Antimicrobial susceptibility testing of *Legionella pneumophila*

The organism

*Legionella pneumophila* is a facultative intracellular, aerobic, Gram-negative bacillus, and the etiological agent of Legionnaires’ disease. Due to its fastidious nature, antimicrobial susceptibility testing is difficult and the correlation between in vitro results and clinical outcome is uncertain.

Antimicrobial resistance

Acquired antimicrobial resistance in *L. pneumophila* is extremely rare [1-4]. However, a clinical isolate resistant to ciprofloxacin due to a single point mutation in the *gyrA* gene, has been reported [5] and further fluoroquinolone resistant strains have been isolated from patients, some during therapy [6].

Treatment

Antimicrobial agents regarded as effective for treatment of legionellosis are macrolides, fluoroquinolones and rifampicin, all achieving adequate intracellular concentrations [7,8]. Agents with poor intracellular penetration (e.g. β-lactam agents and aminoglycosides) are ineffective for treatment despite good in vitro activity. Even with agents regarded as effective poor response to therapy has been described despite the organism appearing susceptible in vitro [9].

Antimicrobial susceptibility testing

There is no gold standard for antimicrobial susceptibility testing of *L. pneumophila*. Different methods, particularly with different media, yield different MICs. The use of media containing charcoal has been subject to debate for a long time and it has been shown that addition of charcoal to the medium will result in elevated MICs for most relevant agents [1, 10]. However, supplementation of medium with charcoal may be necessary to achieve adequate growth.

As correlation between MIC and clinical outcome is uncertain, antimicrobial susceptibility testing should be used for the purpose of detecting resistance mechanisms as indicated by MICs above the epidemiological cut off values (ECOFFs). Sufficient data to establish ECOFFs are not currently available but the published MIC distributions reproduced in the table give an indication of MICs for wild type isolates using gradient tests on the recommended medium, buffered charcoal yeast extract agar supplemented with α-ketoglutarate (BCYE-α), which provides an easy, reproducible and readily available method for routine laboratories [1, 2, 11].

Quality control target MICs and ranges have not been established by EUCAST. However, MICs for *L. pneumophila* ATCC 12821 and ATCC 33152 should be within the wild type [1] and should be used to ensure that growth conditions are adequate. For quality control of gradient strips, follow the instructions of the manufacturer for control of tests on non-fastidious organisms.
Gradient MIC method:

1. Subculture the strain on BCYE-α medium and incubate for 48 hours at 35-37°C in a humidified atmosphere (50-70% relative humidity).
2. Suspend colonies in sterile water to the density of a 0.5 McFarland standard.
3. Inoculate BCYE-α plates by evenly swabbing the entire surface and apply a single gradient strip to each 9 cm plate.
4. Incubate for 48 hours at 35°C in a humidified atmosphere. If there is inadequate growth after 48 hours, the plates can be incubated for an additional 24 hours.
5. Read MICs at the point of intersection of the ellipse with the gradient strip.
6. Compare the MIC for the isolate with the MIC distributions in the table. The likely susceptibility of the isolate may be reported on the basis of whether the MIC is below or equal to the tentative highest MIC for the wild type population.
7. Isolates with MICs above the highest MIC for wild type organisms in the table should be sent to a reference laboratory for confirmation testing.

Table: MIC distributions for *L. pneumophila* (gradient tests by method recommended above)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
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<th>0.016</th>
<th>0.032</th>
<th>0.064</th>
<th>0.125</th>
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<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>≥32</th>
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<td>43</td>
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</table>

Note 1: Reference 1 included serogroup 1 only, reference 2 included serogroups 1, 6, 8 and 10. MICs may be higher for serogroup 1 than for other serogroups [2].

☐ Tentative highest MIC for wild type organisms.
References


