Emergence of mupirocin resistance in multiresistant *Staphylococcus aureus* clinical isolates belonging to Brazilian epidemic clone III::B:A

R.L.B. Ramos*, L.A. Teixeira†, L.R. Ormonde‡, P.L.A. Siqueira*, M.S. Santos§, D. Marangoni§ and A.M.S. Figueiredo†

*Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Prof. Paulo de Gêres, Laboratório de Biologia Molecular de Bactérias, Centro de Ciências da Saúde, Bloco I, Cidade Universitária, Rio de Janeiro, RJ 21941.590, Brazil, †The Rockefeller University, 1230 York Avenue 10021, New York, NY, USA, ‡Instituto de Puericultura Maria Gaia Gesteira, Universidade Federal do Rio de Janeiro and §Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Brazil

Mupirocin is a topical antimicrobial agent that has been successfully used to eradicate methicillin-resistant *Staphylococcus aureus* from the anterior nares and other sites of patients and health care personnel. This report describes the acquisition of a novel mupirocin resistance gene (ileS) by an epidemic MRSA clone that is geographically widespread in Brazil.

Introduction

Hospital-acquired infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have increased in recent years [1, 2]. These isolates are often resistant to many other antimicrobial agents [3, 4] and are sometimes involved in severe infections [5, 6]. Prompt control measures may stop MRSA spread in a hospital, but once strains are established they often become endemic and extraordinary efforts may be necessary to stop nosocomial spread [2]. Colonisation of patients contributes to the increase in morbidity and mortality caused by these resistant bacteria. The selection of MRSA by antibiotic therapy may favour the establishment and predominance of these organisms in hospitals [7].

Mupirocin (pseudomonic acid A) is a topical antimicrobial agent, produced by *Pseudomonas fluorescens*, that has been used successfully to eradicate MRSA from the anterior nares and other sites of colonised patients and health care workers [8]. It has been used mainly in hospitals, with the result that mupirocin resistance has been transmitted both horizontally and vertically among *S. aureus* strains [9].

In Brazil, an epidemic MRSA clone that carries *mecA* polymorph III in combination with Tn554 pattern B, and exhibits pulsed-field gel electrophoresis pattern A (clone III::B:A), has caused hospital outbreaks in seven different cities [4, 10], ranging from Manaus in the north to Porto Alegre 5300 km to the south. This report describes the acquisition of high-level mupirocin resistance by this epidemic, multiresistant *S. aureus* clone.

Materials and methods

Bacterial strains

Thirteen mupirocin-resistant isolates were obtained from Hospital Universitário Clementino Fraga Filho (HUCFF), Rio de Janeiro city, RJ, Brazil. Another three high-level mupirocin-resistant isolates were obtained from two other general hospitals (each <400 beds) in Rio de Janeiro city, where mupirocin has been widely used for MRSA nasal decontamination. The strains were isolated from the anterior nares of different patients during 1995 and 1996. All were identified as *S. aureus* by routine identification tests [11] and stored at −70 °C in sterile glycerol 10%.

Antibiotic sensitivity testing

Disk diffusion tests were performed as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [12]. The following disks (Cecon)
were used: oxacillin 1 μg, cephalothin 30 μg, clindamycin 2 μg, benzylpenicillin 10 U, chloramphenicol 30 μg, ciprofloxacin 5 μg, erythromycin 15 μg, tetracycline 30 μg, gentamicin 10 μg, trimethoprim 1.25 μg plus sulphamethoxazole 23.75 μg, vancomycin 30 μg and rifampicin 5 μg. A mupirocin 5-μg disk was obtained from Oxoid.

The MIC of mupirocin was determined in solid medium by the method recommended by the NCCLS [13]. Trypticase Soy Agar (TSA) containing methicillin (Sigma) 25 mg/L was used to screen for methicillin resistance, as described previously [21].

Molecular methods

The procedures for *S. aureus* DNA preparations were as described previously [15], except that the staphylococcal cell wall was lysed with lysostaphin (90 U/ml). *Escherichia coli* plasmid DNA was prepared with the FlexPrep kit (Pharmacia Biotechnology) as recommended by the manufacturer. The fragment of the *E. coli* plasmid used as probe was purified electrophoretically from an agarose 0.8% gel by use of the Sephaglas Band Prep kit (Pharmacia) as recommended. The fluorescein-labelled probe was obtained by the Enhanced Chemiluminescence (ECL) Gene Labelling System as recommended by the manufacturer (Amerham).

The large plasmid harboured by the mupirocin-resistant strains was excised from an agarose 0.8% gel after electrophoresis. The agarose-inserted plasmid DNA was digested with *Hind*III. The plasmid fragments were purified with the Band Prep kit and dot-blotted with nylon membranes as described previously [14]. The probe used was a 2.0-kb *Xba*I fragment of the plasmid pMZ1 [16].

Chromosomal DNA was digested with *Cla*I. The membranes were hybridised with the mecA probe and re-probed (after boiling) with Tn554. The hybridisation patterns involving mecA or Tn554 were identified by comparison with previously described types [17]. The DNA probe used was a 1.25-kb *Pst*I-*Xba*I fragment of the mecA gene cloned into pTZ219 [18]. A 5.5-kb EcoRV transposon-specific fragment was also used to detect the presence of Tn554 [19].

Genomic DNA was prepared and cut with the *Csu*I restriction enzyme as described previously [20]. Pulsed-field gel electrophoresis (PFGE) was performed as described [20] in a CHEF DR III apparatus, except that the voltage applied was 5.5 V/cm² (BioRad, Richmond, CA, USA). Methods used for staining, photographing, Southern hybridisation and probing of the gels have been described previously [15].

**Results**

The MICs of mupirocin for 16 arbitrarily chosen, high-level mupirocin-resistant isolates from HUCFF and two other Rio de Janeiro hospitals varied from 512 to 2048 mg/L. Their DNA hybridised with the novel ileS gene described previously [16] (Fig. 1).

Most of the strains were susceptible only to vancomycin, except those designated Mup 12–Mup 16, which were also susceptible to chloramphenicol. The mupirocin-resistant isolates were also methicillin resistant (Fig. 2) and were classified as heterogeneous class III or homogeneous class IV. Strains with these profiles produced a confluent lawn on agar containing methicillin 25 mg/L and grew up to the edge of a 1-μg oxacillin disk.

Analysis of the genomic DNA by PFGE indicated a pattern similar to that of the epidemic Brazilian clonal type III::B:A (Fig. 3). The analysis of mecA-*Cla*I and Tn554 polymorphs in three of the isolates showed that they were also mecA-*Cla*I type III and Tn554 type B, similar to MRSA clonal type III::B:A (Fig. 4a, b).

**Discussion**

HUCFF is a 498-bed hospital for adult patients that has medical and surgical care, but no paediatric, obstetric or burns ward. MRSA have been reported in HUCFF

![Fig. 1. Dot-blot hybridisation of HindIII restriction fragments of the large plasmid isolated from the mupirocin-resistant *S. aureus* strains, designated as Mup 01, 02 and 03, with a specific DNA probe for the novel ileS gene cloned in pMZ1 [16]. pMZ1 was used as positive control; total DNA obtained from a mupirocin-susceptible MRSA strain belonging to clonal type III::B:A was the negative control.](image-url)
MuPIROCIN-RESISTANT S. AUREUS IN BRAZIL

Mupirocin cannot effectively bind to isoleucyl tRNA synthetase. Low-level mupirocin resistance (MIC 8–64 mg/L) is probably caused by point mutations in the native gene that codes for the synthetase (ileS) [21]. High-level mupirocin resistance (MIC >500 mg/L) is associated with a novel ileS gene.

A previous study [4] showed that epidemic MRSA isolates belonging to clonal type III::B:A are spread over large distances (5300 km) of Brazil, from the north to the south of the country. Recent studies showed that this clone is present in two further Brazilian states [10, 22, 23], bringing the number of Brazilian cities in which this clone is present to seven. This clone is also present in Argentina and Uruguay (Coimbra et al., unpublished observations). The predominance and spread of a unique MRSA clone between different countries has also been demonstrated in Europe [20, 24].

In the present study, analysis of the genomic DNA of mupirocin-resistant strains by PFGE revealed a pattern similar to that of the epidemic Brazilian clonal type III::B:A (Fig. 3), indicating that the novel ileS gene had been horizontally acquired by MRSA isolates. Acquisition of the novel ileS gene may have occurred after the introduction of mecA and transposon Tn554 into this S. aureus strain, as most isolates so far tested do not yet carry the novel ileS gene [4]. Some strains from clonal type III::B:A have also acquired staphylococcal enterotoxin ent genes [23], suggesting that the horizontal acquisition of genes occurs with considerable frequency among strains of this clonal type.

Purified DNA fragments obtained from the large plasmid observed in the mupirocin-resistant strains hybridised with the mupirocin-specific probe (Fig. 1), but the novel ileS gene was not detected in the chromosomal DNA fragments. These results indicate a plasmid location for the novel ileS gene, but this plasmid has not been characterised further. Mupirocin-resistant S. aureus isolates tested in the UK [21] and the USA [25] also carried the novel ileS gene inserted on a large plasmid. A plasmid encoding high-level mupirocin resistance obtained from Brazilian isolates could be transferred to strain RN8411 by filter-mating [26]. Transfer occurred at low frequencies, and this may explain the vertical spread of the novel ileS gene among isolates belonging to clonal type III::B:A.

Tracing the path of bacterial clones is important in the study of the variability and evolution of bacteria. It may also help epidemiologists to predict and control the extensive spread of a new resistance trait. This study has shown that isolates of the epidemic Brazilian clone III::B:A carrying the novel ileS gene are now present in at least three different hospitals in Rio. Although high-level mupirocin resistance is still rare and the study was thus limited by the number of isolates tested, it is noteworthy that the acquisition of

Fig. 2. Physical location of the mecA gene on SmaI-cut chromosomal DNA from the mupirocin-resistant S. aureus strains Mup 01, 02 and 03 and a mupirocin-susceptible clonal type III::B:A strain. After PFGE and Southern blotting the DNA was hybridised to the mecA-specific probe [18].

since 1987, when the incidence was c. 7.9% of the total S. aureus isolates. The incidence was c. 7.2% in 1988, but increased to 33% in 1989 and has remained at this level (last tested October 1996). Topical mupirocin has been administered in this hospital for MRSA decontamination of patients and health care workers since 1990 and from that time screening for mupirocin resistance has been performed by the disk diffusion test. Very few mupirocin-resistant strains were detected in the hospital until 1994. In 1995 the incidence rose to c. 31% of MRSA isolates; from January 1 1996 to October 20 1996, this incidence was c. 16%.

The molecular mechanisms of mupirocin resistance are not clearly understood. In resistant bacteria,
Fig. 3. PFGE of the SmaI-digested chromosomal DNA from mupirocin-resistant *S. aureus* isolates (Mup 01–Mup 11) and a mupirocin-susceptible MRSA strain belonging to clonal type III::B:A. *S. aureus* isolates Mup 12–16 exhibited a pattern identical to that of Mup 01–11.

Fig. 4. Chromosomal DNA samples from the mupirocin-resistant *S. aureus* strains Mup 01, 02 and 03 and a mupirocin-susceptible clonal type III::B:A were digested with ClaI. After electrophoresis and Southern blotting the DNA was hybridised to: a, the mecA-specific probe [18]; b, the Tn554-derived probe.
ileS gene was restricted to multiresistant S. aureus strains of a particular clonal type.

Because of the epidemic dissemination characteristic of this clone and the fact that mupirocin is widely used for MRSA decontamination in some hospitals in Brazil, the mupirocin resistance gene may spread quickly among MRSA isolates in this country (and possibly in South American neighbours), if measures to control the use of this antibiotic are not observed immediately.

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References