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**Transparency declarations**
None to declare.

**References**


**Keywords**: antimicrobial resistance, extended-spectrum β-lactamases, Gram-negative bacteria

Sir,
Temocillin, a semi-synthetic 6-α-methoxy derivative of ticarcillin, has been shown to be clinically efficacious in infections caused by extended-spectrum β-lactamase (ESBL)- and AmpC-producing Enterobacteriaceae.¹ ² It is licensed in the UK for bacteraemia, urinary tract infections (UTIs) and lower respiratory tract infections where susceptible Gram-negative bacteria are suspected or confirmed.

Currently, only the BSAC has defined temocillin breakpoints for Enterobacteriaceae (susceptible if MIC ≤8 mg/L for systemic infections and ≤32 mg/L for UTIs).³

Here, we report a comparison of three temocillin susceptibility testing methodologies [BD PhoenixTM Automated Microbiology System (instrument version 5.15A, software version 6.01A/V5.15A) (Becton Dickinson, Oxford, UK), Etest (AB Biodisk, Solna, Sweden) and broth microdilution (BMD)]. Our data indicate that, whereas the level of agreement for ‘susceptible’ results is excellent, the Phoenix system overcalls temocillin non-susceptibility.

A total of 281 consecutive clinical Enterobacteriaceae isolates from distinct patients were collected from urine, blood culture, fluid, respiratory and tissue specimens. Isolates comprised Escherichia coli (194), Klebsiella pneumoniae (19), Proteus mirabilis (16), Enterobacter cloacae (9), Serratia marcescens (7), Citrobacter koseri (6), Enterobacter aerogenes (5), Citrobacter freundii (4), Morganella morganii (4), Klebsiella oxytoca (3), Proteus vulgaris (3), Citrobacter farmeri (2), Pantoea agglomerans (2), Serratia liquefaciens (2), Citrobacter braakii (1), Enterobacter gergoviae (1), Klyuyeva sp. (1), Providencia rettgeri (1) and Raoultella ornithinolytica (1). Twenty-seven (9.6%) were ESBL producers, 16 (5.7%) were inducible AmpC β-lactamase producers and 8 (2.8%) were derepressed AmpC β-lactamase producers.

Identification and susceptibility testing were performed on overnight cultures using the BD Phoenix™AP instrument (automated nephelometry) and the BD Phoenix™ Automated Microbiology System (instrument version 5.15A, software version 6.01A/V5.15A) with Gram-negative Phoenix panels (NMIC-84). Temocillin MICs were also determined by BMD in cation-adjusted Mueller–
Hinton broth following the standard ISO method 20776-1:2006, and these results were considered the reference standard against which all other results were compared. Temocillin Etests were used according to the manufacturer’s instructions. Susceptibility to temocillin using the Phoenix system was determined according to BSAC breakpoints for Enterobacteriaceae for systemic infections (susceptible if MIC ≤8 mg/L).3

If the Etest or Phoenix results were within a 2-fold dilution of the BMD MIC for an isolate, the methods were deemed as being in agreement. Unless the Phoenix result advised a retest, e.g. due to insufficient growth, the first test results were used for the evaluation and comparison of the different antimicrobial susceptibility testing (AST) methods. Disagreements between BMD and the Etest or Phoenix methods were then subjected to further investigation by repeating the Phoenix test in duplicate.

Etest MICs showed good correlation with those obtained by BMD—they were within one doubling dilution in 93.6% of isolates.

Overall, there appeared to be discrepant results between the Phoenix system and one of the alternative susceptibility methods for 102 isolates (97 deemed intermediate by Phoenix and 5 deemed resistant) and a discrepancy between Phoenix and both other methods in 93. Therefore, Phoenix AST was repeated, in duplicate, for the 102 isolates to confirm or refute the original Phoenix result.

Of these 102 isolates, the second and third Phoenix AST results were consistent with the original result for 88 (86.3%). However, for 17 (16.7%) samples, the initial Phoenix AST result was not consistent between runs—these results were therefore excluded from our analysis.

Of the 264 remaining isolates, 246 were proven to be susceptible using BMD (MIC ≤8 mg/L) and concordant results were found by Etest in 236 (95.9%). However, the Phoenix system declared only 162 (65.9%) susceptible. Seventeen isolates were ‘susceptible only for UTI’ (MIC >8 to ≤32 mg/L) by BMD and 26 by Etest. In comparison, far more were categorized as ‘susceptible only for UTI’ by Phoenix—96. Only one isolate was found to be resistant (MIC >32 mg/L) by the reference method, and two by Etest, whereas six were declared resistant by the Phoenix method. Table 1 summarizes the results for temocillin susceptibility for all three methods.

In conclusion, this study indicates that ‘susceptible’ results produced by the Phoenix system, using the NMIC-84 card according to the manufacturer’s recommendations, are reliable. However, the majority of isolates found to be ‘susceptible only for UTI’ and ‘resistant’ by Phoenix were actually fully susceptible (MIC ≤8 mg/L) as determined by the reference method. We therefore recommend that isolates categorized as such be retested using another method.

Table 1. Summary of temocillin AST results of 264 isolates comparing the BMD, Etest and Phoenix methods (percentages of total shown in brackets for each method)

<table>
<thead>
<tr>
<th></th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>246 (93.2%)</td>
<td>17 (6.4%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>Etest</td>
<td>236 (89.4%)</td>
<td>26 (9.8%)</td>
<td>2 (0.8%)</td>
</tr>
<tr>
<td>Phoenix</td>
<td>162 (61.4%)</td>
<td>96 (36.4%)</td>
<td>6 (2.3%)</td>
</tr>
</tbody>
</table>

A similar observation has been made with the Vitek 2 system (bioMérieux, Marcy l’Etoile, France), albeit with a lower frequency of error.4 The reason for the high level of discrepancy between Phoenix and BMD or Etest remains speculative, but could be due to instability of the temocillin within the Phoenix panels.

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Pharmacokinetics of maraviroc administered at 150 mg once daily in association with lopinavir/ritonavir in HIV-positive treatment-naive patients

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