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Methicillin-resistant *Staphylococcus aureus*: a 6-month survey in a Lisbon paediatric hospital

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SUMMARY

The prevalence of nasal colonization and infection with methicillin-resistant *Staphylococcus aureus* (MRSA) among patients and staff was studied in a section of a Paediatric Surgical Unit in Lisbon between February and July 1985. Nasal colonization was demonstrated in 41% of burned patients, 5% of non-burned patients and 35% of the nurses. Infection by MRSA occurred in 30% of the burns.

The isolates had identical serological patterns, slight differences on phage typing and were resistant to methicillin, cephalosporins, tetracycline, erythromycin and aminoglycosides. A chloramphenicol resistance plasmid of 3 Md was present in those isolates which were chloramphenicol resistant and a small plasmid of 1·7 Md which coded for constitutive erythromycin resistance was present in many isolates. Gentamicin, tetracycline and inducible erythromycin resistance were chromosomal.

Several reasons for the apparent low virulence of the isolates are discussed. Attempts to control the outbreak by the discharge of colonized or infected patients, improvement of nursing practices and treatment with temporary removal from work of the colonized nurses did not eliminate the organism from the unit.

INTRODUCTION

In recent years methicillin-resistant *Staphylococcus aureus* (MRSA) have become an important cause of hospital infection. The control of MRSA infections is difficult, and several large hospital outbreaks have been contained only by the employment of specially designed isolation units staffed by individuals who are well-versed in this type of nursing. Operation of these units calls for substantial financial commitment (Editorial, 1985).

Few studies have been undertaken to describe the natural history of the spread

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of a MRSA epidemic in a hospital unit. This paper reports a 6-month survey in a Paediatric Surgical Unit in Lisbon.

MATERIAL AND METHODS

The unit has 42 beds distributed in 10 rooms and is tended by a permanent staff of 10 doctors and 20 nurses. Four rooms with a total of 15 beds were selected for entry into the survey.

For a period of 6 months, from February to July 1985, nasal swabs were taken every Wednesday from patients and also from all nurses present in the unit on that day. Specimens obtained by surface swabbing of burned areas were also collected from burned patients being treated in the reference rooms.

S. aureus strains were identified by the coagulase tube test and antibiotic sensitivity testing, phage typing, serology and plasmid analysis were performed.

Antibiotic sensitivities

Sensitivity tests were performed by the diffusion method on DST Agar (Oxoid) with the following antibiotics: penicillin G, methicillin, cefotaxime, ceftazidime, tetracycline, erythromycin, clindamycin, cotrimoxazole, gentamicin, tobramycin, netilmicin, amikacin, neomycin, chloramphenicol, rifampicin and vancomycin. The criteria of the American Society of Microbiology (Barry & Thornsberry, 1985) were followed. Methicillin sensitivity was tested at 30 °C.

Phage typing

Isolates were typed in the Portuguese National Centre (Instituto Bacteriologico Câmara Pestana) using a set of 31 phages at 100 × R.T.D.

Serology

Slide agglutination was performed according to the technique of Pereira (1961).

Plasmid analysis

A representative group of 24 MRSA isolates was submitted for plasmid analysis.

Lysates were prepared by a modification of the method of Wilson, Totten & Baldwin (1978). Lysostaphin (Sigma Chemical Co.) was used at a concentration of 100 μg/ml followed by detergent lysis of protoplasts. After centrifugation the cleared lysate was deproteinated with protease (Sigma Chemical Co.) and the DNA precipitated with isopropanol at −20 °C overnight. Precipitated DNA was dissolved in TES buffer (0·05 M TRIS, 0·005 M-EDTA, 0·05 M-NaCl) and run on 0·8% agarose gels at 35 V overnight. Gels were stained in ethidium bromide (5μg/ml) and the DNA visualized on a C-62 UV-transilluminator (U.V. Products Inc.). Photography was carried out with a Polaroid MP-4 Camera on 665 film.

RESULTS

Nasal colonization and infection among patients

During the survey swabs from 27 burned patients and 94 non-burned patients were cultured. Only 16 (13%) were colonized by MRSA strains (Table 1). Burned

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Table 1. *Nasal MRSA strains isolated from patients and nurses*

	Burned patients	Non-burned patients	Nurses
No. of examined cases	27	94	20
No. of colonized (MRSA) cases	11 (41%)	5 (5%)	7 (35%)
No. of negative (*S. aureus*) swabs	35	104	75
No. of positive (*S. aureus*) swabs	14	60	19
MSSA strains	42	22	28
MRSA strains	91	186	122
Total no. of specimens			

patients were colonized by the organism significantly more often than non-burned patients (41 *v.* 5%, $\chi^2 = 22{\cdot}93$, $P < 0{\cdot}01$). MRSA strains were recovered from eight patients with infected burns (Table 2). The sequence of MRSA nasal colonization of patients during the survey is shown in Fig. 1.

Nasal colonization among staff

The study of 122 nasal swabs from the 20 nurses revealed that four were permanent carriers of MRSA strains throughout the 6-month survey period and three were transient carriers of the organism (Table 1). The 10 doctors were cultured only once on the last month of the survey because of their less extensive contact with patients. No MRSA were isolated.

Characterization of MRSA strains

Antibiotic susceptibility tests revealed that all strains were resistant to penicillin G, methicillin, cefotaxime, ceftazidime, tetracycline, gentamicin, tobramycin, neomycin and erythromycin. In addition, 13·6% of the strains were sensitive to cotrimoxazole, 18·1% to clindamycin, 31·8% to netilmicin and amikacin, 68·1% to chloramphenicol and 95·4% to rifampicin. All strains were sensitive to vancomycin.

Serologically, all strains revealed antigen 13 (Pereira, 1961). Phage typing indicated some slight differences in phage patterns. 27·2% of the strains were untypable at 100 × R.T.D. One strain was lysed by phage 92. The other 68·2% of the isolates were lysed by phages 54, 75, 77, 85, and 89 in four different combinations (85, 75/85, 54/75/77/85 and 75/85/89).

A total of 24 isolates including one from each colonized or infected patient and one from each colonized nurse were submitted for plasmid analysis. The plasmid profiles (Fig. 2) showed that a chloramphenicol resistance plasmid of 3 Md was present in those isolates which were chloramphenicol resistant, and a small plasmid of 1·7 Md was present in isolates which were clindamycin resistant. One strain (24) isolated from a colonized nurse harboured three plasmids of different molecular weights which were not present in any of the other MRSA isolates.

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Table 2. *MRSA strains isolated from burns*

No. of examined patients	27
No. of infected (MRSA) burns	8 (30%)
No. of negative (*S. aureus*) swabs	23
No. of positive (*S. aureus*) swabs	
MSSA strains	9
MRSA strains	15
Total no. of local specimens	47

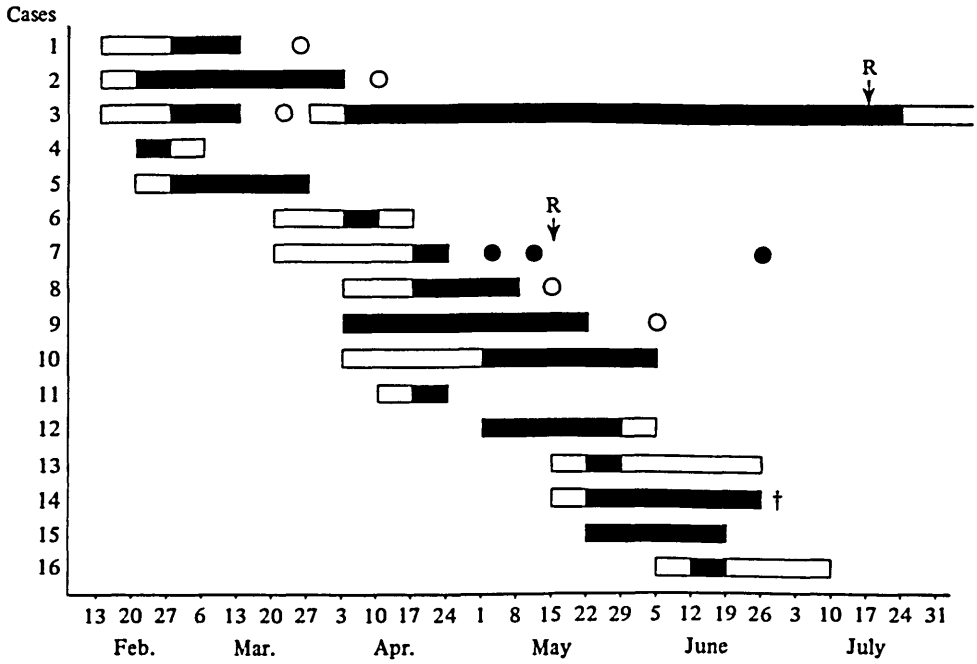

Fig. 1. Sequence of MRSA nasal colonization of patients during the survey. □, negative for MRSA during hospital stay. ■, positive for MRSA during hospital stay. ○, negative for MRSA after discharge. ●, postive for MRSA after discharge. †, dead. R, rifampicin therapy.

Environmental sampling

On the last week of the survey before control measures were introduced, 32 plates containing blood agar and mannitol salt agar were exposed for 30 and 60 min in the four reference rooms during patient care activities. The plates were incubated at 37 °C for 48 h. Twelve *S. aureus* were isolated. None were MRSAs.

Surveillance of patients

Prolonged carriage of MRSA after discharge was uncommon. Ten patients were colonized by MRSA strains on discharge and in six, follow-up cultures were taken at irregular intervals from 8–20 days after discharge. In all but one, MRSA could no longer be recovered.

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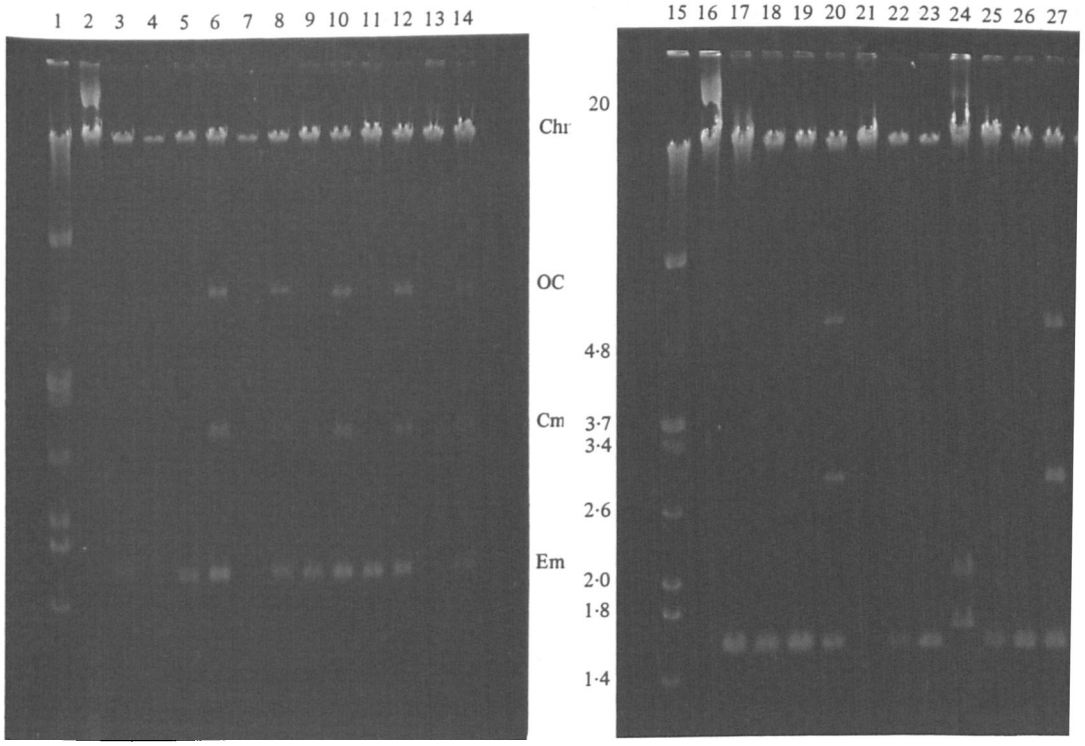

Fig. 2. Agarose gel electrophoresis of 24 MRSA isolates screened for plasmids. Plasmid weights (in Md) are shown on the left. The chromosomal DNA band can be seen migrating at the same position for all strains. Lanes 1, 2 and 15, 16 contain DNA plasmids of known molecular weights from reference strains *E. coli* V517 and *S. aureus* 8325 pI524. Lanes 3, 6, 7 and 9–14 contain MRSA strains isolated from infected burns. Lanes 4, 5 and 8 contain MRSA nasal strains isolated from burned patients. Lanes 17–21 contain MRSA nasal strains isolated from non-burned patients. Lanes 22–28 contain MRSA nasal strains isolated from nurses. Chr, chromosomal DNA. OC, open circular DNA. Cm, chloramphenicol resistance plasmid. Em, erythromycin resistance plasmid.

Control measures

In July 1985, patients known to be infected or colonized by MRSA were sent home. Only one colonized patient could not be discharged. He was treated with oral rifampicin. Follow-up swabs taken 2 and 10 days after rifampicin therapy were negative for MRSA.

An educational program was introduced. The potential seriousness of the problems presented by MRSA was explained to the staff. Improvement of nursing practices was recommended including the use of face masks and careful hand washing with chlorhexidine gluconate before and after any contact with patients. The colonized nurses were temporarily removed from work and were treated with oral rifampicin.

Two months after the survey period, nasal swabs were taken from 11 patients of the four reference rooms as well as from the 10 nurses present in the unit on the same day. A total of 6 MRSA strains were recovered, 4 from patients and 2 from nurses. These nurses had not been found to be colonized during the survey period.

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DISCUSSION

The first report in Portugal of the epidemic spread of an MRSA in a hospital unit occurred 2 years before in the same Paediatric Surgical Unit as that in this study (Melo Cristino, Torres Pereira & Afonso, 1985). The first strain on that occasion was isolated from the nose of a burned patient who was not carrying the organism on admission to the unit and the source of the organism could not be determined. Recommendations were made about control measures but this advice was not carried out. It was therefore expected that in the 1985 survey MRSA strains would be recovered from the unit. This report illustrates the natural history of the spread of an MRSA epidemic over the 6-month period we collected data during which time no active steps were taken to control the outbreak. Steps were only undertaken at the end of the survey period.

Many reports on MRSA have shown that most strains are virulent and are associated with substantial mortality (Boyce *et al.* 1983; Bradley *et al.* 1985; Collopy *et al.* 1984; Cookson *et al.* 1985; Hone *et al.* 1981). However, Linnemann *et al.* (1982) observed that during a hospital MRSA epidemic there was no overall increase in serious infections, and Lacey (1984) suggested that the high incidence of nosocomial infection due to multi-resistant *S. aureus* has probably resulted from environmental and host factors rather than from enhanced virulence of the organism. In this study the MRSA isolates seem to have low virulence. Although 30% of the burns were infected by the strains, no MRSA bacteraemias were detected. One infected patient died, a seriously burned 3-months old infant, but the cause of death could not be directly attributable to the MRSA strain, but rather to a *Pseudomonas aeruginosa* septicaemia.

The only antibiotics that proved to be effective *in vitro* against most of the isolates were vancomycin, rifampicin and chloramphenicol. Vancomycin was not available in the unit and during the hospital stay no infected patient was treated with rifampicin or chloramphenicol.

From only 1 out of 6 colonized patients who had follow-up nasal cultures after discharge was the strain recovered. This patient was treated at home with oral rifampicin to which the organism was sensitive, but the strain could not be eliminated.

Examination of the plasmid profiles showed that although these MRSA strains are gentamicin resistant, they do not have the large gentamicin resistance plasmid usually present in the London and Australian isolates (Bradley *et al.* 1985; Townsend *et al.* 1985), suggesting that genes coding for aminoglycoside resistance are sited on the chromosome, as in some of the recent Dublin isolates (Cafferkey *et al.* 1985). Also, although the isolates came from one hospital unit, they seem not to be a homogeneous group since only some of them possessed a small plasmid of 1·7 Md. This plasmid presumably codes for constitutive erythromycin resistance as curing of clindamycin resistance from these isolates eliminates this plasmid. No such macrolide resistance plasmid has been reported in any of the British, Australian or Dublin MRSA strains (Bradley *et al.* 1985; Cafferkey *et al.* 1985), although a plasmid of the same size has been described in *S.aureus* (Iordanescu & Surdeanu, 1980).

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It is generally agreed that burns units are at a high risk for nosocomial transmission of MRSA, and Crossley, Landesman & Zaske (1979) demonstrated that burn unit personnel exposed to patients with the infection are at increased risk of becoming colonized. The patients colonized or infected with MRSA strains represent perhaps the most important reservoir of the organism (Crossley, Landesman & Zaske, 1979; Rutala *et al.* 1983), which may subsequently be spread within the unit by direct contact transmission or by an airborne mechanism. Although the mode of nosocomial transmission of the organism was not determined during this outbreak, the failure to recover the organism from the air environment in the rooms of infected patients and the high percentage (35%) of permanent or transient MRSA nasal colonization among nurses suggests that nasal carriage played an important role in the transmission and maintenance of this outbreak. Other unidentified factors may also have been important as the improvement of nursing practices and the treatment and temporary removal from work of the colonized nurses proved to be unsuccessful as a control measure.

Because of the lack of isolation facilities in the hospital it was not possible to introduce stricter control measures. This might have been another reason for the failure to control this outbreak which however did not seem to pose much of a threat on the unit. The apparent low virulence of the strains was also supported by their seeming loss of viability outside the hospital environment.

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