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Encapsulation and Capsular Types in Isolates of *Staphylococcus aureus* from Different Sources and Relationship to Phage Types

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The relationship of capsular types of *Staphylococcus aureus* to type of infection, carrier state, and phage type was studied in a collection of 477 isolates from 380 infection sites. Capsular polysaccharides were demonstrated by precipitation and agglutination with 11 monospecific antisera. When only one isolate from each infection was considered, 63% were of type 8 and 16% were of type 5. Of all the isolates tested, over 90% were encapsulated. We did not demonstrate any marked difference in the distribution of capsular types between isolates from the blood stream or purulent processes and isolates from healthy carriers or food. Most isolates from bovine mastitis milk had nontypeable capsules. The capsular type seemed stable in culture, and encapsulation had no apparent influence on susceptibility to phages. Of 27 phage-propagating strains maintained via culture transfer on artificial media over many years, 16 (59%) produced capsules. A striking association between certain phage patterns and capsular types was demonstrated.

In a multitude of pathogenic microorganisms the molecular structure of the outermost cell surface has been shown to be of decisive importance for virulence (e.g., *Salmonella* spp., *Haemophilus influenzae*, *Escherichia coli*, *Streptococcus pneumoniae*, and many others). Surface molecules determine adherence to host tissues and colonization, and through them the organism makes its first contact with cellular and humoral host factors (phagocytes, antibodies, complement, etc). It is therefore surprising that information about the surface layer of such a widespread and important pathogen as *Staphylococcus aureus* is still confusing and contradictory. In recent reviews teichoic acid and protein A are described as the most important surface structures which interfere with the defense mechanism of the host (1, 13, 24). It is stated that "a few strains are encapsulated," but encapsulation may be more common among freshly isolated clinical *S. aureus* (18). Aly et al. (1) stated that teichoic acid mediates the adherence of *S. aureus* to the nasal mucosal cells, but the possibility that capsules may interfere with the contact between teichoic acid and the mucosal lining was not mentioned.

Recently, Karakawa and Vann (15) have described a technique for serological typing of *S. aureus* according to capsular polysaccharides. Preliminary studies indicated that two capsular types are predominant among clinical isolates of varied geographic origin (3). In this study we present results obtained with a large collection of strains from different sources, both infectious and noninfectious. We show that encapsulation is a common feature of most isolates from all clinical sources and that capsular types and phage patterns of *S. aureus* are associated to a high degree. Capsular typing of phage-propagating strains maintained on artificial media over 30 years indicates that capsular types are very stable markers.

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MATERIALS AND METHODS

Bacteria. The prototype strains used for preparation of antisera to capsular polysaccharides of types 1 to 11 are indicated in Table 1. The nonencapsulated strain 32 used for preparation of teichoic acid antiserum and the nonencapsulated strain 57 used to absorb sera were from the collection at the Department of Biochemistry, The Pennsylvania State University. Strain Wood is a protein A-poor strain from the American Type Culture Collection and was used as a nonencapsulated control in agglutination experiments.

The clinical isolates examined for capsular types are listed in Table 2. Only coagulase-positive strains were included. The strains were isolated by different hospital and public health laboratories and by the Mastitis Control Department of the Veterinary Institute. They were all phage typed by the Israeli Reference Laboratory for Staphylococcus. The strains were included only if the enclosed information about the case was sufficient. A total of 135 strains were isolated from blood in 66 cases of sepsis. The cases with multiple isolates are detailed in Table 5. From other staphylococcal infections we examined 198 strains representing 175 infections. In this group, strains obtained from mucosal smears were included only in cases of clear suppurative processes and if no other pathogen was isolated; more than 70% of the samples were from abscesses of soft tissues, whereas isolates obtained from external wounds were excluded as possible contaminants.

Strains from two groups of nose or throat (or both) carriers were included: a group of active hospital staff (medicine, surgery, and gynecology departments) and a group of visitors to a venereal disease outpatient clinic. The latter group was restricted to individuals who had no contact with medical institutions during the last 6 months. A total of 23 strains were isolated from food possibly involved in food poisoning or from food handlers, and 17 strains were isolated from samples of bovine mastitis milk. In addition, we examined the capsular types of 27 strains used for propagat-

TABLE 1. Prototype strains for serotypes of *S. aureus* polysaccharide capsules

Serotype	Strain	Source
1[a]	Dp	Osteomyelitis, Center Community Hospital, State College, Pa.
2[a]	11127-var	Laboratory strain
3	Mardi	As for type 1
4[a]	7007	Burn patient, Japan
5	Reynolds	Blood culture, Kaiser Permanente Hospital, North Hollywood, Calif.
6	C	Clinical isolate, Children's Hospital, Denver, Colo.
7	207	Clinical isolate, University of Gothenberg Hospital, Gothenburg, Sweden
8	Becker	As for type 5
9	91	As for type 7
10	537	As for type 7
11	191	As for type 7

[a] Described in reference 14.

ing typing phages. These strains were originally obtained from the International Subcommittee on Staphylococcus, Colindale, United Kingdom, and maintained in our laboratory for several decades. All strains were sent to the United States on agar slants (stock culture agar; Difco Laboratories, Detroit, Mich.) for capsular typing. Examinations of phage patterns and capsular types were performed blind, on coded cultures.

Phage typing. The isolates were examined for susceptibility to typing phages by the technique of Blair and Williams (7). The following phages were used: lytic group 1, 29, 52, 52A, 79, and 80; lytic group 2, 3A, 3C, 55, and 71; lytic group 3, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 88, 89, and 90; additional phages, 81, 94, 95, 96, and D11 plus HK2 (pool). Strains resistant to all phages at the routine test dilution were reexamined at 100 times the routine test dilution. Strains susceptible to phages of different lytic groups or to phage 81 only were registered as miscellaneous (see Table 6).

Cultivation of prototype *S. aureus* strains. Encapsulated *S. aureus* was grown on Columbia agar (Difco) supplemented with 2% NaCl (CSA medium) for 18 h at 37°C in 5% CO_2. A single colony was tested by slide agglutination with teichoic acid antiserum and typing sera, and positive colonies were subcultured on CSA. Seed cultures of each prototype strain were stored at −20°C in skim milk. Strain 57, used for absorption of typing sera, and teichoic acid-rich strain 32 were maintained on tryptic soy agar (Difco).

Preparation of vaccines. Prototype strains were grown on CSA plates for 18 h at 37°C in 5% CO_2. The cells were suspended by mixing them with a glass rod in 20 ml of 3% Formalin in phosphate-buffered saline (PBS) (pH 7.2) and allowed to stand at room temperature for 18 h. The Formalin-treated cells were washed once in PBS and resuspended in 0.5% Formalin–PBS to give an absorbance of 0.6 at 550 nm. The vaccine was checked for viable organisms on CSA plates and for encapsulation by direct cell agglutination with teichoic acid antiserum (see below). No agglutination at a dilution of 1:20 was considered to be indicative of encapsulation. Vaccines were stored at 4°C.

Preparation of immune sera. New Zealand White rabbits weighing 6 lb (2.7 kg) were infected during the first week with a 0.2-ml subcutaneous injection followed by two 0.1-ml intravenous injections of vaccines composed of the prototype *S. aureus*. The animals were then immunized three times a week with 0.2 ml the second week, 0.3 ml the third week, and 0.4 ml the last week. Five days after the last injection, a serum sample was tested by agglutination with the homologous vaccines. Sera were collected when agglutination titers reached 1,280 or higher, sterile filtered, and stored at 4°C. Anti-teichoic acid serum was obtained by immunization with nonencapsulated strain 32 (9). For the capsular polysaccharide of type 8, we also prepared a monoclonal antiserum from a mouse hybridoma. The concentrated serum was negative with all prototypes except 8, which was agglutinated by a dilution of 1:100. Preparation and characterization of this serum will be described separately.

Typing of *S. aureus*. Bacteria were sent from Israel to the United States on stock culture agar, transferred to a CSA plate, and suspended in 2 ml of PBS (pH 7.4); the suspension was autoclaved (121°C) for 20 min and centrifuged. The supernatants were tested by immunodiffusion as previously described (15), using absorbed typing antisera. When the extracts were nontypeable, the respective cultures were subjected to an agglutination test. In this procedure, bacteria were suspended in 3% Formalin–PBS and kept at room temperature overnight. Cells were washed once and resuspended in PBS (A_{550} = 0.6). Suspensions were tested by tube agglutination with absorbed typing sera. Agglutination at a 1:100 dilution was designated positive. Complete agreement between precipitation and agglutination was found, i.e., strains negative in the immunodiffusion assay were also negative in agglutination tests.

Detection of nonencapsulated strains. Strains that were negative to all 11 typing sera were tested by agglutination

TABLE 2. *S. aureus* isolates included in the study

Source	No. of strains	No. of infections
Purulent or inflammatory processes		
Throat	8	7
Ear	2	2
Nose	15	13
Eye	6	6
Sputum (pneumonia)	4	4
Pus (abscesses)	141	125
Mastitis milk (human)	7	6
Joint punctures	7	6
Peritoneal drainage	1	1
Amniocentesis	2	2
Urine	1	1
Visceral organs (postmortem)	4	2
Blood cultures[a]	135	66
Carriers, throat or nose[b]		
Community	59	57
Hospital staff	45	42
Food	21	21
Food handlers[c]	2	2
Mastitis milk[d] (bovine)	17	17

[a] When strains of different phage patterns were isolated from the same patient they are listed as separate infections. They are detailed under Results.

[b] When strains of different phage patterns were isolated from the same person, they are listed as separate infections.

[c] Only one strain from each item was included.

[d] Seventeen cows from 10 herds.

TABLE 3. Encapsulation of *S. aureus* from different sources[a]

Source	No. with the following capsular types[b]						
	1	5	7	8	10	NT	R
Blood cultures	1	9 (13.6)	1	47 (71.2)		6 (9.1)	2
Purulent processes		29 (16.6)	2	118 (67.4)	4 (2.3)	5 (2.9)	17 (9.7)
Carriers							
Community		4 (7.0)	4 (7.0)	35 (61.4)		9 (15.8)	5 (8.8)
Hospital		12 (28.6)	2	25 (59.5)	2	1	
Food and food handlers		4 (17.4)		12 (52.2)			7 (30.4)
Bovine mastitis		1		2		13 (76.5)	1
Total	1	59 (15.5)	9 (2.3)	239 (62.9)	6 (1.6)	34 (8.9)	32 (8.4)

[a] Only one isolate from each infection is included.
[b] Figures in parentheses indicate percentage of total. NT, Nontypeable; R, nonencapsulated.

with teichoic acid antiserum. Inagglutinability with teichoic acid antibodies suggested encapsulation, whereas agglutination at a 1:100 dilution was interpreted as absence of capsules.

Bound coagulase. A slide test for clumping factor was performed by suspending the cocci in rabbit plasma (33% in saline). The test was considered positive when clumping occurred immediately.

Electron microscopy. Bacterial suspensions were centrifuged at 6,000 × *g* for 10 min. The pellets were mixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h at room temperature.

Thereafter, the cells were postfixed in osmium tetroxide and embedded in Epon 812. Thin sections were cut with an LKB Ultratome III and stained with lead citrate and uranyl acetate. The sections were examined with a JEOL 100-C transmission electron microscope.

For scanning electron microscopy, the fixed cells were allowed to adhere to glass cover slips coated with 1 mg of poly-L-lysine (Bio-Yeda, Israel) per ml for 60 min and were dehydrated sequentially in aqueous solutions of 50, 70, 80, and 100% alcohol, followed by graded absolute alcohol solutions and Freon (50, 75, and 100%). The preparations were dried in a Bomar critical-point drying apparatus with liquid CO_2. The cells were coated with gold in a Technics (Hummer I) sputter and examined with a JEOL JSM-35 scanning electron microscope with a tungsten filament at 35 kV.

Statistical methods. Chi-square analysis was used to examine the significance of differences in the frequency of capsular types in isolates from different sources or of different phage types.

RESULTS

Capsular types of *Staphylococcus aureus* from different sources. The distribution of capsular types among strains from active infections (sepsis, purulent processes) was not essentially different from the distribution among carrier strains (Table 3). Type 8 was represented by 60 to 71% of strains from these sources and type 5 by 14 to 17%. Type 5 was significantly more frequent among hospital staff (28%) than among community carriers (7%) ($P < 0.02$ [chi-square analysis]). Since all hospital carriers were from one hospital (Assaf Harofe Medical Center), the relatively high frequency of type 5 may represent clones prevalent in this institute. Types 7 and 10 each accounted for approximately 2% and type 1 for 0.3% of the strains. The other types (2, 3, 4, 6, 9, and 11) were not demonstrated in this material. About 10% of the strains were nontypeable, i.e., probably encapsulated with other than the 11 prototype capsules. Approximately 10% were not encapsulated, i.e., exhibited agglutination with anti-teichoic acid serum but did not react with any of the antisera to the 11 capsular polysaccharides. Nonencapsulated *S. aureus* was isolated from 2 cases of septicemia and 17 cases of other infectious processes, but these strains were particularly frequent among strains from food (30%).

Among 27 laboratory strains used for propagating typing phages, the frequency of capsular types was different (Table 4). Eleven strains (41%) were of type 5, only 4 (15%) were of type 8, and 11 (41%) were nonencapsulated.

Capsular typing of repeat isolations of *Staphylococcus aureus* from infectious processes. In the category "Purulent processes" of Table 2, 198 strains from 175 patients were examined; the repeat isolations were in all cases of identical capsular type. Among septicemia patients, 27 had more than one positive blood culture (Table 5). In 25 of these patients, the capsular type was identical for all their isolates. Two patients (no. 26 and 27 of Table 5) had *S. aureus* isolates of different phage patterns. The cultures from patient no. 27 (erythema multiform with secondary sepsis) yielded *S. aureus* of phage type 55/71, phage type 85, and a mixture of both, recognizable by different types of hemolysis (25). The type 55/71 strains had type 8 capsules, whereas the type 85 strains had nontypeable capsules. Five of the isolates from patient no. 26 (multiple open fractures after a road accident) were of phage type 3A/3C and were nontypeable for capsular polysaccharides, whereas two of three phage-resistant strains had type 8 capsules and the third strain was unencapsulated. In this latter case, one of the strains had possibly lost the ability to synthesize capsules, either in vitro or in vivo. In general, our results indicate that capsular formation has a high degree of stability in vivo and is not easily lost during cultivation.

Encapsulation and susceptibility to bacteriophages. Because of the reports that encapsulation is incompatible with lysis by bacteriophages (26, 29), we examined the lysis of 28

TABLE 4. Capsular polysaccharides among 27 *S. aureus* propagating strains

Propagating strains for phages	Serotype of capsules
3A, 53, 54, 77, 84, 85, 88, 89, 90, 92, 94.....	5
71, 80, 81, HK2	8
29	7
3C, 6, 42E, 47, 52, 52A/79, 55, 75, 83A, 95, 96...	Nonencapsulated

TABLE 5. Capsular types of multiple isolates of *S. aureus* from blood cultures

Patient no.	No. of isolates	Phage type[a]	Capsular type
1	4	29/42E/81/95/±47/±54	8
2	3	29/88/89	5
3	8	29/53/54/81	5
4	2	52	8
5	2	52/80/±29	8
6	2	3A/±55/±71	8
7, 8	2, 2	3A	8
9, 10	2, 3	3C/71	8
11	2	42E	8
12, 13	2, 2	85/92/±75	8
14	3	92	8
15	2	81	8
16, 17	3, 8	94/96	5
18, 19	2, 2	95/(D11 + HK2)	8
20, 21, 22, 23	2, 2, 2, 8	Resistant	8
24	3	Resistant	5
25	2	Resistant	Nonencapsulated
26	2	Resistant	8
	1	Resistant	Nonencapsulated
	5	3A/3C	Nontypeable
27	5	55/71	8
	8	85	Nontypeable

[a] Phages in parentheses were used as a pool. ± indicates that not all isolates reacted with the phages.

propagating strains on CSA enriched with 400 μg of $CaCl_2 \cdot 2H_2O$ per ml (7). The addition of calcium chloride had no influence on encapsulation since bacteria from this medium were positive for capsular polysaccharides when checked both by agglutination and by precipitation of lysostaphin extracts (9). All typing phage suspensions showed similar titers of PFU on this medium as on calcium chloride nutrient agar for phage typing (7). When specific antiserum was added to this CSA-$CaCl_2$ medium, plaque formation and capsular polysaccharides could be demonstrated side by side (Fig. 1). The propagating strains 80 and 52 were grown on this medium, and the cells were suspended in a 1% anti-type 8 serum (monoclonal), incubated at 37°C for 1 h, washed five times with PBS, and fixed with glutaraldehyde for electron microscopy. Propagating strain 80 retained capsule formation on this medium (Fig. 2), but strain 52 was nonencapsulated (Table 4). Encapsulation and clumping factor could be demonstrated side by side on CSA cultures (Fig. 3).

Phage patterns and capsular types. In Table 6, the capsular types of the *S. aureus* isolates are analyzed in relation to their phage patterns. All encapsulated strains susceptible to group 1 phages or to phage 95 had capsules of type 8. A total of 26 strains were susceptible to phage 96; 25 of them were of capsular type 5, and 1 was unencapsulated. These associations were statistically significant ($P < 0.01$, chi-square test). Nine strains were of capsular type 7, and all of them were resistant to phages; further studies will show whether this is characteristic of type 7 strains. Nontypeable capsules were particularly frequent among phage group 3 isolates (20%).

DISCUSSION

S. aureus is the causative agent of an unusually broad spectrum of pathologic conditions, including circumscript suppurations, blood stream invasion, and a variety of toxic syndromes. Not surprisingly, this organism is equipped with a broad variety of virulence factors (cytotoxic hemolysins, leukocidins, enterotoxins, protein A, teichoic acid, and others). With this background it is impressive that *S. aureus* most often is a harmless commensal on the human body and can be isolated in considerable numbers from 15 to 20% of healthy adults. As far as is known, carrier strains may possess all the virulence factors, including the ability to synthesize specific disease-inducing toxins (10–12, 21). Almost 50 years ago, it was proposed for the first time that encapsulation might be the hallmark for invading strains of *S. aureus* (16), but capsules were demonstrated in a small proportion only of clinical isolates (23, 28, 31). To explain this discrepancy, it was postulated that the ability to form capsules is rapidly lost during culture in artificial media (16, 18). In vivo and in vitro studies have shown the capsules to be antiphagocytic for polymorphonuclear leukocytes and to protect the teichoic acid-mucopeptide complex against antibody and complement (15, 17, 20).

Bacterial capsules are discrete layers of viscous material that lie external to the rigid cell wall, and encapsulation is most definitively demonstrated on a morphological basis (e.g., by electron microscopy). This is, however, inconvenient in the study of large strain collections, particularly due to the special care that must be paid not to remove capsular material by the repeated washing procedures and to avoid shrinkage during fixation and mordanting owing to the high water content of the capsules. Yoshida et al. (32) defined encapsulation of *S. aureus* on the grounds of lack of clumping factor, diffuse growth on serum-soft agar, and phage

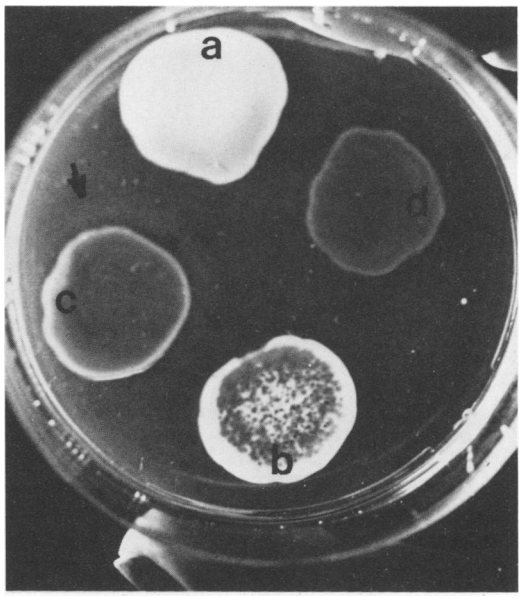

FIG. 1. Capsule formation and phage susceptibility in *S. aureus*. The inoculum was 25 μl of a heavy bacterial suspension, and the medium was CSA with 400 μg of $CaCl_2 \cdot 2H_2O$ per ml and 1% monoclonal anti-type 8 serum. (a) Propagation strain for phage 80; normal area of growth. (b) Same as area a, with the addition of phage 80 before incubation. (c) Same as area a, but after growth, 8 μl of lysostaphin (250 μg/ml) was added. After incubation for 3 h at room temperature and 24 h at 4°C, a ring of immunoprecipitation occurred (arrow). (d) Propagation strain 90 (capsular type 5) treated with lysostaphin as in area c. No immunoprecipitation is evident.

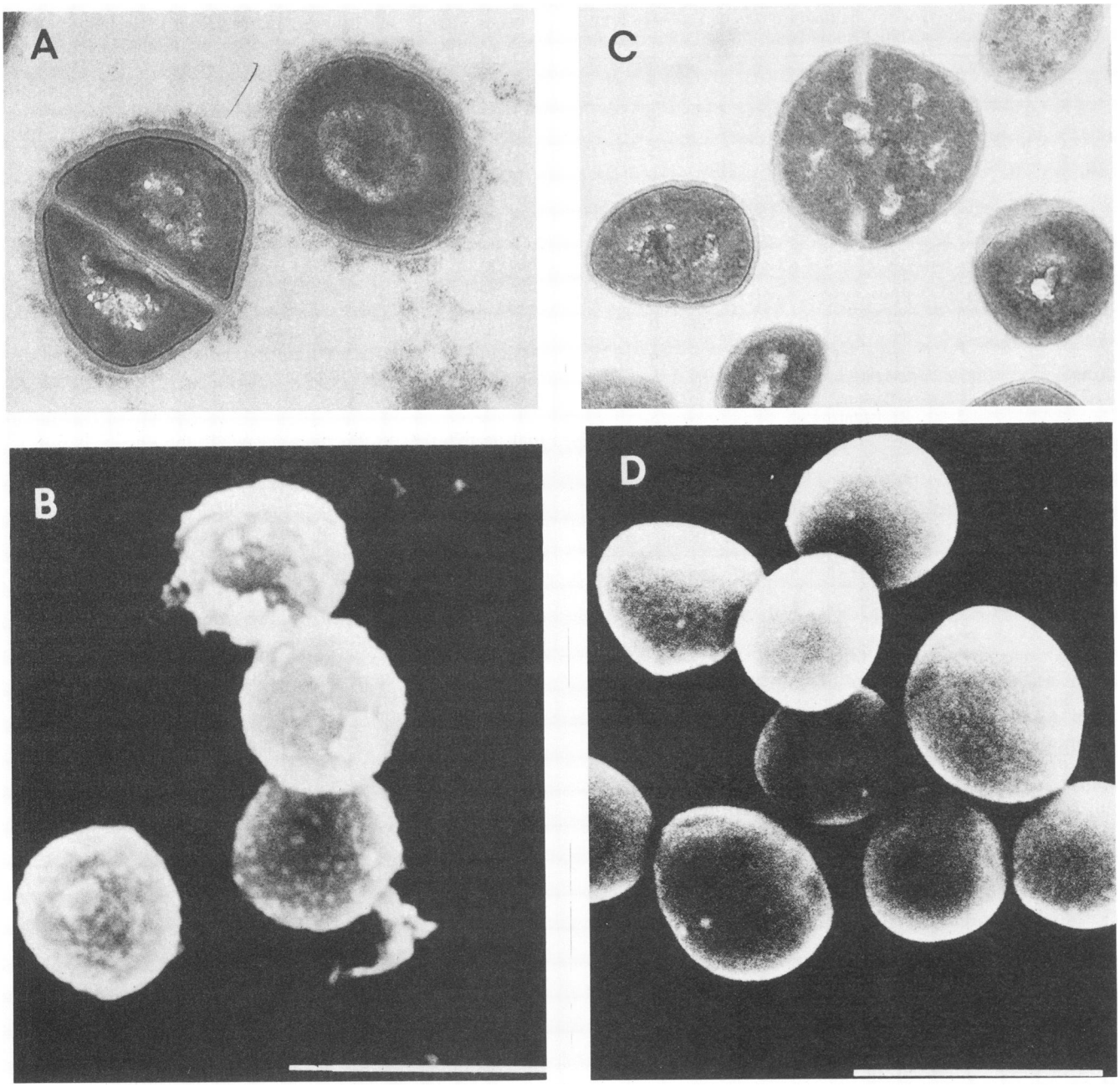

FIG. 2. Electron micrographs of *S. aureus* capsules. Bacteria grown on CSA plus calcium chloride were suspended in PBS plus 1% monoclonal anti-type 8 serum. After incubation (3 h at room temperature), the bacteria were washed and fixed. Shown are thin-section (A; × 26,000) and scanning (B; × 30,000; bar, 1 μm) micrographs of propagation strain for phage 80 (capsular type 8) and thin-section (C; × 26,000) and scanning (D; × 30,000; bar, 1 μm) micrographs of propagation strain for phage 52 (unencapsulated).

nontypeability. Of fresh isolates, 3.8% were reported as encapsulated, but 76.6% of "unencapsulated" strains were stained with fluorescent antisera to capsular polysaccharides in the same way as "encapsulated" strains (32); these strains were claimed to produce particularly thin capsules with "no specific structural feature" (29) or to secrete the capsular substance into the growth medium (32). These notions were not corroborated by our study. Strain 80 was phage typeable, positive for clumping factor (Fig. 3), and equipped with capsules of normal ultrastructure (Fig. 2). The capsular substance probably adhered strictly to the cocci since precipitation in antiserum-containing agar occurred only after lysis of the bacteria with lysostaphin (Fig. 1). It is also noteworthy that the antisera to prototype capsular polysaccharides were obtained by immunization with suspensions of washed bacteria, and they agglutinated washed suspensions of the test organism, but in immunodiffusion they reacted with supernatants only after the suspensions were heated to 121°C for 20 min (see Materials and Methods). Furthermore, isolates that reacted positively with any of the antisera to capsular polysaccharide were negative throughout for agglutination with antiserum to teichoic acid. On this basis we

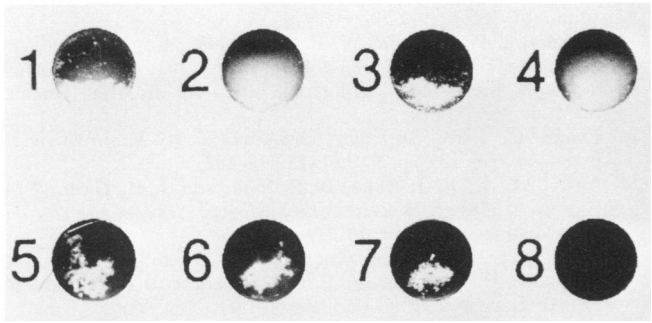

FIG. 3. Encapsulation and clumping factor. The strains for propagation of typing phages 84 (serotype 5), 95 (unencapsulated), and 80 (serotype 8) were grown on CSA for 18 h at 37°C. Antiserum to type 5 capsules agglutinated strain 84 (1) but not 95 (2), and antiserum to type 8 capsules agglutinated strain 80 (3) but not strain 95 (4).

have provisionally described isolates that were negative with all antisera as nontypeable but encapsulated.

According to this criterion encapsulation was demonstrated in 70 to 95% of strains from various sources. Only two capsular types were prevalent, viz., type 8 accounting for 63% and type 5 accounting for 16% of all isolates, whereas types 1, 7, and 10 were rare, and the remaining six types were not observed. We have previously shown (15) that our serotype 2 polysaccharide is serologically identical to the capsular antigen of the Smith diffuse strain (18). Serotype 2 was not recognized among our isolates, but a serotype A corresponding to the Smith diffuse strain was the most prevalent one among *S. aureus* isolates studied by Yoshida et al. (32).

The distribution of capsular types among isolates from active infections and from healthy carriers was similar. Hospital carriers may be transient vehicles for virulent strains picked up on the premises; we therefore studied carrier strains isolated from outpatients with no recent contact with medical institutions. The distribution of capsular types among these strains was similar to the distribution in hospital carrier strains; 61% were of type 8, and only 9% were unencapsulated. The proportion of unencapsulated strains was higher among food strains (30%), but types 8 and 5 were prevalent among these strains too.

Our data do not indicate that capsule formation is lost upon subculture on artificial media. The propagating strains for typing phages have been transferred in culture for decades, yet 60% were encapsulated. The carrier strains from outpatients were isolated between 3 months and 13 years before the examination, and the demonstration of capsules was independent of the age of the culture (data not shown). The strains from infectious processes and food were isolated in a large number of hospital and public health laboratories on different routine media, and they were transferred to nutrient agar in the Reference Laboratory and later to slants of stock culture agar for dispatching from Israel to the United States, where they were typed for capsules. The parcels were in transit for days or weeks without appropriate refrigeration. This is emphasized because Yoshida and Ohmoto (30) have recently claimed that the capsular type of *S. aureus* may change during storage even when freeze-dried. The identical results obtained from almost all repeat isolations from pathologic samples indicate that under the conditions used in this study to maintain the cultures, the capsular types did not alter.

Several authors have claimed that encapsulated *S. aureus* strains are resistant to lysis by phages (27, 31). The capsule was thought to prevent phage absorption (26). In pneumococci, encapsulation has been reported to prevent lysis by bacteriophages (6). We demonstrated lysability of encapsulated strains grown under conditions used for demonstration of capsules, except for the addition of calcium chloride, which is needed for phage adsorption or proliferation (7). We showed that this addition did not alter the serological reaction with anticapsular sera. Furthermore, bacteria harvested from our routine phage-typing medium (7) reacted with monospecific antisera to types 5 and 8 (data not shown).

In the present study, 60% of the isolates were both encapsulated and susceptible to phages, and 65% of the encapsulated strains were typeable by phages. In addition, a surprising correlation between capsular types and phage patterns was revealed (Table 6). Strains susceptible to group 1 phages or to phage 95 were regularly of capsular type 8, and strains lysed by phage 96 had type 5 capsules. These findings were of high statistical significance. We have so far no clue to the genetic or biochemical mechanism(s) involved in this association between the structure of capsular polysaccharide and the susceptibility to certain phages. It may be recalled, though, that bacteriophages may influence the structure of *Salmonella* O-antigens (22) and that lysogeny in *Streptococcus pneumoniae* seems to be restricted to certain capsular types (5); it has been proposed that phages influence capsule production of pneumococci (4). Recently, it has been claimed (19) that 93% of *S. aureus* isolates from bovine

TABLE 6. Capsular types and phage patterns of *S. aureus* isolates

Capsular types[a]	No. of isolates with the following phage pattern[b]								Total
	Group 1	Group 2	Group 3	95/±(D11 + HK2)	(D11 + HK2)	96/±94	Miscellaneous	Phage resistant	
1	0	0	1	0	0	0	0	0	1
5	0	1	12 (26.7)	0	1	25 (96.2)	3	17 (12.6)	59 (15.5)
7	0	0	0	0	0	0	0	9 (6.7)	9 (9.2)
8	59 (86.8)	49 (81.7)	20 (44.4)	15 (93.8)	13 (92.9)	0	9 (56.3)	74 (54.8)	239 (62.9)
10	0	0	0	0	0	0	2	4 (3.0)	6 (1.6)
NT	1	5 (8.3)	9 (20.0)	0	0	0	2	17 (12.6)	34 (8.9)
R	8 (11.8)	5 (8.3)	3	1	0	1	0	14 (10.4)	32 (8.4)

[a] NT, Nontypeable; R, nonencapsulated.

[b] Phages in parentheses were used as a pool. ± indicates that not all isolates reacted with the phages. According to the chi-square test, capsular type 8 was more frequent in phage group 1 strains than in others ($P < 0.001$), in group 2 strains than in others ($P = 0.001$), in phage type 95/±(D11 + HK2) strains than in others ($P < 0.01$), and in (D11 + HK2) strains than in others ($P < 0.02$). Capsular type 5 was more frequent in phage type 96/±94 strains than in others ($P < 0.001$). Numbers in parentheses show percentage of total.

mastitis milk are encapsulated, most of the strains reacting with antiserum to the Smith diffuse strain. Most of the bovine strains examined by us had capsules that were nontypeable with our sera (Table 3). It should be recalled that bovine strains usually are not susceptible to the same phages as are human isolates (8). However, Anderson (2) has rejected the reports of encapsulation of bovine *S. aureus* as based on an erroneous technique; on the basis of India ink staining he concluded that the absence of capsules was characteristic for bovine mastitis strains.

Of all 280 strains studied, 65% were typeable with phages and 83% were typeable with anticapsular sera. However, due to the prevalence of two types only, capsular typing will probably in most cases not be analytical enough for epidemiological purposes. As an adjunct to phage typing, this technique might be helpful, particularly for strains susceptible to phage group 3 or resistant to phages (Table 5).

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LITERATURE CITED

1. **Aly, R., H. R. Shinefield, C. Litz, and H. I. Maibach.** 1980. Role of teichoic acid in the binding of *Staphylococcus aureus* in man. J. Infect. Dis. **141**:463–465.
2. **Anderson, J. C.** 1984. Absence of encapsulation in strains of *Staphylococcus aureus* isolated from bovine mastitis. Res. Vet. Sci. **37**:359–361.
3. **Arbeit, R. D., W. W. Karakawa, W. F. Vann, and J. B. Robbins.** 1984. Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **2**:85–91.
4. **Bernheimer, H. P.** 1977. Lysogeny in pneumococci freshly isolated from man. Science **195**:66–68.
5. **Bernheimer, H. P.** 1979. Lysogenic pneumococci and their bacteriophages. J. Bacteriol. **138**:618–624.
6. **Bernheimer, H. P., and J. G. Tiraby.** 1976. Inhibition of phage infection by pneumococcus capsule. Virology **73**:308–309.
7. **Blair, J. E., and R. E. O. Williams.** 1961. Phage typing of staphylococci. Bull. W.H.O. **24**:771–784.
8. **Davidson, I.** 1961. A set of bacteriophages for typing bovine staphylococci. Res. Vet. Sci. **2**:396–407.
9. **Fournier, J. M., W. F. Vann, and W. W. Karakawa.** 1984. Purification and characterization of *Staphylococcus aureus* type 8 capsular polysaccharide. Infect. Immun. **45**:87–93.
10. **Guinan, M. E., B. B. Dan, R. J. Guidotti, A. L. Reingold, G. P. Schmid, E. J. Bettoli, J. G. Lossik, K. N. Schands, M. A. Kramer, N. T. Hargrett, R. L. Anderson, and C. V. Broome.** 1982. Vaginal colonization with *Staphylococcus aureus* in healthy women: a review of four studies. Ann. Intern. Med. **96**:944–947.
11. **Hedstrom, S. A., and T. Malmquist.** 1982. Sphingomyelinase activity of *Staphylococcus aureus* strains from recurrent furunculosis and other infections. Acta. Pathol. Microbiol. Scand. Sect. B **90**:217–220.
12. **Ivler, D.** 1965. Comparative metabolism of virulent and avirulent staphylococci. Ann. N.Y. Acad. Sci. **128**:62–80.
13. **Kaplan, M. H., and M. J. Tenenbaum.** 1982. *Staphylococcus aureus*: cellular biology and clinical application. Am. J. Med. **72**:248–258.
14. **Karakawa, W. W., and J. A. Kane.** 1972. Characterization of the surface antigen of *Staphylococcus aureus*, strain K-93m. J. Immunol. **108**:1199–1208.
15. **Karakawa, W. W., and W. F. Vann.** 1982. Capsular polysaccharides of *Staphylococcus aureus*, p. 285–293. *In* L. Neinstein and B. N. Fields (ed.), Seminars in infectious diseases. Thieme-Stratton, New York.
16. **Lyons, C.** 1937. Antibacterial immunity to *Staphylococcus pyogenes*. Br. J. Exp. Pathol. **18**:411–422.
17. **Melly, M. A., L. J. Duke, D. F. Liau, and J. H. Hash.** 1974. Biological properties of the encapsulated *Staphylococcus aureus* M. Infect. Immun. **10**:389–397.
18. **Morse, S. I.** 1980. Staphylococci, p. 624–633. *In* B. D. Davis, R. Dulbecco, H. N. Eisen, and H. S. Ginsberg (ed.), Microbiology, 3rd ed. Harper & Row, Publishers, Inc., New York.
19. **Opdebeeck, J. P., and N. L. Norcross.** 1983. Frequency and immunologic cross-reactivity of encapsulated *Staphylococcus aureus* in bovine milk in New York. Am. J. Vet. Res. **44**:986–988.
20. **Peterson, P. K., B. J. Wilkinson, Y. Kim, D. Schmeling, and P. G. Quie.** 1978. Influence of encapsulation on staphylococcal opsonization and phagocytosis by human polymorphonuclear leukocytes. Infect. Immun. **19**:943–949.
21. **Pitz, H. L., J. J. Kirkland, G. G. Bond, E. K. Warner, and G. P. Petty.** 1984. Association of high levels of serum antibody to staphylococcal toxic shock antigen with nasal carriage of toxic shock antigen-producing strains of *Staphylococcus aureus*. Infect. Immun. **43**:954–958.
22. **Robbins, P.W., and T. Uchida.** 1962. Studies on the chemical basis of the phage conversion of O-antigens in the E-group *Salmonella*. Biochemistry **1**:323–335.
23. **Rogers, D. E.** 1966. Experimental observations on staphylococcal disease, p. 279–296. *In* Symposium on staphylococci and staphylococcal infections. Paustwove Wydawnictwo Naukowe, Warszawa, Poland.
24. **Sheagren, J. N.** 1984. *Staphylococcus aureus*. The persistent pathogen. Part one. N. Engl. J. Med. **310**:1368–1373.
25. **Sompolinsky, D., D. Schwartz, Z. Samra, J. Steinmetz, and Y. Siegman-Igra.** 1985. Septicemia with two distinct strains of *Staphylococcus aureus* and dwarf variants of both. Isr. J. Med. Sci. **21**:434–440.
26. **Wilkinson, B., and K. Holmes.** 1979. *Staphylococcus aureus* cell surface capsule as a barrier for bacteriophage adsorption. Infect. Immun. **23**:549–552.
27. **Witte, W.** 1975. Capsule formation in *Staphylococcus aureus* as a reason for non-lysability by phages. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **223**:447–451.
28. **Yoshida, K.** 1971. Demonstration of serologically different capsular types among strains of *Staphylococcus aureus* by the serum-soft agar technique. Infect. Immun. **3**:535–539.
29. **Yoshida, K., and Y. Minegishi.** 1976. Capsular substance production in unencapsulated strains of *Staphylococcus aureus*, p. 359–375. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer, Stuttgart, Federal Republic of Germany.
30. **Yoshida, K., and T. Ohmoto.** 1984. Alteration of capsular type of encapsulated strains of *Staphylococcus aureus* during freeze-drying and storage. Experientia **40**:93–94.
31. **Yoshida, K., M. R. Smith, and Y. Naito.** 1970. Biological and immunological properties of encapsulated strains of *Staphylococcus aureus* from human sources. Infect. Immun. **2**:528–532.
32. **Yoshida, K., M. Takahashi, T. Ohtomo, Y. Minegishi, Y. Ichiman, K. Haga, E. Kono, and C. L. SanClemente.** 1979. Application of fluorescent antibody for detecting capsular substances in *Staphylococcus aureus*. J. Appl. Bacteriol. **46**:147–152.