Antimicrobial susceptibility testing of animal and human isolates of *Clostridium difficile* by broth microdilution

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Introduction

The purpose was to determine the minimum inhibitory concentrations (MICs) of 30 antimicrobials for human and animal isolates of *Clostridium difficile* using broth microdilution.

Material and methods

- 74 strains of *C. difficile* were selected from our collection: 26 human isolates and 48 animal isolates (poultry, pigs, ruminants) of 28 different PCR ribotypes.
- broth microdilution on commercially available plates for monitoring resistance of anaerobic and Gram-positive bacteria (Sensititre Anaerobe Plate ANO2B format and Sensititre Gram-Positive Plate GPALL1F format, (Trek Diagnostic Systems, Ltd., UK)*
- the procedure was done using reduced cation adjusted Mueller-Hinton Broth and supplemented Brucella Broth (Trek Diagnostic Systems, Ltd., UK) to make an inoculum containing 1×10^5 - 1×10^6 cfu/ml.
- incubation of 96-well plates: 48h/37°C, anaerobic conditions
- quality control: *Clostridium difficile* ATCC 700057

* The chosen method is a convenient method to simultaneously determine susceptibilities of a single isolate to several antibiotics, but is currently limited to testing of *Bacteroides* fragilis group according to CLSI. Therefore, it was used for monitoring purposes only.

Table1: ***Origin of C. difficile tested in the study***

Host	No. of strains tested	No. of different PCR ribotypes tested	PCR ribotypes
human	26	15	078, 027, 017, 126, 012, SLO 064, SLO 083, 014/020, 029, 002, SLO 116, SLO 009, SLO 020, SLO 036, 003
poultry	27	11	014/020, 002, 045, SLO 131, 010, 018, SLO160, 046, SLO 080, SLO 020, 001
pigs	17	3	150, 045, 010
ruminants	4	4	056, SLO 061, SLO 151, 010

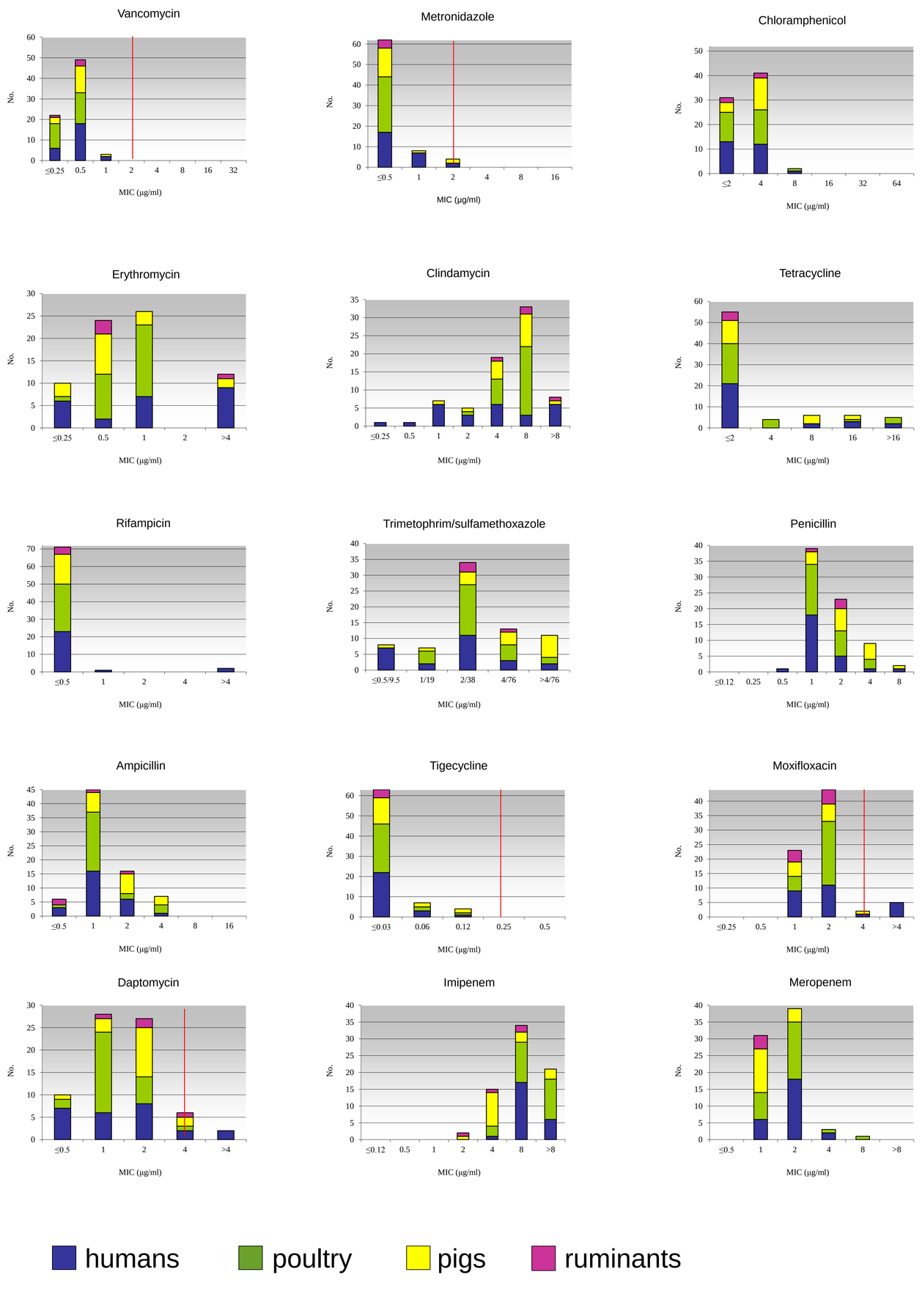
Results and discussion

Table 2*: $MIC_{50/90}$ for human and animal isolates*

Antimicrobial agent	MIC (µg/ml)						
	MIC range	MIC_{50} all	MIC_{50} human	MIC_{50} animal	MIC_{90} all	MIC_{90} human	MIC_{90} animal
Chloramphenicol	≤2 - 8	4	≤2	4	4	4	4
Daptomycin	≤0.5 - >4	1	1	1	4	4	2
Gentamicin	>16	>16	>16	>16	>16	>16	>16
Linezolid	≤1-2	≤1	≤1	≤1	2	2	2
Rifampicin	≤0.5->4	≤0.5	≤0.5	≤0.5	≤0.5	1	≤0.5
Trimethoprim/sulfamethoxazole	≤0.5/0.9 - >4/76	2/38	2/38	2/38	>4/76	>4/76	>4/76
Quinupristin/dalfopristin	≤0.5->4	≤0.5	≤0.5	≤0.5	1	1	1
Tetracycline	≤2->16	≤2	≤2	≤2	16	16	16
Erythromycin	≤0.25->4	0.5	1	0.5	>4	>4	1
Oxacillin	≤0.25->4	≤0.25	≤0.25	≤0.25	>4	>4	>4
Ampicillin	≤0.5-4	1	2	1	1	2	4
Penicillin	0.5-8	1	1	1	4	2	4
Vancomycin	≤0.25-1	0.5	0.5	0.5	0.5	0.5	0.5
Levofloxacin	4->4	4	4	4	>4	>4	>4
Tigecycline	≤0.03-0.12	≤0.03	≤0.03	≤0.03	0.06	0.12	0.06
Moxifloxacin	1->4	2	2	2	2	>4	2
Clindamycin	≤0.25->8	8	4	8	>8	>8	8
Streptomycin	≤1000-1000	≤1000	1000	≤1000	≤1000	≤1000	≤1000
Ciprofloxacin	>2	>2	>2	>2	>2	>2	>2
Nitrofurantoin	≤32	≤32	≤32	≤32	≤32	≤32	≤32
Ampicillin/sulbactam	≤0.5/0.25-2/1	1/0.5	1/0.5	1/0.5	1/0.5	1/0.5	1/0.5
Amoxicillin/clavulanic acid	≤0.5/0.25-2/1	≤0.5/0.25	≤0.5/0.25	≤0.5/0.25	1/0.5	1/0.5	1/0.5
Cefotetan	8-64	16	16	16	16	16	16
Imipenem	2->8	8	8	8	>8	>8	>8
Meropenem	1-8	2	2	1	2	2	2
Cefoxitin	>32	>32	>32	>32	>32	>32	>32
Metronidazole	≤0.5-2	≤0.5	≤0.5	≤0.5	1	1	≤0.5
Piperacillin	≤4-16	8	8	8	8	8	8
Mezlocillin	≤4-8	≤4	≤4	≤4	8	8	≤4
Piperacillin/tazobactam	2/4-16/4	4/4	4/4	4/4	8/4	8/4	8/4

Figure 1: ***Distribution of MICs for selected antimicrobial agents***

** red lines : EUCAST MIC breakpoints valid from 2012-01-01; the breakpoints for metronidazole and vancomycin are based on epidemiological values (ECOFFs), which distinguish wild type isolates from those with reduced susceptibility. For other antimicrobials the red line means ECOFF, it should be used for epidemiological purposes only.*



The emerging resistance to metronidazole and vancomycin, the drugs of choice for human treatment, is of main concern. In our strains, MIC_{90} values were low for both agents. However, one human isolate (PCR ribotype 078) and two isolates from piglets (045) had metronidazole MIC values of 2 µg/mL, which is just at the recently published EUCAST breakpoint.
For rifampicin, two human isolates, both of ribotype 017, displayed MIC >4 µg/mL. One of them also showed decreased susceptibility to chloramphenicol (16 µg/mL), clindamycin (>8 µg/ml), tetracycline (16 µg/ml) and moxifloxacin (>4 µg/ml). Its MIC value for erythromycin was above 4 µg/mL, but the tested range was too narrow to identify high resistance.
MICs of daptomycin were above 4 µg/mL for two human isolates of ribotype 078 and 126, respectively.

There were also animal isolates with decreased susceptibility to multiple antibiotics. These belong to PCR ribotype 046 (poultry isolate) and 150 (toxinotype 0, piglet isolates). One goat isolate of ribotype 010 (non-toxigenic) displayed increased MIC values for erythromycin (>4 µg/mL) and clindamycin (>8 µg/mL).
MICs of clindamycin were distributed within whole tested range (≤0.25- >8 µg/mL), but 66.7 % of animal isolates and 34.6 % of human isolates had MIC ≥8 µg/mL.
The activity of tetracycline was generally high, although 27 % of human strains (among them all of three tested PCR ribotypes 078, and ribotype 017) and 20.8 % of animal strains had MIC ≥8 µg/mL.

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