

Disk diffusion antimicrobial susceptibility testing of *Clostridium difficile*

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Introduction and Purpose

The clinical significance of *Clostridium difficile* and the emergence of decreased susceptibility towards metronidazole and vancomycin have highlighted the importance of antimicrobial susceptibility testing of *C. difficile* and the importance of having a simple testing method for the routine laboratory.

The aim of our study was to evaluate disk diffusion for susceptibility testing of *C. difficile* by comparing disk diffusion zone diameters with MICs from gradient tests.

Methods

Consecutive clinical and toxigenic isolates of *C. difficile* (n=211) were collected from patients with diarrhoea hospitalised at Aarhus University Hospital (n=110) in 2008 and from Odense University Hospital (n=101) in 2010. Furthermore, ten clinical isolates of *C. difficile* from the University Hospital of Wales with known reduced susceptibility to either metronidazole (n=4, MIC 1.5, 2, 2, 3 mg/L, ECOFF \leq 2 mg/L) or vancomycin (n=6, MIC 1.5, 2, 2, 2, 3, 3 mg/L, ECOFF \leq 2 mg/L) were included. MIC determination was performed by gradient test. Etest strips (bioMérieux, Craponne, France) with metronidazole, vancomycin, moxifloxacin, and clindamycin were placed on Brucella Blood Agar supplemented with hemin and vitamin K (Becton Dickinson, Heidelberg, Germany) according to the manufacturer's instructions. Disk diffusion was performed with Oxoid disks (Oxoid, Basingstoke, UK) with vancomycin (5 μ g), metronidazole (5 μ g), moxifloxacin (5 μ g) and clindamycin (2 μ g) on Brucella Blood Agar. Plates were incubated at 37°C in an anaerobic atmosphere (10% CO₂, 10% H₂ and 80% N₂) for 24 hours. The zone diameter was read at 100% inhibition.

Results

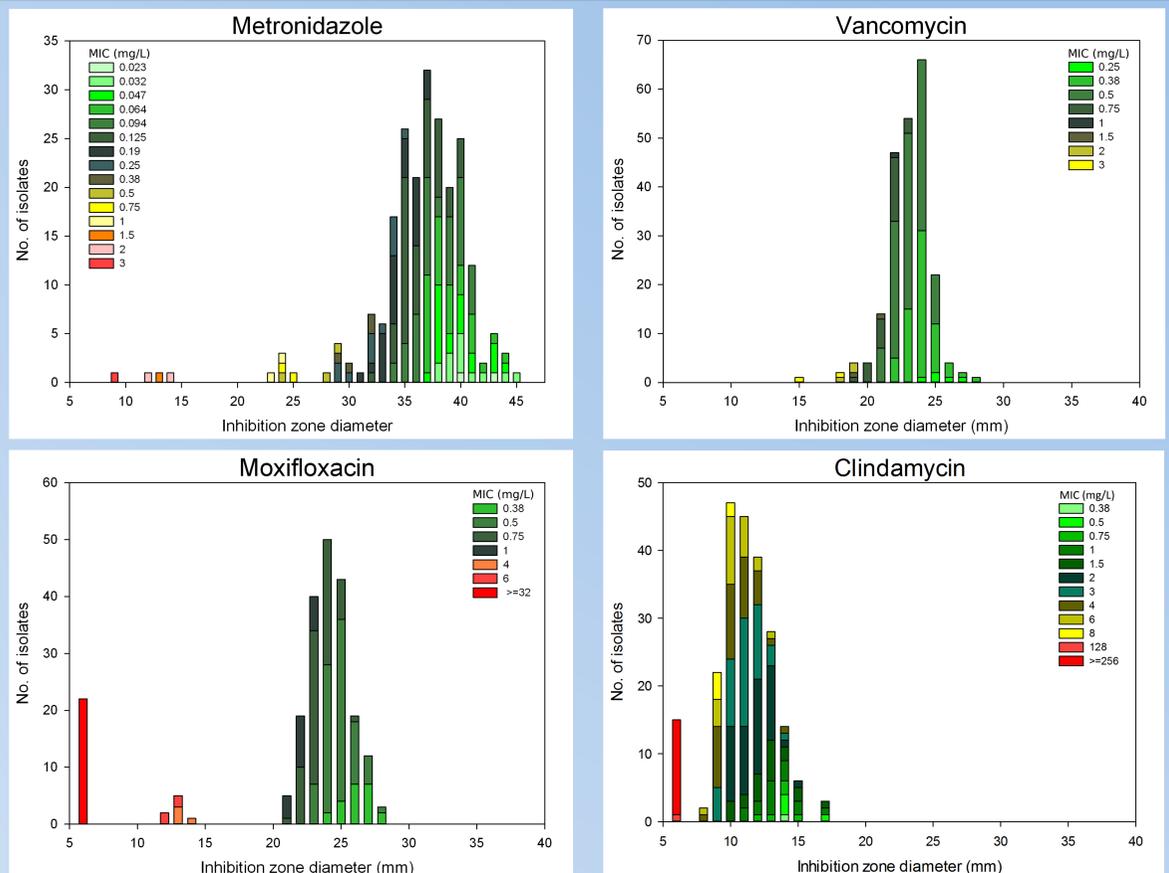


Figure 1. Distribution of inhibition zone diameters (disk diffusion method) and MICs (gradient test) for 221 isolates of *C. difficile* tested against metronidazole (5 μ g disk), vancomycin (5 μ g disk), moxifloxacin (5 μ g disk), and clindamycin (2 μ g disk). Each isolate is represented in the zone diameter histogram in a colour representing its MIC value.

A total of 221 clinical isolates of *C. difficile* from Denmark and Wales were tested. The distribution of inhibition zone diameters and MICs for *C. difficile* isolates towards metronidazole, vancomycin, moxifloxacin, and clindamycin are illustrated in the histograms (Figure 1). The four isolates with known reduced susceptibility to metronidazole were clearly separated from the wild type by 9 mm. The six isolates with known reduced susceptibility to vancomycin had zone diameters between 15 to 19 mm as compared to the consecutive clinical isolates from Denmark which all had zone diameters above 18 mm.

Bivariable regression analysis showed an acceptable to excellent correlation between the log-transformed MIC versus the untransformed zone diameter, thus the R² was 0.75 for metronidazole, 0.55 for vancomycin, 0.97 for moxifloxacin and 0.63 for clindamycin.

Conclusions

For most reference strains, almost regardless of species and antimicrobial agent, the aggregated inhibition zone values, of repeated testing, form a distribution most often covering 6-8 mm [1]. For the 221 clinical isolates of *C. difficile* tested towards metronidazole, vancomycin, moxifloxacin, and clindamycin we found that for each MIC value, the inhibition zones varied from 0 to 8 mm with the majority exhibiting 5 to 6 mm variation. Therefore, we conclude that there is an excellent agreement between disk diffusion zone diameter and the MIC as measured by Etest and consequently we recommend the disk diffusion method as a reliable option for antimicrobial susceptibility testing of *C. difficile*. Zone diameter breakpoints can now be established by EUCAST.

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References

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