Our results are demonstrated in Table 1. *E. coli*, *S. marcescens* and *Enterobacter* spp. isolates were inhibited at MIC values of ≤1, 2 and ≤2 mg/L, respectively. For *K. pneumoniae*, MIC50 and MIC90 values of tigecycline were 0.25 and 2 mg/L, respectively. The Etest results were confirmed by the broth microdilution method. According to US Food and Drug Administration recommendations for tigecycline (susceptible ≤2 mg/L and resistant ≥8 mg/L), all isolates were susceptible and only one (1%) *K. pneumoniae* strain displayed an intermediate MIC of 4 mg/L. When the European Committee on Antimicrobial Susceptibility Testing breakpoint criteria (susceptible ≤1 mg/L and resistant >2 mg/L) were used, the percentage of tigecycline susceptibility decreased. Only 4 (31%) *Enterobacter* spp. and 78 (89%) *K. pneumoniae* isolates tested were susceptible. Furthermore, all *S. marcescens* were characterized as intermediate. The isolates collected in this study were broadly resistant to β-lactams, fluoroquinolones and variably to aminoglycosides.

Currently, multidrug-resistant Gram-negative bacteria remain the most problematic pathogens worldwide, especially in intensive care units. Carbapenem antibiotics were important agents for the management of those infections. Over the past few years, the progressive increase in carbapenem-resistant Gram-negative non-fermentative bacilli as well as the spread of genes encoding carbapenem-hydrolysing enzymes in enterobacterial species is of great concern, leaving limited choices for therapeutic regimens.7

Tigecycline was active against MBL-producing members of the family Enterobacteriaceae, inhibiting 99% of them at a concentration of ≤2 mg/L. A recent study demonstrated that tigecycline was effective against multiresistant *K. pneumoniae* strains producing *Klebsiella pneumoniae* carbapenemase (KPC), an ESBL belonging to molecular class A enzymes with activity against carbapenem.8 In addition, it was active against clinical isolates possessing *bla*TEM, but the number of MBL producers tested was small.9 Our results confirm the in vitro activity of tigecycline against Enterobacteriaceae possessing carbapenemases. Its broad-spectrum activity combined with its stability against common resistance mechanisms and the lack of cross-resistance with other classes of antibiotics1,2 make tigecycline a therapeutic agent for the treatment of infection caused by multi-resistant microorganisms. However, the in vitro results require support from clinical studies.

### Table 1. In vitro activity of tigecycline against 109 MBL-positive Enterobacteriaceae

<table>
<thead>
<tr>
<th>Organism (number of isolates)</th>
<th>MIC (mg/L)</th>
<th>Number of isolates with MIC (mg/L) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
</tbody>
</table>
| *E. coli* (4)                | 0.03–1     | NA | NA | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |<br>0.25–2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |<br>0.12–4 | 0.25 | 2 | 0 | 0 | 25 | 23 | 13 | 17 | 9 | 1<br>
| *S. marcescens* (4)          | 2          | NA | NA | 0 | 0 | 0 | 0 | 0 | 4 | 0 |<br>0.25–2 | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 2 | 9 | 0 |<br>0.12–4 | 0.25 | 2 | 0 | 0 | 25 | 23 | 13 | 17 | 9 | 1<br>
| *Enterobacter* spp. (13)     | 0.25–2     | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 2 | 9 | 0 |<br>0.12–4 | 0.25 | 2 | 0 | 0 | 25 | 23 | 13 | 17 | 9 | 1<br>
| *K. pneumoniae* (88)         | 0.25–2     | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 2 | 9 | 0 |<br>0.12–4 | 0.25 | 2 | 0 | 0 | 25 | 23 | 13 | 17 | 9 | 1<br>

NA, not applicable.<br><sup>a</sup>MICs (MIC<sub>50</sub> and MIC<sub>90</sub>) at which 50% and 90% of isolates tested, respectively, are inhibited.

### Transparency declarations

None to declare.

### References


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### Invasive group B *Streptococcus* isolates showing reduced susceptibility to penicillin in Hong Kong

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Keywords: *Streptococcus agalactiae*, blood culture, penicillin G, benzylpenicillin

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Correspondence

Sir,

Despite being a part of normal intestinal and vaginal flora in some healthy individuals, group B Streptococcus (GBS; Streptococcus agalactiae) is a leading cause of invasive infections in neonates and adults with underlying diseases. The mortality rate of group B streptococcal bacteraemia in adults ranges from 9% to 47% and is significantly associated with age.4 Unless the patient is allergic, penicillin is often used as a first-line treatment as well as maternal intrapartum prophylaxis to prevent the disease in neonates, and resistance is rarely encountered.2–4 However, penicillin tolerance has been previously observed and thought to contribute to the poor therapeutic response seen in some patients with serious GBS infections.5 Resistance to the cephalosporin, cefotaxime, has also been reported in Japan and speculated to be caused by changes in the penicillin-binding proteins.6

In the past 24 months, two blood isolates of GBS (M011 and M046) suspected to have reduced susceptibility to penicillin (zone diameter < 24 mm by the disc diffusion method according to the CLSI criteria7) were referred by the same local hospital to the Microbiology Division, Public Health Laboratory Services Branch, Centre for Health Protection, for confirmation. Both patients involved were male aged over 60 with underlying medical conditions. Serotyping was performed as previously described8 and β-lactamase test by BBL cefinase nitrocefin disc (Becton Dickinson, Franklin Lakes, NJ, USA) following the manufacturer’s instructions. Three methods were employed to determine the MIC of penicillin, namely Etest (AB BIODISC, Solna, Sweden), VITEK 2 automated system with the AST-P534 card and broth microdilution. Manufacturer’s instructions for the two commercial systems, Etest and VITEK2, were followed. Etest was performed using Mueller–Hinton agar supplemented with 5% horse blood. The broth microdilution was done according to the CLSI guidelines7 using Mueller–Hinton broth supplemented with 5% horse blood. Susceptibility to cefotaxime, clindamycin, erythromycin, tetracycline and vancomycin were also determined by the disc diffusion method according to the CLSI criteria.7 Results obtained are summarized in Table 1. The penicillin-susceptible GBS strain ATCC 13813 was included in all the susceptibility tests as control.

The CLSI interpretative criterion for non-susceptibility to penicillin for non-pneumococcal Streptococcus is MIC > 0.12 mg/L.7 Although Etest showed both isolates to be non-susceptible to penicillin, both the AST-P534 card of VITEK 2 and broth microdilution have confirmed only M011 to have the true MIC value of 0.25 mg/L. In fact, the Advanced Expert System (AES) in VITEK 2 only issued an unusual resistance warning for M011. The disc diffusion method and the Etest were sufficiently sensitive to detect reduced penicillin susceptibility in GBS, however, subsequent confirmation by reference methods was necessary. The result congruence between VITEK 2 and broth microdilution was in accord with the finding of a recent study, which showed that the AST-P534 card allows accurate determination of GBS susceptibility to the majority of antimicrobials except erythromycin.9

There is a paucity of information regarding the mechanism behind reduced penicillin susceptibility in GBS. Most work on the penicillin resistance mechanism in streptococci has been done on Streptococcus pneumoniae and Streptococcus pyogenes whereby modification of penicillin-binding proteins was found to be responsible most frequently. However, a recent report has shown that mutational changes in the penicillin-binding protein PBP2X can also lead to reduced susceptibility to penicillin in GBS.10 In the absence of any β-lactamase activity (Table 1), we believe that modification of penicillin-binding proteins is a possible explanation for the reduced penicillin susceptibility seen with our GBS isolates. Nevertheless, further molecular work is needed for confirmation.

The penicillin non-susceptible M011 also exhibited constitutive resistance to tetracycline, clindamycin and erythromycin, in contrast to M046 (Table 1). However, both isolates remained susceptible to cefotaxime and vancomycin. Cross-resistance to macrolides, lincosamides and the streptogramin B compounds (MLSB phenotype, i.e. resistance to clindamycin and erythromycin) in GBS has been shown to be associated with the presence of erm (erythromycin-ribosomal-methylase) enzymes previously, while tetracycline resistance may be due to the presence of the tet(M) gene.4 The patient with M011 isolated from the blood was admitted to the hospital with a high fever and was empirically treated with intravenous amoxicillin–clavulanate for 3 days after which the blood culture result had become negative. The patient recovered fully after 10 days of hospitalization.

Although M046 did not reach the penicillin non-susceptible category, it undoubtedly showed significant reduction in penicillin susceptibility. Noteworthy also is that it belongs to serotype III which is one of the most clinically important serotypes causing most neonatal and invasive GBS diseases in the United States.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Date referred</th>
<th>Patient sex/age</th>
<th>β-Lactamase</th>
<th>Serotype</th>
<th>Penicillin disc diffusion test zone size (mm)</th>
<th>Penicillin G MIC (mg/L)</th>
<th>Resistance phenotype to other agents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VITEK 2 P534</td>
<td>broth microdilution</td>
<td>CTX</td>
</tr>
<tr>
<td>M046</td>
<td>Feb 2007</td>
<td>M/85 negative</td>
<td>III</td>
<td>23</td>
<td>0.19 ≤0.12 0.12</td>
<td>0.12 S</td>
<td>S</td>
</tr>
<tr>
<td>M011</td>
<td>Jul 2005</td>
<td>M/60 negative</td>
<td>VI</td>
<td>23</td>
<td>0.25 0.25 0.25</td>
<td>0.25 S</td>
<td>S</td>
</tr>
<tr>
<td>ATCC</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.032 ≤0.12 0.03</td>
<td>0.03 S</td>
<td>S</td>
</tr>
</tbody>
</table>

* Determined by the disc diffusion method according to the CLSI guidelines: CTX, cefotaxime; CLI, clindamycin; ERY, erythromycin; TET, tetracycline; VAN, vancomycin.
Europe and Asia. Strain M046 was shown to belong to serotype VI, which is more commonly found in Japan than elsewhere.

We present the first report of GBS with confirmed reduced susceptibility to penicillin in Hong Kong. Vigilance in monitoring the level of penicillin resistance in GBS should be maintained and suspected reduced susceptibility should be confirmed by reference methods.

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Transparency declarations

None to declare.

References