Immunochemical Study of Diverse Surface Antigens of a *Staphylococcus aureus* Isolate from an Osteomyelitis Patient and Their Role in In Vitro Phagocytosis

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The cellular antigens of a strain of *Staphylococcus aureus*, isolated from a bone fragment from osteomyelitis, were analyzed immunochemically and by interaction with human phagocytic cells. When this strain was allowed to interact with human polymorphonuclear cells in the presence of antiserum, the strain was shown to have specific antiphagocytic antigens. An acidic polysaccharide consisting of galactose and glucuronic acid was isolated from the cell surface of the organism, and in vitro opsonization tests indicated that this acidic antigen impeded in vitro phagocytosis by human polymorphonuclear cells. It was also observed that antibodies directed against the mucopeptide constituents of homologous and heterologous bacterial cell walls were effective in promoting the in vitro opsonization of the organism. In the presence of antimucopeptide serum and human polymorphonuclear cells, a variant strain was isolated from the wild type, and immunochemical analysis indicated that this strain consisted of galactose and immunodominant amino-galacturonic acid residues. In vitro phagocytosis studies employing this variant strain indicated that the homologous human convalescent serum contained higher levels of opsonins against the variant strain than the original isolate, the wild type. This observation is discussed.

The occurrence of repeated staphylococcal infections in humans and their persistence in many instances suggests an inability of *Staphylococcus aureus* to elicit an in vivo protective immune response in the host (5, 25). In a series of studies by a number of investigators, a picture has emerged which indicates that *S. aureus*, freshly isolated from clinical specimens, generally exhibits more antiphagocytic surface antigens than laboratory-propagated strains (5, 9, 11, 14, 15, 18, 25, 31). For example, fresh isolates were shown to be more resistant to in vitro phagocytosis by polymorphonuclear leukocytes (PMN) than laboratory strains, and this resistance depended upon the antiphagocytic acidic polysaccharides (5, 9, 11, 18, 22). These observations underscore the notion that strains of *S. aureus* have the potential under in vivo conditions to produce antiphagocytic antigens, and these substances may influence the virulence of the organisms (21).

Recently, Peterson and associates have described two significant observations which offer hope for resolving some of the questions associated with acquired immunity to staphylococcal infections (23). These investigators demonstrated that nonimmune sera may contain anti-

mucopeptide antibodies which can initiate the opsonization of organisms possessing exposed mucopeptide moieties. Organisms carrying surface antigens can impede the opsonizing effects of antimucopeptide antibodies by virtue of the fact that these antigens can mask the mucopeptide moieties and thereby block the immunological binding capabilities of specific mucopeptide antibodies. Since most adult human sera possess mucopeptide antibodies (W. W. Karakawa and D. A. Young, unpublished data), it is feasible to assume that encapsulated *S. aureus* strains would appear to have an in vivo survival advantage over strains devoid of surface antigens. In addition, since *S. aureus* has been suggested to possess the genetic potential for producing surface antigens (21), the possibility exists that under in vivo conditions they may selectively form phenotypic variants which are more suited for survival in the host.

In this report, evidence is presented which indicates that an *S. aureus* strain, freshly isolated from a bone fragment obtained from an osteomyelitis patient, has an antiphagocytic galactose-glucuronic acid polymer. In vitro phagocytic studies employing the patient's convalescent serum revealed the presence of two opso-

nins directed against the homologous organism, namely, a galactose-glucuronic acid antibody and a mucopeptide antibody. A variant strain was isolated from a culture of the wild type and was shown to carry an acidic antigen, which consisted of galactose and amino-galacturonic acid. Opsonins directed against this acidic polymer were also demonstrated in the patient's convalescent serum.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* provisionally designated strain Mardi (wild type) was isolated from a sample of bone fragment removed from a patient with osteomyelitis. A variant strain isolated from culture of the wild type grown in the presence of mucopeptide antibodies was also employed in these studies. The remaining strains of organisms were from the collection of the immunochemistry laboratory, Department of Biochemistry, Pennsylvania State University.

Preparation of surface antigens and mucopeptides. Surface antigens were extracted from the wild type and variant strains by methods previously described (7, 11, 17). Mucopeptides were prepared from acid-treated cells by the method described by Krause and McCarty (14). Specific teichoic acids were extracted from cells with cold trichloroacetic acid according to the method of Park and Hancock (20).

Chromatography and gel filtration. The techniques employed for diethylaminoethyl-cellulose chromatography and Bio-Gel filtration were previously described (10).

Analytical methods. Analysis for hexosamine and amino acids was performed by the method of Spackman et al. (28) as described by Karakawa and Kane (9). Total hexose and hexosamine concentrations were determined as previously described (12, 26, 30). Total phosphorus was measured by the method of Chen et al. (1). Uronic acid concentration was calculated by the carbazole method described by Davidson (2). Sodium borohydride (NaBH_4) reduction of polysaccharides was performed by the method of Deuel (3), and the constituents of the polymer were analyzed by gas-liquid chromatography according to the procedure described by Fraser and Mallette (6).

Serological methods. Rabbits were immunized to the various strains of *S. aureus* according to the procedure of McCarty and Lancefield (16). These antisera were used as the source of opsonins in the phagocytosis test. Quantitative precipitin analysis was performed according to a modification of a method by McCarty and Lancefield (16) as described by Karakawa and Krause (10). Double-diffusion analysis in agar was performed according to the method described by Tan and Kunkel (29).

Leukocytes. Heparinized human peripheral blood was sedimented for 45 min at room temperature with 3% Dextran T-250 (Pharmacia, Uppsala, Sweden) to obtain the PMN. PMN were then centrifuged at $160 \times g$ for 5 min, washed with saline containing 100 U of heparin per ml (Sigma Chemical Co., St. Louis, Mo.), and resuspended to a concentration of 10^7 viable cells per ml in RPMI 1640 medium (Microbiological Associates, Bethesda, Md.) supplemented with 5% heat-

inactivated fetal calf serum. Leukocyte counts were made with a Neubauer hemacytometer, and viability was determined by the trypan blue exclusion method.

Phagocytosis test. This method is essentially a modification of the phagocytosis test described by Roberts (24). All tests were performed in sterile siliconized glass screw-cap tubes (13 by 100 mm). Before each test, *S. aureus* organisms from 5-h Todd-Hewitt broth (Difco, Detroit, Mich.) cultures were centrifuged and washed in phosphate-buffered saline (pH 7.2), and the concentration was adjusted to approximately 2.0×10^7 organisms per ml with a Gilford spectrophotometer. The final incubation mixture, consisting of 1.0 ml, usually contained 5.0×10^6 PMN, 10% antiserum, and approximately 2.0×10^6 *S. aureus* organisms. Control tubes were also included to determine the effect of serum or phagocytes alone on the organisms. All tubes were tumbled slowly on a rotating rack (Scientific Industries Inc., Springfield, Mass.) at 37°C , and samples were taken from the phagocytic mixture with an Eppendorf pipette at timed intervals. Each sample was diluted in distilled water to lyse the leukocytes, and the dilutions were plated on Staphylococcus Medium 110 (Difco) to determine colony-forming units. From these data the number of surviving bacteria was calculated and plotted against incubation time on semilogarithmic graph paper.

RESULTS

It has been suggested that the sera of patients with chronic staphylococcal osteomyelitis are virtually free of any heat-stable opsonins directed against the etiological agent of the disease process (19). Illustrated in Fig. 1 are the results of the interaction of *S. aureus* Mardi, isolated from the bone of an osteomyelitis patient, with homologous convalescent and nonimmune human serum in the presence of human PMN. It should be noted that strain Mardi, in the presence of normal rabbit serum, was resistant to phagocytosis by PMN. However, in the presence of homologous convalescent serum, the PMN were markedly effective in phagocytizing the viable organisms. Normal adult human serum and homologous rabbit anti-Mardi serum were also very effective in promoting in vitro phagocytosis by PMN. These results indicate that strain Mardi has surface antigens that can impede in vitro phagocytosis by PMN, and, further, that opsonins directed against the cell surface of these organisms are required for effective bactericidal activities. In addition, since opsonins directed against strain Mardi were observed in the convalescent human serum, this organism may have been associated with the patient's disease process.

Isolation of surface antigens from strain Mardi. Previous studies by Yoshida et al. indicated that *S. aureus*, propagated on artificial laboratory medium, frequently loses certain antigenic surface components (31). In view of this

FIG. 1. The interaction between non-encapsulated *S. aureus* Mardi and human PMN in the presence of normal rabbit serum, convalescent human serum, adult human serum, and rabbit anti-homologous serum.

observation, a representative colony of strain Mardi which survived *in vitro* phagocytosis by PMN (Fig. 1) was isolated and grown in bulk by a previously described method (11). Unwashed cells obtained from 20 liters of culture were extracted sequentially, and each extract was subjected to diethylaminoethyl-cellulose chromatography as previously described (7). Illustrated in Fig. 2 are the results of the chromatographic analysis of an acid hydrolysate of a purified

antigenic preparation obtained from whole cells by the pH 7.0 extraction methods. The results of the chromatographic analysis revealed the presence of galactose and an unknown sugar which was eluted off the ion-exchange column at approximately the same position as xylose (Fig. 2A). However, gas-liquid chromatographic analysis of the alditol acetate derivative of the hydrolysate indicated that the unknown sugar was not xylose. At the present time, the unknown sugar has not been identified. Quantitative chemical analysis indicated that this antigen consisted of 28.6% galactose, 6.3% unknown sugar (reference, xylose), and 32.7% of a carbazole-positive component (reference, glucuronic acid). Since immunoelectrophoresis studies suggested that this polymer was acidic in nature, the antigen was subjected to sodium borohydride reduction and subsequently analyzed (3). Chemical analysis of the acid hydrolysate of the reduced polymer revealed the presence of galactose and glucose, whereas the hydrolysate of the unreduced polymer was devoid of glucose (Fig. 2B). Quantitatively, the reduced polymer consisted of 30.7% galactose, 5.0% unknown sugar, and 46.5% glucose. The remaining constituents probably represented glucuronic acid or degradation products. These results suggest that the acidic polymer consists of glucuronic acid and galactose.

Isolation of cellular constituents of strain Mardi. Teichoic acid consisting of the typical constituents, namely ribitol, glucosamine, alanine, and phosphorus, was readily isolated and

FIG. 2. (A) Carbohydrate analysis of purified untreated acidic antigen. (B) Carbohydrate analysis of the acidic antigen after borohydride reduction. Methods described in the text.

purified from the cold-trichloroacetic acid-extracted cells by the method previously described by Park and Hancock (20). The trichloroacetic acid-treated cells were subsequently extracted with formamide by the method of Krause and McCarty (14). The resulting mucopeptide was purified according to the method of Karakawa and Krause (10). Chemical analysis of the mucopeptide revealed the presence of the following typical constituents: glucosamine ($0.56 \mu\text{mol}/\text{mg}$), lysine ($0.80 \mu\text{mol}/\text{mg}$), glutamic acid ($0.67 \mu\text{mol}/\text{mg}$), alanine ($1.20 \mu\text{mol}/\text{mg}$), and glycine ($2.90 \mu\text{mol}/\text{mg}$).

Illustrated in Fig. 3 are the results of the quantitative precipitin reaction between each of the isolated cellular antigens of strain Mardi and homologous antiserum. Rabbits immunized with strain Mardi consistently produced antibodies that were directed against all of the cellular constituents, namely, the acidic antigen, teichoic acid, and mucopeptide (Fig. 3). It is interesting that all of the animals immunized with this organism consistently formed higher titers of antibodies against the cell wall teichoic acid than the acidic polymer. In addition, most of the antisera contained only low levels of antibodies against mucopeptide. Antisera consisting of these antibodies were subsequently employed in the *in vitro* phagocytosis test.

Immunochemical analysis of the acidic polymer. Illustrated in Fig. 4 are the results of

FIG. 3. Quantitative precipitin reaction between purified teichoic acid, acidic CHO, and mucopeptide isolated from strain Mardi and rabbit anti-homologous serum.

FIG. 4. Quantitative precipitin reaction between both untreated and borohydride-reduced acidic antigen and homologous antiserum.

the quantitative precipitin reaction between the acidic polymer before and after borohydride reduction and homologous antiserum. As depicted in the figure, the unreduced antigen effectively reacted with the antiserum, whereas the reduced polymer gave only a minimal precipitin reaction. These results suggest that the glucuronic acid may represent a major immunodeterminant of the polymer.

Heat-stable opsonins present in rabbit anti-Mardi serum. Previous *in vitro* studies (Fig. 1) suggest the presence of serum opsonins in rabbit anti-Mardi serum. In an extension of this observation, it was noted that rabbits immunized with strain Mardi produced at least three specific antibodies, namely anti-teichoic acid, anti-acidic polymer, and antimucopptide antibodies. *In vitro* phagocytosis studies employing antiserum adsorbed with the three purified cellular antigens indicated that the anti-acidic polymer and antimucopptide antibodies were involved in the opsonization of the organism, whereas the anti-teichoic acid antibodies had minimal opsonizing effects. The ineffectiveness of teichoic acid antibodies as opsonins is consistent with previous observations (9). The capacity of mucopeptide antibodies to opsonize *S. aureus* was previously suggested (23), and the results presented in this study substantiate this view. For example, phagocytosis studies indicated that rabbit anti-Mardi serum (Fig. 5A), adsorbed with purified mucopeptide, was approximately 50% less reactive than the unadsorbed serum. Human serum after adsorption with mucopeptide was also noticeably less reactive than the control antiserum (Fig. 5B). These results sug-

FIG. 5. The interaction between *S. aureus* strain Mardi and human PMN in the presence of nonimmune and immune sera. (A) Rabbit anti-Mardi serum and serum adsorbed with mucopeptide. (B) Human serum and human serum adsorbed with mucopeptide.

gest that antimucopeptide antibodies may be capable of promoting the opsonization of strain Mardi cells. In an attempt to substantiate this view, purified Mardi mucopeptide was solubilized by the method previously described (10), and the mucopeptide specificity of this cellular antigen was tested by a quantitative precipitin reaction with anti-A-variant streptococcal serum, rich in mucopeptide antibodies (8). As illustrated in Fig. 6, Mardi mucopeptide gave a very strong reaction with the anti-A-variant serum; reaction with anti-*S. aureus* D serum and the homologous anti-Mardi serum was less reactive. These results indicate an immunochemical reactivity between the A-variant serum, rich in mucopeptide antibodies, and Mardi staphylococcal mucopeptide. This reactivity may be due to the chemical analogy between streptococcal and staphylococcal mucopeptide, as previously suggested, and not due to staphylococcus-specific antigens associated with the mucopeptide preparation (8). The following experiment substantiates the view that mucopeptide antibodies are capable of opsonizing *S. aureus* strain Mardi and that these antibodies are a common feature of sera derived from animals immunized with bacteria. Depicted in Fig. 7 are the results of the in vitro opsonization of strain Mardi in the presence of *Escherichia coli*, A-variant streptococcal, and homologous antisera by hu-

man PMN. Note that both A-variant antiserum and *E. coli* antiserum were significantly effective in opsonizing strain Mardi. *E. coli* serum adsorbed with Mardi mucopeptide was no longer effective in opsonizing the organism. These results indicate that mucopeptide antibodies are effective in promoting the opsonization of strain Mardi. In view of this observation, it is feasible to assume that the acidic antigen of strain Mardi, although antiphagocytic, represents a possible microcapsule, and that portions of the cell surface consist of an exposed mucopeptide matrix which is accessible for binding by antimucopeptide antibodies. The availability of the mucopeptide site may account for the opsonic effects of mucopeptide opsonins as observed by Peterson et al. (23).

Isolation of a variant strain of Mardi. Park et al. have shown that *S. aureus* grown in medium consisting of a selecting agent such as anti-teichoic acid often formed a variant that can produce a teichuronic acid polymer (21). Strain Mardi was subjected to 3 h of incubation

FIG. 6. Quantitative precipitin reaction between ultrasonically solubilized *S. aureus* strain Mardi mucopeptide and anti-A-variant streptococcal serum, anti-D staphylococcal serum, and rabbit anti-Mardi serum.

FIG. 7. The interaction between *S. aureus* strain Mardi and human PMN in the presence of anti-A-variant streptococcal serum, homologous rabbit antiserum, and *E. coli* antiserum.

in the presence of mucopeptide antibodies and human PMN. In this incubation process, it was assumed that Mardi organisms consisting of exposed mucopeptide would be opsonized, whereas cells containing a masked mucopeptide matrix would be allowed to proliferate. Utilizing this procedure, it was possible to isolate a variant

that formed mucoid colonies. This variant was grown in bulk culture, and the unwashed whole cells were extracted with pH 7.0 buffer at 100°C as previously described for the wild type. Illustrated in Fig. 8 are the results of quantitative precipitin reactions of the pH 7.0-extracted carbohydrate preparations (CHO) of the wild-type and the variant (mutant) Mardi strain with anti-wild type and anti-variant antisera. Note that the variant CHO (Fig. 8A) gave a strong reaction with the homologous serum, whereas the wild type CHO gave only a minimal reaction with the variant antiserum. In contrast (Fig. 8B), the wild-type CHO gave a strong reaction with the homologous serum; however, it should be noted that the variant CHO gave a significant reaction with the wild-type antiserum. Subsequent purification of the variant CHO indicated that the observed precipitin reaction of the variant CHO and wild-type antiserum was due to the presence of small amounts of the galactose-glucuronic acid polymer, a constituent of the wild type. With the presence of the wild-type polymer, these results indicated that the variant may represent a phenotypic variation of the original wild-type culture rather than a laboratory contaminant. In an attempt to determine the possible association of the variant strain and the patient's disease process, a series of 30 colonies were isolated from the original broth culture of the bone fragment. It is interesting that the majority of the colonies were of the wild type; however, five colonies were representative of the variant type. Immunological analysis of the pH

FIG. 8. (A) Quantitative precipitin reaction between the mutant or variant CHO, wild-type CHO, and anti-variant serum. (B) Quantitative precipitin reaction between the variant CHO and wild-type CHO and anti-wild type serum.

7.0 extracts of these cells supported the view that these organisms were immunologically analogous to the variant strain isolated from the wild-type culture. Depicted in Fig. 9 are the results of the *in vitro* phagocytosis of the wild-type and the variant strains by human PMN in the presence of homologous convalescent serum, adult human serum, and pooled human immunoglobulins. Note that the wild type (Fig. 9A) was readily opsonized in the presence of adult human serum. In the presence of Mardi homologous convalescent serum, the degree of opsonization was significant, but not as effective as the adult serum. When the variant strain was used, the adult and pooled immunoglobulins were not effective in opsonizing the organisms. In contrast, Mardi homologous convalescent serum was very effective in opsonizing the variant strain. These results suggest that this individual possessed a high level of opsonins against the variant strain and may have been exposed to this same variant strain or one with a similar surface antigen. It is interesting that the original bone-fragment culture from the patient consisted of a mixed population of organisms, namely, organisms which reacted strongly with rabbit anti-Mardi wild-type serum and rabbit anti-Mardi variant serum. These results clearly underscore the need for careful colony selection for biological assays.

Immunochemical analysis of the surface variations in surface structure may play a role

antigen of the variant strain. Extracts from the variant strain (pH 7.0) were subjected to cetavalon precipitation (17) as previously described, and the extract was purified by ion-exchange chromatography. Gas-liquid chromatography of the acid hydrolysate of both the variant antigen and the borohydride-reduced antigen revealed the presence of galactose and amino-galacturonic acid. Immunochemical studies suggested that amino-galacturonic acid is the major immunodominant determinant. This view was derived from the observation that the reduced polymer was only really reactive with the homologous serum. Further, an immunological cross-reactivity was observed with the variant CHO and the polysaccharide capsule of another osteomyelitis strain, designated strain D, which consisted of galactose and an immunodeterminant amino-galacturonic acid polymer (Fig. 10). Note that the variant CHO (Fig. 10, well 2) reacted with both anti-D and homologous sera to form lines of identity. Similar results were obtained between the D CHO (well 4) and anti-serum. Adsorption studies employing purified D CHO further substantiate the view that both D and the variant carbohydrates are immunochemically identical.

DISCUSSION

It has been suggested that the surface mosaic of *S. aureus* under *in vivo* conditions may be more diverse than once thought and that these

FIG. 9. Interaction between strain Mardi and human PMN in the presence of normal rabbit, human pooled immunoglobulin (γ -globulin), and homologous convalescent serum. (A) Wild-type organism; (B) variant strain.

FIG. 10. Immunodiffusion studies in agar gel between variant CHO, acidic capsule of strain D, anti-strain D serum, and anti-Mardi variant serum. Well 1, anti-D serum; well 2, variant CHO; well 3, anti-variant serum; well 4, strain D CHO.

in repeated staphylococcal infections (4, 25). Yoshida et al. have shown that 4% of fresh clinical isolates of *S. aureus* are encapsulated, but most lose their capsules after a few subcultures on artificial medium (31). These observations suggest a direct relationship between the staphylococcal surface mosaic and the culture conditions.

The results presented in this report support the view that fresh clinical isolates of *S. aureus* possess antiphagocytic surface antigens and that these strains may possess the genetic capacity to form variants under adverse conditions (21). Evidence for this view was obtained from various methods. First, an antiphagocytic surface antigen consisting of galactose and glucuronic acid was isolated from the wild-type strain originating from a patient's bone fragment. Secondly, an acidic antiphagocytic polymer was isolated from a variant strain derived from culture of the wild type grown in the presence of antimucopetide antibodies and human PMN. The variant was suggested to be derived from the wild type rather than a laboratory contaminant, due to the fact that it consisted of small amounts of the antiphagocytic galactose-glucuronic acid polymer associated with the wild type. In addition, this variant strain was also isolated from the original bone-fragment culture. Third, in vitro phagocytosis studies employing the patient's convalescent serum indicated that opsonins with wild-type specificity were present in the patient's convalescent serum, as well as opsonins which were directed against the variant strain. The presence of a high titer of antibodies directed against the variant strain was attributed to the fact that this individual had previous exposure to organisms that carried the variant polysaccharide.

Chemical analysis indicated that the acidic polysaccharide of the wild type consisted of galactose and glucuronic acid. Immunochemical evidence suggested that the polymer, which consisted of immunodominant glucuronic acid antigen, was a significant factor in impeding phagocytosis by PMN. However, it was demonstrated that purified mucopetide was capable of adsorbing a portion of the opsonic activity of the anti-wild type serum. This suggested that the mucopetide matrix may be exposed in the wild type and therefore the antiphagocytic antigen may in fact represent a microcapsule. The availability of the mucopetide in the wild type predisposed these organisms to the opsonic activity of the available mucopetide antibody normally found in serum. This view is supported by the fact that antimucopetide antibodies present in A-variant streptococcus and *E. coli* antisera were shown to be effective in the opsonization of the Mardi wild type. This cross-reactivity between these heterologous bacteria via mucopetide is not surprising in view of the similarity of chemical composition and structure among all bacterial mucopetides. The occurrence of mucopetides in all bacteria insures prolonged and continuous exposure of animals and humans to these substances and provides a means of natural acquisition of antibodies to mucopetide. It is conceivable that the immune response to this family of polymers may play a role in the observed opsonic capacity of normal human serum. In addition, under in vivo conditions, the mucopetide opsonins may play a role in the elimination of the nonencapsulated strains of *S. aureus* and therefore may be a selective factor which can account for the fact that clinical isolates possess great levels of antiphagocytic antigens (27).

In this study, the wild type was subjected to in vitro phagocytosis for 3 h in the presence of antimucopetide serum and human PMN; and a variant strain was isolated which was shown to be completely resistant to the opsonic activity of the mucopetide serum. Antiserum prepared against the variant strain was shown to be opsonic for the homologous organisms and not the wild type. Chemical analysis of the antiphagocytic antigen of the variant strain revealed a polymer consisting of galactose and amino-galacturonic acid; the question as to whether or not the variant was in fact associated with the patient's disease is only speculative. However, in vitro phagocytosis studies in the presence of the patient's convalescent serum and human PMN clearly revealed the existence, in high titer, of opsonins directed against the variant strain. In spite of the initial isolation of the wild type, the

titer of the variant carbohydrate antibodies was higher in the convalescent patient serum than that of the wild-type antiphagocytic carbohydrate antibodies. The presence of the variant type in the original specimen culture can, in part, explain the presence of high levels of anti-variant antibodies in the convalescent serum. This observation clearly suggests that a careful examination of the colony types of original cultures is required for any study dealing with the etiology of type-specific staphylococcal infections.

The results presented in this study substantiate the view that *S. aureus* strains may carry immunologically significant surface antigens under in vivo conditions and may have the potential to produce a variety of teichuronic acids under clinical conditions. Strains which possess these surface structures can mask the available mucopeptide matrix, thereby impeding their opsonization by mucopeptide opsonins and subsequent phagocytosis by PMN. Elucidation of this observation may reveal whether a wide variety of teichuronic acids exist among staphylococci and whether or not phenotypic alteration may play a role in staphylococcal infection and reinfection.

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