

Comparison of Pulsed-Field Gel Electrophoresis and Coagulase Gene Restriction Profile Analysis Techniques in the Molecular Typing of *Staphylococcus aureus*

CHIEN-SHUN CHIOU,* HSIAO-LUN WEI, AND LI-CHU YANG

The Third Branch Office, Center for Disease Control, Department of Health, Taichung City, Taiwan

Received 4 October 1999/Returned for modification 10 November 1999/Accepted 22 March 2000

Pulsed-field gel electrophoresis (PFGE) and coagulase gene restriction profile (CRP) analysis techniques were used to analyze 71 *Staphylococcus aureus* isolates recovered from nine food-borne disease outbreaks. Twenty-two PFGE profiles and 11 CRPs were identified, with discrimination indices of 0.86 and 0.72, respectively. In addition, the variable regions of the coagulase genes of 39 isolates were sequenced and showed extensive identity, indicating that this is not an efficient alternative for the molecular typing of *S. aureus*.

Staphylococcus aureus is one of the major causative agents of food poisoning. In Taiwan, *S. aureus* was responsible for 30% of cases of bacterial food-borne outbreaks between 1986 and 1995 (17). Staphylococcal food poisoning is characterized by nausea, vomiting, abdominal pain, and diarrhea and has an incubation period of 30 min to 8 h after ingestion of the contaminated food (8). Enterotoxins produced by the bacteria are believed to be wholly responsible for the symptoms of food poisoning (3); therefore, only enterotoxigenic strains of *S. aureus* are thought to be able to cause food poisoning. To date, nine *S. aureus* enterotoxins, designated SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ, have been identified (4, 16, 22, 26). The first five of these (SEA through SEE [SEA-E]) can be detected with commercially available antisera. Tests with such antisera are routinely performed with staphylococcal isolates in the laboratories of the Taiwanese health department in order to confirm the source of a food-borne outbreak. However, while these tests can identify SEA-E-producing isolates, they do not address the possibility that non-SEA-E-producing isolates may be the cause of a food-poisoning outbreak. Another problem of *S. aureus* identification is that, because the scale of food-borne outbreaks in which *S. aureus* could be involved is frequently small, only very limited numbers of isolates are usually recovered.

Molecular typing of staphylococcal isolates can provide useful clonality information for confirmation of a staphylococcal food-borne outbreak. Numerous such methods for *S. aureus* typing have been described (5, 6, 7, 12, 19, 23, 25, 27). Among these methods, pulsed-field gel electrophoresis (PFGE) has been demonstrated to have advantages in discriminatory power, typeability, and reproducibility and has been taken as the “gold standard” for the typing of *S. aureus* (2, 18, 20), even though it is labor-intensive and time-consuming. Compared to PFGE, coagulase gene restriction profile (CRP) analysis, a PCR-based method, is easy to perform and has high levels of specimen typeability and reproducibility, and it has been used successfully for the typing of a large number of methicillin-resistant *S. aureus* isolates (9, 13). In this study, we compared PFGE and CRP analysis for the characterization of staphylococcal isolates recovered from nine food-borne outbreaks. The

relationship between the staphylococcal isolates and the food-borne outbreaks is also discussed.

MATERIALS AND METHODS

Bacterial strains. Bacterial isolates were recovered from rectal swabs of patients and from nasal and hand swabs of suspected food handlers from nine food-borne disease outbreaks in central Taiwan between 1995 and 1997. The specimens were streaked onto Baird-Parker agar plates (Merck Taiwan Ltd., Taichung City, Taiwan), and the plates were incubated at 35°C for 24 h. Two or three colonies were picked and subcultured onto nutrient agar plates (Eiken Chemical Co., Tokyo, Japan). The bacteria were tested with staphylase agglutination testing kits (Oxoid Unipath, Hampshire, England), and the bacteria that tested positive were considered to be *S. aureus*. A total of 71 isolates were obtained. The isolates were immediately tested for the presence of enterotoxins (see below) and were then kept in 15% glycerol at –70°C until use.

Detection of toxins by RPLA. Staphylococcal isolates were screened for the expression of SEA, SEB, SEC, SED, and SEE by reverse passive latex agglutination (RPLA) according to the manufacturer’s instructions (Denka Seiken, Co., Tokyo, Japan).

Detection of toxin genes by PCR. Isolates were examined for the presence of *sea*, *seb*, *sec*, *sed*, and *see* by PCR, according to the work of Johnson and colleagues (11).

CRP analysis. Amplification of the repeated region of the coagulase gene by PCR was performed as described by Goh and colleagues (7), except that a new forward primer, primer COAG-5 (5′-GGTATTCGTGAATACAACGATGGA A-3′), located 40 bp upstream from COAG-2, was used in the reaction with primer COAG-3 (5′-AAAGAAAACCACTCACATCA-3′). Restriction profiles were determined by digesting the amplified fragment with *AluI* (Promega Corp., Madison, Wis.) and separating the DNA fragments in 2.5% agarose gels.

DNA sequencing and data analysis. The PCR-amplified DNA was sequenced (done by MB Mission Biotech Corp., Taipei, Taiwan) with COAG-5 as the primer. About 700 bp of each sequence was determined in a single gel run, and 560 bp was used for sequence analysis. The GAP program of the GCG package (version 9) was used to align sequences in order to determine the maximum identity.

PFGE. Preparation and *SmaI* restriction treatment of the genomic DNA for PFGE analysis were performed by a rapid preparation procedure (15). DNA fragments were separated in 0.9% agarose gels at 13°C with a Rotaphor type V apparatus (Biometra, Göttingen, Germany) in 0.5× TBE buffer (0.045 M Tris-borate, 0.1 mM EDTA [pH 8.3]). Electrophoresis was performed at a fixed voltage (190 V), at a 120° fixed angle, and with pulse time intervals from 8 to 14 s for 10 h and then from 19 to 28 s for 12 h. A ladder of bacteriophage λ DNA concatemers (New England BioLabs, Inc., Beverly, Mass.) was included to estimate the sizes of the DNA fragments. The PFGE pattern was interpreted in accordance with a previously published guideline (24).

Statistics. Discriminatory power was evaluated by use of the discrimination index, as described by Hunter (10).

Epidemiological data. Epidemiological data relating to the food-borne disease outbreaks being investigated were obtained from standardized case report forms filled in by the county public health authority. The reports included the dates, times, and locations of suspected food ingestion, the suspected foods, the persons involved in eating and poisoning, and basic information about each patient’s sex, age, date and time of onset, residency, symptoms, and medical treatment.

* Corresponding author. Mailing address: 4F 103 Minchuan Road, Taichung City 403, Taiwan. Phone: 886-4-2225196. Fax: 886-4-2221917. E-mail address: nipmcsc@cdc.gov.tw.

RESULTS

Production of enterotoxins. A total of 71 *S. aureus* isolates were screened for the expression of enterotoxin. First, isolates recovered from the specimens were immediately subjected to screening by RPLA. A total of 17 isolates were identified in this process: 15 isolates from outbreaks 1, 2, and 3 and 2 isolates from outbreak 7, which produced SEC, SED, SEA, and SEA in the four outbreaks, respectively (Table 1). Second, isolates revived from stocks stored at -70°C prior to further molecular characterization were also screened. In the second test, a total of 29 isolates were detected. In addition to the 17 isolates detected in the first screening, an additional 12 isolates from six outbreaks were identified. Ten of these 12 isolates produced SEC, while the other 2 isolates were found to produce SEA and SEB, respectively.

Toxin genes. The types of toxin genes carried by isolates as detected by PCR were concordant with the types of expressed toxins as determined in the second RPLA test (Table 1). Of the 71 isolates, 29 carried one of the toxin genes (*sea*, *seb*, *sec*, *sed*, or *see*), and 42 isolates did not carry *sea*, *seb*, *sec*, *sed*, or *see*.

Genotypes. Screening by PFGE and CRP analysis grouped the 71 isolates into 22 PFGE types and 11 CRP types (Table 1). The PFGE profiles and CRPs are shown in Fig. 1 and 2, respectively. The typeabilities for both techniques were 100%. PFGE produced better discrimination than CRP analysis, with discrimination indices of 0.86 and 0.72, respectively. PFGE further divided CRP-3 into four PFGE types (types 3, 6, 14a, and 14b), CRP-5 into eight PFGE types (types 5, 10a, 10b, 11a, 11b, 11c, 12, and 13), CRP-7 into two PFGE types (types 9 and 17), and CRP-8 into two PFGE types (types 7a and 7b). In contrast, only PFGE type 6 could be separated into two CRPs (CRP-3 and CRP-11). In individual outbreaks, both methods showed the same discriminatory ability for isolates from outbreaks 1, 2, 3, and 7, but PFGE showed a higher discriminatory ability than CRP analysis for isolates from outbreaks 4, 5, 6, 8, and 9 (Table 1).

Variable-region sequences of coagulase genes. To determine whether sequencing of the variable segment of the coagulase gene can be used as an alternative method for molecular typing of *S. aureus* isolates, the 3' variable regions of the coagulase genes of 39 isolates, representing all CRP and PFGE types, were sequenced. Sequences of 560 bp with the same starting base pair were used for analysis and comparison. Eleven unique sequences, designated Seq1 through Seq11, were identified (Table 1). Among the sequences, 63 to 97% identities were found (data not shown). With only a few exceptions, sequences of more than 560 bp from isolates with the same CRP were identical. The most divergence was found in the sequence for the CRP-3 and PFGE type 3 isolate, which showed a 14-bp difference in Seq3. The rest showed mismatches of only 1 to 4 bp.

DISCUSSION

PFGE, because of its great discriminatory power and high degree of specimen typeability, is accepted as the gold standard for the molecular typing of *S. aureus* isolates. It has successfully been used to study the epidemiology of *S. aureus* nosocomial infection and methicillin resistance (12, 25). In this study, PFGE also exhibited superiority as a technique for the identification of *S. aureus* isolates collected from food-borne disease outbreaks. The discrimination index (0.86) for PFGE was found to be much higher than that for CRP analysis (0.72) (Table 1). Nevertheless, PFGE is time-consuming and labor-intensive and can be performed only in reference laboratories with skillful technicians. Due to these drawbacks, PFGE is not

an ideal typing method for health departments undertaking routine analysis of large numbers of *S. aureus* isolates in the investigation of food-borne disease outbreaks in which *S. aureus* is the suspected causative agent.

In contrast, PCR-based CRP analysis is fast and easy to manipulate. As the data in this study show, CRP analysis exhibits the same discriminatory power as PFGE for SEA-E-producing isolates. However, CRP analysis is much less discriminatory than PFGE in the case of non-SEA-E-producing isolates, and its results could therefore lead to the misjudging of the causes of some outbreaks (for example, outbreaks 4 and 5 in this study [Table 1]). Thus, while CRP analysis has been used successfully in several epidemiological studies of *S. aureus* (9, 13), one study has indicated that it is insufficient as a sole method for the typing of staphylococcal isolates (21). Nevertheless, by combining CRP analysis with other fast PCR-based analytical techniques such as protein A gene typing (6) or inter-IS256-PCR typing (5), this methodology may be able to meet the needs of health departments. In any event, the development of a fast and highly discriminatory typing method for *S. aureus* remains an important focus.

It has been hypothesized that the determination of the nucleotide sequence of the highly variable repeated region of the coagulase gene could be an alternative method for the typing of *S. aureus* isolates (21). However, our results from the sequencing of 39 of the 71 isolates show that the variable region of the coagulase gene is much more conserved than expected. Even though isolates were recovered from different disease outbreaks, most isolates with the same CRP differed by only 1 to 4 bp over a 560-bp sequence. This indicates that sequencing of the variable segment of the coagulase gene may not be an efficient method for the typing of *S. aureus* isolates.

In our experience, most staphylococcal food-borne disease outbreaks occur on small scales. As such, in most cases only a few staphylococcal isolates are obtained, leading to difficulty in identifying the causative agent. Since toxigenic strains of *S. aureus* are quite prevalent in the general population (1), it is easy to misdiagnose the cause of a food-borne disease outbreak as being *S. aureus* on the basis of routine enterotoxin analysis of limited isolates. Characterization of staphylococcal isolates recovered from patients and food handlers by various techniques, in particular, molecular typing methods, can provide useful information in investigations of the relatedness of bacterial isolates and disease outbreaks. In outbreaks 1, 2, 3, and 7, only limited numbers of isolates were recovered. Yet, using PFGE and CRP analysis, we traced the contamination sources of two outbreaks (outbreaks 2 and 3) to food handlers and obtained sufficient evidence to identify enterotoxigenic isolates as the causes of the outbreaks.

One of the criteria for confirmation of staphylococcal food poisoning is whether the recovered isolates are enterotoxigenic. As only SEA-E antisera are available for testing, staphylococcal isolates are routinely tested for production of SEA-E only in the laboratories of the public health department in Taiwan. Consequently, non-SEA-E-producing *S. aureus* strains are not considered etiologic organisms for food poisoning. Our typing results indicate that a non-SEA-E-producing strain (CRP-5, PFGE type 11a) may be strongly implicated in outbreak 6 and possibly in a further two outbreaks. Staphylococcal enterotoxins belong to a big superantigen family (14). In addition to SEA-E, four new enterotoxins (SEG, SEH, SEI, and SEJ) have recently been characterized (16, 22, 26). Some non-SEA-E-producing strains presented in our study appear to be implicated in food poisoning and are likely to belong to one of these new groups of enterotoxin producers.

The identification of a new enterotoxin requires complicated

TABLE 1. Phenotypes and genotypes of *S. aureus* isolates from the food-borne disease outbreaks

Outbreak	Date, location	Incubation period (median)	Isolate code ^a	Toxin production ^b		Toxin gene ^c	PFGE profile ^d	CRP	Sequence (no. of bp differences) ^e
				First RPLA	Second RPLA				
1	18 March 1995, Nantou County	1 h–1 h and 10 min (1 h)	398	SEC	SEC	<i>sec</i>	3	3	Seq3 (14)
			400	SEC	SEC	<i>sec</i>	4	4	Seq4
			404	SEC	SEC	<i>sec</i>	4	4	
			405	SEC	SEC	<i>sec</i>	4	4	
2	8 June 1995, Nantou County	4 h and 30 min–9 h and 30 min (6 h)	191	SED	SED	<i>sed</i>	1	1	Seq1
			199	SED	SED	<i>sed</i>	1	1	
			206*	SED	SED	<i>sed</i>	1	1	Seq1
			193	SED	SED	<i>sed</i>	2	2	Seq2
			200	SED	SED	<i>sed</i>	2	2	
3	12 September 1995, Taichung County	1 h and 20 min–2 h and 40 min (2 h and 30 min)	620	SEA	SEA	<i>sea</i>	6	3	Seq3
			621	SEA	SEA	<i>sea</i>	6	3	
			622	SEA	SEA	<i>sea</i>	6	3	
			623	SEA	SEA	<i>sea</i>	6	3	
			625	SEA	SEA	<i>sea</i>	6	3	
			627*	SEA	SEA	<i>sea</i>	6	3	Seq3
4	24 October 1995, Miaoli County	1 h–2 h (2 h)	934	—	—	—	5	5	Seq5
			936	—	—	—	5	5	Seq5
			940	—	—	—	5	5	Seq5
			941	—	—	—	13	5	Seq5
			947	—	—	—	8	6	Seq6
			946	—	SEC	<i>sec</i>	7a	8	Seq8
			953-1*	—	—	—	10a	5	Seq5
			953-2*	—	—	—	10b	5	Seq5
			953-3*	—	—	—	9	7	Seq7
			5	5 January 1996, Taichung County	30 min–20 h (5 h and 30 min)	1120	—	—	—
1121	—	—				—	11a	5	Seq5
1126	—	—				—	11b	5	Seq5
1130	—	—				—	11b	5	Seq5
1131	—	SEC				<i>sec</i>	7a	8	Seq8
6	12 January 1996, Taichung County	1 h–20 h and 30 min (5 h and 30 min)	1172	—	—	—	11a	5	Seq5
			1173	—	—	—	11a	5	Seq5
			1174	—	—	—	11a	5	Seq5 (4)
			1176	—	—	—	11a	5	
			1180	—	—	—	11a	5	
			1181	—	—	—	11a	5	
			1183	—	—	—	11a	5	
			1184	—	—	—	11a	5	
			1185	—	—	—	11a	5	
			1187	—	—	—	11a	5	
			1189	—	—	—	11a	5	
			1190	—	—	—	11a	5	
			1191	—	—	—	11a	5	
			1194	—	—	—	11a	5	
			1195	—	—	—	11a	5	
			1199	—	—	—	11a	5	
			1200	—	—	—	11a	5	
			1202	—	—	—	11a	5	
			1203	—	—	—	11a	5	
1197	—	—	—	12	5	Seq5			
1179	—	SEC	<i>sec</i>	7a	8	Seq8			
1186	—	SEC	<i>sec</i>	7a	8				

Continued on following page

TABLE 1—Continued

Outbreak	Date, location	Incubation period (median)	Isolate code ^a	Toxin production ^b		Toxin gene ^c	PFGE profile ^d	CRP	Sequence (no. of bp differences) ^e
				First RPLA	Second RPLA				
7	8 January 1997, Taichung County	1 h–5 h (2 h and 30 min)	4807	SEA	SEA	<i>sea</i>	14a	3	Seq3
			4811	SEA	SEA	<i>sea</i>	14a	3	
			4813	—	SEC	<i>sec</i>	7b	8	Seq8
8	12 March 1997, Taichung County	4 h–17 h (13 h)	5126	—	—	—	11a	5	Seq5 (2)
			5166	—	—	—	11a	5	Seq5
			5180	—	—	—	11a	5	Seq5 (2)
			5190	—	—	—	6	3	
			5174	—	—	—	14b	3	Seq3 (1)
			5178	—	SEC	<i>sec</i>	7a	8	
			5199	—	—	—	15	9	Seq9
9	17 March 1997, Taichung County	1 h–25 h and 45 min (8 h and 30 min)	5235	—	—	—	17	7	Seq7
			5224	—	—	—	17	7	
			5239	—	—	—	11c	5	Seq5
			5231-1	—	SEB	<i>seb</i>	11c	5	
			5231-2	—	SEC	<i>sec</i>	7a	8	Seq8
			5242-1	—	SEA	<i>sea</i>	6	3	
			5242-2	—	SEC	<i>sec</i>	7a	8	Seq8
			5233	—	SEC	<i>sec</i>	7a	8	
			5222	—	SEC	<i>sec</i>	16	10	Seq10
			5220	—	—	—	6	11	Seq11
Discrimination index							0.86	0.72	

^a *, isolates recovered from food handlers.

^b —, no SEA, SEB, SEC, SED, or SEE was detected by the RPLA test.

^c —, no *sea*, *seb*, *sec*, *sed*, or *see* gene was detected.

^d The major PFGE types are designated by numerals as patterns that differed by at least four DNA fragments; minor PFGE subtypes are designated by letter suffixes as patterns that differed by at most three DNA fragments.

^e Each sequence type differs from the others by less than 97% identity over 560 bp.

biochemical procedures and bioassays, such as emetic responses in rhesus monkeys and stimulation of murine T-cell proliferation (16), which are not easily conducted in general laboratories. Molecular typing provides a way of linking non-SEA-E-producing strains of *S. aureus* with food-poisoning outbreaks and helps to select potential candidates which may be new enterotoxin producers.

In conclusion, molecular typing of *S. aureus* isolates provides useful information in the investigation of food-borne disease

outbreaks. The results of this study demonstrate that PFGE is a more precise method for molecular typing of *S. aureus* isolates than CRP analysis. However, to meet the requirements of health departments undertaking routine typing of large numbers of *S. aureus* isolates, a faster and more discriminatory method of molecular typing is needed. Sequencing of variable regions of the coagulase gene in *S. aureus* isolates would not appear to be an efficient alternative, but other methods combined with CRP analysis may achieve this aim.

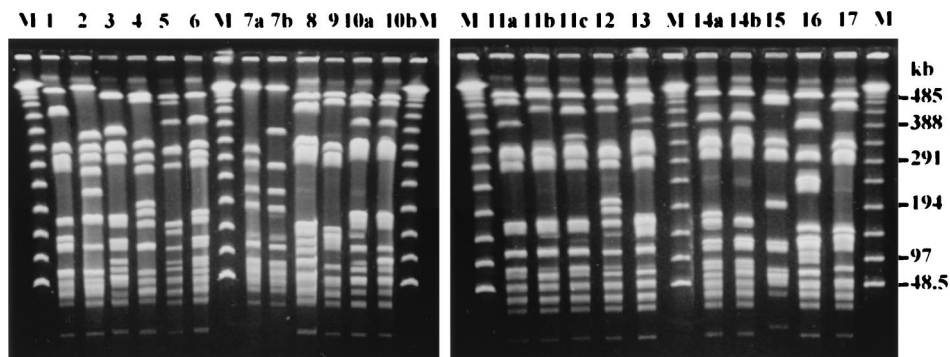


FIG. 1. PFGE profiles of *S. aureus* isolates. Chromosomal DNA was digested with *Sma*I, and the fragments were fractionated on a 0.9% agarose gel. The numbers above the lanes represent the assigned PFGE types and subtypes (1 to 17). Lanes M, bacteriophage lambda molecular weight markers.

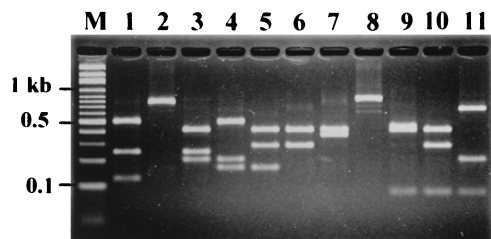


FIG. 2. CRPs of *S. aureus* isolates. PCR amplicons of the 3' region of the coagulase gene were digested with *AluI* and were then fractionated on a 2.5% agarose gel. The numbers above the lanes represent different CRPs (1 to 11). Lane M, molecular weight marker.

ACKNOWLEDGMENTS

This work was supported by grant DOH87-TD-1095 from the Department of Health of Taiwan.

We thank S. Y. Li, of the Vaccine Research & Production Center of the Center for Disease Control, for critical review of this manuscript.

REFERENCES

- al Bustan, M. A., E. E. Udo, and T. D. Chugh. 1996. Nasal carriage of enterotoxin-producing *Staphylococcus aureus* among restaurant workers in Kuwait City. *Epidemiol. Infect.* **116**:319–322.
- Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller. 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J. Clin. Microbiol.* **33**:551–555.
- Bergdoll, M. S. 1989. *Staphylococcus aureus*, p. 464–523. In M. P. Doyle (ed.), *Bacterial foodborne pathogens*. Marcel Dekker, Inc., New York, N.Y.
- Betley, M. J., D. W. Borst, and L. B. Regassa. 1992. Staphylococcal enterotoxins, toxic shock syndrome toxin and streptococcal pyrogenic exotoxins: a comparative study of their molecular biology. *Chem. Immunol.* **55**:1–35.
- Deplano, A., M. Vanechoutte, G. Verschraegen, and M. J. Struelens. 1997. Typing of *Staphylococcus aureus* and *Staphylococcus epidermidis* strains by PCR analysis of inter-IS256 spacer length polymorphisms. *J. Clin. Microbiol.* **35**:2580–2587.
- Frenay, H. M., J. P. Theelen, L. M. Schouls, C. M. Vandembroucke-Grauls, J. Verhoef, W. J. van Leeuwen, and F. R. Mooi. 1994. Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. *J. Clin. Microbiol.* **32**:846–847.
- 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.
- Holmberg, S. D., and P. A. Blake. 1984. Staphylococcal food poisoning in the United States. New facts and old misconceptions. *JAMA* **251**:487–489.
- Hookey, J. V., J. F. Richardson, and B. D. Cookson. 1998. Molecular typing of *Staphylococcus aureus* based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. *J. Clin. Microbiol.* **36**:1083–1089.
- Hunter, P. R. 1990. Reproducibility and indices of discriminatory power of microbial typing methods. *J. Clin. Microbiol.* **28**:1903–1905.
- Johnson, W. M., S. D. Tyler, E. P. Ewan, F. E. Ashton, D. R. Pollard, and K. R. Rozee. 1991. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *J. Clin. Microbiol.* **29**:426–430.
- Kumari, D. N., V. Keer, P. M. Hawkey, P. Parnell, N. Joseph, J. F. Richardson, and B. Cookson. 1997. Comparison and application of ribosome spacer DNA amplicon polymorphisms and pulsed-field gel electrophoresis for differentiation of methicillin-resistant *Staphylococcus aureus* strains. *J. Clin. Microbiol.* **35**:881–885.
- Lawrence, C., M. Cosseron, O. Mimoz, C. Brun-Buisson, Y. Costa, K. Samii, J. Duval, and R. Leclercq. 1996. Use of the coagulase gene typing method for detection of carriers of methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **37**:687–696.
- Marrack, P., and J. Kappler. 1990. The staphylococcal enterotoxins and their relatives. *Science* **248**:705–711.
- Matushek, M. G., M. J. Bonten, and M. K. Hayden. 1996. Rapid preparation of bacterial DNA for pulsed-field gel electrophoresis. *J. Clin. Microbiol.* **34**:2598–2600. (Erratum, **35**:536, 1997.)
- Munson, S. H., M. T. Tremaine, M. J. Betley, and R. A. Welch. 1998. Identification and characterization of staphylococcal enterotoxin types G and I from *Staphylococcus aureus*. *Infect. Immun.* **66**:3337–3348.
- Pan, T. M., T. K. Wang, C. L. Lee, S. W. Chien, and C. B. Horng. 1997. Food-borne disease outbreaks due to bacteria in Taiwan, 1986 to 1995. *J. Clin. Microbiol.* **35**:1260–1262.
- 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.
- Richardson, J. F., P. Aparicio, R. R. Marples, and B. D. Cookson. 1994. Ribotyping of *Staphylococcus aureus*: an assessment using well-defined strains. *Epidemiol. Infect.* **112**:93–101.
- Saulnier, P., C. Bourneix, G. Prevost, and A. Andreumont. 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.
- Schwarzkopf, A., and H. Karch. 1994. Genetic variation in *Staphylococcus aureus* coagulase genes: potential and limits for use as epidemiological marker. *J. Clin. Microbiol.* **32**:2407–2412.
- Su, Y. C., and A. C. Wong. 1995. Identification and purification of a new staphylococcal enterotoxin, H. *Appl. Environ. Microbiol.* **61**:1438–1443.
- Tambic, A., E. G. Power, H. Talsania, R. M. Anthony, and G. L. French. 1997. Analysis of an outbreak of non-phage-typeable methicillin-resistant *Staphylococcus aureus* by using a randomly amplified polymorphic DNA assay. *J. Clin. Microbiol.* **35**:3092–3097.
- Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.
- Yoshida, T., N. Kondo, Y. A. Hanifah, and K. Hiramatsu. 1997. Combined use of ribotyping, PFGE typing and IS431 typing in the discrimination of nosocomial strains of methicillin-resistant *Staphylococcus aureus*. *Microbiol. Immunol.* **41**:687–695.
- Zhang, S., J. J. Iandolo, and G. C. Stewart. 1998. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (*sej*). *FEMS Microbiol. Lett.* **168**:227–233.
- 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.