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Cell surface hydrophobicity and adherence to extra-cellular matrix proteins in two collections of methicillin-resistant *Staphylococcus aureus*

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SUMMARY

Non-specific and specific mechanisms of adherence have been examined in two collections of methicillin-resistant *Staphylococcus aureus* (MRSA). Determination of hydrophobicity by salt aggregation, hydrophobicity indices and of adherence to the extra-cellular matrix proteins fibronectin, vitronectin, laminin and collagen type 1 have failed to reveal any correlation with phage-type, plasmid profile or antibiogram. Further, the strain collections, made over a period of years in two countries, differ markedly in their adherence characteristics; MRSA are heterogeneous in this respect. Such heterogeneity may explain the polarization of views on the epidemicity or 'virulence' of MRSA. With the exception of adherence to collagen a small group of methicillin sensitive *S. aureus* had characteristics intermediate between the two groups of MRSA.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) have attracted much attention for their evident ability to spread once introduced into a hospital ward containing highly susceptible patients. Many authors have pointed out that MRSA are not necessarily more virulent than methicillin-sensitive strains (MSSA), that is they do not cause more serious infection, but that they may be better at the initial colonization which precedes infection [1–4]. A clonal origin for the methicillin resistance gene has been proposed to account for similarities [5].

A prerequisite for colonization is adherence to host tissue [6] and this can be achieved by specific adhesin-receptor mechanisms or by non-specific means. Adhesin-receptor mechanisms include binding to various components of the extra-cellular matrix of host tissues and adhesion of *S. aureus* to collagen [7], laminin [8],

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fibronectin [7, 9–11] and vitronectin [12, 13] has been explored. Among non-specific mechanisms hydrophobic interaction seems to play an important role both in the attachment of bacteria to each other and of bacteria to tissue or foreign bodies [14]. Studies on hydrophobicity have shown for example that pathogenic bacteria adsorbed more strongly to amphiphillic agarose beads than did non-pathogens. In this study, two groups of MRSA were examined using a variety of specific and non-specific adhesion tests.

MATERIALS AND METHODS

Strains

A total of 32 previously characterized MRSA was available from Sweden and 27 from Germany; they were multi-resistant as well as methicillin-resistant. These strains had been collected over a period of years and were generally judged to be epidemiologically independent of each other on the basis of date of isolation, phage-typing pattern using the International Set of phages for *S. aureus* plus two sets of experimental phages, on plasmid profiles and antibiograms. A group of multi-resistant MSSA from a single hospital in the UK was also partially evaluated.

Growth conditions

Organisms were grown at 37 °C on blood agar plates where available or on blood agar base, and stored at 4 °C. Unless otherwise stated, all isolates were cultured on blood agar and grown at 37 °C for 18–20 h prior to use.

Non-specific measures of adherence

All chemicals used were analytical grade.

Salt aggregation testing. Cell hydrophobicity was determined by salting out according to the method of Lindahl and colleagues [15, 16]. Briefly bacterial cells from agar-grown cultures were suspended in phosphate buffered saline (pH 7·2), washed twice and diluted to a concentration of 10^8 cells per ml spectrophotometrically. Bacterial suspensions were mixed with equal volumes of ammonium sulphate solutions of varying molarities on glass slides and observed for aggregation after one minute at room temperature. The lowest molarity at which aggregation was seen was taken to be the hydrophobic value of the isolate. Only 4 of 27 strains had results which differed by one dilution only when assays were repeated after an interval of days.

Hydrophobic interaction chromatography. Retention of bacteria on phenyl-sepharose was determined by a protocol based on the method described by Smyth and colleagues [14]. Phenylsepharose CL4B gel suspensions were equilibrated with 0·01 M sodium phosphate buffer (pH 6·8) containing 4M sodium chloride and poured as columns in pasteur pipettes (height of column 2·0 cm, internal diameter 5 mm).

Bacteria were suspended in the same buffer and adjusted spectrophotometrically to 10% transmission at 620 nm. Five hundred microlitres of the suspension was loaded onto the gel matrix and eluted with 2·5 ml of buffer. The

optical density of the eluate was measured at 540 nm and compared to that of 500 μl of bacterial suspension diluted with 2·5 ml of buffer. Hydrophobicity was expressed as the percentage of bacteria adhering to the column. Results were very variable with the standard deviation ranging from 8–70% of the mean of four or more replicates. All strains were assayed on at least three occasions and the mean reported.

Two-phase partitioning. Three chemical systems were set up to measure the hydrophobicity and the net cell charge of the bacteria in terms of mobility in a two-phase system when the nature of the phases is altered. The three systems set up were dextran:polyethylene glycol (PEG) 600 (8·74:7·13% w/v); dextran sulphate (Dx-s):PEG 600; and dextran:PEG palmitate (PEG-P). The latter two systems were set up by replacing 0·4% of the parent compound. All systems were dissolved on 0·15 M sodium chloride overnight.

Bacteria were washed twice in phosphate buffered saline (pH 7·2) and adjusted to 10^8 cells per ml; 0·9 ml of the phase system was placed into a glass vial and 0·1 ml of bacterial suspension added and vortexed. One hundred microlitres of this sample was placed in a well of a microtitre tray. After standing for 1 h at room temperature to ensure phase separation, 100 μl of the top and bottom phases were pipetted from the vial into the microtitre tray. The phase interface was broken down by adding 200 μl of PBS and samples were read with an ELISA reader at 570 nm.

After adjusting for a 60:40% top:bottom split, the properties of the bacteria were determined with the formulae:

$$G = \frac{\text{bacteria in the bottom phase (\%)}}{\text{bacteria in rest of system (\%)}},$$

$$\text{delta } G = \frac{\text{G(Dx-s or PEG-P)}}{\text{G(rest of system)}}.$$

The log delta G values were plotted.

On 44 occasions assays were repeated after a period of days using a fresh culture; on 5 occasions the charge changed from + to −, on 4 occasions hydrophobicity differed and 4 occasions both differed, though not greatly.

Specific measures of adherence

Particle agglutination assay (PAA). Reagents were prepared for binding experiments according to Naidu and colleagues [7] using collagen type 1, fibronectin, vitronectin and laminin. Briefly latex beads (0·8 μm) were incubated with 100 μg of matrix protein overnight at 37 °C, then washed and resuspended in thimerosal and albumin to ensure that all available binding sites on the beads were coated.

Bacteria were harvested and washed in 0·02 M potassium phosphate buffer (pH 6·8) and resuspended to approximately 10^{10} cell per ml and mixed in equal volumes with latex beads. Clumping was observed after 2 min and scored as high (+++ or ++) or low (+ or −). All isolates were tested for auto-agglutination with phosphate buffer.

Table 1. *Characteristics of five German MRSA with the same International Phage Set phage typing pattern*

Code and year	Phage type*	Antibiogram†	Plasmid profile (Kb)	SAT‡	HIC§	PAA** Fn	PAA** Vn	PAA** Lm
409-1989	75/77 92 SmTcTpS 618/623/630	CmCpEmGmPc	33, 3·1	> 2·0	23	+	+	+
504-1991	75/77 92 SmTcTpS 630+	CpEmGmPc	31, 2·8, 2·5	> 2·0	23	+	+	+
573-1991	75/77 92 TcTpS 630+	CpEmPcSm	28	2	32	−	−	+
574-1991	75/77 92 TcTpS 630	CpEmGmPc	28, 2·8	> 2	24	+	+	+
163-1992	75/77 92 PcTcTpS 616/617/623	CmCpEmGm	2·8, 3·1	0·125	38	−	+	+

* Three phage sets used (*a*) international phage set, (*b*) experimental phage set 88-92, (*c*) experimental phage set 618-630. Results underlined were at 100 × Routine Test Dilution.

† All were methicillin resistant. Other resistances quoted are: Cm, chloramphenicol; Cp, ciprofloxacin; Em, erythromycin; Gm, gentamicin; Pc, penicillin; Sm, streptomycin; Tc, tetracycline; TpS, trimethroprim-sulphonamide.

‡ SAT, salt aggregation test.

§ HIC, hydrophobicity interaction chromatography.

** PAA, particle agglutination assay; Fn, fibronectin; Vn, vitronectin; Lm, laminin.

No replicate assays were performed for PAA as previous studies in Lund had shown these to be highly repeatable.

Chemicals

Fibronectin was purified from human plasma on gelatin-sepharose [17]. Collagen type I (Vitrogen 100^{R}) was obtained from Collagen Corporation, Palo Alto, California. Laminin isolated from an Engelbreth–Holm–Swarm transplantable mouse tumour [18] was kindly supplied by Dr Kaija Valkonen, University of Oulu, Oulu, Finland. Vitronectin was purified from human plasma on heparin-sepharose [19]. Ovalbumin was purchased from Sigma Chemicals Company (Dorset, UK). Thimersol was purchased from ICN (Bucks, UK). Latex beads were from Difco (Surrey, UK) and blood agar base from Oxoid (Unipath, Hants, UK).

Statistical analyses were made using the χ^2 test.

RESULTS

The results were diverse and showed marked heterogeneity between and within the two groups of MRSA. No relationship was evident between phage type or plasmid profile and any of the adherence tests (data not shown). Partial data are shown in Table 1 in which the results of German strains of the same phage type and similar plasmid profile from three separate years are described. The adherence results are clearly diverse.

Table 2. *Salt aggregation tests. Number falling into each group*

SAT value	German MRSA	Swedish MRSA	MSSA
0·125	10	4	14
0·25	0	0	1
1	0	4	0
2	7	17	1
> 2	10	7	3

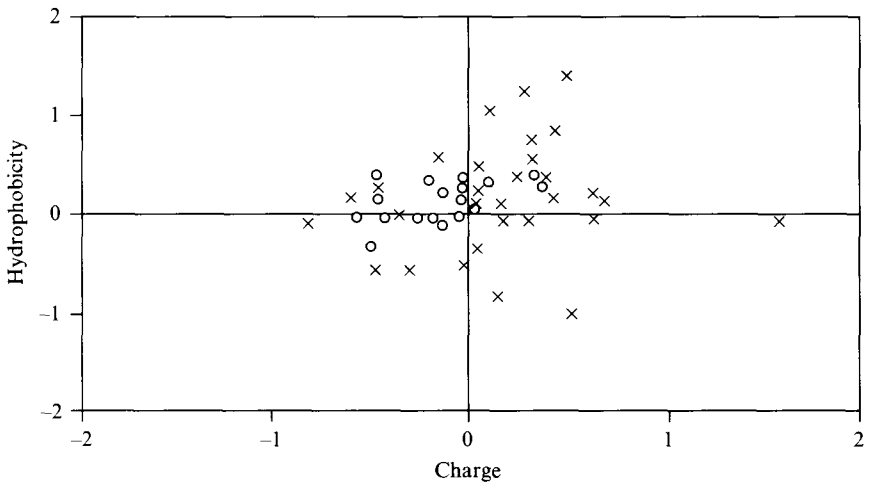

Fig. 1. Relationship between charge and hydrophobicity. ○, German MRSA; ×, Swedish MRSA.

Salt aggregation tests (*SAT*). The SAT results are shown in Table 2, there is no significant difference between German and Swedish MRSA; however, it must be noted that 9 of the 10 lowest values comprised the entire 1992 German MRSA strains.

Hydrophic interaction chromatography (*HIC*). The HIC values were evenly distributed amongst the MRSA strains. Ten of the 26 German MRSA had HIC values of 30% or more (range 11–44%) compared with 15 of the 32 Swedish strains (range 6–45%). One German strain was autoagglutinable. Seven of 12 MSSA had values over 30% adherence.

Two phase partitioning (*TPP*). Figure 1 shows the results for the two groups. It is evident that more of the German MRSA were negatively charged (14/18) than were Swedish MRSA (9/31) ($\chi^2 = 10{\cdot}9$, $P < 0{\cdot}001$). Twelve of 18 German MRSA were judged hydrophobic compared with 18/31 Swedish MRSA. No studies were made on MSSA.

Particle agglutination assays (*PAA*). The results for PAA are shown in Table 3. No adherence was observed for MRSA to collagen type I but all 13 MSSA tested adhered strongly. The two groups of MRSA are clearly different in adherence to fibronectin, vitronectin and laminin. These inter-group differences are statistically significant whether all positive strains or only strongly positive strains are included. (Fibronectin all positives $\chi^2 = 19{\cdot}2$, $P < 0{\cdot}001$, strongly positives only

Table 3. *Adherence to extra-cellular matrix proteins. Number falling into each group*

	German MRSA			Swedish MRSA			MSSA		
	−	+	++	−	+	++	−	+	++
Fibronectin	23	0	4	9	3	20	2	2	15
Vitronectin	17	5	4	8	6	18	—	ND	—
Laminin	14	10	3	4	7	21	0	2	17

* ND, not done.

$\chi^2 = 13{\cdot}8$, $P < 0{\cdot}001$; Vitronectin $\chi^2 = 8{\cdot}65$, $P < 0{\cdot}001$, $\chi^2 = 10{\cdot}8$, $P < 0{\cdot}001$ and laminin $\chi^2 = 10{\cdot}67$, $P < 0{\cdot}001$, $\chi^2 = 18{\cdot}0$, $P < 0{\cdot}001$ respectively).

DISCUSSION

The original intention of this study had been to demonstrate whether MRSA were more or less hydrophobic than MSSA and whether they adhered better to extra-cellular matrix proteins. More evident adherence would have provided a rationale for the apparent greater ability of MRSA to spread and colonize hospital patients.

In view of the variability amongst the MRSA, studies on MSSA were not followed to completion. For salt agglutination (Table 2) the MSSA results more closely resemble German MRSA than Swedish strains; HIC results are consistent with either group whilst in adherence to extra-cellular proteins the MSSA more closely resemble Swedish MRSA, except that all adhered strongly to collagen type I whilst none of the MRSA did so.

The heterogeneity of the results obtained for the two groups of MRSA makes interpretation difficult but does indicate that it cannot simply be assumed that MRSA represent a homogeneous population of strains. This finding reinforces the findings of others that strains bearing methicillin resistance genes are diverse in phenotypic and genotypic characteristics [20–23]. If there was indeed a clonal origin of methicillin-resistance there has evidently been substantial selection of variants or the gene has entered diverse strains. Any study of the special ability of MRSA to adhere to tissue will need to be carried out within the comparatively local context and concentrate on comparisons of MRSA and MSSA in a specific locality at a specific time.

There is no doubt that some workers have experienced extensive outbreaks of severe infection with MRSA; other workers have not had this experience and this has resulted in a polarization of views on the epidemicity or virulence of MRSA. Some reviewers have reminded us that there have been MSSA strains with epidemic potential [1, 2] and it may be that a closely controlled study of either MSSA or MRSA could lead to elucidation of the relationship between adherence characteristics and epidemic spread.

It is clear from the work reported here the MRSA form a group of strains recognized by a particular antibiotic resistant pattern but heterogeneous in terms of their ability to adhere.

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