of *M. morganii*, was detected in two isolates of *E. coli*. DHA-1 is an inducible plasmid-mediated AmpC β-lactamase whose emergence raises concerns because the mortality of patients infected with organisms that produce DHA-1 has been shown to be higher than that of patients infected with organisms that produce CMY-1.2

In conclusion, we have demonstrated that plasmid-mediated AmpC β-lactamases have emerged in Switzerland. The prevalence of 0.16% is still low. However, the occurrence of DHA-1, an inducible type of enzyme, raises clinical concerns. Additionally, a novel plasmid-mediated AmpC β-lactamase, which was designated CMY-31, was found.

**Funding**

This study was funded by the Department of Laboratory Medicine, University Hospital Basel.

**Transparency declarations**

None to declare.

**References**


**Journal of Antimicrobial Chemotherapy**

doi:10.1093/jac/dkm483

Advance Access publication 21 December 2007

**Prevalence and characterization of macrolide-lincomycin-streptogramin B-resistant *Staphylococcus aureus* in Korean hospitals**

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Keywords: macrolide resistance genes, double disc diffusion, non-tertiary hospitals

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Sir,  

Resistance to macrolide, lincosamide and streptogramin B (MLSb) antibiotics in *Staphylococcus* spp. is mediated by a methylase encoded by erythromycin ribosome methylation (*erm*) genes or ATP transporter efflux pumps encoded by the *msr* or *mef* genes. The methylation of the 50S ribosomal subunit by *erm* gene products causes constitutive or inducible resistance to MLSb antibiotics. Constitutively resistant MLSb (MLSbc) strains and inducibly resistant MLSb (MLSbi) strains express *erm* genes, *erm(A)* or/and *erm(C)*. In this study, we investigated and characterized the constitutive and inducible resistance, and the susceptibility to clindamycin of *Staphylococcus aureus* isolated from non-tertiary hospitals.

We collected 508 *S. aureus* strains from 157 hospitals nationwide from January to June 2005 in Korea. Antimicrobial susceptibility to oxacillin, erythromycin, clindamycin and quinupristin/dalfopristin and the MICs were evaluated according to the guidelines of the CLSI. Of the 508 *S. aureus* isolates, 46.1% (234 isolates) were resistant to oxacillin, 55.1% (280 isolates) were resistant to erythromycin, which comprised 98.3% (230/234) methicillin-resistant *S. aureus* (MRSA) strains and 18.2% (50/274) methicillin-susceptible *S. aureus* (MSSA) strains, and 37.4% (190 isolates) were resistant to clindamycin. No strains were susceptible to erythromycin and resistant to clindamycin. One hundred and eighty-six MRSA (80.9%) and four MSSA (274) methicillin-susceptible *S. aureus* strains, 46.1% (46.1%) were MLSbc (resistant to erythromycin and clindamycin; Table 1). D-test according to Steward’s method was used for isolates with erythromycin resistance and clindamycin susceptibility.1 Forty-three MRSA (18.7%) and 40 MSSA (8.0%) were MLSbi (showing the D form on the D-test), and 1 MRSA (0.4%) and 6 MSSA (12.0%) were MLSb8 (susceptible to clindamycin).

The MLSb resistance genes, *erm(A)*, *erm(B)*, *erm(C)*, *msr(B)*, and *msr(C)*, were detected using a PCR method.2 *erm(A)* was detected in 250 strains, *erm(C)* in 14 strains, *erm(B)* in 1 strain, *msr(A)* in 3 strains, *erm(A)* and *erm(C)* in 2 strains, *erm(A)* and *msr(A)* in 3 strains and no genes in 7 strains. Most MLSbc and MLSbi strains carried the *erm(A)* or *erm(C)* gene (Table 1). The *erm(A)* gene (in 250 isolates) was detected far more frequently than *erm(C) (in 14 strains). However, the *erm(C)* gene was distributed more frequently in MLSbc strains (12 isolates) than in MLSbi strains (2 isolates). Using Oligo primers designed by Arthur et al.,3 the PCR products were detected in three MLSbc and MLSbi strains lacking *erm(A)*, *erm(B)* or *erm(C)*. It was possible that the three strains carry an *erm* gene besides *erm(A)*, *erm(B)* or *erm(C)*, *msr(A)*...
was detected in one MRSA isolate and five MSSA isolates, three MLSBc isolates carried msr(A) together with erm(A) and three MLSBk6 contained only msr(A).

We also found msr(A) in three of seven MLSBk6 isolates but did not detect the msr(A) gene in four MLSBk6 strains. To determine whether the four MLSBk6 isolates carried the msr(A) gene, Southern hybridization was performed by the Amersham ECL Direct Nucleic Acid Labeling and Detection system (Amersham, GE Healthcare Life Science, USA), but no fragments were detected (data not shown). Also, any resistance genes encoding ATP transporter efflux pumps [msr(B), msr(C) and msr(D)], mefA/E) genes and the phosphorylase mph(C) gene were not detected in the four strains (data not shown).

The MICs of erythromycin for MLSBk6 and MLSBk6 isolates were ≥256 mg/L, with the exception of one strain with an MIC of 8 mg/L. The MICs of erythromycin for the MLSBk6 strains were 4, 8 or 64 mg/L. The MICs of clindamycin for clindamycin-susceptible and clindamycin-resistant strains, determined by agar dilution, were ≤1 and ≥64 mg/L, respectively (Table 1).

It has been reported that MLSBk6 and MLSBk6 isolates in Staphylococcus carry erm(A), erm(B) or erm(C), and at times erm together with msr(A), but MLSBk6 carry the msr(A) gene.3 Although msr(A) and erm(A) were detected together in MLSBk6, the msr(A) gene was detected in MLSBk6. In Streptococcus, MLSBk6 and MLSBk6 strains carried erm(A) and had MICs of erythromycin of 16–256 mg/L; and MLSBk6 or M phenotype strains had mef(A) and MICs of 16–48 or 8–32 mg/L.5

We propose that MICs of erythromycin of 4–64 mg/L are insufficient to induce resistance to clindamycin, and the erm genes were expressed at high concentrations of erythromycin and the msr(A) gene at low concentrations. If the msr and erm genes are detected together, the erm gene may be expressed more rapidly than the msr gene and the phenotype of the strain may be MLSBk6 or MLSBk6.

In conclusion, the erythromycin resistance rate in S. aureus was high at 55.1%, and 99.6% of erythromycin-resistant MRSA and 88.0% of MSSA strains were MLSBk6 or MLSBk6. Therefore, the treatment of infections caused by erythromycin-resistant S. aureus with clindamycin in non-tertiary hospitals is not effective.

Acknowledgements
We would like to thank the researchers in Seoul Clinical Laboratories (SCL) for sending the Staphylococcus isolates isolated from non-tertiary hospitals to our laboratory.

Funding
This study was supported by a research grant from the Korea Food and Drug Administration (KFDA) in Republic of Korea in 2005.

Transparency declarations
None to declare.

References
Research letters


Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkm488
Advance Access publication 19 December 2007

In vitro activity of ceftobiprole against Burkholderia pseudomallei

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Keywords: B. pseudomallei, melioidosis, cephalosporins

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Sir,

Burkholderia pseudomallei, a Gram-negative bacterium, causes a disease called melioidosis in humans and animals.1 The bacterium is a soil organism found mainly in Southeast Asia and Northern Australia. Antibiotics currently recommended for therapy of melioidosis are ceftazidime, imipenem, meropenem, amoxicillin/clavulanate, trimethoprim/sulfamethoxazole, doxycycline and chloramphenicol.1 A development of resistance of B. pseudomallei to the aforementioned antibiotics has been recognized;2,3 hence, a search for new agents effective against B. pseudomallei is needed.

Ceftobiprole is a novel parenteral cephalosporin whose broad spectrum of activity includes most clinically important Gram-positive and Gram-negative bacteria.4

One hundred and fifteen strains of ceftazidime-susceptible B. pseudomallei from different infected patients were selected from our collection. All strains were identified as B. pseudomallei by API 20NE (bioMérieux, France). In vitro susceptibility was determined by Kirby-Bauer disc diffusion for all 115 strains and Etest for 5 randomly chosen strains. Paper discs containing 30 μg ceftobiprole per disc (MASTDISC) and Etest strips of ceftobiprole at concentrations of 0.016–256 mg/L were provided by Janssen-Cilag (Thailand). The disc diffusion test was repeated for six strains of B. pseudomallei in order to determine the reproducibility of the test. The methodology for susceptibility testing was performed by direct colony suspension according to guidelines suggested by the CLSI.5 Quality control was performed by testing susceptibility of Pseudomonas aeruginosa ATCC 27853. The proposed breakpoints for inhibition zone diameters of ceftobiprole are ≥20 mm for susceptible, 17–19 mm for intermediate and ≤16 mm for resistant. The proposed breakpoints for MICs of ceftobiprole are ≤4 mg/L for susceptible, 8 mg/L for intermediate and ≥16 mg/L for resistant.

The inhibition zone diameter of ceftobiprole against P. aeruginosa ATCC 27853 was within the reference limits. The distribution of inhibition zone diameters of ceftobiprole against B. pseudomallei is shown in Table 1. Inhibition zone diameters of ≥20, 17–19 and ≤16 mm were observed in 46 (40%), 55 (47.8%) and 14 (12.2%) strains, respectively. Four strains of B. pseudomallei with inhibition zone diameters of 15–19 mm on the initial disc diffusion test had identical inhibition zone diameters on the second test. Another two strains with an inhibition zone diameter of >20 mm had 1 mm difference in inhibition zone diameter on the second test, but the inhibition zone diameters from both tests were still within susceptible values. Four B. pseudomallei strains with an inhibition zone diameter of 17–19 mm had MICs of ceftobiprole of 6–8 mg/L, whereas a strain with an inhibition zone diameter of 16 mm had an MIC of 16 mg/L.

Our findings indicate that the in vitro activity of ceftobiprole against B. pseudomallei determined by Kirby-Bauer disc diffusion is reproducible and correlates with that determined by Etest. Ceftobiprole has less in vitro activity than ceftazidime against B. pseudomallei, and only 40% of B. pseudomallei strains are susceptible to ceftobiprole.

Acknowledgements

We thank Janssen-Cilag (Thailand) and The Thailand Research Fund for supporting the study.

Funding

V. T. is a recipient of Senior Researcher Scholar of the Thailand Research Fund. Janssen-Cilag (Thailand) provided ceftobiprole susceptibility discs and Etest strips for this study.

Transparency declarations

None to declare.

References


Table 1. Distribution of ceftobiprole inhibition zone diameter for 115 strains of B. pseudomallei

<table>
<thead>
<tr>
<th>Inhibition zone diameter (mm)</th>
<th>No. of strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>15</td>
<td>5 (4.3)</td>
</tr>
<tr>
<td>16</td>
<td>8 (7.0)</td>
</tr>
<tr>
<td>17</td>
<td>25 (21.7)</td>
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<tr>
<td>18</td>
<td>19 (16.5)</td>
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<td>19</td>
<td>11 (9.6)</td>
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<td>20</td>
<td>30 (26.1)</td>
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<td>21</td>
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</tr>
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</tr>
<tr>
<td>23</td>
<td>4 (3.5)</td>
</tr>
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<td>25</td>
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