JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2000, p. 877–880 Vol. 38, No. 2
0095-1137/00/$04.00+0

PCR-Based Assay for Discrimination between Invasive and Contaminating *Staphylococcus epidermidis* Strains

NOËLLE BARBIER FREBOURG,* SOPHIE LEFEBVRE, STÉPHANIE BAERT, AND JEAN-FRANÇOIS LEMELAND

Groupe de Recherche sur les Antimicrobiens et Microorganismes, C.H.U. de Rouen, Hôpital Charles Nicolle, 76031 Rouen Cedex, France

Received 21 July 1999/Returned for modification 23 September 1999/Accepted 7 November 1999

The discrimination between *Staphylococcus epidermidis* strains that contaminate and infect blood cultures is a daily challenge for clinical laboratories. The results of PCR detection of putative virulence genes were compared for contaminating strains, sepsis-related strains, catheter strains, and saprophytic strains. Multiplex PCR was used to explore the *atlE* gene, which is involved in initial adherence, the intercellular adhesion gene cluster (*ica*), which mediates the formation of the biofilm, and the *agrA*, *sarA*, and *mecA* genes, which might contribute to the pathogenicity of *S. epidermidis*. Whereas the *atlE*, *agrA*, and *sarA* genes were almost ubiquitously amplified, the *ica* and *mecA* genes were detected significantly more in infecting strains than in contaminating strains ($P \leq 0.02$) and thus appeared to be related to the potential virulence of *S. epidermidis*.

During the last decade, *Staphylococcus epidermidis* and other coagulase-negative staphylococci have emerged as a major cause of nosocomial infections. These organisms, which constitute a major component of the normal skin and mucosal microflora, are particularly responsible for catheter- and medical device-related sepsis (13). They also frequently contaminate blood cultures, making their interpretation a major concern for clinicians and for analytical laboratories. Although the decision for therapy relies mostly on the observation of sepsis symptoms, other criteria are often considered, such as the number of positive blood cultures and the similarity of their antibiotic resistance profiles (15). However, these criteria may be controversial in many cases: antibiotic resistance profiles may differ for isogenic strains, whereas infections may involve strains that are isolated only once. Moreover, numeric criteria may not be available for anemic or pediatric patients, who cannot undergo multiple venous punctures. The purpose of this study was to assess whether the person making the clinical decision may benefit from genetic data, such as the detection of genes encoding putative virulence factors.

The pathogenesis of *S. epidermidis* catheter-related infections mostly relies on adherence to polymer surfaces (4). The bacterial biofilm is produced in a two-step manner: the initial bacterial attachment to the surface is followed by biofilm formation, consisting of bacterial proliferation, intercellular adhesion, and extracellular slime substance production (9). A recent study demonstrated that the primary attachment of *S. epidermidis* to a polystyrene surface is related to a cell surface protein exhibiting vitronectin-binding activity. This protein is encoded by the chromosomal *atlE* gene and exhibits a high similarity to the major autolysin of *Staphylococcus aureus* (11). In other respects, investigation of the second stage of biofilm formation demonstrated that cell aggregation and biofilm accumulation were mediated by the products of the chromosomal *ica* gene locus, which comprises three intercellular adhesion genes (*icaA*, *icaB*, and *icaC*) organized in an operon structure and which leads to the biosynthesis of polysaccharide intercellular adhesin (10). Besides virulence factors that are involved in adherence and biofilm formation, homologs of the *sar* and *agr* loci of *S. aureus* have recently been characterized for *S. epidermidis* (7, 17, 20; W. J. B. Van Wamel, J. Verhoef, and A. C. Fluid, Abstr. 96th Gen. Meet. Am. Soc. Microbiol., abstr. B-338, p. 213, 1996). Whereas the products of these genes mediate the production of major virulence factors in *S. aureus* (12, 18), the functions of their homologs in *S. epidermidis* are still unknown. Indeed, the *agr* and *sar* loci might also be implicated in the regulation of the expression of the chromosomal *mecA* gene, which is responsible for methicillin resistance (16). Based on the lack of *mecA* transcription in phase variants (16) and on the absence of mutation in the *mecI* gene and *mecA* promoter or operator region in methicillin-resistant *S. epidermidis* (14), one can speculate on the role of the *agr* and *sar* loci in the possible coregulation of resistance and of virulence.

The pathogenicity of *S. epidermidis* may rely on the presence or absence of candidate genes that are involved in the virulence process. This was recently shown for the *ica* gene locus, which proved to be almost exclusively present in sepsis-causing strains and not detectable in saprophytic isolates (21). The purpose of the present study was to investigate whether the presence of *ica* and also that of the *atlE*, *agrA*, *sarA*, and *mecA* genes might discriminate between virulent *S. epidermidis* strains that cause real sepsis and nonvirulent *S. epidermidis* strains that contaminate blood cultures. To address this question, multiplex PCR amplification was used to compare the genetic backgrounds of strains collected from presumed sepsis and from catheter-related infections with those of blood culture-contaminating strains and of healthy carriage strains. Between September 1998 and March 1999, 138 *S. epidermidis* isolates were collected from 122 patients hospitalized in the University Hospital of Rouen, France, and from 16 healthy volunteers. The strains were intentionally selected for inclusion into four clinical groups. Group S included 39 strains that were potentially involved in a sepsis because they infected at least three distinct blood cultures or at least two blood cultures and one concomitant entry site and because the different isolates of each patient shared the same antibiotic resistance profile. Group C included 39 strains that were considered contaminating strains because they were isolated from only one blood

* Corresponding author. Mailing address: C.H.U. de Rouen, Hôpital Charles Nicolle, Laboratoire de Bactériologie, 1, rue de Germont, 76031 Rouen Cedex, France. Phone: 33 2 32 88 80 52. Fax: 33 2 32 88 80 24. E-mail: Bacteriologie@chu-rouen.fr.

TABLE 1. DNA sequences of amplification primers

Target gene(s)	Primer[a]	DNA sequence (5′–3′)	Product size (bp)	Reference
atlE	*atlE*-F	CAA CTG CTC AAC CGA GAA CA	682	This work
	atlE-R	TTT GTA GAT GTT GTG CCC CA		
icaAB	*icaAB*-F	TTA TCA ATG CCG CAG TTG TC	546	This work
	icaAB-R	GTT TAA CGC GAG TGC GCT AT		
sarA	*sarA*-F	TGG TCA CTT ATG CTG ACA GAT T	313	This work
	sarA-R	TTT GCT TCT GTG ATA CGG TTG		
agrA	*agrA*-F	CAA CAA CGA AAC ATG GTG CT	923	This work
	agrA-R	TGT CAT CGA AAA TGG TTA CTT TG		
mecA	*mecA*-F	GTA GAA ATG ACT GAA CGT CCG ATA A	310	8
	mecA-R	CCA ATT CCA CAT TGT TTC GGT CTA A		
16S rRNA	91E-F	GGA ATT CAA A(T/G)G AAT TGA CGG GGG C	478	19
	13B-R	CGG GAT CCC AGG CCC GGG AAC GTA TTC AC		

[a] F, forward primer; R, reward primer.

culture and because they were not associated with any other *S. epidermidis*-positive cultures from potential entry sites of patients. Group K comprised 44 strains that significantly colonized intravascular devices. In our hospital, quantitative cultures of catheters are performed by rinsing the distal 6-cm segment of the catheter with 1 ml of broth and inoculating 100 μl of the broth on blood agar, and the cultures are considered significant when the bacterial count is $\geq 10^3$ CFU/ml (3). Finally, 40 healthy volunteers, who did not attend the hospital, were asked to place their fingers on blood agar in order to collect saprophytic strains. However, *S. epidermidis* strains were isolated from only 16 of these individuals; the remaining 24 were colonized by other species of coagulase-negative staphylococci. Therefore, 16 saprophytic strains were included in group H as control strains for subsequent molecular analysis. All the strains of the study were identified by colony morphology, Gram stain characteristics, and results of the Pastorex Staph Plus test (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) and the APISTAPH system (bioMérieux, La Balme les Grottes, France), performed according to the manufacturers' recommendations. In addition to the 138 strains of the study, 8 clinical isolates belonging to other coagulase-negative staphylococci species were analyzed to assess the specificities of the PCR primers.

Four pairs of primers were designed for amplification of fragments of the *atlE*, *icaA*, *icaB*, *sarA*, and *agrA* genes of *S. epidermidis*, with the help of previously published sequences (7, 10, 12, 17). The *ica* primers were designed to amplify the *icaA* and *icaB* genes of the *ica* locus, while Ziebuhr et al. used a Southern blot probe specific to the *icaAB* portion of the locus in a previous study (21). For amplification of the *mecA* gene, primers previously designed by Geha et al. (8) were used. For amplification of an internal control, we used universal primers targeting 16S rRNA genes (19). The nucleotide sequences of the primers are presented in Table 1. For DNA extraction, 10 μl of a 2× McFarland standard suspension of staphylococcal cells was placed in the amplification tube and submitted to a cell lysis program of a GeneAmp PCR system 2400 (Perkin-Elmer Cetus, Norwalk, Conn.). Subsequently, 40 μl of the PCR reagent mixture was added to the PCR tube to initiate amplification. The PCR reagent mixture consisted of 200 μM (each) dATP, dTTP, dCTP, and dGTP; 10 mM Tris (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 1.25 U of *Taq* polymerase (Perkin-Elmer Cetus) and 0.5 to 1 μM each PCR primer. Multiplex PCR was performed for combined amplification of the (i) *atlE*, *icaA*, *icaB*, and 16S rRNA genes; (ii) *agrA*, *sarA*, and 16S rRNA genes; and (iii) *mecA* and 16S rRNA genes. Therefore, each PCR included amplification of the 16S rRNA gene as an internal control. Each PCR was performed twice for confirmation of the results, and each experiment included a PCR-positive control strain and a negative control, consisting of the PCR mixture without bacterial DNA. The optimal primer concentration for multiplex amplification was 0.5 μM, with the exception of that for the *agrA* and 16S rRNA primers, which required a concentration of 1 μM. The optimal annealing temperature for all multiplex amplifications appeared to be 55°C. Finally, DNA amplifications were carried out with the following thermal cycling profile: an initial denaturation at 94°C for 2 min, followed by 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min), and ending with a final extension at 72°C for 5 min. Amplification products were analyzed by agarose gel electrophoresis. Examples of the different multiplex amplifications are shown in Fig. 1. All the strains were amplifiable. With the exception of the *mecA* and universal 16S rRNA primers, all the PCR primers used in this study appeared to be specific to the *S. epidermidis* species. Under the high-stringency conditions, the *atlE*, *icaAB*, *agrA*, and *sarA* primers did not generate any PCR product for the following species: *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus lugdunensis*, *Staphylococcus saprophyticus*, *Staphylococcus xylosus*, and *Staphylococcus simulans*. Statistical analysis of the PCR results was performed by using the chi-square test and the Fisher exact test.

The *atlE* gene, which encodes a vitronectin-binding cell surface protein involved in primary attachment (11), was ubiquitously amplified in *S. epidermidis* strains. The *agrA* and *sarA* genes may also be involved in the virulence of *S. epidermidis*, as was suggested by previous findings that insertional mutations in *agr* and mutations in both *agr* and *sar* respectively attenuate and nearly abolish the virulence of *S. aureus* in experimental endophthalmitis (2). Indeed, the *agrA* and *sarA* genes were amplified in almost all the infecting and contaminating strains, with the exception of only one catheter isolate lacking *agrA*, one sepsis isolate lacking *sarA*, and one contaminating isolate

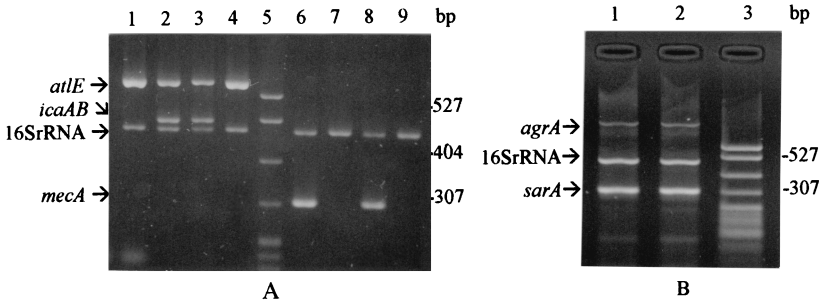

FIG. 1. Multiplex amplifications of representative *S. epidermidis* strains. (A) Multiplex PCR of *altE*, *icaAB*, and 16S rRNA gene fragments (lanes 1 to 4) and of *mecA* and 16S rRNA gene fragments (lanes 6 to 9). Lanes 1, 4, 7, and 8, blood culture-contaminating isolates; lanes 2, 3, and 6, sepsis-related isolates; lane 5, molecular weight marker (pBR322 DNA-*Msp*I digest); lane 9, healthy volunteer isolate. (B) Multiplex PCR of *agrA*, 16S rRNA, and *sarA* gene fragments. Lane 1, sepsis-related isolate; lane 2, blood culture-contaminating isolate; lane 3, molecular weight marker (pBR322 DNA-*Msp*I digest).

lacking both amplicons. As the great majority of strains harbor these genes, negative amplifications in a few isolates may be related to mutations of the annealing sequences and should be confirmed by Southern blot analysis. Although *atlE*, *agrA*, and *sarA* were quite ubiquitously found in the different groups of strains, the potential functions of their products in virulence cannot be excluded and neither can the possibility of point mutations or abnormal gene transcription in noninvasive strains.

In contrast, the amplification of the *icaA*, *icaB*, and *mecA* genes revealed striking differences between the different groups of strains. First, the *ica* locus was detected significantly more in infecting strains than in contaminating strains ($P \leq 0.003$) (Table 2). These results are in agreement with those of a recent study in which *S. epidermidis* strains from clinical material were shown to differ from saprophytic strains by the presence of the *icaA* and *icaB* genes, their capacity for phase variation, their abilities to adhere to polymer and autoaggregate, and in their colony morphology on Congo red agar (21). Although phenotypic markers, such as culture on Congo red agar, also reflect the potential virulence of the strains and although phenotypic testing may be easier to perform than molecular analysis, the determination of phenotypes is hampered by the capacity of phase variants to change specific phenotypic features rapidly (5). Moreover, test tube adherence and in vitro slime production were shown to be of minor usefulness in guiding clinical decisions (15). Therefore, based on these previous findings, the detection of the *ica* gene locus is the most reliable means to address the discrimination of virulent and nonvirulent strains. In their study, Ziebuhr et al. demonstrated, by Southern hybridization with an *icaAB* probe, that the *ica* gene cluster was present in 44 of 52 (85%) blood culture isolates versus in only 2 of 36 (6%) saprophytic strains collected from healthy volunteers. This higher sensitivity for the detection of *ica*-positive infecting strains (85% versus 68.2 and 76.9% in our work) is relevant to the better sensitivity of Southern blot analysis, while PCR detection might be adversely affected by minor mutations. Incidentally, we found that the rate of strains carrying the *ica* locus among healthy volunteers (37.5%) was higher than the rate reported by Ziebuhr et al. (6%). Although the reason for this difference remains unclear, our result suggests that virulence factors can somehow be present in community strains and are not specific to nosocomial isolates. The innovative feature of the present study is the comparison of the *ica* PCR results between strains that potentially contaminate blood cultures and strains that potentially infect blood cultures or intravascular devices. The presence of the *ica* gene locus appeared to be statistically related to the potential virulence of the strains (Table 2). Although the detection of *ica* is neither sensitive nor specific enough to guide fully the clinical decision, it might be helpful when associated with other clinical and biological criteria of septicemia (15). In this aspect, further prospective investigations are needed and should include genetic, phenotypic, and clinical data. Moreover, as the PCR primers used in the present study appeared specific to *S. epidermidis*, further investigations are needed to establish the presence and determine the DNA sequences of *ica* homologs in various coagulase-negative species, such as *S. haemolyticus*, that are increasingly involved in sepsis (13).

Finally, the last candidate gene of the present work for discrimination between contaminating and invasive strains was the *mecA* gene, which controls the synthesis of the additional penicillin-binding protein PBP2′ in methicillin-resistant staphylococci. It is known that methicillin resistance is documented more often in disease-causing isolates than in colonizing isolates (1). Moreover, the lack of *mecA* transcription in slime-negative phase variants of methicillin-resistant *S. epidermidis* has suggested the possible implication of *mecA* gene regulation in pathogenicity (16). In the present study, *mecA* was found in almost half of the blood culture-contaminating strains and in more than 75% of the invasive strains. Despite the wide distribution of *mecA* among nosocomial staphylococci, the difference between the contaminating and invasive groups of strains was statistically significant for the presence of the *mecA* gene ($P \leq 0.02$), in agreement with the results of a previous study

TABLE 2. Results of *icaAB* and *mecA* gene amplification grouped by the origins of the strains

Origin of strains[a]	No. of strains	% Positive[b] for:		
		icaAB	*mecA*	*icaAB* + *mecA*
S	39	30 (76.9) A	30 (76.9) C	25 (64.1) E
K	44	30 (68.2) B	37 (84.0) D	29 (65.9) F
C	39	11 (28.2) A, B	19 (48.7) C, D	8 (20.5) E, F
H	16	6 (37.5)	4 (25.0)	1 (6.2)

[a] Group S contains sepsis strains infecting at least three blood cultures or two blood cultures and one concomitant entry site and displaying the same antibiotic resistance profile for each patient. Group K contains strains significantly colonizing intravascular devices. Group C contains contaminating strains isolated from only one blood culture and not associated with positive culture of entry sites. Group H contains strains sampled from the hands of healthy volunteers.

[b] Values with the same letter are statistically significantly different from each other as follows: A, $P = 10^{-5}$; B, $P = 0.003$; C, $P = 0.02$; D, $P = 10^{-3}$; E, $P = 5 \times 10^{-4}$; and F, $P = 5 \times 10^{-5}$.

reporting a higher rate of methicillin resistance in disease-causing strains than in colonizing isolates (1). However, although the presence of *mecA* was concordant with that of the *ica* locus for most of the invasive strains, the presence of one or both of these genes in 22 (56%) contaminating strains hampered the interest in a combined *ica-mecA* PCR. In any case, although the detection of *mecA* does not augment the interest in the detection of *ica* for the diagnosis of infection, it remains significant information for empiric antibiotic therapy. Unexpectedly, the *mecA* gene was amplified from four (25%) saprophytic strains sampled from the hands of healthy volunteers who did not attend the hospital. These data suggest the presence of methicillin-resistant *S. epidermidis* in the general population and might be somehow related to the increased incidence of methicillin-resistant *S. aureus* in the community (6). Whether the presence of the *mecA* gene in *S. epidermidis* strains of the healthy population reflects the dissemination of hospital strains or the role of antibiotics in food remains to be elucidated.

In conclusion, this study demonstrates the ability of the detection of the *ica* and *mecA* gene loci to discriminate between contaminating and infecting *S. epidermidis* strains. Although the *ica* and *mecA* PCRs lack sensitivity and specificity and cannot be considered biological tests, they may potentiate the clinical criteria used for the diagnosis of septicemia or catheter-related infections.

We gratefully thank Jean-François Menard for the performance of the statistical tests.

REFERENCES

1. **Archer, G. L.** 1991. Alteration of cutaneous staphylococcal flora as a consequence of antimicrobial prophylaxis. Rev. Infect. Dis. **13**(Suppl. 10)**:**805–809.
2. **Booth, M. C., A. L. Cheung, K. L. Hatter, B. D. Jett, M. C. Callegan, and M. S. Gilmore.** 1997. Staphylococcal accessory gene regulator (*sar*) in conjunction with *agr* contributes to *Staphylococcus aureus* virulence in endophthalmitis. Infect. Immun. **65:**1550–1556.
3. **Brun-Buisson, C., F. Abrouk, P. Legrand, Y. Huet, S. Larabi, and M. Rapin.** 1987. Diagnosis of central venous catheter-related sepsis. Critical level of quantitative tip cultures. Arch. Intern. Med. **147:**873–877.
4. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. **37:**318–326.
5. **Christensen, G. D., L. M. Baddour, B. M. Madison, J. T. Parisi, S. N. Abraham, D. L. Hasty, J. H. Lowrance, J. A. Josephs, and W. A. Simpson.** 1990. Colonial morphology of staphylococci on Memphis agar: phase variation of slime production, resistance to β-lactam antibiotics, and virulence. J. Infect. Dis. **161:**1153–1159.
6. **Collignon, P.** 1999. Increased incidence of methicillin-resistant strains of *Staphylococcus aureus* in the community. J. Infect. Dis. **179:**1592.
7. **Fluckiger, U., C. Wolz, and A. L. Cheung.** 1998. Characterization of a *sar* homolog of *Staphylococcus epidermidis*. Infect. Immun. **66:**2871–2878.
8. **Geha, D. J., J. R. Uhl, C. A. Gustaferro, and D. H. Persing.** 1994. Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. J. Clin. Microbiol. **32:**1768–1772.
9. **Heilmann, C., C. Gerke, F. Perdreau-Remington, and F. Götz.** 1996. Characterization of Tn*917* insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect. Immun. **64:**277–282.
10. **Heilmann, C., O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, and F. Götz.** 1996. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol. Microbiol. **20:**1083–1091.
11. **Heilmann, C., M. Hussain, G. Peters, and F. Götz.** 1997. Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. Mol. Microbiol. **24:**1013–1024.
12. **Heinrichs, J. H., M. G. Bayer, and A. L. Cheung.** 1996. Characterization of the *sar* locus and its interaction with *agr* in *Staphylococcus aureus*. J. Bacteriol. **178:**418–423.
13. **Kloos, W. E., and T. L. Bannerman.** 1994. Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. **7:**117–140.
14. **Kobayashi, N., K. Taniguchi, and S. Arasawa.** 1998. Analysis of diversity of mutations in the *mecI* gene and *mecA* promoter/operator region of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **42:**717–720.
15. **Kotilainen, P.** 1990. Association of coagulase-negative staphylococcal slime production and adherence with the development and outcome of adult septicemias. J. Clin. Microbiol. **28:**2779–2785.
16. **Mempel, M., H. Feucht, W. Ziebuhr, M. Endres, R. Laufs, and L. Grüter.** 1994. Lack of *mecA* transcription in slime-negative phase variants of methicillin-resistant *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **38:**1251–1255.
17. **Otto, M., R. Suusmuth, G. Jung, and F. Götz.** 1998. Structure of the pheromone peptide of the *Staphylococcus epidermidis agr* system. FEBS Lett. **424:**89–94.
18. **Peng, H. L., R. P. Novick, B. Kreiswirth, J. Kornblum, and P. Schlievert.** 1998. Cloning, characterization, and sequencing of an accessory gene regulator (*agr*) in *Staphylococcus aureus*. J. Bacteriol. **170:**4365–4372.
19. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N. Engl. J. Med. **327:**293–301.
20. **Vandenesch, F., S. J. Projan, B. Kreiswirth, J. Etienne, and R. P. Novick.** 1993. Agr-related sequences in *Staphylococcus lugdunensis*. FEMS Microbiol. Lett. **111:**115–122.
21. **Ziebuhr, W., C. Heilmann, F. Götz, P. Meyer, K. Wilms, E. Straube, and J. Hacker.** 1997. Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. Infect. Immun. **65:**890–896.