EDITORIAL

Choosing the best antimicrobial for the job

Tim Nuttall discusses some factors to consider when interpreting the results of antimicrobial susceptibility tests

WHEN selecting an antibiotic to treat an infection, a key factor is the antimicrobial susceptibility of the bacteria. This can be assessed using qualitative (eg, disc diffusion) or quantitative (eg, minimum inhibitory concentration [MIC]) tests. However, interpreting the results of these tests is not always straightforward.

Kirby-Bauer disc diffusion tests use antibiotic-impregnated paper discs placed on agar plates (Fig 1a). If the bacteria are susceptible to an antibiotic, a clear halo (the zone of inhibition) develops around the disc. The zone of inhibition is compared with international standards to determine whether the bacteria are susceptible or resistant to that antibiotic. The size of the zone of inhibition by itself is meaningless and should not be used to determine how susceptible or resistant the bacterial isolate is.

The MIC is the lowest concentration of an antibiotic that completely inhibits growth of the bacteria. Broth dilution methods culture the bacteria with doubling dilutions of each antibiotic. E-strips such as Etest (bioMérieux) and MIC evaluator system (Oxoid) use paper strips impregnated with antibiotics at differing concentrations along the strip. These produce an elliptical zone of inhibition, with the MIC read as the concentration where the edge of the zone of inhibition touches the strip (Fig 1b).

The isolate will be reported as susceptible or resistant, based on accepted standards, but, unlike disc diffusion methods, the results also reveal how sensitive the organism is. A low MIC by itself does not correlate with increased efficacy as the susceptibility range for each organism-drug combination varies. Nevertheless, the more dilutions of a drug that still inhibit bacteria growth (ie, the lower the MIC), the more sensitive that bacterial isolate is to the drug.

Fig 2 shows the antimicrobial sensitivity pattern from broth dilution tests (performed by Idexx Laboratories) for a *Pseudomonas aeruginosa* isolate from a case of otitis externa in a dog. The letters under the reference range refer to the tested range in doubling dilutions. In this example, the bacteria
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FIG 1: (a) Kirby-Bauer disc diffusion test. An isolate of Escherichia coli has been incubated with several antibiotic impregnated discs. The zones of inhibition around each disc will be measured and compared to agreed standards to determine whether the isolate is sensitive or resistant to each antibiotic. (b) The e-strip minimum inhibitory concentration (MIC) assay. An E coli isolate has been incubated with two e-strips: one impregnated with enrofloxacin and one with both ceftazidime alone and ceftazidime in combination with clavulanic acid (TZL). The MIC for enrofloxacin is 8 to 12 µg/ml. In contrast, the isolate appears to be sensitive to even low concentrations of ceftazidime with or without clavulanic acid.

FIG 2: Results from a broth dilution test (performed by Idexx Laboratories). A Pseudomonas aeruginosa isolate was incubated in broth with doubling dilutions of different antibiotics. The minimum inhibitory concentration is read as the minimum dilution that prevented bacterial growth. This is compared to accepted standards to determine how sensitive or resistant this isolate is to each antibiotic

<table>
<thead>
<tr>
<th>Isolate 1: Pseudomonas aeruginosa</th>
<th>Antibiotic</th>
<th>Result</th>
<th>MIC (µg/ml)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin Resistant</td>
<td>0.25</td>
<td>s</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Marbofloxacin Resistant</td>
<td>0.5</td>
<td>s</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pot Sulphonamide Resistant</td>
<td>&gt;320</td>
<td>ss</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Gentamicin Resistant</td>
<td>&gt;16</td>
<td>ss</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Amikacin Resistant</td>
<td>&gt;64</td>
<td>s</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime SENSITIVE</td>
<td>&lt;8</td>
<td>S</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Pipercillin SENSITIVE</td>
<td>&lt;8</td>
<td>S</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Carbenicillin Intermediate</td>
<td>256</td>
<td>s</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>Ticarcillin SENSITIVE</td>
<td>64</td>
<td>S</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Tobramycin SENSITIVE</td>
<td>4</td>
<td>S</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

were grown in the presence of 0.25 µg/ml, 0.5 µg/ml, 1.0 µg/ml and 2.0 µg/ml enrofloxacin. The lower case letters refer to the accepted standards and the upper case letters refer to the actual MIC. In this case, the MIC for enrofloxacin is more than or equal to 2.0 µg/ml (the highest tested concentration). The breakpoint for resistance is the highest plasma concentration of the drug that can safely be achieved; this is the lowest concentration in the ‘r’ zone in the reference range (2.0 µg/ml for marbofloxacin, 80 µg/ml for trimethoprim potentiated sulfonamide and so on).

If the MIC falls within the ‘r’ zone, then it is unlikely that the drug will attain a therapeutic concentration in the target tissue. Treatment is therefore unlikely to be successful and the organism should be regarded as resistant to that antibiotic. If, however, the MIC falls within the ‘s’ zone, then it is likely that the drug will exceed the therapeutic concentration in the target tissue. Treatment is likely to be successful, and the organism can be regarded as sensitive to that antibiotic.

The subtlety lies in where the MIC is in the ‘s’ zone. For ticarcillin the MIC is only one dilution below the breakpoint. For piperacillin, in contrast, the MIC is at least four dilutions below the breakpoint. This Pseudomonas isolate is therefore more sensitive to piperacillin than it is to ticarcillin. Other factors, including pharmacodynamics and administration problems, could mean a suboptimal concentration of ticarcillin at the target site. This is less likely to occur with piperacillin, and this would be a better choice of drug in this case.

Intermediate (i) susceptibility is sometimes used when the MIC is near to the limit of serum or tissue concentrations following standard doses. It can also be used as a ‘buffer’ to prevent classification errors associated with technical variables. Generally, these isolates should be regarded as resistant. However, an antibiotic may be successful if used at a high dose and/or it concentrates in the target tissue, thereby exceeding the MIC.

 MICs are usually reported in µg/ml ranges as the tests assume that the antimicrobial will be administered systemically. Topical therapy, which delivers mg/ml antibiotic concentrations, can overcome apparent in vitro resistance. Using antimicrobial sensitivity tests to predict the response of topical therapy can therefore be misleading. The identity of the organism can guide the choice of antimicrobial, but decisions should be based on clinical signs and cytology.

Disc diffusion tests may give misleading results – for example β-lactam and cephalosporin susceptibility or resistance in vitro is poorly predictive of the presence of meticillin-resistant staphylococci (MRSA) or extended spectrum β-lactamase (ESBL) Escherichia coli. Further tests using PBP2a latex bead agglutination tests or PCR for mecA and other resistance genes are used to confirm the identity of suspect isolates. MRS and ESBL E. coli should be regarded as resistant to all β-lactams and cephalosporins irrespective of any apparent in vitro sensitivity.

Many MRS isolates exhibit inducible clindamycin resistance in vivo despite apparent in vitro sensitivity. This is associated with the presence of erm genes, which can be tested for using D-zone tests (where the zone of inhibition around a clindamycin/erythromycin disc is smaller than that around a clindamycin disc) or PCR.

It is important to realise that in vitro susceptibility tests do not necessarily predict the clinical outcome. In human medicine there is a ‘90-60’ rule: 90 per cent of infections with susceptible bacteria respond to therapy, but infections with resistant isolates will respond to an ‘inappropriate’ antibiotic about 60 per cent of the time.

This can be explained by flaws and limitations in susceptibility tests, variations in pharmacodynamics and tissue distribution, dosing or other compliance errors, underlying diseases and/or immune status. In addition to the MIC, understanding the nature of the infection, the pharmacokinetic properties of the antibiotic and patient factors will help achieve a successful outcome.

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