Comparison of the E test with a conventional agar dilution method in evaluating the in vitro activity of moxifloxacin

J. M. Andrews and R. Wise

D Department of Antimicrobial Chemotherapy, City Hospital NHS Trust, Dudley Road, Birmingham B18 7QH, UK

Tel: +44-121-554-3801; Fax: +44-121-551-7763.

Sir,

The E test was introduced in 1988 as an alternative method of determining MICs. Although it is used principally to confirm resistance, rather than as a primary susceptibility testing method, the pharmaceutical industry is increasingly adopting it as a means of evaluating new antimicrobial agents; these data being used to confirm or suggest tentative MIC breakpoints. In the present study we have compared the MICs of moxifloxacin determined by two methodologies: according to recommendations of the British Society for Antimicrobial Chemotherapy (BSAC), and with the E test adapted for use in the UK.

Moxifloxacin was provided by Bayer AG (Wuppertal, Germany). The 149 clinical isolates studied comprised 11 strains of Bacteroides fragilis, 17 of anaerobic streptococci, 12 of H. hemophilus influenzae, 11 of Streptococcus pneumoniae, 12 of M. orallis catalarrhalis, 52 of Enterobacteriaceae, 12 of Pseudomonas aeruginosa and 22 of staphylococci; 11 NCTC/ATCC strains were included as controls.

The media used were those recommended by the BSAC, i.e. Iso-Sensitest agar (ISTA; Oxoid, Basingstoke, UK) for facultative anaerobes and Wilkins & Chalgren agar (WCA; Oxoid) for anaerobes. Where appropriate, ISTA was supplemented with 5% defibrinated horse blood (DFB) and/or β-nicotinamide adenine dinucleotide (Sigma Diagnostics, Poole, UK) in a concentration of 20 mg/L and WCA was supplemented with 5% DFB. The inoculum for the agar dilution method was 10⁴ cfu/spot, and that for the E test was a suspension with a density equivalent to that of a 0.5 McFarland standard. Incubation was at 35–37°C for 18–20 h in air, except in the case of fastidious organisms, for which the atmosphere was enriched with 4–6% CO₂, and anaerobes, which were incubated in an anaerobic cabinet.

Susceptibility categories were assigned according to the following tentative MIC breakpoints: susceptible ≤1 mg/L and resistant ≥2 mg/L; the categories for P. aeruginosa were susceptible ≤1 mg/L, intermediate susceptibility 2–4 mg/L and resistant ≥8 mg/L.

The data were analysed by the Mann–Whitney test with a standard statistical package (GraphPad Instat, San Diego, CA, USA). A standard statistical package was also used to compare the data for the 52 Enterobacteriaceae isolates and the 52 Enterobacteriaceae isolates were analysed firstly, according to frequency distribution based on the E test gradient and secondly, according to the conventional doubling dilution range based on a unit of 1 mg/L.

The MICs for the control strains, as determined by the E test, were invariably lower than those obtained with the agar dilution method, although the differences were within two two-fold dilutions, except for the two P. aeruginosa strains, ATCC 27853 and NCTC 10662 (MICs of 0.75 and 0.38 mg/L, respectively, with the E test and 2 mg/L in both cases with the agar dilution method).

When the cumulative percentages of MICs determined by the two methods were analysed, taking account of both the gradient and agar dilution MIC ranges, the differences in both cases were statistically significant (P < 0.0001 and 0.002, respectively). Statistically significant differences were also observed for four groups of isolates: the Entero-bacteriaceae (P = 0.0001), P. aeruginosa (P = 0.002), H. influenzae (P = 0.04) and anaerobic streptococci (P = 0.01). The differences between the results for the staphylococci and the S. pneumoniae isolates did not quite reach statistical significance (P = 0.05).

Except for one strain of B. fragilis (for which the MICs obtained with the E test and agar dilution method were 1.5 and 1 mg/L, respectively), the MICs for all isolates determined by the E test were consistently lower than those determined by the agar dilution method. On 11 occasions these differences were associated with discrepancies in the assigned susceptibility categories. Based on an MIC breakpoint of 1 mg/L, one strain of B. fragilis (referred to above) was classified as susceptible by the agar dilution method and resistant by the E test, one strain of S. pneumoniae was classified as susceptible by the E test and resistant by the agar dilution method (MICs of 1 and 4 mg/L, respectively) and four staphylococci were classified as susceptible by the E test (MICs of 0.75–1 mg/L) and resistant by the agar dilution method (MICs of 2 mg/L). Similarly, the MICs for five P. aeruginosa strains were between 0.38 and 0.75 mg/L as determined by the E test, which placed them in the susceptible category, whereas the MICs obtained with the agar dilution method were 2 mg/L, which placed them in the intermediate susceptibility category.
Differences between results obtained with the E test and the agar dilution method have been reported previously. In the present study we have demonstrated that different techniques used to determine MICs do not necessarily yield the same results, that the differences between the results may be statistically significant, that differences vary depending on the genus of bacteria being studied and, perhaps most importantly, that isolates shown to have reduced susceptibilities by the agar dilution method might be classified as susceptible when their MICs are determined by the E test. The implications of these findings are four-fold: incorrect identification of clinical isolates as susceptible by the E test, a situation that could potentially have adverse effects on response to treatment; acquisition of data that are used to confirm tentative MIC breakpoints by a methodology not used to derive the breakpoints in the first place; detection of subtle changes in susceptibilities by a method (the E test) that is not sufficiently sensitive for this purpose; and the appropriateness of combining MIC data obtained using the agar dilution method with those obtained using the E test in multicentre surveillance studies designed to detect differences in the incidences of antimicrobial resistance.

References

