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RESISTANCE TO VARIOUS CHEMOTHERAPEUTICS OF BACTEROIDES STRAINS ISOLATED FROM CLINICAL SPECIMENS IN ISTANBUL

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SUMMARY

Resistance to various chemotherapeutics of 201 *Bacteroides* strains isolated from clinical specimens was reported. The term *Bacteroides* was used as it was used before the creation of *Prevotella* and *Porphyromonas* genera, so includes strains of these genera as well. Ornidazole, amoxicillin+clavulanic acid, ampicillin+sulbactam and chloramphenicol were found to be the most effective chemotherapeutics to which only 4-14 % of the strains were resistant. Tetracycline, cefuroxime, cephalothin, ampicillin, penicillin G and ceftazidime were found to be effective on less than half of the strains.

ÖZET

İstanbul'da muayene maddelerinden izole edilen Bacteroides suşlarının çeşitli kemoterapötiklere direnci.

Muayene maddelerinden izole edilen 201 *Bacteroides* suşunun çeşitli kemoterapötiklere direnç oranları bildirilmiştir. *Bacteroides* deyimi *Prevotella* ve *Porphyromonas* cinslerinin oluşturulmasından önceki bu cinslere ait suşları da kapsayan anlamı ile kullanılmıştır. Ornidazol, amoksisilin+klavulanik asit, ampisilin+sulbaktam ve kloramfenikol, suşların sadece % 4-14'ünün direnç gösterdiği en etkili kemoterapötikler olmuştur. Tetrasiklin, sefuroksim, sefalotin, ampisilin, penisilin G ve seftazidim ise suşların yarıdan azına etkili bulunmuştur.

INTRODUCTION

Bacteroides species are important human pathogens. These organisms are usually considered together because they appear to be more resistant to antibiotics than other anaerobic bacteria.

In this study, resistance to various chemotherapeutics of 201 strains of *Bacteroides* spp. isolated from clinical specimens was reported.

MATERIALS AND METHODS

Bacteroides strains were isolated and identified in routine methods. The term *Bacteroides* was used as it was used before the recent classification that proposes some new bacterial genera for Gram-negative, rod-shaped, nonsporeforming anaerobic bacilli; thus, the strains from *Prevotella* and *Porphyromonas* genera according to the new taxonomy were also included. Broth-disk elution method (11, 16) was used for susceptibility testing in tryptic soy broth supplemented with yeast extract, cysteine hydrochloride, vitamin K_1 and hemin. Desired concentrations of

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chemotherapeutics were obtained in 5 ml broth by dropping appropriate numbers of disks with a known amount of the chemotherapeutic as shown in table 1. Each tube was inoculated with one drop of 24-48 hours old culture adjusted to the turbidity of McFarland No 0.5 and incubated in anaerobic conditions in 37°C for 48 hours. Any growth within 48 hours was accepted as the sign of resistance. A broth tube with no chemotherapeutic was also inoculated for each strain as growth control. Only 60 strains were tested for 4 chemotherapeutics shown in table 2 while all of 201 strains were tested for the remaning 15 chemotherapeutics.

Table 1. Preparation of broth-disk tubes for anaerobic susceptibility testing.

Antimicrobial agents	Contents of each disk (µg)	No.of disks in 5 ml medium	Final concentration (µg/ml)
Penicillin G	10 U	1	2 U
Ampicillin	10	2	4
Azidocillin	10	4	8
Mezlocillin	75	4	60
Cephalothin	30	1	6
Cefuroxime	30	3	18
Ceftriaxone	30	3	18
Cefoperazone	75	2	30
Cefotaxime	30	3	18
Ceftazidime	30	3	18
Ceftizoxime	30	3	18
Amoxicillin+clavulanic acid	30 (20+10)	1	6
Ampicillin+sulbactam	20 (10+10)	8	32
Erythromycin	15	1	3
Clindamycin	2	4	1.6
Chloramphenicol	30	2	12
Rifampicin	30	1	6
Tetracycline	5	3	3
Ornidazole	40	1	8

RESULTS

The numbers and percentages of resistant strains for each chemotherapeutic were shown in table 2. As it is seen from the table, higher percentages of resistance were observed for ceftazidime (70 %), penicillin G (65 %), ampicillin (63 %), cephalothin (58 %), cefuroxime (55 %), tetracycline (52 %), and lower percentages for ornidazole (4 %), amoxicillin+clavulanic acid (9 %), ampicillin+sulbactam (13 %), and chloramphenicol(14 %).

DISCUSSION

A considerable portion of anaerobic infections are caused by *Bacteroides* species. There are some reports indicating the increase of antibiotic resistance in *Bacteroides*. For example Pelaez et al (22) reported that the resistance rates in *B.fragilis* strains increased from 62 % in 1988 to 96 % in 1990 for penicillin, from 0 % to 45 % for ticarcillin, from 61 % to 84 % for tetracycline, from 36 % to 55 % for clindamycin, from 2 % to 11 % for metronidazole in Spain. Hurlbut et al (14) found a novel beta-lactamase in a *B.distasonis* strain that enabled the bacterium to

Table 2. Resistance in 201 Bacteroides strains to various chemotherapeutics.

Antimicrobial agent	Number of resistant strains	Resistance rates (%)
Penicillin G	131	65
Ampicillin	126	63
Azidocillin	77	38
Mezlocillin	60	30
Cephalothin	116	58
Cefuroxime	110	55
Ceftriaxone	85	42
Cefoperazone	69	34
Cefotaxime	73	36
Ceftazidime* (n=60)	42	70
Ceftizoxime* (n=60)	18	30
Ampicillin+sulbactam* (n=60)	8	13
Amoxicillin+clavulanic acid	19	9
Erythromycin	94	47
Chloramphenicol	29	14
Tetracycline* (n=60)	31	52
Clindamycin	60	30
Rifampicin	57	28
Ornidazole		

*Antimicrobial agents tested for 60 strains.

become resistant to all commonly used beta-lactam antibiotics including imipenem, and in conjunction with significant permeability barrier to clavulanic acid and sulbactam, resulted in loss of synergism between beta-lactamase inhibitors and beta-lactam antibiotics. On the other hand, O'Keefe et al (21) found lower geometric means of MICs for cefoxitin and cefotaxime in *B.fragilis* strains isolated in 1986 than those in strains isolated in 1979-1982, together with almost equal geometric means for some other antibiotics in two groups of strains.

Most of the *B.fragilis* and many of the other *Bacteroides* strains produce beta-lactamases which enable them to be resistant to penicillin, ampicillin and amoxicillin (13). Penicillin resistance was reported to be 94 % (18) or 97 % (7) in the *B.fragilis* group strains and to be 80.4 % (23) in *Bacteroides* spp. Munro (19) found 88 % ampicillin resistance in the *B.fragilis* group and 23 % in other *Bacteroides* strains. In some other studies the ratios of ampicillin resistant *B.fragilis* and *Bacteroides* strains were reported to be 96 % and 70 % (1) or 95 % and 24 % (24). Our results showed 65 % penicillin and 63 % ampicillin resistance which fell within the limits reported by other authors.

Beta-lactamase inhibitors such as sulbactam and clavulanic acid lower the resistance rates to beta-lactam antibiotics significantly in *Bacteroides* strains as they do in many other bacterial genera. Aldridge et al (3) reported that the addition of a beta-lactamase inhibitor enhanced the activity of the various beta-lactam agents from 4-fold to 16-fold overall. Jacobs et al (15) found that clavulanic acid lowered the geometric mean MICs of amoxicillin from 46.7 mg/l to 0.6 mg/l and the number of strains resistant to amoxicillin from 90.5 % to 10 %. In some studies the resistance to ampicillin/sulbactam or amoxicillin/clavulanic acid was reported to be less than 1 % (7, 8, 27). In others, higher percentages of resistance up to 26 % were reported in strains of the *B.fragilis* group (4, 23). In our study, 9 % resistance for amoxicillin/clavulanic acid and 13 % resistance for ampicillin/sulbactam were recorded in *Bacteroides* strains.

The resistance to other penicillin group drugs, mezlocillin and azidocillin, was found to be 30 % and 38 %, respectively, in our strains. Imipenem, although not used in this study, was found as the most active beta-lactam antibiotic in many studies. Some authors found it effective on all strains in their series (7, 17, 18, 21). Some others found 1 or 2 imipenem resistant strains among some hundreds, being the resistance rates usually less than 1 % (10, 15, 22).

In cephalosporin and cephamycin groups, ceftizoxime and cefoxitin are known as the most active ones on anaerobic bacteria. For ceftizoxime resistance rates ranging from 8 % (2) to 55 % (17), and for cefoxitin from 5 % (2) to 26 % (7) were reported for strains of the *B.fragilis* group. In other *Bacteroides* spp, these ratios were found to be as low as 3 and 1 %, respectively (17). The resistance to other cephalosporins were also reported with great discrepancies by different authors. For example Cuchural et al (10) and Aldridge and Henderberg (2) found 66 % and 27-30 % resistance for cefoperazone, 53 % and 13-17 % resistance for cefotaxime in strains of the *B.fragilis* group. 29-33 % resistance for ceftriaxone in *B.fragilis* strains (2) and 36 % resistance for cefuroxime (26), 87 % resistance for ceftazidime (10) were recorded in the literature. In our study, including all *Bacteroides* strains according to past few years' nomenclature, the most effective cephalosporin among used ones was ceftizoxime with 30 % resistance and the least effective one was ceftazidime with 70 % resistance. The percentages of resistant strains were found from 34 % to 58 % for cefoperazone, cefotaxime, ceftriaxone, cefuroxime and cephalothin. Although no combination of a cephalosporin and a beta-lactamase inhibitor was used in this study, it should be mentioned that the activity of such combinations, e.g. cefoperazone and sulbactam (8, 13), or cefazolin and sulbactam or clavulanic acid (12) were found to be much higher than cephalosporins alone.

Erythromycin was the only macrolide antibiotic in this study and 47 % of the strains were found to be resistant. Phillips et al (24) reported, in 1981, 9 % resistance for the *B.fragilis* group and 6 % resistance for other *Bacteroides* strains, while Pierard et al (23), found, in 1988, 63 % of *Bacteroides* strains resistant to erythromycin.

Tetracyclines are not among frequently used antibiotics in recent years. In 1981, Acar et al (1) and Cuchural et al (9) reported 60 to 70 % resistance in *Bacteroides* strains while Phillips et al (24) reported only half of these rates. In recent years, Lee et al (18) found 84 % resistance to tetracycline in *B.fragilis* strains and Pierard et al (23) 50.5 % resistance to doxycycline in *Bacteroides* strains. In Spain, the 61 % tetracycline resistance in 1988 in the *B.fragilis* group strains increased to 76 % in 1989 and to 84 % in 1990 (22). Our finding of 52% tetracycline resistance in *Bacteroides* strains is somewhat lower than the results of other authors.

We found only 28 % of our strains to be resistant to rifampicin, the activity of which was not studied frequently for *Bacteroides* strains in literature. Phillips et al (24) reported resistant strains as 1 % in the *B.fragilis* group and 16 % in other *Bacteroides* strains in 1981.

Chloramphenicol, clindamycin and metronidazole are known as having a high level of antibacterial activity on anaerobic bacteria. Many authors did not met any chloramphenicol resistant *B.fragilis* or non-fragilis *Bacteroides* strain in their works (7, 9, 17, 18, 22, 23, 27). In a paper about the mechanisms of resistance to chloramphenicol and metronidazole, Britz (6) referred to *Bacteroides* strains resistant to 25 mg/l of chloramphenicol and to plasmid-mediated chloramphenicol resistance in a *B.ochraceus* strain. Phillips et al (24) did not find any

chloramphenicol resistant strain in 123 strains of the *B.fragilis* group and found 1 resistant in 94 other *Bacteroides* strains. On the other hand, Acar et al (1) reported 6.2 % chloramphenicol resistance in *Bacteroides* spp. Our finding of 14 % chloramphenicol resistance exceeds the ratios recorded in these reports.

Clindamycin, a lincosamide antibiotic, is extensively used for the therapy of or prophylaxis for anaerobic infections. Some workers did not meet clindamycin resistance in *Bacteroides* strains (5, 20). Some met resistant strains among the *B.fragilis* group but not in other *Bacteroides* (19, 24). Many papers inform less than 10 % (7, 9, 12, 23) or from 10 % to 24 % (1, 2, 3, 18, 27) clindamycin resistance. Pelaez et al (22) reported 36 %, 45 % and 55 % resistance in strains of the *B.fragilis* group in 1988, 1989 and 1990, respectively, in Spain where the antibiotic resistance is known to be prevalent as in Turkey. 30 % clindamycin resistance found in our strains is between the results obtained in Spain and other countries.

Metronidazole is a chemotherapeutic from 5-nitroimidazole derivatives which is uniquely bactericidal for anaerobic bacteria. In too many studies no metronidazole-resistant *B.fragilis* or *Bacteroides* strain was encountered (2, 3, 7, 9, 13, 15, 18, 19, 20, 27). There are some reports informing about 2 % metronidazole resistance in *Bacteroides* strains (17, 23, 24). Acar et al (1) reported 11 % and 12 % resistance in *B.fragilis* and other *Bacteroides* strains, respectively. The percentages of metronidazole-resistant strains increased from 2 % in 1988 to 11 % in 1990 in Spain (22). In our study we used ornidazole, a close relative of metronidazole and a little different 5-nitroimidazole derivative which has been marketed in Turkey since many years, and found it to be the most effective drug among used ones in this study with 4 % resistant strains.

As a result, ornidazole, amoxicillin-clavulanic acid, ampicillin-sulbactam and chloramphenicol were found as the most effective drugs on *Bacteroides* strains.

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