SHORT ARTICLE

THE EXPRESSION OF CAPSULE IN SERUM-SOFT AGAR BY *STAPHYLOCOCCUS AUREUS* ISOLATED FROM HUMAN CLINICAL SOURCES

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SUMMARY. *Staphylococcus aureus* isolates from human clinical sources were incubated for various times in modified 110 medium and tested for production of capsule by the serum-soft agar technique. Ten (5.7%) of 175 isolates were encapsulated after incubation for 24 h. A more detailed examination of 77 isolates showed that incubation period affected the production of capsule. After 2 h, 31% were encapsulated, but after 6 h and 24 h this decreased to 17% and 4% respectively. Rapid passage *in vitro* induced the expression of capsule in four of 50 unencapsulated strains. Only three of 20 encapsulated strains could be typed with standard antisera.

INTRODUCTION

The prevalence of encapsulated *Staphylococcus aureus* from human clinical sources, as shown by negative staining or the serum-soft agar technique, is considered to be rare. Examination of large numbers of hospital isolates by the latter method in two studies yielded 4.2% and 3.8% encapsulated organisms respectively (Yoshida *et al.*, 1970; Yoshida *et al.*, 1979). Similar levels were reported for *S. aureus* strains isolated from bovine mastitis (Yokomizo *et al.*, 1977). Recently, when milk from cows with mastitis was inoculated directly into serum-soft agar (SSA) 90% of the *S. aureus* isolates showed evidence of capsules but the majority lost their capsule on subculture (Norcross and Opdebeeck, 1983; Opdebeeck and Norcross, 1983). Furthermore, capsular antigen has been identified by fluorescence microscopy on strains considered to be negative when assessed in SSA (Yoshida *et al.*, 1979). However, by appropriate manipulation of the growth environment, expression of the capsule in SSA has been induced in strains not previously exhibiting this characteristic (Yoshida *et al.*, 1974; Opdebeeck *et al.*, unpublished observation). In view of these findings, the prevalence of capsules in *S. aureus* strains from man was further studied.

MATERIALS AND METHODS

Bacteria. Swabs from various clinical sources, collected over a 3-month period, were refrigerated after primary routine culture. Those confirmed as yielding *S. aureus* were aseptically clipped 1.5 cm above the cotton into 2 ml of modified Staphylococcus Medium 110 (Yoshida and Ekstedt, 1968) and incubated at 37°C for 24 h. The resulting cultures were plated on to 10% sheep-blood agar and the identity of *S. aureus* strains was confirmed by colony morphology and a positive coagulase test. The Smith diffuse strain was obtained from Dr C. L. San Clemente, Lansing, Michigan, and strain 305 from Dr F.H. Newbould, Guelph, Ontario.

Assessment of capsule. Serum-soft agar (SSA) was prepared from modified 110 medium by adding agar 0.15% w/v (Difco) and sterile rabbit serum 1% w/v. This serum was tested for the absence of capsular antibodies with the Smith strain. Tubes containing 2 ml of SSA were seeded by a straight wire with organisms from the swabs after incubation for various times in modified 110 medium. Inoculation in this way yielded a small number of colonies (usually < 50) so that

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the test could be read easily. The medium was thoroughly mixed on a vortex mixer to distribute the organisms throughout the SSA, overlaid with a 2% w/v agar plug and incubated at 37°C for 18 h. The colony morphology was then assessed as diffuse or compact. For the purpose of this paper, the diffuse colony form (as shown by the Smith diffuse strain) was considered to represent capsule formation. In all tests, the Smith diffuse strain and strain 305 were used as positive (diffuse) and negative (compact) controls respectively. Of the 175 isolates tested, all were evaluated for capsule formation after incubation for 24 h in modified 110 medium, but 77 isolates were also evaluated after incubation for 2 and 6 h. These strains were isolated from skin and surgical wounds (47), abscesses (17) and other miscellaneous infections (13).

Serotyping of isolates. SSA in which normal rabbit serum 1% v/v was replaced by rabbit antiserum 1% v/v was used as described by Yoshida (1971). In this test, diffuse colonies are converted to the compact type in the presence of specific antiserum. The antisera used were anti-Smith serum, prepared as described by Norcross and Opdebeeck (1983) and anti-B and -C sera obtained from Dr K. Yoshida, School of Medicine, Kawasaki, Japan. Anti-Smith serum is equivalent to the anti-A serum of Yoshida (1971).

Antibiotic-resistance patterns were determined by growing each isolate on sensitivity test agar containing (per ml): penicillin 0.1 and 1.0 I.U., cloxacillin 4 µg, ampicillin 2 and 15 µg, erythromycin 1 and 5 µg, carbenicillin 50 and 100 µg, trimethoprim 1 and 2 µg, chloramphenicol 7.5 µg, gentamicin and tobramycin 4 and 8 µg, kanamycin 3.75 µg, and amikacin 8 and 16 µg. Strains which grew on the higher, or single, concentration of antibiotic were termed resistant (R) and those inhibited by both concentrations were termed sensitive (S).

RESULTS

When 175 *S. aureus* isolates were incubated for 24 h in modified 110 medium and then inoculated into SSA, only 10 showed the diffuse colony form indicative of capsule formation (table I). However, in the subgroup of 77 cultures taken at random, 24 were positive for capsule production after incubation for 2 h whereas after 6 h and 24 h only 13 and three cultures were positive. There was some variation in expression of capsule in that 27 (35%) strains showed evidence of capsule production at one or more of the three evaluation times.

The 50 strains which grew consistently as compact colonies in SSA were reassessed after three rapid passages, each with incubation for 2 h, through modified 110 medium. Only four of these isolates (8%) showed diffuse colony morphology after this treatment.

Table II shows the relationship between encapsulation and the five antibiotic sensitivity patterns which were found in the 77 isolates. The 31 diffuse isolates classified in this way included those four which were induced to express encapsulation by the rapid passage method described above. Although group-1 strains showed a high level of encapsulation (48.8%) it was not significantly different from that found in the other groups.

Of the 20 diffuse isolates subjected to serotyping in SSA, only three were typable; one by Smith and B antisera, one by Smith and C antisera and one by B and C antisera.

TABLE I
Expression of capsule by S. aureus in serum-soft agar

Incubation time* (h)	Number of isolates examined	Number of encapsulated isolates† (%)
2	77	24 (31.2)
6	77	13 (16.9)
24	77	3 (4.05)
24	175‡	10 (5.7)

* Swabs were incubated in 2 ml of modified 110 medium before inoculation of organisms into SSA.

† Isolates expressing diffuse colony morphology in SSA.

‡ This group includes the 77 isolates which were randomly selected for complete evaluation.

TABLE II

The relationship between antibiotic sensitivity and encapsulation of S. aureus

Group	Antibiotic-sensitivity patterns											Number of isolates	Number of encapsulated isolates (%)
	P	Cl	A	E	Ca	T	Ch	G	To	K	Am		
1	R	S	R	S	R	S	S	S	S	S	S	59	27 (45.8)
2	S	S	S	S	S	S	S	S	S	S	S	8	1 (12.5)
3	R	R	R	R	R	R	R	R	S	R	S	7	2 (28.6)
4	R	S	R	R	R	S	S	S	S	S	S	2	0 (0)
5	R	S	R	R	R	R	S	S	S	S	S	1	1 (100)

P = penicillin, Cl = cloxacillin, A = ampicillin, E = erythromycin, Ca = carbenicillin, T = trimethoprim, Ch = chloramphenicol, G = gentamicin, To = tobramycin, k = kanamycin, Am = amikacin.

DISCUSSION

After laboratory subculture, 35% of 77 isolates showed evidence of encapsulation when assessed by the SSA technique, a prevalence which is significantly higher than that reported previously from human clinical cases (Yoshida, 1971; Yoshida *et al.*, 1979). Nonetheless, this was still considerably less than was reported for isolates from bovine mastitis of which >90% were encapsulated when examined by a similar technique (Opdebeeck and Norcross, 1983). The majority of human encapsulated isolates which have been serotyped belong to group A (A being synonymous with Smith) (Yoshida, 1971; Yoshida *et al.*, 1974). This is consistent with work on isolates from the bovine mammary gland of which 73% were classified (either uni- or polyvalently) as belonging to the Smith serotype (Opdebeeck and Norcross, 1983). In the present study, the Smith antiserum converted two of the three typable strains from the diffuse to the compact colony form. Although most bovine encapsulated isolates were typable (Opdebeeck and Norcross, 1983), human strains have tended to be poorly typable by the A/Smith-B-C system (Yoshida *et al.*, 1974).

A significant decrease in the number of strains showing encapsulation during the growth of organisms *in vitro* during a 24-h period confirms previous observations that the capsule is rapidly lost *in vitro* (Opdebeeck and Norcross, 1983; Norcross and Opdebeeck, 1983). This could be explained if organisms produced a repressor substance during replication *in vitro*, which subsequently inhibited the expression of encapsulation. Alternatively, factor(s) required for capsule production may only be readily available *in vivo*. Labile factor(s) present in serum, milk and mastitic milk have been shown to enhance the production of capsule (Opdebeeck *et al.*, unpublished observation). The formation of a capsule by *S. aureus* may thus result from a balance between various environmental factors that act as inhibitors or stimulators. The prevalence of encapsulation *in vivo* may be higher than reported here. Rapid passage stimulated capsule production in four of the 50 compact isolates but only 9 (33.3%) of 27 strains confirmed as capsule-produced in the primary tests could be induced by this method to re-express capsules lost after prolonged storage. Yoshida *et al.* (1974 and 1979), using fluorescence microscopy, showed the presence of small quantities of capsular antigen on some compact strains and these may be the strains that can be induced to express encapsulation by rapid passage. This was more difficult to achieve than with bovine isolates, and media other than modified 110 medium may be more successful for human isolates.

There was no relationship between encapsulation and the clinical origin of the strain, but most showed a similar pattern of resistance to antibiotics. This indicates that encapsulation may be associated with particular groups of *S. aureus*, but further studies would be required to confirm this.

The role of the capsule of *S. aureus* in pathogenesis is still uncertain, although in strains such as Smith it has antiphagocytic properties (Peterson *et al.*, 1978; Wilkinson *et al.*, 1979) and capsular antisera are protective in mice. The capsule on the Smith strain is large and easily observed while on other strains that produce diffuse colonies in SSA it is not, yet such strains show evidence of additional surface components by fluorescence microscopy (Yoshida *et al.*,

1979) and by electronmicroscopy (Norcross and Opdebeeck, 1983). Electronmicroscopy and biochemical analysis of the surface components of microbes has created confusion in terminology (Bowles and Marsh, 1982), and controversy over the definition of encapsulation of *S. aureus* is long standing (Mudd, 1965). It seems likely that the diffuse colony in SSA is due directly or indirectly to the production of capsular polysaccharide antigens as described by Karakawa and Vann (1982), and as visualised in lesions by Mayberry-Carson *et al.* (1984). The high prevalence of encapsulation in isolates from bovine mastitis (Opdebeeck and Norcross, 1983) compared with that of human isolates may reflect their different ecology. In man, the organism is generally regarded as an opportunist pathogen with a low level of infectivity and the primary econiche is the anterior nares, while in the dairy cow it survives primarily within the mammary gland and this constitutes the major reservoir. Although the presence of factors in unheated milk and serum enhance diffuse colony morphology in SSA (Opdebeeck *et al.*, unpublished observation), it is still not clear whether these changes are significant in pathogenesis.

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