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Capsule Expression by Bovine Isolates of *Staphylococcus aureus* from Argentina: Genetic and Epidemiologic Analyses

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***Staphylococcus aureus* is an important cause of bovine mastitis worldwide, and effective preventive or therapeutic modalities are lacking. Although most human *S. aureus* isolates produce capsular polysaccharides (CPs), few reports have described the prevalence of capsules on bovine isolates. This information is important for the rational design of a vaccine for the prevention of staphylococcal mastitis. We serotyped 195 *S. aureus* strains isolated between 1989 and 1997 from the milk of mastitic cows in Argentina. Only 14 (7.1%) of the strains were serotype 5, and all were recovered between 1989 and 1992. Thirteen serotype 8 strains were identified, and 12 of these were isolated between 1991 and 1994. The remaining 168 isolates were nonreactive (NR) with CP serotype 5 (CP5)- or CP8-specific antibodies. Hybridization studies performed with genomic DNA from eight NR strains revealed that only three of them carried the capsule genes. Pulsed-field gel electrophoresis (PFGE) performed with 127 of the 195 *S. aureus* isolates revealed that most (86%) strains belonged to one of four major PFGE groups. Although 8 of 14 CP5 isolates showed a common PFGE pattern (arbitrarily defined as A1), 31 other A1 isolates from the same time period (1989 to 1992) were not CP5 positive. In contrast, only nine PFGE type B3 isolates were recovered between 1990 and 1994, and eight of these were positive for CP8 ($P < 0.0003$). The results of this study underscore the variability in capsule expression by *S. aureus* strains isolated from different geographical regions and cast doubt on the roles of CP5 and CP8 in the pathogenesis and immunoprophylaxis of bovine mastitis in Argentina.**

Mastitis is an infectious disease of dairy ruminants that affects milk production and quality. This disease has been singled out as the most significant cause of economic loss to the dairy industry. Although several bacterial pathogens can cause the disease, *Staphylococcus aureus* has emerged as one of the most prevalent ones, and once it is established in the mammary gland of the milking animal, it is very difficult to eradicate (23). *S. aureus* strains are able to produce capsular polysaccharide (CP) in vivo (14) or under defined culture conditions (14, 33). The capsule has been shown to promote *S. aureus* virulence in several animal infection models (24, 35), and capsular antibodies have been shown to protect rodents against lethality, endocarditis, bacteremia, and metastatic infection of the liver, spleen, and kidneys (2, 5, 22).

Eleven CP serotypes were described with the use of polyclonal antisera, and subsequent surveys revealed that a high percentage of *S. aureus* isolates from different human sources were encapsulated (10, 29, 32). Whereas there is general agreement that CP serotype 5 (CP5) and CP8 are the most prevalent ones in humans, variable serotype prevalence has been reported in ruminants from different geographical regions of the world. In fact, 70% of 212 *S. aureus* bovine isolates from France belonged to CP5 or CP8 (27), but only 3 of 17 isolates from 10 different herds in Israel were CP5 or CP8 (32). In 1991 Naidu et al. (21) reported that 70% of 100 *S. aureus* isolates from bovines with mastitis were either CP5 or CP8 producers. The investigators, however, did not disclose the geographical origins of their isolates. In recent studies, Guidry et al. (8, 9) evaluated the prevalence of serotype 5 and 8 *S. aureus* strains in milk from bovines in the United States and Europe. Those investigators showed that 41% of the U.S. isolates were serotype 5 or 8, whereas in Europe approximately 70% of the strains were typeable with antibodies to CP5 and CP8. No such information is available from Central or South America. This study was aimed at evaluating the prevalence of *S. aureus* CP5 and CP8 in milk from bovines with mastitis in Argentina and to ascertain the clonal relationships among these isolates. This information is critical for the rational design of a vaccine for the prevention of *S. aureus* bovine mastitis.

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MATERIALS AND METHODS

Bacterial isolates and cultures. Between 1989 and 1997, 195 *S. aureus* isolates were obtained from the milk of cows with mastitis from herds located in 22 districts of Argentina. The shortest distance between herds in two adjacent districts was 20 miles, and the greatest distance between herds was 700 miles. The isolates included in this study are representative of those that cause bovine mastitis in Argentina as a whole since they were obtained from the major dairy regions of that country. *S. aureus* was identified by a standard procedure of the microbiology laboratory (11) that includes isolation on Chapman agar (Difco Laboratories, Detroit, Mich.) and tests for production of coagulase, clumping factor, acetoin, and acid (aerobically) from trehalose and maltose. Isolates were stored in brain heart infusion (Difco) medium with 20% glycerol at −20°C until use. Five unrelated strains that did not react with antibodies to CP5 or CP8 and that did not carry the capsule gene cluster, as assessed by hybridization, were confirmed to be *S. aureus*, by amplification of a 108-bp *S. aureus*-specific fragment by PCR (17). Simultaneous amplification of a 241-bp DNA fragment from a highly conserved region of the bacterial 16S rRNA gene ensured the adequacy of the PCR assay.

PFGE. A total of 127 *S. aureus* isolates were characterized by pulsed-field gel electrophoresis (PFGE) with the CHEF DR-III system (Bio-Rad, Hercules, Calif.) by a standard protocol (18). In brief, *S. aureus* was cultured and plugs were prepared. DNA was digested with *Sma*I, and DNA fragments were resolved by electrophoresis in 0.8% agarose gels run over 18 h at 6 V/cm and 13°C. The

TABLE 1. Hybridization of genomic DNAs from NR *S. aureus* isolates with *cap* gene fragments[a]

District	Strain	CP type	Hybridization to:			PFGE type
			cap5ABCD	*cap5IJK*	*Cap5LMNOP*	
Control (United States)	Becker	8	+	−	+	ND[b]
Control (United States)	Newman	5	+	+	+	ND
Basavilbaso	MB-067	NR	−	−	−	B2
San Francisco	MB6-3-996	NR	+	+	+	A4
Brandsen	MB-101	NR	+	+	+	B3
Rafaela	MB2-1790	NR	−	−	−	A8
Olavarría	MB-327	NR	−	−	−	A13
Lincoln	MBD-014	NR	−	−	−	A14
Tres Arroyos	MB-099	NR	−	−	−	A2
Tandil	MB-319	NR	+	+	+	A1

[a] Strain Becker but none of the bovine strains hybridized to the *cap8HIJK* (type 8-specific) gene probe.
[b] ND, not done.

included angle was 120°, and initial and final switch times were 1 and 30 s, respectively. *S. aureus* NCTC 8325 was included in each gel for quality control. The gels were stained with 2 μg of ethidium bromide per ml and were scanned with the Bio-Rad Gel Doc system by using the Molecular Analyst Software (Bio-Rad). For the final band analysis, relative positions were established visually on thermal paper prints of the gels and were compared with those generated with bacteriophage lambda ladder DNA concatemers (New England Biolabs, Beverly, Mass.). To evaluate the clonal relationship among isolates, the criteria of Tenover and coworkers (34) were used. PFGE patterns that differed at seven or more bands were recorded as types and were identified with a capital letter. Patterns that differed at two to six bands were recorded as different subtypes of the pattern with the highest prevalence and identified with a capital letter (type) followed by an arabic numeral.

Numerical analysis. The similarity among PFGE types was evaluated by use of the Dice coefficient (4). The resultant matrix was analyzed by the unweighted pair group method of analysis (31).

Capsule serotyping. CP typing was performed for 195 *S. aureus* bovine isolates by a colony immunoblot method with CP5- or CP8-specific antibodies as described previously (13). The reactivities of the bovine isolates were evaluated by comparison to those of control *S. aureus* strains (type 1, 2, 5, and 8 and nontypeable isolates) included on each filter membrane. Positive reactions were scored as 2+ to 4+. Each clinical isolate was tested at least twice. Isolates with no reaction to CP5 and CP8 antibodies were defined as nonreactive (NR).

DNA hybridization experiments. The genes involved in the biosynthesis of CP5 and CP8 are chromosomal and allelic (30). Each gene cluster contains 16 open reading frames (ORFs), named *cap5A* through *cap5P* for CP5 and *cap8A* through *cap8P* for CP8. The predicted amino acid sequences of 12 of the 16 ORFs of the *cap5* and *cap8* gene clusters are almost identical. However, four ORFs located in the central region [*cap5(8)H* through *cap5(8)K*] bear no homology to each other and are type specific. By probing genomic staphylococcal DNA with different DNA fragments from within the capsule gene clusters, one can establish whether NR *S. aureus* isolates carry the genes for serotype 5 or 8 capsule expression. Genomic DNA was extracted from eight NR bovine isolates of *S. aureus* (listed in Table 1) that had been shown to be epidemiologically unrelated. DNAs from *S. aureus* strains Newman (serotype 5) and Becker (serotype 8) were included in the hybridization studies as positive controls. DNA from each strain was digested with *Hin*dIII (Life Technology, Gaithersburg, Md.) and was electrophoresed in a 0.8% agarose gel. The DNA was transferred to a nylon membrane (Gene Screen; NEN Research Products, Boston, Mass.) and probed sequentially with cloned DNA fragments (*cap5ABCD*, *cap5IJK*, *cap8HIJK*, or *cap5LMNOP*) that were enzyme labeled with AlkPhos Direct (Amersham Life Science, Inc., Arlington Heights, Ill.). Membrane hybridization and washing were performed as directed by the manufacturer at 60°C. Membrane stripping and autoradiography were carried out according to the manufacturer's recommendations.

RESULTS

CP typing and prevalence. Fourteen of 195 *S. aureus* isolates (7.1%) expressed CP5, whereas 13 (6.6%) were CP8. Surprisingly, and in a departure from previous reports, 168 isolates (86.3%) did not react with antibodies to CP5 or CP8 in the immunoblot assay. Among the 22 districts investigated, the 14 CP5 strains were found in 9 districts and the 13 CP8 strains were found in 8 districts. There was an overlap in four districts, where both CP5- and CP8-bearing isolates were detected (Table 2). NR *S. aureus* strains were isolated in almost every district (21 of 22) but were not recovered in one district from which a single CP8 strain was recovered.

Our serotyping experiments were performed only with antibodies to CP5 and CP8 since strains that express CP1 and CP2 are extremely rare (13, 32) and the capsules from the other putative serotypes have never been chemically characterized. In addition, CP5 and CP8 are the only serotypes considered for construction of a purified component vaccine (6). We performed hybridization experiments with eight epidemiologically unrelated NR isolates in order to determine whether these strains carried the genes known to be involved in capsule synthesis. Genomic DNAs from three of the eight NR strains hybridized to *cap5ABCD*, genes that are conserved among serotypes 1, 2, 5, and 8. DNAs from the same three strains also reacted with *cap5LMNOP*, a DNA fragment that is common to both the *cap5* and *cap8* gene clusters, and *cap5IJK*, a DNA fragment that is *cap5* specific. These results suggest that these three NR strains carry an intact *cap5* gene cluster, although

TABLE 2. Distribution of CP5- and CP8-producing *S. aureus* isolates among 22 districts of Argentina

District	No. of isolates			
	Total	CP5	CP8	NR
Ameghino	6	1		5
América	1			1
Basavilbaso	4	1		3
Bolívar	11		5	6
Brandsen	20	2	1	17
Cañuelas	2		1	1
Entre Ríos	4		1	3
French	1			1
General Rodríguez	10			10
Gualeguaychú	14		1	13
La Delia	4			4
La Vacherie	22	3	2	17
Las Heras	11	2		9
Lincoln	14			14
Luján	20	2	1	17
Mercedes	3			3
Nueve de Julio	8	1		7
Olavarría	6			6
Rafaela	8			8
San Francisco	1			1
Tandil	19	1	1	17
Tres Arroyos	6	1		5
Total	195	14	13	168

TABLE 3. *S. aureus* isolates with positive reactions to anti-CP5 or anti-CP8 antibodies in an immunoblot assay

Strain	Source (district)	Date (mo/yr)	CP type	PFGE type
MB 320	La Vacherie	3/90	5	A1
MB 113	Tres Arroyos	4/90	5	A1
MB 339	Las Heras	4/90	5	A1
MB 304	Ameghino	10/89	5	A1
MB 305	9 de Julio	11/89	5	A1
MB 337	La Vacherie	4/90	5	A1
MB 313	Brandsen	11/89	5	A1
MB 334	La Vacherie	4/90	5	A1
MB 311	Las Heras	11/89	5	A3
MBb 18	Brandsen	3/92	5	A3
MB 094	Tandil	11/89	5	A17
MBa 3	Basavilbaso	6/91	5	B4
MB 111	Luján	4/90	5	D
MBb 35	Luján	8/92	5	D
MBb 13	Bolívar	3/92	8	A2
MB 008	Cañuelas	5/94	8	A2
MBC 212	Gualeguaychú	6/94	8	A14
MB 019	Luján	6/94	8	B3
MB 107	Bolívar	2/90	8	B3
MBa 12	Entre Ríos	11/91	8	B3
MBb 11	La Vacherie	2/92	8	B3
MBb 19	Brandsen	3/92	8	B3
MBb 22	Bolívar	4/92	8	B3
MB 018	Bolívar	6/94	8	B3
MB 022	Bolívar	6/94	8	B3
MB 451	Tandil	6/91	8	C
MBa 004	La Vacherie	7/91	8	C

they do not express detectable levels of CP5. The remaining five strains tested did not hybridize to any of the DNA probes, indicating that they do not carry genes similar to those responsible for synthesis of CP1, CP2, CP5, or CP8.

Clonal relationships among *S. aureus* isolates. All isolates that reacted with antibodies to CP5 and CP8 ($n = 27$) plus 100 isolates selected at random were analyzed by PFGE. Those 127 isolates were distributed into four clusters arbitrarily identified as A, B, C, and D, and most isolates (86%) were type A. The levels of similarity of clones B, C, and D to clone A according to the Dice coefficient were 66, 33, and 71%, respectively. Type A was discriminated by PFGE band analysis into 17 subtypes with more than 80% similarity; subtype A1 was the most prevalent (44 isolates).

Eight of 14 CP5 *S. aureus* isolates obtained between 1989 and 1992 were subtype A1 (Table 3). To establish whether CP5 was associated with PFGE type A1, the number of CP5 A1 isolates/total number of CP5 isolates (8/14) was compared with the number of A1 isolates/total number of isolates (39/94) from 1989 to 1992 that were subjected to PFGE. Statistical evaluation of these proportions (Fisher's exact test; GraphPad PRISM, version 2.0) showed that there was a random association between the PFGE type and CP5 expression ($P = 0.387$). A similar conclusion was drawn when the distribution of serotype 5 *S. aureus* strains within all PFGE type A isolates (subtypes were not considered) was investigated. The eight CP5 A1 isolates were found in six different locations, yet these eight isolates exhibited identical PFGE band patterns.

Seventy-five isolates obtained between 1990 and 1994 were typed by PFGE analysis; nine of these were PFGE type B3, and eight produced CP8. Following the same rationale described above for serotype 5 *S. aureus* isolates, the number of CP8 B3 isolates/total number of CP8 isolates (8/13) was statistically compared with the number of B3 isolates/total number of isolates examined by PFGE (9/75). The analysis showed that there was a significant difference ($P = 0.0003$, Fisher's exact test) between these proportions, which indicates that the expression of a CP8 phenotype was associated with PFGE genotype B3. NR *S. aureus* isolates appeared uniformly distributed among PFGE subtypes. There was no association of the NR phenotype with a given genotype, location, or period under scrutiny.

TABLE 4. Prevalence of CP types 5 and 8 *S. aureus* over time

Year	No. (%) of isolates			
	Total	CP5[a]	CP8[b]	NR
1989-1990	69[c]	11 (15.9)	1 (1.5)	57 (82.6)
1991-1992	47	3 (6.4)	7 (14.9)	37 (78.7)
1993-1994	30	0	5 (16.7)	25 (83.3)
1995-1996	35	0	0	35 (100)
1997	14	0	0	14 (100)
Total	195	14	13	168

[a] The decrease in the prevalence of CP5 isolates from 1989-1990 to 1993-1994 (11/69 to 0/30) was significant ($P = 0.014$, Fisher's exact test).
[b] The increase in the prevalence of CP8 isolates from 1989-1990 to 1991-1992 (1/68 to 7/40) was significant ($P = 0.007$, Fisher's exact test). Later, the prevalence of CP8 isolates from 1991-1992 to 1995-1996 decreased significantly from 7/40 to 0/35 ($P = 0.017$, Fisher's exact test).
[c] Forty-four isolates from 1989 plus 25 isolates from 1990.

CP prevalence variation over time. *S. aureus* strains that express CP5 or CP8 were not represented equally during the period from 1989 to 1997 (Table 4). The prevalence of serotype 5 isolates decreased from 15.9% of 69 total isolates in 1989-1990 to 0% of 30 isolates in 1993-1994. Conversely, the prevalence of serotype 8 isolates was 1.5% in 1989-1990, increased to 16.7% in 1993-1994, and decreased to 0% thereafter. The prevalence of NR isolates did not vary markedly from 1989-1990 to 1997. Isolates that were indistinguishable from each other by their CP types and PFGE types were found in the same location over several years. Such is the case of PFGE type B3, which persisted over a 4-year period in infected animals of the Bolivar district. When the prevalence of different subtypes over time without distinction of isolate source was investigated, the results revealed an initial high prevalence of PFGE type A1 (CP5 plus NR strains) before 1992, followed by a decline of subtype A1 isolates and an increase in PFGE subtypes A13 (NR) and B3 (primarily CP8) after 1993. Analysis of 22 isolates from a single district (La Vacherie) revealed that the PFGE types shifted from seven A1 isolates and no A13 isolates in 1989-1990 to three A13 isolates and no A1 isolates in 1995-1996, suggesting a trend similar to that obtained by analysis of all 127 isolates.

DISCUSSION

CPs, most commonly those of serotype 5 or 8, are produced by most human *S. aureus* isolates independently of their source. Studies on the prevalence of encapsulated strains in bovines have reported a different situation, with a variable prevalence of strains that react with antibodies to either CP5 or CP8 in different countries (9, 32). To our knowledge, this is the first study to describe the prevalence of CP5 and CP8 in clinical bovine isolates in a South American country. Moreover, this is the first study to evaluate clonal relationships among bovine *S. aureus* isolates that produce CP5 and CP8.

The results from this study show that the low prevalence of bovine *S. aureus* strains that produce CP5 or CP8 in Argentina resembles that observed by analysis of a small number of iso-

lates from Israel (32) and differs from the prevalences reported in Europe and the United States (9, 27). The prevalence of NR *S. aureus* strains in Argentina was surprisingly high, and one question that arose from our results was whether those strains carried the genes required for capsule expression, as has been shown for the NR strain *S. aureus* 8325-4 (3, 15, 37). This strain carries an intact copy of the *cap5* gene cluster, but a mutation in *cap5E* renders it capsule negative (37). Three of eight bovine strains tested carried the genetic information required to produce CP5 but did not express this polysaccharide. The remaining five selected *S. aureus* strains did not hybridize to any of the known capsule genes. The slight differences in PFGE band patterns (six bands or less compared with those of other *S. aureus* strains and the considerable genealogical distance between *S. aureus* and other coagulase-positive *Staphylococcus* species (26) indicated that the nonhybridizing strains were *S. aureus*. Because most *Staphylococcus* species other than *S. aureus* do not express serotype 5 or 8 capsules (28), the identification of these five strains was confirmed by amplification of DNA sequences specific for *S. aureus*.

The fact that most of the *S. aureus* strains isolated from the milk of diseased cows from Argentina were nonreactive with capsular antibodies casts doubt on the role of serotype 5 and 8 CPs in bovine mastitis. Whereas other investigations have shown that the capsule enhances bacterial virulence in animal models of *S. aureus* lethality, bacteremia, and septic arthritis (24, 35), the capsule actually attenuates virulence in a rat model of staphylococcal endocarditis (2). Studies that address the role of the serotype 5 and 8 capsules in animal models of mastitis are lacking. Mamo et al. (16) reported that bovine *S. aureus* isolates cultivated in milk whey produced a periodate-sensitive capsular material that correlated with virulence in a mouse model of mastitis. However, the composition of this capsular substance was never reported. Watson and Watson (38) showed that *S. aureus* strains grown in milk expressed a "pseudocapsule" that had immunoprotective properties in cows. Again, the chemical nature of this material was never reported. Thus, much remains to be explained concerning what role, if any, *S. aureus* surface polysaccharides play in bovine mastitis. Indeed, many NR bovine strains of *S. aureus* react with antibodies to a newly described staphylococcal surface polysaccharide composed of poly-*N*-succinyl glucosamine (19). The prevalence of this new antigen or other capsular substances among Argentine isolates remains to be determined.

Most currently marketed or experimental vaccines for the prevention of staphylococcal bovine mastitis are based on killed *S. aureus* cells, are administered by the parenteral route, and contain capsular polysaccharide antigens as key components (7, 25; D. L. Watson and C. L. Schwartzkoff, Proc. Int. Symp. Bovine Mastitis, p. 73, 1990). Many factors are likely to contribute to the limited success of these vaccines, including the incorporation of irrelevant capsular antigens not representative of strains circulating in the target population. Furthermore, it is not clear that a vaccine that elicits a systemic immune response will be effective against staphylococcal infection confined to the udder. A vaccine for the prevention of bovine mastitis needs careful tailoring, and a careful characterization of the prevalent strains in the target population is essential.

There are certain *S. aureus* traits, such as resistance to methicillin, that are restricted to a limited number of clones isolated from humans worldwide (12). Moreover, a certain genome pattern defined by PFGE analysis with *Sma*I was found to be associated with expression of staphylococcal enterotoxin A in *S. aureus* isolated from food-borne outbreaks occurring in geographically distant locations (36). We have found that CP8 was significantly associated with PFGE subtype B3. The interpretation of this finding can be seen from two opposite viewpoints. On one hand, four of the eight CP8 B3 *S. aureus* strains were isolated from different herds within the same district from February 1990 to June 1994. The epidemiological relatedness of isolates from herds within the same district cannot be ruled out since undisclosed vectors may have spread the CP8 B3 strain to neighboring localities within the district over a period of 4 years. On the other hand, CP8 isolates were also obtained from herds as far as 700 miles apart, and several of these isolates exhibited identical fingerprints as assessed by PFGE analysis. An epidemiological association between eight CP8 B3 isolates obtained from five different districts is possible but highly improbable. Expression of CP8 may be restricted to a limited number of clones in the geographical region under scrutiny. The presence of these clones in distant districts of Argentina may indicate a close genealogical origin of the strains involved, although they may have no epidemiological relatedness. Another explanation for this finding is that PFGE analysis may not be a procedure discriminative enough to genealogically discriminate those clones that express CP8.

A prevalent PFGE type (type A) was found in Argentina. It may have disseminated from unique sources of the bacterium or may indicate an increased ability of this organism to infect and survive in the bovine environment (1). In addition, repeated isolation of a bacterial subtype appears to be due to persistence in the diseased animal rather than reinfection (20). The source in Argentina from which PFGE subtype A1 isolates may have disseminated remains unknown. Even though 57% of the CP5 isolates were PFGE subtype A1, association of CP5 with this subtype appeared to be a random event. It is likely that the addition of other molecular identification procedures may permit us to uncover genealogical differences between epidemiologically unrelated, CP5-positive *S. aureus* isolates.

In conclusion, this study shows that in Argentina the prevalence of *S. aureus* CP5 and CP8 isolated from bovine milk is very low (14%). Our results, together with those previously reported, indicate that there are remarkable differences in the prevalence of CP serotypes 5 and 8 among bovine strains of *S. aureus* from different geographical sources. These data suggest that type 5 and 8 capsular antigens may not be good candidates for a vaccine for the prevention of bovine mastitis in Argentina. Further studies are required to establish whether the staphylococcal CPs play a role in the pathogenesis of *S. aureus* bovine mastitis.

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REFERENCES

1. **Aarestrup, F. M., H. C. Wegener, and V. T. Rosdahl.** 1999. Evaluation of phenotypic and genotypic methods for epidemiological typing of *Staphylococcus aureus* isolates from bovine mastitis in Denmark. Vet. Microbiol. **45:**139–150.
2. **Baddour, L. M., C. Lowrance, A. Albus, J. H. Lowrance, S. K. Anderson, and J. C. Lee.** 1992. *Staphylococcus aureus* microcapsule expression attenuates bacterial virulence in a rat model of experimental endocarditis. J. Infect. Dis. **165:**749–753.
3. **Bhasin, N., A. Albus, F. Michon, P. J. Livolsi, J. S. Park, and J. C. Lee.** 1998. Identification of a gene essential for O-acetylation of the *Staphylococcus aureus* type 5 capsular polysaccharide. Mol. Microbiol. **27:**9–21.
4. **Dice, L. R.** 1945. Measures of the amount of ecological association between species. Ecology **26:**297–302.
5. **Fattom, A. I., J. Sarwar, A. Ortiz, and R. Naso.** 1996. A *Staphylococcus aureus* capsular polysaccharide (CP) vaccine and CP-specific antibodies protect mice against bacterial challenge. Infect. Immun. **64:**1659–1665.

6. **Fattom, A. I., J. Sarwar, L. Basham, S. Ennifar, and R. Naso.** 1998. Antigenic determinants of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharide vaccines. Infect. Immun. **66:**4588–4592.
7. **Giraudo, J. A., A. Calzolari, H. Rampone, A. Rampone, A. T. Giraudo, C. Bogni, A. Larriestra, and R. Nagel.** 1997. Field trials of a vaccine against bovine mastitis. 1. Evaluation in heifers. J. Dairy Sci. **80:**845–853.
8. **Guidry, A., A. Fattom, A. Patel, and C. O'Brien.** 1997. Prevalence of capsular serotypes among *Staphylococcus aureus* isolates from cows with mastitis in the United States. Vet. Microbiol. **59:**53–58.
9. **Guidry, A., A. Fattom, A. Patel, C. O'Brien, S. Shepherd, and J. Lohuis.** 1998. Serotyping scheme for *Staphylococcus aureus* isolated from cows with mastitis. Am. J. Vet. Res. **59:**1537–1539.
10. **Karakawa, W. W., and W. F. Vann.** 1992. Capsular polysaccharides of *Staphylococcus aureus*. Semin. Infect. Dis. **4:**285–293.
11. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282–298. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
12. **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 *Staphylococcus aureus*. Science **259:**227–230.
13. **Lee, J. C., M. J. Liu, J. Parsonnet, and R. D. Arbeit.** 1990. Expression of type-8 capsular polysaccharide and production of toxic shock syndrome toxin-1 are associated among vaginal isolates of *Staphylococcus aureus*. J. Clin. Microbiol. **28:**2612–2615.
14. **Lee, J. C., S. Takeda, P. J. Livolsi, and L. C. Paoletti.** 1993. Effects of in vitro and in vivo growth conditions on expression of type 8 capsular polysaccharide by *Staphylococcus aureus*. Infect. Immun. **61:**1853–1858.
15. **Lee, J. C., S. Xu, A. Albus, and P. J. Livolsi.** 1994. Genetic analysis of type 5 capsular polysaccharide expression by *Staphylococcus aureus*. J. Bacteriol. **176:**4883–4889.
16. **Mamo, W., M. Lindahl, and P. Jonsson.** 1991. Enhanced virulence of *Staphylococcus aureus* from bovine mastitis induced by growth in milk whey. Vet. Microbiol. **27:**371–384.
17. **Martineau, F., F. J. Picard, P. H. Roy, M. Ouellette, and M. G. Bergeron.** 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. J. Clin. Microbiol. **36:**618–623.
18. **Maslow, J. N., A. M. Slutsky, and R. A. Arbeit.** 1993. Application 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 application. ASM Press, Washington, D.C.
19. **McKenney, D., K. L. Pouliot, Y. Wang, V. Murthy, M. Ulrich, G. Doring, J. C. Lee, D. A. Goldmann, and G. B. Pier.** 1999. Broadly protective vaccine for *Staphylococcus aureus* based on an in vivo-expressed antigen. Science **284:**1523–1527.
20. **Myllys, V., J. Ridell, J. Bjorkroth, I. Biese, and S. Pyorala.** 1997. Persistence in bovine mastitis of *Staphylococcus aureus* clones as assessed by random amplified polymorphic DNA analysis, ribotyping and biotyping. Vet. Microbiol. **51:**245–251.
21. **Naidu, A. S., A. Forsgren, S. Kalfas, J. L. Watts and J. M. Fournier.** 1991. Comparison between lactoferrin and subepithelial matrix protein binding in *Staphylococcus aureus* associated with bovine mastitis. J. Dairy Sci. **74:**3353–3359.
22. **Nemeth, J., and J. C. Lee.** 1995. Antibodies to capsular polysaccharides are not protective against experimental *Staphylococcus aureus* endocarditis. Infect. Immun. **63:**375–380.
23. **Nickerson, S. C., W. E. Owens, and R. L. Bodie.** 1995. Mastitis in dairy heifers: initial studies on prevalence and control. J. Dairy Sci. **78:**1607–1618.
24. **Nilsson, I. M., J. C. Lee, T. Bremell, C. Ryden, and A. Tarkowski.** 1997. The role of staphylococcal polysaccharide microcapsule expression in septicemia and septic arthritis. Infect. Immun. **65:**4216–4221.
25. **Nordhaug, M. L., L. L. Nesse, N. L. Norcross, and R. Gudding.** 1994. A field trial with an experimental vaccine against *Staphylococcus aureus* mastitis in cattle. 1. Clinical parameters. J. Dairy Sci. **77:**1267–1275.
26. **Pantucek, R., F. Gotz, J. Doskar, and S. Rosypal.** 1996. Genomic variability of *Staphylococcus aureus* and the other coagulase-positive *Staphylococcus* species estimated by macrorestriction analysis using pulsed field gel electrophoresis. Int. J. Syst. Bacteriol. **46:**216–222.
27. **Poutrel, B., A. Boutonnier, L. Sutra, and J. M. Fournier.** 1988. Prevalence of capsular polysaccharide types 5 and 8 among *Staphylococcus aureus* isolates from cow, goat, and ewe milk. J. Clin. Microbiol. **26:**38–40.
28. **Poutrel, B., C. Mendolia, L. Sutra, and J. M. Fournier.** 1990. Reactivity of coagulase-negative staphylococci isolated from cow and goat milk with monoclonal antibodies to *Staphylococcus aureus* capsular polysaccharide types 5 and 8. J. Clin. Microbiol. **28:**358–360.
29. **Ryding, U., J. I. Flock, M. Flock, B. Söderquist, and B. Christensson.** 1997. Expression of collagen-binding protein and types 5 and 8 capsular polysaccharide in clinical isolates of *Staphylococcus aureus*. J. Infect. Dis. **176:**1096–1099.
30. **Sau, S., N. Bhasin, E. R. Wann, J. C. Lee, T. J. Foster, and C. Y. Lee.** 1997. The *Staphylococcus aureus* allelic genetic loci for serotype 5 and 8 capsule expression contain the type-specific genes flanked by common genes. Microbiology **143:**2395–2405.
31. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman & Co., San Francisco, Calif.
32. **Sompolinsky, D., Z. Samura, W. W. Karakawa, W. F. Vann, R. Schneerson, and Z. Malik.** 1985. Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. J. Clin. Microbiol. **22:**828–834.
33. **Stringfellow, W. T., B. Dassy, M. Lieb, and J. M. Fournier.** 1991. *Staphylococcus aureus* growth and type 5 capsular polysaccharide production in synthetic media. Appl. Environ. Microbiol. **57:**618–621.
34. **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.
35. **Thakker, M., J. S. Park, V. Carey, and J. C. Lee.** 1998. *Staphylococcus aureus* serotype 5 capsular polysaccharide is antiphagocytic and enhances bacterial virulence in a murine bacteremia model. Infect. Immun. **66:**5183–5189.
36. **Tsen, H. Y., G. K. Yu, and H. H. Yu.** 1997. Comparison of type A enterotoxigenic *Staphylococcus aureus* strains isolated from geographically far distant locations by pulsed field gel electrophoresis. J. Appl. Microbiol. **82:**485–493.
37. **Wann, E. R., B. Dassy, J. M. Fournier, and T. J. Foster.** 1999. Genetic analysis of the *cap5* locus of *Staphylococcus aureus*. FEMS Microbiol. Lett. **170:**97–103.
38. **Watson, D. L., and N. A. Watson.** 1989. Expression of pseudocapsule by *Staphylococcus aureus*: influence of cultural conditions and relevance to mastitis. Res. Vet. Sci. **47:**152–157.