Point Surveillance of *Staphylococcus aureus* Carriage among Medical Staff in Infectious Diseases Hospital, Kuwait

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Key Words

Staphylococcus aureus · Nasal and throat carriage · Plasmid analysis · Antibiotic susceptibility ·

Abstract

Objective: To investigate the carriage of *Staphylococcus aureus* among doctors and nurses in Infectious Diseases Hospital, Kuwait, following the detection of 3 cases of methicillin-resistant *S. aureus* (MRSA). **Materials and Methods:** A total of 260 nasal and throat swabs were obtained from 19 doctors and 111 nurses and cultured for the carriage of *S. aureus*. Forty-three *S. aureus* were identified based on their growth characteristics on man-nitol-salt agar, catalase and tube coagulase and DNA hydrolysis. The isolates were tested for susceptibility to antibacterial agents and typed by phage typing; plasmid analysis and pulsed-field electrophoresis were carried out to determine their relatedness. **Results:** Of the 19 doctors, 4 (21%) had nasal carriage while only 1 of them had a throat carriage. Sixteen (14.4%) nurses carried *S. aureus* in their noses and 20 (18%) in their throats. The combined nasal carriage rate for both doctors and nurses was 15.8%, and combined throat carriage was 16.6%. None of them carried MRSA. The isolates were resistant to penicillin G (90%), tetracycline (23.3%), ery-

thromycin (9.3%) and cadmium (100%). Typing of the isolates showed a variety of phage types, plasmid and pulsed-field gel electrophoresis patterns. **Discussion:** None of the doctors or nurses carried MRSA. Typing of the methicillin-susceptible strains that they carried demonstrated that the *S. aureus* were different, indicating an absence of a dominant clone capable of spreading. It is important to maintain a low carriage of *S. aureus* among health-care workers.

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Introduction

Staphylococcus aureus has long been recognized as an important pathogen in human diseases. Staphylococcal infections occur regularly in hospitalized patients with severe consequences despite antibiotic therapy. Due to an increasing number of infections caused by methicillin-resistant *S. aureus* (MRSA) strains, which are now most often multiply resistant to antibacterial agents, therapy has become problematic. Therefore, prevention of staphylococcal infections is now more important than ever, especially following the reports of the isolation of vancomycin intermediate resistant *S. aureus* strains [1–3] and the very recent report of vancomycin-resistant *S. aureus* [4]. Nasal

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carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of *S. aureus* infection. Several studies have consistently documented that the carriage of *S. aureus* in the anterior nares is an important human reservoir for *S. aureus* [5–9] and has been established as a major risk factor for the development of both community-acquired and nosocomial infections [10, 11]. Dissemination of *S. aureus* within hospitals by nasopharyngeal carriage of the organism by health-care workers is also acquiring special importance as an infection control problem, especially in situations where cases of MRSA have been detected. The control of nasal carriage of *S. aureus* among patients and hospital staff is important to prevent suppurative diseases and decrease the incidence of post-operative wound infections [12].

The Infectious Diseases Hospital (IDH) Kuwait is a 150-bed facility for treating infectious diseases. It has no long-term care facility. The IDH had been free of MRSA despite their frequent isolation in other hospitals in Kuwait [13–15]. In September 1996, MRSA was isolated from a patient transferred to the Intensive Care Unit (ICU) from a Medical Ward. Following the discovery of the MRSA in this patient, other patients in the ICU and the Medical Wards were screened for MRSA to detect colonization or infection. The screening yielded MRSA in 2 additional patients in the Medical Ward. Consequently, doctors and nurses in the ICU and Medical Wards were screened for nasal or throat carriage of MRSA in an attempt to determine if any of the medical and nursing staff in these wards was an MRSA carrier. This paper presents the findings of a point surveillance study of *S. aureus* carriage among doctors and nurses in a facility where MRSA was newly isolated.

Materials and Methods

Study Population

In November 1996 following the discovery of methicillin-resistant *S. aureus* in patients in a previously MRSA-free hospital, nasal and throat swabs were obtained from 130 medical staff (19 medical doctors and 111 nurses) of the IDH Kuwait to determine if they carried MRSA. All the participants were healthy adults. Nasal and throat swab specimens were obtained by using sterile dry cotton wool swabs (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, UK). For the nasal swab both the left and right anterior nares were swabbed by rubbing the swab 4 times around the inside of each nostril while applying an even pressure and rotating the swab without interruption. Throat swabs were obtained by holding down the tongue with a tongue blade and firmly rubbing the swab against the tonsils area. The swabs were immediately placed in Stuart's transport medium (Transwab) and transported to the laboratory.

Fig. 1. PFGE of methicillin-resistant *S. aureus* patterns obtained from patients. Lanes 1–3, isolates 74, 75, 77 from patient 1. Lanes 4–6, isolates 79, 90 and 92 from patient 2. Notice the similarity in the patterns of isolates in lanes 1–6. Lanes 7–9, isolates 169, 170 and 172 from patient 3.

Isolation and Identification of *S. aureus*

Within 4 h of obtaining the nasal and throat swabs, they were inoculated onto Columbia blood agar plates (5% sheep blood) and phenol-red mannitol-salt agar plates (Becton Dickinson). The swabs were inoculated in nutrient broth containing 6.5% NaCl incubated at 37°C for 24 h, for subsequent subculture onto solid media. All solid media were incubated at 37°C for 48 h. Gram-positive, catalase-positive cocci were identified as *S. aureus* in accordance with their growth characteristics on mannitol-salt agar, catalase reaction, tube coagulase test and DNA hydrolysis (DNase test).

Antimicrobial Susceptibility Testing

Susceptibility testing to antibiotics was determined by the disk diffusion method on Mueller-Hinton agar [16]. The following antibiotics were tested: penicillin G, oxacillin, vancomycin, teicoplanin, erythromycin, clindamycin, tetracycline, chloramphenicol, gentamicin, kanamycin, streptomycin, trimethoprim, fusidic acid, ciprofloxacin and rifampin, linezolid and mupirocin. Susceptibility to heavy

Table 1. Characteristics of MRSA from 3 patients in IDH, Kuwait

Strain No.	Resistance profiles	Plasmid profile	Phage types	
			International Set	Supplementary Set
74	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8	6/42E/54/75/85/81	NT
75	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8	6/42E/54/75/85/81	MR12/38/M3/MR25/56B/MR12
77	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8	6/75	MR12
78	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8	6/42E/75/81/94	MR12/33/38/MR25/56B
79	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8	6/42E/94	33/MR25
90	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8	85	MR12/33/M5/622/M3
91	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8, 2.0	42E	MR12/33/622/38/M3
169	Mc, Pc, Gm, Tc, Fa, Cip, Cd	25.7, 3.8, 2.0	6/47/75	MR12/33/30/622/38
170	Mc, Pc, Gm, Sm, Em, Cip, Cd	27.5, 3.8, 2.0	6/47/75/85	MR12/33/30/622/38
172	Mc, Pc, Gm, Tc, Fa, Cd	27.5, 3.8, 2.0	52/79/3C/55/6/75	MR12/33/M5/56B
191	Mc, Km, Tc, Fa, Cip, Cd	27.5, 3.8, 2.0	79/47/75/85	MR12/33/38/M3/MR8
192	Mc, Gm, Sm, Em, Cl, Cm, Tc, Tp, Fa, Tp, Cip, Cd	27.5, 3.8	6/47/54/75/85	MR12/38
216	Mc, Pc, Gm, Tc, Fa, Cip, Cd	27.5, 3.8	6/85	MR12/56B

Isolates 74–78 were from patient 1 and were isolated from ear swab, left foot ulcer, nasal swab and axillary and right foot ulcer. Isolates 79, 90, 192 and 216 were from patient 2 and were obtained from vaginal swab, nasal swab, wound swab and throat swab. Isolates 91, 169, 170, 172 and 191 were from patient 3 and were obtained from throat swab, tracheal aspirate, urethral catheter and nasogastric tube.

Mc = Methicillin; Pc = benzyl penicillin; Gm = gentamicin; Sm = streptomycin; Km = kanamycin; Em = erythromycin; Cl = clindamycin; Cm = chloramphenicol; Tp = trimethoprim; Tc = tetracycline; Cip = ciprofloxacin; Fa = fusidic acid; Cd = cadmium.

Table 2. Health carrier rates of *S. aureus* among medical staff in IDH

Occupation	Nasal carriers	Throat carriers	Nasal and throat carriers
Doctors (n = 19)	4 (21.0%)	1 (5.3%)	1 (5.3%)
Nurses (n = 111)	16 (14.4%)	20 (18.1%)	3 (2.7%)

metal ions, cadmium, mercuric chloride and nucleic acid-binding compounds, propamidine isethionate and ethidium bromide was tested using 6-mm disks impregnated with cadmium acetate (130 µg), propamidine isethionate (50 µg) and ethidium bromide (60 µg). Penicillinase production was determined with nitrocefin (Oxoid) according to the manufacturer's instructions. *S. aureus* strain ATCC25923 was used as a control strain.

Phage Typing

The 43 *S. aureus* isolates were typed by a combination of phage typing, plasmid contents and pulsed-field gel electrophoresis (PFGE) to determine if they were related.

Bacteriophage typing was performed using the International Set of typing phages and Supplementary phages supplied by the Staphylococcal Reference Laboratory, Laboratory of Hospital Infection of the Public Health Laboratory Service, Colindale, UK. Typing was performed at 100 × RTD and results were reported to conform to the standards as published previously [17].

Plasmid Isolation

Plasmids were isolated by the cetyltrimethylammonium bromide method, separated by agarose gel electrophoresis and sized as described previously [14].

Pulsed-Field Gel Electrophoresis

PFGE was carried out as published previously [14].

Results

Characterization of MRSA Isolates

The characteristics of the MRSA isolated from the 3 patients are presented in table 1. Plasmid analysis and PFGE indicated that a single strain colonized different sites in each patient. The results also demonstrated that isolates from 2 patients had identical PFGE patterns and were closely related to those from the third patient with only three PFGE band differences (fig. 1).

Screening of Doctors and Nurses

The results showed that none of the doctors and nurses carried MRSA. However, 4 (21%) doctors and 16 (14.4%) nurses carried *S. aureus* in their noses, while 1 (5.3%) doctor and 20 (18%) nurses carried the organism in their throats (table 2). The combined nasal and throat carriage rate for both doctors and nurses was 33.0%. Only 4 work-

Fig. 2. Plasmid content of representative *S. aureus* from nurses and doctors. No plasmid was detected in isolates in lanes F, O and Q. Isolates in lanes B, I, J, K, L, N, and P contained only one plasmid. The rest contained more than two plasmids.

ers (1 doctor and 3 nurses) had both nasal and throat carriage of *S. aureus*.

Characterization of the S. aureus Isolates

The 43 *S. aureus* isolates from the doctors and nurses were all susceptible to vancomycin, teicoplanin, mupirocin, linezolid, ciprofloxacin, fusidic acid, rifampicin, mercuric chloride, propamidine isethionate and ethidium bromide. They were resistant to cadmium ion. Thirty-nine (90%) of the isolates were resistant to penicillin G and produced penicillinase. Ten isolates (23.2%) were resistant to tetracycline, 4 isolates (9.3%) were resistant to erythromycin and 2 isolates were resistant to kanamycin and trimethoprim, 1 isolate was resistant to chloramphenicol.

Thirty-seven isolates contained detectable plasmid DNA. The plasmids varied in number and size as shown in figure 2. Twenty-one isolates carried a single plasmid while the rest carried two or more plasmids.

Results of phage typing using both phages of the International Set of typing phages and Supplementary phages showed that more of the isolates were typed with the Supplementary phages than with those of the International Set of typing phages. Ten isolates could not be typed by both sets of phages. Thirteen isolates were typed by the Supplementary phages but not by the International Set of phages. Of those that were typed by the International Set of phages, 11 were lysed by group III phages, 4 were lysed by group II phages. Of the supplementary phages, 17 isolates were lysed by phage 56B while 12 were lysed by phage MR25. PFGE (not shown) yielded different unrelated patterns, indicating a lack of a dominant clone. In addition, PFGE failed to demonstrate a clonal relatedness between the MRSA and the *S. aureus* isolates from the doctors and nurses.

Discussion

This study investigated the nasal and throat carriage of *S. aureus* among doctors and nurses following the detection of MRSA in 3 patients in a facility that had been previously free of MRSA. The MRSA from the 3 patients were closely related as demonstrated by their resistance patterns, plasmid contents (table 1) and PFGE pattern (fig. 1). It appears that the MRSA was introduced into the medical ward by one of the patients. However, it was difficult to ascertain which of the patients was the index case. No MRSA was isolated from any of the doctors and nurses, suggesting that they were not the source of the MRSA obtained from the patients. The absence of MRSA carriage among the health-care workers is in contrast to reports of other studies where MRSA carriage was high among health-care workers [18], but is consistent with the fact that IDH had previously been free of MRSA. However, 43 (33.0%) of the 130 personnel screened carried methicillin-susceptible *S. aureus* in their noses or throats. The carriage rate of *S. aureus* among the doctors and nurses in this study was similar to the carriage rate reported among a healthy nonhospitalized population in Portugal [19] and Kuwait [20], but was lower than the 41% carriage rate detected among health-care workers in two West African hospitals [21] and higher than the 23% carriage rate among health-care workers in a German hospital [22].

This study has highlighted the importance of screening multiple sites in patients and health-care workers for *S. aureus* carriage. Screening of the nose alone would have detected *S. aureus* in only 4 of the 19 doctors and 18 of the

111 nurses. Similarly, screening of the throat alone would have yielded *S. aureus* in only 1 doctor and 20 nurses. This is significant since only 3 nurses and 1 doctor carried the organism in both nose and throat. This observation suggests that *S. aureus* carriage may have been underreported in studies where only nasal carriage has been investigated.

Results of antibiotic susceptibility testing showed that 90% of the isolates were resistant to benzyl penicillin while a small number of isolates were resistant to erythromycin, kanamycin and trimethoprim. This is reassuring because it shows that a range of therapeutic options is available for clearing these organisms. Nasal carriage of penicillin-resistant *S. aureus* has been successfully cleared with dicloxacillin, a penicillinase-stable penicillin [23]. Similarly, mupirocin is useful in the eradication of nasal carriage of *S. aureus* [24, 25]. It is interesting that all of the isolates, including the penicillin-susceptible isolates, were resistant to cadmium ions. The determinants for penicillin resistance in *S. aureus* are usually carried on plasmids that also carry resistance to cadmium and other antimicrobial agents [26]. However, a plasmid carrying cadmium resistance without penicillin resistance has recently been isolated from a penicillin-susceptible *S. aureus* [27] similar to the isolates in this study.

Typing is an important tool for defining the relatedness of microorganisms. Phage typing, plasmid content and PFGE patterns have been used extensively to type *S. aureus* isolates [14, 15, 28, 29]. In this study, results of phage typing indicated that the majority of the isolates were susceptible to the Supplementary phages compared to those that were susceptible to the International Set of typing phages. The results justified the use for these new phages for typing *S. aureus* isolates. Nevertheless, phage typing was still limited as a typing tool since it failed to type all the strains. The results of typing the isolates based on plas-

mid content and PFGE patterns demonstrated that the isolates were different and unrelated. The results also failed to demonstrate a clonal relationship between the MRSA obtained from patients and *S. aureus* from the doctors and nurses. Furthermore, only very few of those screened carried the organism in both noses and throats. The absence of a dominant or dominant clones among the *S. aureus* carried by the doctors and nurses was reassuring as it signifies the absence of epidemic clones capable of rapid spread. However, it is important to maintain very low *S. aureus* carriage in health-care workers because of the clinical implications of such carriage.

Conclusion

We investigated the throat and nasal carriage of *S. aureus* among doctors and nurses following the detection of MRSA in 3 patients in a facility that had been previously free of MRSA. The results demonstrated that none of the doctors or nurses carried MRSA and were not responsible for the MRSA transmission. However, they carried methicillin-susceptible *S. aureus*. Typing of the methicillin-susceptible strains that they carried demonstrated that they were different and not related to the MRSA isolates carried by the patients, indicating an absence of a dominant clone capable of spreading. The study highlighted the significance of screening more than a single site for *S. aureus* colonization.

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