β-LACTAMASE ACTIVITY OF ANAEROBIC *BACTEROIDES* STRAINS ISOLATED FROM CLINICAL SAMPLES AND THEIR SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

Güven Uraz and Lale Turkmen

Microbiology Department, Faculty of Science and Arts
Gazi University, Ankara, Turkey

SUMMARY

β-lactamase production and susceptibility to 13 antimicrobial agents (penicillin-G, amoxycillin, amoxycillin/clavulanic acid, cefoxitin, imipenem, clindamycin, metronidazole, piperacillin, ticarcillin, rifampicin, chloramphenicol, tetracycline and erythromycin) of 32 isolated *Bacteroides* strains were determined. The strains included 23 isolates of *B. frugilis*, 2 *B. thetaioatomicron*, 2 *B. ovatus*, 1 *B. distasonis*, 1 *B. capillosus*, 1 *B. uniformis*, 1 *B. ureolyticus* and 1 *B. merdae*. β-Lactamase production was detected in 65% of the *Bacteroides* using the nitrocefin test, All the antibiotic agents showed excellent activity against β-lactamase negative strains (for tetracycline, ticarcillin and clindamycin, 90% were susceptible, whereas for the other drugs, 100% were susceptible). β-Lactamase-positive *Bacteroides* strains showed 95% susceptibility to metronidazole and rifampicin; 90% susceptibility to piperacillin and cefoxitin; 85% susceptibility to tetracycline and erythromycin; 80% susceptibility to clindamycin and amoxycillin/clavulanic acid, and 76% susceptibility to ticarcillin. All β–lactamase-positive strains were found to be susceptible to imipenem and chloramphenicol.

KEY WORDS

Bacteroides, β-lactamase, antibiotic susceptibility tests

INTRODUCTION

The role of anaerobic bacteria in infections has been increasingly recognised. Differences in their resistance to antimicrobial agents which are genus- and species-specific have necessitated their definite identification /1/. *Bacteroides* species are important anaerobic pathogens for two reasons: They are the microorganisms most frequently isolated from suppurative anaerobic infections, and they are the anaerobic bacteria with the broadest spectrum of resistance to commonly used antimicrobial agents /2/.

The object of this study was to isolate *Bacteroides* strains from clinical material and to determine the β-lactamase activity and the antibiotic susceptibility of each isolate.

MATERIALS AND METHODS

147 different clinical samples from anaerobic infections that were delivered to the clinical microbiology laboratory between 1995 and 1997 were studied. After inoculating these samples into the non-selective enriched *Brucella* blood agar and CDC anaerobe blood agar media, as well as the selective enriched *Bacteroides* bile esculin agar, kanamycin-vancomycin bile esculin agar and phenylethyl alcohol agar media, bacteria were isolated /3/. Anaerobes were identified at the species level by a combination of the Rapid ID 32 A system (BioMerieux, France) and the API 20 A system (BioMerieux) /l,4/.

β-Lactamase activity was determined in all isolates using the chromogenic cephalosporin (nitrocefin) method /5/. The susceptibility of all the isolated *Bacteroides* strains, both β-lactamase positive and negative, to 13 antimicrobial agents was determined with the ATB system (BioMerieux) which is an API system /6/.

RESULTS

In our research, 32 *Bacteroides* strains were isolated from the 147 different clinical samples taken from anaerobic infections (Table 1).

It was determined by the chromogenic cephalosporin (nitrocefin) method that 21 (65%) of these 32 isolated and identified *Bacteroides* isolates were β-lactamase positive, and the other 11 (34%) were β-lactamase negative (Table 2).

TABLE 1

Isolated *Bacteroides* strains and their statistical distribution

***Bacteroides* strains**	**Number of times isolated**
Bacteroides fragilis	23
Bacteroides thetaiotaomicron	2
Bacteroides ovatus	2
Bacteroides distasonis	1
Bacteroides uniformis	1
Bacteroides merdae	1
Bacteroides ureolyticus	1
Bacteroides capillosus	1
Total	**32**

It should be noted that *B. fragilis* is the most commonly isolated *Bacteroides* strain from clinical material.

TABLE 2

β-Lactamase enzyme activity in 32 isolated *Bacteroides* strains

***Bacteroides* strains**	**β-lactamase positive**	**β-lactamase negative**	**No. of isolates**
B. fragilis	16	7	23
B. thetaiotaomicron	1	1	2
B. ovatus	2	-	2
B. distasonis	-	1	1
B. capillosus	1	-	1
B. ureolyticus	-	1	1
B. uniformis	-	1	1
B. merdae	1	-	1
Total	**21**	**11**	**32**

Table 3 shows the susceptibility and resistance of each *Bacteroides* strain isolated to 13 antimicrobial agents according to their β-lactamase positive or negative activity.

DISCUSSION

Identification of anaerobic bacteria in specimens taken from sites of infection due to mixed organisms can be time-consuming and expensive. Laboratories should limit anaerobic workups by testing only those specimens that have been properly collected and transported to the laboratory. Use of selective and differential media for initial processing can provide rapid and relevant information to the clinician. Anaerobes isolated from normally sterile sites and sites of serious infection should always be completely identified. The *Bacteroides fragilis* group of organisms should always be identified because of their virulence and resistance to many antimicrobial agents /7/.

In every stage of our study from collecting the material to identification anaerobic conditions were addressed with the utmost care and *Bacteroides* strains were isolated from the clinical material with the most accurate methods. Selective and non-selective media were used to facilitate and increase anaerobe isolation, and the API 20 A and Rapid ID 32 A systems were used for their identification. In addition, the chromogenic cephalosporin (nitrocefin) method was used to determine β-lactamase activity. The popularity of these methods in recent studies /3,5/ was the main reason for us to use them in the identification and isolation of anaerobes. A commonly used fourth method, which we also used, was API ATB, the antibacterial susceptibility test for anaerobic bacteria /6/.

The results of the susceptibility tests indicate that in Turkey β-lactamase negative strains have 100% susceptibility to both penicillin-G and amoxycillin, whereas the β-lactamase positive strains have 100% resistance to penicillin-G and 80% resistance to amoxycillin (see Table 3).

It was also observed that resistance to tetracycline is not at a critical level in Turkey, unlike in many other countries. However, further to information acquired from the literature, resistance to clindamycin, ticarcillin, amoxycillin/clavulanic acid and erythromycin is at a very high level /2,8/ (see Table 3). In addition, it was also observed that resistance to piperacillin, cefoxitin, metronidazole and

TABLE 3

The susceptibility and resistance of *Bacteroides* strains to antimicrobial agents

Antimicrobial agents	β-lactamase positive Bacteroides strains				β-lactamase negative Bacteroides strains			
	Susceptible	%	Resistant	%	Susceptible	%	Resistant	%
Penicillin G	-	-	21	100.0	7	100.0	-	-
Amoxycillin	4	19.0	17	80.9	11	100.0	-	-
Tetracycline	18	85.7	3	14.2	10	90.9	1	9.0
Chloramphenicol	21	100.0	-	-	11	100.0	-	-
Clindamycin	17	80.9	4	19.0	10	90.9	1	9.0
Metronidazole	20	95.2	1	4.7	11	100.0	-	-
Cefoxitin	19	90.4	2	9.5	11	100.0	-	-
Ticarcillin	16	76.1	5	23.8	10	90.9	1	9.0
Piperacillin	19	90.4	2	9.5	11	100.0	-	-
İmipenem	21	100.0	-	-	11	100.0	-	-
Rifampicin	20	95.2	1	4.7	11	100.0	-	-
Erythromycin	18	85.7	3	14.2	11	100.0	-	-
Amoxycillin/clavulanic acid	17	80.9	4	19.0	11	100.0	-	-

rifampicin is very low, while no resistance to imipenem and chloramphenicol was observed (see Table 3). These results conform with information in the literature /8-10/. Therefore these latter antibiotics should be efficient in the treatment of anaerobic infections caused by *Bacteroides* species.

In conclusion, it is necessary to identify the strain of *Bacteroides* present, and antibiotic susceptibility tests should be performed before attempting to cure the disease.

REFERENCES

1. Kitch TT, Appelbaum PC. Accuracy and reproducibility of the 4-hour ATB 32 A method for anaerobe identification. J Clin Microbiol 1989; 27 : 2509-2513.
2. Rasmussen BA, Bush K, Tally FP. Antimicrobial resistance in *Bacteroides*. Clin Infect Dis 1993: 16 (Suppl 4): 390-400.
3. Goldstein EJC, Citron DM, Goldman RJ. National hospital survey of anaerobic culture and susceptibility testing methods: Results and recommendations for improvement. J Clin Microbiol 1992; 30: 1529-1534.
4. Murray PR, Weber CJ, Niles AN. Comparative evaluation of three identification systems for anaerobes. J Clin Microbiol 1985; 22: 52-55.
5. Finegold SM. Susceptibility testing of anaerobic bacteria. J Clin Microbiol 1988; 26: 1253-1256.
6. Grollier G, Burucoa C, Bonnin M, De Rautlin de la Roy Y. Identification and susceptibility testing for obligate anaerobic bacteria using a semi-automated API ATB plus system. Ann Biol Clin 1992; 50: 393-397.
7. Citron DM, Appelbaum PC. How far should a clinical laboratory go in identifying anaerobic isolates and who should pay? Clin Infect Dis 1993; 16 (Suppl 4): 435-438.
8. Sanders CV, Aldridge KE. Current antimicrobial therapy of anaerobic infections. Eur J Clin Microbiol Infect Dis 1992; 11: 999-1011.
9. Debreuil D, Breuil J, Dublanchet A, Sedallian A. Survey of the susceptibility patterns of *Bacteroides fragilis* group strains in France from 1977 to 1992. Eur J Clin Microbiol Infect Dis 1992; 11: 1094-1099.
10. Mastrantonia P, Spigaglia P, Sebastianelli A. Susceptibility patterns and characterization of beta-lactamases in clinical isolates of *Bacteroides fragilis*. Eur J Clin Microbiol Infect Dis 1994; 13: 475-480.