Tropical Medicine and International Health

VOLUME 3 NO 7 PP 576–583 JULY 1998

Molecular, antibiogram and serological typing of *Staphylococcus aureus* isolates recovered from Al-Makased hospital in East Jerusalem

Tamer Essawi[1], Tarek Na'was[2], Abbass Hawwari[3], Suzan Wadi[1], Anwar Doudin[4] and Ali Ibrahim Fattom[3]

1 *Department of Biology and Biochemistry, Birzeit University, Birzeit, Palestine*
2 *Natural Science Division, Lebanese American University, Beirut, Lebanon*
3 *W. W. Karakawa Microbial Pathogenesis Laboratory, Nabi, Rockville (MD), USA*
4 *Al-Makased Hospital, East Jerusalem, Palestine*

Summary *Staphylococcus aureus* is a major cause of nosocomial infections and a risk in patients who have either undergone surgery or are on haemodialysis. The *S. aureus* infections in patients admitted to the clinical departments of Al-Makased Charitable Hospital in Jerusalem during a period of one year were investigated. Isolates included were from blood, surgical wounds, or other nonsuperficial sites. Of 63 isolates available for analysis, 46 (73.0%) expressed type 8 capsular polysaccharide; 13 (20.7%), type 5 capsular polysaccharide; only 4 isolates (6.3%) did not express type 5 or type 8 antibodies. The strains fitted in 7 different antibiogram types, with the type showing resistance only to penicillin and ampicillin prevalent in 34 out of 63 isolates (54.0%). Of the 12 methicillin-resistant *S. aureus* (MRSA) isolates (19.1%), 8(66.7%) possessed the type 8 capsule and 4(33.7%) the type 5 capsule. Pulsed-field gel electrophoresis of all isolates with the restriction-endonuclease enzymes Sma I revealed 34 patterns demonstrating that no single methicillin-sensitive *S. aureus* strain was endemic in the hospital. However, all MRSA isolates with a type 8 capsule showed identical PFGE patterns using the 2 restriction-endonuclease enzymes Sma I and SST II. Moreover, type 5 isolates showed identical patterns (one isolate differed from the rest with one band only). These data suggest and confirm the clonality of type 5 and type 8 MRSA isolates. Analysing the results of the capsular and antibiogram typing schemes in conjunction proved useful and suggested that such an analysis can be employed as a helpful epidemiological tool in hospitals with limited resources.

keywords *Staphylococcus aureus*, nosocomial infection, epidemiology, pulsed-field-gel electrophoresis

correspondence Dr Ali Fattom, Nabi Rockville, 12280 Wilkins Avenue, Rockville, MD 20852, USA. E-mail: afattom@nabi.com

Introduction

Staphylococcus aureus, a major cause of hospital-acquired infections, causes serious systemic infections including bacteraemia, endocarditis and osteomyelitis. Virtually all nosocomial strains of *S. aureus* produce at least one of a group of enzymes known as the beta-lactamases which render the organisms resistant to penicillin and/or other β–lactam antibiotics. These infections have been associated with outbreaks caused by multidrug resistant strains of *S. aureus*, mainly by methicillin-resistant *S. aureus* (MRSA) (Musher & McKenizie 1977; Bennet & Hall 1988; Weinstein 1991). MRSA strains were first isolated in England after the introduction of semisynthetic penicillins in 1959. Their spread was soon noted in the United States and across the world (Anonymous 1961; Lacey 1987). Reports of MRSA outbreaks in tertiary hospitals, community teaching hospitals, nursing homes and rehabilitation facilities (CDC 1981; McGowan 1988; Storch *et al*. 1990) soon followed.

The outburst of infections caused by MRSA created obstacles for physicians and infection control personnel in health care centres around the world. The main problem was failure of treatment due to the multiple resistance of these *S. aureus* strains. Patients acquiring an infection caused by MRSA are usually treated with vancomycin, a toxic and relatively expensive antibiotic. It is, however, feared that other gram-positive cocci groups, which have developed vancomycin resistance such as the enterococci (Uttley *et al*.

1989; Al Obeid *et al*. 1990), may transmit the gene(s) responsible for this property to *S. aureus*, leaving few, if any, options for antimicrobial chemotherapy (Schwalbe *et al*. 1987). In fact, *S. aureus* isolates which exhibit an increased resistance to vancomycin have been reported in the USA and Japan (CDC 1997a,b).

Epidemiologically, MRSA is introduced to hospital settings through different routes, most commonly through a patient infected with MRSA who then serves as a singular *S. aureus* infection reservoir (Colley *et al*. 1965; Peacock *et al*. 1980). Cross-infection involving hospital staff is another mode of transmission (Ward *et al*. 1981). Due to the difficulties created by the inability to control the spread of the organism in an outbreak and to treat the patients infected by it, efforts must be made to detect MRSA eruption early and to promptly implement infection control measures that may limit the consequences of a flare-up (Boyce *et al*. 1981; McManus *et al*. 1989; Mulligan & Arbeit 1991). Proper monitoring of hospitals for emergence of *S. aureus* (and MRSA) infections may occur only if extensive epidemiological studies are performed.

Reports about the occurrence and prevalence of infections caused by *S. aureus* including MRSA in hospitals in Palestine were practically nonexistent. We chose Al-Makased Hospital, the largest and best equipped hospital in East Jerusalem, which serves as a reference hospital for patients suffering from profound medical conditions, for our study. The objectives were surveillance of *S. aureus* infections, determination of the prevalence of MRSA in infection isolates in hospital wards, and phenotypic as well as genotypic characterization of *S. aureus* isolates.

Materials and methods

Study isolates

Sixty-three *S. aureus* isolates were recovered from clinical specimens submitted to the clinical microbiology laboratory of Al-Makased Hospital between November 1993 and November 1994. They included blood and pus from nonobvious pyogenic infections that occurred in patients admitted to different hospital departments. In some cases, multiple isolates from one patient were tested and invariably found identical. Therefore one isolate was used for inclusion in the study in these cases. Specimens were registered and processed and the isolated pathogens were identified by standard procedures (Kloos & Lambe 1991). The *S. aureus* isolates retrieved were sent to the research laboratory (NABI – Rockville), on blood agar plates (BBL, Cockeysville, MD), where their identity was reconfirmed using standard procedures (Kloos & Lambe 1991). The isolates were then stored at −70 °C in microbank ampules (Pro-Lab Diagnostics, Ontario, Canada) for further analysis at NABI, Rockville, USA.

Capsular polysaccharide typing

Capsular serotyping of the isolates was performed by direct cell agglutination and immuno-precipitation of cell extracts. The organisms were grown at 37°C under 5% CO_2 on Columbia broth (Difco Laboratories, Detroit, Michigan) supplemented with 1.5% $CaCl_2$ and 0.5% $MgCl_2$. Samples were removed from the flasks and tested for capsulation as follows: rabbit antiteichoic acid sera, prepared with the noncapsulated Wood strain (Karakawa *et al*. 1985), were diluted with saline to 1/512 to give lower titre preparations. Samples were tested for direct agglutination with the different preparations. Cultures which did not show agglutination at a titre of $<$ 1:4 of the original sera were considered fully capsulated and could be tested for capsular typing. Two different rabbit sera were used in the direct agglutination assay to identify the capsular type of each isolate: sera from animals vaccinated with a whole cell vaccine (Karakawa *et al*. 1985), and sera prepared in rabbits against either type 5 or type 8 conjugated vaccine (Fattom *et al*. 1990, 1993). Monoclonal antibodies to capsular types 5 and 8 produced and standardized by Nabi (Rockville, Maryland) were used to confirm the capsular serotype of the isolates.

Antimicrobial susceptibility testing

The susceptibility of all isolates to different antimicrobials was tested by the disk-agar diffusion method in accordance with the National Committee for Clinical Laboratory Standards (NCCLS 1993). The antimicrobial disks used were tetracycline (30 μg/ml), oxacillin (1 μg/ml), penicillin (10 U/ml), erythromycin (15 μg/ml), ampicillin (10 μg/ml), vancomycin (30 μg/ml), clindamycin (2 μg/ml), cephalothin (30 μg/ml) and gentamicin (10 μg/ml) (Becton Dickinson Microbiology system, BBL, Cockeysville, MD). The results of these tests were recorded after 18–24 h of incubation at 35 °C. A standard strain of *S. aureus* (ATCC 25923) was used as a control.

Detection of MRSA

Two standard methods were used for the detection of MRSA. The disk agar diffusion method using a 1 μg oxacillin disk incubated at 35 °C for 24 h and checking after 48 h (NCCLS 1993), and ability to grow on Mueller-Hinton agar supplemented with 4% NaCl, and 6 μg/ml of oxacillin (MRSA screen agar, BBL, Cockeysville, MD) after incubation at 35 °C for 24 h (McDougal & Thornsberry 1984; Working Party 1986).

Table 1 Capsular types of the *Staphylococcus aureus* clinical isolates from patients at Al-Makased hospital as stratified by hospital source

	Capsular type			
Hospital department	*T5*	*T8*	*N**	Total
Surgical ICU	0	3	1	4
Internal medicine	3	6	1	10
Neonatology	1	9	0	10
Obstetrics	0	2	1	3
Paediatrics	1	6	1	8
Orthopedic surgery	5	13	0	18
General surgery	3	7	0	10
Total	13	46	4	63

*Non 5 or 8 capsular type.

β–lactamase production test

The ability of the isolates to produce β–lactamase was tested by using several similar colonies from a fresh culture to smear disks impregnated with the chromogenic cephalosporin, nitrocephin (cefinase disks BBL, Cockeysville, MD). A positive result was recorded upon the development of a yellow to dark red colour change, within a period of one hour, on the area where the culture was applied. Confirmatory tests were performed by the disk agar diffusion method using amoxicillin clavulanic acid (AMC30) and ampicillin-sulbactam (SAM20) disks (Difco laboratories, Detroit, MI) as recommended (NCCLS 1993).

Genomic DNA analysis by pulsed field gel electrophoresis (PFGE)

S. aureus genomic DNA was extracted from overnight agar cultures inoculated (initial O. $D._{540}$ = 0.1) into 10 ml of brain heart infusion broth (BBL, Cockeysville, MD.) and incubated with shaking at 37 °C to a final O. $D._{540}$ = 1.0 as described by Murray *et al*. (1990) and modified by Wanger *et al*. (1992). The DNA size standards used were a bacteriophage lambda ladder consisting of concatemers starting at 48.5 kbp and increasing to approximately 1000 kbp (Bio-Rad Laboratories, Hercules, CA). The gels were stained with ethidium bromide, rinsed and photographed under UV light.

Results

Hospital source and capsular typing of the isolates

Sixty-three clinical *S. aureus* isolates from the different clinical departments of Al-Makased Hospital in Jerusalem were sent to the research laboratory during the period November 1993 to November 1994. The infected patients were distributed in the different hospital departments as follows: 28 in orthopaedics and general surgery, 18 in paediatrics and neonatology, 10 in internal medicine, 4 in the surgical intensive care unit and 3 in obstetrics. Table 1 shows the capsular types of the *S. aureus* isolates obtained from each hospital department. Type 8 isolates were predominant as they constituted 73% (46/63) of all isolates with type 5 noted in 20.7% (13/63) of the isolates. The remaining 4 isolates (6.3%) were not typed by the type 5 or the type 8 antisera. Most isolates (93.%) were typed as either having the type 5 or the type 8 capsule; however, isolates with the type 8 capsule predominated over other types in all hospital departments.

Antibiogram typing

The antibiotic susceptibility of the 63 isolates was tested by disc-agar diffusion method. Table 2 shows the antibiogram types and Table 3 indicates that the majority of the isolates (34/63; 54%) were of antibiogram type F, i.e. resistant only to penicillin and ampicillin. Antibiogram F isolates were recovered from all the clinical departments of our hospital except the surgical ICU. The multiresistant strains belonging to type A, sensitive only to vancomycin, were mainly isolated

Table 2 *Staphylococcus aureus* antibiogram types identified at Al-Makased Hospital

Type	Tet	Ox	P	E	Am	Va	Cc	Cf	Gm
A	r	r	r	r	r	s	r	r	r
B	r	r	r	r	r	s	s	r	r
C	r	s	r	r	r	s	s	s	s
D	r	s	r	s	r	s	s	s	s
E	s	s	r	r	r	s	s	s	s
F	s	s	r	s	r	s	s	s	s
G	s	s	s	s	s	s	s	s	s

Abbreviations: Tet, tetracycline; Ox, oxacillin; P, penicillin G; E, erythromycin; Am, ampicillin; Va, vancomycin; Cc, clindamycin; Cf, cephalothin; Gm, gentamicin; s, susceptible; r, resistant.

Table 3 Antibiogram types of the *Staphylococcus aureus* isolates retrieved from the different hospital departments

Department	Antibiogram type							
	A	*B*	*C*	*D*	*E*	*F*	*G*	*Total*
Surgical ICU	0	0	1	3	0	0	0	4
Internal medicine	0	1	1	2	0	6	0	10
Neonatology	5	0	0	0	1	4	0	10
Obstetrics	0	0	0	0	0	3	0	3
Paediatrics	2	0	1	1	0	3	1	8
Orthopedic surgery	3	0	0	1	0	13	1	18
General surgery	1	0	0	4	0	5	0	10
Total	11	1	3	11	1	34	2	63

Table 4 *Staphylococcus aureus* isolates included in the study distributed by capsular and antibiogram type

Capsular	Antibiogram type							
Type	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*Total*
5	4	0	2	2	1	4	0	13
8	7	1	1	8	0	28	1	46
N	0	0	0	1	0	2	1	4
Total	11	1	3	11	1	34	2	63

from two adjacent wards: neonatology and paediatrics (7/11; 63.6%). The 4 other multiresistant isolates were recovered from two other adjacent wards: orthopaedic and general surgery. Antibiogram type D, of strains resistant to ampicillin, penicillin and tetracycline, was as prevalent in the clinical isolates as was the multiresistant antibiogram A. Antibiogram type G isolates, susceptible to all antimicrobials tested, including penicillin, were not common and appeared only in 2 (3.2%) isolates. Table 4 lists the distribution of the *S. aureus* isolates by capsular and antibiogram types. The biggest cluster of strains is the one showing the capsular type 8 and antibiogram type F, constituting 44.4% (28/63).The multiresistant strains in antibiogram type A more commonly (63.6%) possessed the type 8 capsule.

Table 5 The 12 methicillin-resistant Staphylococcus aureus isolates as stratified by hospital source, date of isolation and capsular type

Hospital department	Date of isolation	Type 5	Anti-biogram	Type 8
Neonatology	11/93	0	A,A	2
	1/94	0	A,A	2
	2/94	0	A	1
Paediatrics	11/93	0	A	1
	11/94	0	A	1
Orthopedic Surgery	2/94	2	A,A	0
	3/94	1	A	0
General Surgery	3/94	1	A	0
Internal Medicine	12/93	0	B	1
Total		4		8

Methicillin-resistant infection isolates

The numbers of MRSA (antibiograms A and B) isolated from the different hospital locations are given in Table 5. Of the 12 (19.1%) isolates identified as MRSA, 66.7% (8/12) possessed a type 8 capsule, while the remaining 4 (33.3%) had a type 5 capsule. Table 5 also shows that, unlike the type 8 MRSA strains which were isolated at different intervals during our study and from most of our hospital departments, type 5 MRSA appeared within a specific period (2/94 and 3/94) and in only two adjacent wards of the orthopaedic and general surgery departments.

Pulse field gel electrophoresis

To investigate the relatedness of the isolates genotypically, PFGE of all isolates was performed using the restriction-endonuclease enzyme *Sma I*. Thirty-four distinct patterns without significant clustering were recovered, suggesting lack of clonal relatedness among the isolates included in the study. However, different results were obtained when the 12 MRSA isolates were analysed distinctly. On

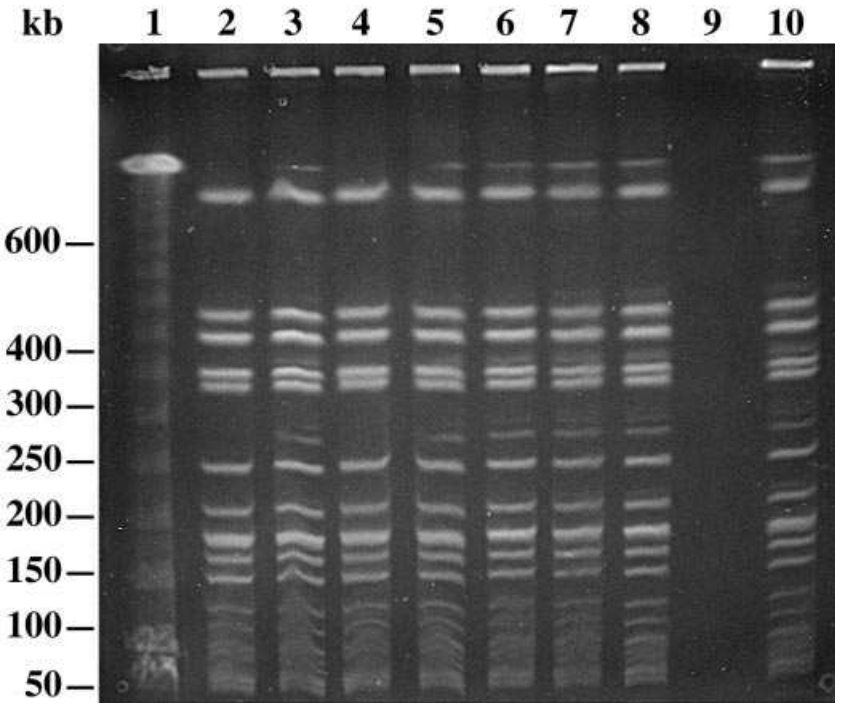

Figure 1 CHEF Characterization of MRSA isolates by pulsed-field gel electrophoresis. Chromosomal DNA was digested with endonuclease *Sma I* (lanes 2–10) and separated by pulsed-field gel electrophoresis apparatus then photographed under UV light. The 7 similar patterns shown (lanes 2–8) represent the type 8 isolates, while lane 10 represents a nasal isolate from an attending nurse. Lane 1 contains size markers.

digestion with *Sma I,* all isolates with type 8 capsules showed similar patterns (Figure 1). The isolates with type 5 capsules, however, showed two similar DNA banding patterns, with three of the capsular type 5 isolates having identical DNA patterns and the fourth having one different band (data not shown). The type 8 isolates had identical patterns to that obtained from a nasal isolate from an attending nurse.

Discussion

Serious *S. aureus* infections in hospitalized patients are caused by auto-inoculation from a common carriage site, usually the anterior nares, or by transfer from another carrier such as a patient or health care worker. Health care workers were often implicated as a potential reservoir for nosocomial outbreaks of MRSA (Na'was & Fakhoury 1991; Scully *et al*. 1992), a dangerous organism that causes hospital outbreaks which are difficult to control, costly, and very dangerous to high risk patients (Lamb 1991).

Most of the *S. aureus* clinical isolates possessed capsular polysaccharides of multiple distinct serological types. Since their discovery and the establishment of the capsular typing scheme (Arbeit *et al*. 1984; Karakawa *et al*. 1985), numerous studies have used the new scheme for the typing of infectious isolates of man and animals. Of the 13 known capsular types, types 5 and 8 comprise the majority of all clinical isolates from man, sheep, cows, poultry and other animals (Poutrel *et al*.1993; Daum *et al*. 1994), thus limiting the usefulness of the capsular polysaccharide typing scheme as an epidemiological tool to identify outbreaks in hospitals and to establish relatedness of isolates.

Advances in molecular biology and the subsequent introduction and widespread use of more sophisticated methodologies such as PFGE, which discriminates isolates based on their genetic profile, have diverted attention away from the development and potential utility of other simple typing schemes such as capsular polysaccharide and antibiogram typing. As a result, medical microbiology laboratories in developing countries, where resources are limited, have been further limited to play a role in the effort to properly detect epidemics involving *S. aureus* and to develop appropriate and cost-effective infection control measures. In certain countries antibiotics are freely dispensed over the counter (OTC) and hospitals lack policies regarding their use. Due to these problems, establishing a simple and accurate tool for the detection of MRSA outbreaks has become essential to effective infection control in these countries.

For the first time in the Al-Makased hospital in Jerusalem, the capsular polysaccharide serotyping scheme was used to investigate the distribution of capsular types among the *S. aureus* clinical isolates from patients in all hospital departments. The majority of isolates (93.7%), were, as expected, capsulated by either a type 5 or a type 8 capsule, with obvious prevalence of type 8 (73.0%).

Methicillin-resistant *S. aureus* isolates constituted 19.1% (12/63) of the *S. aureus* isolates included in this study. A correlation of methicillin resistance with capsular type 5 was previously implied, based on the fact that the majority of MRSA isolates from Europe and the United States were of type 5 capsules (Fournier *et al*. 1987; Wanger *et al*. 1992; Na'was *et al*. 1998). In our setting, most MRSA isolates were of type 8 (66.7%) and not of type 5 (33.4%), suggesting that methicillin resistance is not related to the capsular type of MRSA isolates, and that the *S. aureus* capsular type 8 can acquire this property. Of the 8 MRSA with a type 8 capsule, 7 (87.5%) were isolated from patients in two adjacent wards (neonatology and paediatrics). The capsular type 5 MRSA isolates were obtained, however, from patients in two other adjacent wards, the general and orthopedic surgery wards. It is important to note that strains with similar capsular type may have different antibiogram patterns. However, it is highly likely that strains with similar capsular and antibiogram type would show similar PFGE patterns, too. Previous data (based on a combination of capsular and antibiogram typing) suggested a possible common source for each of the capsular MRSA clusters which was confirmed by PFGE (see below).

To further investigate the source and relatedness of these isolates, we performed PFGE on all isolates. The 34 patterns of PFGE obtained indicated, as reported for other settings, that different genotypes were present (Struelens *et al*. 1992; Schlichting *et al*. 1993). When the PFGE patterns of the MRSA isolates, using two different restriction-endonuclease enzymes, were studied, all isolates with a type 8 capsule were found to have identical PFGE profiles, indicating that these isolates were of the same clone. The MRSA with the type 5 capsules, on the other hand, all had identical PFGE profiles with the exception of one isolate that differed in one band only.

The role of nasal carriage in dissiminating *S. aureus* nosocomial infection is well established (Sewell *et al*. 1982; Yu *et al*. 1986). The clonal relationship of MRSA isolates suggest a common source of these infection isolates which is possibly hospital-based (Ward *et al*. 1981). We noted that from April to October 1994 the *S. aureus* isolates in our clinical microbiology laboratory did not include any MRSA isolates. Upon investigation, we realized that during the month of April, another study was performed, where anterior nares of the hospital physicians and nurses were swabbed and cultured for determination of *S. aureus* nasal carriage. Eight nurses were positive for nasal carriage of MRSA, 2 from neonatology, 4 from orthopedic surgery, and 2 from paediatrics. They were treated with vancomycin until carriage was terminated. This agrees with the distribution of cases of MRSA isolates we obtained in our study, and suggests that the probable common source of the MRSA strains was the nares of carriers of the organism.

The only isolate we were able to obtain from these nasal MRSA isolates was from a nurse in the paediatric ward. The strain was a type 8 isolate that gave an identical PFGE profile to the infection (outbreak) isolates with the same capsular type, thus proving our theory concerning the source of the MRSA strains in our hospital, and supporting published data (Sewell *et al*. 1982; Yu *et al*. 1986; Casewell & Hill 1986) for other hospitals. Moreover, the abscence of MRSA infections in the hospital following the treatment of *S. aureus* carriers with antibiotics and consequently their clearance strongly suggest the role of nasal carriage among medical personnel in dissimination of *S. aureus* infection in hospitalized patients.

The data presented in this paper confirms the value of using capsular typing in conjunction with antibiogram typing as a simple, cheap, and efficient strategy in pin-pointing epidemics or outbreaks caused by multiresistant *S. aureus* strains in hospitals. Moreover, the combined method may serve as a convenient and cheap way for periodic monitoring of nasal carriers and identification of the main reservoirs of MRSA in hospitals, in order to control the spread of these dangerous organisms.

Acknowledgements

We thank Suzan Zughaier, Maher Zughaier and Suleiman Turkman (Al-Makased Hospital) for their technical assistance. We also thank Lisa Basham and Sara Shepherd (NABI, Rockville) for critically reviewing the manuscript.

References

Al-Obeid S, Collatz E & Gutman L (1990) Mechanism of resistance to vancomycin in *Entrococcus faecium* D366 and *Enterococcus faecalis* A256. *Antimicrobial Agents and Chemotherapy* **4,** 252–256.

Anonymous (1961) A new penicillin. *British Medical Journal* **1,** 124–125.

Arbeit R, Karakawa WW, Vann WF & Robbins JB (1984) Predominance of two newly described capsular polysaccharide types among clinical isolates of *Staphylococcus aureus*. *Diagnostic Microbiology and Infectious Diseases* **2,** 85–91.

Bennet PM & Hall LMC (1988) Characterization of methicillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology* **27,** 117–123.

Boyce JM, Landry M, Deetz TR *et al*. (1981) Epidemiologic studies of an outbreak of nosocomial methicillin-resistant *Staphylococcus aureus* infections. *Infection Control* **2,** 110–116.

Casewell MW & Hill RLR (1986) The carrier state: methicillin-resistant *Staphylococcus aureus*. *The Journal of Antimicrobial Chemotherapy* **18** (Suppl. A), 1–12.

Centers for Disease Control (1981) Methicillin-resistant *Staphylococcus aureus* in the United States. *Morbidity Mortality Weekly Report* **30,** 140–147.

Centers for Disease Control & Prevention (1997a) Reduced

susceptibility of *S. aureus* to vancomycin – Japan, 1996. *Morbidity Mortality Weekly Report* **46,** 624–626.

Centers for Disease Control & Prevention (1997b) *S. aureus* with reduced susceptibility to vancomycin – United States. *Morbidity Mortality Weekly Report* **46,** 765–766.

Colley EW, McNicol MW & Bracken PM (1965) Methicillin-resistant staphylococci in a general hospital. *Lancet* **1,** 595–597.

Daum RS, Fattom A & Karakawa WW (1994) Capsular Polysaccharides serotypes of coagulase-positive staphylococci associated with tenosynovotis, osteomyelitis, and other invasive infections in chicken and turkeys: Evidence for new capsular types. *Avian Diseases* **38,** 762–771.

Fattom A, Schneerson R, Szu SC, Vann WF, Shiloach J, Karakawa WW *et al*. (1990) Synthesis and immunologic properties in mice of vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides conjugated to *Pseudomonas aeruginosa* exotoxin A. *Infection and Immunity* **58,** 2367–2374.

Fattom A, Schneerson R, Watson DC, Karakawa WW, Fitzgerald D, Pastan I *et al*. (1993) Laboratory and clinical evaluation of conjugate vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides bound to *Pseudomonas aeruginosa* recombinant exoprotein A. *Infection and Immunity* **61,** 1023–1032.

Fournier JM, Bouvet A, Boutonnier A, Audurier A, Goldstein F, Pierre J *et al*. (1987) Predominance of capsular polysaccharide type 5 among Oxacillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* **25,** 1932–1933.

Karakawa WW, Fournier JM, Vann WF, Arbeit R, Schneerson RS & Robbins JB (1985) Methods for the serological typing of the capsular polysaccharides *Staphylococcus aureus*. *Journal of Clinical Microbiology* **22,** 445–447.

Kloos WE, Lambe DW Jr (1991) *Staphylococcus*. In *Manual of Clinical Microbiology*, 5th edn. (eds. Balows *et al*.) American Society for Microbiology, Washington, DC, pp. 222–237.

Lacey RW (1987) Multi-resistant *Staphylococcus aureus*—a suitable case for inactivity? *Journal of Hospital Infections* **9,** 103–105.

Lamb YU (1991) Overview of the role of mupirocin. *Journal of Hospital Infections* **10** (Suppl. B), 27–38.

Mc Dougal LK & Thornsberry C (1984) New recommendations for disk diffusion antimicrobial susceptibility tests for methicillin-resistant (heteroresistant) staphylococci. *Journal of Clinical Microbiology* **19,** 482–488.

McGowan JE (1988) Gram-positive bacteria; Spread and antimicrobial resistance in university and community hospitals in the USA. *The Journal of Antimicrobial Chemotherapy* **21** (Suppl. C), 49–45.

McManus AT, Manson AD Jr, McManus WF & Pruitt BA (1989) What's in a name? Is methicillin-resistant *Staphylococcus aureus* just another *S. aureus* when treated with vancomycin? *Archives of Surgery* **124,** 1456–1459.

Mulligan ME & Arbeit RD (1991) Epidemiologic and clinical utility of typing systems for differentiating among strains of Methicillin-resistant *Staphylococcus aureus*. *Infection Control and Hospital Epidemiology* **12,** 120–128.

Murray BE, Singh KV, Heath JD, Sharma BR & Weinstock GM (1990) Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. *Journal of Clinical Microbiology* **28,** 2059–2063.

Musher DM & McKenizie SO (1977) Infections due to *Staphylococcus aureus*. *Medicine* **56,** 383–409.

Na'was T & Fakhoury J (1991) Nasal Carriage of methicillin-resistant *Staphylococcus aureus* by hospital staff in North Jordan. *Journal of Hospital Infections* **17,** 223–229.

Na'was T, Hawwari A, Hendrix E, Hebden J, Edelman R, Martin M, Campbell W, Naso R, Schwalbe R & Fattom AI (1998) Phenotypic and genotypic characterization of nosocomial *S. aureus* isolates from trauma patients. *Journal of Clinical Microbiology* **36,** 414–420.

National Committee for Clinical Laboratory Standards (1993) Approved Standard M2- A5. *Performance standards for antimicrobial disk susceptibility tests*, 5th edn. Villanova, PA.

Peacock JE Jr, Marsik FJ & Wenzel RP (1980) Methicillin-resistant *Staphylococcus aureus:* Introduction and spread within a hospital. *Annals of Internal Medicine* **93,** 526–532.

Poutrel B & Sutra L (1993) Type 5 and 8 capsular polysaccharides are expressed by *Staphylococcus aureus* isolates from rabbits, poultry, pigs, and horses. *Journal of Clinical Microbiology* **31,** 467–469.

Schlichting C, Branger C, Fournier JM, Witte W, Boutonnier A, Wolz C *et al*. (1993) Typing of by pulsed-field gel electrophoresis, zymotyping, capsular typing and phage typing: resolution of clonal relationships. *Journal of Clinical Microbiology* **31,** 227–232.

Schwalbe RS, Stapleton JS & Gilligan PH (1987) Emergence of vancomycin resistance in coagulase-negative staphylococci. *New England Journal of Medicine* **316,** 927–931.

Scully BE, Briones F, Gu J-W & Neu HC (1992) Mupiricon treatment of nasal staphylococcal colonization. *Archives of Internal Medicine* **152,** 353–356.

Sewell CM, Clarridge J, Lacke C, Weinman EJ & Young EJ (1982) Staphylococcal nasal carriage and subsequent infection in peritoneal dialysis patients. *Journal of the American Medical Association* **248,** 1493–1495.

Storch GA, Radcliff JL, Meyer PL & Hinrichs JH (1990) Methicillin-resistant *Staphylococcus aureus* in a nursing home. *Infection Control and Hospital Epidemiology* **11,** 600–603.

Struelens MJ, Deplano A, Godard C, Maes N & Serruys E (1992) Epidemiologic typing and delineation of genetic relatedness of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis of genomic DNA by using pulsed-field gel electrophoresis. *Journal of Clinical Microbiology* **30,** 2599–2605.

Uttley AH, George RC, Naidoo J, Woodford AP, Johnson CH, Collins D *et al*. (1989) High level vancomycin-resistance enterococci causing hospital infections. *Epidemiology and Infection* **103,** 173–181.

Wanger AR, Morris SL, Ericsson C, Singh KV & Larocco MT (1992) Latex agglutination-negative methicillin-resistant *Staphylococcus aureus* recovered from neonates: Epidemiologic Features and Comparison of typing methods. *Journal of Clinical Microbiology* **30,** 2583–2588.

Ward TT, Winn RE Jr, Hartstein AI & Sewell DL (1981) Observations relating to an inter-hospital outbreak of methicillin resistant *Staphylococcus aureus*: role of antimicrobial therapy in infection control. *Infection Control* **2,** 453–459.

Weinstein RA (1991) Epidemiology and control of nosocomial

infections in adult intensive care units. *American Journal of Medicine* **91** (Suppl. 3B), 179–184.

Working party of the hospital Infection Society & British Society for Antimicrobial Chemotherapy. (1986) Guidelines for the control of epidemic methicillin resistant *Staphylococcus aureus* **7**, 193–201.

Yu VL, Goetz A, Wagener M, Smith PB, Rihs JD, Hanchett J & Zuraleff JJ (1986) *Staphylococcus aureus* nasal carriage and infection in patients on hemodialysis: Efficacy of antibiotic prophylaxis. *New England Journal of Medicine* **315** (2), 91–96.