Unveiling the role of MRSA ST398 as a zoonotic foodborne pathogen requires more research.

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Transparency declarations

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References


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Molecular characterization of group B streptococci with reduced penicillin susceptibility recurrently isolated from a sacral decubitus ulcer

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Penicillin is the first-line antibiotic for group B streptococci (GBS) in disease therapy as well as in intrapartum chemoprophylaxis because resistance to this agent has so far not been reported among GBS clinical isolates. However, we have recently reported GBS isolates with reduced penicillin susceptibility (PRGBS), where altered penicillin-binding protein (PBP) 2X demonstrates a major contribution to the reduction of β-lactam susceptibility. We have also shown a diversity of mutations in PBP genes among PRGBS, while those genes of the penicillin-susceptible strains were highly conserved, irrespective of their isolation dates. Those PRGBS isolates have mostly been recovered from respiratory specimens. Here, we report a case of a lasting sacral decubitus ulcer from which PRGBS were isolated repeatedly, together with the molecular characteristics of the isolates.

A 58-year-old man underwent descending graft replacement of a Stanford type B dissecting aortic aneurysm at our medical centre. After the operation, the patient developed paraplegia, which was not improved after axillofemoral bypass surgery. He was then transferred to a special physical rehabilitation centre. During his prolonged rehabilitation stay, a severe decubitus ulcer developed in the sacral area, wound bleeding was seen and he was referred to our centre. He had not received antimicrobial treatment for more than 3 months except for a 1 day intravenous injection of piperacillin (2000 mg/day) due to the occurrence of a fever just 3 days before hospital admission. Bacterial culture of the specimen taken at the surface of the decubitus ulcer at his initial visit yielded heavy growth of GBS as well as small numbers of group G streptococci and Proteus mirabilis. GBS was still predominant together with S. aureus, Staphylococcus pneumoniae, and G429D substitution, which corresponds to the G422D substitution, where the latter two were new substitutions for PRGBS. The PRGBS isolates shared three substitutions, G398A, G329V and G429D, where the latter two were new substitutions for PRGBS. The nucleotide and deduced amino acid sequences of PBP 2X from the two PRGBS isolates, which were detected at intervals, were PRGBS with a penicillin MIC of 0.25 mg/L determined by a broth microdilution method with a MicroScan MICrOFAST panel type 3J system (Siemens Healthcare Diagnostics Inc., Tokyo, Japan) as described previously. MICs of ampicillin, cefazolin and cefotaxime were 0.25, 2 and 1 mg/L, respectively (Table 1). PFGE patterns of Apal-digested DNA of these two isolates were identical. Multilocus sequence typing (MLST) was performed using specific primers as described previously. Amplification of seven housekeeping genes, adhP, pheS, atr, glnA, sdhA, glcK and tkt, by PCR was carried out using PrimeSTAR HS DNA polymerase (Takara Shuzo Co., Kyoto, Japan) with reaction conditions of one cycle of 98°C for 1 min, followed by 30 cycles of 98°C for 10 s, 55°C for 15 s and 72°C for 1 min, and finally one cycle of 72°C for 7 min. PCR products were purified and sequenced. Allelic profile assignment and sequence type (ST) determinations were made using the GBS MLST databases (http://pubmlst.org/sagalactiae). Both PRGBS were found to have ST-1 with allelic profile 1121122, which has been a heterogeneous lineage comprising mainly serotype V and various other serotypes, and has been identified among carriage and invasive isolates. Thus, those PRGBS were regarded as genetically identical by PFGE and MLST.

The MICs of antimicrobials for PRGBS isolates are presented in Table 1. Penicillin, ampicillin, cefazolin, and cefotaxime showed MICs of 0.25, 0.25, 2, and 1 mg/L, respectively. Cefotiam, Cefixime, Cefepime, Cefozopran, Meropenem, Clarithromycin, Erythromycin and Clindamycin showed MICs of >4, 1, 2, 1, 0.25, ≤0.12, ≤0.12, ≤0.12, and >8 mg/L, respectively. Clindamycin, which is often used for conventional post-operative treatment in our medical centre, only showed MIC of 0.25 mg/L.

In this case, PRGBS was consecutively and predominantly isolated from a sacral decubitus ulcer as a cause of mixed infection, and thus was found to be capable of surviving persistently at the site of infection, for >3 weeks. It remains unclear whether or not the results of in vitro penicillin non-susceptibility of PRGBS would predict the clinical significance of this kind of microbe in antimicrobial chemotherapy. Despite the relatively high MIC of cefazolin, 2 mg/L for this PRGBS, 4 day intravenous administration of cefazolin (2000 mg/day) seemed effective in this case, but accumulation of clinical data on an appropriate antimicrobial therapeutic strategy would be essential, especially in the cases of sepsis or meningitis in both neonates and elderly individuals.

### Table 1. MICs of antimicrobials for PRGBS isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.25</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.25</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>2</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1</td>
</tr>
<tr>
<td>Cefotiam</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Cefixime</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Cefepime</td>
<td>2</td>
</tr>
<tr>
<td>Cefozopran</td>
<td>1</td>
</tr>
<tr>
<td>Cefidoren</td>
<td>0.25</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.25</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤0.12</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤0.12</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤0.12</td>
</tr>
<tr>
<td>Levoloxacin</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>
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Plasmid-mediated ArmA and RmtB 16S rRNA methylases in Escherichia coli isolated from chickens

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Sir,

High-level aminoglycoside resistance mediated by the production of 16S rRNA methylase has been increasingly reported among various Gram-negative pathogens. Six 16S rRNA methylase genes have been previously identified: armA, rmtA, rmtB, rmtC, rmtD and npmA.1 However, in food animals, only two studies have described the presence of the armA and rmtB genes in pigs, respectively.2,3 In China, aminoglycoside antibiotics are widely used for the prevention and control of Escherichia coli infections of chickens. However, it remained unknown if 16S rRNA methylase genes were present in E. coli isolated from chickens.

Between March and May 2008, a total of 120 individual E. coli strains were isolated from the livers (n = 154) of diseased and dead chickens in four farms (Farms A–D) in Henan Province, China. Among them, 12 isolates exhibited high-level resistance to the aminoglycoside antibiotic amikacin (MICs > 512 mg/L). These isolates were screened by PCR for the six known types of 16S rRNA methylase genes. The genes that produced positive results were further confirmed by DNA sequencing of the amplimers. In addition, phenotypic and genotypic tests for extended-spectrum β-lactamases in 16S rRNA methylase-positive isolates were performed according to CLSI recommendations and using PCR assays as previously described.4,5 Overall, the armA and rmtB genes were detected in 3 and 9, respectively, of the 12 isolates that had high-level amikacin resistance. No positive amplimers were found to be present for the other four 16S rRNA methylase genes (Table 1). Multiplex PCR was performed to determine whether the 12 isolates were commensals (A and B1) or were associated with phylogroups exhibiting extraintestinal virulence (B2 and D).6 Five of them were found to be associated with extraintestinal virulence (Table 1).

Genetic relationships of the E. coli isolates that produced 16S rRNA methylase were assessed by PFGE after digestion with XbaI. The PFGE patterns differentiated these samples (n = 12) into seven major pulsotypes, indicating that both horizontal and vertical transfer could have played an important role in the dissemination of the 16S rRNA methylase genes (Table 1).

The 16S rRNA methylase resistance determinants from armA- or rmtB-positive isolates were transferred to E. coli J53Az2 and DH10B by conjugation and electroporation, respectively, to investigate whether the two determinants were localized on plasmids and whether transfer of these genes increased the resistance of the recipient E. coli to antimicrobials. Southern hybridization of digested plasmid DNA from the isolates and their transconjugants/transconjugants was performed with digoxigenin-labelled probes specific for armA and rmtB, respectively. As shown in Table 1, armA and rmtB genes could transfer among armA- and rmtB-positive isolates by conjugation and electroporation. Also, increases in MICs of multiple classes of antimicrobials were found for both the transconjugants and transformants, suggesting that co-transferred resistance to other antimicrobial agents could occur on these plasmids. Hybridization with the rmtB-specific probe indicated that rmtB genes from three isolates (isolates 63, 3 and 73) were localized on two differently sized digested fragments. Interestingly, hybridization with the armA-specific probe revealed that one isolate (isolate 14) contained two copies of the