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Molecular typing of *Staphylococcus aureus* clinical isolates by pulsed-field gel electrophoresis, staphylococcal cassette chromosome *mec* type determination and dissemination of antibiotic resistance genes

Leticia Millán Laplana [a], Mª Pilar Goñi Cepero [a], Joaquim Ruiz [b], Paula Cerdá Zolezzi [a], Mª Carmen Rubio Calvo [a,c], Melisa Canales Erazo [a], Rafael Gómez-Lus [a,*]

[a] *Department of Microbiology, Preventive Medicine and Public Health, Faculty of Medicine, University of Zaragoza, C/Domingo Miral s/n, 50009 Zaragoza, Spain*

[b] *Centro de Salud Internacional, IDIBAPS, Hospital Clinic, C/Villarroel 170, 08036 Barcelona, Spain*

[c] *Microbiology Service, Clinical University Hospital 'Lozano Blesa', C/San Juan Bosco 19, 50009 Zaragoza, Spain*

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Abstract

Pulsed-field gel electrophoresis (PFGE) of *Sma*I macrorestriction fragments of genomic DNA as well as staphylococcal cassette chromosome *mec* (SCC*mec*) typing for *mecA*-carrying isolates were used to study the distribution of clonal types among 177 *Staphylococcus aureus* clinical isolates recovered in a Spanish hospital between 2000 and 2003. Five major clonal types (P1 to P5) were identified by PFGE, with one of them (P1) comprising the majority of strains (47.5%). According to SCC*mec* typing, SCC*mec* type IVA was the most prevalent type, showing increasing prevalence in the hospital setting with respect to other pandemic clones. One SCC*mec* pattern was detected in different PFGE types, which demonstrates that the latter is a major discriminative typing method. Three novel SCC*mec* elements or variants were found, each in a different PFGE type. Oxacillin (methicillin)-resistant and -susceptible *S. aureus* (MRSA and MSSA, respectively) strains were detected showing identical PFGE patterns, suggesting horizontal transfer of *mecA* to MSSA and/or *mecA* deletion from MRSA. Persistence of several *S. aureus* clones throughout the years within the same hospital environment was also observed.

Keywords: *Staphylococcus aureus*; PFGE; SCC*mec*; *mecA*; Antibiotic resistance genes; Antimicrobial susceptibility

1. Introduction

Nosocomial infections due to multidrug-resistant *Staphylococcus aureus* are an important health problem worldwide [1]. In Spain, *S. aureus* blood isolates show a high prevalence of resistance to oxacillin, ciprofloxacin and erythromycin as well as a high prevalence of multiresistance [2]. Since the first report of methicillin-resistant *S. aureus* (MRSA) in 1961, MRSA has become a major threat worldwide, with an increasing incidence. It is an important clinical problem, since MRSA are often multiresistant and therapeutic options are limited [3]. The prevalence of oxacillin-resistant *S. aureus* is lower in Spain than in other European countries such as the UK, Ireland, Portugal and Italy, and it has increased from 1.5% in 1986 to 31.2% in 2002, although it has not increased in recent years [2–4]. However, large differences have been reported between Spanish hospitals, with 34% MRSA isolates from a hospital in Sevilla, 16.8% for one in Tenerife and 9% for another in Barcelona [3,5].

Multiple DNA-based methods have been introduced to type *S. aureus* strains genetically and to track the dissemination of MRSA clones. Whereas multilocus sequence typing (MLST) has been proven to be the most suitable method for long-term and global epidemiological studies, pulsed-field gel electrophoresis (PFGE) is the method of choice for short-term or local epidemiological studies of *S. aureus* [6,7]. Moreover, complete characterisation of MRSA also

* Corresponding author. Tel.: +34 976 761 692; fax: +34 976 761 693.
E-mail address: gomezlus@unizar.es (R. Gómez-Lus).

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requires identification of the structural types of staphylococcal cassette chromosome *mec* (SCC*mec*), the mobile element that carries the methicillin resistance determinant *mecA* [8,9]. Three types of SCC*mec* (types I, II and III) were originally described in hospital-acquired MRSA strains [9,10]. A fourth type (type IV) has been described, first in community-acquired MRSA isolates and then in several MRSA backgrounds, including hospital isolates [9,11–13]. Recently, another type (type V) has been reported in a community-acquired MRSA strain isolated in Australia [14]. In 2005, seven novel variants of SCC*mec* were reported [15], whereas in 2006 an SCC*mec* variant similar in size to type IV but with a new *ccrAB* allotype was proposed to be renamed SCC*mec* type VI [16]. Acquisition of SCC*mec* has occurred on multiple occasions and at least five different methicillin-susceptible *S. aureus* (MSSA) phylogenetic lineages have acquired the element. Hence, MRSA has arisen multiple independent times by lateral transfer of the SCC*mec* element into MSSA precursors [13,17–21].

In this work, we studied the epidemic relationship between 177 clinical *S. aureus* isolates by molecular typing using PFGE in combination with SCC*mec* typing for *mecA*-carrying isolates. In addition, the distribution of resistance genes for various antibiotics was analysed.

2. Materials and methods

2.1. Bacterial isolates and growth conditions

From October 2000 to February 2003, 177 erythromycin- and/or oxacillin-resistant *S. aureus* clinical isolates were collected in the Microbiology Service of the Clinical University Hospital 'Lozano Blesa' (Zaragoza, Spain). Initial identification was based on colony and microscopic morphology as well as agglutination tests with the Pastorex® Staph Plus kit (Bio-Rad, Marnes-la-Coquette, France). The isolates were cultured on Mueller–Hinton agar (Bio-Rad) and stored frozen (−80 °C) in sterile skimmed milk. *Staphylococcus aureus* ATCC 29213 was used as the control strain.

2.2. Antibiotic susceptibility testing

Susceptibility tests were performed by the disk diffusion method [22] with erythromycin, azithromycin, clindamycin, oxacillin, gentamicin, kanamycin, neomycin, streptomycin, tetracycline, minocycline, chloramphenicol, vancomycin (Bio-Rad) and miokamycin (Neo-Sensitabs, Taastrup, Denmark). Results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [23] and the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) [24] for miokamycin, neomycin and streptomycin. Erythromycin, miokamycin and clindamycin were tested by the disk diffusion method to differentiate the macrolide–lincosamide–streptogramin B (MLS) resistance phenotype as constitutive, inducible or MS [25].

Minimum inhibitory concentrations (MICs) for erythromycin, clindamycin, gentamicin, tetracycline, minocycline, chloramphenicol (Sigma, St Louis, MO), azithromycin (Pfizer, Madrid, Spain), miokamycin (Menarini SA, Badalona, Spain), telithromycin, quinupristin/dalfopristin (Synercid®; Aventis, Madrid, Spain), oxacillin (SmithKline Beecham S.A., Toledo, Spain), kanamycin (Amersham Life Science, China), linezolid (Pharmacia & Upjohn Inc., Kalamazoo, MI), and vancomycin (DISTA, S.A., Alcobendas, Madrid, Spain) were determined by the agar dilution method [26]. CLSI [27] breakpoints were used for MIC interpretation for all drugs except for miokamycin, which was interpreted according to the CA-SFM [24].

2.3. Polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from staphylococcal cultures [28] and used as a template for amplification. The presence of *mecA* was tested according to the PCR assay described by Louie et al. [29]. The ribosomal methylases encoded by *erm*(A), *erm*(B) and *erm*(C) [30] and the macrolide efflux pump encoded by *msr*(A) [31] were determined by PCR. Oligonucleotide primers and conditions for *aph*(3′)-*IIIa, ant*(4′)-*Ia* and *ant*(6) were those published by Van de Klundert and Wiegenthart [32], Schmitz et al. [33] and Swenson et al. [34], respectively. Primers for *aac*(6′)/*aph*(2″) (forward 5′-CAAgAgCAATAAgggCATAC-3′ and reverse 5′-CAATAgTTTCAATAggATAA-3′) were designed by us from published GenBank sequences (accession no. M18086, M29261) to provide a PCR product of 936 bp. PCR conditions consisted of an initial cycle of 5 min of denaturation at 94 °C, followed by 32 cycles of 30 s of denaturation at 94 °C, 45 s of annealing at 44 °C, 2 min of elongation at 72 °C and a final cycle of 10 min of elongation at 72 °C. The PCR mix contained 2.5 mM $MgCl_2$, 0.3 mM nucleotide triphosphates, 0.5 μM of each primer and 1.5 U *Taq* polymerase. Primers and conditions for amplification of *tet*(K) and *tet*(M) were those described by Trzcinski et al. [35] and Doherty et al. [36], respectively. cat_{pC194} was detected with oligonucleotides catD 5′-gAA ACA TAA AAC AAg AAg gA-3′ and catR 5′-ATA gAA AgA gAA AAA gCA TT-3′ designed from the sequence of staphylococcal plasmid pC194 (accession no. NC_002013). The PCR reaction was performed at the following temperature profiles: 32 cycles of 94 °C for 30 s, 46 °C for 45 s and 72 °C for 2 min, with an initial cycle of 5 min of denaturation at 94 °C and one cycle of 10 min of elongation at 72 °C. The PCR mix contained 3 mM $MgCl_2$, 0.5 mM nucleotide triphosphates, 1 μM of each primer and 1.75 U *Taq* polymerase. All PCR amplifications were performed in a Perkin-Elmer Cetus DNA Thermal Cycler (Perkin-Elmer, Norwalk, CT) and the products were analysed by electrophoresis through 1.5% agarose gels. PCR reagents were purchased from Promega (Madison, WI).