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In Vitro Activities of 10 Antimicrobial Agents against Bacterial Vaginosis-Associated Anaerobic Isolates from Pregnant Japanese and Thai Women

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The in vitro activities of 10 antimicrobial agents against 159 bacterial vaginosis-associated anaerobic isolates from pregnant Japanese and Thai women were determined. Clindamycin, imipenem, cefmetazole, amoxicillin, amoxicillin-clavulanate, and metronidazole were highly active against all anaerobic isolates except *Prevotella bivia* and *Mobiluncus* species, which were resistant to amoxicillin and metronidazole, respectively. Cefotiam, ceftazidime, and ofloxacin were variably effective, while cefaclor was the least effective agent.

Many recent studies have found increased risks of preterm labor, preterm birth, premature rupture of the membranes, and chorioamnionitis among women with bacterial vaginosis (BV), particularly for those women from whom anaerobes, especially *Prevotella* spp., *Porphyromonas* spp., *Peptostreptococcus* spp., and *Mobiluncus* spp., were recovered (3–6). Reductions in the rates of these adverse effects are most likely to be achieved when specific therapy can be provided to pregnant women at risk (7). Unfortunately, in Thailand, the nature of the susceptibility pattern of anaerobic isolates has been documented rarely, if ever. Because of geographic variations in the prevalence of etiologic organisms and their patterns of antimicrobial susceptibility, coupled with emerging antimicrobial resistance, susceptibility testing of locally recovered isolates is required to provide proper treatment of pregnancy complications.

We recently investigated the vaginal microflora associated with BV in pregnant Japanese and Thai women (9). Therefore, the purpose of this study was to determine the in vitro antimicrobial susceptibilities of the most frequently isolated BV-associated anaerobes from pregnant Japanese and Thai women.

A total of 159 vaginal isolates of anaerobic bacteria obtained from pregnant Japanese and Thai women were used for susceptibility testing. All isolates were identified at the Institute of Anaerobic Bacteriology, Gifu, Japan, by a combination of standard methods (10), the RapID/ANA II system (Innovative Diagnostic Systems, Atlanta, Ga.), and gas-liquid chromatography. The isolates were stored in skim milk at −75°C for subsequent susceptibility testing. MICs were determined by an agar dilution procedure outlined by the National Committee for Clinical Laboratory Standards (8). β-Lactamase production was assessed by the cefinase disk method (BBL, Becton Dickinson, Cockeysville, Maryland).

Inocula of 10^5 CFU per spot were delivered with a multipoint inoculator (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto brucella HK agar (Kyokuto Pharmaceutical, Tokyo, Japan) supplemented with 5% laked sheep blood. Plates were incubated at 37°C for 48 h except for those with *Mobiluncus* spp., which were incubated for 72 h in an anaerobic chamber (Hirasawa, Tokyo, Japan) with an atmosphere of 82% N_2, 10% CO_2, and 8% H_2. The MIC was defined as the lowest concentration permitting the growth of fewer than five colonies. *Bacteroides fragilis* ATCC 25285 and *B. fragilis* GAI 5562 were used as controls. The following antimicrobial agents were provided in standard powders from pharmaceutical manufacturers located in Japan: amoxicillin (Meiji Seika Kaisha, Tokyo), amoxicillin-clavulanate (SmithKline Beecham Pharmaceutical, Tokyo), cefaclor (Shionogi & Co. Ltd., Osaka), cefotiam (Takeda Chemical Industries Ltd., Tokyo), cefmetazole (Sankyo Company Ltd., Tokyo), ceftazidime (Nippon Glaxo, Ltd., Tokyo), clindamycin (Japan Upjohn, Tokyo), imipenem (Banyu-Pharmaceutical, Tokyo), ofloxacin (Daiichi Pharmaceutical, Tokyo), and metronidazole (Rhone-Poulenc Japan, Tokyo).

Although the medium used was not Wilkins-Chalgren agar, the medium on which the breakpoint standards of the National Committee for Clinical Laboratory Standards are set, the brucella HK agar used in this study yielded endpoints for control organisms equivalent to those obtained on Wilkins-Chalgren agar.

Since the susceptibility patterns of most of the recovered anaerobes were generally similar for the pregnant Japanese and Thai women tested in this study, the susceptibility results from the two groups were combined. The MIC ranges, the MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of isolates were inhibited, and the percentages of susceptible isolates at the breakpoints are shown in Table 1. Percent susceptibility data are not presented for cefotiam, ceftazidime, ofloxacin, and cefaclor because no approved breakpoints for anaerobic bacteria are presently available for these antimicrobial agents. As can be seen, 34 of 36 isolates of *Prevotella bivia* and 1 of 25 isolates of *Porphyromonas asaccharolytica* produced β-lactamase according to the cefinase disk method. By contrast, none of the gram-positive bacteria produced this enzyme.

Clindamycin, imipenem, and cefmetazole were highly active against all isolates and had MIC_{90}s of ≤0.06 to 0.5, ≤0.06 to 1, and 0.125 to 4 μg/ml, respectively. Amoxicillin was also active against all isolates except *P. bivia* and had MIC_{90}s of ≤0.06 to 0.5 μg/ml. The activity of amoxicillin-clavulanate was equivalent to that of clindamycin, cefmetazole, and imipenem for all

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TABLE 1. In vitro activity of 10 antimicrobial agents against bacterial vaginosis-associated anaerobic microflora isolated from pregnant Japanese and Thai women

Antimicrobial agent	Microorganism (no. of β-lactamase-positive isolates/total no. of isolates)[a]	MIC (μg/ml)			% Susceptible[b]
		Range	50%	90%	
Clindamycin	*P. bivia* (34/36)	≤0.06	≤0.06	≤0.06	100, 100, 100
	P. asaccharolytica (1/25)	≤0.06–0.25	≤0.06	≤0.06	100, 100, 100
	Peptostreptococcus asaccharolyticus (0/39)	≤0.06–0.5	≤0.06	0.25	100, 100, 100
	P. magnus (0/33)	≤0.06–2	0.125	0.5	97, 100, 100
	Peptostreptococcus anaerobius (0/10)	≤0.06	≤0.06	≤0.06	100, 100, 100
	Mobiluncus spp. (0/16)	≤0.06	≤0.06	≤0.06	100, 100, 100
Imipenem	*P. bivia*	≤0.06–0.125	≤0.06	≤0.06	100, 100, 100
	P. asaccharolytica	≤0.06–0.5	≤0.06	0.125	100, 100, 100
	P. asaccharolyticus	≤0.06	≤0.06	≤0.06	100, 100, 100
	P. magnus	≤0.06–1	0.125	0.5	100, 100, 100
	P. anaerobius	≤0.06–0.5	0.125	0.5	100, 100, 100
	Mobiluncus spp.	0.25–1	0.5	1	100, 100, 100
Cefmetazole	*P. bivia*	0.5–4	2	4	100, 100, 100
	P. asaccharolytica	≤0.06–1	≤0.06	1	100, 100, 100
	P. asaccharolyticus	≤0.06–0.25	≤0.06	0.125	100, 100, 100
	P. magnus	≤0.06–1	0.25	1	100, 100, 100
	P. anaerobius	≤0.06–4	0.5	4	100, 100, 100
	Mobiluncus spp.	0.5–2	1	2	100, 100, 100
Amoxicillin	*P. bivia*	≤0.06–128	32	128	28, 31, 36
	P. asaccharolytica	≤0.06–4	≤0.06	0.25	100, 100, 100
	P. asaccharolyticus	≤0.06–125	≤0.06	≤0.06	100, 100, 100
	P. magnus	≤0.06–1	0.125	0.25	100, 100, 100
	P. anaerobius	≤0.06–0.5	0.25	0.5	100, 100, 100
	Mobiluncus spp.	≤0.06–0.125	≤0.06	0.125	100, 100, 100
Amoxicillin-clavulanate	*P. bivia*	≤0.06–4	2	4	89, 100, 100
	P. asaccharolytica	≤0.06–0.125	≤0.06	≤0.06	100, 100, 100
	P. asaccharolyticus	≤0.06–0.125	≤0.06	≤0.06	100, 100, 100
	P. magnus	≤0.06–1	0.125	0.25	100, 100, 100
	P. anaerobius	≤0.06–0.5	0.25	0.5	100, 100, 100
	Mobiluncus spp.	≤0.06–0.125	≤0.06	0.125	100, 100, 100
Metronidazole	*P. bivia*	0.125–2	0.25	0.5	100, 100, 100
	P. asaccharolytica	≤0.06–4	≤0.06	4	84, 100, 100
	P. asaccharolyticus	0.25–1	1	1	100, 100, 100
	P. magnus	0.125–2	0.5	1	100, 100, 100
	P. anaerobius	0.125–0.5	0.25	0.5	100, 100, 100
	Mobiluncus spp.	16–>128	32	>128	0, 0, 69
Cefotiam	*P. bivia*	1–128	32	64	
	P. asaccharolytica	≤0.06–16	≤0.06	2	
	P. asaccharolyticus	≤0.06–4	0.5	1	
	P. magnus	≤0.06–8	2	4	
	P. anaerobius	0.25–16	1	16	
	Mobiluncus spp.	1–4	2	4	
Ceftazidime	*P. bivia*	1–128	32	128	
	P. asaccharolytica	≤0.06–8	0.125	1	
	P. asaccharolyticus	≤0.06–32	0.25	0.5	
	P. magnus	≤0.06–64	8	16	
	P. anaerobius	0.5–16	1	16	
	Mobiluncus spp.	8–64	16	64	
Ofloxacin	*P. bivia*	≤0.06–8	4	8	
	P. asaccharolytica	0.25–4	1	1	
	P. asaccharolyticus	≤0.06–16	4	8	
	P. magnus	0.25–64	0.5	4	
	P. anaerobius	0.5	0.5	0.5	
	Mobiluncus spp.	1–4	1	1	
Cefaclor	*P. bivia*	0.25–>128	128	>128	
	P. asaccharolytica	≤0.06–8	0.125	4	
	P. asaccharolyticus	0.125–4	1	2	
	P. magnus	≤0.06–>128	8	16	
	P. anaerobius	0.125–4	1	2	
	Mobiluncus spp.	2–8	4	8	

[a] β-Lactamase-positive isolates were identified by the cefinase disk method. The numbers of isolates recovered from the pregnant Japanese women were as follows: *P. bivia*, 12; *P. asaccharolytica*, 9; *P. asaccharolyticus*, 7; *P. magnus*, 11; *P. anaerobius*, 3; and *Mobiluncus* spp., 2.

[b] At 1 dilution below the breakpoint, at the breakpoint, and at 1 dilution above the breakpoint. The breakpoints of clindamycin, imipenem, cefmetazole, amoxicillin, amoxicillin-clavulanate, and metronidazole were 2, 4, 16, 4, 4-2, and 8 μg/ml, respectively. Breakpoints of the other agents for anaerobes have not yet been approved by the National Committee for Clinical Laboratory Standards.

isolates. Metronidazole was active against more than 98% of the isolates (except *Mobiluncus* spp.) and had MIC_{90}s of 0.5 to 4 μg/ml. Cefotiam, ceftazidime, and ofloxacin were variably effective. Cefaclor was the least effective agent.

Among the gram-negative rods investigated in this study, all isolates of *P. bivia* except one produced β-lactamase. The presence of β-lactamase-producing bacteria in the vaginal flora is of concern because of the potential for indirect pathogenicity resulting from the secretion of free β-lactamase at this site (1). Amoxicillin is considered safe for use during pregnancy, but in clinical studies it has been reported to be ineffective in pregnant women (2). Its failure may be due to the presence of β-lactamase-producing *P. bivia* or *Prevotella* spp. in the vagina, as we found in this study. As it is shown in the present study that amoxicillin-clavulanate had a MIC_{90} lower than that of amoxicillin alone, the combination use of a β-lactamase inhibitor and amoxicillin may solve this problem. Presently, the most effective agent for the treatment of BV is metronidazole, which has excellent activity against most anaerobic bacteria. However, mutagenic and carcinogenic effects of metronidazole are of concern especially during pregnancy. Furthermore, *Mobiluncus* spp., which are highly associated with BV (4, 9), are usually resistant to this agent. Although none of the isolates tested except *Mobiluncus* spp. were found to be resistant to metronidazole, slightly higher MIC ranges of metronidazole were detected against isolates from the Thai than from the Japanese pregnant women (i.e., the MIC ranges against Thai isolates were higher than those against Japanese isolates for *P. asaccharolytica* and *Peptostreptococcus magnus* [$\leq$0.06 to 4 versus $\leq$0.06 μg/ml and 0.125 to 2 versus 0.25 μg/ml, respectively]). This may reflect differences in the frequency of use of this antibiotic since metronidazole is prohibited for the treatment of anaerobic infections in Japan but not in Thailand. However, the small number of Japanese isolates in the present study may also affect this finding. Clindamycin has recently been shown to be equally if not more effective than metronidazole. Clindamycin has been found to be effective in limited clinical trials with pregnant women (7, 11). Considering the spectrum of organisms involved in BV, several of the newer penicillins as well as cephalosporins, penicillin and β-lactamase inhibitor combinations, and quinolones may be successful in the treatment of BV. Our study found that imipenem, cefmetazole, and amoxicillin-clavulanate were highly active against all isolates. The use of these agents is promising for the treatment of BV during pregnancy. BV should be detected and treated early in pregnancy to reduce the risks of chorioamnionic infection and prematurity (3, 7). Further study is certainly warranted for the future clinical trials needed to evaluate whether treatment of BV during pregnancy will reduce these adverse outcomes.

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