Correspondence

imipenem-free medium (Mueller–Hinton agar) and the Etest was repeated on both imipenem-free Mueller–Hinton agar and Mueller–Hinton agar containing imipenem at a concentration equivalent to 0.25 × MIC for the parent strain. The MICs for the organisms were invariably the same as those for the parent strains, irrespective of the medium. Also in common with the parent strains, discrete colonies were observed within the zones of inhibition. It was therefore decided to disregard the presence of these colonies when recording the endpoint of susceptibility testing with the Etest.

The correlation (MICs differing by no more than one two-fold dilution) for each comparison of the methods of determining MICs was as follows: MBD versus AD, 92%; MBD versus Etest, 92%; and AD versus Etest, 94%. When the susceptibility categories determined by the three methods were compared, the numbers of minor errors were as follows: MBD versus AD, eight; MBD versus Etest, three; and AD versus Etest, 11. There were no major or very major errors. The relatively high incidence of minor errors might be explained by the large number of strains for which the MICs of imipenem were close to the breakpoint of intermediate susceptibility (8 mg/L).

The MICs for each of the three control strains, as determined by the Etest method, were within one two-fold dilution throughout the 8 month study period, thereby confirming that the imipenem Etest strips are highly stable.

In conclusion, the results of the three methods of determining the MICs of imipenem for A. baumannii isolates agreed closely (≥92% correlation), and all of these techniques can therefore be used with confidence. However, the reliability, simplicity and speed of the Etest make it particularly well-suited to this purpose and give it an advantage over the other techniques. Further studies to ascertain the significance and clinical relevance of colonies growing within the zones of inhibition around the Etest strips are currently under way.

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References


Failure to detect significant in-vitro activity of artemether and β-arteether against bacteria and fungi by an agar dilution test

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Sir,
The use of artemisinin and its derivatives to treat patients with malaria has increased during the past few years. However, much of this use has not been supported by robust pharmacokinetic data, and it is only recently that significant effort has been made to ensure that some of these agents meet international standards.

The detection of artemisinin-type compounds in biological fluids poses practical problems. Currently available assay methods, which include the ‘gold standard’, a high-

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performance liquid chromatography electrochemical detection method (HPLC-ECD), and a semi-quantitative bioassay with *Plasmodium falciparum* as the test organism, are laborious, expensive and can be carried out efficiently in only a few laboratories throughout the world. A simple and inexpensive method for estimating the concentrations of these drugs in biological fluids would obviously be of great benefit. The present study was therefore undertaken to investigate whether a microbiological assay method that measures total drug activity might be appropriate for this purpose; agar plates are readily available, the methodology is standardized and the test is relatively cheap. As the success of such an assay depends on identifying a susceptible test organism, we determined the in-vitro activity of artemisinin against a wide range of candidate bacteria and yeasts. In addition, because ferrohaem-induced decomposition is needed to activate artemisinin-type compounds in the malaria parasite, we attempted to determine if the addition of cytochrome, iron or haemin to the culture medium increased the sensitivity of the assay.

Artemether and β-arteether were supplied by Sapec SA, Barbengo, Switzerland. Stock solutions (184.32 mg of each compound in 20 mL of methanol) were prepared immediately before testing and then diluted in acetone to give final concentrations of 576 mg/L. Cytochrome c (Sigma Chemicals, Milan, Italy) and iron (Aldridge Chemical Co., Milan, Italy) were dissolved in distilled water just before each experiment. Haemin (Sigma) was dissolved in acetic acid to give a final concentration of 5%. The final pH of the cytochrome c was 7.7 and that of iron was 2.9.

The 86 bacterial strains and 68 of the 72 yeast strains were clinical isolates which had been held at −70°C in our culture collection. The remaining four yeasts included *Candida albicans ATCC 90028, Candida glabrata ATCC 90030, Candida krusei ATCC 6258* and *Candida parapsilosis ATCC 9018*. The bacteria tested belonged to the following families: Enterobacteriaceae and Aeromonadaceae (22 strains), Pseuodomadaceae (10), Neisseriaceae (seven), non-fermenting aerobic Gram-negative bacilli (AGNB) (six), Gram-positive cocci (22) and Gram-positive aerobic bacilli (19). The fungal strains were as follows: *Candida* spp. (27), *C. albicans* (five), *C. glabrata* (three), *Candida guilliermondii* (one), *C. krusei* (one), *Candida kefyr* (three), *Candida lusitaniae* (one), *C. parapsilosis* (11), *Candida tropicalis* (two), other yeasts (47), *Cryptococcus neoformans* (19), *Hansenula anomala* (one), *Hanseniaspora uvarum* (one), *Saccharomyces cerevisiae* (16), *Torulopsis* spp. (two); *Torulopsis candida* (one), *Torulopsis inconspicua* (two) and *Trichosporon capitatum* (five).

The MICs of artemether and β-arteether were determined by an agar dilution method. Columbia agar base (Unipath Ltd, Basingstoke, UK) supplemented with 5% blood was used for Enterobacteriaceae, AGNB, Gram-positive bacilli, staphylococci and yeasts; chocolate agar (GC agar base, Unipath Ltd) supplemented with 1% Isovitalex (BBL, Cockeysville, MD, USA) was used for Neisseriaceae, *Haemophilus* spp. and streptococci. The final drug concentrations ranged from 32 mg/L to 512 mg/L. The bacterial inoculum was prepared from an 18 h culture (or 72 h for *Nocardia* spp. and *C. neoformans*) on solid media; colonies were suspended in nutrient broth to give 10^12 cfu/mL and these suspensions were then diluted 1 in 100. A Steers multipoint inoculator was used to deliver 10^−10^5 cfu of each organism to the agar surface. The plates were incubated at 35°C for 18 h in an aerobic atmosphere for aerobic strains (72 h for *Nocardia* spp.), for 18 h at 35°C in an atmosphere enriched with 10% CO_2 for organisms inoculated on to chocolate agar and at 30°C for 48 h (72 h for *C. neoformans*) in an aerobic atmosphere for yeasts. The MIC was defined as the lowest concentration of each drug that allowed no visible growth. All tests were carried out in duplicate.

Both artemether and β-arteether exhibited, at best, only marginal activities against the bacteria and fungi tested and there were no significant differences between the two drugs in terms of the MICs. The bacteria for which the activities of these agents were marginal (MICs = 64–256 mg/L) included certain Gram-positive organisms, such as *Nocardia asteroides, Oerskovia* spp. and some corynebacteria, aquatic strains (e.g. *Pseudomonas vesiculosus*) and bacteria that are part of the normal flora but that are occasionally pathogenic, including *Neisseria lactamica, Neisseria subflava, Haemophilus influenzae, Streptococcus agalactiae* and *Streptococcus pneumoniae*. For the remaining strains, the MICs were >512 mg/L. The activities against the yeast tested were equally poor, irrespective of whether or not the medium was supplemented with cytochrome c or iron (range of MICs = 64–>512 mg/L and 32–>512 mg/L, respectively): the inclusion of haemin in acetic acid in the medium did not support fungal growth.

The methodology for determining MICs for yeasts has not yet been standardized. Although the National Committee for Clinical Laboratory Standards (NCCLS) recently approved an operational procedure for determining MICs with a broth dilution test, this technique could not be used in the present study because of difficulties associated with the drugs tested and the media supplements.

An agar dilution method with bacteria and yeasts as the test organisms has not been shown here to be suitable for assaying artemisinin-type compounds in biological fluids. Artemether and β-arteether were, at best, marginally active at concentrations well in excess of both therapeutic concentrations and those that can be detected with the *P. falciparum* bioassay. The IC_{50} of β-artemether and β-arteether were 1.74 nM (range 1.34–1.81 nM) and 1.61 nM (range 1.57–1.92 nM), respectively. The lower limit of detection with the *P. falciparum* bioassay is 3.5 ng/mL (D. Kyle, personal communication).

Although the agar dilution method was not shown to be suitable for monitoring drug concentrations, this study, to our knowledge, is the first attempt to investigate the activi-
tary of artemisinin-type compounds against bacteria and fungi.

References


The failure of a once-daily vancomycin dosing regimen in patients with normal renal function

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Sir,

Although the use of a once-daily vancomycin dosing regimen in patients with normal renal function has been reported previously, the efficacy and safety of this strategy remain unconfirmed. As a quality performance project, our hospital developed a once-daily vancomycin dosing policy, based on the administration of a single daily dose of 20 mg/kg to patients with creatinine clearances of >35 mL/min. I describe here two patients with normal renal function in whom clinical and microbiological failure were demonstrated whilst they were receiving this regimen. The patients were cured of their infections only when the therapy was switched to the conventional 12 hourly dosing interval.

The first patient was a 60 year old otherwise healthy male who had undergone a vascular surgical procedure. On the fifth hospital day, he developed a fever of 38.1°C and an elevated white blood cell (WBC) count; a source of infection was not immediately obvious. Over the next few days, his temperature continued to rise and the insertion site of the right subclavian triple-lumen catheter became erythematous and discharged cloudy fluid. Both the catheter tip, following removal, and a swab of the insertion site were cultured, and, when a Gram stain of purulent fluid obtained from the insertion site revealed large numbers of Gram-positive cocci, treatment with vancomycin 1 g once daily was initiated (the patient’s renal function being normal). Semi-quantitative culture of the catheter tip subsequently yielded >15 colonies of a strain of Enterococcus faecalis, the same organism also being isolated from the insertion site. Five days after starting therapy, the vancomycin trough concentration was 2.5 mg/L and the dosage of vancomycin was increased to 1.25 g daily. The dosage was increased further to 1.5 g daily on the next day when the trough concentration was reported to be 3.8 mg/L. However, the patient’s temperature and WBC count continued to rise and he exhibited features of hyperdynamic sepsis. Intubation and mechanical ventilation became necessary because of progressive respiratory distress. Extensive diagnostic investigations failed to reveal an additional source of infection and, on the seventh day of vancomycin therapy, the dosage was changed to 1 g bd. A dramatic fall in the WBC count and temperature ensued and the patient was successfully extubated on day 11. Vancomycin was continued for a total of 14 days, the remainder of the patient’s hospital course being uneventful.

The second patient was a 48 year old male who had undergone an oesophagectomy for carcinoma of the lower third of the oesophagus. In the early post-operative period, he developed clinical features consistent with a right lower lobe pneumonia and a mini-bronchoalveolar lavage (BAL) and protected specimen brush (PSB) sampling were performed. These specimens yielded $6 \times 10^6$, $7 \times 10^5$ cfu/L respectively of a methicillin-susceptible strain of Staphylococcus aureus. As the patient was alleged to be allergic to penicillins, vancomycin 1.4 g once daily was commenced; his renal function was normal. A vancomycin trough concentration obtained 48 h later was only 4.1 g/L and the dosage was therefore increased, first to 1.6 g and then to 1.8 g once daily. However, the patient’s temperature continued to rise and a chest X-ray revealed bilateral lower lobe infiltrates. The BAL and PSB sampling were repeated, the cultures again yielding a methicillin-susceptible strain of S. aureus ($>1 \times 10^7$ and $1.5 \times 10^6$ cfu/L respectively). On the seventh day, the dosage of vancomycin was changed to 1 g bd. This was followed by a reduction in the volume of the patient’s respiratory secretions, normalization of the temperature and WBC count and eradication of the S.