ANTIBACTERIAL ACTIVITY OF TEICOPLANIN AGAINST *CLOSTRIDIUM DIFFICILE*

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Abstract. The *in vitro* inhibitory action of teicoplanin, vancomycin, metronidazole and clindamycin against clinical isolates of *Clostridium difficile* was investigated. Minimum inhibitory concentrations (MICs) were determined using E test. Teicoplanin (MIC range 0.023-0.75 μg/ml), vancomycin (MIC range 0.5-3 μg/ml) and metronidazole (MIC range 0.19-1 μg/ml) were all very active against the isolates examined. No resistant strains of *C. difficile* to those three antimicrobial agents were observed, whereas resistance to clindamycin was found in 39.5% of the tested strains. Teicoplanin was about 4-times more potent than vancomycin. It appears to be a more promising antimicrobial for treatment of *C. difficile* enteric disease.

INTRODUCTION

Clostridium difficile-associated diarrhea and colitis are increasingly common diseases in the general population, immunocompetent patients and AIDS patients (Wongwanich *et al*, 1990; Cozart *et al*, 1993; Cappell and Philogene, 1993). The choice of antibiotics available for the treatment of the diseases is still limited. Only vancomycin, metronidazole or bacitracin are antimicrobials of choice for the treatment of *C. difficile* infections (Bartlett, 1985; Chang *et al*, 1980). However, a number of reports have indicated that 20-35% of patients treated with vancomycin or metronidazole experience recurrent diarrhea (Wilcox and Spencer, 1992; Teasley *et al*, 1983; Bartlett *et al*, 1980; Walters *et al*, 1983; Wistrom *et al*, 1994). The poor *in vitro* activity of bacitracin against *C. difficile* has also been reported in our previous study (Kusum and Wongwanich, 1994). Recently, teicoplanin has been considered the drug of choice for the treatment of infections caused by *C. difficile* in developed countries (Wistrom *et al*, 1994), but has not been reported in this developing region. This report documents the antibacterial activity of teicoplanin against clinical isolates of *C. difficile*.

MATERIALS AND METHODS

Bacterial strains: 38 strains of *C. difficile* were isolated from colitis and diarrheal patients. Colonies with clostridial morphology were identified by their biochemical reaction profiles as described previously (Holdeman *et al*, 1977). Identification of *C. difficile* isolates was confirmed by their positive reactions for leucine arylamidase activity test (Phan-Urai *et al*, 1994) and *C. difficile* latex agglutination test (CD D-1 kit, Mitsubishi Chemical Industries, Tokyo).

Determination of MICs: Antibacterial activity, expressed as the MIC in μg/ml, of teicoplanin, vancomycin, metronidazole and clindamycin was determined by the E test (AB Biodisk, Sweden) in 5% sheep blood agar or brain heart infusion agar, according to the technical procedures described by the manufacturer. The E test is a plastic strip (5 by 50 mm; antibiotic carrier) with a continuous gradient of antibiotic immobilized on one side and an MIC interpretative scale corresponding to 15 two-fold MIC dilutions on the other side. The range of concentrations for teicoplanin, vancomycin and clindamycin was 0.016 to 256 μg/ml or for metronidazole was 0.002 to 32 μg/ml. Turbidity of the inoculum was adjusted to equal the density of a McFarland no. 0.5 standard in brain heart infusion broth. The tested plates were incubated at 35°C for 24-48 hours in an anaerobic chamber with an atmosphere of 85% N_2, 10% H_2, and 5% CO_2. The MIC was recorded as the MIC value at the point of intersection between the edge of inhibition zone and the E test strip.

Resistance cutoff points: Resistance was defined as a MIC of ≥ 16 μg/ml for teicoplanin, vancomycin and metronidazole or ≥ 4 μg/ml for clindamycin (NCCLS, 1994).

Table 1

In vitro activity of 4 antibiotics against 38 isolates of *Clostridium difficile.*

Antibiotic	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant
Teicoplanin	0.023-0.75	0.38	0.5	0
Vancomycin	0.5 -3	1.0	2.0	0
Metronidazole	0.19 -1.0	0.38	0.5	0
Clindamycin	0.38 -≥ 256	2.0	≥ 256	39.5

RESULTS

MICs of teicoplanin, vancomycin, metronidazole and clindamycin for 38 clinical isolates of *C. difficile* are shown in Table 1. Teicoplanin, vancomycin and metronidazole were highly active against these isolates (MIC_{90s} of 0.5, 2.0 and 0.5 μg/ml, respectively). There were no resistant to those three antimicrobial agents. Fifteen (39.5%) isolates of *C. difficile* characterized by moderate (MIC 4 μg/ml) or high (MIC ≥ 256 μg/ml) level resistance to clindamycin were found.

DISCUSSION

Antimicrobial option for treatment of *C. difficile* infections include vancomycin, metronidazole or bacitracin. Comparative clinical trials indicate that those drugs are therapeutically equivalent to each other, although most authorities still recommend vancomycin as preferred drug for seriously ill patients (Cozart *et al*, 1993; Bartlett, 1985). Its use in oral treatment was attempted because it is not absorbed well from the bowel. Although metronidazole has advantage of being considerably less expensive. One concern about oral metronidazole, since it acheives extremely high blood levels and is absorbed high in the gastrointestinal tract, was that fecal levels would actually be fairly low. The low *in vitro* activity of bacitracin against *C. difficile* has been reported in previous study (Kusum and Wongwanich, 1994). Recently, teicoplanin is considered the drug of choice for the treatment of infections caused by *C. difficile*. It is a new non-absorbable glycopeptide antibiotic, structurally related to vancomycin (Babul and Pasko, 1988). It has a pharmacokinetic profile which is superior to that of vancomycin, characterized by a long half-life with lower toxicity and the additional benefit of virtual absence of administration effects (Verbist *et al*, 1984, Stille *et al*, 1988). The clinical response to oral teicoplanin treatment of *C. difficile* infections is very good and rapid (Wistrom *et al*, 1994; de Lalla *et al*, 1992). Relapse after treatment with teicoplanin at a dosage of 100 mg twice a day for 10 days has been reported for 7.7% of patients and post treatment asymptomatic carriage of *C. difficile* has also been found in 7.7% of patients. Whereas a higher recurrence rate (20-35%) in patients treated with vancomycin or metronidazole has been reported (Wilcox and Spencer, 1992; Teasley *et al*, 1983; Bartlett *et al*, 1980; Walters *et al*, 1983; Wistrom *et al*, 1994). Asymptomatic persistence or relapse of *C. difficile* has been found in approximately 20% of patients with clinical cure using vancomycin (Wistrom *et al*, 1994).

The *in vitro* activity of teicoplanin has been shown to be high against *C. difficile*, with MICs ranging from < 0.007 to 2.0 mg/l, compared with 0.125 to 4.0 mg/l for vancomycin (Babul and Pasko, 1988; Newsom *et al*, 1985; Bartoloni *et al*, 1990). The results of this study confirm the activity of vancomycin (MICs range 0.5-3 μg/ml) and metronidazole (MICs range 0.19-1.0 μg/ml) against *C. difficile* and show that teicoplanin (MICs range 0.023-0.75 μg/ml), a new glycopeptide antimicrobial, has approximately 4 times higher potency than vancomycin against the same strains. There were no strains resistant to those three antibiotics. Amongst the antimicrobials tested, clindamycin exhibited very high MICs (MIC_{90} > 256 μg/ml) for *C. difficile*. These results are in agreement with those of most investigators in developed countries (Newsom *et al*, 1985; Bartoloni *et al*, 1990; Sheikh *et al*, 1993).

Standard methods of antimicrobial susceptibility testing are based either on dilution or diffusion techniques. E test is based on a combination of the concepts of both dilution and diffusion tests. E test MIC values have been shown to be reproducible and directly proportional to MIC values from the NCCLS reference agar dilution procedure. Agreement of 86-94% between E test and agar dilution for quantitative susceptibility testing of anaerobic bacteria had been shown by Citron *et al* (1991). It has been found to be a reliable method for determining the susceptibility testing of all anaerobes, other aerobic and facultatively anaerobic bacteria (Finegold, 1988; Jorgensen *et al*, 1991). We also found that E test was easy to perform and read. Most of the tested *C. difficile* grew and displayed endpoints after overnight incubation, with very little change in the MIC after additional incubation.

The excellent *in vitro* activity of teicoplanin against *C. difficile* shows promising potential and warrants clinical trials to determine the most suitable dosage of this agent for therapy of infections involving *C. difficile* in this region.

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