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Phenotypic and genetic characteristics of fluoroquinolone- and methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: Fluoroquinolone resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) has increased in recent years. The objective of this study was to characterise two MRSA populations, one susceptible to fluoroquinolones and other resistant identifying the clonal types and the differential characteristics of both MRSA populations.
Methods: Molecular typing using PFGE, MLST, *spa* and SSC*mec* was performed on 192 MRSA strains isolated from 2009 to 2011, 49 only oxacillin-resistant (OX-R) and 143 oxacillin and levofloxacin-resistant (OX-R-LEV-R). Mutations that conferred resistance to fluoroquinolones, hypermutable phenotypes and the presence of eight microbial surface components recognising adhesive matrix molecules (MSCRAMMs) were also studied.
Results: A statistically significant increase in the OX-R-LEV-R phenotype was observed ($p < 0.05$). The most common clone of the OX-R isolates was sequence type (ST) 8 (32.6%), followed by ST72 (26.5%) and ST5 (26.5%). In the OX-R-LEV-R phenotype, the ST5 clone was the most common (65.7%), followed by ST72 (15.4%), and ST125 (12.6%). All isolates except the ST398 clone carried the SCC*mec*IVc. Clones ST5, ST72, ST125, and ST30 had hypermutable phenotypes. The ST72 clone and the ST30 clone in the OX-R phenotype harboured the highest number of MSCRAMMs.
Conclusion: ST5 and ST72 clones were the most frequent clones identified in OX-R-LEV-R phenotype. Both clones showed a hypermutable phenotype that favours their selection as the fluoroquinolone resistant clones. The genetic relationships identified indicate that OX-R-LEV-R clones have evolved from OX-R MRSA clones.

Características fenotípicas y genéticas de *Staphylococcus aureus* resistente a meticilina y a fluoroquinolonas

Palabras clave:
Staphylococcus aureus resistente a meticilina
Fluoroquinolonas
Resistencia
Componentes de la superficie microbiana que reconocen adhesinas de la matriz extracelular

RESUMEN

Introducción: La resistencia a fluoroquinolonas en *Staphylococcus aureus* resistente a meticilina (SARM) se ha incrementado en los últimos años. El objetivo de este estudio consistió en caracterizar 2 poblaciones de SARM, una sensible a fluoroquinolonas y otra resistente identificando los tipos clonales y las características diferenciales entre los mismos.
Métodos: En un total de 192 SARM aislados entre los años 2009-2011, 49 solo oxacilina resistentes (OX-R) y 143 oxacilina y levofloxacino resistentes (OX-R-LEV-R), se realizó el tipado molecular mediante PFGE, MLST, *spa* y SSC*mec*. Además se estudiaron las mutaciones que confieren resistencia a las fluoroquinolonas, los fenotipos hipermutadores y la presencia de 8 componentes de la superficie microbiana que reconocen adhesinas de la matriz extracelular.

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Resultados: En el periodo de estudio se detectó un incremento estadísticamente significativo del fenotipo OX-R-LEV-R ($p < 0,05$). Entre los OX-R el clon ST8 (32,6%) fue el más frecuente seguido de los clones ST72 (26,5%) y ST5 (26,5%). Entre los aislados del fenotipo OX-R-LEV-R, el clon ST5 fue el más frecuente (65,7%), seguido de los clones ST72 (15,4%) y ST125 (12,6%). Todos los aislamientos, excepto el clon ST398, portaban el SCC*mec*-IVc. Los clones ST5, ST30, ST72 y ST125 presentaron un fenotipo hipermutador. Los clones ST72 y ST30 OX-R son los que poseen una mayor dotación de componentes de la superficie microbiana que reconocen adhesinas de la matriz extracelular.

Conclusión: Los clones ST5 y ST72 fueron los más frecuentes en el fenotipo OX-R-LEV-R. Ambos clones poseían un fenotipo hipermutador. La estrecha relación genética entre los clones OX-R y OX-R-LEV-R pertenecientes al mismo ST sugiere que estos últimos han evolucionado a partir de una población OX-R preexistente.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a large number of infections.[1] Its ability to adapt and acquire resistance to different antimicrobials has possibly favoured its spread both in hospitals and in the community.[1] Methicillin resistance in *S. aureus* is coded by the *mec* gene, included in a genomic island called the staphylococcal cassette chromosome *mec* (SCC*mec*). The fluoroquinolones are among the therapeutic agents whose activity is not affected by SCC*mec*. However, rates of fluoroquinolone resistance have increased,[2] mainly due to mutations in genes *gyrA* and *grlA*.[2]

S. aureus expresses surface proteins that are essential for its success both as a commensal and as a pathogen. The most important group of these proteins are the MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules).[3]

The objective of this study was to analyse the increase in MRSA resistant to fluoroquinolones and its possible relation with a clonal selection. In addition, the characteristics of the MRSA isolates studied were analysed including the phenotypic hypermutability of the identified clones and the MSCRAMMs content.

Materials and methods

Selection of microorganisms and patients for inclusion in the study

The University Hospital Complex of Pontevedra has a catchment population of 296,463. We selected two groups of MRSA isolates from the years 2009 to 2011: (i) those that presented resistance only to beta-lactams (oxacillin, OX-R), being susceptible to all other groups of antimicrobials; and (ii) those with resistance to oxacillin and to levofloxacin OX-R-LEV-R, being susceptible to all other antimicrobial groups. Only one isolate per patient was analysed in the study. A total of 192 isolates were studied. The following patient data were recorded: sex, age, type of sample, underlying diseases and risk factors for MRSA infection. The criteria of the Centers for Disease Control and Prevention (CDC) were applied to determine the origin of the MRSA infection.[4,5]

Bacterial identification and susceptibility to antimicrobials

The biochemical characteristics and antibiotic susceptibility of the isolates were studied using the commercially available Wider system (Gram Positive MIC/ID Panel, Francisco Soria Melguizo, S.A.). The minimum inhibitory concentrations (MICs) for fluoroquinolones were determined using the gradient diffusion (epsilon-test) method. Resistance to methicillin was detected using a 30 μg cefoxitin disc. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were followed for the determination of breakpoints and resistance to methicillin.[6]

Molecular typing of the isolates

For the SCC*mec*, PFGE, and *spa* gene typing, the 192 strains included in the study were analysed.

Analysis of the staphylococcal cassette chromosome mec (SCCmec): a multiplex polymerase chain reaction (PCR) was employed to identify the *mecA* gene and SCC*mec*[7] type and subtype.

Pulsed-field gel electrophoresis (PFGE): cells were processed following the protocol proposed by Murchan et al.[8] Gels were analysed using the GelCompar II software (Applied Maths NV), applying the Dice similarity coefficient with an optimisation of 0.5% and tolerance 1%. Banding patterns were assigned to pulsotypes (using uppercase Latin letters) and subtypes (using numerical subindices). Pulsotypes were considered to be different if the coefficient of similarity was less than 80%. Subtypes were defined as pulsotypes with a coefficient of similarity between 80% and 95%.[9]

spa gene typing: amplification and sequencing of the X region of the *spa* gene were performed under the conditions described by Harmsen et al.[10] The *spa* type was established using Ridom StaphType software (Ridom GmbH, Würzburg, Germany).

Multilocus sequence typing (MLST): the Enright et al.[11] recommendations were followed. A total of 17 strains were studied including one isolate from each of the identified pulsotypes and *spa* types. The MLSTs of the strains not analysed by this method were deduced from the *spa* types.

Analysis of the frequency of mutation in the isolates

The frequency of mutations for rifampicin and streptomycin were performed under the conditions described by Trong et al.[12] One isolate from each clonal type and one from each phenotype were selected and processed in triplicate. In summary, five OX-R strains including one of each ST5, ST72, ST30, ST8, and ST398, and another five OX-R-LEV-R strains including one of each ST5, ST72, ST30, ST125, and ST22 were studied.

Analysis of mutations in genes coding for fluoroquinolone resistance

The amplification of genes *gyrA* and *grlA* was carried out under the conditions described by Schmitz et al.[13] A total of 30 strains belonging to the ST5 clone, 22 strains belonging to the ST72, 18 strains from the ST125, 8 strains from the ST22, and 1 strain from the ST30 were studied. Sequence analysis was performed using the BioEdit software, version 7.1.9 (Ibis Therapeutics, Isis Pharmaceuticals, Inc.).

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