Rapid Diagnostic Tests for Antimicrobial Resistance: Comparability of Results and Interpretation of Data

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Susceptibility Testing - comparability of results.

• The reference (gold standard) method against which results should be compared.

• The inherent variation of the test methodology – category agreement and essential agreement.

• Test parameters
  • Sensitivity
  • Specificity
  • Very major errors
  • Major errors
  • Clinical predictive value

• Sources of Information.
Susceptibility Testing – interpretation of data.

- Test parameters
  - Sensitivity
  - Specificity
  - Very major errors
  - Major errors
  - Clinical predictive value
- Complicating factors
Sources of Information:


• National susceptibility testing committees.
Reference method – broth dilution determination of minimum inhibitory concentration (MIC).

- Reference method (ISO 20776-1).
Minimum Inhibitory Concentration. Broth Dilution MIC.

| Antimicrobial concentration | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 |

Final inoculum usually $10^5$ cfu/ml in broth, $10^4$ cfu/ spot on solid media. **The MIC is defined as the lowest concentration of antibiotic at which there is no visible growth of the organism.**
Complicating Factors:

- Detection of resistance genes that are not expressed.
- Complex regulation of resistance gene expression.
  - Inducible resistance
  - Constitutive resistance
- Variation in the regulation of a resistance mechanism – efflux pumps.
- The selection of isolates included in the panel tested.
Complicating Factors:

• Inducible resistance – only expressed in the presence of an inducing antimicrobial.
• Constitutive resistance – expressed permanently.
Features of the MIC distribution (from EUCAST):

Ciprofloxacin / Campylobacter jejuni

International MIC Distribution - Reference Database 2015-08-17

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

MIC: Epidemiological cut-off (ECOFF): 0.5 mg/L
Wildtype (WT) organisms: ≤ 0.5 mg/L

12212 observations (43 data sources)
In fact, repeatedly testing the SAME isolate gives the same WT distribution:

Ciprofloxacin / Campylobacter jejuni
International MIC Distribution - Reference Database 2015-08-17

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.
Inherent Variation in the Reference Test Methodology – ESSENTIAL AGREEMENT.

• There is inherent variation in the reference test method.
• This can be at least partly accounted for in test comparisons by use of the term essential agreement.
• MIC result obtained by new method which is within one dilution of the result obtained by the reference method.
• \[
\text{Number with essential agreement} \times 100 \\
\text{Total number examined}
\]
Features of the MIC distribution (from EUCAST):

Breakpoint $R > 0.5$ – clearly separates resistant and wild-type susceptible populations.

MIC distributions include collated data from multiple sources, geographical areas and time periods and cannot be used to infer rates of resistance.
Features of the MIC distribution (from EUCAST):

If the breakpoint was > 0.06 and split the wild-type population, you could not clearly and consistently separate resistant and susceptible populations because of the inherent test variation.
Category Agreement.

- Examines agreement between sensitive/intermediate/resistant results with the reference method.

\[
\frac{\text{Number of isolates with category agreement}}{\text{Total number tested}} \times 100
\]
Essential Agreement – an example.

- Compared an identification and susceptibility testing system with broth microdilution.
- Overall essential agreement ($\pm 1 \log_2$ dilution) for 3,719 organism / antimicrobial combinations was 95.6%
- Essential agreement was calculated for groups of organisms studied (88.0% for non-fermentative Gram-negative rods).
- Essential agreement was calculated for groups of organisms and individual antimicrobials (84.0% for Enterobacteriaceae versus imipenem).

<table>
<thead>
<tr>
<th></th>
<th>&gt;-2</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>+1</th>
<th>+2</th>
<th>&gt;+2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>0</td>
<td>2.1</td>
<td>97.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amox/clav</td>
<td>0</td>
<td>0.7</td>
<td>7.9</td>
<td>85.5</td>
<td>3.6</td>
<td>1.4</td>
<td>0.7</td>
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</tbody>
</table>
Category Agreement – an example.


• Evaluation of susceptibility of clinical isolates of *Enterobacteriaceae* and nonfermentative Gram-negative bacilli to cefoperazone/sulbactam.

• Used agar dilution as the reference method and compared disc diffusion and two automated systems against it.

• *E. coli* (N=150) categorical agreement for cefoperazone-sulbactam:

<table>
<thead>
<tr>
<th></th>
<th>Categorical agreement</th>
</tr>
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<tbody>
<tr>
<td>Disc Diffusion</td>
<td>97.3</td>
</tr>
<tr>
<td>Automated system 1</td>
<td>97.3</td>
</tr>
<tr>
<td>Automated system 2</td>
<td>84</td>
</tr>
</tbody>
</table>
Sensitivity and Specificity.

- Sensitivity is the proportion of true positives correctly identified by the test.
- Specificity is the proportion of true negatives identified by the test.
- Provide one of the approaches to quantifying the diagnostic ability of a test.
- May be applied to assess degree of categorical agreement (non-susceptible versus susceptible) of different methods.
Examples from the literature...

- Multi-centre assessment of Carba NP test – hydrolysis of imipenem produces acid, affects a pH indicator.
- Genotype result (carbapenemase resistance gene detection) used as the gold standard.
- Specificity for all non-Enterobacteriaceae was 100.0% at each of seven sites.
- Sensitivity 60.0–87.5% across seven sites.
Sensitivity and Specificity.

- Evaluation of susceptibility of clinical isolates of Enterobacteriaceae and nonfermentative Gram-negative bacilli to cefoperazone/sulbactam.
- Used agar dilution as the reference method and compared disc diffusion and two automated systems against it.
- *E. coli* (N=150) versus cefoperazone-sulbactam compared to reference method

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<td>99.3</td>
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<tr>
<td>Automated 2</td>
<td>44.4</td>
<td>87.2</td>
</tr>
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</table>
Predictive Values.

- We need to know the probability that the test used will give us the correct diagnosis.
- Sensitivity and specificity don’t provide this.
- Positive predictive value is the proportion of patients/tests with positive/resistant results which are correctly diagnosed.
- Negative predictive value is the proportion of patients with negative/susceptible test results which are correctly diagnosed.
- Dependent on prevalence of item of interest; if rare can be more certain that negative result is negative, but less sure that positive result is positive…

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<th>Positive predictive value</th>
<th>Negative predictive value</th>
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<td>44.4</td>
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<td>18.2</td>
<td>96.1</td>
</tr>
</tbody>
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Text in footer: Sensitivity Specificity Positive predictive value Negative predictive value

Disc Diffusion 100 98.6 81.8 100
Automated 1 88.9 99.3 88.9 99.3
Automated 2 44.4 87.2 18.2 96.1
Errors.

• Used because there are particular features relevant to antimicrobial treatment.

• If the test result is susceptible but the organism is resistant, then the patient may die, because you may choose a treatment which may not work.

• If the test result is resistant, but the organism is susceptible, then the patient will not be given this antimicrobial and will (hopefully!) receive a suitable alternative and survive.

• These ideas are captured by
  • Very major errors (also called very major discrepancy)
  • Major errors (also called major discrepancy)
  • Minor errors (also called minor discrepancy)
Very Major Error/ Discrepancy.

- Reference test result **resistant**, method under comparison result **sensitive**.
- The patient might be inappropriately treated and die.

Number of tests giving a very major error \times 100
Number of bacteria resistant by reference method
Major Error/ Discrepancy.

• Reference test result sensitive, method under comparison result resistant.
• The patient will not be inappropriately treated but the category result is wrong.

Number of tests giving a major error x 100
Number of bacteria susceptible by reference method
Minor Error/ Discrepancy.

- Reference test result **intermediate**, method under comparison result **sensitive or resistant**.
- Or vice versa.

\[
\frac{\text{Number of tests giving a minor error}}{\text{Number of bacteria tested by reference method}} \times 100
\]
Errors/ Discrepancies.

• Advice levels are provided in some of the references mentioned earlier.

• Rates are set for what may be considered an acceptable error level in each category.

• ISO, quoted in Shio-Shin et al. (see earlier).
  • Very Major Error ≤ 1.5%
  • Major Error ≤ 3%
  • Minor Error ≤ 10%

• BSAC
  • Very major error considered < 1% when setting breakpoints.
Clinical Predictive Value.

- Clinical response of patient considering susceptible or resistant test result.

<table>
<thead>
<tr>
<th>MIC</th>
<th>Number of Patients</th>
<th>% Cured or Improved</th>
<th>% Eradication</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 4 (S)</td>
<td>~1000</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>8 (S)</td>
<td>~250</td>
<td>90</td>
<td>86</td>
</tr>
<tr>
<td>16 (I)</td>
<td>~150</td>
<td>77</td>
<td>75</td>
</tr>
<tr>
<td>32 (I)</td>
<td>70</td>
<td>84</td>
<td>71</td>
</tr>
<tr>
<td>64 (R)</td>
<td>20</td>
<td>64</td>
<td>50</td>
</tr>
</tbody>
</table>
Thank you for listening…