Short communication

MRSA Pediatric clone expressing \textit{ermC} plus \textit{lnuA} genes causing nosocomial transmission and healthcare workers colonization in a neonatal intensive care unit

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\textbf{A B S T R A C T}

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is a major cause of both nosocomial and community-acquired infections. We describe an outbreak caused by the MRSA Pediatric clone expressing an unusual lincosamide resistant phenotype. Between January and May 2006, an MRSA outbreak was detected at the Neonatal Unit of Hospital Interzonal General de Agudos “Evita”, Buenos Aires Province, Argentina that affected ten patients. Seven isolates from seven patients plus five MRSA recovered from healthcare workers (nasal carriage) were studied. Two phenotypes were observed: (i) ELCi (10), resistance to erythromycin and lincomycin and inducible resistance to clindamycin; (ii) ELiCi (2), resistance to erythromycin and inducible resistance to lincomycin and clindamycin. All 12 MRSA were resistant to oxacillin, erythromycin and gentamicin. Isolates expressing the ELCi-phenotype showed lincomycin MIC values between 16 and 32 mg/L while the remaining 2 isolates with ELiCi-phenotype presented a MIC value of 0.5 mg/L. No differences were observed between the clindamycin MIC values in both phenotypes, ranging 0.25–0.5 mg/L. Isolates showing ELCi-phenotype harbored \textit{ermC} plus \textit{lnuA} genes, and the other two only \textit{ermC} gene. All 12 isolates were genetically related and belonged to the Pediatric clone (ST100) harboring a new variant of SCC\textit{mec}IV. This is the first MRSA outbreak expressing an unusual ELCi phenotype due to a combination of \textit{ermC} plus \textit{lnuA} genes.

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1. Introduction

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is a major cause of both nosocomial and community-acquired infections, with reported high level of morbidity and mortality within infected patients, especially in newborns and children. Outbreaks of infection caused by MRSA have been reported even in neonatal units (Lin et al., 2007; Regev-Yochay et al., 2005; El Helali et al., 2005; Giuffre et al., 2012). Macrolides and lincosamides are therapeutic options for some MRSA infections. The main mechanisms of resistance to macrolides and lincosamides in staphylococci are mediated by target site modification, efflux and, less frequent, drug inactivation (Roberts, 2008). ErmA and ErmC methylases, codified by \textit{ermA} and \textit{ermC} genes, respectively, have been widely described in this genera and confer cross resistance to macrolides, lincosamides and streptogramins B (\textit{ MLS\textsubscript{B}}), which could be expressed in a constitutive (\textit{cMLS\textsubscript{B}}) or inducible (\textit{iMLS\textsubscript{B}}) way. MsrA is an ABC putative efflux pump that confers resistance only to 14- and 15-member ring macrolides. Ribosomal mutations have also been described but as an unusual mechanism. Lincosamide nucleotidyltransferase (\textit{lnuA}) enzyme has activity over lincosamides and confers a characteristic L phenotype of resistance to lincomycin and susceptibility to clindamycin (Leclercq et al., 1985). \textit{lnuA} gene has been described in different species of staphylococci and occasionally in clinical \textit{S. aureus} (Novotna et al., 2005; Lozano et al., 2012).

2. Materials and methods

2.1. Bacterial isolates

Twelve MRSA isolates were studied, six from blood, one from suppuration and five recovered from nasal carriage of health care workers.
Rifampin-resistant *S. aureus* RN4220 was used as recipient strain for biparental conjugation and electroporation assays. Transformants selection was performed in brain–heart infusion agar containing 4 mg/L of lincomycin.

### 2.2. Susceptibility assays

Minimal inhibitory concentrations were determined by agar dilution according the CLSI guidelines (CLSI, 2013).

Using the triple disk assay (Novotna et al., 2005) with clindamycin (C)–erythromycin (E)–lincomycin (L) we defined two phenotypes: (i) ELiCi, resistance to erythromycin and lincomycin and inducible resistance to clindamycin; (ii) ELCi, resistance to erythromycin and inducible resistance to lincomycin and clindamycin.

Methicillin-resistance was confirmed by the detection of the PBP2a protein using Slidex MRSA Detection (Denka Seiken, Japan).

### 2.3. Molecular methods

PCR detection for *ermC* and *ermA* genes was performed using standard protocols and primers previously described (Sutcliffe et al., 1996). Primers for detection of *lnu* gene were *lnu*-F, 5'-GGC GTA GAT GTA TTA ACT GG-3' and *lnu*-R, 5'-AAC TTG AGC TTC TTT GGA A-3', yielding a 321 bp amplicon. Primers for sequencing the *lnu* gene were *lnu*-F1174, 5'-CTA TTC GTC ACA AAA GAC AAA A-3' and *lnu*-R2342, 5'-CCG CCT TAA ATT TAA AAA TAA A-3'.

Presence of the Panton-Valentine leukocidin (pvl) toxin was performed using primers described by Lina et al. (1999). PCR multiplex described by Milheirico et al. (2007) was used for SCCmec typing.

DNA sequence of *lnu* amplicon was determined using BigDye terminator 3.1 (Applied Biosystem, Foster City, CA, USA).

The genetic relation was evaluated by pulsed-field gel electrophoresis (PFGE) by *SmaI* digestion (Corso et al., 1998). Sequence type was determined according the protocol described in the MLST web page (http://saureus.mlst.net/).

### 3. Results and discussion

Between January and May 2006, MRSA-causing infections were isolated from ten patients at the Neonatal Unit of Hospital Intergalional de Aguadass “Evita”, Buenos Aires Province, Argentina (Table 1). Seven MRSA isolates (one per patient) were available for further studies. Nasal carriage was evaluated in 39 health care workers, of which: 6 (15%) isolates were MRSA, 3 (7.5%) methicillin-resistant, and 7 (18%) gram-negative bacilli (one *Klebsiella pneumoniae* producing an extended-spectrum beta-lactamase). Five out of six MRSA strains recovered from the health care workers were studied. Immediate infection control measures were applied to control the outbreak, including review of invasive procedures, contact precautions for handling patients, and mupirocin treatment for nasal decolonization of health care workers.

Ten out of twelve MRSA isolates expressed the ELiCi-phenotype while the remaining 2 showed the ELCi-phenotype (Fig. 1 and Table 1). All 12 MRSA were resistant to (MIC range in mg/L) oxacillin (4–64), erythromycin (>512) and gentamicin (32–128), and susceptible to ciprofloxacin (0.25), vancomycin (0.5–1), teicoplanin (1–2), rifampin (0.5–2), tetracycline (0.25–0.5), minocyclin (0.5), chloramphenicol (8), and trimethoprim–sulfamethoxazole (0.06).

The ten MRSA isolates expressing the ELCi-phenotype showed lincomycin MICs values between 16 and 32 mg/L while the remaining 2 isolates (ELiCi-phenotype) presented a MIC value of 0.5 mg/L (Table 1). No differences were observed between the clindamycin MIC values in both phenotypes, ranging 0.25–0.5 mg/L, which were into the category of susceptibility. MRSA isolates harbors the *ermC* gene, and those expressing ELCi phenotype were also positive for *lnuA* gene (Table 1).

The MRSA isolates were genetically related (>89% of similarity, up to two bands of difference), and were subdivided in three subtypes: A1 (8 strains), A2 (3) and A3 (1). The isolates were negative for the Pvl toxin, belonged to ST100 and harbored a new variant of SCCmecIV, named SCCmeclIVNv, recently described by Sola et al. (2012). The ST100 is a single locus variant of ST5 that is representative of the Pediatric clone (clonal complex 5), which was reported as an epidemic clone in our country (Corso et al., 1998). This MRSA-ST100 clone harboring the SCCmeclIVNv and negative for Pvl virulence factor was described in Argentina

Table 1

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sample</th>
<th>Date</th>
<th>Resistance phenotype</th>
<th>MIC (mg/L)</th>
<th>PCR</th>
<th>Smal-PFGE</th>
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<tr>
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<td></td>
<td></td>
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<td>CLI</td>
<td>LIN</td>
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<tr>
<td>6286</td>
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<td>&gt;512</td>
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</tbody>
</table>

NC, nasal carriage; phenotype ELiCi, resistance to erythromycin and inducible resistance to lincomycin and clindamycin; phenotype ELCi, resistance to erythromycin and lincomycin and inducible resistance to clindamycin; ERY, erythromycin; CLI, clindamycin; LIN, lincomycin.

Fig. 1. Triple disc diffusion assay. MRSA M6275 expresses the ELiCi-phenotype, and M6276 expresses the ELCi-phenotype. Abbreviations: C, clindamycin; E, erythromycin; L, lincomycin.
associated with hospital-acquired infections in children (Sola et al., 2006, 2012), and also in Switzerland but harboring a particular mosaic SCCmec structure (Heusser et al., 2007). Conjugation assays were repeatedly unsuccessful. Plasmid extractions were electroproporated into S. aureus RN4220. Transformant strains displayed the L phenotype and were positive for *lnuA* gene and negative for *ermC* gene. *lnuA* gene was previously reported to be carried on small cryptic plasmids, 2–4 Kb approx., in different staphylococcal species (Leclercq et al., 1985; Loeza-Lara et al., 2004; Luthje et al., 2007). The *lnuA* sequence (486 bp) of our isolates showed 100% similarity with that described in pSAP015B plasmid from *S. aureus* CDC61 (accession number GQ900502). Little is known about the prevalence of *lnuA* gene in staphylococci clinical isolates, although it seems to be more frequent in coagulase-negative staphylococci, suggesting a possible reservoir for this uncommon gene (Lina et al., 1999). Moreover, during the last years we detected an increasing number of staphylococci harboring *lnuA* gene suggesting a silent change in lincomamide resistance epidemiology (personal communication). Moreover, the finding of two MRSA isolates harboring only the *ermC* gene, suggesting the possible occurrence of a contemporary event of acquisition/loss of the *lnuA* gene.

4. Conclusions

Clindamycin represents an important option for several reasons, (i) is available in both intravenous and oral formulations, (ii) has a remarkable distribution into skin and soft-tissue structures, and (iii) community-acquired MRSA (CA-MRSA), which has rapidly emerged in recent years as a cause of skin and soft-tissue infections, is frequently susceptible to several antibiotics, including clindamycin. Some reports about clindamycin treatment failure of *S. aureus* were described, but they were not associated (or investigated) with the presence of *lnu* genes (Levin et al., 2005; Siberry et al., 2003). The limited use of lincomycin in the clinical laboratory and the apparent clindamycin susceptibility could underestimate the real prevalence of this mechanism of resistance.

To the best of our knowledge this is the first MRSA outbreak in a neonatal unit expressing an unusual ELCi phenotype due to a combination of *ermC* plus *lnuA* genes. Cross-communication between health care workers and patients was observed, reinforcing the implementation and maintenance of measures for control and prevention of MRSA infections in healthcare facilities.

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References