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Molecular Characterization of Epidemic Ciprofloxacin- and Methicillin-Resistant *Staphylococcus aureus* Strains Colonizing Patients in an Intensive Care Unit

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Eighteen methicillin-resistant *Staphylococcus aureus* (MRSA) samples isolated from patients and the environment in an intensive care unit (ICU) during a routine surveillance were tested for antimicrobial resistance and typed by pulsed-field gel electrophoresis. Three pulsed-field patterns were observed. Sixteen were ciprofloxacin resistant and had identical pulsed-field patterns. The results suggested that a ciprofloxacin-resistant MRSA clone had contaminated the environment and spread among patients. This study demonstrates the application of infection control surveillance combined with strain typing in detecting MRSA colonization in the ICU where it was not known to exist.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is being introduced from the community into the hospitals (5, 7, 17). These are posing new problems for infection control practitioners since patients coming from the communities may not be subjected to the rigorous preadmission screening used for previously hospitalized patients (12). Besides being multiply resistant to common antistaphylococcal agents, some MRSA strains spread more readily than others once introduced into hospitals and are often difficult to eradicate once established there (12, 14). Transmission of MRSA occurs primarily from colonized or infected patients to other patients or staff and vice versa (1, 2, 8, 9, 11). The environment also contributes to MRSA transmission (4). Hospitals, determined to prevent MRSA outbreaks, have adopted measures to enhance their early detection in patients and to institute appropriate control measures, which are the cornerstone for controlling MRSA spread (12, 14, 18, 19). This often involves strain typing to identify those causing the outbreak (3, 13, 17).

The Mubarak Al Kabeer Hospital, Kuwait, is a 500-bed teaching hospital affiliated to the Faculty of Medicine, Kuwait University. It consists of general medical, urology, pediatrics, surgical, dialysis, and renal transplantation wards; an intensive care unit (ICU); and outpatient departments. The ICU has five rooms with three beds each. The incidence of MRSA in this hospital is low. It constituted only 6% of all *S. aureus* isolated in the hospital in 1994 (unpublished results) and has been isolated infrequently from the ICU. Consequently, patients are not usually screened for MRSA on admission to the ICU. Nevertheless, the importance of keeping the ICU free of MRSA is well appreciated. Consequently a decision was taken to conduct microbiological surveillance of patients, staff, and environment in the ICU for MRSA at regular 3-month intervals to detect any newly introduced MRSA. This study was initiated in response to that decision. The first such surveillance study was conducted between 17 and 24 January 1995. All medical and nursing staff, patients, and environmental sites were screened for MRSA. Swabs were taken from the nose, groin, axillae, skin lesions (if present), catheter sites, and urine from patients with urinary catheters. Nasal swabs only were taken from staff. Environmental sites swabbed were floors, beds, shelves, and medicine trolleys. Prior to the screening a patient was transferred from the ICU to a medical ward. Subsequently patients and staff in the medical ward were screened for MRSA.

Surveillance swabs were cultured on mannitol salt agar (MSA; UniPath, Basingstoke, United Kingdom) and incubated at 37°C for 48 h. Cultures were identified as *S. aureus* based on growth characteristics on MSA plates (yellow colonies), Gram reaction, and positive results for coagulase, catalase, and DNase tests. All isolates were stored in skim milk at −80°C until processed.

A total of 18 MRSA samples were isolated from five patients in two rooms (rooms 1 and 2) in the ICU and from two patients in the medical ward (Table 1). MRSA strains were isolated from the nose in all seven patients and from additional sites in six patients. MRSA was isolated from the floor and a bedside in room 2 of the ICU (Table 1). No MRSA was grown from urine or catheter sites or from the staff. Colonized patients were transferred to an isolation ward and treated by their physicians until they were discharged. Those that were not colonized were transferred to the other rooms in the ICU. The two rooms were closed and cleaned with Hycolin and were reopened after repeated swabs failed to grow MRSA.

The 18 MRSA samples were tested for resistance to antimicrobial agents and typed by pulsed-field gel electrophoresis (PFGE) to determine their relatedness. Susceptibility testing was performed by the disk diffusion method as described previously (10) using commercial disks (Oxoid; UniPath). For sensitivity to heavy metals and nucleic acid-binding compounds, 6-mm-diameter disks were impregnated with the following agents: cadmium acetate (50 μg), propamidine isethionate (50 μg), ethidium bromide (60 μg). All of the samples were susceptible to vancomycin, teicoplanin, mupirocin, rifampicin, clindamycin, minocycline, novobiocin, and propamidine isethionate but resistant to methicillin, gentamicin, kanamycin, and the agents shown in Table 1. They were divided into three groups based on their resistance patterns. Group 1 consisted of 16 ciprofloxacin-resistant isolates. Half of these were also resistant to chloramphenicol. Groups 2 and 3, consisting of one isolate each, were sensitive to ciprofloxacin but differed from

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TABLE 1. Properties of MRSA isolates

Patient[a] (isolation date [day/mo/yr])	Strain	Ward (room)	Site	Resistance profile[b]	PFGE type
1 (17/1/95)	MB357	ICU (1)	Nostril	Em Tc Tp Cf Cd	1
1 (17/1/95)	MB358	ICU (1)	Axilla	Em Tc Tp Cf Cd	1
1 (17/1/95)	MB359	ICU (1)	Groin	Em Tc Tp Cf Cd	1
2 (18/1/95)	MB360	ICU (2)	Nostril	Em Tc Tp Cf Cd Cm	1
2 (18/1/95)	MB361	ICU (2)	Axilla	Em Tc Tp Cf Cd Cm	1
2 (18/1/95)	MB362	ICU (2)	Groin	Em Tc Tp Cf Cd Cm	1
3 (17/1/95)	MB363	ICU (1)	Nostril	Em Tp Cd Cm	2
3 (17/1/95)	MB364	ICU (1)	Axilla	Em Tc Tp Cf Cd Cm	1
— (18/1/95)	MB365	ICU (2)	Shelf	Em Tc Tp Cf Cd Cm	1
4 (23/1/95)	MB366	MW[c]	Nostril	Em Tc Tp Cf Cd	1
4 (23/1/95)	MB367	MW	Groin	Em Tc Tp Cf Cd	1
4 (23/1/95)	MB368	MW	Groin	Em Tc Tp Cf Cd	1
5 (24/1/95)	MB369	MW	Nostril	Em Tc Tp Cf Cd	1
5 (24/1/95)	MB370	MW	Groin	Em Tc Tp Cf Cd	1
6 (24/1/95)	MB371	ICU (2)	Nostril	Em Tc Tp Cf Cd Cm	1
6 (18/1/95)	MB372	ICU (2)	Groin	Em Tc Cd	3
— (18/1/95)	MB373	ICU (2)	Bedside	Em Tc Tp Cf Cd Cm	1
7 (17/1/95)	MB374	ICU (1)	Nostril	Em Tc Tp Cf Cd Cm	1

[a] —, sample was from environment rather than patient.
[b] Abbreviations: Em, erythromycin; Tc, tetracycline; Tp, trimethoprim; Cf, ciprofloxacin; Cm, chloramphenicol; Cd, cadmium.
[c] MW, medical ward.

each other in their resistance to tetracycline and chloramphenicol.

For PFGE, cells were treated as reported previously (16) and digested with *Sma*I (Sigma Chemical Co.) according to the manufacturer's instructions. Electrophoresis was performed in 1% (wt/vol) agarose (pulsed-field grade; BioRad Laboratories, Richmond, Calif.) by using the CHEF-DR II system (BioRad Laboratories) at 14°C for 20 h in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0) at 6 V/cm. The pulse times were 5 s (initial) and 40 s (final). Differences between isolates were determined by visual comparison of the bands. Isolates were considered to be related if they did not differ by more than three bands (15). An MRSA strain isolated previously from one of the wards was compared with the 18 isolates. No MRSA isolated previously from the ICU was available for comparison. The 18 MRSA samples gave three PFGE patterns and all of the 16 ciprofloxacin-resistant isolates had identical PFGE patterns (Type 1, Table 1) and were unrelated to isolates MB363 and MB372 (Fig. 1).

The demonstration by PFGE that 16 of the MRSA samples were identical suggested that they had a common origin. This implied that although three different clones of MRSA colonized the patients, only one clone was successful in spreading. This ability to spread among patients qualifies this clone as an epidemic MRSA strain.

The origin of this MRSA clone is unknown. Unfortunately, its relationship to MRSA isolated previously in the ICU could not be determined as none was available and the isolate from outside the ICU and medical ward was unrelated to the epidemic clone (Fig. 1). Also, failure to detect MRSA in any of the staffs in the two wards eliminated the possibility of a staff-patient transmission circuit. Therefore, isolates could have been introduced by a patient from the community. Community MRSAs are becoming increasingly important in hospital infections (5, 7, 17). Alternatively, the floor could have served as a reservoir from which an earlier MRSA could have colonized new patients, similar to the report of Layton et al. (4).

Although the importance of infection control surveillance in controlling established MRSA outbreaks is well established (12, 18, 19), this study has demonstrated the usefulness of infection control surveillance conducted at regular intervals in combination with PFGE typing in detecting MRSA colonization of patients in the ICU where it was unknown to exist. Following the detection of the MRSA the colonized patients were isolated and treated while the rooms were cleaned and disinfected. This program has now been adopted as routine practice in the ICU. The early detection of the MRSA in these patients resulted in the timely arrest of a potential outbreak of MRSA infections. This notion derives from the observation that all seven patients had nasal colonization which has been shown to precede the development of staphylococcal infections in long-term care facilities because most such infections arise from the patients' endogenous strains (6, 9). Also, colonization of patients has contributed to the transmission of MRSA be-

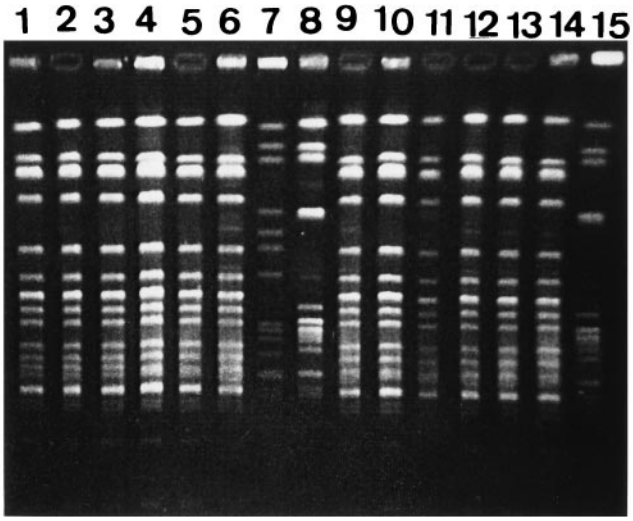

FIG. 1. Pulsed-field patterns of representative MRSA isolates. Lanes 1, 2, 3, 4, 5, 6, 8, 8, 10, 11, 12, 13, and 14, PFGE pattern 1 (see Table 1); lane 7, PFGE pattern 2 (MB363); lane 8, PFGE pattern 3 (MB372); lane 15, ciprofloxacin-sensitive MRSA isolate from another ward.

tween patients and staff (6, 8, 9, 11). This experience suggests that there may be similar cases in other institutions with no known cases of MRSA infections. MRSA could be imported by a colonized patient or staff into an institution with no known problem. Screening patients prior to admission (12) or isolating them till bacteriological culture results are known (14) has been used to prevent the introduction and establishment of MRSA in some institutions. However, this can be very expensive (15). In the absence of such established procedures, screening of patients and staff at regular 1- to 3-month intervals is recommended as a useful alternative. The isolates can then be typed with PFGE or a similar method and the results can be used to assess changes in the composition of MRSA strains in the unit. The exercise can be designed to suit the available manpower and resources of the particular institution.

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