First clinical isolate in Europe of clindamycin-resistant group B Streptococcus mediated by the Inu(B) gene

ABSTRACT

We characterize the mechanisms implicated in an unusual phenotype of resistance to macrolides-lincosamides (no halos of inhibition around clindamycin and lincomycin discs, and a 15 mm halo around erythromycin disc) in a Streptococcus agalactiae isolate recovered in Spain. The presence of macrolide or lincosamide resistance genes [erm(A), erm(B), erm(C), erm(T), mef(A), mrs(A), Inu(A), Inu(B), Isa(B), Isa(C) and vga(C)] was investigated by PCR and sequencing. The strain showed a resistant phenotype to erythromycin and clindamycin (MIC = 2 mg/L and MIC = 8 mg/L, respectively) and the presence of Inu(B) and mef(A) genes was demonstrated. Clinical microbiology laboratories should be aware of this unusual phenotype due to the association of two mechanisms mediated by Inu(B) and mef(A) genes. This constitute, to our knowledge, the first report of Inu(B) in S. agalactiae in human isolates in Europe.

Key words: nucleotidyltransferase, lincosamide resistance, macrolide resistance, clindamycin resistance, Streptococcus agalactiae.

INTRODUCTION

Group B Streptococcus (GBS, Streptococcus agalactiae) is a commensal microorganism of the gastrointestinal and genitourinary tracts. In some circumstances, it is able to cause invasive infections in neonates, pregnant women, and non-pregnant adults with underlying medical conditions, such as diabetes mellitus, or immunosuppression. The vaginal and/or rectal colonisation in pregnant women represents an important risk factor to develop sepsis, pneumonia and meningitis in neonates. Penicillin G and ampicillin are the antibiotics of choice for intrapartum prophylaxis and treatment of invasive infections, while clindamycin is the recommended agent for patients who are allergic to beta-lactams. However, although S. agalactiae remains almost always susceptible to penicillins, there is a significant and rising resistance to macrolides and lincosamides.

Two major resistance mechanisms to macrolides and lincosamides have been reported: alteration of the antibiotic target site and active drug efflux pump. Ribosomal alteration is mediated by ribosomal methylases encoded by erm(B) and/or erm(A) genes. The drug efflux pump by a membrane-bound protein is encoded by the mef(A) gene. Expression of erm(B) and/or erm(A) confer cross-resistance to all macrolides, lincosamides, and streptogramins B (MLS\textsubscript{B} phenotype), and this mechanism of resistance can be expressed constitutively (MLS\textsubscript{B}c) or inducibly (MLS\textsubscript{B}i), while expression of mef(A) confers resistance to 14- and 15-membered ring macrolides (M phenotype, erythromycin resistant but clindamycin susceptible).
A third unusual mechanism of resistance has been previously reported, mediated by lincosamide nucleotidyltransferases encoded by \textit{lnu} genes, responsible for the enzymatic inactivation of lincosamides by a mechanism of adenylation \textsuperscript{5-7}. Inactivation of lincosamides mediated by \textit{lnu} genes was firstly described in \textit{Enterococcus faecium} HM1025 strain\textsuperscript{8}. The L-phenotype (erythromycin susceptible but clindamycin resistant) mediated by \textit{lnu}(B) expression has been recently described in \textit{S. agalactiae} in specific areas from Korea\textsuperscript{7}, Latin-America\textsuperscript{5} and Canada\textsuperscript{6}, while, to our knowledge, no cases have been previously reported in strains from humans in Europe.

The aim of this report is to describe the emergence in Europe of \textit{S. agalactiae} harbouring the \textit{lnu}(B) gene.

**MATERIAL AND METHODS**

\textit{S. agalactiae} isolate 12/30482 was recovered from a vaginorectal exudate of a pregnant woman in a Spanish hospital (Madrid, Spain) and tested for clindamycin and erythromycin susceptibility by using disc-diffusion and epsilon test methods. Minimal inhibitory concentrations of penicillin, cefotaxime, vancomycin, erythromycin, clindamycin, tetracycline, and levofloxacin were determined by microdilution using WIDER system, being the results interpreted according to EUCAST\textsuperscript{9}.

DNA extraction of isolate 12/30482 was carried out by InstaGene Matrix (BioRad, Hercules, CA, USA). The presence of the genes \textit{erm}(A), \textit{erm}(B), \textit{erm}(C), \textit{erm}(T), \textit{mef}(A), \textit{mrs}(A), \textit{lnu}(A), \textit{lnu}(B), \textit{lsa}(B), \textit{lsa}(C), and \textit{vga}(C) was analysed by PCR using primers and conditions previously described (table 1). Multilocus sequence typing (MLST) was performed by PCR/sequencing\textsuperscript{10}, and molecular capsular typing was developed by multiplex-PCR\textsuperscript{11}.

**RESULTS**

The \textit{S. agalactiae} isolate 12/30482 was resistant to clindamycin and erythromycin by disc-diffusion. In order to study the resistant profile to macrolides and lincosamides, a triple disc diffusion assay with clindamycin-erythromycin-lincomycin was developed. No halos of inhibition around clindamycin and lincomycin discs were observed, while a 15 mm halo around erythromycin disc was visualized, indicating a phenotype different from the \textit{erm}-mediated phenotypes (MLS\textsubscript{B}c or MLS\textsubscript{Bi}) previously reported and suggesting that this strain had

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer 5‘–3’</th>
<th>Annealing temperature (ºC)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>\textit{erm}(A)</td>
<td>F: TCTAAAAAGCATGTAACAAAGA&lt;br&gt;R: CTTCGATGTTTATATATAAG</td>
<td>52</td>
<td>645</td>
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<tr>
<td>\textit{erm}(B)</td>
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<td>52</td>
<td>639</td>
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<td>52</td>
<td>642</td>
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<tr>
<td>\textit{erm}(T)</td>
<td>F: ATTTGCTTGAAGGAAAAGGTC&lt;br&gt;R: GCTTGAATAAAATGTTTG</td>
<td>45</td>
<td>550</td>
<td>15</td>
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<tr>
<td>\textit{mef}(A)</td>
<td>F: AGATATCAATTATACAGATG&lt;br&gt;R: TTCTTCTGTAGTTAAAAGTTG</td>
<td>52</td>
<td>348</td>
<td>14</td>
</tr>
<tr>
<td>\textit{mrs}(A)</td>
<td>F: GCAAATGGTGTAGGAAGACAA&lt;br&gt;R: ATCATGTGATGTAAGAAACAAATT</td>
<td>55</td>
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<tr>
<td>\textit{lnu}(A)</td>
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<td>944</td>
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<td>\textit{vga}(C)</td>
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<td>58</td>
<td>671</td>
<td>15</td>
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</tbody>
</table>
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In conclusion, the emergence of S. agalactiae harbouring the inu(B) gene in Europe is described for the first time. Nevertheless, more clinical isolates need to be detected and more studies need to be done to know how this mechanism is acquired, because S. agalactiae is a common commensal microorganism and could exchange genetic material with other bacteria of the human microbiota.

REFERENCES

First clinical isolate in Europe of clindamycin-resistant group B Streptococcus mediated by the *lnu*(B) gene


