Clostridium difficile-associated infection can result in diarrhoeal illness, colitis and death and is a major cause of morbidity and mortality in the UK. The main antibiotic treatments are metronidazole and vancomycin, but there is a lack of evidence for the superiority of one over the other. Metronidazole is usually 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.

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) added. 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 10^3 cfu/spot, confirmed by viable counts. All plates were incubated for 48 h 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 MIC90 of metronidazole was 1 mg/L and the MIC90 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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### Transparency declarations

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### References


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

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Keywords: synergy, fractional inhibitory concentration index, antibiotic-associated diarrhoea

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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. The main antibiotic treatments are metronidazole and vancomycin, but there is a lack of evidence for the superiority of one over the other. 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. 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.

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### References


Detecting streptomycin in apples from orchards treated for fire blight

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Sir,

Antibiotic use, both in terms of quantity and length of application, is the major contributor to the selection and propagation of resistant strains.1 In the European Union, antibiotics can also be authorized as pesticides to treat plants. Until 31 December 2004, streptomycin was a registered pesticide. In its decision 2004/129/EC, the Commission decided not to include streptomycin in the appendix of the council’s guideline 91/414/EEC. In effect, this led to the ban on using pesticides containing streptomycin. Article 8, paragraph 4 RL 91/414/EEC permits limited and controlled use of streptomycin as a pesticide. Austria has made use of this exception, and since 2005 has authorized pesticides containing streptomycin to be used in cases of ‘clear and present danger’.

In 2008, in Austria a total amount of 2.5 kg of streptomycin (active ingredient) was used in human medicine, 1400 kg in veterinary medicine and 34 kg as a pesticide, the latter directed against fire blight (F. Allerberger and L. Girsch, unpublished results). Fire blight is a destructive bacterial disease of apple trees and pear trees caused by Erwinia amylovora. Up to now, it was assumed that the application of streptomycin to apple trees in bloom did not lead to any detectable antibiotic contamination of the fruit harvested ~6 months later. Until 31 August 2008, the legal limit for streptomycin in apples was 0.05 mg/kg, according to the directive on maximum residual values of pesticides in or on foodstuffs of plant or animal origin. Since 1 September 2008, the maximum level has been considered to be 0.01 mg/kg, according to (EC) Regulation No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and the amending Council Directive 91/414/EEC.

In 2008, we studied apples from orchards treated and not treated with streptomycin using liquid chromatography/tandem mass spectrometry and detected streptomycin in 20 of 41 samples from treated orchards versus none in 14 samples from untreated orchards. Eighty-six days elapsed between the last antibiotic application on trees in blossom (18 May 2008) and the first sampling of apples (12 August 2008). Samples from treated and untreated orchards were stratified for apple cultivars, orchard location and time of sampling. The concentration of streptomycin in positive specimens ranged from detectable below the level of quantification (LOQ; 6.6 μg/kg) to 18.4 μg/kg; the limit of detection was 1.9 μg/kg. Of the 11 samples from orchards treated once with streptomycin, 4 yielded detectable levels of streptomycin (all detectable below LOQ); of the 5 samples from orchards treated twice, one was positive (detectable below LOQ); and of the 25 samples from orchards treated three times, 15 yielded streptomycin (range: detectable below LOQ to 18.4 μg/kg). The application frequency is highly regulated according to the actual weather conditions (humidity and temperature) in the affected orchard. Our results clearly show that the use of streptomycin as a pesticide can lead to detectable concentrations of streptomycin in apples. Subsequent studies comparing fruit flesh, core and skin revealed concentrations in the apple core two to three times higher than those in the flesh and concentrations in the skin three to four times higher than those in the flesh.

Streptomyces griseus, the microorganism producing streptomycin, is considered a ubiquitous soil bacterium and therefore the natural antibiotic streptomycin occurs widely in the environment. Nevertheless, the use of streptomycin and other antibiotics in agriculture increases selective pressure (selection pressure) on the human body. Resistant strains could develop due to the potential intake of antibiotics (streptomycin) via the food chain, thereby possibly transferring resistance to potentially pathogenic bacteria. As the amount of streptomycin reaching the human gut is lower than the MIC for gut flora, there should be no concern. In the absence of a common European acceptable daily intake (AID) value for streptomycin, Austria has decreed an AID of 0.03 mg/kg of body mass per day. Nevertheless, the use of streptomycin as a pesticide must be scrutinized critically, even though the development of resistant human pathogenic strains via this route is not very likely, and, in addition, streptomycin is no longer of relevant importance in daily medical practice as an antibiotic in Austria.2 To prevent human risk related to food consumption, the risk associated with antibiotic treatment has to be assessed and communication about antimicrobial resistance caused by non-human use needs to be developed.3 Considering the proverb ‘an apple a day keeps the doctor away’ the challenge of communicating about risk may be paramount.

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**Transparency declarations**

None to declare.

**References**


Research letters