Heterogeneity of metallo-β-lactamases in clinical isolates of Chryseobacterium meningosepticum from Hangzhou, China

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Objectives: To determine the distribution and heterogeneity of metallo-β-lactamases (MBLs) responsible for imipenem resistance in Chryseobacterium meningosepticum.

Methods: Clinical C. meningosepticum isolates (n = 170) were collected from hospitals in Hangzhou, China. Production of MBLs was investigated by determination of imipenem MICs, and by using both a three-dimensional test and a 2-mercaptoethylacetic acid inhibitory test. Genes encoding BlaB and GOB MBLs were amplified by PCR, sequenced and compared with genes in GenBank.

Results: More than 95% of the 170 isolates showed high (MIC > 16 mg/L) or intermediate resistance to imipenem, but only 94 isolates (55%) were shown phenotypically to produce MBLs (imipenem MIC range, 8–256 mg/L), with MBL genes detected in 93 of these. Among them, 83 isolates had blαB alleles and 65 isolates had blαGOB alleles; 38 isolates possessed one MBL gene and 55 isolates contained two genes. The major blαB alleles encoded BlaB-2, -3 and -11, while the major blαGOB alleles encoded GOB-2, -4, -8 and -10. MBLs or their genes were not detected in 76 (45%) isolates, including many that were highly resistant to imipenem.

Conclusions: High levels and rates of imipenem resistance in C. meningosepticum from Hangzhou often result from the presence of heterogeneous BlaB and/or GOB MBLs, although undefined carbapenem resistance mechanisms also exist. Susceptibility testing and screening for MBLs should be conducted in order to inform effective treatment for C. meningosepticum infections.

Keywords: imipenem, resistance, genotypes, phenotypes

Introduction

Chryseobacterium meningosepticum is the most clinically important species of the genus Chryseobacterium, causing neonatal meningitis, pneumonia, sepsis and soft tissue and other tissue infections. The mortality rate for neonatal meningitis can be as high as 57%.1 Chryseobacteria are resistant to multiple antibiotics, especially to β-lactams.1,2 Many possess two different types of β-lactamases, namely class A extended-spectrum β-lactamases and class B metallo-β-lactamases (MBLs); the latter confer resistance to carbapenems, which are widely used to treat infections caused by multidrug-resistant Gram-negative bacteria.1-3

Two types of MBL, BlaB and GOB, have been identified in isolates of C. meningosepticum.1,6 Although they have similar molecular weights and pls, these two enzyme types show only very low molecular similarity.1,2 Sequencing and analysis of genes encoding BlaB and GOB has revealed heterogeneity, with up to 12 blαGOB and 14 blαB alleles identified and registered in GenBank.1,6 However, there have been no systematic studies to determine the distribution and heterogeneity of BlaB versus GOB MBLs in a large collection of clinical isolates of C. meningosepticum.

In this study, we collected a large number of clinical isolates of antibiotic-resistant C. meningosepticum from hospitals in Hangzhou, China, and sought MBLs by phenotypic methods. The genotypes and heterogeneity of genes encoding MBLs were also analysed.

Materials and methods

Bacterial strains

Clinical isolates of C. meningosepticum (n = 170) were collected from major hospitals in Hangzhou, China. Identification and susceptibility...
testing was conducted using automated systems (VITEK, BioMerieux, Marcy l’Etoile, France and/or Phoenix, Becton Dickinson, MD, USA). The MICs of a range of antibiotics were determined using the agar dilution method according to CLSI (NCCLS) recommendations.

**Screening and confirmation of MBLs**

Two phenotypic methods were used to screen isolates for MBLs. For the three-dimensional (3-D) tests, crude enzyme extracts were prepared from isolates by repeated freeze-thawing. Both crude enzyme extract (40 μL) only, and enzyme extract (30 μL) plus 10 μL of 0.3 M EDTA were loaded in slots in a plate of Mueller–Hinton agar, in the centre of which was placed a paper disc containing 10 μg of imipenem (Oxoid, Basingstoke, UK). *Escherichia coli* ATCC 25922 was inoculated as the indicator isolate. After overnight incubation, extracts that gave larger inhibition zones in the presence of EDTA and showed growth in the absence of EDTA were judged to contain MBLs. Production of MBLs was confirmed by the method developed by Arakawa et al. Two imipenem discs were placed on a Mueller–Hinton agar plate on to which a 2-mercaptopropionic acid disc. MBL production was assessed after incubation at 35°C for 18–24 h, and judged positive if the imipenem inhibition zone was enlarged on the side toward the 2-mercaptopropionic acid disc.

**PCR amplification and sequencing of bla genes**

Consensus primer pairs that detect all reported *blaB* and *bla<sub>GOB</sub>* MBL genes were used for PCR amplification of the target genes. The PCR products were cloned into a pGEM-T-easy-Vector (Promega, Madison, USA) and recombinant plasmids were transformed into *E. coli* DH5α. The transformants were selected on ampicillin-containing (50 mg/L) Luria–Bertani agar plates. Inserts were sequenced using an ABIPRISM 377 DNA Sequencer (ABI, MN, USA) and results were compared with existing *blaB* and *bla<sub>GOB</sub>* sequences in the GenBank database.

**Results and discussion**

**Screening *C. meningosepticum* isolates for phenotypic MBL production**

The susceptibilities of *C. meningosepticum* isolates to imipenem and 13 other antibiotics were determined. The distribution of imipenem MICs for the 170 isolates is shown in Figure 1. Only eight (4.6%) isolates were susceptible to imipenem *in vitro* (MICs ≤ 4 mg/L), while 140 (>76%) isolates were highly resistant (MICs ≥ 16 mg/L) (Figure 1). The proportion of imipenem-resistant *C. meningosepticum* isolates in our collection was higher than has been reported from hospitals in other parts of the world. However, only 94 (55%) isolates produced metallo-carbapenemases as determined by the 3-D test and a 2-mercapto propionic acid inhibitory test. A wide range of imipenem MICs (8–256 mg/L; Figure 1). Thirty-eight isolates possessed one MBL gene and 55 isolates contained two distinct MBL genes, which may contribute to the high-level imipenem resistance of some isolates.

**PCR amplification and heterogeneity of MBL genes**

We amplified *blaB* and/or *bla<sub>GOB</sub>* sequences from 93 of the 94 *C. meningosepticum* isolates that were phenotypic MBL producers (Table 1); 83 isolates had *blaB* alleles and 65 had *bla<sub>GOB</sub>* alleles. The major *blaB* genotypes encoded BlaB-2 (43%), -3 (20%) and -11 (26%) enzymes, while the major *bla<sub>GOB</sub>* genotypes encoded GOB-2, -4, -8 and -10; GOB-8 and GOB-10 composed 61.5% (40/65) of all GOB types. MBLs were detected in isolates over a wide range of imipenem MICs (8–256 mg/L; Figure 1). Thirty-eight isolates possessed one MBL gene and 55 isolates contained two distinct MBL genes, which may contribute to the high-level imipenem resistance of some isolates.

**Table 1. Summary of MBLs found in *C. meningosepticum***

<table>
<thead>
<tr>
<th>MBL Type</th>
<th>GOB Type</th>
<th>No. of Isolates</th>
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<tbody>
<tr>
<td>B-2</td>
<td>GOB-2</td>
<td>3</td>
</tr>
<tr>
<td>B-3</td>
<td>GOB-4</td>
<td>2</td>
</tr>
<tr>
<td>B-5</td>
<td>GOB-8</td>
<td>5</td>
</tr>
<tr>
<td>B-11</td>
<td>GOB-10</td>
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</tr>
<tr>
<td>B-1</td>
<td>GOB-4</td>
<td>1</td>
</tr>
<tr>
<td>B-1</td>
<td>GOB-8</td>
<td>3</td>
</tr>
<tr>
<td>B-2</td>
<td>GOB-1</td>
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<tr>
<td>B-2</td>
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<tr>
<td>B-2</td>
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</tr>
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</tr>
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<td>B-3</td>
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</tr>
<tr>
<td>B-11</td>
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<td>B-11</td>
<td>GOB-8</td>
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<td>B-11</td>
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<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>93</td>
</tr>
</tbody>
</table>
spread of common strains. On the other hand, the MBLs in isolates from the 2nd Affiliated Hospital showed greater heterogeneity; GOB-8, -4, -2 and -1, which were all identified in isolates from other hospitals, and also GOB-6 and GOB-5, which were not detected in other hospitals.

**Novel mechanisms of carbapenem resistance**

We found no phenotypic evidence of MBL production for 76 (45%) *C. meningosepticum* isolates, and failed to amplify either *blaB* or *blaGOB* sequences, even though many of these isolates were highly resistant to imipenem (Figure 1). These results suggest the presence of other carbapenem resistance mechanisms in *C. meningosepticum* isolates from Hangzhou.

It is clear that *C. meningosepticum* isolates from Hangzhou are highly resistant to imipenem, and that many isolates produce BlaB- and/or GOB-type MBLs. Therefore, in cases of serious infection by *C. meningosepticum*, susceptibility testing and screening for MBLs should be conducted in order to inform effective treatment. Carbapenems, which are widely used for the treatment of Gram-negative bacteria including Chryseobacteria, may not be a good choice for *C. meningosepticum*. Isolates from our region show greater susceptibility to combinations of cefoperazone/sulbactam (70–85%), piperacillin/tazobactam (65–80%) and trimethoprim/sulfamethoxazole (50–70%) *in vitro* (data not shown). These agents may be more effective options and have been used in our hospital for *C. meningosepticum* infection. In addition, vancomycin and rifampicin, which are more frequently used for Gram-positive bacteria, are an even better choice (not shown).10

**Transparency declarations**

None to declare.

**References**


