

Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance

MANISHA MEHROTRA, GEHUA WANG, AND WENDY M. JOHNSON*

Special Project Unit, Bureau of Microbiology, Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba, Canada

Received 30 August 1999/Returned for modification 27 October 1999/Accepted 5 December 1999

A multiplex PCR assay for detection of genes for staphylococcal enterotoxins A to E (*entA*, *entB*, *entC*, *entD*, and *entE*), toxic shock syndrome toxin 1 (*tst*), exfoliative toxins A and B (*etaA* and *etaB*), and intrinsic methicillin resistance (*mecA*) was developed. Detection of *femA* was used as an internal positive control. The multiplex PCR assay combined the primers for *sea* to *see* and *femA* in one set and those for *eta*, *etb*, *tst*, *mecA*, and *femA* in the other set. Validation of the assay was performed using 176 human isolates of *Staphylococcus aureus*. This assay offers a very specific, quick, reliable, and inexpensive alternative to conventional PCR assays used in clinical laboratories to identify various staphylococcal toxin genes.

It is well documented that strains of *Staphylococcus aureus* produce a variety of extracellular protein toxins, including enterotoxins, toxic shock syndrome toxin 1 (TSST-1), exfoliative toxin (ET), hemolysins, and coagulase (15). *S. aureus* is one of the most commonly found pathogenic bacteria and is hard to eliminate from the human environment. It is responsible for many nosocomial infections, besides being the main causative agent of food intoxication by virtue of its variety of enterotoxins (15).

According to serological classification, to date six staphylococcal enterotoxin (SE) groups have been recognized: SEA, SEB, SEC, SED, and SEE (15) and the recently described SEH (22, 27). These enterotoxins are small peptides (26 to 29 kDa) and have a great deal of similarity at the amino acid level (21). They are the main source of food poisoning and cause intensive intestinal peristalsis. The toxic shock syndrome of humans and animals is caused by the presence of *S. aureus* isolates producing TSST-1. The enterotoxins, as well as TSST-1, belong to a family of superantigens (25). The two ETs ETA and ETB, in conjunction or independently, are implicated in the cause of staphylococcal scalded-skin syndrome (15).

Over the last few decades, there has been an enormous increase and emergence of *S. aureus* strains resistant to the antibiotic methicillin (MRSA strains), particularly in nosocomial settings (13). The intrinsic resistance to these antibiotics is attributed to the presence of *mecA*, whose product is a 78-kDa protein called penicillin binding protein 2a (14, 28). Identification of *mecA* in such MRSA strains has led to some knowledge regarding the use of the antibiotic vancomycin (9). The *femA* gene encodes a factor which is essential for methicillin resistance and is universally present in all *S. aureus* isolates. The *femA* gene product, a 48-kDa protein, has been implicated in cell wall metabolism and is found in large amounts in actively growing cultures (17, 29).

For epidemiological surveillance, the methods most frequently used for the detection of staphylococcal toxins are immunodiffusion, agglutination, radioimmunoassay, and en-

zyme-linked immunosorbent assay (15, 18). Among the techniques used to identify toxin genotypes, DNA-DNA hybridization and PCR have been reported to be very successful and reliable, and our laboratory previously designed specific primers for the successful and reliable detection of SEs, TSST-1, and ETs by PCR (18).

There are several reports in the literature describing the use of multiplex PCR for detection of MRSA strains, but most of these techniques are designed to detect only two or three gene fragments (1, 8, 12, 24, 26, 29, 30). In this report, we describe two multiplex PCR primer sets which can detect the presence of 10 staphylococcal genes simultaneously, in just two reactions. As an internal positive control for each reaction, we incorporated primers specifically designated to amplify *femA*, which has been reported to be specific to *S. aureus* (29). Validation of the multiplex PCR primers was performed and interpreted using 176 isolates of *S. aureus* which were first characterized for their toxin gene profiles by using individual primers (18). We conclude that the multiplex primer sets described here are reliable and specific in detecting the toxin genes of *S. aureus*.

MATERIALS AND METHODS

Bacterial strains and culture media. A total of 176 *S. aureus* strains were used in this study. Of these, 107 strains were isolated from nasal swabs of a control (healthy) population, 47 strains were from The Netherlands, and 19 MRSA strains were from a national surveillance study in Canada. The remaining three strains were isolated from clinical specimens. All strains were stored on suitable maintenance media in the culture collection in the National Laboratory for Bacteriology, Laboratory Center for Disease Control. The control strains had been previously defined in terms of toxigenicity with respect to enterotoxins, ETs, and TSST-1. The following strains were used as positive controls in this study: ATCC 13565 (SEA), ATCC 14458 (SEB), ATCC 19095 (SEC), 90-S-1025 (SED), ATCC 27664 (SEE), 88-S-8902 (ETA), 88-S-8620 (ETB), 92-S-1344 (TSST-1), and 95-S-739 (*mecA*). The non-ATCC strains mentioned above were obtained from the culture collection of the Laboratory Center for Disease Control and were characterized for their toxin production by using semiquantitative reversed passive latex agglutination toxin detection kits (SET-RPLA and TST-RPLA; Oxoid Ltd., Basingstoke, Hampshire, England). The two ET-producing strains were originally identified by immunodiffusion tests (18). Disk diffusion tests for MRSA isolates were performed as described previously (21a). Bacterial cultures were grown in brain heart infusion broth prior to extraction of total DNA.

DNA isolation. Total DNA was isolated from 0.5 ml of brain heart infusion broth culture grown overnight for all the bacterial strains used in the study. The procedure used for DNA isolation has been described previously (18). DNA samples were dissolved in Tris-EDTA buffer (10 mM Tris chloride, 1 mM EDTA

* Corresponding author. Present address: Cangene Corporation, 26 Henlow Bay, Winnipeg, Manitoba, Canada R3Y 1G4. Phone: (204) 275-4301. Fax: (204) 275-4289. E-mail: wjohnson@cangene.com.

TABLE 1. Nucleotide sequences, gene locations, and anticipated sizes of PCR products for the *S. aureus* gene-specific oligonucleotide primers used in this study

Gene ^a	Primer	Oligonucleotide sequence (5'-3')	Location within gene	Size of amplified product (bp)	Multiplex PCR set
<i>sea</i>	GSEAR-1	GGTTATCAATGTGCGGGTGG	349-368	102	A
	GSEAR-2	CGGCACITTTTTTCTCTTCGG	431-450		
<i>seb</i>	GSEBR-1	GTATGGTGGTGTAAGTACGAGC	666-685	164	A
	GSEBR-2	CCAAATAGTGACGAGTTAGG	810-829		
<i>sec</i>	GSECR-1	AGATGAAGTAGTTGATGTGTATGG	432-455	451	A
	GSECR-2	CACACTTTTAGAATCAACCG	863-882		
<i>sed</i>	GSEDR-1	CCAATAATAGGAGAAAATAAAAG	492-514	278	A
	GSEDR-2	ATTGGTATTTTTTTTCGTTTC	750-769		
<i>see</i>	GSEER-1	AGGTTTTTTCACAGTTCATCC	237-257	209	A
	GSEER-2	CTTTTTTTTCTTCGGTCAATC	425-445		
<i>femA</i>	GFEMAR-1	AAAAAAGCACATAACAAGCG	1444-1463	132	A or B
	GFEMAR-2	GATAAAGAAGAAACCAGCAG	1556-1575		
<i>mecA</i>	GMECAR-1	ACTGCTATCCACCCTCAAAC	1182-1201	163	B
	GMECAR-2	CTGGTGAAGTTGTAATCTGG	1325-1344		
<i>eta</i>	GETAR-1	GCAGGTGTTGATTTAGCATT	775-794	93	B
	GETAR-2	AGATGTCCCTATTTTTGCTG	848-867		
<i>etb</i>	GETBR-1	ACAAGCAAAAAGAATACAGCG	509-528	226	B
	GETBR-2	GTTTTTGCTGCTTCTCTTG	715-734		
<i>tst</i>	GTSSTR-1	ACCCCTGTTCCCTTATCATC	88-107	326	B
	GTSSTR-2	TTTTTCAGTATTTGTAACGCC	394-413		

^a Nucleotide sequence and locations were derived from the published sequences for *sea* (5), *seb* (19), *sec* (7), *sed* (2), *see* (11), *mecA* (23), *femA* (4), *eta* (20), *etb* (16), and *tst* (6).

[pH 8.0]), and the concentration was determined as micrograms per milliliter according to A_{260} values. Template DNA in amounts ranging from 10 to 1,000 ng was used in the study.

Primers. Oligonucleotides ranging from 18- to 24-mers were selected from the published DNA sequences of the *S. aureus* genes (Table 1) using Oligo software (version 3.4). Synthesis of oligonucleotides was carried out at the DNA Core Facility at the Laboratory Center for Disease Control. For multiplex PCRs, two primer sets were prepared: set A was designed to amplify *sea*, *seb*, *sec*, *sed*, *see*, and *femA*, whereas set B was designed to amplify *mecA*, *eta*, *etb*, and *tst* as well as *femA*. The primer sequences used in the multiplex PCRs are described in Table 1.

Multiplex PCR conditions. Two sets of primer mixes were prepared according to the master mixes of components from the GeneAmp kit (Perkin-Elmer, Norwalk, Conn.), with slight modifications to the given instructions. Multiplex primer set A contained 200 μ M deoxynucleoside triphosphates; 5 μ l of 10 \times reaction buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl); 1.5 mM MgCl₂; 20 pmol (each) of *sea*, *seb*, *sec*, *see*, and *femA* primers; 40 pmol of *sed* primer; 2.5 U of Taq DNA polymerase (Ampli-Taq DNA polymerase; Perkin-Elmer), and 10 to 1,000 ng of template DNA. The volume of this mix was adjusted to 50 μ l with sterile water. Multiplex primer set B included the same constituents as in set A except for the MgCl₂ concentration (2.0 mM) and the primers, which were used at 50 pmol for *eta* and 20 pmol each for *etb*, *tst*, *mecA*, and *femA*. Evaporation of the reaction mixture was prevented by addition of 100 μ l of sterile mineral oil. DNA amplification was carried out in a Perkin-Elmer thermocycler with the following thermal cycling profile: an initial denaturation at 94°C for 5 min was followed by 35 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 7 min.

RESULTS

Multiplex PCR for detection of selected staphylococcal genes. The reaction conditions for the multiplex PCR assay were optimized to ensure that all of the target gene sequences were satisfactorily amplified. The primers were designed to target the coding regions of the genes; care was taken to avoid

areas of homology within the structural genes for the enterotoxins. The primers used in each set had almost equal annealing temperatures, which reduced the possibility of occurrence of unwanted bands originating from nonspecific amplification. Figure 1 shows the presence of the amplified products after agarose gel electrophoresis, when DNA extracted from a representative toxigenic *S. aureus* strain (positive control) was used as the template in the PCR using the multiplex primer sets. Reliable amplification of six bands in set A (for *sea*, *seb*, *sec*, *sed*, *see*, and *femA*) was obtained when a mixture of DNAs from the same strains was tested (Fig. 1). Similarly, five bands were obtained when a mixture of DNAs from the corresponding strains in set B (for *eta*, *etb*, *tst*, *mecA*, and *femA*) was tested (Fig. 1). The sizes of the amplicons obtained from the various control strains corresponded to the predicted sizes (Table 1). As a negative control, both sets were tested with sterile water, and no amplicons were observed (Fig. 1). Genomic DNA in amounts ranging from 10 to 1,000 ng/reaction was used, with no apparent change in sensitivity and ability to detect all of the genes in the sample (data not shown).

To further test the specificity of the assay, DNA templates from one *Campylobacter jejuni* strain and two *Escherichia coli* strains were used with the multiplex PCR primer sets (A and B), and no amplified products were obtained (data not shown).

To substantiate the multiplex PCR technique, 176 strains of *S. aureus* that were tested by multiplex PCR were also screened for the presence of individual toxin genes by using the method described previously (18). Full agreement of the two methods was observed. In order to test for 10 toxin genes individually, a total of 1,760 PCRs were required, whereas when multiplex

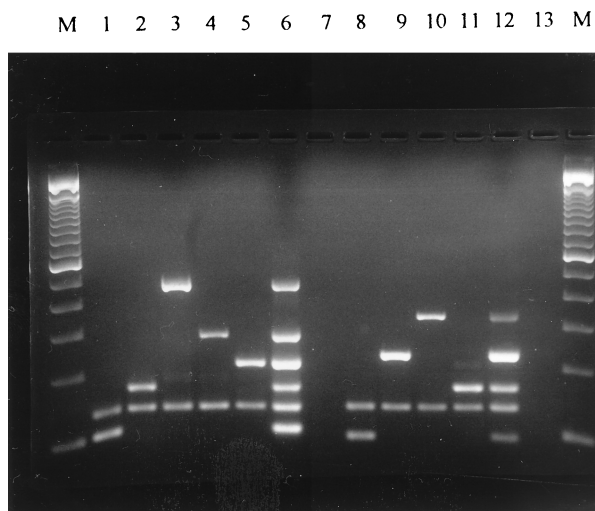


FIG. 1. Agarose gel electrophoresis patterns showing multiplex PCR amplification products for the *S. aureus* genes. Lanes M, DNA molecular size marker (100-bp ladder; Bethesda Research Laboratories Inc., Gaithersburg, Md.); lanes 1 to 7, PCR amplicons from primer set A; lanes 8 to 13, PCR amplicons from set B. Lanes: 1, *sea* plus *femA*; 2, *seb* plus *femA*; 3, *sec* plus *femA*; 4, *sed* plus *femA*; 5, *see* plus *femA*; 6, *sea*, *seb*, *sec*, *sed*, *see*, and *femA* simultaneously; 7, negative control; 8, *eta* plus *femA*; 9, *etb* plus *femA*; 10, *tst* plus *femA*; 11, *mecA* plus *femA*; 12, *eta*, *etb*, *tst*, *mecA*, and *femA*; 13, negative control.

primer sets were used, only 352 PCRs were needed. The multiplex primer sets were seen to be very reliable, since no discrepancy was observed in the results obtained using the two methods. An internal control of *femA* was present in all of the samples, confirming the presence of *S. aureus* and also validating the PCR conditions. Other workers have used primers for 16S rRNA as the internal control when designing multiplex primers to detect *mecA* (24, 26).

Primary validation of the amplicons. The sizes of the amplicons obtained by the multiplex primer sets were identical to those predicted from the design of the primers (Tables 1 and 2). The amplicons from the control strains were subjected to further confirmation and characterization by digestion with restriction endonucleases with cleavage sites within the amplicon. The restriction enzymes used and the predicted product sizes are given in Table 2. Enzyme fragments with the anticipated sizes were obtained in each case (data not shown).

Analysis of the results. Among the 176 strains tested, 107 strains were collected from nasal swabs of healthy humans. Of these 107 strains, 21 (19.6%) were positive for *sea*, 26 (24.3%)

were positive for the TSST-1 gene (*tst*), 6 (5.6%) were found to be *seb* positive, 8 (7.5%) were positive for *sec*, and 2 (1.9%) contained the gene for *sed*. None were positive for *see*, *eta*, *etb*, or *mecA*. Of the 47 strains obtained from The Netherlands, 6 were positive for *sea*, 1 was positive for *seb*, 3 were positive for *sec*, 4 were positive for *eta*, and 11 were positive for *tst*. Among the three clinical isolates tested, all were positive for *eta* as well as *etb*.

Among the 19 strains obtained from the national MRSA surveillance study, 18 were *mecA* positive, indicating that *mecA* is responsible for methicillin resistance in those strains. The single remaining oxacillin-resistant isolate (which was *mecA* negative) must be methicillin resistant by virtue of some other mechanism (9). Of these MRSA isolates, three were positive for *sea*, two were positive for *seb*, three were positive for *sec*, two were positive for *sed*, three were positive for *see*, and one contained the gene for TSST-1. All of the 176 samples tested contained the *femA* gene.

DISCUSSION

We have described a multiplex PCR-based diagnostic protocol to detect the genes for enterotoxins A to E, ETA, ETB, and TSST-1 and the *mecA* gene in DNA extracted from human isolates of *S. aureus*. This procedure is an improvement over our previously described PCR protocols, where individual primers were used to identify the staphylococcal toxin genes (18). The multiplex PCR primer sets were shown to be very specific, reliable, and, most importantly, very efficient in detection of all 10 genes. As an internal control, *femA* was found to be present in all of the strains studied. The gene product of *femA* has been suggested to have a role in cell wall metabolism and is reported to be present in all *S. aureus* species during the active growth phase (17, 29).

Six pairs of primers were used in multiplex primer set A to target the structural genes for enterotoxins A to E (*sea*, *seb*, *sec*, *sed*, and *see*), along with *femA*. In the multiplex primer set B, five pairs of primers were mixed together to target the structural genes for *mecA*, *eta*, *etb*, and *tst*, along with *femA*. All of the primers were gene specific, as demonstrated by restriction fragment lengths obtained after specific restriction endonuclease digestion of the amplicons. The multiplex primers were shown to be specific for *S. aureus*, since no amplification product was obtained when either *C. jejuni* or *E. coli* DNA was used as the template.

In this study, the toxin genotypes of *S. aureus* strains isolated from healthy human carriers are also demonstrated. Of 107 such isolates tested, 24.3% possessed the gene for TSST-1 and 19.6% were positive for SEA. These results are in accordance with previous findings that many healthy individuals are carriers of toxin-producing strains of *S. aureus* (10).

The use of multiplex PCR to characterize staphylococcal strains and their resistance to methicillin has been well documented (1, 8, 12, 29, 30). Those reports focus on detection of the gene responsible for methicillin resistance (*mecA*) along with either the *femA*, 16S rRNA, *nuc*, or IS431 gene as a positive control(s) (1, 8, 12, 29). A recent study describes the use of two multiplex PCR assays for detection of *S. aureus* exotoxin genes: one is designed to detect the enterotoxin genes, and the other is designed to detect the *tst*, *eta*, and *etb* genes (3). Becker et al. have used DNA enzyme immunoassays to validate the specificity of the PCR products, using oligonucleotide probes derived from the sequences of the *S. aureus* toxin genes (3).

The study described in this paper provides detailed information about *S. aureus* toxin genes as well as *mecA*. The inclusion

TABLE 2. Predicted sizes of restriction fragments and enzymes used for restriction fragment length polymorphism analysis to validate the amplified products of multiplex PCR

Gene	Amplicon size (bp)	Multiplex primer set	Restriction enzyme used	Expected sizes (bp) of restriction fragments
<i>sea</i>	102	A	<i>AluI</i>	65, 37
<i>seb</i>	164	A	<i>MaeIII</i>	120, 31, 13
<i>sec</i>	451	A	<i>NdeI</i>	307, 144
<i>sed</i>	278	A	<i>MboII</i>	175, 103
<i>see</i>	209	A	<i>AccI</i>	119, 90
<i>femA</i>	132	A or B	<i>AccI</i>	78, 54
<i>eta</i>	93	B	<i>MboI</i>	57, 36
<i>etb</i>	226	B	<i>HpaII</i>	159, 67
<i>tst</i>	326	B	<i>MboI</i>	187, 139
<i>mecA</i>	163	B	<i>MaeIII</i>	84, 79

of an internal positive control (*femA*) in the reaction provides assurance against false-negative results. Use of this multiplex PCR assay will help provide the information required for appropriate therapy and infection control during outbreaks of *S. aureus*. It is important to recognize that this technique only will identify strains harboring the toxin genes and is independent of the expression and secretion of the toxin. To verify toxin production by any given isolate, time- and labor-intensive immunological methods may be used to detect the excreted toxins.

Considering the low cost and much shorter time required to detect the 10 genes of *S. aureus* by multiplex PCR, we believe this to be a powerful tool for studying the genotypes of staphylococcal isolates. This procedure was specially developed to fit into the daily work pattern of a routine clinical laboratory, since genotypic detection of drug resistance and the presence of toxin genes is becoming an important component of the diagnostic inventory of such laboratories.

ACKNOWLEDGMENTS

We are grateful to J. W. Cohen Tervaert for the strains from The Netherlands and to M. Mamelak for the strains from healthy controls. We also thank Russell Easy and Hugh Cai for technical assistance and M. R. Mulvey for critical review and reading of the manuscript.

M.M. was funded by a postdoctoral fellowship from a grant to W.M.J. by the Canadian Bacterial Disease Network.

REFERENCES

- Barski, P., L. Piechowicz, J. Galinski, and J. Kur. 1996. Rapid assay for detection of methicillin-resistant *Staphylococcus aureus* using multiplex PCR. *Mol. Cell. Probes* **10**:471-475.
- Bayles, K. W., and J. J. Iandolo. 1989. Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. *J. Bacteriol.* **171**:4799-4806.
- Becker, K., R. Roth, and G. Peters. 1998. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin gene. *J. Clin. Microbiol.* **36**:2548-2553.
- Berger-Bachi, B., L. Barberis-Maino, A. Strassle, and F. H. Kayser. 1989. FemA, a host-mediated factor essential for methicillin resistance in *Staphylococcus aureus*: molecular cloning and characterization. *Mol. Gen. Genet.* **219**:263-269.
- Betley, M. J., and J. J. Mekalanos. 1988. Nucleotide sequence of the type A staphylococcal enterotoxin gene. *J. Bacteriol.* **170**:34-41.
- Blomster-Hautamaa, D. A., B. N. Kreiswirth, J. S. Kornblum, R. P. Novick, and P. M. Schlievert. 1986. The nucleotide and partial amino acid sequence of toxic shock syndrome toxin-1. *J. Biol. Chem.* **261**:15783-15786.
- Bohach, G. A., and P. M. Schlievert. 1987. Nucleotide sequence of the staphylococcal enterotoxin C₁ gene and relatedness to other pyrogenic toxins. *Mol. Gen. Genet.* **209**:15-20.
- Brakstad, O. G., J. A. Maeland, and Y. Tveten. 1993. Multiplex polymerase chain reaction for detection of genes for *Staphylococcus aureus* thermonuclease and methicillin resistance and correlation with oxacillin resistance. *APMIS* **101**:681-688.
- Chambers, H. F. 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin. Microbiol. Rev.* **10**:781-791.
- Chance, T. D. 1996. Toxic shock syndrome: role of the environment, the host and the microorganism. *Br. J. Biomed. Sci.* **53**:284-289.
- Couch, J. L., M. T. Soltis, and M. J. Betley. 1988. Cloning and nucleotide sequence of the type E staphylococcal enterotoxin gene. *J. Bacteriol.* **170**:2954-2960.
- Geha, D. L., J. R. Uhl, C. A. Gustafiero, and D. H. Persing. 1994. Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. *J. Clin. Microbiol.* **32**:1768-1772.
- Haley, R. W., A. W. Hightower, R. F. Khabbaz, C. Thorsberry, W. J. Martone, J. R. Allen, and J. M. Hughes. 1982. The emergence of methicillin-resistant *Staphylococcus aureus* infections in United States hospitals. *Ann. Intern. Med.* **97**:297-308.
- Hartman, B. J., and A. Tomasz. 1984. Low-affinity binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. *J. Bacteriol.* **158**:513-516.
- Iandolo, J. J. 1989. Genetic analysis of extracellular toxins of *Staphylococcus aureus*. *Annu. Rev. Microbiol.* **43**:375-402.
- Jackson, M. P., and J. J. Iandolo. 1986. Sequence of the exfoliative toxin B gene of *Staphylococcus aureus*. *J. Bacteriol.* **167**:726-728.
- Johnson, S., K. Dominique, and H. Labischinski. 1995. FemA of *Staphylococcus aureus*: isolation and immunodetection. *FEMS Microbiol. Lett.* **132**:221-228.
- 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.
- Jones, C. L., and S. A. Khan. 1986. Nucleotide sequence of the enterotoxin B gene from *Staphylococcus aureus*. *J. Bacteriol.* **166**:29-33.
- Lee, C. Y., J. J. Schmidt, A. D. Johnson-Winegar, L. Spero, and J. J. Iandolo. 1987. Sequence determination and comparison of the exfoliative toxin A and toxin B genes from *Staphylococcus aureus*. *J. Bacteriol.* **169**:3904-3909.
- Marrack, P., and J. Kappler. 1990. The staphylococcal enterotoxins and their relatives. *Science* **248**:705.
- National Committee for Clinical Laboratory Standards. 1993. Document M2-A5. Performance standards for antimicrobial disk susceptibility tests, 5th ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- Ren, K., J. D. Bannan, V. Pancholi, A. L. Cheung, J. C. Robbins, V. A. Fischetti, and J. B. Zabriskie. 1994. Characterization and biological properties of a new staphylococcal exotoxin. *J. Exp. Med.* **180**:1675-1683.
- Ryffel, C., W. Tesch, I. Birch-Machin, P. E. Reynolds, L. Barberis-Maino, F. H. Kayser, and B. Berger-Bachi. 1990. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Gene* **94**:137-138.
- Salisbury, S. M., L. M. Sabatini, and C. A. Spiegel. 1997. Identification of methicillin-resistant staphylococci by multiplex polymerase chain reaction assay. *Am. J. Clin. Pathol.* **107**:368-373.
- Schlievert, P. M. 1993. Role of superantigens in human diseases. *J. Infect. Dis.* **167**:997-1002.
- Schmitz, F.-J., C. R. Mackenzie, B. Hofmann, J. Verhoef, M. Finken-Eigen, H.-P. Heinz, and K. Kohrer. 1997. Specific information concerning taxonomy, pathogenicity, and methicillin resistance of staphylococci obtained by a multiplex PCR. *J. Med. Microbiol.* **46**:773-778.
- Su, Y., and A. C. Lee Wong. 1995. Identification and purification of a new staphylococcal enterotoxin. *H. Appl. Environ. Microbiol.* **61**:1438-1443.
- Utsui, Y., and T. Yokota. 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **28**:397-403.
- Vannuffel, P., J. Gigi, H. Ezzedine, B. Vandercam, M. Delmee, G. Wauters, and J. Gala. 1995. Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. *J. Clin. Microbiol.* **33**:2864-2867.
- Zambardi, G., M. E. Reverdy, S. Bland, M. Bes, J. Freney, and J. Fleurette. 1994. Laboratory diagnosis of oxacillin resistance in *Staphylococcus aureus* by a multiplex-polymerase chain reaction assay. *Diagn. Microbiol. Infect. Dis.* **19**:25-31.