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Short Communication

Chlorhexidine and mupirocin susceptibilities in methicillin-resistant *Staphylococcus aureus* isolates from bacteraemia and nasal colonisation

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ABSTRACT

Chlorhexidine and mupirocin have been increasingly used in healthcare facilities to eradicate methicillin-resistant *Staphylococcus aureus* (MRSA) carriage. The aim of this study was to determine the prevalence and mechanisms of chlorhexidine and mupirocin resistance in MRSA from invasive infections and colonisation. MRSA isolates obtained from blood and nasal samples between 2012 and 2014 were analysed. Susceptibility to mupirocin was determined by disk diffusion and Etest and susceptibility to chlorhexidine by broth microdilution. The presence of *mupA* and *qac* (*A*/*B* and *C*) genes was investigated by PCR. Molecular typing was performed in high-level mupirocin-resistant (HLMR) isolates. Mupirocin resistance was identified in 15.6% of blood isolates (10.9% HLMR) and 15.1% of nasal isolates (12.0% HLMR). Presence of the *mupA* gene was confirmed in all HLMR isolates. For blood isolates, chlorhexidine minimum inhibitory concentrations (MICs) ranged from ≤0.125 to 4 mg/L and minimum bactericidal concentrations (MBCs) from ≤0.125 to 8 mg/L. In nasal isolates, chlorhexidine MICs and MBCs ranged from ≤0.125 to 2 mg/L. The *qacA*/*B* gene was detected in 2.2% of MRSA isolates (chlorhexidine MIC range 0.25–2 mg/L) and the *qacC* gene in 8.2% (chlorhexidine MIC range ≤0.125–1 mg/L). The prevalence of *qacC* was 18.9% in HLMR isolates and 3.6% in mupirocin-susceptible isolates ($P = 0.009$). Most of the HLMR isolates (97.1%) belonged to ST125 clone. These results suggest that chlorhexidine has a higher potential to prevent infections caused by MRSA. In contrast, mupirocin treatment should be used cautiously to avoid the spread of HLMR MRSA.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major problem in healthcare facilities. Many strategies can be used to reduce the risk of MRSA transmission and infection. Intranasal mupirocin (MUP) and chlorhexidine (CHX) gluconate baths are widely used to decolonise MRSA carriers [1].

CHX gluconate is a hexamethylene biguanide cationic biocide compound with rapid bactericidal action against a variety of Gram-positive and Gram-negative micro-organisms [2]. It has been suggested that decreased susceptibility to CHX is mediated primarily through multidrug efflux pumps encoded by the *qacA*, *qacB* and *smr* (*qacC*) genes [3]. These genes are mainly found on plasmids and are associated with resistance to other biocides [4]. Some studies define CHX resistance on the basis of MRSA isolates possessing *qac* genes [5–7].

MUP (pseudomonic acid A) is a topical antibacterial agent that interferes with protein synthesis by competitively inhibiting bacterial isoleucyl-tRNA synthetase [8]. High-level MUP resistance is conferred by the *mupA* gene, which is carried on a plasmid that may also contain resistance determinants to other antimicrobial agents [9]. Recently, a new determinant of high-level mupirocin resistance, *mupB*, has been identified [10]. Both *mupA* and *mupB* are *ileS* genes imported from other species. A possible association between the presence of *qac* genes and resistance to MUP has been suggested [6,11].

Since MUP became available in the 1980s, its widespread use has been linked to increasing rates of resistance. Moreover, the

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increased use of CHX has raised concerns about the possible emergence of CHX-resistant strains. The aim of this study was to determine the prevalence and mechanisms of CHX and MUP resistance in MRSA in two epidemiological scenarios in hospitalised patients, with invasive infections represented by blood isolates and colonisation represented by nasal isolates.

2. Materials and methods

2.1. Hospital setting and bacterial isolates

This was an observational study conducted at Hospital Universitario 12 de Octubre, a 1300-bed facility serving a population of 550,000 in southern Madrid (Spain). MRSA isolates were collected during 2012–2014 from two groups of adult patients. Group I comprised all hospitalised patients with bacteraemia ($n = 64$). Group II included all isolates ($n = 358$) obtained from nasal swab samples used to detect MRSA nasal carriage in patients who were admitted to the hospital with a previous history of MRSA infection/colonisation, or from the surveillance studies at admission to the intensive care units and haemodialysis or surgery wards.

2.2. Chlorhexidine and mupirocin susceptibility testing

Isolation and identification of *S. aureus* were based upon standard microbiological procedures. Identification and antimicrobial susceptibility testing of blood isolates were performed using a MicroScan® WalkAway® System (Siemens, West Sacramento, CA). Resistance was defined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. All isolates were screened for resistance to MUP on Mueller–Hinton agar with a 5 μg disk (Oxoid Ltd., Thermo Scientific, Waltham, MA). A zone of inhibition of ≤13 mm in diameter was considered to reflect MUP resistance. MUP-resistant organisms underwent minimum inhibitory concentration (MIC) analysis by the Etest strip method (bioMérieux, Marcy-l'Étoile, France) in order to classify in high-level MUP resistance (MIC > 256 mg/L) or low-level MUP resistance (MIC > 1–≤256 mg/L).

CHX susceptibility testing was performed using the broth microdilution method for 134 isolates, comprising all 64 blood isolates and a selection of 70 nasal isolates [30 high-level MUP-resistant (HLMR), 10 low-level MUP-resistant (LLMR) and 30 MUP-susceptible]. CHX digluconate (Sigma–Aldrich, St Louis, MO) was prepared in sterile distilled water at a concentration of 100 mg/mL prior to further dilution in broth [12]. The concentration range was 0.125–128 mg/L. CHX resistance was defined as an MIC ≥4 mg/L [2]. Minimum bactericidal concentrations (MBCs) were determined by subculturing 10 μL from each well without visible bacterial growth on blood agar plates (Soria Melguizo, Madrid, Spain). After 48 h of incubation at 37 °C, the dilution yielding three colonies or fewer was scored as the MBC. *S. aureus* isolate ATCC 29213 was used for quality control.

2.3. Detection of mecA, mupA and qac genes

All isolates were confirmed as MRSA by PCR detection of the *mecA* gene. PCR was also performed on all HLMR and LLMR isolates to detect the plasmid-associated *ileS2* gene (*mupA*) [13]. The presence of *qac* (*A*/*B* and *C*) genes was determined by PCR using previously published primers [6].

2.4. Molecular typing

Molecular typing was performed on a selection of HLMR isolates by pulsed-field gel electrophoresis (PFGE). Computer-assisted analysis of electropherograms was carried out with BioNumerics software (Applied Maths, Kortrijk, Belgium). A 1.8% tolerance was used for comparison of DNA patterns, and PFGE types were defined using a similarity coefficient of 0.75. Representative isolates were analysed by multilocus sequence typing (MLST), and the sequence types (STs) were assigned using the MLST website (http://www.mlst.net).

2.5. Statistical analysis

Data are represented as percentages. Statistical analysis was performed using IBM SPSS Statistics for Windows v.20.0 (IBM Corp., Armonk, NY).

3. Results

MUP resistance was identified in 15.6% (10/64) of blood isolates [3 (4.7%) LLMR and 7 (10.9%) HLMR] and in 15.1% (54/358) of nasal isolates [11 (3.1%) LLMR and 43 (12.0%) HLMR]. Presence of the *mupA* gene was confirmed in all HLMR isolates; none of the LLMR isolates were positive for the *mupA* gene.

CHX reduced susceptibility was found in 1.6% (1/64) of blood isolates (MIC = 4 mg/L and MBC = 8 mg/L). None of the MRSA nasal isolates showed MICs ≥4 mg/L. Of the 134 isolates tested, all had CHX MICs ≤4 mg/L (range ≤0.125–4 mg/L; MIC_{50} and MIC_{90}, 1 mg/L) and MBCs ≤8 mg/L (range ≤0.125–8 mg/L; MBC_{50} and MBC_{90}, 1 and 2 mg/L, respectively) (Fig. 1). Of the 64 blood isolates, all had CHX MICs ≤4 mg/L (range ≤0.125–4 mg/L; MIC_{50} and MIC_{90}, 0.5 and 1 mg/L, respectively) and MBCs ≤8 mg/L (range ≤0.125–8 mg/L; MBC_{50} and MBC_{90}, 1 and 2 mg/L, respectively) (Fig. 1). Of the 70 nasal isolates, all had CHX MICs and MBCs that were ≤2 mg/L (range ≤0.125–2 mg/L). The MIC_{50} and MIC_{90} values were 1 mg/L and the MBC_{50} and MBC_{90} values were 1 and 2 mg/L, respectively (Fig. 1).

Among the 134 MRSA isolates, 3 isolates (2.2%) harboured the *qacA*/*B* gene, all from nasal samples. The CHX MIC ranged from 0.25 to 2 mg/L. The *qacC* gene was detected in 11 isolates (8.2%), including 1 from blood and 10 from nasal samples. The CHX MIC of these isolates ranged from ≤0.125 to 1 mg/L. One nasal isolate harboured *qacA*/*B* and *qacC* genes and its CHX MIC and MBC were 1 and 2 mg/L, respectively. The MRSA blood isolate that had a CHX MIC of 4 mg/L did not harbour *qacA*/*B* and *qacC* genes.

The possible relationship between MIC and MBC distribution of CHX and the presence of *qac* genes was analysed (Supplementary Fig. S1). The distribution was similar both in *qac*-positive and *qac*-negative MRSA isolates. Furthermore, the relationship between CHX susceptibility, as measured by MIC/MBC and by the presence of *qac* genes, and MUP susceptibility was investigated (Table 1). *qac* genes were detected in 21.6% (8/37) of HLMR isolates, 15.4% (2/13) of LLMR isolates and 3.6% (3/84) of MUP-susceptible isolates. The presence of *qacC* was more frequent in HLMR isolates (18.9%; 7/37) than in MUP-susceptible isolates (3.6%; 3/84) ($P = 0.009$). In addition, the presence of *qacC* was 14.3% (10/70) in nasal isolates and 1.6% (1/64) in blood isolates ($P = 0.009$).

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.jgar.2015.11.005.

Thirty-five HLMR isolates available for PFGE (30 from nasal samples and 5 from blood) were grouped into two types (Fig. 2). One of them grouped 34 (97.1%) of HLMR isolates (all 5 blood isolates and 29 nasal isolates). Nine representative isolates belonging to this PFGE type were identified by MLST as ST125. The presence of *qac* genes was observed in seven isolates belonging to the major clone.