Performance of the Oxoid M.I.C.Evaluator™ Strips compared with the Etest® assay and BSAC agar dilution

Shazad Mushtaq1, Marina Warner1, Jonathan Cloke2, Mariya Afzal-Shah1 and David M. Livermore1*

1Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, UK; 2Thermo Fisher Scientific, Basingstoke, Hampshire, UK

*Corresponding author. Tel: +44-20-8327-7223; Fax: +44-20-8327-6264; E-mail: david.livermore@hpa.org.uk

Received 29 January 2010; returned 16 March 2010; revised 10 May 2010; accepted 10 May 2010

Objectives: The Oxoid M.I.C.Evaluator™ (M.I.C.ETM; Thermo Fisher Scientific) comprises an antibiotic gradient on a plastic support. We compared its performance with Etest®—a similar product—using BSAC agar dilution as a reference.

Methods: Parallel MIC tests were performed by M.I.C.Evaluator, Etest and agar dilution on Iso-Sensitest™ agar. In total, 9354 organism/strip combinations were tested by each method, using 1017 bacteria representing clinically important fastidious and non-fastidious species.

Results: Essential agreement of strip MIC values (+1 doubling dilution) with the agar dilution reference, with off-scale results excluded, was 89.9% for M.I.C.Evaluator versus 89.5% for Etest (P < 0.05). These proportions were similar, at 89.5% and 89.3% (P < 0.05), respectively, if off-scale values were counted as agreeing if they could agree (e.g. a strip MIC > 32 mg/L and an agar dilution MIC of 128 mg/L). For both strips, agreement with agar dilution was best for non-fastidious genera, Moraxella, Listeria, Pasteurella and Campylobacter spp. and weaker for streptococci, anaerobes, Neisseria spp. and, especially, Haemophilus influenzae. Many 'disagreements', especially for H. influenzae, concerned organisms unequivocally resistant by all methods (e.g. ampicillin MIC 256 mg/L by agar dilution, 16 or 32 mg/L by both strips); nevertheless both strips underestimated imipenem MICs for Proteus. There was no difference between the two strip types in the proportion of agreements with agar dilution (P < 0.05); nevertheless their results agreed better with each other than with agar dilution (P < 0.01).

Conclusions: The M.I.C.Evaluator performed almost identically to the Etest, giving good agreement with BSAC agar dilution.

Keywords: susceptibility testing, sensitivity testing, antibiotic gradient strips

Introduction

Discs and automated systems with truncated MIC ranges are adequate for most routine susceptibility testing, but precise MIC determinations are needed in difficult settings, such as endocarditis and pneumococcal meningitis. MICs are also needed to guide the treatment of infections caused by multiresistant pathogens, where pharmacodynamically based dose adjustment may be sought. Finally, MICs are needed for organisms or antibiotic/organism combinations where disc testing is demonstrably unreliable, e.g. anaerobes, penicillin against most α-haemolytic streptococci except pneumococci, and glycopeptides against Staphylococcus aureus, where diffusion tests fail to discriminate strains with vancomycin MICs of 8 mg/L,1 let alone those with the small reductions in susceptibility (MICs 2–4 mg/L) now being associated with poor clinical outcomes.2

Classical MIC determinations on agar or in broth are routinely performed by specialist centres, but are inconvenient for diagnostic laboratories, where only a few isolates require these investigations and where the range of drugs to be tested varies with each isolate. Rather, diagnostic laboratories find it more convenient to perform their few MIC tests using pre-formed antibiotic gradients, such as Etest® (AB bioMérieux, Marcy l’Etoile, France). These are versatile and give results in good agreement with the CLSI broth microdilution method, against which they are calibrated.3 Their agreement with other methods, such as that of the BSAC is less well validated, but is asserted to be acceptable by the BSAC.4
The Oxoid M.I.C.Evaluator® Strip (Thermo Fisher Scientific, Basingstoke, UK) is a new gradient strip for MIC determinations, asserted by the manufacturer to be suitable for use on Iso-Sensitest agar as well as Mueller–Hinton agar. We compared its performance with that of the Etest Strip and the BSAC agar dilution methods.

**Materials and methods**

**Bacteria**

The same 1017 bacterial strains and antibiotics were tested using M.I.C.Evaluator Strips, Etests and the BSAC agar dilution method, with a total of 9354 antibiotic/strain combinations. The organisms were recent clinical isolates, selected to represent a wide range of species and susceptibility profiles, as summarized in Table 1. Controls comprised Escherichia coli NCTC 10418 and ATCC® 25922, Pseudomonas aeruginosa ATCC® 27853, Staphylococcus aureus ATCC® 25923, ATCC® 9213™ and ATCC® 43300™, Streptococcus pneumoniae ATCC® 49619™ and ATCC® 49620™, Haemophilus influenzae ATCC® 49247™, Neisseria gonorrhoeae ATCC® 49226™, Enterococcus faecalis ATCC® 29212™, Bacteroides fragilis ATCC® 25285™ and Bacteroides thetaiotaomicron ATCC® 29741™, all provided as Culti-Loops® (Thermo Fisher Scientific).

**Susceptibility tests**

Tests with both the M.I.C.Evaluator and Etest Strips were performed on Iso-Sensitest agar (Thermo Fisher Scientific) with the supplements, incubation periods and conditions specified in the BSAC agar dilution method, except that: (i) based on advice for Etests, p-nitrophenyl glycerol was omitted from the media for strip tests with Proteaeae, whereas it was included, at 50 mg/L, as an anti-swarming agent in agar dilution MIC determinations; and (ii) based on the package insert for the M.I.C.Evaluator Strips, Iso-Sensitest agar was used throughout for tests with imipenem, although the BSAC advocates Mueller–Hinton agar for this and other carbapenems. Oxacillin strips were tested on Mueller–Hinton agar supplemented with 2% NaCl as per the BSAC agar dilution method. For all isolates the inocula for strip tests were matched to a 0.5 McFarland standard. Results were read in accordance with the manufacturers’ directions, which are essentially identical for both strip products. For bactericidal antibiotics, the MIC was taken as the point of termination of all growth; for bacteriostatic agents as the point of 80% inhibition. In those cases where growth terminated between two points on the strip scale, the MIC was rounded to the higher value. The following strip types were used: 0.002–32 µg strips of ciprofloxacin, cefotaxime, imipenem, levofloxacin and penicillin G; 0.015–256 µg strips of amoxicillin, amoxi- clin/clavulanic acid, ampicillin, cefotaxime, erythromycin, gentamicin, linezolid, metronidazole, oxacillin, penicillin G, tetracycline and vancomycin; and 0.06–1024 µg strips of gentamicin. Storage was at 2–8°C for the M.I.C.Evaluator Strips and −20°C for Etests. Boxes were allowed to equilibrate at room temperature for at least 1 h before opening.

Agar dilution MICs were determined by the BSAC method, as current in 2007, on oblong 12×8 cm plates, inoculated using a 96-point inoculator. Results were read with an automated optical reader (Perceptive Instruments, Haverhill, UK) and were manually corrected as necessary.

**Data handling**

Data analysis was performed in Microsoft Office Excel®, by comparing the number of doubling dilutions difference between each of the gradient test strip results and the reference method. Intermediate MIC values (1.5, 3 or 6 mg/L etc.) read from the M.I.C.Evaluator Strip or Etest scales were rounded up to the next highest value on the standard doubling dilution scale (i.e. 2, 4 or 8 mg/L) for calculations.
|                     | Enterobacteriaceae (250) | Non-fermenters (76) | Staphylococci (154) | Enterococci (100) | Streptococci (161) | Neisseria (56) | Haemophilus (56) | Anaerobes (101) | Moraxella (21) | Listeria (21) | Pasteurella (10) | Campylobacter (22) |
|---------------------|--------------------------|---------------------|---------------------|------------------|------------------|---------------|----------------|----------------|--------------|--------------|--------------|-------------------|
| Erythromycin range  | 0.06 to >256             | 0.06 to >256        | 1–64                | 0.06–0.12        | 0.25–4           |
| MIC<sub>50</sub>    | 0.5                      | 0.12                | 8                   | 0.12             | 0.5             |
| MIC<sub>90</sub>    | >256                     | 32                  | 16                  | 0.12             | 1              |
| Gentamicin range    | 0.25–512                 | 0.125 to >1024      | 0.016 to >1024      | 0.25             | 16              |
| MIC<sub>50</sub>    | 0.5                      | 0.12                | 2 to >1024          | 32               | 16              |
| MIC<sub>90</sub>    | >1024                    | >1024               | 64                  | 16               | 1              |
| Imipenem range      | 0.06–4                   | 0.03 to >32         | 0.25–2<sup>m</sup>  | 0.002–0.5        | 0.06–2          | 0.008–4       |
| MIC<sub>50</sub>    | 0.25                     | 2                   | 1<sup>m</sup>       | 0.016            | 0.5             | 0.12           |
| MIC<sub>90</sub>    | 2                        | 32                  | 2                   | 0.12             | 2              | 1              |
| Levofloxacin range  | 0.015 to >32             | 0.12 to >32         | 0.5–4               | 0.008–4          |
| MIC<sub>50</sub>    | 0.125                    | 0.5                 | 1                   | 0.015            |
| CIC<sub>90</sub>    | 16                       | 2                   | 2                   | 0.06             |
| Linezolid range     | 0.5–32                   | 1–64                | 0.5–2               |
| MIC<sub>50</sub>    | 2                        | 2                   | 1                   |
| MIC<sub>90</sub>    | 2                        | 16                  | 2                   |
| Oxacillin range     | 0.06 to >256             | 0.03–16             |
| MIC<sub>50</sub>    | 8                        | 0.5                 |
| MIC<sub>90</sub>    | >256                     | 8                   |
| Penicillin G range  | 0.016–128                | 0.004–2             | 0.015–64            | 0.008 to >512    | 0.016–0.25     |
| MIC<sub>50</sub>    | 8                        | 0.03                | 0.12                | 4                | 0.12           |
| MIC<sub>90</sub>    | 128                      | 1                   | 1                   | 128              | 0.12           |
| Tetracycline range  | 0.12–256                 | 0.125–128           | 0.25–16             | 0.12–16          |
| MIC<sub>50</sub>    | 0.5                      | 0.5                 | 0.25                | 0.5              |
| MIC<sub>90</sub>    | 64                       | 64                  | 4                   | 2                |
| Vancomycin range    | 0.5–4                    | 0.5 to >256         | 0.25–1              |
| MIC<sub>50</sub>    | 1                        | 8                   | 0.5                 |
| MIC<sub>90</sub>    | 2                        | >256                | 0.5                 |
| Metronidazole range | 0.015 to >256            |
| MIC<sub>50</sub>    | 0.5                      |
| MIC<sub>90</sub>    | 1                        |

<sup>a</sup>Comprising 50 <i>Citrobacter</i> spp., 50 <i>Enterobacter</i> spp., 50 <i>E. coli</i>, 50 <i>Klebsiella</i> spp., 29 <i>Proteus</i> spp. and 21 <i>Salmonella</i> spp.

<sup>b</sup>Comprising 26 <i>Acinetobacter</i> spp., 42 <i>P. aeruginosa</i> and 8 <i>Pseudomonas</i> spp.

<sup>c</sup>Comprising 104 <i>S. aureus</i> and 50 coagulase-negative staphylococci.

<sup>d</sup>Comprising 50 <i>Enterococcus faecium</i>, 40 <i>E. faecalis</i> and 10 other named <i>Enterococcus</i> spp.

<sup>e</sup>Comprising 34 <i>Streptococcus</i> Lancefield A, 34 <i>Streptococcus</i> Lancefield B, 39 <i>S. pneumoniae</i> and 54 other α-haemolytic streptococci.

<sup>f</sup>Comprising 27 <i>N. gonorrhoeae</i> and 29 <i>Neisseria meningitidis</i>.

<sup>g</sup>Comprising 46 <i>H. influenzae</i> and 10 <i>Haemophilus parainfluenzae</i>.

<sup>h</sup>Comprising 32 <i>Clostridium</i> spp., 46 <i>Bacteroides</i> spp., 8 <i>Anaerococcus</i> spp., 7 <i>Peptophilus</i> spp., 3 <i>Finegaldia</i> spp. 3 <i>Peptostreptococcus</i> spp. and 2 <i>Parvimonas</i> spp.

<sup>i</sup>Comprising 10 <i>Moraxella catarrhalis</i>.

<sup>j</sup>Comprising 11 <i>Listeria monocytogenes</i>, 9 <i>Listeria innocua</i> and 1 <i>Listeria ivanovii</i>.

<sup>k</sup>Comprising 7 <i>Pasteurella multocida</i> and 3 unidentified <i>Pasteurella</i> spp.

<sup>l</sup>Comprising 12 <i>Campylobacter jejuni</i>, 4 <i>Campylobacter coli</i>, 5 <i>Campylobacter fetus</i> and 1 <i>Campylobacter lari</i>.

<sup>m</sup>Only <i>E. faecalis</i> tested.
‘Essential agreement’ was defined as the percentage of cases where the MIC result by the test method was within one doubling dilution of that by the BSAC reference method among cases where both values were on-scale.

‘Potential agreement’ was defined as the percentage of cases where the MIC result by the test method was within one doubling dilution of the reference method or was off-scale by one method and potentially in agreement with the reference method (e.g. >32 mg/L by one method and 256 mg/L by the other). Either essential or potential agreement could appear higher because the denominators and the numerators both varied between the two measurements. Correlation between MICs by different methods was calculated using log2 MICs, with off-scale values excluded. Comparisons of performance were by $\chi^2$ test.

**Results and discussion**

*Performance of M.I.C.Evaluator and Etest Strips versus BSAC agar dilution*

A total of 1017 isolates and 18 strip types were tested, with 77 antibiotic/organism combinations represented. Essential agreement was achieved for 89.9% of tests with the M.I.C.Evaluator versus 89.5% for the Etest ($P > 0.05$) (Tables 2 and 3); potential agreement was achieved in 89.5% for the M.I.C.Evaluator versus 89.3% for the Etest ($P > 0.05$) (Tables 4 and 5). The proportions of strip-type/organism combinations for which various target levels of agreement with the agar dilution reference ($\geq 95\%$, $\geq 90\%$, $\geq 85\%$ etc.) was achieved did not differ significantly between the two strip types ($P > 0.05$) irrespective of the target criterion selected (Table 6).

It should be added that these ‘global’ agreement rates included antibiotic/organism combinations, e.g. cefotaxime and oxacillin against MRSA and benzylpenicillin against penicillinase-producing S. aureus, where MICs are notoriously fickle and would not ordinarily be determined. Agreement in these cases was poorer than for most other agents tested against staphylococci, and global agreement rates rose if they were excluded from the analysis (Tables 2 and 4).

Even for antibiotic/organism combinations where essential agreement was $< 90\%$, the results of the strip tests mostly correlated well with BSAC agar dilution (Tables 7 and 8). Exceptions, with poorer correlation ($r \leq 0.7$), were: (i) imipenem against Enterobacteriaceae, where a distortion arose owing to the behaviour of Proteaeae, as described below; (ii) cases where high-drug-content strips were tested against species groups where low-content strips were more appropriate, as with cefotaxime against Neisseria spp.; and (iii)—most especially—(Tables 7 and 8), cases where MICs by the reference method were clustered over four or fewer drug dilutions, i.e. over a scarcely wider collective range than the $\pm 1$ doubling dilution range conventionally accepted as experimental variation when the MIC for a single organism is repeatedly determined by the same method.

In general, agreement between the two strip tests and the BSAC reference method was best for non-fastidious organisms, including staphylococci, enterococci, Enterobacteriaceae and non-fermenters, and for members of the genera Moraxella, Listeria, Pasteurella and Campylobacter (Tables 2–5). For these organisms, and taking all antibiotics combined, there was 89% essential agreement with the BSAC reference for the

**Table 2.** ‘Essential agreement’ (%) between M.I.C.Evaluator (M.I.C.E) or Etest and agar dilution ($\pm 1$ doubling dilution*), counting only on-scale values; non-fastidious species

|                | Enterobacteriaceae | Non-fermenters | Staphylococci | Enterococci |
|----------------|--------------------|----------------|--------------|-------------|
| M.I.C.E        | Etest              | M.I.C.E        | Etest        | M.I.C.E     | Etest  |
| Amoxicillin/clavulanic acid | 86.9 | 91.3 |                |             |        |
| Amoxicillin    | 87.3               | 88.7           |              |             |        |
| Ampicillin     | 87.6               | 87.6           |              |             |        |
| Cefotaxime 32  | 92.7               | 87.6           |              |             |        |
| Cefotaxime 256 | 91.1               | 85.8           |              |             |        |
| Ciprofloxacin  | 95.8               | 96.2           | 100          | 100         |        |
| Erythromycin   | 93.7               | 93.6           | 93.5         | 93.5        |        |
| Gentamicin 256 | 91.6               | 93.6           | 93.5         | 93.5        |        |
| Gentamicin 1024| 94.9               | 91.8           | 95.3         | 93.8        |        |
| Imipenem       | 81.7               | 82.5           | 96           | 88          |        |
| Levofloxacin   | 94.3               | 92.1           |              |             |        |
| Linezolid      | 99.3               | 99.3           |              |             |        |
| Oxacillin      | 83.2*              | 74.7*          |              |             |        |
| Penicillin G 32| 87.2*              | 80.2*          |              |             |        |
| Penicillin G 256| 83.9*             | 83*            |              |             |        |
| Tetracycline   | 98.7               | 98.7           |              |             |        |
| Vancomycin     | 95.6               | 96.2           | 93.3         | 93.3        |        |
| Overall        | 90.4               | 89.7           | 96.2         | 93.8        |        |
| Excluding asterisked values | 97.0 | 97.8 |                  |             |        |

*aOnly E. faecalis tested.*
### Table 3. ‘Essential agreement’ (%) between M.I.C.Evaluator (M.I.C.E) or Etest and agar dilution (+1 doubling dilution), counting only on-scale values: fastidious species

|                      | Streptococci | Neisseria | Haemophilus | Anaerobes | Moraxella | Listeria | Pasteurella | Campylobacter |
|----------------------|--------------|-----------|-------------|-----------|-----------|----------|-------------|---------------|
|                      | M.I.C.E | Etest | M.I.C.E | Etest | M.I.C.E | Etest | M.I.C.E | Etest | M.I.C.E | Etest | M.I.C.E | Etest | M.I.C.E | Etest |
| Amoxicillin/clavulanic acid |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| Amoxicillin          | 92.7     | 84.1    | 59.4    | 42.4    | 95.2     | 100     | 95.2     | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     |
| Ampicillin           | 94.2     | 94.2    | 83.9    | 85.7    | 95.2     | 100     | 95.2     | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     |
| Cefotaxime 32        | 92       | 88.3    | 83.9    | 85.7    | 95.2     | 100     | 95.2     | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     |
| Cefotaxime 256       | 88.3     | 82.1    | 57.1    | 71.4    | 88.5     | 100     | 95.2     | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     |
| Ciprofloxacin        | 96.2     | 95.5    | 98      | 98      | 88       | 100     | 95.2     | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     |
| Erythromycin         | 60.9     | 66.2    | 97.6    | 95.2    | 100      | 95.4    | 90.9     | 90.9    | 100     | 95.4    | 90.9     | 90.9    | 100     | 95.4    | 90.9    |
| Gentamicin 256       | 88.9     | 93.2    | 54.8    | 58      | 46.5     | 48.8    | 90       | 90      | 100     | 100     | 100     | 100     | 100     | 100     | 100     |
| Gentamicin 1024      | 81.6     | 94.4    | 97.5    | 96.9    | 100      | 97.7    | 97.5     | 97.7    | 100     | 100     | 100     | 100     | 100     | 100     | 100     |
| Imipenem             | 89.5     | 71.1    | 84.1    | 81.3    | 84.1     | 100     | 95.2     | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     |
| Levofloxacin         | 73       | 77.9    | 84.1    | 81.3    | 84.1     | 100     | 95.2     | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     | 90.4    | 100     |
| Overall              | 84.4     | 82.9    | 81.2    | 85.6    | 76.9     | 74.4    | 79.7     | 80.4    | 100     | 97.6    | 97.7    | 95.0    | 95.0    | 95.5    | 95.5    | 95.5    | 95.5    |

### Table 4. ‘Potential agreement’ (%) between M.I.C.Evaluator (M.I.C.E) or Etest Strips and agar dilution (+1 doubling dilution), counting all potential agreements as agreement: non-fastidious species

|                      | Enterobacteriaceae | Non-fermenters | Staphylococci | Enterococci |
|----------------------|---------------------|----------------|---------------|-------------|
|                      | M.I.C.E | Etest | M.I.C.E | Etest | M.I.C.E | Etest | M.I.C.E | Etest |
| Amoxicillin/clavulanic acid |         |         |         |         |         |         |         |         |
| Amoxicillin          | 78.3     | 83.7    | 70.5    | 79.4    | 95.2     | 100     | 95.2     | 100     |
| Ampicillin           | 91.1     | 91.1    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Cefotaxime 32        | 89.9     | 84.1    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Cefotaxime 256       | 89.1     | 84.8    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Ciprofloxacin        | 95.7     | 94.1    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Erythromycin         | 77.9     | 94.9    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Gentamicin 256       | 91.4     | 93     | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Gentamicin 1024      | 94.9     | 91.8    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Imipenem             | 89.1     | 97.7    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Levofloxacin         | 88.3     | 84.5    | 77.9    | 94.9    | 77.9     | 94.9    | 77.9     | 94.9    |
| Overall              | 89.0     | 87.9    | 73.8    | 91.7    | 90.0     | 90.0    | 90.0     | 90.0    |

*Poorer potential than essential agreement (see Table 2) for levofloxacin versus staphylococci reflects 25 isolates with MICs of 16 mg/L by agar dilution, but >32 mg/L by both strip methods.

*Only E. faecalis tested.*
M.I.C.Evaluator and Etest versus BSAC MIC tests

Table 5. ‘Potential agreement’ (%) between M.I.C.Evaluator (M.I.C.E) or Etest Strips and agar dilution (+1 doubling dilution), counting all potential agreements as agreement: fastidious species

| Streptococci | Neisseria | Haemophilus | Anaerobes | Moraxella | Listeria | Pasteurella | Campylobacter |
|--------------|-----------|-------------|-----------|-----------|----------|-------------|---------------|
| M.I.C.E     | Etest     | M.I.C.E     | Etest     | M.I.C.E   | Etest    | M.I.C.E     | Etest         |
| Amoxicillin| 72        | 62.7        | 88.1      | 91.8      | 100      | 100         | 90.9          |
| Amoxicillin/clavulanic acid | 56.8 | 43 |
| Amoxicillin | 93.2      | 85.2        | 83.9      | 85.7      | 72.1     | 55.8        | 95.4          |
| Ampicillin  | 94.4      | 94.4        | 82.1      | 82.1      | 83.7     | 55.8        | 100           |
| Cefotaxime 32 | 92  | 88.3        | 82.1      | 87.5      | 76.7     | 88.3        | 90.9          |
| Cefotaxime 256 | 92 | 88.3       | 82.1      | 87.5      | 76.7     | 88.3        | 90.9          |
| Ciprofloxacin | 94.4 | 93.2        | 94.6      | 94.6      | 58.1     | 86          | 90.9          |
| Erythromycin| 63.1      | 68          | 97.6      | 93        | 100      | 95.4        | 90.9          |
| Gentamicin 256 | 88.9 | 93.2        | 94.6      | 94.6      | 58.1     | 86          | 90.9          |
| Levofloxacin| 97.8      | 96.9        | 97.7      | 97.7      | 97.7     | 95.4        | 90.9          |
| Linezolid   | 98.1      | 98.7        | 97.7      | 97.7      | 97.7     | 95.4        | 90.9          |
| Oxacillin   | 89.5      | 71.1        | 97.7      | 97.7      | 97.7     | 95.4        | 90.9          |
| Penicillin G 32 | 61.9 | 68          | 97.2      | 89.2      | 84.5     | 89          | 90.9          |
| Penicillin G 256 | 63.8 | 74.8        | 80.3      | 80.3      | 91.8     | 90          | 90.9          |
| Tetracycline| 76.6      | 76.6        | 98.2      | 96.4      | 97.7     | 95.4        | 90.9          |
| Vancomycin  | 73        | 77.9        | 97.7      | 97.7      | 97.7     | 95.4        | 90.9          |
| Metronidazole | 84.5 | 81.8        |           |           |          |             |               |
| Overall     | 84.7      | 83.4        | 84.9      | 88.0      | 75.9     | 72.7        | 83.6          |

Table 6. Agreement of MICs by strip methods with BSAC agar dilution, based on 77 strip/organism combinations

| M.I.C.Evaluator Strips and >87% for Etest Strips. Essential agreement was less good, at between 83% and 88% for all antibiotics combined, for anaerobes, Neisseria spp. and Streptococcus spp. and poorest, at 72.7%–75.9%, for H. influenzae. Agreement rates for H. influenzae were lowered by imipenem (which would rarely be tested against the species) and, more importantly, by amoxicillin and ampicillin. Many of the underlying disagreements with these latter drugs were for β-lactamase-positive isolates found highly resistant by all methods, but with substantially higher MICs by one method than another (e.g. agar dilution amoxicillin MIC 256 mg/L, but only 32 mg/L by the strip methods). MICs are rarely determined for such unequivocal isolates in clinical practice. Among amoxicillin-borderline H. influenzae isolates—where strip tests are more likely to be used—four of seven with amoxicillin MICs of 1 mg/L by agar dilution (i.e. just susceptible) were found susceptible using both strip types, with MICs of 0.5–1 mg/L, whereas three proved resistant, with MICs of 1.5–2 mg/L; among five with agar dilution MICs of 2–4 mg/L (i.e. just resistant), one was found susceptible to amoxicillin with both strip types, with MICs of 0.5–1 mg/L, one was resistant, with MICs of 8 mg/L (Etest) and 16 mg/L (M.I.C.Evaluator) and three gave mixed results, with MICs of 1 mg/L with one strip and 1.5 mg/L with the other. Tristram similarly tested M.I.C. Evaluator ampicillin strips against H. influenzae, though taking CLSI broth microdilution as a reference, and found 90% agreement (+1 doubling dilution) for ampicillin-susceptible isolates and those with β-lactamase-negative ampicillin resistance, versus only 65%–75% for those with β-lactamase.

Tests with penicillin against streptococci also deserve comment, being another case where gradient MIC test strips are particularly useful—e.g. for pneumococci with borderline resistance and for endocarditis isolates—but where agreement appeared rather low, at 62%–68%. These figures, however, exaggerate the ‘disagreements’, which largely concerned β-haemolytic streptococci with penicillin MICs of ≤0.008 mg/L by agar dilution and 0.023 (rounded to 0.03) mg/L by the strip methods—a difference of no practical consequence. Penicillin MICs by Etest and M.I.C.Evaluator Strips for pneumococci with agar dilution MICs from 0.12 to 2 mg/L are shown in Table 9, indicating perfect essential agreement for the two high-content strips, though rather poorer for the low-content M.I.C.Evaluator Strip.

Agreement rates for Enterobacteriaceae were poorer with imipenem than with other strip types. This was largely owing to the strips giving ~8-fold lower imipenem MICs for Proteus spp.
Table 7. Correlation coefficients between MICs determined by strip methods and those found by BSAC agar dilution: non-fastidious species

|                          | Enterobacteriaceae | Non-fermenters | Staphylococci | Enterococci |
|--------------------------|--------------------|----------------|---------------|-------------|
|                          | M.I.C. Etest       | M.I.C. Etest   | M.I.C. Etest  | M.I.C. Etest |
| Amoxicillin/clavulanic acid | 0.949              | 0.891          |               |             |
| Amoxicillin              | 0.927              | 0.94           |               |             |
| Ampicillin               | 0.906              | 0.898          |               |             |
| Cefotaxime 32            | 0.951              | 0.939          |               |             |
| Cefotaxime 256           | 0.962              | 0.957          |               |             |
| Ciprofloxacin            | 0.924              | 0.916          | 0.972         | 0.971       |
| Erythromycin             | 0.906              | 0.913          |               |             |
| Gentamicin 256           | 0.939              | 0.916          | 0.946         | 0.944       |
| Gentamicin 1024          | 0.957              | 0.949          | 0.964         | 0.961       |
| Imipenem                 | 0.562<sup>a</sup>  | 0.427<sup>a</sup> | 0.951 | 0.922 |
| Levofloxacin             | 0.951              | 0.95           |               |             |
| Linezolid                | 0.9                | 0.9            | 0.947         | 0.941       |
| Oxacillin                | 0.907              | 0.906          |               |             |
| Penicillin G 32          | 0.9                | 0.9            |               |             |
| Penicillin G 256         | 0.958              | 0.958          |               |             |
| Tetracycline             | 0.962              | 0.975          |               |             |
| Vancomycin               | 0.655<sup>b</sup>  | 0.7            | 0.955         | 0.969       |
| Overall                  | 0.903              | 0.878          | 0.958         | 0.949       |

M.I.C.E, M.I.C.Evaluator.

<sup>a</sup>Most of the less-susceptible organisms were Proteaeae, for which there was poor correlation between BSAC and strip methods.

<sup>b</sup>Poor correlation (≤ 0.7) explained by >90% agar dilution MICs being spread over ≤4 dilutions.

Table 8. Correlation coefficients between MICs determined by strip methods and those found by BSAC agar dilution: fastidious species

|                          | Streptococci | Neisseria | Haemophilus | Anaerobes | Moraxella | Listeria | Pasteurella | Campylobacter |
|--------------------------|--------------|-----------|-------------|-----------|-----------|----------|-------------|---------------|
|                          | M.I.C. Etest | M.I.C. Etest | M.I.C. Etest | M.I.C. Etest | M.I.C. Etest | M.I.C. Etest | M.I.C. Etest | M.I.C. Etest |
| Amoxicillin/clavulanic acid | 0.7         | 0.6       | 0.9         | 0.9       | 0.9       | 0.939    |               |               |
| Amoxicillin              | 0.926        | 0.915     | 0.762       | 0.653     |           |          |             |               |
| Ampicillin               | 0.937        | 0.942     | 0.9         | 0.9       | 0.895     | 0.853    |             |               |
| Cefotaxime 32            | 0.9          | 0.9       | 0.655       | 0.717     | 0.9       | 0.9       |             |               |
| Cefotaxime 256           | 0.9          | 0.9       | 0.5         | 0.6       | 0.8       | 0.9       |             |               |
| Ciprofloxacin            | 0.78         | 0.757     | 0.920       | 0.939     | 0.6<sup>a</sup> | 0.6<sup>a</sup> | 0.931 | 0.95 |
| Erythromycin             | 0.763        | 0.838     | 0.9         | 0.9       |          |          | 0.445<sup>a</sup> | 0.488<sup>a</sup> |
| Gentamicin 256           | 0.707        | 0.719     |             |           |          |          | 0.681<sup>a</sup> | 0.677<sup>a</sup> |
| Gentamicin 1024          | 0.703        | 0.763     |             |           |          |          |               |               |
| Imipenem                 | 0.989        | 0.991     | 0.7<sup>a</sup> | 0.6<sup>a</sup> | 0.793 | 0.724 |               |               |
| Levofloxacin             | 0.522<sup>a</sup> | 0.47<sup>a</sup> | 0.9          | 0.9       |           | |               |               |
| Linezolid                | 0.656<sup>a</sup> | 0.654<sup>a</sup> |               |           |          |          |               |               |
| Oxacillin                | 0.916        | 0.934     |             |           |          |          |               |               |
| Penicillin G 32          | 0.9          | 0.9       | 0.917       | 0.916     | 0.946     | 0.939    | 0.936       | 0.948         |
| Penicillin G 256         | 0.9          | 0.9       | 0.930       | 0.938     | 0.946     | 0.95     | 0.798       | 0.867         |
| Tetracycline             | 0.919        | 0.958     | 0.9         | 0.9       | 0.949     | 0.928    |             |               |
| Vancomycin               | 0.285<sup>a</sup> | 0.386<sup>a</sup> |               |           |          |          |               |               |
| Metronidazole            |               |           |             |           |          |          | 0.56        | 0.5           |

M.I.C.E, M.I.C.Evaluator.

<sup>a</sup>Poor correlation (≤ 0.7) explained by >90% agar dilution MICs being spread over ≤4 dilutions.
Agreement was far better for other species, as shown by the geometric mean MIC values (Table 10); these are a valid summary parameter since the MICs were normally distributed and unimodal within each species. It is possible that higher MICs by agar dilution for Proteaeae reflected the inclusion of p-nitrophenyl glycerol to prevent swarming, but we can find no published assertion that this compound interferes with the activity of imipenem; moreover, broth dilution MICs, e.g. by the CLSI method, are similarly high for Proteus spp. with no anti-swarming agent present. An alternative explanation, perhaps more likely, is simply that in seeking to ignore swarming into the zone of inhibition, the recorder tends to underestimate the real MICs.

Other specific concerns could be identified, e.g. there were 19 enterococci with gentamicin MICs of 256–512 mg/L, counting as high-level resistant by BSAC criteria, but nine of these gave MICs ≤ 128 mg/L by Etest, counting as susceptible, and five did so by M.I.C.Evaluator. Only 2/34 enterococci with gentamicin MICs of ≥ 1024 mg/L gave MICs ≤ 128 mg/L by the strip methods, each of them with both products, whilst 2/51 enterococci with a gentamicin MIC ≤ 128 mg/L by agar dilution appeared resistant, with MICs ≥ 256 mg/L in strip tests, one with the Etest only and one with both products.

\section*{Agreement between M.I.C.Evaluator and Etest Strip results}

Concordance between the M.I.C.Evaluator Strip and Etest Strip results was excellent, with >95% potential agreement (±1 dilution) for 54 of 77 strip/organism combinations and 90%–95% for another 12 (Table 11). These proportions are significantly better than between either strip type and agar dilution (P<0.01, χ² test; compare Table 6). The only antibiotic/organism combinations with <85% agreement between the two strips were amoxicillin/clavulanic acid against both Enterobacteriaceae (70.6%) and Haemophilus spp. (81.3%) and both ampicillin and amoxicillin against Haemophilus spp. (67.4% and 68.1%, respectively). In the case of ampicillin and amoxicillin, virtually
all the underlying disagreements related to strains that were resistant by all three methods, while most (43/75) >1 dilution disagreements for amoxicillin/clavulanic acid against Enterobacteriaceae related to Enterobacter and Citrobacter spp., both of which are inherently resistant to the drug combination.

Conclusions

Etests are in widespread use for custom MIC determinations in diagnostic laboratories. They have been calibrated to give equivalent MICs to those found by CLSI methodology using Mueller–Hinton agar, and there is a voluminous literature to support their accuracy when used in this manner. The manufacturer (AB Biodisk at the time of this study, now bioMérieux) does not advocate their use on other media, and there are no substantial performance studies on Iso-Sensitest agar, which is the standard medium for the BSAC dilution method. The BSAC nevertheless states that, in general, Etests can be used on Iso-Sensitest agar, with BSAC/EUCAST breakpoints, so long as inocula equivalent to a 0.5 McFarland are used.

The M.I.C.Evaluator Strip has been developed as an alternative strip-based MIC method, which the manufacturer indicates to be suitable for use on either Mueller–Hinton or Iso-Sensitest agar. As with the Etest Strip, it comprises a laminated plastic support carrying a double series of antibiotic-impregnated droplets of diminishing content. Once placed on agar, the strip rapidly releases the antibiotic, delivering a stable gradient. After incubation, the MIC can be read off against a printed scale. The present data show that, used on Iso-Sensitest agar, both M.I.C.Evaluator and Etest Strips gave essentially equivalent results to one another and acceptable agreement with BSAC agar dilution.

Acknowledgements

We are grateful to colleagues at the Centre for Infections for contributing isolates to the study.

Funding

The work was supported by Thermo Fisher Scientific, Basingstoke, UK.

Transparency declarations

D. M. L. has received conference support from Thermo Fisher Scientific, bioMérieux and AB Biodisk, he has received grants and conference support from numerous pharmaceutical companies, he holds shares in AstraZeneca, Merck, Pfizer, Dechra and GlaxoSmithKline and, as Enduring Attorney, managed further holdings in GlaxoSmithKline and Eco Animal Health. D. M. L. is employed by the HPA and sits on the BSAC’s Working Party on Susceptibility Testing, and is influenced by their views. J. C. is an employee of Thermo Fisher Scientific. Other authors: none to declare.

References

1. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in Staphylococcus aureus. Emerg Infect Dis 2001; 7: 327–32.
2 Sakoulas G, Moise-Broder PA, Schentag J et al. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant Staphylococcus aureus bacteremia. J Clin Microbiol 2004; 42: 2398–402.
3 Etest Technical Manual. 2009. http://www.abbiodisk.com/pdf/etm_index.htm (10 December 2009, date last accessed).
4 Use of Gradient Tests for Determination of MICs by BSAC Methodology. http://www.bsac.org.uk/_db/_documents/etest_may_2007.pdf (3 June 2010, date last accessed).
5 Andrews JM. BSAC standardized disc susceptibility testing method (version 6). J Antimicrob Chemother 2007; 60: 20–41.
6 Tristram SG. A comparison of Etest, M.I.C.Evaluator strips and CLSI broth microdilution for determining β-lactam antimicrobial susceptibility in Haemophilus influenzae. J Antimicrob Chemother 2008; 62: 1464–6.
7 Hoogkamp-Korstanje JA, Roelofs-Willemse J. Antimicrobial resistance in Gram-negative bacteria from Intensive Care Units and Urology Services. A nationwide study in The Netherlands 1995–2000. Int J Antimicrob Agents 2003; 21: 547–56.