Reduced susceptibility of multidrug-resistant
Acinetobacter baumannii to tigecycline in
combination with 1-(1-naphthylmethyl)-piperazine
is not a pH-dependent phenomenon

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Sir,

Acinetobacter baumannii has emerged as an important cause of
nosocomial infection in the immunocompromised and critically
ill. The organism frequently exhibits multidrug resistance with
only polymyxin derivatives and the glycylcycline antibiotic tige-
cycline retaining significant activity in vitro. Resistance to these
agents has been reported1 and in the case of tigecycline is
thought to be mediated by the AdeABC resistance-nodulation-
division efflux pump. Recently, we described a curious phenom-
phenon whereby strains of A. baumannii belonging to the
multidrug-resistant OXA-23 clone 1 appeared to decrease in sus-
cceptibility to tigecycline in the presence of the efflux pump
inhibitor 1-(1-naphthylmethyl)-piperazine (NMP). The converse
was observed when NMP was combined with doxycycline, tetra-
cycline or minocycline.2 In an attempt to explain this finding,
we considered whether low pH could contribute to this obser-
vation, as the activity of many tetracyclines is known to be
enhanced by pH3 and the preparation of NMP requires acidifica-
tion of the solvent (0.2 M HCl, pH 2). We were unable to detect
any significant changes in the pH of the medium when NMP
was added to Iso-Sensitest agar (Oxoid, Basingstoke, UK), pre-
sumably due to the low volume (640 mL of 10000 mg/L stock
solution per 100 mL of agar) and the buffering capacity of the
medium. We therefore proceeded to study the effect of directly
supplementing antimicrobial discs with NMP and the NMP
solvent. In disc diffusion tests, blunting of the zones of inhibi-
tion was observed when discs containing 64 µg of NMP were
placed 10 mm from 15 µg tigecycline discs (Figure 1a). When
this was repeated using minocycline, the converse occurred with
enhancement of the zone size adjacent to the NMP-containing
disc (Figure 1b). Addition of solvent alone to discs placed
10 mm from tigecycline discs had no effect on zone sizes
(Figure 1c).

It is well documented that in vitro testing of the susceptibility
of A. baumannii to tigecycline is highly dependent on the
method used and the level of manganese supplementation.4
However, the addition of acidic substances in disc diffusion tests
had no effect on tigecycline susceptibility and certainly did not
account for the paradoxical decrease in susceptibility observed
with NMP versus other tetracyclines.

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nothing to declare.

Figure 1. Disc diffusion tests with tigecycline (TGC), minocycline (MIN) and NMP versus A. baumannii OXA-23 clone 1. Blunting (a) of the tigecycline and expansion (b) of the minocycline zone size when placed adjacent to a disc containing 64 µg of NMP. No effect (c) on tigecycline zone size when placed adjacent to a disc containing the NMP dissolving solution alone.
Clostridium difficile-associated infection can result in diarrhoeal disease. Antibiotics such as metronidazole and vancomycin are often prescribed in combination, for rifampicin and metronidazole, and was conducted as a randomized trial. That study found no advantage in this combination.

Antimicrobial interaction was examined using a standard agar-dilution chequerboard method. The agar medium used was Brazier’s cefoxitin cycloserine egg yolk agar (BioConnections, UK) without the selective supplement (i.e. cefoxitin/cycloserine)

Both agents were tested at a concentration range of 0.125–2 mg/L alone and in combination, giving a total of 36 culture plates. All plates were multipoint inoculated with 15 distinct ribotypes of C. difficile (including 2 NCTC strains and 13 clinical isolates) as well as Bacteroides fragilis NCTC 8650 and Staphylococcus aureus NCTC 6571 as controls of known susceptibility. The final inoculum was 10^4 cfu/spot, confirmed by viable counts. All plates were incubated for 48 hr at 37°C in an anaerobic cabinet. The MIC of each agent and the fractional inhibitory concentration index (FICI) for each strain were then calculated.

The MIC of metronidazole was 1 mg/L and the MIC of vancomycin was 0.5 mg/L. For all test strains of C. difficile, the FICI was limited to a narrow range between 1.5 and 3, consistently indicating indifference between the two agents. While the corresponding in vivo effect cannot be inferred from these results, the results provide some reassurance that if combination treatment is chosen, there is no in vitro evidence that this would be less effective than using a single agent.

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References


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In vitro effect of metronidazole and vancomycin in combination on Clostridium difficile

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Sir, Clostridium difficile-associated infection can result in diarrhoeal illness, colitis and death and is a major cause of morbidity and mortality in the UK.1 The main antibiotic treatments are metronidazole and vancomycin, but there is a lack of evidence for the superiority of one over the other.2 Metronidazole is usually preferred for first-line treatment of non-severe infection due to its lower cost and the potential of widespread vancomycin use to promote resistance in other organisms. Vancomycin is recommended in severe infection due to better clinical outcomes, although metronidazole resistance is not thought to be responsible for this.3 However, administration in such patients can often be problematic and both drugs may sometimes be prescribed in combination, so that if the enteral route becomes unavailable, parenteral metronidazole can be given (the efficacy of which has not been properly assessed).

We sought to examine the in vitro effect of these drugs in combination on C. difficile, to detect synergy or antagonism. Only one study has previously looked at the effect of antibiotics in combination, for rifampicin and metronidazole, and was conducted as a randomized trial. That study found no advantage in this combination.4

Antimicrobial interaction was examined using a standard agar-dilution chequerboard method. The agar medium used was Brazier’s cefoxitin cycloserine egg yolk agar (BioConnections, UK) without the selective supplement (i.e. cefoxitin/cycloserine)