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Cell Wall Composition and Associated Properties of Methicillin-Resistant *Staphylococcus aureus* Strains

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Methicillin-resistant (MR) *Staphylococcus aureus* strains have previously been reported to be deficient in surface negative charge; this has been correlated with methicillin resistance and ascribed to a deficiency of teichoic acid at the cell surface (A. W. Hill and A. M. James, Microbios **6**:157-167, 1972). Teichoic acid was present in walls of MR organisms as revealed by appreciable phosphate levels and detection of ribitol residues. Phosphate levels in walls from five MR strains (0.54 to 0.77 μmol/mg of wall) were lower than in three unrelated methicillin-sensitive (MS) strains (0.86 to 1.0 μmol/mg of wall). However, two MS strains derived from two of the MR strains had wall phosphate levels very similar to those of the MR strains. No evidence for unusual wall polymers was found. Simple deficiency of wall teichoic acid does not result in methicillin resistance since an independently isolated teichoic acid-deficient strain (0.1 μmol of phosphate per mg of wall) was not methicillin resistant. In studies of biological properties possibly related to wall teichoic acid, it was discovered that walls isolated from MR organisms grown in the presence of methicillin autolyzed more rapidly than those isolated from organisms grown in the absence of the drug. Since methicillin resistance is enhanced by NaCl and suppressed by ethylenediaminetetraacetate, the effects of these compounds on autolysis of isolated walls were studied. NaCl (1.0 M) and ethylenediaminetetraacetate (1.0 mM) inhibited the autolysis of walls isolated from MR and MS strains. An MR strain bound phage 47, 52A, and 3A only slightly less well than their respective propagating strains.

The mechanism whereby certain *Staphylococcus aureus* strains resist the antibacterial effects of methicillin (and other β-lactamase-resistant penicillins) remains unknown almost 20 years after these drugs were first introduced. Methicillin resistance, or intrinsic resistance as it is sometimes termed, does not appear to be due to enzymatic destruction of the antibiotic. Dyke (4) could find no evidence for production of "methicillinase" activity by 108 epidemiologically distinct methicillin-resistant (MR) *S. aureus* strains. Also, β-lactamase (EC 3.5.2.6)-negative variants of MR *S. aureus* strains retain their resistance to methicillin (5, 25).

James and his co-workers (12-14, 19) have repeatedly stressed that based on cell electrophoresis measurements, MR *S. aureus* strains are deficient in surface negative charge. This has been ascribed to a deficiency of teichoic acid at the cell surface, the presence and absence of which is associated with methicillin sensitivity and resistance, respectively. In the hope that this may be a clue to the molecular mechanisms of methicillin resistance, we isolated cell walls from several MR and methicillin-sensitive (MS) *S. aureus* strains to see if walls from MR organisms were chemically deficient in teichoic acid. Also, in view of the involvement of teichoic acid in the staphylococcal phage receptor site (2), and in autolytic activity in other species (see 28), we have examined the phage typing and binding and autolytic properties of MR and MS strains.

A common characteristic of newly isolated MR *S. aureus* strains is that only 1 cell in 10^5 can give rise to a colony on methicillin-containing agar (22). Various chemical and physical factors alter the degree of resistance expression; e.g. high salt and low temperature allow most of the cells in a culture to express resistance (form colonies on methicillin-containing agar), whereas ethylenediaminetetraacetate (EDTA) decreases resistance expression (22). In view of these findings the MR strains were grown at 30°C in the presence of methicillin in order to have a more homogeneous population for isolation and analysis of walls. The effects of NaCl

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and EDTA on the autolysis of isolated walls were tested since this was a convenient system in which to look for effects of these substances which might be correlated with their effects on methicillin resistance in whole organisms.

MATERIALS AND METHODS

Strains. The following *S.aureus* strains have been maintained in the laboratory of one of us (L.D.S.) for several years: MR—Col (β-lactamase negative), Meuse, Kas, and 592; MS—Oxford. The MR and MS strains 5814R and 5814S, originally isolated from a strain of mixed population (21), were obtained from Ferenc Rozgonyi, Medical University, Debrecen, Hungary. MR strain DU4916 was provided by R. Lacey, North Cambridgeshire Hospital, Wisbech, U.K., and its MS counterpart, DU4916S, was given by J. J. Iandolo, Kansas State University, Manhattan. *S. aureus* H was obtained from Sir James Baddiley's laboratory, University of Newcastle Upon Tyne, Newcastle Upon Tyne, U.K. and teichoic acid-deficient mutant 52A5 was provided by J. T. Park, Tufts University, Boston, Mass. Strain MS1 was a recent clinical isolate from the University of Minnesota Hospital. The strains were maintained on Trypticase soy agar (Difco Laboratories, Detroit, Mich.) slants at 2°C. We are grateful to these workers for their kind gifts of strains.

Cultural conditions. Cells were grown in the peptone-yeast extract-phosphate-glucose (PYK) medium of Gilpin et al. (8) in volumes of 100 or 500 ml in 250- or 1-liter Erlenmeyer flasks, respectively, with shaking (220 rpm) at 30 or 37°C in either the presence or absence of methicillin.

Antibiotic MIC determinations. Organisms were grown standing in test tubes at 37°C for about 6 h before determination of antibiotic minimum inhibitory concentration (MIC) in PYK medium with glucose omitted. The turbidity of the culture was adjusted to an absorbance at 625 nm of about 0.08 (McFarland standard no. 0.5). Fifty microliters of a 1:100 dilution of this was added to 50-μl amounts of PYK medium minus glucose containing twofold dilutions of antibiotics in a Microtiter (7). The final inoculum concentration was about 10^5 colony-forming units/ml. Incubation was at 30 or 37°C with examination after 24 and 48 h, and the MIC was read as the first concentration showing no turbidity. D-Cycloserine was purchased from Sigma Chemical Co., St. Louis, Mo. The other antibiotics were the generous gifts of their respective manufacturers to L. D. Sabath.

Population analyses. The percentage of the population expressing methicillin resistance was estimated by comparing the colony count on drug-free PYK agar with that obtained on PYK agar supplemented with methicillin (50 μg/ml).

β-Lactamase production. β-Lactamase production was determined by the Haight-Finland (11) modification of the Gots test (9).

Cell wall preparation and analysis. The methods for cell wall preparation and analysis have been detailed in previous publications from this laboratory (10, 20). Briefly, organisms were broken by shaking with glass beads, autolytic activity was destroyed by heating crude walls at 100°C for 15 min, and walls were purified by ribonuclease, deoxyribonuclease, trypsin, and 40% phenol treatments. Amino acids and amino sugars in walls were estimated on the amino acid analyzer after hydrolysis in 6 M HCl for 18 h at 105°C. Phosphorus and hexosamine were estimated colorimetrically as described in references 17 and 18. The method of Dische (3) was used to test for the presence of uronic acid. Teichoic acids were extracted by heating walls in 10% (wt/vol) trichloroacetic acid at 60°C for 90 min. Trichloroacetic acid was removed by five ether extractions, and the teichoic acid-containing solution was made 2 M in HCl and hydrolyzed at 105°C for 3 h. Both untreated and alkaline phosphatase-treated hydrolysates were examined by paper chromatography for reducing sugars and amino compounds (10, 20). The teichoic acid extract was made 4 M in HCl and hydrolyzed for 4 h at 105°C for estimation of hexosamine. Walls retaining autolytic enzyme activity were prepared by omitting heating and other treatments during preparation. They were washed once with water and twice with 0.01 M KPO_4 buffer (pH 7.0) and stored at −15°C.

Phage typing and binding. Organisms were typed at routine test dilution (RTD) and 100 RTD, using the international set of human *S. aureus* phages, by the method of Blair and Williams (1). Irreversible phage adsorption at 37°C was measured by incubating phage lysate (0.1 ml, 10^5 plaque-forming units) with 0.9 ml of a heat-killed (70°C, 30 min) *S. aureus* culture grown in Trypticase soy broth (Difco) supplemented with 0.4 mg of $CaCl_2$ per ml for 18 h at 37°C. Samples (0.1 ml) were removed at various times and were diluted 1:50 in Trypticase soy–$CaCl_2$ broth to stop further adsorption. Samples (0.1 ml) of appropriate dilutions were assayed for plaque-forming units by mixing with 0.1 ml of a live overnight broth culture of the homologous propagating strain in 3.0 ml of molten, soft (0.7%, wt/vol) Trypticase soy agar (Difco) supplemented with 0.4 mg of $CaCl_2$ per ml at 52°C. This was overlaid onto a Trypticase soy agar plate, allowed to solidify, and incubated overnight at 30°C.

Wall autolytic activity. Walls retaining autolytic enzyme activity were resuspended in 0.01 M KPO_4 buffer (pH 7.0) containing various experimental additions at an absorbance at 625 nm of 0.5 to 0.7. Absorbance readings at 625 nm were taken in a Spectronic 20 (Bausch & Lomb Scientific Optical Products Division, Rochester, N.Y.) at hourly intervals during incubation at 37°C.

RESULTS

The primary question this study set out to answer was whether the walls of MR *S. aureus* strains were chemically deficient in teichoic acid. For this purpose several strains were selected for study, some of which may have advantages for studying the methicillin resistance phenomenon. For example, MS counterparts to MR strains DU4916 and 5814R were available; also, strain DU4916 has been extensively studied from a genetic viewpoint and shows a high expression of methicillin resistance (15). Strain Col is β-lactamase negative and is thus a model of pure

intrinsic resistance. Several MS strains were studied for comparison; Oxford and H are two well-known laboratory strains, and MS1 was a recent clinical isolate.

Antibiotic resistance patterns. As part of a general characterization of the strains (16), their susceptibilities to a range of antibiotics including various cell wall antibiotics were determined (Table 1). The production of β-lactamase activity and enterotoxin B was also determined. The MR strains all had high MICs for methicillin, particularly when the determinations were carried out at 30°C or when incubation time was extended to 48 h (data not shown; 22). The MICs of strains Meuse and 5814R, in particular, increased with extended times or lowered temperatures of incubation. However, under our experimental conditions strain 5814R was not as resistant to methicillin as in those of Rozgonyi (21). Lower incubation temperatures and longer incubation times did not increase the methicillin MICs for MS strains. The MR strains were resistant to other β-lactamase-resistant penicillins tested and to benzylpenicillin and cephalothin. Strain Col had moderate resistance to benzylpenicillin even though a β-lactamase was not produced (Table 1); the other MR strains all produced β-lactamase. Strains 5814S and MS1 were β-lactamase-producing MS strains. When other cell wall antibiotics were tested, cycloserine, bacitracin, novobiocin, and vancomycin, the MR strains did not show increased resistance as compared with the MS organisms. As is often found in MR *S. aureus* (16), the MR strains were resistant to tetracycline, streptomycin, and sulfamethoxazole. The MICs of the MS strains are shown for comparison; Oxford and H were not resistant to most of the antibiotics tested. The MS strains 5814S and DU4916S which were derived from strains 5814R and DU4916 retained resistance to sulfamethoxazole, streptomycin, and tetracycline. All of the MR organisms produced enterotoxin B as determined by the methods described in reference 26 (J. J. Iandolo, personal communication), whereas none of the MS strains did. Enterotoxin B production and methicillin resistance often occur in the same strains (16).

Cell wall composition. The chemical analyses of walls of the organisms are shown in Table 2. All of the MR strains were grown at 30°C in the presence of methicillin (50 μg/ml) to increase resistance expression and hence have a more homogeneous population for chemical analysis (22). These conditions did result in relatively homogeneous populations (Table 2), where most of the inoculum of MR organisms formed a colony in the presence of methicillin. It was anticipated that these conditions should reveal whether or not the walls of MR organisms were chemically deficient in teichoic acid since correlation between increased methicillin resistance and decreased surface charge at this temperature has been shown (13). Colorimetric estimation revealed appreciable amounts of wall phosphate, and typical teichoic acid hydrolysis products (20), including ribitol, were detected on chromatography of a hydrolysate of a hot trichloroacetic acid extract of walls. These observations clearly establish that the walls of these MR strains contain a ribitol teichoic acid which is a species characteristic of *S. aureus* (10). However, the phosphate levels were lower in the walls of the MR strains (0.54 to 0.77 μmol/mg [dry weight]) than in MS strains Oxford, H, and MS1 (0.86 to 1.0 μmol/mg). Strains DU4916S and 5814S had phosphate levels very similar to those of their MR counterpart strains. The walls of the teichoic acid-deficient mutant, strain 52A5, contained 0.1 μmol of phosphate per mg.

TABLE 1. *Antibiotic susceptibilities and β-lactamase production of MR and MS S. aureus strains*

Strain	Antibiotic[a] MIC (μg/ml)[b]													β-Lactamase
	Met	Naf	Oxa	Ben	Cep	Chl	Tet	Str	Sul	Bac	Cyc	Van	Nov	
MR														
Col	800	400	1,600	25	200	1,600	100	>1,600	>1,600	100	200	<0.8	0.8	−
Meuse	25	12.5	25	>1,600	25	25	100	>1,600	>1,600	12.5	25	<0.8	<0.8	+
592	1,600	100	400	400	50	50	100	>1,600	>1,600	50	25	0.8	0.8	+
5814R	25	6.2	12.5	400	3.1	3.1	100	1,600	>1,600	6.2	25	<0.8	<0.8	+
DU4916	1,600	400	800	400	200	200	100	>1,600	>1,600	25	100	<0.8	0.8	+
MS														
Oxford	3.1	<0.8	<0.8	<0.8	<0.8	50	<0.8	3.1	200	100	50	<0.8	<0.8	−
H	3.1	<0.8	<0.8	<0.8	<0.8	6.2	<0.8	>1,600	400	100	50	<0.8	<0.8	−
MS1	12.5	1.6	1.6	1,600	1.6	12.5	<0.8	3.1	800	50	100	<0.8	<0.8	+
5814S	1.6	<1.6	<1.6	>1,600	<0.8	25	25	400	>1,600	3.1	6.2	<0.8	<0.8	+
DU4916S	<0.8	<1.6	<0.8	<0.8	<0.8	1.6	100	1,600	>1,600	3.1	12.5	<0.8	<0.8	−

[a] Abbreviations: Met, methicillin; Naf, nafcillin; Oxa, oxacillin; Ben, benzylpenicillin; Cep, cephalothin; Chl, chloramphenicol; Tet, tetracycline; Str, streptomycin; Sul, sulfamethoxazole; Bac, bacitracin; Cyc, cycloserine; Van, vancomycin; Nov, novobiocin.
[b] Values after 24 h of incubation at 37°C.

TABLE 2. *Cell wall composition of MR and MS S. aureus strains*[a]

Strain	% Resistance expression	Wall component (μmol/mg)[b]							Trichloroacetic acid extract		% Wt of wall[c] as:	
		Pi	Mur	Glu	Gly	Ala	$GlcNH_2$	Lys	Ribitol	Hexosamine-Pi ratio	Teichoic acid	Peptidoglycan
MR												
Col	NT[d]	0.59	0.40	0.51	2.4	1.4	0.73	0.47	NT	0.86	24.2	58.0
Meuse	76	0.77	0.29	0.50	1.7	1.3	0.53	0.52	+	1.3	31.5	56.7
Kas	39	0.64	0.23	0.46	1.3	1.2	0.39	0.43	+	NT	26.2	51.2
592	116	0.70	0.37	0.56	1.7	1.4	0.58	0.52	+	1.0	28.7	63.7
5814R	14	0.54	0.34	0.41	2.0	1.0	0.53	0.39	+	0.89	22.1	46.8
DU4916	114	0.56	0.20	0.39	1.4	0.95	0.36	0.38	+	0.82	22.9	43.9
MS												
Oxford	0	0.86	0.29	0.50	2.0	1.0	0.53	0.46	+	0.97	35.2	56.9
H	0	1.0	0.18	0.28	1.8	0.85	0.68	0.44	+	NT	41.0	50.5
MS1	0	0.91	0.32	0.59	2.7	1.3	0.62	0.61	+	1.2	36.9	67.3
5814S	0	0.62	0.19	0.38	1.2	0.79	0.41	0.32	+	0.87	25.4	43.4
DU4916S	0	0.52	0.33	0.53	2.3	1.2	0.70	0.53	NT	0.88	21.3	60.3

[a] MR organisms were grown with methicillin (50 μg/ml) for 24 h at 30°C; MS organisms were grown for 18 h at 30°C, except for H where the data were taken from reference 19. Inocula of MR organisms were previously grown with the antibiotic (50 μg/ml) for 24 h at 30°C.

[b] Abbreviations: Pi, phosphate; Mur, muramic acid; Glu, glutamic acid; Gly, glycine; Ala, alanine; $GlcNH_2$, glucosamine; Lys, lysine.

[c] Calculated using a formula weight of 410 for teichoic acid, 1,138 for peptidoglycan, and the glutamic or lysine value (20).

[d] NT, Not tested.

In an experiment where the organism was grown at 30°C in the absence of methicillin, walls of strain 5814R contained 0.56 μmol of phosphate per mg compared to 0.54 and 0.62 μmol of phosphate per mg of walls of methicillin-grown 5814R and non-methicillin-grown 5814S, respectively. This indicates that wall phosphate levels are not artificially depressed due to growth in the presence of the drug. If it is assumed that ribitol and phosphate are equimolar (20) and each ribitol residue is substituted with an *N*-acetyl-D-glucosamine residue (since the hexosamine and phosphate ratios in the hot trichloroacetic acid extract approach unity), then the approximate weight of the wall accounted for by teichoic acid can be calculated. In the MR strains teichoic acid accounts for 22 to 31.5% of wall weight and from 35 to 40% of wall weight of strains Oxford, MS1, and H (Table 2).

Amino acid analysis of walls revealed typical components of the *S. aureus* peptidoglycan. Some of the MR strains had somewhat lower glycine levels than is usual for *S. aureus* (10). This may be related to growth in the presence of methicillin. For several of the strains 90% or more of the weight of the wall could be accounted for as peptidoglycan and teichoic acid components. In strains DU4916, 5814R, and 5814S only about 70% of wall weight was accounted for.

James and Al-Salihi (14) have speculated that additional or different wall polymers may be present in the walls of MR organisms. In some species, including *S. aureus*, uronic acid-containing polymers replace teichoic acid in walls when synthesis of teichoic acid is induced by phosphate limitation (6). The walls and trichloroacetic acid extracts of walls were negative in the uronic acid estimation. However, this method would not apparently serve to detect aminouronic acids (3). Examination of the chromatograms of the wall hot trichloroacetic acid hydrolysate did not reveal any unusual components. Neither were unusual components noted on the amino acid analyzer. Amino acid analysis of walls of strains 5814R and 5814S that had not been heated or subjected to cleaning treatments did not reveal large amounts of nonpeptidoglycan amino acids to be present. This makes it unlikely that large amounts of protein are present in the walls of MR strains. Thus, there was no readily apparent evidence for the presence of unusual wall polymers.

In view of the potential relationship of wall teichoic acid to methicillin resistance, the methicillin susceptibility of the teichoic acid-deficient mutant was determined. This strain was sensitive to 3.1 μg of methicillin per ml and resistance could not be produced by longer incubation times or lower incubation temperatures.

Biological properties of MR and MS strains. Since the walls of the MR organisms

were not grossly different in chemical composition from those of MS strains, we sought to examine some biological properties which may be more sensitive indicators of subtle alterations in wall features. We chose to examine rates of autolysis of isolated walls since teichoic acid-deficient mutants of some species are deficient in autolytic activity. Also, phage typing and binding properties were examined in view of the involvement of teichoic acid in the *S. aureus* phage receptor site.

Initially, the rates of autolysis and effects of various agents thereon were compared in walls isolated from methicillin-grown MR strains and MS strains grown in the absence of the drug (Fig. 1b, d, e, and f). In each case the presence of 1.0 M NaCl and 1.0 and 0.1 mM (data not shown) EDTA substantially inhibited autolysis. The presence of 0.1 M NaCl (data not shown) had little effect on the rate of autolysis, whereas 1.0 mM $MgCl_2$ slightly increased the rate of autolysis. When the rates of autolysis of walls isolated from MR strains grown in the absence of the antibiotic were measured (Fig. 1a and c), it was discovered that growth in the presence of methicillin had yielded walls more prone to autolysis. When two MS strains, 5814S and H, were grown with subinhibitory methicillin concentrations (0.5 μg/ml), the rates of wall autolysis noted were similar to those of walls isolated from non-drug-grown organisms (Fig. 1g and h).

Thus, NaCl and EDTA inhibited autolysis in all strains examined; i.e., there was no special effect of NaCl or EDTA on wall autolysis in MR strains that could be correlated with their effect on methicillin resistance in such strains. Furthermore, 1.0 M NaCl did not activate wall autolytic activity as has been reported for the teichoic acid-deficient mutant (30). It may be that in *S. aureus* high salt concentrations dissociate autolysin from the wall, and the inhibitory effects of EDTA might mean a divalent

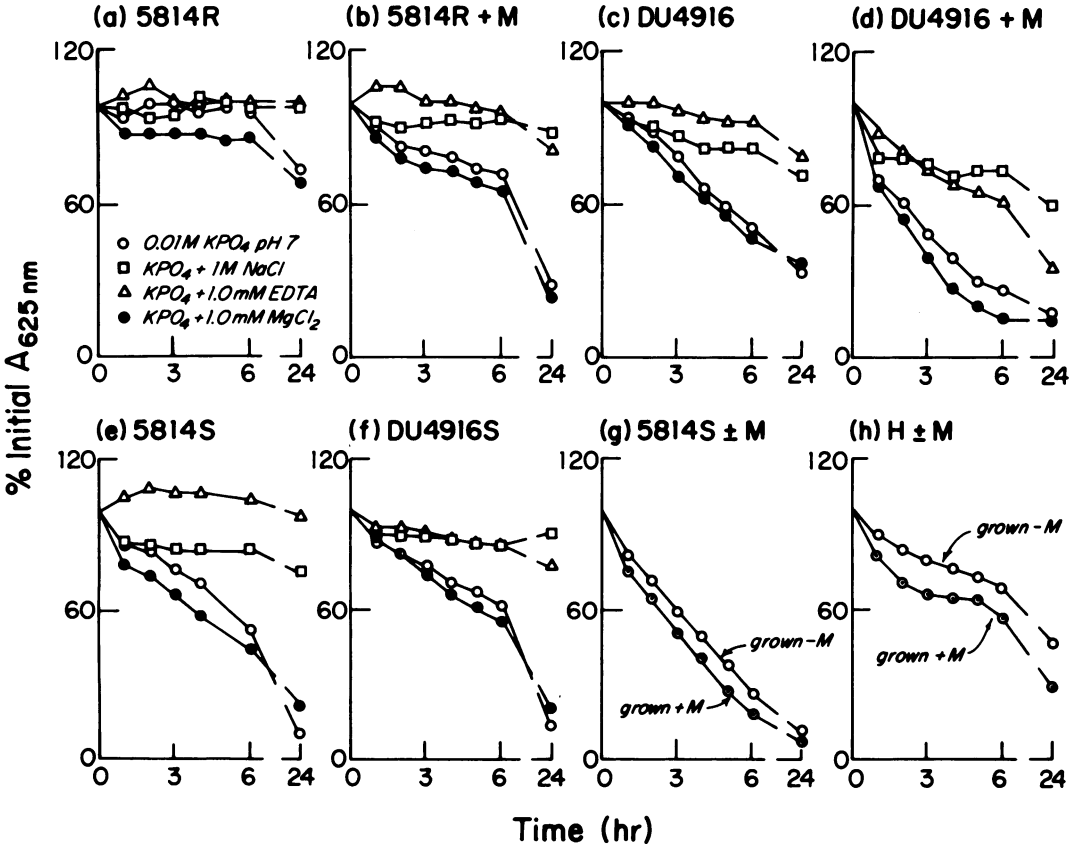

FIG. 1. *Autolysis of walls of MR and MS S. aureus strains. Walls were isolated from MR organisms grown for 24 h at 30°C in the presence (+M) or absence of methicillin (50 μg/ml). Walls were isolated from MS organisms grown for 18 h at 30°C in the presence or absence of methicillin (0.5 μg/ml). $A_{625\,nm}$, Absorbance at 625 nm.*

cation is necessary for wall autolysis.

Strain Col was lysed by phages 77, 84, and 85 as revealed by plaque formation at the RTD in a phage-typing experiment (1). Strains Meuse, Kas, 592, and DU4916 were not susceptible to any of the international human *S. aureus* phages in our hands since they were not lysed at the RTD or 100 RTD (1). Rozgonyi (21) has reported that strain 5814R is susceptible to phage 77 at 100 RTD. These results indicate that, at least, phage are able to bind to the walls of strains Col and 5814R since lytic reactions are observed with some phages. In Table 3 the binding of phages 3A, 52A, and 47 to the nontypable MR strain 592 was compared with binding to their respective propagating strains. Whereas the phage in each case was bound more rapidly and heavily by its propagating strain, the binding of each phage to strain 592 was not much less. Thus, these results make it unlikely that the non-typability of strain 592 is due to inability of phage to bind to its cell surface.

DISCUSSION

The wall composition of several MR *S. aureus* strains, well characterized in antibiotic resistance patterns and quite homogeneous in expression of methicillin resistance, has been compared with that of several MS *S. aureus* strains. The walls of the MR organisms were not grossly deficient in teichoic acid as measured by wall phosphate levels and detection of ribitol residues on paper chromatograms. Somewhat lower wall phosphate levels were found in the MR organisms compared to three unrelated MS organisms. However, MS isolates from two of the MR strains, which were hence of close genetic backgrounds, had wall phosphate levels very similar to those of their MR counterparts. The MR strains had fivefold-higher or greater wall phosphate levels than a bona fide teichoic acid-deficient mutant. To determine the significance of the somewhat lower wall phosphate levels observed, a large number of MR and MS *S. aureus* strains would have to be examined. During the course of this work Vernon and Russell (27) have reported that walls of two MR strains had lower teichoic acid levels than an MS strain based on the weight of wall material removed by cold trichloroacetic acid extraction of isolated walls.

TABLE 3. *Binding of phage by propagating strains and MR S. aureus strain 592*

Time (min)	% Phage unbound					
	Phage 47		Phage 3A		Phage 52A	
	PS[a] 47	592	PS 3A	592	PS 52A	592
5	4.0	21	12	28	2.1	9.0
10	0.5	8	6	11	1.9	8.5

[a] PS, Propagating strain.

Initially, it seemed that a clear demonstration of chemical deficiency of teichoic acid in walls of MR organisms may be realized. Hill and James (13) reported that surface charge decreased with lowering temperature, and this correlated with increased methicillin resistance. Also, a methicillin-dependent, resistant derivative of *Pediococcus cerevisiae*, produced by repeated subcultivation of the parent in the presence of methicillin, had walls that were clearly deficient in teichoic acid (28). This study rules out the possibility that the reported decreased surface negative charge of MR *S. aureus* strains is due to a total absence of teichoic acid in the wall of such organisms. It remains possible that deficiency of surface charge is due to a different arrangement of teichoic acid in the walls of MR strains. Alternatively, only a small reduction in the actual amount of teichoic acid may cause a large alteration in surface charge, or other cell surface polymers are present that modify the charge of the organisms. All these possibilities would require more detailed investigation. Here, experiments of limited scope failed to turn up evidence for additional cell surface ploymers.

However, it is clear that lack of teichoic acid in the walls of an organism does not by itself confer methicillin resistance, since the teichoic acid-deficient mutant studied could not be shown to be methicillin resistant. Also, the two pairs of MS and MR strains showed little difference in their wall phosphate levels, thus showing that there is not a simple correlation between wall teichoic acid content and methicillin resistance. Perhaps this result is not surprising since it has been reported that in spite of the heterogeneity of expression of methicillin resistance, all cells of a heterogeneous MR strain have identical electrokinetic properties (12).

In our studies of potentially teichoic acid-related biological properties we did not find any clear-cut evidence for reduced wall autolysis rates or reduced phage binding in MR strains. Strain 5814R walls autolyzed poorly when the organism was grown without methicillin, but strain DU4916 walls autolyzed readily. However, these studies led to the discovery that growth of these two MR strains in the presence of methicillin yielded walls which showed about a doubled rate of autolysis. The explanation and significance of this phenomenon, and its relationship to the mechanism of methicillin resistance (if any), must await further work. Also, since phage can bind to the walls of MR strains, this

implies that teichoic acid in the walls of these organisms is still able to contribute to the phage receptor site.

However, additional definition of the surface properties of MR strains would seem to be worthwhile since a number of studies continue to point in the direction of altered surface properties of MR strains. Sabath et al. (24) have reported that at pH 5.2 MR strains are susceptible to most penicillins and cephalosporins to which they are resistant at pH 7.4. Cephaloridine is an exception to this in that the strains are resistant to this drug at either pH. Since most β-lactam antibiotics are acids, but a neutral molecule, cephaloridine, does not show pH-dependent variation in activity, this phenomenon may be based in some special surface charge properties of MR strains. MR *S. aureus* strains are also reported to be less susceptible to lysostaphin (23) and to be deficient in protein A (27).

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