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Spread of the Brazilian epidemic clone of a multiresistant MRSA in two cities in Argentina

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognised as an important cause of nosocomial infection. The spread of some MRSA epidemic clones is well documented. In Brazil, and more recently in Portugal, a considerable number of hospital infections has been caused by a unique multiresistant MRSA clone designated as the Brazilian epidemic clone. This paper describes the spread of this clone in hospitals in two cities in Argentina.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged and spread globally over the years since the first clinical use of methicillin. Once MRSA is introduced into a hospital it usually becomes endemic, despite the implementation of infection control measures [1, 2] and is an important cause of nosocomial infection [2, 3].

MRSA hospital isolates have been analysed by various chromosomal typing techniques [4]. These data have shown the predominance of some epidemic MRSA clones either in hospitals located in the same geographic area [5, 6], or separated by large distances [7–9]. Besides the rapid spread of these MRSA clones, epidemic MRSA are frequently multiresistant to antibiotics and susceptible only to vancomycin [6, 9]. Furthermore, MRSA isolates showing reduced vancomycin susceptibility have recently been reported in Japan and the USA [10, 11].

In Brazil, it has been demonstrated that a multiresistant

MRSA clone (Brazilian epidemic clone) is widespread geographically [9]. This clone has caused outbreaks in different cities ranging from Manaus in the north to Porto Alegre in the south (5300 km apart). The great majority of MRSA strains isolated from Brazilian hospitals so far have shared a common pulsed-field gel electrophoresis (PFGE) pattern and the same *ClaI*-*mecA* and *ClaI*-*Tn554* patterns [9]. Recently, the intercontinental spread of the Brazilian epidemic clone to Portugal was demonstrated [7].

This study reports the spread of the Brazilian epidemic clone in Argentina and the persistence of this clone in two hospitals in Rio de Janeiro during the last 5–6 years.

Materials and methods

Bacterial isolates

A total of 80 isolates of MRSA was obtained from infections or nasal colonisation during the period 1992–1997 from three hospitals in two Argentine cities, Buenos Aires and Posadas (Provincia de Misiones), and from two hospitals in Rio de Janeiro, Brazil. Nineteen of these isolates were from the Instituto de Investigaciones Médicas Alfredo Lanari, a 100-bed teaching hospital associated with the

Universidad de Buenos Aires, specialising in nephrology and renal transplantation. Fourteen isolates were from the Hospital de Clínicas José de San Martín, a 500-bed general teaching hospital, also associated with the Universidad de Buenos Aires. These two hospitals are c. 7 km apart in Buenos Aires. Eighteen MRSA isolates were from the Hospital Regional Dr Ramón Madariaga in Posadas, Provincia de Misiones, about 1100 km from Buenos Aires. This general hospital serves Posadas City and neighbouring cities, including some in Paraguay. Ten other MRSA isolates were from the Hospital da Força Aérea do Galeão in Rio de Janeiro. This is a 120-bed hospital offering surgical and medical care (except obstetrics). Finally, 19 isolates were from the Hospital Universitário Clementino Fraga Filho, a 500-bed general teaching hospital in Rio de Janeiro.

Disk diffusion test

Disk diffusion tests were performed as recommended by the National Committee for Clinical Laboratory Standards [12]. The antimicrobial drugs tested were: oxacillin 1 µg, penicillin 10 U, cephalothin 30 µg, clindamycin 2 µg, chloramphenicol 30 µg, ciprofloxacin 5 µg, erythromycin 15 µg, tetracycline 30 µg, gentamicin 10 µg, rifampin 5 µg, vancomycin 30 µg and trimethoprim-sulphamethoxazole 1.25–23.75 µg.

DNA preparation

All the procedures for *S. aureus* DNA preparation were as described previously [13], except that the staphylococcal cell wall was lysed by lysostaphin 90 U/ml. *Escherichia coli* plasmid DNA was prepared with the Flex-Prep kit (Pharmacia Biotechnology) as recommended by the manufacturer. The DNA fragment of *E. coli* plasmid (used as probe) was purified from an agarose 0.8% gel DNA electrophoresis with the Sephaglas Band Prep kit (Pharmacia), as recommended by the manufacturer.

PFGE

Preparation of cells and SmaI digestion of genomic DNA were performed as described previously [9]. PFGE was performed in a counter-clamped homogeneous electric field (CHEF) DR III apparatus (BioRad) for 23 h at 11°C. Running conditions were: the voltage was set at 5.5 V/cm, ramped with an initial forward time of 1 s and a final forward time of 30 s. After electrophoresis, gels were stained with ethidium bromide (1 µg/ml) for 30 min and photographed over a UV transilluminator after washing for 1 h in distilled water. Southern blotting of the DNA gels was as described previously [13]. The DNA was hybridised with a specific IS257 probe, a 630-bp HindIII fragment cloned into pIB 1644 [14]. Bacterial clones were defined as proposed by Tenover et al. [4].

Determination of ClaI-mecA polymorphism

Chromosomal DNA from all MRSA isolates was digested with ClaI and, after Southern blotting, the membranes were hybridised with a specific mecA probe, a 1250-bp PstI-XbaI fragment of the mecA gene cloned into pTZ219 [15].

Probe preparation

The procedure to obtain the fluorescein-labelled probe by the Enhanced Chemiluminescence (ECL) Gene Labelling System was performed as recommended by the manufacturer (Amersham).

Results

Antibiotic resistance phenotype

The 80 MRSA isolates studied were divided into two groups according to their antibiotic susceptibility patterns. The majority of the isolates (72) were susceptible only to vancomycin or to vancomycin and a combination of one or two of chloramphenicol, rifampin and ciprofloxacin. The second group (eight isolates) were susceptible to vancomycin and to five to six other drugs (Table 1).

PFGE patterns

To confirm the persistence of the Brazilian epidemic clone in hospitals in Rio de Janeiro, the PFGE patterns of 29 isolates that were colonising or causing infection in patients from two hospitals in this city were analysed. All the isolates from these Brazilian hospitals had a PFGE pattern designated A₁ that was indistinguishable from the pattern of the Brazilian epidemic clone (Table 1).

The analysis of the PFGE patterns of the 18 MRSA isolates from Hospital Ramón Madariaga in Posadas, Provincia de Misiones, Argentina, during 1995 and 1996, showed four major patterns, designated A–D. Eight of these isolates had a pattern, designated A, indistinguishable from that of strains belonging to the Brazilian epidemic clone. Six other isolates had another major pattern designated B, two had a pattern named C and two had pattern D. Strains designated pattern C had only four PFGE bands different from the Brazilian epidemic clone and thus they are closely related MRSA isolates (Fig. 1a, b; Table 1).

Strains belonging to the Brazilian epidemic clone and its subclones (strains differing by only one to three PFGE bands) were also found among the isolates from Instituto de Investigaciones Médicas in Buenos Aires (nine of 19 isolates). In addition, four isolates with pattern D were shown to cause infection in this hospital. Other sporadic clones were identified among

Table 1. Antibiotic susceptibility and PFGE patterns of the MRSA strains isolated from Brazil and Argentina

City	Hospital	Antibiotic susceptibility	PFGE pattern	Number of isolates
RJ	HFAG	Vc	A ₁	10
RJ	HUCFF	Vc	A ₁	8
RJ	HUCFF	VcCo	A ₁	5
RJ	HUCFF	VcCip	A ₁	2
RJ	HUCFF	VcRf	A ₁	4
Posadas	HM	Vc	A ₁	7
Posadas	HM	VcCoRf	A ₁	1
Posadas	HM	Vc	B ₁	2
Posadas	HM	VcCoClSftCipTc	B ₁	1
Posadas	HM	VcCoClSftCipTc	B ₂	1
Posadas	HM	VcCoClSftCipTc	B ₃	2
Posadas	HM	VcCo	C ₁	1
Posadas	HM	VcCo	C ₂	1
Posadas	HM	VcCoClSft	D	2
BA	IIM	Vc	A ₁	5
BA	IIM	VcCo	A ₃	1
BA	IIM	Vc	A ₄	2
BA	IIM	Vc	A ₆	1
BA	IIM	Vc	D	1
BA	IIM	VcCo	D	1
BA	IIM	VcCoClSftTc	D	2
BA	IIM	VcCipRf	E ₁	1
BA	IIM	Vc	E ₂	1
BA	IIM	VcCipRf	E ₂	1
BA	IIM	VcCo	F	1
BA	IIM	Vc	G	1
BA	IIM	Vc	H ₂	1
BA	HC	Vc	A ₁	5
BA	HC	Vc	A ₂	2
BA	HC	Vc	A ₅	1
BA	HC	Vc	C ₁	2
BA	HC	Vc	C ₂	1
BA	HC	VcRfCip	D	1
BA	HC	Vc	H ₁	1
BA	HC	VcRf	I	1

HFAG, Hospital de Força Aérea do Galeão; HUCFF, Hospital Universitário Clementino Fraga Filho; HM, Hospital Ramón Madariaga; IIM, Instituto de Investigaciones Médicas; HC, Hospital de Clínicas José de San Martín.

RJ, Rio de Janeiro; BA, Buenos Aires.

Vc, vancomycin; Co, chloramphenicol; Cip, ciprofloxacin; Rf, rifampin; Cl, clindamycin; Sft, sulphamethoxazole-trimethoprim; Tc, tetracycline.

the isolates analysed and were designated as patterns E–H (Table 1).

Isolates with PFGE patterns similar to that of the Brazilian epidemic clone, and also subclones of this clone, were observed to cause localised and disseminated infections in eight of 14 patients in another hospital in Buenos Aires (Hospital de Clínicas José de San Martín), and strains with patterns C (observed in the hospital in Posadas city) and D (observed in Posadas and in the Instituto de Investigaciones Médicas, Buenos Aires) were also shown to cause infection in patients in this hospital (Table 1). Sporadic isolates with PFGE pattern H caused infections in patients in this hospital and in the Instituto de Investigaciones Médicas de Buenos Aires. Finally, another clone, designated as clone I, was also isolated (Table 1).

mecA polymorphism and IS257 insertion pattern

All isolates from Argentina that had a PFGE pattern indistinguishable from, or very similar to, that of the

Brazilian epidemic clone also had the same physical location for the *mecA* gene in *S. aureus* chromosome. Furthermore, the isolates had a similar pattern of insertion for IS257 (Fig. 2a, b).

Discussion

DNA typing techniques are useful tools for distinguishing different bacterial strains of the same species [4]. Epidemiological studies using molecular analysis have provided new insights into the origin and evolution of pathogenic bacteria. This approach was used to study the spread and evolution of a unique MRSA clone in Brazil [9, 16, 17]. This study looked for this clonal type in another South American country, Argentina. After restriction with the endonuclease *Sma*I, the fragment patterns of the genomic DNA of 51 MRSA isolates from three Argentinian hospitals and 29 MRSA isolates from two Brazilian hospitals were determined by PFGE. This showed that 25 (49%) of the 51 Argentinian isolates belonged to PFGE pattern A (a pattern similar to that of the Brazilian epidemic clone),

Fig. 1. PFGE patterns of the MRSA isolates from Argentinian and Brazilian hospitals. (a) Isolates with a pattern similar to the Brazilian epidemic clone. Lane 1, λ ladder; 2, MRSA strain BMB5292 (Brazilian epidemic clone [9]); 3–5, MRSA isolates from Argentinian hospitals; 6, MRSA strain BMB5292; 7, 8, isolates from two Brazilian hospitals; 9, MRSA strain BMB5292; 10, λ ladder; 11, λ low mol. range. (b) MRSA isolates exhibiting PFGE patterns A–E. Lane 1, λ ladder; 2, subclone of the Brazilian epidemic clone (PFGE pattern A); 3, PFGE pattern C; 4, PFGE pattern A; 5, 6, pattern D; 7, pattern C; 8, pattern A; 9, pattern B; 10, pattern A; 11 pattern C; 12, pattern A; 13, pattern L; 14, pattern D; 15, λ ladder.

Fig. 2. Polymorphism of the *mec* region among MRSA strains isolated from Argentina and Brazil, exhibiting PFGE pattern A. (a) *mecA*-*Cla*I polymorphism. Lane 1, λ -HindIII; 2–4, isolates from the three hospitals in Argentina; 5, 6, isolates from the two hospitals in Rio de Janeiro; 7, strain BMB5292, a representative of the Brazilian epidemic clone. (b) IS257 insertion pattern. Lanes 1–3, isolates from Argentina; 4, BMB5292 (Brazilian epidemic clone); 5, 6, isolates from Rio de Janeiro.

six (12%) belonged to pattern B, five (10%) to pattern C (very closely related to the Brazilian epidemic clone), seven (14%) to pattern D and the remaining eight isolates belonged to five sporadic patterns (E, F, G, H and I). PFGE pattern A was observed in all three Argentinian hospitals studied and strains with patterns C, D and H had caused infection in patients in hospitals in Posadas city and in Buenos Aires.

The spread of some MRSA clones over large distances may reflect an enhanced ability to spread and colonise patients in hospitals. Although some studies have compared the virulence of epidemic and sporadic MRSA clones, no conclusive data have been published so far. It was shown that some epidemic and non-epidemic MRSA isolates could be discriminated on the basis of protein A gene polymorphism. However, the relationship between virulence and increased number of protein A repeat domains is unclear [18].

The 29 MRSA isolates from Rio de Janeiro hospitals, all belonging to the Brazilian epidemic clone, reflected the predominance and persistence of this unique MRSA clone in Rio in the last 5–6 years [9].

To confirm that the strains with PFGE pattern A from three hospitals in Argentina belonged to the Brazilian clone, the *mecA* region of these isolates was investigated with two DNA probes: (i) a *mecA* probe and (ii) an IS257-specific probe. All isolates tested exhibited the same *mecA* IS257 patterns as that in the Brazilian epidemic clone [9]. These results confirm that the Brazilian epidemic clone has also spread and seems to predominate in some Argentinian hospitals. More recently, the spread of MRSA isolates from the Brazilian epidemic clone in Portugal and its rapid displacement of the Iberian clone (an epidemic MRSA clone that was found in Spain, Portugal, Italy and Scotland) by the Brazilian clone, has been reported [7, 19].

Hitherto, the spread and predominance of some specific clones of MRSA over large geographic areas have been poorly understood. The potential social and economic impact of MRSA infection caused by isolates belonging to the Brazilian epidemic clone can be predicted by its alarmingly rapid spread and by its ability to acquire new genes, including high-level mupirocin resistance [16].

Thus, it is recommended that South American and European health authorities pay special attention to the rapid spread of the Brazilian epidemic clone in hospitals. Further studies are needed to investigate what factors endow isolates of this MRSA clone type with their remarkable ability to spread.

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