วารสารวิชาการสาธารณสุข
ปีที่ 3 ฉบับที่ 3 ก.ค.–ก.ย. 2537

Journal of Health Science
Vol. 3 No.3 Jul.-Sep. 1994

นิพนธ์ต้นฉบับ

Original Article

ความไวของเชื้อ *Clostridium difficile* ต่อยาต้านจุลชีพ 16 ชนิด

Susceptibility of *Clostridium difficile* to Sixteen Antimicrobial Agents

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บทคัดย่อ

ได้นำ *Clostridium difficile* 28 สายพันธุ์และ *Clostridium species* อื่นๆ 11 สายพันธุ์มาทดสอบความไวของเชื้อต่อยาต้านจุลชีพ 16 ชนิด ได้แก่ ampicillin, bacitracin, carbenicillin, cefazolin, cefoperazone, cefoxitin, chloramphenicol, clindamycin, erythromycin, metronidazole, penicillin, piperacillin, rifampin, tetracycline, ticarcillin และ vancomycin โดยใช้วิธี broth-disk เชื้อ *Clostridium* ทั้งหมดแยกได้จากผู้ป่วยลำไส้อักเสบและอุจจาระร่วง การทดสอบพบว่า *C. difficile* ไม่ดื้อต่อยา carbenicillin, metronidazole และ vancomycin แต่มี *C. difficile* 27 สายพันธุ์ (96.4%) ที่ดื้อต่อยาหลายชนิด (multiple drug resistance) ตั้งแต่ 3 ถึง 11 ชนิด ส่วนเชื้อ *Clostridium species* อื่นๆทุกสายพันธุ์มีความไวต่อยา cefoperazone, piperacillin และ ticarcillin แต่มีการดื้อต่อยาหลายชนิดตั้งแต่ 2 ถึง 5 ชนิดจำนวน 7 สายพันธุ์ (63.6%) ดังนั้น metronidazole และ vancomycin น่าจะเป็นยาตัวเลือกที่ใช้รักษาโรคลำไส้อักเสบและอุจจาระร่วงที่มีสาเหตุจากเชื้อ *Clostridium difficile* ได้ดี

ABSTRACT

The broth-disk method was used to determine the activities of ampicillin, bacitracin, carbenicillin, cefazolin, cefoperazone, cefoxitin, chloramphenicol, clindamycin, erythromycin, metronidazole, penicillin, piperacillin, rifampin, tetracycline, ticarcillin, and vancomycin against 28 strains of *Clostridium difficile* and 11 strains of other clostridium species. All strains were isolated from colitis and diarrhoeal patients. Carbenicillin, metronidazole and vancomycin were found to be the most active agents against *C. difficile*. None resistant strains of *C. difficile* to those three antimicrobial agents were observed. Twenty-seven strains (96.4%) of *C. difficile* showed multiple drug resistant against three to eleven of other tested antimicrobial agents. All other clostridium species were susceptible to cefoperazone, piperacillin and ticarcillin. Seven strains (63.6%) had multiple drug resistant against two to six tested antimicrobial agents.

INTRODUCTION

Clostridium difficile is increasingly common enteric pathogen, usually causing pseudomembranous colitis, antibiotic-associated diarrhoea, antibiotic-associated colitis, and diarrhoea unrelated to antimicrobial therapy. An antibiotic therapy is indicated in case of severe infection by *C. difficile*.[1] Classically, vancomycin and metronidazole are considered the drug of choice for the treatment of infections. A bacitracin has also been used for treatment of *C. difficile* diarrhoea and antibiotic-associated enterocolitis.[2,3] Few studies have been carried out to determine the *in vitro* susceptibilities of *C. difficile*.[4,5] However, there was no report on the antimicrobial susceptibility pattern of clinically isolated *C. difficile* in Thailand. Clinicians still rely upon previous studies from developed countries to initiate antimicrobial therapy when *C. difficile* are suspected pathogens.

This report documents the *in vitro* susceptibility patterns of recently clinical isolated *C. difficile* and other clostridium species to sixteen antimicrobial agents.

MATERIALS AND METHODS

Bacterial strains

The 28 strains of *C. difficile* and 11 strains of other clostridium species were isolated from colitis and diarrhoeal patients. Colonies with clostridial morphology were identified by their biochemical reaction profiles as described previously.[6] Identifying *C. difficile* isolates was confirmed by their positive reactions for leucine arylamidase activity test [7] and *C. difficile* latex agglutination test (C.D. D-1 kit), Mitsubishi Chemical Industries, Tokyo).

Susceptibility testing

The susceptibility test was performed by broth-disk method.[8,9] Briefly, screw-cap tube contained 5 ml of brain-heart infusion broth (BHI) supplemented with hemin (0.5 ml of a 1% solution per liter) and vitamin K1 solution (0.1 ml of a 1% solution per liter) was kept in anaerobic glove box (in 85% N_2, 10% H_2, 5% CO_2 atmosphere) for 4-18 h prior to use. Before inoculation, antimicrobial agents were added to each tube of BHI broth using aseptic technique. Single commercial disks (BBL, DIFCO) were used for susceptibility test. The number of antimicrobial disks to add to each tube of 5 ml brain-heart infusion broth was as follow:

1 disk of ampicllin,
1 disk of bacitracin,
6 disks of carbenicillin,
3 disks of cefazolin,
4 disks of cefoperazone,
3 disks of cefoxitin,
3 disks of chloramphenicol,
10 disks of clindamycin,
1 disk of erythromycin,
1 disk of metronidazole,
8 disks of penicillin,
3 disks of piperacillin,
2 disks of rifampin,
1 disk of tetracycline,
4 disks of ticarcillin, and
1 disk of vancomycin.

Inoculated each tube with 0.1 ml of actively growing BHI broth culture of the organism to be tested (24-48 hours old). Incubated anaerobically for 24-48 hours. In tubes showing 50% or more of turbidity of the growth control tube (without antimicrobial), the organism was considered resistant, whereas in antimicrobial tubes showing no turbidity or less than 50% of the control tube,

the organism was considered susceptible. The susceptibility test was considered indeterminate if the turbidity was approximately 50% of that of the control. Indeterminate results were reported as resistant.

RESULTS

Table 1 showed the rates of resistance to antimicrobial agents of 28 *C. difficile* and 11 other clostridium species. All strains of *C. difficile* were susceptible to carbenicillin, metronidazole and vancomycin. Among the penicillins, piperacillin showed the greatest activity against *C. difficile* (resistance in 3.6% of strains). Ticarcillin, penicillin and ampicillin were less active, with resistance rates of 3.6% - 82.1%. Cephalosporins showed poor activity against *C. difficile* (resistance in 50% - 92.9% of strains). A high degree of resistance to bacitracin, clindamycin, erythromycin and tetracycline was seen in 85.7%, 64.3%, 60.7%, and 53.6% of *C. difficile*, respectively. Chloramphenicol and rifampin had intermediate activity against *C. difficile*, with resistance rates of 21.4% and 14.3%.

Twenty-six profiles of multiple drug resistance of 27 *C. difficile* isolates were observed (Table 2). Only one strain was resistant to a single tested agent, bacitracin.

The majority of strains of other *Clostridium species* were susceptible to all tested antimicrobial agents except to bacitracin and erythromycin. Two strains (18.1%) of *Clostridium species* showed no resistance profile to the tested antimicrobial agents.

DISCUSSION

C. difficile is becoming a common cause of bacterial diarrhoea in general population in Thailand. It ranks second after *Salmonella spp.*[10] The role of *C. difficile* in patient with AIDS, where it is a major recognized cause of antibiotic-associated diarrhoea and colitis, has been defined in developed countries,[11,12,13] but has not been reported in this region.

Antimicrobial option for patients with *C. difficile* colitis or diarrhoea include vancomycin, metronidazole or bacitracin. Comparative clinical trials indicate that those drugs are therapeutically equivalent to each other, although most authorities still recommen vancomycin as the preferred drug for seriously ill patients.[2,11,12] In this study, vancomycin and metronidazole were the most potent antimicrobial agents against *C. difficile.* There were no resistant to either agent. Our results confirm and extend the data regarding the *in vitro* activity of vancomycin and metronidazole against *C. difficile* isolated in this region. Bacitracin has been reported to be effective in treatment of antibiotic-associated colitis due to *Clostridium difficile.*[3] However, in our study, it was not active against *C. difficile*, 85.7% of tested strains were resistant. It is of interest that carbenicillin showed very good activity against *C. difficile* comparable with that of vancomycin and metronidazole, which has not been recorded in previous studies.[4,5] Carbenicillin was also most active against other *Clostridium species,* and no resistance strains were noted. Cefoxitin was the least active of the tested antimicrobial agents against *C. difficile.* Reports of isolation of cefoxitin-resistant and clindamycin-resistant *C. difficile* have been published previously.[5] Wexler et al[4] reported that 50% of *C. difficile* were susceptible to clindamycin and tetracycline. We observed fewer strains susceptible to those agents. Although a few strains of

Table 1 Rates of resistance to antimicrobial agents of *C. difficile* and other clostridium species

Organism (No. of isolates)	Antimicrobial agent	Resistant strains No.	(%)
C. difficile (28)	Ampicillin	23	(82.1)
	Bacitracin	24	(85.7)
	Carbenicillin	0	
	Cefazolin	14	(50)
	Cefoperazone	14	(50)
	Cefoxitin	26	(92.9)
	Chloramphenicol	6	(21.4
	Clindamycin	18	(64.3)
	Erythromycin	17	(60.7)
	Metronidazole	0	
	Penicillin	9	(32.1)
	Piperacillin	1	(3.6)
	Rifampin	4	(14.3)
	Tetracycline	15	(53.6)
	Ticarcillin	7	(25)
	Vancomycin	0	
Other clostridium species (11)	Ampicillin	1	(9.1)
	Bacitracin	7	(63.6)
	Carbenicillin	0	
	Cefazolin	1	(9.1)
	Cefoperazone	0	
	Cefoxitin	2	(18.2)
	Chloramphenicol	1	(9.1)
	Clindamycin	2	(18.2)
	Erythromycin	6	(54.5)
	Metronidazole	2	(18.2)
	Penicillin	1	(9.1)
	Piperacillin	0	
	Rifampin	3	(27.3)
	Tetracycline	3	(27.3)
	Ticarcillin	0	
	Vancomycin	1	(9.1)

Table 2 Resistance profiles of *Clostridium difficile* and other *Clostridium species* isolates

Resistance profiles of indicated isolates (No. of resistant isolates)	
Clostridium *difficile*	other *Clostridium species*
Am, B, CZ, CEP, Fox, CC, E, P, PIP, Te, TIC (1)	B, Fox, E, RA, Te, VA (1)
Am, B, CZ, CEP, Fox, C, CC, E, Te (1)	B, C, E, Met, RA, Te (1)
Am, B, CEP, Fox, CC, E, P, RA, Te (1)	B, CZ, CC, Met, RA (1)
Am, B, Fox, C, CC, E, P, RA, Te (1)	B, Fox, P (1)
Am, B, CZ, CEP, Fox, CC, Te, TIC (1)	B, CC, E (1)
Am, CZ, CEP, Fox, CC, E, Te, TIC (1)	B, E (1)
Am, B, CZ, CEP, Fox, E, P, Te (1)	B, Te (1)
B, CZ, CEP, Fox, C, CC, E, TIC (1)	E (2)
B, CEP, Fox, C, CC, E, RA, TIC (1)	
Am, B, CZ, Fox, C, CC, Te (1)	
Am, CZ, CEP, Fox, C, CC, TIC (1)	
Am, B, CZ, CEP, Fox, CC, E (1)	
Am, B, CZ, CEP, Fox, CC, Te (1)	
Am, B, Fox, CC, E,Te, TIC (7)	
Am, B, CZ, Fox, E, P (1)	
Am, B, Fox, E, P, Te (1)	
Am, CZ, CEP, Fox, E, P (1)	
Am, B, CEP, Fox, CC (1)	
Am, B, CZ, Fox, P (1)	
Am, B, CEP, Fox, E (1)	
Am, B, Fox, CC, P (1)	
Am, B, Fox, CC, Te (1)	
Am, B, Fox, E, Te (1)	
B, Fox, CC, E, Te (1)	
CZ, CC, E, RA,Te(1)	
Am, B, Fox (2)	
B (1)	

Am, ampicillin; B, bacitracin; CZ, cofazolin; CEP, cefoperazone; Fox, cefoxitin; C, chloramphenicol; CC, clindamycin; E, erythromycin; Met, metronidazole; P, penicillin; PIP, piperacillin; RA, rifampin; Te, tetracycline; TIC, ticarcillin; VA, vacomycin.

other *Clostridium species* were resistant to metronidazole and vancomycin, the over all resistance rate of *Clostridium species* to the other agents was lower than that of *C. difficile.* These results are in agreement with those of most investigators.[4,5,14] However,the *C. difficile* isolates in this study were resistant to a wide range of antimicrobial agents with several variable resistance profiles (27 resistance profiles of 28 isolates). The difference in resistance profiles may reflect the variation in antimicrobial usage between regions and countries. The uncontrolled used of antibiotics in this developing region may contribute to the increased incidence of multiple drug resistance.

Our data show the susceptibility patterns of *C. difficile* and other clostridium species which may use for consideration on treatment of colitis or diarrhoea in this region. Metronidazole and vancomycin remain the drugs of choice for the treatment of severe *C. difficile* infections. Furthermore, carbenicillin showed very active against *C. difficile*. Clinical trials may be required to determine the role of vancomycin, metronidazloe or carbenicillin in infections caused by *C. difficile* in this region.

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