21 November, 2019

Warning against the use of gradient tests for benzylpenicillin MIC in *Streptococcus pneumoniae*.

EUCAST benzylpenicillin breakpoints in *Streptococcus pneumoniae* are ≤0.06 mg/L, R>2 mg/L for indications other than meningitis. Isolates which are positive in the screen for beta-lactam resistance (with the oxacillin 1 µg disk) have benzylpenicillin MIC values above 0.06 mg/L and are categorized either “Susceptible, increased exposure”, in which case isolates can be treated with benzylpenicillin if dosing is adjusted according to the MIC value, or resistant (R>2 mg/L), in which case benzylpenicillin, and often many other beta-lactam agents, should be avoided for treatment. Laboratories must be able to perform correct MIC determination on screen positive isolates and this is especially important in isolates for which benzylpenicillin MICs are in the range 0.5 – 4 mg/L.

Following questions from NEQAS, EARS-Net and EUCAST users, the EDL investigated the accuracy of benzylpenicillin gradient tests (Etest™, bioMerieux; MTS™, Liofilchem). Both gradient tests were tested on in-house prepared MH-F agar from Oxoid (Thermo Fisher Scientific) och BBL (BD). Broth microdilution using Mueller-Hinton-F (MH-F) broth was used as the reference.

Both gradient tests were found to frequently underestimate MIC values by one or more doubling dilutions. In the area around the R breakpoint (0.5 – 4 mg/L), and with some variation between the MH-F media and the two tests, 0 – 37% of values were on the reference MIC, 63 – 100 % were below and 0-10 % of the values above the reference MIC.

**Conclusion:** Available gradient tests (Etest™ and MTS™) systematically underestimate benzylpenicillin MIC values in *S. pneumoniae*. This is especially detrimental in the important area close to the R breakpoint. Laboratories using gradient tests must be aware of this and MIC values of 0.5 - 2 mg/L should be checked with broth microdilution.

A beta-lactam intended for use in meningitis should always be tested using broth microdilution if the oxacillin screen is positive.

**Disk diffusion:** The oxacillin 1 µg disk screening test can be used to (1) screen for beta-lactam resistance but (2) also to exclude resistance to several beta-lactam agents.

(1) *S. pneumoniae* with an oxacillin 1 µg -inhibition zone <20 mm have beta-lactam resistance mechanisms and only a benzylpenicillin MIC can determine the degree of resistance to benzylpenicillin.

(2) *S. pneumoniae* with an oxacillin 1 µg -inhibition zone ≥28 mm are not resistant to either of ampicillin, amoxicillin and amoxicillin-clavulanic acid, piperacillin and piperacillin-tazobactam, cefotaxime, ceftriaxone, cefepime, ceftaroline or ceftobiprole. These can without further testing, be reported “susceptible”. However, isolates with zone diameters <8 mm are not automatically resistant to these agents.
Investigating the performance of gradient tests (Etest from bioMerieux; MTS from Liofilchem) on two different media vs. broth microdilution for the determination of benzylpenicillin MICs.

Two other materials (from Sweden and France; not shown here) exhibited the same but slightly worse principle results.

Thermofisher Scientific has stopped marketing M.I.C.E which is why these were not part of the investigation.

The EUCAST Development Laboratory, November 2019
### PCG MICs for gradient tests vs. BMD MICs
#### EDL study

<table>
<thead>
<tr>
<th>Etest, BBL MH-F</th>
<th>Etest, Oxoid MH-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2 dilutions lower</td>
<td>0</td>
</tr>
<tr>
<td>2 dilutions lower</td>
<td>2</td>
</tr>
<tr>
<td>1 dilution lower</td>
<td>18</td>
</tr>
<tr>
<td>Identical</td>
<td>33</td>
</tr>
<tr>
<td>1 dilution higher</td>
<td>4</td>
</tr>
<tr>
<td>2 dilutions higher</td>
<td>0</td>
</tr>
<tr>
<td>&gt;2 dilutions higher</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MTS, BBL MH-F</th>
<th>MTS, Oxoid MH-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2 dilutions lower</td>
<td></td>
</tr>
<tr>
<td>2 dilutions lower</td>
<td>6</td>
</tr>
<tr>
<td>1 dilution lower</td>
<td>33</td>
</tr>
<tr>
<td>Identical</td>
<td>17</td>
</tr>
<tr>
<td>1 dilution higher</td>
<td>1</td>
</tr>
<tr>
<td>2 dilutions higher</td>
<td></td>
</tr>
<tr>
<td>&gt;2 dilutions higher</td>
<td></td>
</tr>
</tbody>
</table>

For queries and advice – contact the EDL (erika.matuschek@eucast.org or gunnar.kahlmeter@eucast.org)