

Virulence of *Staphylococcus aureus* Mutants Altered in Type 5 Capsule Production

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Most clinical isolates of *Staphylococcus aureus* produce microcapsules (uronic acid-containing extracellular polysaccharides) that are detectable by serologic methods but are not visible by negative staining. Among the 11 reported serotypes, capsule types 5 and 8 comprise ~75% of all isolates. Transposon mutagenesis was performed on *S. aureus* to create mutants altered in capsule expression. Tn918 was introduced into the capsule type 5 strain Reynolds by filter mating, and a capsule-deficient transconjugate, JL236, was isolated. The wild-type strain was transformed with JL236 chromosomal DNA to confirm that transfer of the appropriate-size chromosomal fragment containing Tn918 generated a capsule-deficient transformant. Strain Reynolds was mutagenized with ethyl methanesulfonate to obtain a capsule-negative mutant (strain JL240). Capsular phenotypes were determined by colony immunoblots, antibody adsorption experiments, and transmission electron microscopy. The virulences of the parental and mutant strains in mice were compared. The 50% lethal doses for strains Reynolds, JL236, and JL240 were similar ($10^{8.59}$, $10^{8.98}$, and $10^{8.93}$ CFU, respectively). Animals injected intraperitoneally with either wild-type or mutant strains had comparable levels of bacteremia at 3 and 24 h after challenge. Quantitative cultures of blood and kidneys from animals challenged intravenously with sublethal doses of the *S. aureus* strains also showed no differences in bacterial clearance or renal abscess formation. These studies indicate that the type 5 *S. aureus* microcapsule does not promote bacterial virulence in the animal models tested.

Microorganisms that cause invasive disease often produce extracellular capsular polysaccharides. Capsules from a number of bacterial pathogens have been shown to enhance virulence and to elicit protective antibodies (28). Although capsule production by staphylococci was first recognized in 1930 by Gilbert (9), only recently has the prevalence of encapsulation among *Staphylococcus aureus* strains been appreciated. This is due largely to the efforts of Karakawa and Vann, who serologically and biochemically distinguished 11 capsular types among clinical isolates (15, 29). Recent studies by a number of investigators have focused on the chemical and biologic properties of the *S. aureus* capsule and on the prevalence of capsule expression among staphylococcal isolates from many sources (1–3, 12, 14–16, 26, 29).

Encapsulated *S. aureus* strains produce extracellular polysaccharides that are usually composed of aminouronic acid sugars and fucosamine (15). Highly encapsulated, mucoid strains belong to serotype 1 or 2 and are rarely isolated (2, 29). Most clinical isolates are microencapsulated, i.e., they form nonmucoid colonies and their capsules are not made apparent by negative stains such as India ink (2, 15). Type 5 and 8 strains predominate, constituting ~22 and ~53%, respectively, of isolates examined (1, 2, 12, 15, 26).

We previously examined the biologic properties associated with type 1 capsule expression by using Tn551 insertional mutagenesis of a highly encapsulated, mucoid strain of *S. aureus* (16). The highly encapsulated parent strain was more virulent for mice than either a microencapsulated type 1 mutant or a nonencapsulated mutant. Only the highly encapsulated strain showed enhanced lethality for mice and

required specific capsular antibodies for in vitro complement-mediated opsonophagocytic killing. The microencapsulated mutant was not more virulent in mice than the nonencapsulated mutant strain, and neither mutant required capsular antibodies for in vitro complement-mediated opsonophagocytosis. These studies not only established the importance of the type 1 capsule in staphylococcal virulence but also suggested that microencapsulated strains of *S. aureus* do not share the biologic properties (enhanced virulence and resistance to phagocytosis) associated with highly encapsulated *S. aureus* strains.

Because type 5 strains represent a large proportion of clinical isolates of *S. aureus*, we wanted to determine whether the microcapsules produced by these strains promoted staphylococcal virulence. In this report, we describe in vitro mutagenesis experiments to alter capsule expression and in vivo studies to evaluate the virulence of the strains in mice. Our results indicate that microencapsulated type 5 strains of *S. aureus* are not more virulent for mice than capsule-negative or capsule-deficient mutants.

MATERIALS AND METHODS

Bacteria and antibodies. The bacterial strains used in this study were maintained in skim milk at -70°C and are listed in Table 1. Strain Reynolds, the prototype capsule type 5 strain of *S. aureus*, was marked with antibiotic resistance before mutagenesis was performed. Rifampin resistance (Rif^r) was acquired by serial passage of the bacteria on tryptic soy agar (TSA; BBL Microbiology Systems, Cockeysville, Md.) plates containing 1, 3, or 5 μg of rifampin per ml. Chloramphenicol resistance (Cm^r) was obtained by transforming strain Reynolds with pSK265 (13). Staphylococci were grown in Columbia broth (Difco Laboratories, Detroit, Mich.) modified by the addition of 0.1% D-glucose,

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TABLE 1. Bacterial strains used for this study

Species and strain	Genotype or marker	Capsule type	Source
<i>S. aureus</i>			
Reynolds	None	5	W. Vann (15)
Cm ^r	pSK265	5	This study
Rif ^r	Rif ^r	5	This study
Sm ^r	Sm ^r	5	This study
JL236	Cm ^r Reynolds::Tn918	5 (scant)	This study
JL240	Rif ^r Reynolds, EMS	Negative	This study
NT857	None	Negative	This study
Becker	None	8	W. Vann (15)
<i>E. faecalis</i>			
FA378	pAM373::Tn918		D. Clewell (5)

1% yeast extract, and 0.5% NaCl (15) unless otherwise indicated. Capsular polysaccharide type 5 (CP5), purified from strain Reynolds (7), was kindly provided by J.-M. Fournier, Pasteur Institute, Paris, France. Polyclonal antiserum was raised in rabbits immunized three times per week for 3 weeks with heat-killed (70°C for 1 h) *S. aureus*. Monoclonal antibodies (MAbs) specific for CP5 were described previously (12, 22).

Mutagenesis. We introduced Tn918, a 16-kb conjugative tetracycline resistance transposon, into Cm^r strain Reynolds by the filter mating technique of Clewell et al. (5). Suspensions of the donor, *Enterococcus faecalis* FA378(pAM373::Tn918), and of the recipient were mixed at a ratio of 1:10 and collected on a membrane filter (type HA, pore size 0.45 µm. Millipore Corp., Bedford, Mass.). The filters were placed on TSA plates containing 5% sheep erythrocytes, and the plates were incubated at 37°C overnight. Bacterial colonies scraped from the filter were suspended in broth and plated on TSA plates containing tetracycline (5 µg/ml) and chloramphenicol (15 µg/ml) to isolate *S. aureus* transconjugants.

Strain Reynolds (Rif^r) was mutagenized chemically with ethyl methanesulfonate (EMS). The bacteria were grown to logarithmic phase in a chemically defined medium (21), and 2 ml of the culture was incubated with 0.03 ml of EMS for 2 h at 37°C. Samples were diluted in brain heart infusion broth, incubated overnight at 37°C, and spread on TSA plates to form single colonies.

Colony immunoblot. To screen mutagenized *S. aureus* cells for loss of capsule production, we used a colony immunoblot method described previously (18). TSA or Mueller-Hinton salt (3) plates were inoculated with 100 to 500 colonies, including control types 5 and 8 and nontypeable isolates. After overnight incubation at 37°C, colonies were transferred to nitrocellulose filters (82.5 mm; Schleicher & Schuell, Keene, N.H.) and fixed by heating at 60°C for 15 min. The filters were washed twice in 5 mM sodium phosphate-0.85% sodium chloride, pH 7.5 (phosphate-buffered saline [PBS]) before they were immersed in 0.01 M phosphate buffer-0.1% trypsin (Sigma Chemical Co., St. Louis, Mo.), pH 8.0, for 1 h at 37°C to remove protein A. The nitrocellulose filters were again washed and then blocked for 1 h with 0.05% skim milk (BBL). After the filters were washed three times in PBS-0.05% Tween 20 (PBS-Tween), rabbit antisera raised to heat-killed bacteria or capsule type-specific MAbs were incubated with each filter for 2 h at 37°C. After the filters were washed again in PBS-Tween, horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse immunoglobulin (Organon Teknika-

Cappel, West Chester, Pa.) was incubated with each filter for 2 h at 37°C. After three washes, the filters were immersed in a substrate solution containing 0.6 mg of 4-chloro-1-naphthol per ml and 0.1% H₂O₂. Purple color developed within 15 min and was visually scored 0 to 4+. Mutant colonies scored 0 to 1+ were tested at least three times with antibodies specific for type 5 capsules.

Electroporation. To ensure that the single Tn918 insert introduced into strain Reynolds was responsible for the capsule-deficient phenotype of mutant JL236, we used electroporation to transform the altered capsule phenotype into the parental strain. A spontaneous streptomycin-resistant (Sm^r) mutant of strain Reynolds was chosen as the recipient strain, and competent cells were prepared by the method of Augustin and Götz (4). The cells were thawed for 5 min at room temperature, and 50 µl of cells (7.7 × 10⁸ CFU) was incubated for 30 min at room temperature with 2 to 11 µg of JL236 chromosomal DNA. The samples were transferred to a cuvette with a 0.2-cm electrode gap and pulsed once at 2.5 kV with a 25-µF capacitor and a 100-Ω parallel resistor (12.5-kV/cm field strength for 2.5 ms). Samples were immediately resuspended in 950 µl of SMMP50 medium (4) containing tetracycline (1 µg/ml), transferred to a culture tube, and incubated at 37°C with shaking for 90 min. Transformants were selected on TSA plates containing tetracycline (5 µg/ml).

Southern blot hybridizations. To determine the number of transposon insertions in the mutant strains, we isolated chromosomal DNA from wild-type and mutant *S. aureus* strains. Preparation of bacterial DNA, nick translations, and hybridizations were performed as previously described (16). Chromosomal DNA was digested with restriction enzymes (New England BioLabs, Beverly, Mass.) under conditions suggested by the manufacturer. Chromosomal digests were hybridized to ³²P-labeled pAM120, a 21.2-kb *Escherichia coli* plasmid that carries Tn916 (8). The sequence of Tn916 is closely related to that of Tn918, and the two elements cross-hybridize efficiently (5).

Phenotypic characterization. To exclude the possibility that factors other than capsule expression might be altered by mutagenesis, the *S. aureus* strains were phenotypically characterized as described previously (16). Immunodiffusion was used to detect production of enterotoxin B by organisms cultivated by the membrane-over-agar method (11). Production of alpha-toxin was evaluated by measuring zones of hemolysis around colonies grown on TSA plates containing 5% defibrinated rabbit blood. Coagulase in *S. aureus* culture supernatants was quantitated as described by Phonimdaeng et al. (25). Cell wall protein A content was measured by bacterial agglutination with immunoglobulin-coated sheep erythrocytes (23). Lipase and protease production were evaluated on egg yolk agar plates (BBL).

Antibody adsorption studies. To confirm the capsular phenotype of the mutants, we tested their ability to adsorb capsular antibodies from rabbit immune serum. Bacteria grown overnight on TSA plates at 37°C were suspended and washed in PBS. Pellets adjusted to contain the same number of CFU were heat killed for 1 h at 70°C before they were incubated with 1 ml of 0.01 M phosphate buffer-0.1% trypsin, pH 8.0, for 1 h at 37°C to remove protein A. After three washes in PBS, dilutions of each bacterial suspension were incubated with rabbit serum at 4°C overnight. Sera were clarified by centrifugation, filter sterilized, and added to wells of microtiter plates coated with CP5 (1 to 2 µg/ml) that was coupled to poly-L-lysine by the cyanuric chloride method (10). Capsular antibody levels were measured by an

indirect enzyme-linked immunosorbent assay (ELISA) method described previously (17).

Electron microscopy. F(ab')₂ fragments of immunoglobulin G (IgG) were prepared from rabbit serum raised to strain Reynolds by using an F(ab')₂ preparation kit (Pierce Chemical Co., Rockford, Ill.). Following pepsin digestion, the sample was passed over a protein A column (to remove undigested IgG and Fc fragments) and dialyzed against PBS. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to verify that F(ab')₂ fragments were recovered by this procedure.

Overnight cultures of *S. aureus* Reynolds, JL236, and JL240 were fixed with 1% glutaraldehyde-0.1% CaCl₂ for 30 min at 4°C. The bacteria were recovered by centrifugation, washed, and incubated for 2 h at ambient temperature with F(ab')₂ fragments of IgG (2.4 mg of protein) prepared from serum raised to strain Reynolds. The cells were washed five times with PBS and then incubated overnight at 4°C with ferritin-labeled F(ab')₂ fragments of goat anti-rabbit IgG, generously provided by Shilu Xu, Boston, Mass. Samples were washed again and processed further for electron microscopy as previously described (16).

Animal experiments. Female C57BL/6J mice, 8 to 10 weeks old, were obtained from Jackson Laboratories (Bar Harbor, Maine). These inbred animals provide a reliable model of *S. aureus*-induced bacteremia and renal abscess formation (16). Female CD-1 mice, 8 to 10 weeks old, were obtained from Charles River Laboratories (Kingston, Mass.). These outbred animals are useful for 50% lethal dose (LD₅₀) determinations, and they are less expensive than the inbred strains of mice. The animals were injected intraperitoneally (i.p.) or intravenously (i.v.) in the tail vein with *S. aureus* strains grown to logarithmic growth phase. Lethality, bacteremia, and renal abscess formation were evaluated as previously described (16).

Statistical analysis. Data from quantitative bacterial cultures were analyzed by the unpaired Student *t* test. LD₅₀s were calculated by the method of Reed and Muench (27).

RESULTS

Isolation of type 5 capsule-negative mutants. We used filter matings to transfer Tn918 from the donor *E. faecalis* to the type 5 strain Reynolds (Cm^r). In a series of matings, the transfer frequency ranged from 2.8×10^{-6} to 3.0×10^{-5} *S. aureus* transconjugants per donor cell. Transconjugants were screened by colony immunoblots with CP5-specific polyclonal serum or MAbs. Of 10,000 colonies screened, 0 were negative and 4 reacted weakly with type 5 polyclonal serum. Three of the mutants produced serologically altered type 5 capsules (unpublished observations) and will be the subject of a separate communication. The fourth mutant, JL236, was weakly reactive by immunoblot or immunodiffusion with either rabbit antiserum raised to strain Reynolds or an IgG MAb (831S [12]) specific for the type 5 capsule. It was nonreactive with three capsule type 5 IgM MAbs (17-20, 17-23 [22], and 814S [12]) and an IgG MAb (17-7 [22]). Mutant JL236, designated capsule deficient, was characterized further.

Since we were not successful in creating a capsular polysaccharide-negative mutant from the type 5 strain by using transposon mutagenesis, we chemically mutagenized strain Reynolds (Rif^r) to derive a strain that produced no capsule. Of 1,300 EMS-treated colonies that were tested, 11 (0.8%) were nonreactive by immunoblot or rocket immunoelectrophoresis (12) with either polyclonal antibodies or

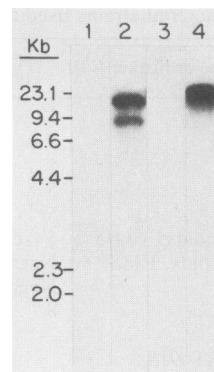


FIG. 1. Autoradiogram obtained from Southern blot analysis of chromosomal digests of *S. aureus* DNA. The molecular weight standards (left) are *Hind*III digests of phage lambda DNA. *Hind*III digests of chromosomal DNA from Cm^r strain Reynolds (lane 1) and from JL236 (lane 2) and *Eco*RI digests of chromosomal DNA from Cm^r strain Reynolds (lane 3) and from JL236 (lane 4) were hybridized to ³²P-labeled pAM120 (which contains Tn916).

MAbs to the type 5 capsule. A single Rif^r EMS mutant, designated JL240, was studied further.

Southern blot analysis. DNA from the type 5 insertional mutant JL236 was analyzed by Southern blot to ensure that it had a single transposon insertion. Digests of chromosomal DNA were hybridized with pAM120, a 21.2-kb *E. coli* plasmid that carries Tn916 (8). The radiolabeled probe did not hybridize to DNA prepared from the parent strain Reynolds (Cm^r), but it did hybridize to a single *Eco*RI band (>23 kb) and to two *Hind*III fragments (14.3 and 9.6 kb) of JL236 DNA (Fig. 1). These data indicate that JL236 contains a single transposon insertion, since Tn918 has no *Eco*RI sites and has a single *Hind*III site (5).

Electroporation. To show that Tn918 was responsible for the capsule-deficient phenotype of strain JL236, we used electroporation to transform JL236 chromosomal DNA into Sm^r strain Reynolds. Of eight Tc^r Sm^r transformants obtained, seven were reactive with capsule type 5-specific polyclonal antibodies or MAbs on colony immunoblots. One transformant, JL293, reacted weakly with polyclonal antiserum raised to strain Reynolds and was nonreactive with CP5-specific IgM MAb 17-20 (22). This pattern of serologic reactivity was identical to that observed with the original Tn918 mutant, JL236. Southern blots were performed on DNA digests of JL236 and of six of the eight transformants (Fig. 2). Tn916 hybridized to similar-size *Hind*III fragments of transformant JL293 and mutant JL236. It is likely that transformant JL293 underwent a double homologous recombination event with sequences flanking the transposon, replacing a portion of the wild-type capsule genes with DNA insertionally inactivated by Tn918. Five capsule-positive transformants had Tn918 inserted in *Hind*III fragments that did not correspond to fragments of JL236 DNA that contained the transposon. These transformants may have been the result of transposition events that occurred as Tn918 entered the recipient cells.

Phenotypic characterization. Of 115 phenotypic parameters examined, the mutant strains JL236 and JL240 differed from the wild-type isolate only in capsule production and antibiotic resistance (due to Tn918 insertion). The strains all tested positive for enterotoxin B, DNase, lipase, and protease and negative for toxic shock syndrome toxin 1 and staphylokinase. The parent and the mutant strains had

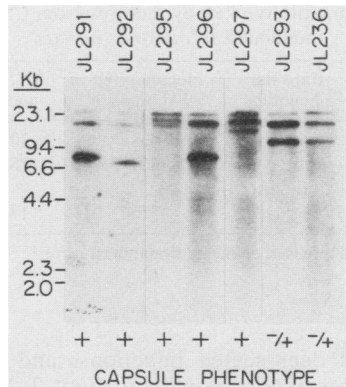


FIG. 2. Autoradiogram obtained from a Southern blot of *Hind*III-digested *S. aureus* genomic DNA after hybridization with 32 P-labeled pAM120. The molecular weight standards (left) are *Hind*III digests of phage lambda DNA. Strains JL291, JL292, JL295, JL296, JL297, and JL293 were obtained by transforming Sm^r strain Reynolds with chromosomal DNA from mutant JL236. The capsular phenotypes were determined by colony immunoblots with polyclonal antibodies and MAbs specific for CP5. +, Reactive; -/+, weakly reactive.

similar doubling times and produced comparable levels of alpha and beta hemolysins, coagulase, and protein A. Metabolic enzymes and carbohydrate utilization profiles of the parent and the mutant strains were identical.

Electron microscopy. We used transmission electron microscopy to verify the capsular phenotypes of the wild-type and mutant *S. aureus* strains. Strain Reynolds elaborated a microcapsule that was visualized by using $F(ab')_2$ fragments of capsular antibodies and ferritin-conjugated $F(ab')_2$ secondary antibodies (Fig. 3). JL236 produced a scant amount of the type 5 capsule, whereas JL240 appeared nonencapsulated.

Antibody adsorption studies. To confirm the capsular phenotypes of the mutant strains, we tested their ability to adsorb capsular antibodies from polyclonal serum. Antibody binding to CP5-coated ELISA plates was not diminished by adsorption of rabbit serum raised to strain Reynolds with more than 10^9 CFU of a nontypeable strain or of the heterologous type 8 strain Becker (Fig. 4). In contrast, sera adsorbed with 10^6 CFU of strain Reynolds (Cm^r) showed

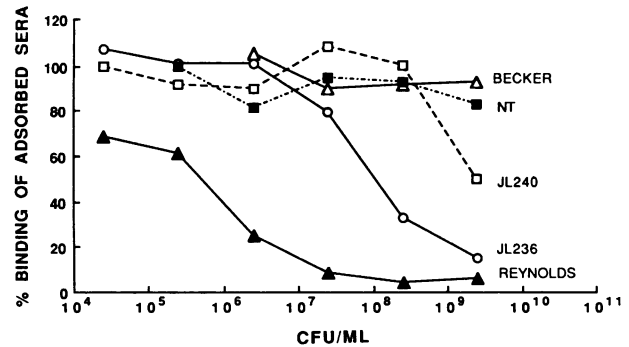


FIG. 4. Antibody adsorption experiments in which trypsinized bacterial suspensions were used to adsorb antibodies to the type 5 capsule. Tenfold serial dilutions of bacterial cells were incubated overnight with rabbit serum (diluted 1:20,000) raised to the type 5 strain Reynolds. Microtiter plates were coated with purified CP5 (1 to 2 μ g/ml) coupled to poly-L-lysine. The data represent the average of two to five determinations for each bacterial strain. NT, Strain NT857.

less than 50% binding. Suspensions of 2.5×10^8 CFU of the capsule-deficient strain JL236 adsorbed $\sim 67\%$ of the capsular antibodies, which is consistent with the scant amounts of CP5 visualized on this strain by transmission electron microscopy. After adsorption with the EMS mutant JL240 at 2.5×10^9 CFU/ml, the type 5 rabbit sera exhibited $\sim 50\%$ binding to purified CP5.

Mouse lethality. To determine the influence of the microcapsule on lethality, CD-1 mice were injected i.p. with either the wild-type or the mutant strains. LD_{50} s for strains Reynolds (Cm^r), JL236 (Tn9/8 capsule-deficient mutant), and JL240 (EMS capsule-negative mutant) were similar ($10^{8.59}$, $10^{8.98}$, and $10^{8.93}$ CFU, respectively).

Mouse bacteremia. To determine whether the parental and mutant strains differed in invasiveness, bacteria were injected i.p. into groups of 20 CD-1 mice. Quantitative blood cultures were made for 10 mice at 3 h after challenge with each strain and for another 10 mice at 24 h after challenge with each strain. As shown in Table 2, significant differences between the bacteremia levels of mice challenged with the microencapsulated strain of *S. aureus* and those of mice challenged with the mutant strains of *S. aureus* were not seen.

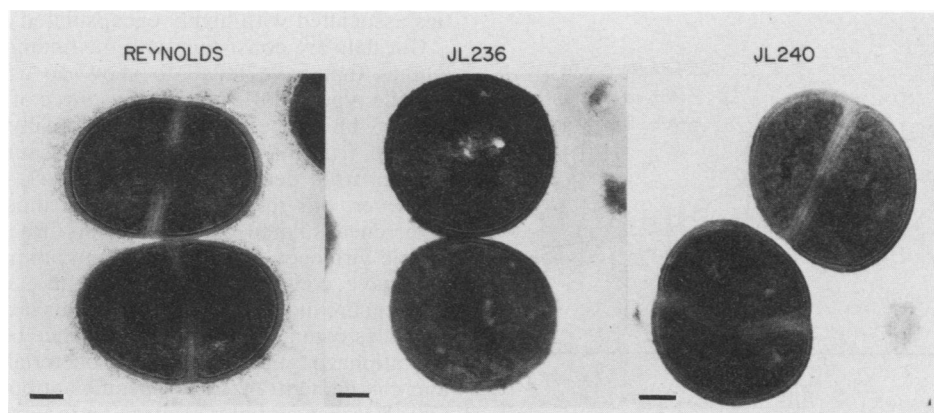


FIG. 3. Transmission electron micrographs of *S. aureus* Reynolds, JL236, and JL240 treated with $F(ab')_2$ fragments of antibodies raised to strain Reynolds and with ferritin-conjugated $F(ab')_2$ fragments of goat anti-rabbit IgG. Bars, 0.2 μ m.

TABLE 2. Quantitative cultures of blood from CD-1 mice inoculated i.p. with *S. aureus* strains^a

Challenge strain	Capsular phenotype	Inoculum (10 ⁸ CFU/mouse)	<i>S. aureus</i> bacteremia after inoculation (log CFU/ml) ^b	
			3 h	24 h
Reynolds (Cm ^r)	Type 5	6.2	2.85 ± 0.26	2.86 ± 0.46
JL236 (Tn918)	Type 5	7.0	3.06 ± 0.13	2.60 ± 0.22
JL240 (EMS)	Negative	7.3	2.43 ± 0.21	1.99 ± 0.16

^a Two groups of 10 mice were inoculated with each challenge strain; one group was bled at 3 h, and one was bled at 24 h.

^b Each value is expressed as mean ± standard error.

Bacteremia and renal abscess formation following i.v. challenge. C57BL/6J mice were inoculated i.v. with sublethal doses (~5 × 10⁶ CFU) of either the prototype or the mutant *S. aureus* strains. To evaluate differences in bacterial clearance, quantitative blood cultures were made each day for alternating groups of 8 to 10 mice. No significant differences between mice challenged with the wild-type strain of *S. aureus* and mice challenged with mutant strains of *S. aureus* were observed (Fig. 5). The prototype microencapsulated strains did not persist in the bloodstream at higher levels or for longer periods of time than the capsule-deficient or capsule-negative mutant. On day 7, the animals were killed and their kidneys were excised, homogenized, and quantitatively cultured. Both wild-type and mutant *S. aureus* strains proliferated in the kidneys, inducing renal abscess formation. The mean bacterial counts in the kidneys of mice injected with strain Reynolds (Cm^r), JL236, or JL240 did not differ significantly (Table 3).

To ensure that the wild-type and mutant strains were not changing their capsular phenotypes *in vivo*, we performed immunoblots on colonies plated directly from blood and kidneys of infected animals. Approximately 100 colonies of strain Reynolds tested positive for capsule production, but none of approximately 5,000 colonies of strain JL236 or JL240 tested positive for the type 5 capsule.

DISCUSSION

Eleven capsular serotypes of *S. aureus* have been distinguished by using polyclonal antibodies or MAbs (29). The prevalence of capsular types 5 and 8 has been observed in strains from diverse geographic areas, humans and animals,

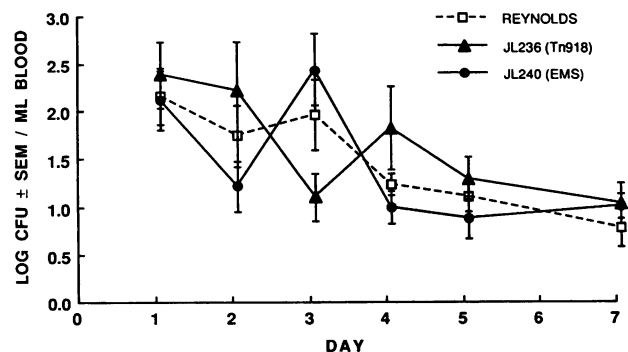


FIG. 5. Recovery of viable *S. aureus* from blood samples from 8 to 10 C57BL/6J mice challenged i.v. with ~5 × 10⁶ CFU of strain Reynolds (Cm^r), JL236, or JL240. SEM, Standard error of the mean.

TABLE 3. Quantitative kidney cultures from C57BL/6J mice inoculated i.v. with *S. aureus*

Challenge strain	Capsular phenotype	Inoculum (10 ⁶ CFU/mouse) ^a	No. of mice	Log CFU/g of kidney ^a
Reynolds (Cm ^r)	Type 5	4.3	20	5.61 ± 0.60
JL236 (Tn918)	Type 5	4.4	18	5.73 ± 0.62
JL240 (EMS)	deficient			
	Negative	5.6	17	6.17 ± 0.80

^a Each value is expressed as mean ± standard error.

superficial and deep-seated infections, and the skin and mucosal surfaces of normal individuals (1, 2, 12, 15, 26, 29). A few subpopulations of *S. aureus* have been associated with either the type 5 or the type 8 capsular phenotype. Notably, oxacillin-resistant *S. aureus* strains and bovine milk isolates from France are predominately capsule type 5 (2, 6, 26, 30), whereas more than 90% of vaginal, toxic shock syndrome toxin 1-positive strains are capsule type 8 (18). Thus, type 5 and type 8 microcapsules are commonly expressed by *S. aureus* isolates, and the distribution of capsule types is similar among isolates obtained from both pathologic and commensal sources.

In this study, we used transposon and chemical mutagenesis to create strains of *S. aureus* differing in capsule expression. We used filter mating to introduce Tn918 into Cm^r strain Reynolds. However, we isolated no transconjugants that were serologically capsule negative. Mutant JL236 produced scant amounts of capsule that were serologically detectable by rocket immunoelectrophoresis (unpublished observations), antibody adsorption, and electron microscopy. We included JL236 in our bacterial virulence studies; we also included an EMS mutant, JL240, that was capsule negative by rocket immunoelectrophoresis, antibody adsorption, and electron microscopy.

In mouse models of staphylococcal infection, we observed that the type 5 strain was not more virulent than a capsule-negative or capsule-deficient mutant. These conclusions are based on LD₅₀ determinations as well as on bacterial clearance studies of the blood and kidneys of infected animals. The results are compatible with our previous studies showing that a microencapsulated type 1 strain was not more virulent for mice than an isogenic, nonencapsulated strain (16). Only the highly encapsulated, parental type 1 strain showed enhanced virulence in mice. These data suggest that microencapsulated strains do not share the biologic properties associated with highly encapsulated *S. aureus* strains.

Our data are consistent with the findings of epidemiologic studies that have failed to show an association between capsule type and the pathologic source of *S. aureus* isolates. Type 5 and type 8 strains are equally prevalent among isolates from the skin or mucosal surfaces and among isolates from deep-seated infections (1, 2, 12, 15, 18, 29). However, our results contrast with those of studies using other encapsulated microorganisms. Bacterial capsules are classic virulence determinants, and animal models of infection have confirmed the premise that most encapsulated bacteria are more virulent than strains lacking capsules (28). Capsules commonly promote virulence because they impart an antiphagocytic nature to the bacterial surface, an effect that can be neutralized by specific antibodies to the capsule (28). Karakawa et al. (14) used an *in vitro* phagocytic assay to assess whether *S. aureus* microcapsules were antiphagocytic. They reported that type 5 and type 8 *S. aureus* strains

were opsonized for phagocytosis only in the presence of specific capsular antibodies. Serum samples from rabbits immunized with a nonencapsulated strain contained high levels of teichoic acid antibodies, but these sera were not opsonic for a type 8 strain. Moreover, type 5 and type 8 antisera were not opsonic for strains of the heterologous capsule type. These experiments suggest that *S. aureus* microcapsules, like the large capsules expressed by highly encapsulated staphylococci, impede phagocytosis. However, their assays were performed with heat-inactivated sera, so complement was not available in their assay mixture to opsonize the bacteria. We have observed that microencapsulated type 5 and type 8 strains can be opsonized for phagocytosis by either complement or antibodies (unpublished observations), unlike mucoid, capsule type 1 and type 2 strains, which require both complement and capsular antibodies for opsonophagocytic killing (16, 24).

Staphylococcal microcapsules are surface associated, limited in antigenic specificity, and prevalent among clinical isolates. Our results suggest that *S. aureus* microcapsules do not enhance bacterial virulence in the animal models tested, but these polysaccharides may have other, as yet undefined functions. Capsular antigens may be important targets for immunization, since antibodies to the microcapsule are opsonic (14). Whether *S. aureus* microcapsules promote or impede bacterial adherence to human cells or prosthetic devices is not known. Few studies have addressed the role that the *S. aureus* capsule plays in adherence; however, most of the evidence suggests that the large capsule elaborated by mucoid strains masks cell wall-associated adhesin molecules (19, 20, 31). Adherence studies comparing microencapsulated and nonencapsulated *S. aureus* strains have not yet been reported.

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