Introduction

The antibiotic resistant pattern could differ in different hosts and while human isolates are regularly surveilled for antimicrobial susceptibility, the information on animal C. difficile strains is more deficient and has been not often systematically determined (1,2,3).

Materials and methods

Bacterial isolates

31 fecal isolates of C. difficile from pigs (21), calves (5), dogs (4) and a horse were collected and identified previously (4). The bacteria were recovered from frozen storage (−70°C). The aim of this study was to determine the overall growth and purity the isolates were subcultured by at least two serial transfers on 5% sheep blood agar prior to testing.

Broth microdilution

The method was performed on commercially available 96-well broth microdilution plates for monitoring resistance of anaerobic and Gram-positive bacteria. Anaerobe 10: 47-50. * MIC50 and MIC90 are minimum inhibitory concentrations at which 50% and 90% of the isolates, respectively, were inhibited.

Discussion

From the viewpoint of antibiotic therapy the emerging resistance to metronidazole, and vancomycin is the main concern, as the agents are the drugs of choice for human treatment. Resistance to other antibiotics is also important as it enables the growth in the presence of increased antibiotic levels that disrupt the normal gut flora.

All tested isolates were inhibited by concentrations that did not exceed 0.5 µg/ml for vancomycin and 1 µg/ml for metronidazole.

Some studies found correlation with resistance to chloramphenicol and erythromycin, clindamycin and tetracycline, the pattern found almost exclusively in serogroup C (6). In our study, decrease resistance to chloramphenicol (16 µg/ml) was observed in two isolates and one of them also had decreased susceptibility for clindamycin (2 µg/ml), tetracycline (8 µg/ml), rifampin (≥4 µg/ml) and moxifloxacin (≥4 µg/ml).

The colonies were selected from 48 hour anaerobic culture, the inoculation was done in aerobic atmosphere, but the organisms were not exposed to air for more than 1.5 minutes.

The procedure was done as recommended by the producer, using reduced cation adjusted Mueller-Hinton Broth and supplemented Broth (ThiK Diagnostic Systems, Ltd., UK) to make an inoculum containing 1×10^7-1×10^8 cfu/ml. The plates were incubated at 35°C for 48 hours in anaerobic conditions (GENBox anaerobic jar and GENBox Anaer generators, BioMerieux, France), up to three in a stack. The MIC endpoints were determined where no growth or the most significant reduction of growth was observed.

Quality Control

The following control strains were included: Bacteroides fragilis ATCC 25285, Bacteroides thetaiotaomicron ATCC 29545, Clostridium perfringens ATCC 29741, Enterococcus faecalis ATCC 29213 (aerobic control for Gram-positive plate). C. difficile ATCC 8446 was included for the internal control of the procedure. The tests for ten isolates were performed in duplicate. The inoculum suspension was checked for purity by subculturing onto 5% sheep blood agar plates and incubated anaerobically and aerobically. All plates include control wells.

Results

Broth microdilution

Certain antimicrobial agents (chloramphenicol, tetracycline, ampicillin, penicillin, clindamycin) were included in both panels, for testing anaerobes and Gram-positive organisms. The MICs did not differ for more than one two-fold dilution for any of the antimicrobial agents. The results of anaerobe panel were chosen for presentation of these results. Also, duplicates did not differ for more than one two-fold dilution for any of the antimicrobial agents. The results used were the first duplicate for presentation of the results.

The results for MIC determination, MIC50, and MIC90 are given in Table 1.

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The strain was isolated from a dog and belongs to toxinotype 0 (unpublished data), which is commonly found in serogroup C. MICs for clindamycin, erythromycin and trimethoprim/sulfamethoxazole were distributed within whole tested range, which is consistent to findings of a wide range of susceptibility in those antimicrobial agents. MIC for tetracycline showed bimodal distribution: 38% of isolates had MICs ≥25 µg/ml, and 42% of isolates had MICs ≤4 µg/ml. Rifampin showed high level activity against all C. difficile isolates but one, with MIC ≤0.25 µg/ml.

The broth microdilution method in this study showed as reproducible and reliable, MIC endpoints were generally clearly determined. However, in the case of some antimicrobial agents, the dilution range was too narrow to make conclusions on MIC values or to identify resistant strains.

References