ANTIBIOTIC SUSCEPTIBILITY AND BETA-LACTAMASE PRODUCTION OF FAECAL AND CLINICAL ISOLATES OF BACTEROIDES SPECIES IN SINGAPORE

T W Soong
B Ho
E H Yap

SYNOPSIS

Eight faecal and 22 clinical isolates were identified as Bacteroides species by the API 20A microsystem and gas liquid chromatography (GLC).

Studies of antibiotic sensitivity showed that all the issolates were sensitive to chloramphenicol. More than 86% of the 30 isolates were resistant to B-lactam antibiotics, carbenicillin, ampicillin and penicillin G. Varid sensitivities against 5 other antibiotics were also determined. The minimum inhibitory concentrations (MICs) against the 8 antibiotics ranged from <0.125 ug/ml to >128.0 ug/ml. Beta-lactamase was produced by 29 out of 30 isolates. All clinical isolates were beta-lactamase producers.

In experiments on resistance to heavy metal ions, all 30 isolates were resistant to cadmium. More than 93% were resistant to mercury and zinc but resistance to lead and arsenate was variable.

INTRODUCTION

Bacteroides are strict anaerobic bacteria, Gram-negative non-spore forming bacilli. They are chemoorganotrophs, metabolise carbohydrates and peptone.

In man and other animals, many species of Bacteroides are opportunistic pathogens, not normally occuring outside the body except perhaps in sewage.

Bacteroides can cause severe infections mostly in proximity to the mucosal surfaces. Some of the infections caused by Bacteroides respond to surgical drainage alone, or, together with chemotherapy.

Gorbach and Barlett (1) reported that the Bacteroides fragilis group comprising B. fragilis, B. thetaiotaomicron, B. vulgatus, B. ovatus and B. distasonis were resistant to the B-lactam antibiotics and 60% were resistant to tetracycline. Clindamycin is still useful as a therapeutic agent as few resistant isolates have been documented (2). Chloramphenicol is one of the most effective drugs for the treatment of anaerobic infections, but an increasing number of Bacteroides have failed to respond to the drug. Significant resistance of Bacteroides to chloramphenicol has been recorded. Cefoxitin still shows high activity, in vitro, against most anaerobes, including B. fragilis.

Beta-lactamase is the primary cause of penicillin and cephalosporin resistance in bacteria. There are many different B-lactamases and they vary considerably with respect to sustrate specificity. Penicillinase and cephalosporinase are B-lactamases that hydrolyse the B-lactam bond of penicillin and cephalosporin respectively.

Wallace et al (3) found that 7 of their 8 plasmid-containing Bacteroides species were resistant to cadmium which may indicate a possible association between cadmium resistance and a specific plasmid.

The present study is to investigate the effect of various antibiotics against local strains of Bacteroides. Beta-lactamase production and resistance of these strains to heavy metal ions were studied.

Microbiology Department
National University of Singapore
Kent Ridge
Singapore 0511

T W Soong, B.Sc. (Hons)
Research Assistant

B Ho, Ph.D.
Lecturer

E H Yap, Ph.D.
Senior Lecturer

MATERIALS AND METHODS

Source of Bacteroides Species: Twenty-two clinical isolates were obtained from the Pathology Laboratory, Singapore General Hospital. Eight species were isolated from human faeces of staff members of the Microbiology Department, National University of Singapore.

Kanamycin-vancomycin blood agar, KVA (4), was the selective medium used to isolate Bacteroides from the faeces.

Growth and Maintenance of Bacteroides: Bacteroides were grown on Schaedler agar (SA) consisting of Schaedler broth (Difco) with 1.5% technical agar no. 3 (Oxoid), supplemented with 10 ug/ml vitamin K_1, 5 ug/ml haemin and 5% defibrinated rabbit blood.

The organisms were maintained in Schaedler broth foetal calf serum (SBFS) comprising 50% Schaedler broth and 50% foetal calf serum (Gibco) and deep frozen at -70°C.

All the cultures were incubated in an anaerobic chamber (Forma Scientific) at 35° in an atmosphere of 85% N_2, 10% H_2 and 5% CO_2.

Identification: The morphology, aerotolerance, motility, growth in 20% bile and Gram-nature of the organisms were studied. The organisms were identified using the API 20A microsystem and GLC (5).

Additional tests such as fluorescence under long-wave UV light and black pigment production (up to 21 days) were carried out to identify the B. melaninogenicus group.

ANTIBIOTIC SUSCEPTIBILITIES

Disc Sensitivity Test. A modified Kirby-Bauer (6) method was used. Two drops of overnight SBFS culture were diluted in 2 ml sterile 0.89% saline and swabbed in 3 planes on the SA surface.

The antibiotic discs used were carbenicillin 100 ug, penicillin G 10 units, ampicillin 10 ug, cefoxitin 30 ug, chloramphericol 30 ug, clindamycin 2 ug, erythromycin 10 ug and tetracycline 30 ug.

Minimum Inhibitory Concentrations. The agar dilution method of Thornsberry and Swenson (7) was employed using a range of 0.125 ug/ml to 128.0 ug/ml of antibiotics. The control organisms were E. coli ATCC 25922 and S. aureus ATCC 25923. A Denley Multipoint Inoculator A400 was used. Control plates without anti-biotics were used to ensure the viability of the organisms. Two drops of overnight culture were diluted in 2 ml of 0.89% saline and spotted on dry pre-reduced SA plates.

Resistance to Heavy Metal Ions. The heavy metal ions and their concentrations were selected according to a study made by Novick and Roth (3) on the plasmid-linked resistance to inorganic salts in S. aureus.

The metal ions investigated were arsenate (0.1M), cadmium (0.001M), lead (0.1M), mercury (0.001M) and zinc (0.1M). Results were read after 24 hours incubation. Sensitive strains showed clear zones of inhibition whilst resistant strains grew around the discs.

Detection of B-lactamase. The chromogenic cephalosporin spot test of Montgomery et al (9) was used. Chromogenic cephalosporin solution was prepared by dissolving 5.0 mg of the compound (87/312, Nitrocefin, Glaxo) in 0.5 ml of dimethyl sulphoxide and diluted in 9.5 ml of 0.1M phosphate buffer (pH 7.0). Colonies grown on SA or Mueller-Hinton Agar (MHA) were picked and applied onto paper disc soaked with the chromogenic cephalosporin solution. Any change in colour was observed within 10 mins. A change in colour from yellow to red indicated B-lactamase production. Klebsiella K_1 and S. aureus ATCC 25923 were used as the positive and negative controls respectively.

QUANTITATIVE DETERMINATION OF PENICILLINASE ACTIVITY

Test organisms, grown overnight at 35°C in 20 ml of Brain Heart Infusion (BHI) broth, were harvested, washed with 0.89% saline and resuspended in 1.0 ml 0.05M phosphate buffer (pH 7.0). The cells were disrupted by sonication to release B-lactamase and then centrifuged at 12,000 rpm for 20 mins. Bijou bottles containing 1 ml of penicillin G at a concentration of 10 ug/ml were incubated with ten-fold dilutions of the supernatant at room temperature for 1 h. The amount of penicillin G left unhydrolysed was measured by bioassay, with a range of antibiotic standards from 1.25 ug/ml to 10 ug/ml. The hydrolysis of the penicillin G by the B-lactamase extract was calculated from the amount of antibiotics that was hydrolysed (10).

RESULTS

Isolation and Identification. The 22 clinical isolates were obtained in pure culture from the Pathology Laboratory (PL). The 8 faecal isolates were successfully isolated and purified on KVA. All the isolates were Gram-negative, non-motile rods.

Only B. assacharolyticus (PL4) failed to grow in 20% bile. PL4 gave a brick-red fluorescence under long-wave UV light. This fluorescence diminished completely by the fourth day when the colonies turned black. The absorption peak of the red pigment of B. assacharolyticus was measured by a spectrophotometer (Pye Unicam PU 8800) and found to peak at 410.6 nm (Fig. 1).

The isolates were positively identified with the API 20A microsystem (Table 1). Only the GLC result for B. assacharolyticus (PL4)) was helpful in confirming the identification of this isolate (Fig. 2).

TABLE 1.
FAECAL AND CLINICAL ISOLATES OF BACTEROIDES

TEST ISOLATES	SPECIES	ORIGIN
W1	B. thetaiotaomicron	Human faeces
W3	B. ovatus	Human faeces
W4	B. ovatus	Human faeces
A8	B. vulgatus	Human faeces
M10	B. vulgatus	Human faeces
J11	B. thetaiotaomicron	Human faeces
J12	B. ovatus	Human faeces
J13	B. eggerthii	Human faeces
*PL1	B. fragilis	Wound swab
PL2	B. fragilis	Groin swab
PL3	B. fragilis	Wound swab
PL3	B. assacharolyticus	Pus from vagina
PL5	B. thetaiotaomicron	— not known —
PL6	B. fragilis	Pleural fluid
PL7	B. ovatus	Abscess cavity
*PL8	B. fragilis	Pus from right leg
PL9	B. fragilis	Swab from finger
PL10	B. fragilis	Pus from abscess
PL11	B. fragilis	Swab from big toe
PL12	B. fragilis	Perineal abscess
PL13	B. fragilis	Perineal abscess
PL14	B. fragilis	Blood
PL15	B. fragilis	Wound swab
PL16	B. fragilis	Wound swab
PL17	B. fragilis	High vagina swab
PL18	B. fragilis	Wound swab
PL19	B. thetaiotaomicron	Wound swab
PL20	B. fragilis	High vagina swab
PL21	B. fragilis	Perineal abscess
PL22	B. fragilis	Pus from brain

*PL — Pathology Laboratory, Singapore General Hospital.

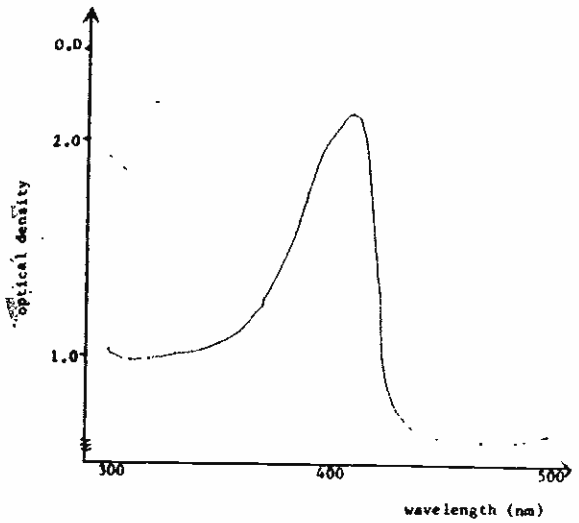

Fig. 1 Measurement of the absorption peak of pigment produced by B. assacharolyticus (410.6 nm).

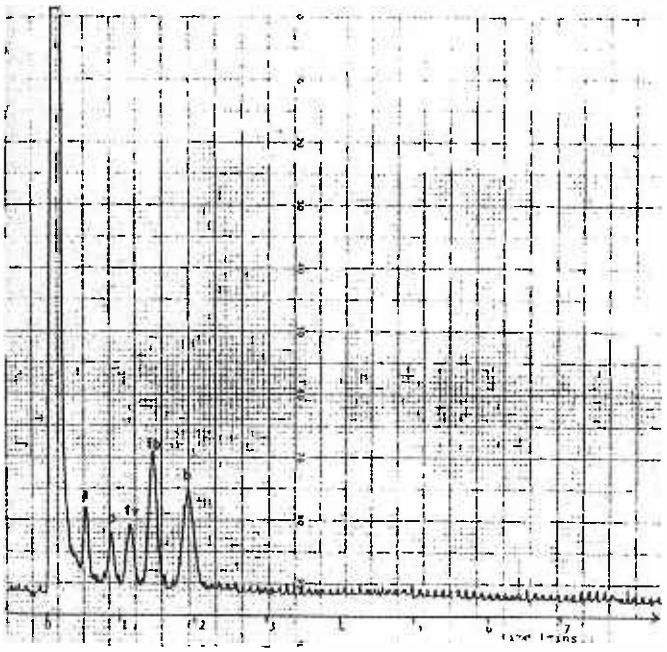

Fig. 2 Profile of volatile fatty acids by GLC analysis of B. assacharolyticus (PL4)

(Conditions: Injection port temperature 160 C
Detector temperature 160 C
Column temperature 160 C
Bridge current 200 mA
Attenuator at "1"
Chart speed 2 cm/min)

a = acetic acid
p = propionic acid
iv = isovaleric acid
ib = isobutyric acid
b = butyric acid

ANTIBIOTIC SUSCEPTIBILITIES

Disc Sensitivity Test. All 30 isolates were sensitive to chloramphenicol (Table 2). The susceptibilites of these isolates to the other 7 antibiotics were variable.

Minimum Inhibitory Concentrations. The MICs ranged from <0.125 ug/ml to >128.0 ug/ml (Table 3). All 30 isolates showed an MIC of ≤ 4.0 ug/ml for chloramphenicol whilst 24 isolates demonstrated an MIC of ≤ 4.0 ug/ml for cefoxitin. Of the 22 clinical isolateds, 15 showed MICs of ≥ 128.0 ug/ml for erythromycin and 10 showed MICs of ≥ 128.0 ug/ml for tetracycline. The MIC for carbenicillin, penicillin G, ampicillin and erythromycin of B. fragilis (PL1) was >128.0 ug/ml. B. fragilis (PL2) was the only isolate to show considerable resistance to clindamycin with an MIC of 128.0 ug/ml. B. eggerthii (J13) showed low MICs for carbenicillin (0.5 ug/ml), penicillin G (0.25 ug/ml) ampicillin (0.25 ug/ml),clindamycin (0.25 ug/ml) and cefoxitin (<0.125 ug/ml). B. assacharolyticus (PL4) showed MICs of <0.125 ug/ml for cefoxitin, clindamycin and erythromycin. Isolates PL4 and A8 were sensitive to all 8 antibiotics.

TABLE 2.
RESULTS OF ANTIBIOTIC DISC SENSITIVITY TEST OF BACTEROIDES

Isolates \ Antibiotics	PY	P	PN	FOX	C	DA	E	Te
W1	S	S	S	S	S	S*	S*	R
W3	S	S*	R	R	S	R	R	R
W4	S	R	S*	R**	S	R	S*	S
A8	S	S	S	S	S	S	S	S
M10	S	R	S*	R	S	S	S	R
J11	S	R	R	R**	S	R	R	R**
J12	S	R	R	S	S	R	S	R**
J13	S	S	S	S	S	S	R	S
PL1	R	R	R	S	S	S*	R	R
PL2	S	R	S*	S	S	R	R	R
PL3	S	R	R	R	S	S*	R	S
PL4	S	S	S	S	S	S	S	S
PL5	S	S	S	S	S	S	S*	S
PL6	S	R	S	S	S	S*	S*	S
PL7	S	R	R	R	S	S*	S*	S
PL8	S	R	R	S	S	S	R	R
PL9	S	R	R	S	S	S*	R	R
PL10	S	R	R	S	S	S	R	R
PL11	S	R	R	S	S	S	R	R
PL12	S	R	R	S	S	S	R	R
PL13	S	R	R	S	S	R	R	R
PL14	S	R	R	S	S	R	R	R
PL15	S	R	R	S	S	S	R	S
PL16	S	R	R	S	S	S	R	S
PL17	S	R	R	S	S	R	R	R
PL18	S	R	R	S	S	S	R	S
PL19	S	S	S	S	S	S	R	R
PL20	S	S*	S*	S	S	S	R	R
PL21	S	R	R	S	S	S	R	R
PL22	S	R	R	S	S	S	R	S*

Key R = resistant S = sensitive
carbenicillin PY 100 ug chloramphenicol C 30 ug
penicillin G P 10 units clindamycin DA 2 ug
ampicillin PN 10 ug erythromycin E 10 ug
cefoxitin FOX 30 ug tetracycline Te 30 ug

* should be resistant (according to MIC)
** should be sensitive (results)

TABLE 3.
RESULTS OF MICS FOR BACTEROIDES AND CONTROL ORGANISMS

ANTIBIOTICS (ug/ml) / ISOLATES	PY	P	PN	FOX	C	DA	E	Te
W1	16.0	4.0	16.0	1.0	2.0	16.0	64.0	64.0
W3	128.0	32.0	64.0	16.0	2.0	8.0	64.0	64.0
W4	128.0	64.0	32.0	16.0	2.0	4.0	64.0	1.0
A8	2.0	8.0	4.0	2.0	1.0	<0.125	16.0	32.0
M10	64.0	16.0	16.0	8.0	2.0	0.5	16.0	64.0
J11	128.0	32.0	64.0	16.0	2.0	8.0	32.0	16.0
J12	64.0	32.0	32.0	8.0	1.0	8.0	8.0	16.0
J13	0.5	0.25	0.25	<0.125	1.0	0.25	16.0	32.0
PL1	>128.0	>128.0	>128.0	4.0	2.0	8.0	>128.0	64.0
PL2	128.0	32.0	32.0	4.0	2.0	128.0	128.0	64.0
PL3	32.0	16.0	16.0	4.0	2.0	4.0	128.0	32.0
PL4	32.0	8.0	8.0	<0.125	0.5	<0.125	<0.125	16.0
PL5	32.0	2.0	4.0	1.0	2.0	0.5	64.0	16.0
PL6	64.0	16.0	16.0	2.0	1.0	4.0	64.0	0.5
PL7	128.0	32.0	32.0	4.0	2.0	8.0	128.0	1.0
PL8	64.0	32.0	64.0	16.0	4.0	2.0	128.0	>128.0
PL9	32.0	8.0	16.0	4.0	4.0	16.0	128.0	128.0
PL10	64.0	8.0	32.0	4.0	4.0	4.0	64.0	64.0
PL11	64.0	8.0	32.0	4.0	4.0	4.0	64.0	64.0
PL12	16.0	8.0	16.0	4.0	4.0	2.0	64.0	>128.0
PL13	64.0	16.0	32.0	4.0	4.0	16.0	>128.0	>128.0
PL14	64.0	16.0	32.0	4.0	4.0	16.0	>128.0	>128.0
PL15	64.0	16.0	32.0	4.0	4.0	2.0	128.0	2.0
PL16	64.0	16.0	32.0	4.0	2.0	2.0	128.0	2.0
PL17	32.0	8.0	16.0	4.0	4.0	16.0	128.0	>128.0
PL18	32.0	16.0	32.0	4.0	2.0	2.0	128.0	2.0
PL19	4.0	0.25	2.0	2.0	2.0	4.0	32.0	128.0
PL20	32.0	32.0	32.0	4.0	2.0	4.0	128.0	128.0
PL21	16.0	16.0	16.0	4.0	2.0	0.5	128.0	128.0
PL22	16.0	32.0	32.0	4.0	2.0	4.0	128.0	>128.0
E. coli 25922	<0.125	<0.125	<0.125	<0.125	1.0	0.25	2.0	0.25
S. aureus ATCC 25923	0.5	<0.125	<0.25	<0.125	1.0	0.25	1.0	0.25

TABLE 4.
RESISTANCE OF BACTEROIDES TO HEAVY METAL IONS

TESTS / ISOLATES	$Cd^{2}+$ (0.001M)	$Hg^{2}+$ (0.001M)	$Zn^{2}+$ (0.1M)	$Pb^{2}+$ (0.1M)	$ArO_4^{3}-$ (0.1M)
W1	R	R	S	S	S
W3	R	R	R	R	R
W4	R	R	R	R	R
A8	R	R	R	S	S
M10	R	R	R	S	S
J11	R	R	R	R	S
J12	R	R	R	R	R
J13	R	R	R	S	S
PL1	R	R	R	S	S
PL2	R	R	R	S	S
PL3	R	R	R	R	S
PL4	R	S	R	S	S
PL5	R	S	R	S	S
PL6	R	R	R	R	S
PL7	R	R	R	R	R
PL8	R	R	R	R	S
PL9	R	R	R	R	S
PL10	R	R	R	R	S
PL11	R	R	R	R	S
PL12	R	R	R	R	S
PL13	R	R	R	R	S
PL14	R	R	R	R	S
PL15	R	R	R	R	S
PL16	R	R	R	R	S
PL17	R	R	R	R	S
PL18	R	R	R	R	S
PL19	R	R	R	R	S
PL20	R	R	R	R	S
PL21	R	R	R	R	S
PL22	R	R	- R	R	S

TABLE 5.
B-LACTAMASE ACTIVITY OF BACTEROIDES

TESTS / ISOLATES	B-LACTAMASE PRODUCTION	MIC PEN. G (ug/ml)	RATE OF HYDROLYSIS OF PEN. G (ug/ml/hr)
W1	+	4.0	2.4×10^{2}
W3	+	32.0	3.6×10^{2}
W4	+	64.0	4.1×10^{2}
A8	+	8.0	2.5×10^{2}
M10	+	16.0	4.3×10^{2}
J11	+	32.0	4.0×10^{2}
J12	+	32.0	4.0×10^{2}
J13	–	0.25	0
PL1	+	128	$>2.0 \times 10^{4}$
PL2	+	32	4.8×10^{2}
PL3	+	16.0	4.3×10^{2}
PL4	+	8.0	2.9×10^{2}
PL5	+	2.0	2.3×10^{2}
PL6	+	16.0	4.6×10^{2}
PL7	+	32.0	3.8×10^{2}
PL8	+	32.0	9.7×10^{2}
PL9	+	8.0	1.8×10^{2}
PL10	+	8.0	4.7×10^{2}
PL11	+	8.0	1.0×10^{2}
PL12	+	8.0	3.9×10^{2}
PL13	+	16.0	3.5×10^{2}
PL14	+	16.0	2.5×10^{2}
PL15	+	16.0	4.4×10^{2}
PL16	+	16.0	2.3×10^{2}
PL17	+	8.0	2.9×10^{2}
PL18	+	16.0	9.7×10^{2}
PL19	+	0.25	2.5×10^{2}
PL20	+	32.0	6.1×10^{2}
PL21	+	16.0	4.7×10^{2}
PL22	+	32.0	6.1×10^{2}
Klebsiella K1	+		
S. aureus ATCC 25923	–		

Resistance to Heavy Metal Ions (Table 4). All 30 isolates were resistant to cadmium. all except B. thetaiotaomicron (W1) were sensitive to mercury, whereas, the other 28 isolates were resistant. All the 4 B. ovatus strains (W3, W4, J12 and PL7) were resistant to arsenate.

Determination of B-lactamase Production (Table 5). By using the chromogenic cephalosporin spot test, 29 out of the 30 isolates were detected as B-lactamase producers. The red colour produced by the B-lactamase producers could be more easily observed when colonies were picked from mHA plates than from SA plates.

In the study of hydrolysis of penicillin G, 29 isolates were found to be penicillinase producers. Twenty six of these penicillinase producers were resistant to $\geqslant$8.0 ug/ml of penicillin G. B. fragilis (PL1) with the highest MIC of >128.0 ug/ml for penicillin G showed the highest penicillinase activity of $>2.0 \times 10^4$ ug penicillin G hydrolysed/ml enzyme extract/hour. This penicillinase activity was at least ten-fold greater than the other 28 penicillinase producers. The rate of hydrolysis of penicillin G for these other 28 penicillinase producers ranged from 1.0×10^2 to 9.7×10^2 ug penicillin g hydrolysed/ml enzyme extract/hour.

B. eggerthii (J13) with MICs of <0.125 ug/ml for cefoxitin and 0.25 ug/ml for penicillin G was found to be a non-B-lactamase producer.

DISCUSSION

The API 20A microsystem was found to be useful for the differentiation and identification of the genus Bacteroides. It was convenient and easy to use.

GLC was more helpful in speciating the B. melaninogenicus group which comprises B. melaninogenicus, B. assacharolyticus and B. intermedius. The different volatile fatty acids produced by each species of this group made speciation easy.

B. fragilis is by far the most prevalent in local infections as 18 out of 22 clinical isolates belong to this species. B. vulgatus and B. thetaiotaomicron, as mentioned by Davis et al (11), are usually numerically dominant in faecal isolates. In this study 50% of the faecal isolates belong to these two groups.

Thornsberry and Swenson (7) and Sutter and Washington (12) recommended that the agar disc diffusion methods of Kirby-Bauer should not be used for anaerobes. This was because the Kirby-Bauer technique was intended only for rapidly growing aerobic or facultatively anaerobic bacteria. In this study, only about 9.0% of the disc sensitivity test results showed no correlation with the MIC results. The disc sensitivity test could therefore be used as a guide for determining the range of concentration of antibiotics to be used for the estimation of MICs.

The B-lactam antibiotics, carbenicillin, ampicillin and penicillin G were ineffective against Bacteroides in vitro as more than 86% of the isolates were resistant (MIC $\geqslant$8.0 ug/ml). This was due to the production of B-lactamase by the organisms

Contrary to the report by Willis et al (13), all the 30 Bacteroides species tested were sensitive to chloramphenicol (MIC $\leqslant$4.0 ug/ml).This would suggest that chloramphenicol may still be useful in the treatment of Bacteroides infections in Singapore. The main objection to the use of chloramphenicol is its potential activity as a bone marrow depressant and should therefore not be recommended for common use (13).

B. fragilis (PL2) was the only isolate with significant resistance to clindamycin in vitro (MIC = 128mg). Of the other 29 isolates, about 80% showed MIC of $\leqslant$ 8.0 ug/ml for clindamycin. Even though clindamycin has been found to be effective against Bacteroides in vitro, its usage for the treatment of minor Bacteroides infections should be discouraged since its side effects include diarrhoea,a marked disturbance of the gut flora and pseudomembranous colitis (13).

Tetracycline was once regarded as the antibiotic of choice against Bacteroides infections but now half of the Bacteroides were reported to be resistant (11).This study found that 80% of the organisms tested were resistant to tetracycline (MIC $\geqslant$16.0 ug/ml) indicating that tetracycline would, in most cases, not be useful in treating Bacteroides infections.

All the organisms were sensitive to cefoxitin (MIC $\leqslant$ 16.0 ug/ml). This result is in agreement with Willis et al (13) that cefoxitin is highly active in vitro against Bacteroides.

Since more than 76% of the test organisms were resistant to erythromycin (MIC $\geqslant$64.0 ug/ml), this antibiotic should not be recommended for treatment of Bacteroides infections.

The chromogenic cephalosporin spot test was both simple and reproducible. Out of 30 organisms tested, 9 were cephalosporinase producers. These 29 cephalosporinase producers were also penicillinase producers (Table 5) indicating that Bacteroides which produce cephalosporinase also produce penicillinase.

Generally, a Bacteroides species having a higher MIC for penicillin G also showed a higher rate of hydrolysis for this antibiotic. This indicated that Bacteroides which showed a higher MIC for penicillin G would produce quantitatively more penicillinase to hydrolyse the antibiotic.

There was a correlation between penicillin resistance of Bacteroides species and penicillinase production since 26 of th 29 penicillinase producers were also penicillin resistant (MIC $\geqslant$8.0 ug/ml).

Plasmids have been isolated from 13 of the Bacteroides species. Work is now in progress to determine any possible association between these plasmids and resistance to antibiotics and heavy metal ions.

ACKNOWLEDGEMENT

This work was supported by grants from the Singapore Turf Club and the Shaw Foundation.

We are grateful to Mrs. P.Y. Tan of the Pathology Department, Singapore General Hospital, for the clinical isolates of Bacteroides and Miss Gurupatham Caroline for typing this manuscript.

REFERENCES

1. Gorbach SL, Barlett JG: Anaerobic infections. N Engl J Med 1974; 290, 1177-84, 1237-54, 1289-94.
2. Tally FP, Syndman DR, Gorbach SL, Malamy MH: Plasmid-mediated transferable resistance to clindamycin and erythromycin in Bacteroides fragilis. J of Inf Dis 1979; 139: 83-8.
3. Wallace BL, Bradley JE, Rogolsky M: Plasmid analyses in clinical isolates of Bacteroides fragilis and other Bacteroides species. J Clin Microbiol 1981; 14: 383-8.
4. Sutter VL, Citron DM, Finegold SM: Wadsworth anaerobe bacteriology manual. St. Louis, Missouri, 1980.
5. Holdeman LV, Cato EP, Moore WEC: Anaerobe laboratory manual, ed. 4. Blacksbury Va., Vikrginia Polytechnic Institute and State University, 1977.
6. Bauer AW, Kirby WMM, Sherris JC, Turk M: Antibiotic susceptibility testing by a standardised single disk method. Am J Clin Pathol 1966; 45: 493-6.
7. Thornsberry C,Swenson JM: Antimicrobial susceptibility testing of anaerobes. Am J Clin Pathol 1978; 9: 43-8.
8. Novick RP, Roth C: Plasmid-linked resistance to inorganic salts in Staphylococcus aureus. J of Bacteriol 1968; 95: 1335-42.
9. Montgomery K, Raymundo L, Drew WL: Chromogenic cephalosporin spot test to detect beta-lactamase in clinically significant bacteria. J of Clin Microbiol 1979; 9: 205-7.
10. Chen HY, Williams JD: Temocillin compared to ampicillin against Haemophilus influenzae and with other penicillins against intestinal aerobic gram-negative rods. J of Antimicrobial Chemotherapy 1982; 10: 279-87.
11. Davis BD, Dulbecco R, Eisen HN, Ginsberg HS: Microbiology. USA, Harper and Row, 1981.
12. Sutter VL, Washington JA: Susceptibility testing of anaerobes. In: Lennett EH, Spaulding EH, Truant JP, eds. Manual of Clinical Microbiology. Washington DC: American Society for Microbiology, 1974: 436-8.
13. Willis AT, Jones PH, Reilly S: Management of anaerobic infections, prevention and treatment. England, Research Studies Press, 1981.