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## Highly epidemic strains of methicillin-resistant *Staphylococcus aureus* (MRSA) do not differ from other MRSA or methicillin-sensitive strains in capsule formation, Protein A content or adherence to HEp-2 cells

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**Abstract** During the 1990s, two strains of epidemic methicillin-resistant *Staphylococcus aureus*, designated 'phage types EMRSA-15 and EMRSA-16, have emerged as significant hospital pathogens. They have resisted standard methods of control and spread widely amongst in the UK, often becoming endemic, while the incidence of other epidemic types of MRSA has either declined or not changed. This suggests that EMRSA-15 and EMRSA-16 possess special properties that favour their dissemination and survival. In order to investigate this hypothesis, a study was undertaken that examined methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*, including EMRSA types 1, 2, 3, 15 and 16, for capsule formation, the amount of bound protein A produced, and quantitative adherence to the human continuous epithelial cell line HEp-2. Although all these properties varied amongst the strains examined, there was no relationship between any of them and methicillin resistance or epidemic type, and, incidentally, no relationship between cell-wall bound protein A content and adherence.

### Introduction

In recent years, methicillin- and multiply antibiotic-resistant strains of *Staphylococcus aureus* (MRSA) have caused outbreaks of hospital infection throughout the world. Some MRSA strains have particular abilities to spread in hospitals (and sometimes into the community) and have been called "epidemic methicillin-resistant *Staphylococcus aureus*" (EMRSA) [1-3]. New strains designated EMRSA-15 and EMRSA-16 appeared for the first time in the North and Midlands of England in the early 1990s, and since then have spread throughout the UK [4-7]. The dramatic spread of these two strains over the last six years has changed the UK from a country where MRSA was only a sporadic problem to one where it is now endemic, necessitating a major revision of national guidelines for control [8]. EMRSA-3, which is now the third most common epidemic strain, has changed little in its incidence during the 1990s, suggesting that the emergence of the highly epidemic EMRSA-15 and EMRSA-16 strains is the result of organism factors rather than changes in medical practice or patient casemix. One possible organism factor involved in colonisation and spread might be an enhanced ability of MRSA to colonise human mucosa.

The factors involved in staphylococcal colonisation of nasal epithelium are poorly understood. The organisms must adhere or bind to the cells (or to nasal mucin [9]) and resist the normal epithelial defensive and cleansing mechanisms [10]. A number of staphylococcal adherence factors related to invasion and infection have been identified, but their role in colonisation of intact epithelium is unclear. These factors

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include those expressed by human cells, such as vitronectin, fibronectin, fibrinogen, elastin, collagen and heparan sulphate [11-15], and those expressed by the bacterium, including lipoteichoic acid, teichoic acid, protein A [16-18] and capsular polysaccharides [19].

Several workers have investigated the adherence of staphylococci to human nasal epithelial cells or to cultured cell lines. Duckworth & Jordens [20] compared the ability EMRSA-1 and other MRSA and MSSA to adhere to HEp-2 cells, and Ward [21] measured adherence of MRSA and MSSA to nasal mucosa, but neither study found any significant differences. Poston et al. [22] investigated the adherence of EMRSA-1 and other MRSA strains to HEp-2, Vero and mesothelial cells and showed no significant differences from results previously reported with MSSA strains [23]. However, they noted that deletion of the *mec* gene from one of the EMRSA-1 strains was associated with co-deletion of *agr* and *spa*, reduced production of protein A and reduced adherence.

In order to determine whether highly epidemic strains of EMRSA differ in their abilities to adhere to human epithelial cells in vitro, we have measured the adherence of a variety of EMRSA and MSSA to cultured HEp-2 cells, using <sup>3</sup>H-thymidine labelled bacteria in a quantitative assay. We also performed parallel microscopic studies to assess the patterns of adherence and the presence or absence of a capsule, and we measured cell wall-bound protein A concentrations.

## Materials and Methods

**Bacterial Strains.** We used 13 clinical isolates of MRSA collected in the late 1980s and early 1990s during outbreaks at Guy's and St. Thomas' hospitals in London. They were selected so as to include examples of the common British epidemic strains EMRSA-1, EMRSA-2, EMRSA-3, EMRSA-15 and EMRSA-16. EMRSA-1 and EMRSA-16 are highly epidemic and several isolates of these from different years were examined; EMRSA-15 is also highly epidemic, but only one isolate of this type was available. Organisms were identified by standard methods, including the determination of tube-coagulase, and DNAase-production. Methicillin-susceptibility was identified by the formation of a 5mm diameter clear zone around a 5µg methicillin disc on blood agar plates incubated for 24h at 30°C; methicillin-resistance strains showed no inhibition zone. The MRSA strains were kindly 'phage typed by the Staphylococcal Reference Laboratory of the CPHL (Table 1). *Staphylococcus aureus* control strains were Wood 46, a methicillin-sensitive strain which is known to have relatively low adherence to HEp-2 cells [23]; Cowan I, another methicillin-sensitive strain known to have relatively high adherence to HEp-2 cells and which produces relatively large amounts of protein A [17], and the 'Oxford staphylococcus', NCTC 6571, another methicillin-sensitive strain.

**Human Cell Line.** This was human larynx carcinoma, continuous epithelial cell line HEp-2, (Imperial Laboratories, UK), that has been used previously in studies of staphylococcal adherence in our laboratory [23].

**Chemicals, Media, Reagents and Plastic Ware.** [methyl-<sup>3</sup>H] thymidine was obtained from Amersham International, UK; Hisafe Optiphase scintillation cocktail from Wallac, UK; brain heart infusion (BHI), Mueller Hinton broth (MHB), Lauria Bertani broth (LB) and tryptose soy broth (TSB) from Unipath, UK; Trypan blue and Giemsa stains and recombinant protein A from Sigma-Aldrich, UK; and the following tissue culture materials were from Imperial Laboratories, UK: Earls Balanced Salt (EBS) solution, Dulbecco's phosphate-buffered saline (PBS), foetal bovine serum (FBS), Eagle's minimum essential medium with Earl's salt (EMEM), L-glutamine, penicillin and streptomycin, and 0.25 % trypsin with 0.02% EDTA in PBS without Ca<sup>2+</sup> and Mg<sup>2+</sup>. Tissue culture plastic ware and 50ml polyethylene centrifuge tubes were from Corning Costar Corporation, Cambridge, USA; Immulon 2 microtiter plates from Dynex Technologies, UK;

peroxidase conjugated antibody to goat IgG, peroxidase substrate system 3,3',5,5'-tetramethylbenzidine (TMB) and wash solution containing 0.002M imidazole and 0.02% Tween 20 in buffered saline from Kirkegaard & Perry Laboratories, USA; and India Ink from Windsor & Newton, UK.

*Radiolabelling of Bacteria.* Bacterial strains were radiolabelled as follows: five or six colonies from a fresh, 16 h culture on blood agar were picked by touching with a sterile wire loop and transferred to 2.5 ml TSB in a sterile 50 ml Corning tube. The bacterial cells were resuspended by vortexing and incubated overnight at 37°C in the presence of 0.925 Mega becquerel <sup>3</sup>H-thymidine, in an orbital shaker at 175 rpm. The radiolabelled bacterial cells were harvested by centrifugation and washed twice in 20 ml of PBS. The washed bacterial cells were centrifuged, the pellet resuspended in EBS to an optical density (OD) of 0.4 OD<sub>540</sub> to produce about 10<sup>8</sup> cells/ml [23].

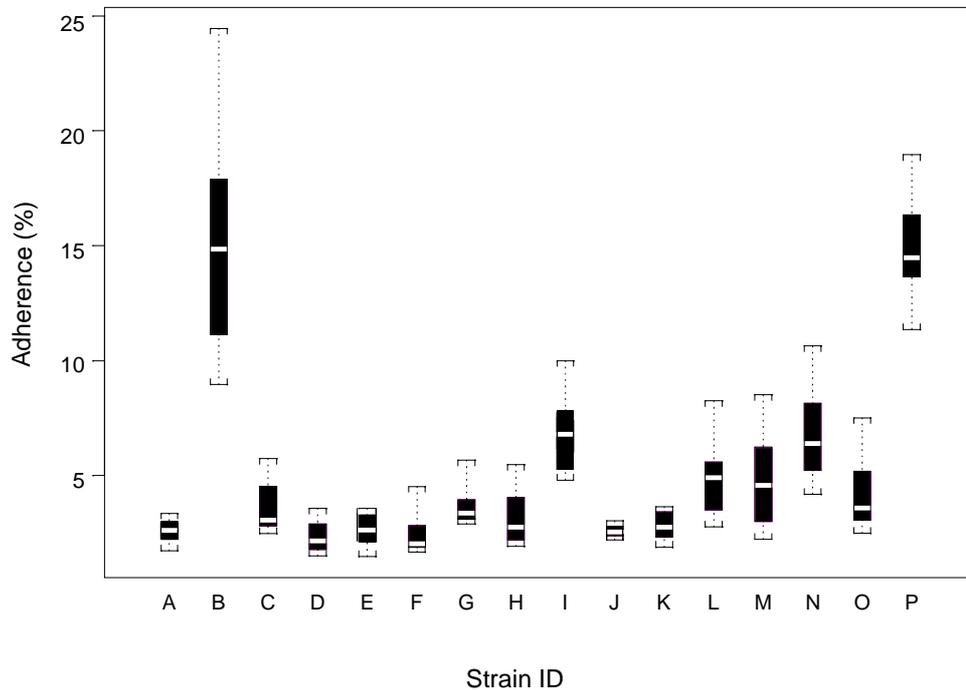
*Determination of Specific Activity of <sup>3</sup>H-labelled Bacteria.* For each strain, two 100 µl aliquotes of the <sup>3</sup>H-labelled bacterial suspension was transferred to a 1.5 ml Eppendorf tube containing 1 ml scintillant, and the radioactivity measured in a Micro Beta Trilux liquid scintillation and luminescence counter (Wallac).

*Cell Culture and Adherence Assay.* 10<sup>5</sup> cells/ml of HEP-2 cells suspended in EMEM were incubated for 48 h at 37°C in 24 well tissue culture plates to form confluent monolayers at the bottom of each well. The confluency and integrity of each monolayer was confirmed microscopically. Spent media was gently aspirated and the monolayer washed in 1 ml EBS. 0.5 ml of the <sup>3</sup>H-labelled bacterial suspension was added to each well and the plate incubated at 37°C in a humid 5% CO<sub>2</sub> incubator for 90 min. After incubation, the monolayer was washed twice with 1 ml fresh EBS and then lysed by adding 250 µl of pre-warmed trypsin/EDTA solution and incubating at 37°C for 15 min. Following complete detachment and solubilisation of the monolayer, 1 ml of scintillant was added and the mixture mixed in an orbital shaker until a clear solution was obtained. The radioactivity of each well was then counted in the Microbeta Trilux counter by inserting cross-talk elimination tubes into each well and using the Costar Software program with insert protocol (Wallac). The percentage of adherence was calculated as follows:

$$\% \text{ of adherence} = \frac{\text{mean cpm of the lysed monolayer with adherent bacteria} \times 100}{\text{mean cpm of the total bacterial suspension added}}$$

For each strain, eight replicates made from separate subcultures were tested and the median percentage adherence was used in comparisons.

*Pairwise Comparison With negative Control Strain Wood 46.* Figure 1 gives box-and-whisker plots associated with the adherence data in Table 1, and the 16 strains given in the table were compared using the Kruskal-Wallis method. This gave a *P* value of  $3.6 \times 10^{-7}$ , indicating that at least one of the strains was displaying adherence values significantly different from the rest. In order to identify which pairs of strains were significantly different, we used a multiple-comparison method [24]. This approach takes account of all the data present without compounding Type I errors. For this purpose, we used the non-parametric Dwass method [25], as described by Sokal and Rohlf [26]. According to the Dwass method, a pair of samples is significantly different at the  $\alpha$  level if the Mann-Whitney *U* statistic for the pair is greater than or equal to the critical value  $U_{\alpha[g,n]}$ . This critical value is dependent on the number of groups *g* and on the number of values *n* in each group. With *n*=8, the largest possible value for *U* is 64, but  $U_{0.05[16,8]}=64.3$ , which makes  $U < U_{0.05[16,8]}$  for all possible *U*. Therefore, in order to increase the power of the test, we omitted strains Wood 46 and 229.8 from this analysis on the grounds that their distribution was somewhat similar to that for strain GH177. This gave  $U_{0.05[14,8]}=63.9$ ; consequently, two strains are significantly different at the 5% level if their box-and-whisker plots shown in Figure 1 do not overlap.



**Figure 1** Box-and-whisker plots for replicate adherence experiments of *Staphylococcus aureus* isolates A-P shown in Table 1. See text for explanation

*Spearman-Rank Correlation Analysis.* A scatter plot of median percent adherence versus protein A concentration is given in Figure 2. The amount of correlation between median percent adherence and protein A concentration was assessed by the Spearman rank test.

*Determination of Pattern of Adherence by Light Microscopy.* HEp-2 cell monolayers were grown on 13 mm diameter, poly-L-lysine treated glass coverslips by placing the coverslips in the wells of tissue culture plates, seeding them with  $10^5$  HEp-2 cells and incubating to confluency as described above. A bacterial adherence assay was performed as before, but instead of lysing the cells with trypsin, the coverslips were removed, dehydrated with alcohol and stained with either Giemsa or crystal violet. The stained coverslips with their epithelial monolayers and adherent bacteria were examined by light microscopy at x400 and x1000 magnifications.

*Examination of Capsules by Light Microscopy.* According to Wilkinson's [27] practical definition, a capsule is a covering layer outside the cell wall, demonstrable by light microscopy and having a thickness greater than 200 nm and a definite external surface. We investigated all the bacterial strains used in this study for the existence of macrocapsules by the method of Duguid [28]. Fresh colonies grown on blood agar were suspended in PBS to a density of about  $2 \times 10^8$  cfu/ml, and mixed with India Ink in ratio of 2:1. This was examined under glass coverslips using phase contrast microscopy at x600 and x1500 magnifications.

*Protein A Quantitation.* Cell bound protein A content of the isolates was determined by a modification of the enzyme immunoassay (EIA) of Takeuchi et al. [29, 30]. Briefly, 100  $\mu$ l of a formalin-treated

bacterial suspension containing about  $2-3 \times 10^8$  cfu/ml was coated onto wells in 96 well microtiter plates by incubating at  $37^\circ\text{C}$  for 16h. After washing, 100  $\mu\text{l}$  of peroxidase-conjugated antibody to goat IgG was added at a 1:2000 dilution and incubated for 1 h at room temperature. (This antibody binds with protein A). This was followed by thorough washing of the wells, the addition of 100  $\mu\text{l}$  TMB substrate solution, a short incubation until the development of a blue colour and then stopping the reaction by the addition of 100  $\mu\text{l}$  1M phosphoric acid. The optical density of the resulting yellow-coloured reaction mixture was measured at 450 nm using a Multiskan MCC 340 MK II EIA plate reader (Flow Laboratories, UK). Appropriate concentrations of recombinant protein A were used as standards in each plate and the quantity of protein A in each strain was calculated from the standard curve.

## Results

*Microscopic Assessment of Adherence.* At 1000x magnification, the microscopic field typically covers an area of  $0.01\text{mm}^2$  and encompasses a minimum of 100 epithelial cells. In such fields, regardless of the staphylococcal strain investigated, we observed a great variation in the adherence of bacteria to HEp-2 cells, varying from a few tens to many hundreds of bacteria per cell. Furthermore, the distribution of the adherent bacteria was not uniform across each Hep-2 cell, but was concentrated close to the intercellular junctions. Furthermore, even a known low-adhering strain such as Wood 46 usually showed a large number of bacteria adhering to individual HEp-2 cells at some location in the field. Thus, it was not possible to make a quantitative assessment of adherence by microscopic analysis alone.

*Specific Activity of  $^3\text{H}$ -thymidine Labelled Strains of *Staphylococcus aureus*.* There were considerable differences in the amount of  $^3\text{H}$ -thymidine incorporation amongst the various strains studied, and it was also found that this varied with the culture medium used. However, with TSB the amount of incorporation was consistent for a particular strain, and this medium was therefore used throughout the study.

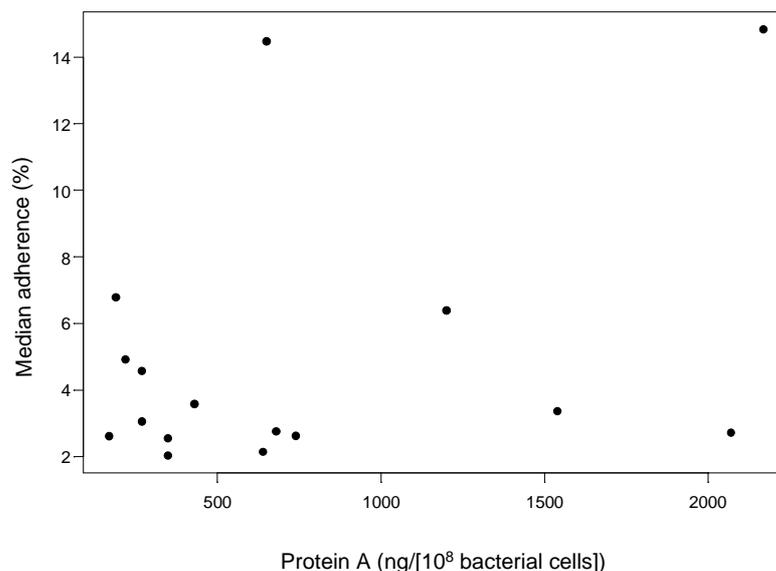
*Adherence of Different *Staphylococcus aureus* Strains.* The eight replicate percentage adherence results and the median values for each strain are shown in Table 1. Median adherence values ranged from 2.02% to 14.85%. The control strains Wood 46 (a known low-adherer) and Cowan I (a high-adherer) had values of 2.61 and 14.85 respectively. Figure 1 shows the box-and-whisker plots for the data in Table 1.

### *Statistical analysis*

Using this analysis, Strains Cowan I (MSSA, mean adhesion 14.85%), GH13 (EMRSA-1, 14.48%), 114.1 (EMRSA-16, 6.78%), and 99.6 (EMRSA-1, 6.39%) had significantly increased adherence compared with the 'negative' control strain Wood 46 (MSSA, 2.61%). Thus, amongst the 16 strains tested, one MSSA strain had high adherence (Cowan I), as did two out of four EMRSA-1 strains and one of five EMRSA-16 strains. The single strains of EMRSA-2, EMRSA-3, EMRSA-15, non-typable MRSA, and the other MSSA strain (the Oxford staphylococcus) were not significantly different from the Wood 46 'negative' control.

### *Relationship Between Adherence and Cell-Wall Bound Protein A Content*

Cell-wall bound protein A content varied amongst the 16 strains from 2170 ng per  $10^8$  bacterial cells for the high-adherer control MSSA strain Cowan I, to 170 ng per  $10^8$  cells for the low-adherer control MSSA strain Wood 46. However, amongst the remaining strains, there was no association between protein A content and adherence, as shown by the scatter plot in Figure 2 and the Spearman rank correlation coefficient of 0.158 ( $P > 0.2$ ). Furthermore, there was no association between protein A production and methicillin resistance.



**Figure 2** Scatter plot of mean percentage adherence versus cell-wall bound protein A content for the *Staphylococcus aureus* isolates tested.

*Capsules and Adherence.* No macrocapsule was detected in any of the strains examined except for 114.1 (EMRSA-16) and 86900 (EMRSA-1), which had thin capsular structures. None of the strains produced a mucoid colonial morphology on blood agar.

## Discussion

Although others have used microscopic visual analysis for quantifying bacterial adherence to mammalian cells [9, 31, 32], we found this an unsatisfactory method. Accurate quantitation was difficult because adherence varied between individual cells and between different microscopic fields, a phenomenon that has been noted by others [23]. The radiometric system developed by Wyatt et al., [23] is more objective and reproducible and preferred for these studies. We used a modification of the Wyatt method in which the final specific activity of the cell lysate was measured directly in the tissue culture well itself. This is a simpler procedure and less prone to errors than having to remove lysates from the wells for radioanalysis. The problem of labelled bacteria adhering to the side walls of the wells was solved by using cross-talk eliminator inserts.

The expression of adhesion molecules in *Staphylococcus aureus* may be dependent on the growth phase and the composition of the culture medium [33, 34]. This is another source of variation that must be taken into account in adhesion experiments. We used TSB medium and stationary phase cells in this study.

Different mammalian cells differ in their expression of various staphylococcal adherence receptors, and therefore the cell line used in adherence studies will affect the results. Fibronectin is one of the many binding sites (receptors) for *Staphylococcus aureus* [11-13, 16]. The transformed cell line HEp-2, lacks fibronectin [35-37]. However, Wyatt et al., [23] reported that the addition of fibrinectin to the adherence assays did not

significantly reduce the adherence of Cowan I to HEp-2 monolayers, and HEp-2 has been used in other studies of *Staphylococcus aureus* adhesion [20, 22]. For these reasons we believe HEp-2 is a suitable cell line for these studies.

With this methodology, the measurement of bacterial adherence was quantitatively reproducible, as shown by the inter-quartile range values (IQR) for eight replicates for each strain (Table 1). The mean adherence values for the control low-adhering and high adhering MSSA strains Wood 46 and Cowan I were 2.61% and 14.85% respectively, which are in accordance with the expected results. In addition to the MSSA Cowan I control strain, two out of the four EMRSA-1 strains tested had significantly increased adherence compared with Wood 46, as did one of the five EMRSA-16 strains. Adherence values for the single strains of EMRSA-2, EMRSA-3, EMRSA-15, non-typable MRSA, and the Oxford staphylococcus MSSA control strain were not significantly different from that of Wood 46. Thus in this study of 16 strains of *Staphylococcus aureus* there was no relationship between adherence to HEp-2 cells and methicillin-resistance, 'phage type or apparent clinical epidemicity. Furthermore, adherence values varied significantly amongst different isolates of EMRSA-1 and EMRSA-16. Thus, adherence in this assay is affected by factors other than methicillin resistance and epidemic type.

One factor that has been associated with adherence of staphylococci is protein A expression. Protein A is an extracellularly secreted and cell-wall associated protein of *Staphylococcus aureus* which interacts with immunoglobulin molecules in a pseudo-immune Fc reaction [38]. Various studies have suggested that Protein A expression is associated with increased adhesion to human cells [17, 22, 39], reduced susceptibility to opsonisation [18] and increased virulence [40]. The highly adherent *Staphylococcus aureus* strain Cowan I is known to produce higher amounts of protein A than the low-adhering Wood 46 [22] and is estimated to contain 80,000 IgG binding sites per organism [41]. Freney et al. [42] found that epidemic strains of MRSA had increased Protein A gene polymorphism, but van Belkum et al., [43] found no relation between gene polymorphism or protein A expression and colonisation of nasal epithelium. Poston et al. [22] demonstrated that *mec<sup>-</sup> agr<sup>-</sup> and spa<sup>-</sup>* mutants of a *spa<sup>+</sup>* MRSA strain had less protein A and adhered less well to cell lines. However, in another strain, a *mec<sup>-</sup>* mutant retained the parent levels of protein A while also showing decreased adherence. Finally, van Wamel et al., [44] found a significant difference between the ability of epidemic and sporadic strains of MRSA to bind vitronectin and Fc fragments of immunoglobulin G.

Protein A content has not been previously measured in human, clinical epidemic strains of MRSA. In the present study we measured cell wall-bound protein A quantitatively. As expected, the high- and low-adhering control strains had high and low levels of protein A respectively. However, there was no association between cell-wall bound protein A content and either adherence or methicillin resistance amongst the other *Staphylococcus aureus* strains tested.

Approximately 90% of *Staphylococcus aureus* isolates are said to produce capsular polysaccharides [45]. Encapsulated strains are more virulent in mice models than unencapsulated ones [46] and are less susceptible to phagocytosis [47]. However, the role of capsules in adherence is not clear. The present study showed no relationship between capsules and adherence since only two strains of the 16 examined showed microscopic evidence of capsule formation, and none had a macroscopic mucoid colonial morphology.

From the results of this study, we must conclude that while different *Staphylococcus aureus* strains show significant variation in their adherence to Hep-2 cells in vitro, there is no association between adherence and methicillin resistance, cell-wall bound protein A content, capsule formation or epidemic type. Specifically, we found no increase in adherence or protein A expression amongst the highly epidemic EMRSA strains EMRSA-15 and EMRSA-16.

## References

1. Cooke EM, Marples RR: Outbreaks of staphylococcal infection. Public Health Laboratory Services Microbiology Digest (1985) 2:62-64
2. Cookson B, Phillips I: Epidemic methicillin-resistant *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy (1988) 21, Supplement C: 57-65
3. Phillips I: Epidemicity of methicillin-resistant *Staphylococcus aureus*. In: Coello R, Casewell MW (eds): Methicillin-resistant *Staphylococcus aureus*. Wells Medical: Tunbridge Wells: (1992): pp 29–32
8. Report of a combined working party of the British Society for Antimicrobial Chemotherapy, the Hospital Infection Society and the Infection Control Nurses Association. Revised guidelines for the control of methicillin-resistant *Staphylococcus aureus* infection in hospitals. Journal of Hospital Infection (1998) 39:253-290
4. Cox RA, Conquest C, Mallagham C, Marples RR: Major outbreak of methicillin-resistant *Staphylococcus aureus* caused by a new phage type (EMRSA-16). Journal of Hospital Infection (1995) 29: 87-106
5. Johnson AP, James D: continuing increase in invasive methicillin-resistant infection (letter) Lancet (1997) 350:1710
6. Anonymous: Epidemic MRSA. Communicable Disease Report Weekly (1997) 7: 192
7. Anonymous: Communicable Disease Report Weekly (1999) 9: 65,68
9. Shuter J, Hatcher VB, Lowy FD: *Staphylococcus aureus* binding to human nasal mucin. Infection and Immunity (1996) 64:310-318
10. Ofek I, Doyle RJ.(eds) Bacterial adhesion to cells and tissues. Chapman and Hall, New York and London,(1994)
11. Kuusela P. Fibronectin binds to *Staphylococcus aureus*. Nature (1978) 276: 718-720
12. Vandaux P, Suzuki R, Waldvogel FA, Morgenthaler JJ, Nydegger UE: Foreign body infection: role of fibronectin as a ligand for the adherence of *Staphylococcus aureus*. Journal of Infectious Diseases (1984) 150: 1546-1553
13. Wadstrom T: Molecular aspects on pathogenesis of wound and foreign body infections due to staphylococci. Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene Series A (1987) 266:191-211
14. Wadstrom T, Paulsson M, Ljungh A: Molecular pathogenesis of staphylococcal infections: microbial adhesion to extracellular matrix and colonisation of wounded tissues and biomaterial surfaces. In: Mollby R, Flock JI, Nord CE, Christensson B (eds): Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart ( 1994). pp 343-352
15. Ljungh A, Wadstrom T: Binding of extracellular matrix proteins by microbes. In : Doyle RJ, Ofek I (eds): Methods of enzymology. Microbial adhesion. Academic Press, New York (1995). Volume 253: 501-514
16. Froman G, Switalski LM, Speziale P, Hook M: Isolation and characterization of a fibronectin receptor from *Staphylococcus aureus*. Journal of Biological Chemistry (1987) 262:6564-6571
17. Haagen IA, Heezius HC, Verkooyen RP, Verhoef J, Verbrugh HA: Adherence of peritonitis causing staphylococci to human peritoneal mesothelial cell monolayers. Journal of Infectious Diseases (1990) 161: 266- 273
18. Foster TJ, McDevitt D: Surface-associated proteins of *Staphylococcus aureus*: their possible roles in virulence. Federation of European Microbiological Societies Microbiology Letters (1994) 118: 199-206

19. Soell M, Diab M, Archipoff G, Beretz A, Herbelin C, Poutrel B, Klein J: Capsular polysaccharide type 5 and 8 of *Staphylococcus aureus* bind specifically to human epithelial (KB) cells, endothelial cells, and monocytes and induce release of cytokines. *Infection and Immunity* (1995) 63:1380-1386
20. Duckworth GJ, Jordens JZ: Adherence and survival properties of an epidemic methicillin-resistant strain of *Staphylococcus aureus* compared with those of methicillin sensitive strains. *Journal of Medical Microbiology* (1990) 32:195-200
21. Ward TT: Comparison of in vitro adherence of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* to human nasal epithelial cells. *Journal of Infectious Diseases* (1992) 166: 400-404
22. Poston SM, Glancey GR, Wyatt JE, Hogan T, Foster TJ: Co-elimination of *mec* and *spa* genes in *Staphylococcus aureus* and the effect of *agr* and protein A production on bacterial adherence to cell monolayers. *Journal of Medical Microbiology* (1993) 39: 422-428
23. Wyatt JE, Poston SM, Noble WC: Adherence of *Staphylococcus aureus* to cell monolayers. *Journal of Applied Bacteriology* (1990) 69: 834-844
24. Hsu JC: Multiple comparisons. Chapman & Hall, London (1996) pp119-180
25. Dwass M: Some *k*-sample rank-order tests. In: Olkin I, Ghurye S, Hoeffding W, Madow W, Mann H (eds): *Contributions to probability and statistics: essays in honor of Harold Hotelling*. Stanford University Press, Stanford (1960) pp198-202
26. Sokal RR, Rohlf FJ: *Biometry*. Freeman, New York (1995) pp 432-433
27. Wilkinson JF: The extracellular polysaccharides of bacteria. *Bacteriological Review* (1958) 22: 46-73
28. Duguid JP: The demonstration of bacterial capsules and slime. *Journal of Pathological Bacteriology* (1951) 63: 673-685
29. Takeuchi S, Kobayashi Y, Mori Y: Assay of protein A in *Staphylococcus hyicus* subsp. *hyicus* by ELISA and immunoelectron microscopy. *Veterinary Microbiology* (1990) 25: 297-302
30. Takeuchi S, Matuda K, Sasano K: Protein A in *Staphylococcus aureus* isolates from pigs. *Journal of Veterinary Medical Science* (1995) 57: 581- 582
31. Aly R, Shinefield HI, Strauss WG, Maibach HI: Bacterial adherence to nasal mucosal cells. *Infection and Immunity* (1977) 17: 546-549
32. Martino PD, Bertin Y, Girardeau JP, Livrelli V, Joly B, Michaud AD: Molecular characterization and adhesive properties of CF29K, an adhesin of *Klebsiella pneumoniae* strains involved in nosocomial infections. *Infection and Immunity* (1995) 63: 4336-4344
33. Ekstedt RD: Immune responses to surface antigens of *Staphylococcus aureus* and their role in resistance to staphylococcal diseases. *Annals of the New York Academy of Sciences* (1974) 236:203-219
34. Cheung AL, Fischetti VA: Variation in the expression of cell wall proteins of *Staphylococcus aureus* grown on solid and liquid media. *Infection and Immunity* (1988) 56: 1061-1065
35. Hynes, RO: Alteration of cell surface proteins by viral transformation and by proteolysis. *Proceedings of the National Academy of Sciences USA* (1973) 70: 3170-3174
36. Ruoslahti E, Vaheri A: Novel human serum protein from fibroblast plasma membrane. *Nature* (1974) 248: 789-791
37. Proctor RA: The staphylococcal fibronectin receptor: evidence for its importance in invasive infections. *Reviews of Infectious Diseases* (1987) 9 Suppl 4: S335-S340

38. Forsgren A, Ghetie V, Lindmark R, Sjoquist JP: Protein A and its exploitation. In: Easmon CSF, Adlam C (eds): *Staphylococci and staphylococcal infections*. Academic Press, London (1983) pp 429-480
39. Teranishi H, Shimizu A, Kawano J, Kimura S: Comparative adhesion of protein A-positive and protein A-negative strains of porcine *Staphylococcus hyicus* subsp. *hyicus* to Vero cells. *Japanese Journal of Veterinary Sciences* (1988) 50: 825-827
40. Patel AH, Nowlan P, Weavers ED, Foster T: Virulence of protein A- deficient and alpha-toxin-deficient mutants of *Staphylococcus aureus* isolates by allele replacement. *Infection and Immunity* (1987) 55:3103-3110
41. Kronvall G, Quie PG, Williams RC Jr: Quantitation of staphylococcal proteinA: determination of equilibrium constant and number of protein A residues on bacteria. *Journal of Immunology* (1970) 104:273-278
42. Frenay HME, Theelen JPG, Schouls LM, Vandenbroucke G, Verhoef J, van Leeuwen WJ, Mooi FR: Discrimination of epidemic and nonepidemic methicillin resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. *Journal of Clinical Microbiology* (1994) 32: 846-847
43. van Belkum A, Eriksen NHR, Sijmons M, Leeuwen WV, Bergh MVD, Kluytmans J, Espersen F, Verbrugh H: Coagulase and protein A polymorphism do not contribute to persistence of nasal colonisation by *Staphylococcus aureus*. *Journal of Medical Microbiology* (1997) 46: 222-232
44. van Wamel WJB, Fluit ADC, Wadstrom T, van Dijk H, Verhoef J, Vandenbroucke-Grauls CMJE: Phenotypic characterisation of epidemic versus sporadic strains of methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* (1995) 33: 1769-1774
45. Sompolinsky D, Samra Z, Karakawa WW, Vann WF, Schneerson R, Malik Z: Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. *Journal of Clinical Microbiology* (1985) 22: 828-834
46. Wiley BB, Maverakis NH: Capsule production and virulence among strains of *Staphylococcus aureus*. *Annals of the New York Academy of Sciences* (1974) 236:221-232
47. Peterson PK, Wilkinson BJ, Kim Y, Schmeling D, Quie PG: Influence of encapsulation on staphylococcal opsonisation and phagocytosis by human polymorphonuclear leukocytes. *Infection and Immunity* (1978) 19:943-949