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Isolation and molecular characterization of methicillin-resistant coagulase-negative staphylococci from nasal flora of healthy humans at three community institutions in Rio de Janeiro City

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SUMMARY

We describe the isolation and molecular characterization of methicillin-resistant coagulase-negative staphylococci (MRCNS) from the nasal flora of healthy humans from three institutions located in Rio de Janeiro City. Swabs were obtained from the nares of students attending a non-residential public school and adults from two military quarters. Isolates of staphylococci were tested for the presence of the *mecA* gene by hybridization with a specific probe. *S. epidermidis* was the most frequent MRCNS (38 of the total 45 CNS isolated). Twenty-five percent of nasal staphylococcal carriers studied were colonized with MRCNS. Pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested genomic DNA was carried out to study the clonality of the methicillin-resistant *S. epidermidis* (MRSE) isolates. In addition to cross-colonization among individuals belonging to the same institution, familial cross-colonization appeared to contribute to the spread of the methicillin-resistant isolates among two inter-communicable institutions. Indeed, the wide genomic diversity among the MRSE flora suggests that the spread of the *mecA* gene among these isolates might also have occurred via horizontal transmission. Despite the limited number of institutions analysed, it is reasonable to conclude that our data do not represent a situation unique to the three organizations but may reflect other communities in Rio with respect to transmission of MRCNS.

INTRODUCTION

Coagulase-negative staphylococci (CNS) are natural inhabitants of human skin but are also among the most frequently isolated bacteria in clinical specimens [1]. Recently, CNS have emerged as a significant cause of hospital-acquired infections [1, 2]. The increased use of medical devices, such as intravascular catheters and other medical prostheses in seriously ill and immunocompromised patients, is an important contributing factor for the increased isolation of these bacteria in opportunist infections [3]. CNS have been reported to account for 25% of all nosocomial bacteraemia cases and for about 45% of these infections in intensive care units (ICU) in Europe [4]. *Staphylococcus epidermidis* is the dominant species but *S. haemolyticus* is also of importance [1].

Infections caused by methicillin-resistant coagulase-negative staphylococci (MRCNS) represent a therapeutic challenge and these strains may account for 60–90% of all CNS isolated in hospitals [4–6]. The *mecA* gene determining methicillin resistance is widely disseminated among CNS species including *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. simulans*, *S. saprophyticus*, *S. sciuri*, *S. capitis*, *S. warneri* and *S.*

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caprae [7–13]. This widespread distribution of *mecA* might be due to horizontal transmission among CNS and *S. aureus* isolates [8, 14]. Frequently, CNS are multiresistant to antimicrobial agents but susceptible to vancomycin although isolates with decreased susceptibility to vancomycin and teicoplanin have been described [15–17].

Antimicrobial resistance is recognized as a substantial problem for a number of community-acquired infectious diseases. However, few studies have attempted to assess the extent of colonization by resistant bacteria of healthy individuals in the community [18, 19]. The normal non-pathogenic flora of ambulatory and hospitalized individuals may represent a large and constant reservoir of resistance genes which are potentially transferable to virulent bacteria [1, 14]. Thus, tracing the dissemination of resistant bacterial populations and their genomic diversity may inform the epidemiological and evolutionary overview of their antimicrobial resistance and provide new insights into means of control. There are no recent data in the literature on the frequency of methicillin resistance among the CNS nasal flora of healthy individuals. We therefore investigated the dissemination of the *mecA* gene among CNS nasal isolates from healthy individuals in three institutions, located in Rio de Janeiro City, and studied their genomic diversity using pulsed-field gel electrophoresis (PFGE) of chromosomal DNA digests.

METHODS

Nasal sampling

We analysed 316 paired nasal swabs randomly obtained from 57 healthy children (6–13 years old) in four classes from a non-residential public school located in Rio de Janeiro City and from 101 adults (18–49 years old) from two military barracks. Barrack 1 (50 adults) and barrack 2 (51 adults) are both located in Rio and the latter is situated about 50 m from the public school. Approximately one-third of the military personnel at barrack 2 sent their children to the public school. Barrack 1 is totally independent from barrack 2 and from the public school, and is about 6 km from barrack 2. The population studied represented 10% of the total individuals of these three communities. Nasal swabs were transported to the laboratory and processed immediately. One of each pair of swabs from each individual was put into 2 ml of trypticase soy broth (TSB) containing 7·5% NaCl (w/v) and 10 mg/l of methicillin. The broth was incubated at 35 °C for 24–48 h and subcultured on trypticase soy agar (TSA) which was incubated at 35 °C for 18 h. The second swab was streaked directly on TSA and incubated at 35 °C for 18 h. Staphylococci were presumptively identified by Gram-stain and by catalase and coagulase tests. Identification of methicillin-resistant strains was confirmed in the Autoscan, Microscan System (Dade Behringer, Sacramento, USA).

Methicillin resistance

Methicillin-resistant isolates were identified by subculturing 100 μl of a turbid broth culture on TSA containing 25 mg/l of methicillin, incubated at 35 °C for 24–48 h [20–22]. Isolates were stored in 12% glycerol broth at −70 °C. Chromosomal DNA from each isolate was prepared as described previously [23], with a lysis mixture containing mutanolysin (65 U/ml), lysozyme (7×10^4 U/ml) and BRIJ 35 (0·05%; v/v).

E. coli plasmid (pMF13) [24] was purified using a Flex-Prep kit (Amersham-Pharmacia Biotechnology, Piscataway, USA). The *Xba*I–*Pst*I DNA fragment of 1196 bp of pMF13 was excised from 0·8% agarose gel using the Sephaglas Band Prep kit (Amersham-Pharmacia) and fluorescein-labelled probe by the enhanced chemiluminescence (ECL) gene labelling system (Amersham-Pharmacia). Chromosomal DNA was assayed for the *mecA* gene by dot–blot hybridization procedures using the ECL detection system.

PFGE

Chromosomal DNA digested with the restriction endonuclease *Sma*I was separated by PFGE as described previously [25]. DNA banding patterns were analysed as follows. Isolates with identical DNA patterns were assigned the same capital letter; isolates varying by 1–6 bands from this were considered variants of a unique pattern and were differentiated by a number. Strains differing by more than six bands were assigned different capital letters and considered unrelated.

Susceptibility tests

Disk diffusion tests were carried out as recommended by the National Committee for Clinical Laboratory

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Standards (NCCLS) [26]. The following antimicrobial agents (Cecon, São Paulo, Brazil) were tested: oxacillin 1 μg, penicillin 10 U, clindamycin 2 μg, chloramphenicol 30 μg, ofloxacin 5 μg, erythromycin 15 μg, tetracycline 30 μg, gentamicin 10 μg, trimethoprim-sulfamethoxazole 1·25–23·75 μg, vancomycin 30 μg and rifampin 5 μg. Mupirocin (5 μg) disks were purchased from Oxoid, Brasil Ltda., São Paulo, Brazil.

RESULTS

Carriers

Enrichment of nasal swabs in methicillin broth allowed the recovery of 38 MRCNS isolates (24%) from the 158 volunteers. However, direct plating of swabs on antibiotic free medium yielded only 4·4% of MRCNS from the sample population. Only 7 of 158 (4%) individuals had been hospitalized in the 6-month period prior to the study. Eight (5%) had received antibiotics within that period and 31 (20%) had had familial contact with hospitalized persons or health care personnel. Although none of the individuals colonized with MRCNS had been in hospitals or had contact with patients, 4 of the 8 that had previous antimicrobial therapy were colonized with methicillin-resistant isolates.

Methicillin-resistance

All but 7 of 45 isolates that hybridized with the *mecA* probe were identified as *S. epidermidis*. Four were *S. haemolyticus* and the remainder were *S. hominis*, *S. warneri* and *S. equorum*. There was therefore an exact correlation between growth on the screening agar containing 25 mg/l methicillin and detection of *mecA* gene by hybridization with the specific probe. With rare exceptions, MRCNS isolates were susceptible to the majority of non β-lactam antimicrobials tested. However, resistance was observed to tetracycline (62%), erythromycin (42%) and trimethoprim-sulfamethoxazole (27%). Multiresistance to 5 groups of drugs was exhibited by 12 isolates.

Genomic diversity

A total of 17 unique DNA patterns (A-Q) was discerned among the 29 MRSE isolates studied. (Table 1, Fig. 1). Evidence for cross-colonization among individuals at the same institution was found since isolates with the same or closely related DNA profiles (differing by 1–3 DNA bands) were recovered from the same institution. Cross colonization was verified for patterns B and N (isolated from 4 persons at barrack 2), F and J (5 individuals from barrack 1) and B and E (5 persons from the public school). Variants of the patterns B and D were also identified among isolates from individuals at barrack 2 and the public school. Sharing of isolates with specific DNA patterns was not observed between individuals from barrack 1 and the school or from barrack 1 and barrack 2.

Table 1. *Genomic diversity of 28 methicillin-resistant* S. epidermidis *isolates obtained from the nasal flora of healthy humans from three institutions in Rio de Janeiro, Brazil*

Strain	Institution	PFGE pattern*
MR01	Public school	A
MR03		B_1
MR04		B_2
MR05		C
MR06		D
MR07		E_1
MR08		D
MR10		E_2
MR11		E_3
MR15	Barrack 1	F
MR16		G
MR17		H
MR18		F
MR19		I
MR20		J_1
MR21		J_2
MR22		K
MR23		L
MR24		J_3
MR29	Barrack 2	B_3
MR30		M
MR31		B_4
MR32		N_1
MR33		O
MR37		N_2
MR39		P
MR40		Q
MR41		D

* PFGE pattern; pulsed-field gel electrophoresis patterns of the genomic DNA digested with the *Sma*I restriction enzyme.

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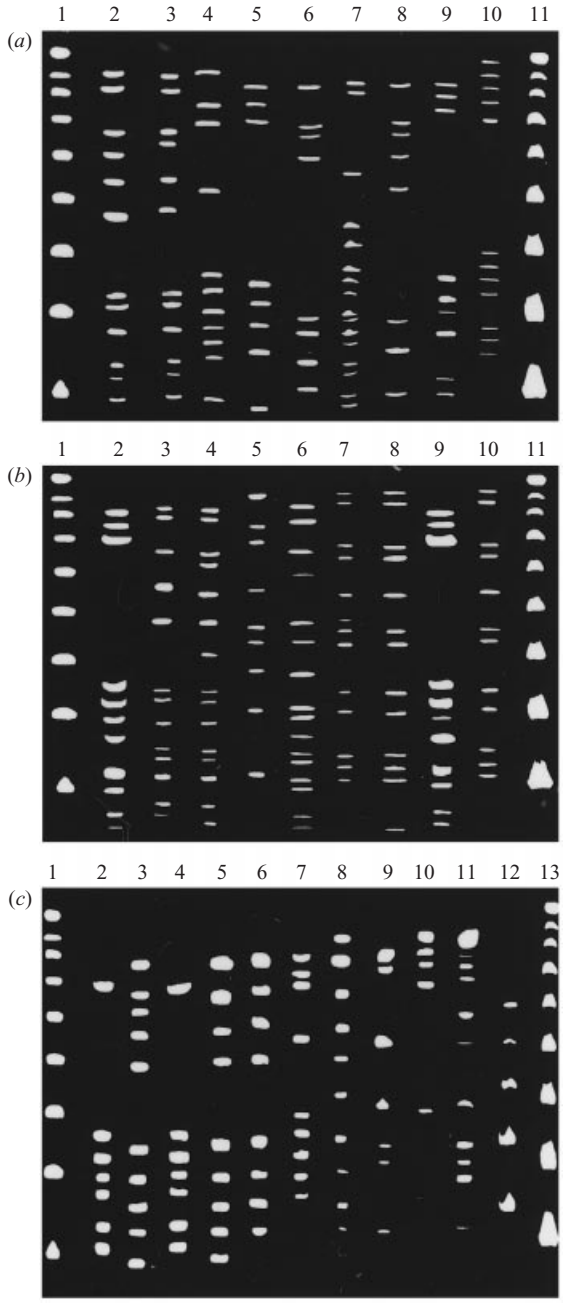

Fig. 1. PFGE patterns of methicillin-resistant *Staphylococcus epidermidis* (MRSE) isolates from healthy carriers. (*a*) MRSE isolates from barrack 2. Lanes 1 and 11, λ ladder (smallest fragment = 100 bp); Lane 2, strain MR29, PFGE pattern B_3; Lane 3, strain MR31, pattern B_4; lane 4, MR30, pattern M; lane 5, MR40, pattern Q; lane 6, MR32, pattern N_1; lane 7, MR39, pattern P; lane 8, MR37, pattern N_2; lane 9, MR41, pattern D; lane 10, MR33, pattern O. (*b*) MRSE isolates from school pupils. Lane 1 and 11, λ ladder; lane 2, strain MR6, PFGE pattern D; lane 3, strain MR4, pattern B_2; lane 4, MR3, pattern B_1; lane 5, MR1, pattern A; lane 6, MR5, pattern C; lane 7, MR7, pattern E_1; lane 8, MR11, pattern E_3; lane 9, MR8, pattern D; lane 10, MR10, pattern E_2. (*c*) MRSE isolates from barrack 1. Lane 1 and 13, λ ladder; lane 2, strain MR18, PFGE pattern F; lane 3, strain MR24, pattern J_2; lane 4, MR15, pattern F; lane 5, MR20, pattern J_1; lane 6, MR21, pattern J_2; lane 7, MR19, pattern I; lane 8, MR23, pattern L; lane 9, MR16, pattern G; lane 10, MR22, pattern K; lane 11, MR17, pattern H; lane 12, λ low range.

DISCUSSION

The emergence of multiresistant bacteria has increased alarmingly in the last three decades and recently, bacteria resistant to all available antimicrobial agents have been reported [6]. This has paralleled the increase of patient populations at high risk of infection due to the frequency and extent of invasive medical interventions and the prolonged survival of many patients with chronic debilitating diseases [6]. In this study we have shown that 24% of 158 healthy adults and children were colonized with MRCNS in the nose and methicillin resistant *S. epidermidis* (MRSE) accounted for about 84% of the total MRCNS isolates recovered. The methicillin screening agar has previously been shown to be very sensitive for the detection of methicillin resistance among staphylococci [22]. Heterogeneous resistant isolates (belonging to classes I and II [22]) that have a low frequency of subpopulations expressing methicillin resistance (about 10^{-5}–10^{-8}) were detected by this test, although these isolates may fail to be detected by some NCCLS recommended tests [20, 22]. The combination of a high inoculum (that allows the detection of small resistant subpopulations) and 25 mg/l of methicillin (a concentration that impairs the growth of borderline resistant strains but allows the growth of frankly resistant isolates) are the factors responsible for the reliability of this test [22]. Our finding of an absolute correlation between growth on the agar medium and the presence of the *mecA* gene underlines the value of this test for the screening of isolates.

Considerable genomic diversity was evident among the MRSE isolates and some cross-colonization between individuals within a community was indicated. In addition, there was a suggestion of familial cross-transmission as strains of the same DNA pattern type were recovered from school children and soldiers from a barrack nearby. This is explained by the fact that about one-third of the students attending the school were the children of personnel from the barrack 2.

In a survey carried out in two hospitals in Rio de Janeiro, (Coimbra MVS, Rocha FS, Figueiredo AMS, unpublished), it was found that MRCNS accounted

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for about 25% and 33% of urinary tract infections caused by CNS in hospital outpatients. These results are in accordance with the 24% MRCNS carriage rate identified in the present study. Furthermore a recent study from the USA [27], carried out with patients from skilled nursing facilities (SNF), reported an overall prevalence of 40% MRCNS colonization. However, 49% of newly admitted patients and 60% of SNF nursing personnel were also colonized with MRCNS. Thus, it is reasonable to speculate that at least a proportion of the individuals sampled here might have been colonized previously in a hospital facility or through cross-colonization by contact with nursing personnel. *S. epidermidis* was the most frequent staphylococcal species isolated (84%; 38/45) and apart from methicillin resistance, isolates from healthy carriers also exhibited varying degrees of resistance to tetracycline, erythromycin and trimethoprim-sulfamethoxazole. To our knowledge there has been no published survey of the pattern of antibacterial drug usage in Rio de Janeiro. The absence of rigid controls of antibiotic usage in the city is likely to be an important selective pressure for the high level of methicillin resistance observed. An association between the use of cloxacillin and cephalosporins and emergence of methicillin resistance in staphylococci has previously been reported [10].

It is important to note that most of the MRCNS carriers had low levels of colonization. Only 4·4% of the carriers were detected without broth enrichment of swabs followed by culture on methicillin-containing agar, which is consistent with low bacterial numbers. Assuming a population of 10^2–10^3 c.f.u./ml of MRCNS in a culture of 10^7–10^8 c.f.u./ml of susceptible bacteria, this would explain the increase of the isolation rate to 24% following enrichment in methicillin broth. Previously, Kernodle et al. [28], using a 6 μg nafcillin-containing medium to detect small numbers of methicillin-resistant staphylococci, suggested that the high percentage of MRCNS strains detected on the skin or in the nares of patients after cardiac surgery was derived from methicillin-resistant organisms present in a site preoperatively in much smaller numbers. These organisms emerged in high numbers as a result of surgical antimicrobial prophylaxis.

In summary, we have shown that a quarter of healthy individuals in three separate institutions harboured MRCNS in the nose. Cross-colonization among members of a community and also familial cross-contamination may have played a role in the spread of resistant clones. Due to the wide diversity of DNA profiles among the predominant MRSE isolates it is probable that the spread of the *mec* locus among these strains might also have occurred via horizontal transmission.

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