymorphisms obtained by using IS*257/431* sequences as a probe (3). DNA was extracted and hybridized as described previously (22), except that hybridization was performed by using target DNA cleaved with *Bgl*II and probed with a 250-bp internal fragment of IS*257/431* DNA (3). It primarily targeted multiresistant staphylococcal isolates. The designation NH (no homology) was used to indicate no binding of the DNA probe to the target nucleic acid. Patterns that differed only in a single band were designated subtypes.

FIGE. Field inversion gel electrophoresis (FIGE) was performed as described by Goering and Winters (14). DNA samples were digested in duplicate with *Sma*I and electrophoresed through agarose gels with switching times to separate fragments of ≥50 kb on one gel and fragments of <50 kb on a second gel (44). Three or more band differences were interpreted as indicative of strain differences. An isolate that demonstrated a change in a single band when it was compared with another isolate was considered to be a subtype of the first isolate.

Immunoblotting. Immunoblotting was performed as described by Tsang et al. (41). Briefly, EDTA extracts of *S. aureus* cell surface proteins were prepared from cultures grown for 24 h at 35°C in brain heart infusion broth (Becton Dickinson Microbiology Systems). Samples were subjected to polyacrylamide gel electrophoresis (5% stacking gel and 12.5% running gel) for 6 h and were transferred to nitrocellulose in a Trans blot chamber (Bio-Rad Laboratories, Richmond, Calif.) as described previously (41). The antibody source was a 1:50 dilution of pooled human serum (from 50 patients), and detection of protein bands was achieved by using peroxidase-conjugated goat anti-human immunoglobulin G, which was subsequently reacted with a mixture of 50 mg of 3,3′-diaminobenzidine and 0.1 ml of 3% hydrogen peroxide.

MLEE. MLEE was performed as described by Selander et al. (38) by using the following enzymes: alcohol dehydrogenase (EC 1.1.1.1), mannitol 1-phosphate dehydrogenase (EC 1.1.1.17), lactate dehydrogenase (EC 1.1.1.27), hydroxybutyrate dehydrogenase (EC 1.1.1.30), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), diaphorase (NADH) (EC 1.6.99.3), catalase (EC 1.11.1.6), nucleoside phosphorylase (EC 2.4.2.1), creatine kinase (EC 2.7.3.2), esterase (β-naphthyl propionate) (EC 3.1.1.1), mannose phosphate isomerase (EC 5.3.1.8), and phosphoglucose isomerase (EC 5.3.1.9). Organisms were sonicated by using a XL2020 programmable sonicator (Heat Systems, Inc.) fitted with a cup probe. Sonicates were filter sterilized through a 0.22-μm-pore-size low-protein-binding filter (Millex-GV; Millipore) before analysis. Electrophoretic variants of each enzyme assayed were considered to be alleles of that enzyme and were assigned different numbers. Each unique combination of alleles was designated an electrophoretic type. Genetic relationships among electrophoretic types were determined by the average linkage method of clustering from a matrix of pairwise coefficients of weighted distance (38, 39) by using a SAS program described by Jacobs (19). Strains were delineated at a genetic distance of 0.1 and were assigned letter and number designations reflecting their relative relatedness. No subtypes were designated.

PFGE. PFGE was performed as described by Maslow et al. (27) by using the enzyme *Sma*I, and the results were interpreted as described by Arbeit et al. (1). All isolates within a set were compared in a single gel; each set was evaluated by at least two independent electrophoretic runs. Gels were stained with ethidium bromide and photographed under UV illumination, and the profiles were compared visually. Isolates with identical restriction profiles were assigned the same type. Isolates that differed by one or two band shifts consistent with a single genetic event (e.g., a point mutation resulting in the loss or the gain of a restriction site, an insertion, a deletion, or a chromosomal inversion) were assigned a subtype; isolates with more than one such differences were considered to be different types.

PCR amplification and restriction analysis of the staphylococcal coagulase gene. Strains were analyzed as described previously by Goh et al. (16) by PCR, a nested primer technique, and *Alu*I digestion. The outer primers were COAG-1 (ATACTCAACCGACGACACCG) and COAG-4 (GATTTTGGATGAAGCGGATT) (GenBank accession number D00184), and the inner primers were COAG-2 (CGAGACCAAGATTCAACAAG) and COAG-3 (AAAGA AAACCACTCACATCA).

A binary code based on the presence or absence of 243-, 324-, 405-, and 486-bp *Alu*I digest fragments was used to type most strains. However, not all strains with the same binary code type were identical. For these strains, a decimal code referring to the presence of a 162-bp *Alu*I fragment was used (i.e., 2.1).

Restriction digestion of plasmid DNA. Plasmid DNAs were obtained from all isolates by the method of Pfaller et al. (33) and were digested separately with *Hin*dIII and *Eco*RI. The digestion products of both digests were electrophoresed through 0.7% agarose gels, stained with ethidium bromide, and photographed by using a midrange UV light source. The patterns produced by both enzymes were used to determine a composite strain type. Strains showing greater than two band differences were given different letters. Strains without plasmids were considered to be nontypeable.

Ribotyping. Ribotyping was performed independently by two laboratories. In the first laboratory, ribotyping was performed with the restriction enzyme *Hin*dIII as described previously by McDougal et al. (28), except that organisms were grown overnight in Mueller-Hinton broth and lysostaphin was substituted for lysozyme. rRNA was labeled with [γ-^{32}P]ATP (specific activity, 3,000 Ci/mmol). Isolates showing two or more band differences were given different types; those with a single band difference (either size or number of bands) were considered subtypes.

Ribotyping was performed in the second laboratory with the restriction enzyme *Cla*I as described by Maslow et al. (25) by using a [^{32}P]ATP-labeled gene probe (DNA that encodes rRNA) from the *Escherichia coli rrnB* operon.

Study protocol. Sixty isolates of staphylococci were sent to each participating laboratory in three sets of 20. The investigators tested each set of 20 isolates independently to determine which of the isolates in each set were related. All

TABLE 2. Number of major types, subtypes, and nontypeable isolates by set and number of isolates correctly identified and misclassified by each typing method

Method	No. of isolates in set:									Total no. of types[a]	No. classified correctly[b]	No. misclassified[c]
	A			B			C					
	Types	Subtypes	Nontypeable	Types	Subtypes	Nontypeable	Types	Subtypes	Nontypeable			
Phage typing	9		3	7			5		2	18	25	4
Antibiogram	11	4		5	3		6	3		21	26	6
Biotype	6	8		4	8		6	11		23	17	2
Plasmids	9		4	10			5		1	20	23	0
*Hin*dIII ribotyping	7			6	1		2	2		16	27	7
*Cla*I ribotyping	6	1		5	1		3			9	29	7
IS typing	5	1	4	2	1	9	1		11	9	16	3
RFLP typing	10		1	7			4			17	28	3
Coagulase gene PCR	3		1	4	1		2	1		7	28	8
PFGE	11	3		6	3		3	1		25	28	7
FIGE	11	3		6	5		4			25	27	3
Immunoblotting	5	7		4	8		4			23	28	6
MLEE	11			7			7			21	26	4

[a] Total number of types and subtypes among the 60 isolates examined.
[b] Number of isolates identified correctly as outbreak-related (n = 29).
[c] Number of unrelated isolates reported to have same strain type as outbreak-related strains.

primary data were sent to CDC for confirmation and further analysis. The total number of strain types and the presence of strains in multiple sets were determined independently by investigators at CDC after analysis of the primary typing data.

RESULTS

Typeability of strains. Fifty-nine isolates of *S. aureus* and 1 isolate of *S. intermedius* were typed by 12 methods. The total number of types, subtypes, and nontypeable strains identified by each method are given in Table 2. All isolates, including the *S. intermedius* isolate, were typeable by antibiogram, biotype, and immunoblot analyses, MLEE, ribotype analysis, PFGE, and FIGE; all *S. aureus* isolates were typeable by coagulase gene PCR. Phage typing was nonreactive for four *S. aureus* isolates, and five isolates, including the *S. intermedius* strain, lacked plasmids for analysis. Among the set of four RFLP probes, the *agr* probe typed all *S. aureus* isolates and the *mec* probe typed all oxacillin-resistant isolates; the remaining two probes in this set, those for Tn*554* and *aph*(2″)-*aac*(6′), typed most oxacillin-resistant isolates, but only a minority of oxacillin-susceptible isolates. The IS*257*/*431* probe typed all but two of the oxacillin-resistant isolates, but none of the oxacillin-susceptible isolates. Consequently, IS typing was not evaluable for isolates from outbreaks II and IV, both of which were due to oxacillin-susceptible isolates.

Four methods, i.e., phage typing, plasmid restriction analysis, MLEE, and RFLP typing, assigned each isolate to a distinct type without the use of subtypes. Isolates assigned to the same type or related subtypes were considered to be epidemiologically related; isolates assigned to different types were considered to be unrelated.

Reproducibility. To check the reproducibilities of the methods, two organisms were included twice in set A and one organism was included twice in set C. The *S. aureus* type strain ATCC 12600 was included in each set. Of the duplicate strains in set A (SA-01 and SA-09), antibiograms, plasmid restriction, and PFGE all showed slight variations in their respective typing results for one of the duplicate isolates. In the second set of replicates (SA-02 and S-15), phage typing, RFLP typing, immunotyping, and MLEE showed some variability. The replicates in set C were identical by all typing methods. The results for the three *S. aureus* ATCC 12600 replicates must be interpreted with caution, since several of the methods used different codes for each set. However, the primary data show that RFLP typing classified one of the three isolates as different in the Tn*554* locus, immunotyping reported one isolate as a subtype of the others, while all other methods classified the isolates as identical.

Discriminatory power. Since phage typing has been the standard epidemiologic tool for *S. aureus* for many years, we wanted to test the ability of the various techniques to differentiate strains with a common phage type that were known to be epidemiologically unrelated. Therefore, three *S. aureus* isolates of phage type 47/54/75/77/83A, which were sent to CDC from three different states in 3 different years, were included in set A. Antibiograms, plasmid restriction analysis, FIGE, immunotyping, and MLEE classified each as different (FIGE showed only minor variations), while biotyping, both ribotyping techniques, IS typing, RFLP typing, PFGE, and coagulase gene PCR classified all the isolates as the same strain.

With regard to the ability of the techniques to discriminate the 29 outbreak-related strains from the other isolates in the study, antibiograms grouped 26 of 29 outbreak-related isolates together, but included 6 additional strains in the outbreak-related clusters. By using the standard rules of interpretation, phage typing correctly classified 25 of 29 outbreak strains but included 4 unrelated strains with the clusters. For biotyping, if type A4 from outbreak I was considered correct, then 17 of 29 outbreak strains were correctly identified and 2 unrelated isolates were incorrectly included in the clusters.

Among the DNA-based techniques, plasmid restriction analysis was relatively insensitive, recognizing only 23 of 29 outbreak-related isolates; however, no unrelated isolates were incorrectly classified, which means that this technique had the highest degree of specificity of any of the typing methods. *Cla*I ribotyping, on the other hand, correctly

identified all 29 outbreak-related isolates, which made it the most sensitive technique, but it also incorrectly identified an additional 7 strains as being related to the clusters. *Hin*dIII ribotyping identified 27 of 29 outbreak-related isolates but also included 7 additional unrelated isolates as part of the clusters.

IS typing could type only 17 of 29 outbreak-related isolates (the other isolates were oxacillin susceptible), and of these, 16 were correctly identified. Three additional isolates were incorrectly identified as belonging to the cluster. RFLP typing grouped 28 of 29 isolates correctly, but included 3 additional unrelated isolates in the cluster. PCR coagulase gene typing correctly identified 28 of 29 outbreak-related isolates, but included an additional 8 unrelated isolates in the clusters.

Among the alternative electrophoretic methods, PFGE correctly identified 28 of 29 organisms in the outbreak-related cluster, but incorrectly identified an additional 7 isolates as being related to the clusters. FIGE, on the other hand, correctly identified 27 of 29 outbreak-related isolates, but included 3 unrelated isolates.

Finally, immunoblotting correctly identified 28 of 29 outbreak-related strains, but incorrectly identified 6 isolates as related to the clusters, and MLEE correctly identified 26 of 29 outbreak-related isolates, but included 4 additional isolates in the clusters.

Ease of use and interpretation. Antibiograms and biotypes were the simplest methods to perform and their results were the simplest to interpret; no equipment was required. Phage typing was easy to perform, but titering and maintaining stocks were very laborious. Interpretation of phage typing results was difficult when multiple, related patterns were observed. An inoculator for dispensing the phages to the bacterial lawns proved to be invaluable for reproducibility.

Of the DNA-based methods, plasmid restriction analysis was the easiest to perform; only several relatively inexpensive pieces of equipment were needed, including an electrophoresis chamber, a power supply, a transilluminator, and a camera system. Interpretation required merging of two sets of patterns (*Hin*dIII and *Eco*RI); however, this required only minimal subjective interpretive steps. Ribotyping, PFGE, and FIGE generated patterns of 15 to 20 bands which occasionally included partial restriction products, doublet bands representing two or more fragments of approximately the same size, and faintly staining bands of low molecular size. These problems made interpretation of the banding patterns more subjective than interpretation of plasmid restriction patterns or the RFLP and IS typing schemes, which resulted in fewer bands per pattern. The more complex the pattern, the greater the expertise required for interpretation. PFGE and FIGE both required preparation of DNA in agarose blocks and the use of specialized electrophoretic equipment. PFGE equipment costs several thousands of dollars more than FIGE equipment, but banding PFGE patterns are typically more distinct than FIGE patterns. Unlike PFGE, FIGE required two separate gels to resolve high- and low-molecular-size fragments; however, in several instances, the low-molecular-size FIGE gel allowed differentiation of strains that appeared to be identical by PFGE.

The methods involving DNA probing (ribotyping, RFLP typing, and IS typing) all required electrophoretic equipment, an apparatus for transferring the electrophoresed DNA onto a membrane, and procedures for labeling probes and visualizing the results. Although nonradioactive labeling procedures are readily available, these procedures were still highly labor intensive and time-consuming.

The enzyme banding patterns generated by starch gel electrophoresis during MLEE were easy to interpret, but the overall analysis required the application of sophisticated algorithms and computer software not readily available in most laboratories. This makes this technique inaccessible to most clinical laboratories.

Immunoblotting involved both polyacrylamide gel protein electrophoresis and preparation of Western blots (immunoblots), the latter of which can be difficult for those unaccustomed to protein analysis techniques. Typically, the banding patterns produced were very complex, differentiation of strains from subtypes was often subjective, and the promising results may be directly related to the experience of the investigator.

DISCUSSION

The goal of the present study was to identify the strengths and weaknesses of 12 different typing techniques for *S. aureus* to determine which methods would be best suited for clinical and research microbiology laboratories. The "gold standard" for these studies was the epidemiologic data collected during the four outbreak investigations from which 29 of the study isolates were taken and data from an investigation of an outbreak in two nursing homes. For the strains from the nursing homes and for two strains from outbreak II, the epidemiologic data did not match the majority of the typing data. This disparity emphasizes the need to collect and analyze epidemiologic and laboratory data together when investigating a cluster of isolates.

Of the two traditional techniques most accessible to clinical laboratories, antibiograms and biotyping, we found that antibiogram typing worked reasonably well, but only when zone diameters and not the categorical interpretations of susceptible, intermediate, or resistant were used as strain markers. The antibiograms obtained by using interpretive categories have proved to be problematic in past studies (2, 9, 13, 17, 30). In our study, most subtypes, i.e., changes in disk diffusion zone sizes for a single antimicrobial agent, were not significant. Therefore, changes in the zone sizes around disks for two or more antimicrobial agents must be observed before two isolates can be considered to be different strains. This highlights the instability of resistance patterns in *S. aureus*, which is probably at least partially related to changes in plasmid content (see below). Although we used 12 antimicrobial agents, trospectomycin was not helpful in discriminating among strains and could be eliminated. Antibiogram typing is the least expensive typing method and could be considered, especially in small laboratories, as an initial screen to determine strain relatedness.

Although biotyping works well for coagulase-negative staphylococci (18), it recognized too many subgroups within the *S. aureus* outbreak clusters for it to be useful in the present study. Biotyping results did not correlate well with either the epidemiologic data or the results of the other typing methods. While biotyping could be used to subtype isolates within clusters into smaller groups, it is not clear that this would have epidemiologic significance.

Bacteriophage typing has been used for typing *S. aureus* for many years, but its limitations are clearly recognized (5, 20, 30, 37, 42, 45). In the present study, 4 of the 59 *S. aureus* isolates were nonreactive by phage typing, and 3 produced results during the study that differed from the original typing results. Approximately 20% of isolates submitted to CDC for

typing are nonreactive by using the international phage typing set (16a); other studies suggest much higher percentages (5, 17, 20, 30, 37, 42). Given our results and the time and labor required to maintain phage stocks and propagating strains, we concluded that bacteriophage typing is not a cost-effective method of typing *S. aureus* for most clinical laboratories, particularly since other available methods can be used to type a broader range of other microorganisms.

Plasmid analysis was the first DNA-based method to be applied to *S. aureus*, and it has been used in a number of outbreak investigations (2, 12, 17, 29, 34, 44, 46). Although used extensively, the relative stability of staphylococcal plasmids has often been debated. In our study, the specificity of the technique was high; none of the epidemiologically unrelated isolates were misclassified. However, plasmid typing showed only moderate reproducibility. One replicate (SA-09) within one of the duplicate pairs demonstrated the loss of a plasmid, and 5 of 29 outbreak-related isolates had an altered plasmid profile. Both the loss of plasmids, as suggested in set C (SC-11), and the acquisition of additional plasmids, as in set B for isolates SB-01 and SB-16, posed problems. The latter two strains were identified in the present study as being outbreak related by nine typing techniques, but were called unrelated in the original outbreak investigation since the patients were not hospitalized during the outbreak period and the strains showed additional plasmid DNA. Thus, while these isolates were deemed to be unrelated to the cluster epidemiologically, they appeared to be related in terms of their other characteristics. Whether further studies would have been able to establish an epidemiologic link among these patients outside of the hospital is unclear. To determine more precisely the loss or acquisition of a plasmid during the course of an epidemiologic study, plasmid restriction analysis can be supplemented with examination of unrestricted plasmid preparations.

RFLP typing with a variety of DNA and RNA probes has been used with increasing frequency to type bacterial strains. The most widely applicable procedure is ribotyping (5), although some investigators have questioned its effectiveness for differentiating among isolates of methicillin-resistant *S. aureus* (35). In our study, two different laboratories performed ribotyping, each using a slightly different probe and different restriction enzymes to digest the DNA. The results highlight some of the potential limitations of this procedure. Ribotyping with *Cla*I digests of DNA probed with a cloned *Escherichia coli* ribosomal operon was the only technique that identified all 29 outbreak-related isolates, but it was also one of the least discriminatory techniques, assigning strains from outbreaks II and III to the same type and misclassifying 7 additional isolates as outbreak related. The use of labeled rRNA to probe *Hin*dIII digests was slightly less consistent than *Cla*I ribotyping, but it misclassified an equal number of isolates.

Differences in ribotype and in RFLP and IS typing results generally reflect random DNA mutations that alter the distribution of restriction sites within and adjacent to the loci being probed. Such changes are relatively infrequent within bacterial rDNA operons and other coding sequences, but are more common in noncoding flanking regions (35). As with many other typing systems, however, the most appropriate criterion for interpreting ribotype banding patterns remains unresolved. In a study by Blumberg et al. (5), ribotypes that varied in the size of a single band were considered to represent different strains. In our study, we assigned *Cla*I and *Hin*dIII ribotypes that differed by a single band to subtypes. Such differences in interpretation may account for some of the differences in the results.

Immunoblotting is a technique that is relatively easy to perform (41), is widely applicable to many bacterial species, and, as shown here, was successful in differentiating outbreak-related from unrelated strains as long as the subtype data were ignored. However, a stricter interpretation of results, which would not classify D1 and D2 or A5 and A6 as similar, would result in a much lower degree of sensitivity. Thus, the method used for interpretation of the blots is the key to this technique, since the banding patterns produced are complex and may be difficult to interpret by individuals who do not perform Western blots routinely. The requirement for human serum for the Western blots is another disadvantage.

As the use of PCR becomes more widespread in clinical laboratories, the use of typing techniques based on this technology may become more appealing. Saulnier et al. (36) have reported that results generated by PCR by using a random priming technique are less discriminatory than PFGE. However, it is possible that other primers or different amplification conditions may yet prove to be useful. Although we did not undertake arbitrarily primed PCR in our study, we investigated restriction analysis of amplified DNA generated by PCR amplification of coagulase gene sequences. This technique was highly sensitive in detecting outbreak-related strains (28 of 29); however, it failed to exclude eight of the nonrelated isolates from the clusters. The low number of types seen within the study (total of seven) is a disadvantage. If the number of bands generated by restriction analysis could be expanded by the use of other enzymes, the technique may be more useful.

Although many of the reports of strain typing focus on methicillin-resistant *S. aureus*, methicillin-susceptible isolates remain a common cause of nosocomial infections. For that reason, both methicillin (oxacillin)-susceptible and methicillin-resistant isolates were included in the present study. Two additional Southern blot methods were tested in the current study, one that used IS*431/257* sequences as probes (IS typing) and another that used a collection of four probes (RFLP typing). These techniques were primarily designed to type methicillin-resistant strains and proved to be relatively effective with such isolates. However, these methods were ineffective with methicillin-susceptible strains, which, by definition, lack the *mec* gene, carry the *aph*(2″)-*aac*(6′) gene only infrequently, and carry Tn*554* or IS*431/257* rarely (22). This emphasizes the importance of choosing the typing techniques that are most appropriate for examining isolates. In this case, probes directed at mobile genetic elements that can be plasmid associated [e.g., IS*431/257*, Tn*554*, and *aph*(2″)-*aac*(6′)] may show pattern differences that reflect changes in plasmid content rather than true strain differences. Such plasmid-related changes may explain the various results obtained by these methods for some outbreak isolates (e.g., SC-11) or replicates (SA-02 and SA-15).

The diversity detected by each individual probe was relatively limited; only two *mec* genotypes, three Tn*554* genotypes, four *agr* genotypes, and two gentamicin resistance genotypes were observed among the isolates tested. Thus, the additional discriminatory power achieved by using multiple probes must be offset by the extra cost and effort involved.

The final two methods used in the present study, FIGE and PFGE, were developed for resolving large (50- to 700-kb) fragments of DNA obtained by digesting whole-cell DNA with restriction enzymes that cleave DNA infre-

quently. This general approach has proved to be useful for a wide variety of bacterial species (27). PFGE has been recommended as the typing method of choice for methicillin-resistant *S. aureus* by several groups of investigators (6, 35–37, 40), although FIGE also has its supporters (44).

In the present study, in comparison with PFGE, FIGE misclassified fewer nonrelated strains, differentiated subtle differences among the 47/54/75/77/83A strains, but excluded one of the outbreak III isolates (SC-11). Given that both techniques used the enzyme *Sma*I, the differences in results are intriguing but may reflect the ability of FIGE to resolve differences in smaller fragments, differences in interpretation, or spontaneous variation among organisms from the same culture. The fact that both methods can be used to type virtually all bacteria and yeasts is a major advantage in choosing one of these technologies for the clinical laboratory. The major issue with the electrophoretic techniques, as with most typing techniques, is the lack of standards for interpreting the results. While Prevost and coworkers (35) attempted to address this issue by setting up criteria for interpretation of pulsed-field gels, few other investigators have followed these rules. Until standardized rules of interpretation are published, the same data may be interpreted in different ways by different investigators.

MLEE is a technique frequently used for studying populations of organisms, but it is rarely, if ever, used in the clinical laboratory (38). In our study, only 12 enzymes were used to type the isolates, and we arbitrarily chose a genetic distance of 0.1 to indicate strain differences. In fact, the 12-enzyme set worked well and was able to differentiate most outbreak-related from unrelated isolates. However, the time and labor involved in this technique do not make it practical for use in the routine outbreak investigations that would be undertaken in a clinical laboratory.

In summary, we analyzed the typeability, reproducibility, discriminatory power, ease of use, and ease of interpretation of 12 typing methods by using a well-characterized collection of 60 staphylococcal isolates. No typing method clearly prevailed among the others, and ultimately, a combination of two methods may be most efficacious. One method that would be sensitive enough to include all potential patients or sources may be used for screening isolates early in an epidemiologic study, and another method for detailed strain differentiation may be used later. The choice depends on the resources available to the laboratory and the level of expertise of the personnel involved in the testing. Microbiologists should not expect physicians or infection control personnel to interpret strain typing data without assistance. Thus, the strengths and weaknesses of the techniques used should be understood before any results are generated.

REFERENCES

1. **Arbeit, R. D., M. Arthur, R. D. Dunn, C. Kim, R. K. Selander, and R. Goldstein.** 1990. Resolution of recent evolutionary divergence among *Escherichia coli* from related lineages: the application of pulsed field gel electrophoresis to molecular epidemiology. J. Infect. Dis. **161**:230–235.
2. **Archer, G. L., and C. G. Mayhall.** 1983. Comparison of epidemiologic markers used in the investigation of an outbreak of methicillin-resistant *Staphylococcus aureus* infections. J. Clin. Microbiol. **18**:395–399.
3. **Barberis-Maino, L., B. Berger-Bachi, H. Weber, W. D. Beck, and F. H. Kayser.** 1987. IS*431*, a staphylococcal insertion sequence-like element related to IS*26* from *Proteus vulgaris*. Gene **59**:107–113.
4. **Blair, J. E., and R. E. O. Williams.** 1961. Phage typing of staphylococci. Bull. W.H.O. **24**:771–778.
5. **Blumberg, H. M., D. Rimland, J. A. Kiehlbauch, P. M. Terry, and I. K. Wachsmuth.** 1992. Epidemiologic typing of *Staphylococcus aureus* by DNA restriction fragment length polymorphisms of rRNA genes: elucidation of the clonal nature of a group of bacteriophage-nontypeable, ciprofloxacin-resistant, methicillin-susceptible *S. aureus* isolates. J. Clin. Microbiol. **30**:362–369.
6. **Carles-Nurit, M. J., B. Christophle, S. Broche, A. Gouby, N. Bouziges, and M. Ramuz.** 1992. DNA polymorphisms in methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus*. J. Clin. Microbiol. **30**:2092–2096.
7. **Centers for Disease Control.** 1990. Postsurgical infections associated with an extrinsically contaminated intravenous anaesthetic agent—California, Illinois, Maine, Michigan, 1990. Morbid. Mortal. Weekly Rep. **39**:426–433.
8. **Coia, J. E., F. Thomson-Carter, D. Baird, and D. J. Platt.** 1990. Characterization of methicillin-resistant *Staphylococcus aureus* by biotyping, immunoblotting, and restriction enzyme fragmentation patterns. J. Med. Microbiol. **31**:125–132.
9. **Cookson, B., and I. Phillips.** 1988. Epidemic methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **21**(Suppl. C):57–65.
10. **Costas, M., B. D. Cookson, H. G. Talsania, and R. J. Owen.** 1989. Numerical analysis of electrophoretic protein patterns of methicillin-resistant strains of *Staphylococcus aureus*. J. Clin. Microbiol. **27**:2574–2581.
11. **Garner, J. S., W. R. Jarvis, T. G. Emori, T. C. Horan, and J. M. Hughes.** 1988. CDC definitions for nosocomial infections, 1988. Am. J. Infect. Control. **16**:128–140.
12. **Gaston, M. A., P. S. Duff, J. Naidoo, K. Ellis, J. I. S. Roberts, J. F. Richardson, R. R. Marples, and E. M. Cooke.** 1988. Evaluation of electrophoretic methods for typing methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **26**:189–197.
13. **Gillespie, M. T., B. R. Lyon, and R. A. Skurray.** 1990. Typing of methicillin-resistant *Staphylococcus aureus* by antibiotic resistance phenotypes. J. Med. Microbiol. **31**:57–64.
14. **Goering, R. V., and M. A. Winters.** 1992. Rapid method for epidemiological evaluation of gram-positive cocci by field inversion gel electrophoresis. J. Clin. Microbiol. **30**:577–580.
15. **Goetz, M. B., M. E. Mulligan, R. Kwok, H. O'Brien, C. Caballes, and J. P. Garcia.** 1992. Management and epidemiologic analyses of an outbreak due to methicillin-resistant *Staphylococcus aureus*. Am. J. Med. **92**:607–614.
16. **Goh, S.-H., S. K. Byrne, J. L. Zhang, and A. W. Chow.** 1992. Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms. J. Clin. Microbiol. **30**:1642–1645.

16a. **Hancock, G.** Unpublished data.

17. **Hartstein, A. I., V. H. Morthland, S. Eng, G. L. Archer, F. D. Schoenknecht, and A. L. Rashad.** 1989. Restriction enzyme analysis of plasmid DNA and bacteriophage typing of paired *Staphylococcus aureus* blood culture isolates. J. Clin. Microbiol. **27**:1874–1879.
18. **Hébert, G. A., R. C. Cooksey, N. C. Clark, B. C. Hill, W. R. Jarvis, and C. Thornsberry.** 1988. Biotyping coagulase-negative staphylococci. J. Clin. Microbiol. **26**:1950–1956.
19. **Jacobs, D.** 1990. SAS/GRAPH software and numerical taxonomy, p. 1413–1418. Proceedings of the 15th Annual Users Group International Conference. SAS Institute, Cary, N.C.
20. **Khalifa, K. I., A. A. Heiba, and G. Hancock.** 1989. Nontypeable bacteriophage patterns of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J. Clin. Microbiol. **27**:2249–2251.
21. **Kloos, W. E., and W. D. Lambe, Jr.** 1991. Staphylococcus, p. 222–227. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
22. **Kreiswirth, B., J. Kornblum, R. D. Arbeit, W. Eisner, J. N. Maslow, A. McGeer, D. E. Low, and R. P. Novick.** 1993. Evidence for a clonal origin of methicillin resistance in *Staph-*

ylococcus aureus. Science **259:**227–230.

23. **Kreiswirth, B., A. McGeeral, J. Kornblum, A. E. Simor, W. Eisner, R. Poon, J. Righter, I. Campbell, and D. E. Low.** 1990. The use of variable gene probes to investigate a multihospital outbreak of MRSA, p. 521–530. *In* R. P. Novick (ed.), Molecular biology of the staphylococci. VCH Publishers, New York.
24. **Maple, P. A. C., J. M. T. Hamilton-Miller, and W. Brumfitt.** 1989. World-wide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. Lancet **i:**537–540.
25. **Maslow, J. N., S. Brecher, K. S. Adams, A. Durbin, S. Loring, and R. D. Arbeit.** 1993. The relationship between indole production and the differentiation of *Klebsiella* species: indole-positive and negative isolates of *Klebsiella* determined to be clonal. J. Clin. Microbiol. **31:**2000–2003.
26. **Maslow, J. N., M. E. Mulligan, and R. D. Arbeit.** 1993. Molecular epidemiology: the application of contemporary techniques to typing bacteria. Clin. Infect. Dis. **17:**153–162.
27. **Maslow, J. N., A. M. Slutsky, and R. D. Arbeit.** 1993. Applications of pulsed-field gel electrophoresis to molecular epidemiology, p. 563–572. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
28. **McDougal, L. K., R. Facklam, M. Reeves, S. Hunter, J. M. Swenson, B. Hill, and F. C. Tenover.** 1992. Analysis of multiply antimicrobial-resistant isolates of *Streptococcus pneumoniae* from the United States. Antimicrob. Agents Chemother. **36:** 2177–2184.
29. **Mulligan, M. E., and R. D. Arbeit.** 1991. Epidemiologic and clinical utility among typing systems for differentiating among strains of methicillin-resistant *Staphylococcus aureus*. Infect. Control Hosp. Epidemiol. **12:**20–28.
30. **Mulligan, M. E., R. Y. Y. Kwok, D. M. Citron, J. F. John, Jr., and P. B. Smith.** 1988. Immunoblots, antimicrobial resistance, and bacteriophage typing of oxacillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **26:**2395–2401.
31. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Approved standard, M7-A2, vol. 10. National Committee for Clinical Laboratory Standards, Villanova, Pa.
32. **National Committee for Clinical Laboratory Standards.** 1990. Performance standards for antimicrobial disk susceptibility tests, 4th ed. Approved standard, M2-A4, vol. 10. National Committee for Clinical Laboratory Standards, Villanova, Pa.
33. **Pfaller, M., R. J. Hollis, and M. J. Bale.** 1992. Plasmid fingerprinting of staphylococci, p. 10.3.1–10.3.6. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
34. **Pfaller, M. A., D. S. Wakefield, R. Hollis, M. Fredrickson, E. Evans, and R. M. Massanari.** 1991. The clinical microbiology laboratory as an aid in infection control. The application of molecular techniques in epidemiologic studies of methicillin-resistant *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **14:**209–217.
35. **Prevost, G., B. Jaulhac, and Y. Piemont.** 1992. DNA fingerprinting by pulsed-field gel electrophoresis is more effective than ribotyping in distinguishing among methicillin-resistant *Staphylococcus aureus* isolates. J. Clin. Microbiol. **30:**967–973.
36. **Saulnier, P., C. Bourneix, G. Prevost, and A. Andremont.** 1993. Random amplified polymorphic DNA assay is less discriminant than pulsed-field gel electrophoresis for typing strains of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **31:** 982–985.
37. **Schlichting, C., C. Branger, J.-M. Fournier, W. Witte, A. Boutonnier, C. Wolz, P. Goullet, and G. Doring.** 1993. Typing of *Staphylococcus aureus* by pulsed-field gel electrophoresis, zymotyping, capsular typing, and phage typing: resolution of clonal relationships. J. Clin. Microbiol. **31:**227–232.
38. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51:**873–884.
39. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy, p. 230–234. W. H. Freeman & Co., San Francisco.
40. **Struelens, M. J., A. Deplano, C. Godard, N. Maes, and E. Serruys.** 1992. Epidemiologic typing and delineation of genetic relatedness of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis of genomic DNA by using pulsed-field gel electrophoresis. J. Clin. Microbiol. **30:**2599–2605.
41. **Tsang, V. C. W., J. M. Peralta, and A. R. Simons.** 1983. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by electrophoresis. Methods Enzymol. **92:**377–391.
42. **van Belkum, A., R. Bax, P. Peerbooms, W. H. F. Goessens, N. van Leeuwen, and W. G. V. Quint.** 1993. Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for discriminating methicillin-resistant *Staphylococcus aureus* strains. J. Clin. Microbiol. **31:**798–803.
43. **Wenzel, R. P., M. D. Nettleman, R. N. Jones, and M. A. Pfaller.** 1991. Methicillin-resistant *Staphylococcus aureus*: implications for the 1990s and effective control measures. Am. J. Med. **91**(suppl. 3B)**:**221S–227S.
44. **Winters, M. A., R. V. Goering, S. E. Boon, R. Morin, M. Sorensen, and L. Snyder.** 1993. Epidemiological analysis of methicillin-resistant *Staphylococcus aureus* comparing plasmid typing with chromosomal analysis by field inversion gel electrophoresis. Med. Microbiol. Lett. **2:**33–41.
45. **Zierdt, C. H., I. K. Hosein, R. Shively, and J. D. MacLowry.** 1992. Phage pattern-specific oxacillin-resistant and borderline oxacillin-resistant *Staphylococcus aureus* in U.S. hospitals: epidemiologic significance. J. Clin. Microbiol. **30:**252–254.
46. **Zuccarelli, A. J., I. Roy, G. P. Harding, and J. J. Couperus.** 1990. Diversity and stability of restriction enzyme profiles of plasmid DNA from methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **28:**97–102.