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EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE OF *B. fragilis* GROUP ORGANISMS ISOLATED FROM CLINICAL SPECIMEN AND HUMAN INTESTINAL MICROBIOTA

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SUMMARY

Epidemiological aspects and the antimicrobial susceptibility profile of the *Bacteroides fragilis* group isolated from clinical and human intestinal specimens were examined in this study. *B. fragilis* group strains were isolated from 46 (37%) of 124 clinical specimens and the source of the samples was: Blood culture (3), intraabdominal infection (27), brain abscess (2), soft tissue infection (17), respiratory sinus (3), pleural aspirate (9), breast abscess (3), surgical infected wound (22), pelvic inflammatory disease (22), chronic otitis media (9) and miscellaneous (7). Intraabdominal and soft tissue infections were responsible for more than half of the clinical isolates. Susceptibility to penicillin, cefoxitin, tetracycline, metronidazole, chloramphenicol and clindamycin was examined. All isolates were susceptible to metronidazole and chloramphenicol. For clindamycin and cefoxitin the resistance rates observed were 21.7% and 10.9% respectively. Susceptibility profiles varied among the different species tested. A total of 37 species of *B. fragilis* group isolated from intestinal microbiota of individuals who had no antimicrobial therapy for at least 1 month before the sampling was also examined. All strains were also susceptible to chloramphenicol and motronidazole and the resistance rates to clindamycin and cefoxitin were 19.4% and 5.4% respectively. A few institutions, in Brazil, have monitored the antimicrobial susceptibility of *B. fragilis* group strains isolated from anaerobic infections. The resistance rates to cefoxitin and clindamycin and the variation in susceptibility patterns among the species isolated in this study emphasize the need for monitoring of susceptibility patterns of *B. fragilis* group organisms isolated, especially at our University Hospitals.

KEYWORDS: *Bacteroides fragilis* group; Antimicrobial resistance; Anaerobic bacteria.

INTRODUCTION

The role of *Bacteroides fragilis* group organisms in human infections is well established. Almost a century has passed since the studies of VEILLON & ZUBER (1898)[27], and extensive studies have been performed to reaffirm the importance of those bacteria in pathological process such as intraabdominal, pelvic and soft tissue infections. In the last three decades other questions involving antimicrobial resistance and the epidemiology of *B. fragilis* group infections have been raised.

The *B. fragilis* group antimicrobial resistance has been documented since 1970 and consequently changes

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in therapeutic schemes have occurred. Tetracycline, once considered an important drug in the treatment of *B. fragilis* group infections, is no longer used[2]. Clindamycin and cefoxitin resistance has also been reported and most recently, resistance to chloramphenicol, metronidazole and imipenem, which are drugs considered of first line in the treatment of *B. fragilis* group infections has been described[2,6,16,17,19]. Several studies have shown variation of the *B. fragilis* group susceptibility patterns among species, and they have demonstrated that even within a given region susceptibility is not predictable[2,12,13].

The epidemiological aspects of *B. fragilis* group infections, particulary those related to nosocomial infections are not well known yet. Serogrouping schemes for the epidemiological study of the *B. fragilis* infections have been described[9], but questions about transmission, cross-infection and intraspecies differences in hospital infections remain without an answer.

With the interest of delineating the antimicrobial resistance profile of *B. fragilis* group organisms in our region and of discussing the epidemiological aspects of the clinical infections caused by these important anaerobic bacteria, a study was carried out with four objectives as follows:

1. To determine the isolation pattern of *B. fragilis* group species from samples collected at the University Hospital of Universidade Federal do Ceará (UFC).
2. To determine the antimicrobial resistance profile of the *B. fragilis* strains isolated.
3. To compare resistance patterns at this setting with those reported in the literature.
4. To make some considerations about the epidemiological aspects that involve the infections caused by this group of bacteria.

MATERIALS AND METHODS

The samples used in this study consisted of clinical and fecal samples.

Clinical isolates

Between July 1993 and December 1994, 124 clinical specimens from 124 inpatients assisted at the University Hospital (UFC) were received by the Microbiology Laboratory. The source of the 124 clinical specimens processed in this study was: Blood culture (3), intraabdominal infection (27), brain abscess (2), soft tissue infection (17), respiratory sinus (3), plural aspirate (9), breast abscess (3), surgical infected wound (22), pelvic inflammatory disease (22), chronic otitis media (9), miscellaneous (7).

Bacteroides fragilis group organisms were isolated from 46 of the 124 clinical specimens processed (all organisms isolated and used in the susceptibility studies were from clinical single-patients isolates). Most of the isolates came from intraabdominal infection (15); other sources included surgical infected wound (10), soft tissue infection (7), chronic otitis media (7), pelvic inflammatory disease (3), pleural aspirate (2) and miscellaneous (2).

Fecal samples

In the same period, 37 nonduplicated microorganisms of the *B. fragilis* group were isolated from fecal samples obtained from 37 outpatients with no previous antimicrobial therapy for at least 30 days before the sampling.

Epidemiology

Analysis was made of the frequency of isolation of *B. fragilis* group organisms from clinical and fecal samples and of the body sites from which clinical specimens were isolated.

The antimicrobial susceptibility profile of the *B. fragilis* group organisms was delineated and the resistance rates were examined and compared between the two groups of samples studied.

Specimen transport and processing

Samples of exudate and body fluids were collected with a swab and introduced into a transport medium (modified Cary & Blair) or were aspirated with a needle and syringe and injected into an oxygen-free transport tube or sometimes, the specimens were transported in a syringe with a needle introduced into a rubber lid in order to maintain an anaerobic environment.

Fecal samples from each patient were collected into sterile plastic containers.

After collection, the specimens were sent to the laboratory and processed as soon as they arrived. Anaerobic processing consisted of the following steps:

1. Plating of the specimens onto *Bacteroides* base bile-esculin agar and phenylethyl alcohol-sheep blood agar.
2. Inoculation into supplemented and (pre-reduced anaerobically sterilized) Brain Heart Infusion broth PRAS.
3. Incubation at 37°C for 48h in anaerobic jars. The anaerobic environment was obtained using

a gaseous mixture (80%N_2, 10%CO2, 10%H_2) or commercially available kits for anaerobiosis (DIFCO, OXOID).

4. Identification of the anaerobic isolates as described in HOLDEMAN et al. (1977)[14] and SEBALD & PETIT (1994)[23] manuals.
5. Gram staining of the original specimen.
6. Maintenance of isolates at -25°C in 10% skim milk or at -15°C in 40% glycerol GC medium[2] until the susceptibility tests were done.

Susceptibility tests

All the strains isolated from clinical specimen and human intestinal microbiota were tested for susceptibility to Penicillin G (Sigma Chemical Co. St. Louis, MO), Cefoxitin (Merck Sharp & Dohme, Rahway, NJ) and Chloramphenicol, Clindamycin, Metronidazole, Tetracycline (Sigma Chemical Co. St. Louis, MO). Antimicrobial susceptibility tests were performed by the agar dilution method as standardized by the National Committee for Clinical Laboratory Standards (NCCLS)[20] using Wilkins-Chalgren agar. The agar dilution tests plates were inoculated with a Steers replicator and all the procedures followed the recomendation of the NCCLS manual. Reference strains of *B. fragilis* (ATCC 25285) and *B. thetaiotaomicron* (ATCC 29741) were included in each experiment to assess the reliability of the method. Resistance was defined as follow: MIC ≥8µg/ml for penicillin and clindamycin; MIC ≥16µg/ml for tetracycline; MIC ≥32µg/ml for metronidazole and chloramphenicol and MIC ≥64µg/ml for cefoxitin (NCCLS, 1993).

RESULTS

Clinical isolates

Bacteroides fragilis group strains were isolated from 46 (37%) of the 124 clinical specimens. Infected wound, intra-abdominal and soft tissue infections were responsible for more than half of all the clinical isolates (Table 1). The species most frequently isolated was *B. fragilis* (68.0%) followed by *B. thetaiotaomicron* (21.7%), *B. vulgatus* (10.8%) and *B. distasonis* (6.5%).

Human intestinal microbiota

The following species were isolated from the 37 fecal samples: *B. fragilis* (19), *B. vulgatus* (06), *B. ovatus* (04), *B. distasonis* (04), *B. merdae* (03) and *B. thetaiotaomicron* (01).

Antimicrobial susceptibility

The isolates were uniformly susceptible to chloramphenicol and metronidazole. The resistance rates to penicillin, tetracycline, clindamycin and cefoxitin for the *B. fragilis* group strains isolated from clinical specimens were 93.5%, 80.5%, 21.7% and 10.9% respectively (Table 2). Of the total of 37 strains of *B. fragilis* group strains isolated from fecal samples, 78.4% were resistant to penicillin and 73% were resistant to tetracycline. The resistance rates to clindamycin and cefoxitin were 19% and 5.4% respectively.

In a significance level of 0.05, the resistance rates to clindamycin and tetracycline were similar for the *B. fragilis* group isolated from clinical specimens and human intestinal microbiota. So, we can observe that the strains isolated from human intestinal microbiota and clinical specimens had similar resistance rates for clindamycin and tetracycline ($Z_{clindamicina} = 0.228$, $Z_{tetraciclina} = 0.76$; p=0.05).

The resistance rates to penicillin and cefoxitin were different for the *B. fragilis* group strains isolated from the two groups of patients. The strains isolated from human intestinal microbiota were less resistant to those antimicrobials than the ones isolated from clinical specimens ($Z_{penicillin} = 2.0$, $Z_{cefoxitin} = 4.3$; p=0.05).

TABLE 1

Frequency of the *Bacteroides fragilis* group strains isolated from various body sites of 46 patients

Species	Intraabdominal infection		Surgical infected wound		Soft tissue infection		Chronic otitis media		Pelvic inflammatory disease		Pleural aspirate		Miscellaneous		Total	
	n°	%	n°	%	n°	%	n°	%	n°	%	n°	%	n°	%		
B. fragilis	9	60	7	70	3	42.9	4	57.2	1	33.3	2	100	2	100	28	68
B. thetaiotaomicron	4	26.7	2	20	2	28.6	1	14.3	1	33.3	0	0	0	0	10	21.7
B. vulgatus	1	6.6	1	10	2	28.6	1	14.3	0	0	0	0	0	0	5	10.8
B. distasonis	1	6.6	0	0	0	0	1	14.3	1	33.3	0	0	0	0	3	6.5
Total	15	32.6	10	21.7	7	15.2	7	15	3	6.6	2	4.3	2	4.3	46	100

Percentages given in each specimen category are based on total number of isolates from that source; percentages in total categories are based on the total number of isolates (46).

TABLE 2
Resistance rates of 46 *B. fragilis* group species isolated from clinical specimens and 37 human intestinal microbiota strains

Species	N° of isolates		% of resistance							
			Penicillin		Cefoxitin		Clindamycin		Tetracycline	
	CS	HIM	CS	HIM	CS	HIM	CS	HIM	CS	HIM
B. fragilis group	46	37	93.5	78.4	10.9	5.4	21.7	19	80.5	73
B. fragilis	28	19	89	79	3.5	0	7.2	5.2	75	73.7
B. thetaiotaomicron	10	1	100	100	50	0	50	0	90	100
B. vulgatus	5	6	100	66.7	0	50	20	16.7	80	50
B. distasonis	3	4	100	100	100	50	66.7	75	100	100
B. ovatus	0	4	-	75	-	0	-	25	-	75
B. merdae	0	3	-	66.7	-	0	-	0	-	100

CS: Clinical specimens; HIM: Human intestinal microbiota.

In Table 2, we can also observe the existence of resistance rates variation between the species of the *B. fragilis* group for the antimicrobials tested. Although the number of strains tested was small, it seems clear that *B. fragilis* was the species most susceptible to the drugs studied.

DISCUSSION

The *B. fragilis* group strains are the anaerobic microorganisms most commonly associated with a wide variety of clinical infections, especially *B. fragilis* species. In the last decades gradual changes in the antimicrobial resistance profile of these important pathogens have been reported (Ref). Analysis of the *B. fragilis* group strains isolated from clinical specimens and of the resistance profile to six antibiotics frequently used for the treatment of anaerobic infections in our region is accomplished in this study. Considering the endogenous origin of these important bacteria to anaerobic infections and also knowing the resistance transferability among bacteria, mainly at the intestinal level, a study of the antomicrobial resistance profile of the *B. fragilis* group strains isolated from that microbiota was also carried out.

In this study, *B. fragilis* was the species most frequently recovered from clinical specimens followed by *B. thetaiotaomicron*, *B. vulgatus* and *B. distasonis*. Infected wound, intraabdominal and soft tissue infections were the isolation sites from where the microorganisms were most frequently isolated.

CUCHURAL et al. (1988)[7], reported the susceptibility of 1229 *B. fragilis* group strains obtained from clinical infections in eight centers in the United States. Similar to our study, intraabdominal and soft tissue infections were the isolation sites from where the species were recovered most frequently. *B. fragilis* was also the most common isolate followed by *B. thetaiotaomicron* and *B. distasonis* species. These authors have suggested that *B. fragilis* is more invasive than other members of the group. Although differences in virulence between the species of the *B. fragilis* group have been described, distinct types of *B. fragilis* associated with disease are not known yet[9,24].

We found that *B. fragilis* was the species most frequently isolated from human intestinal microbiota followed by *B. vulgatus*, *B. distasonis*, *B. ovatus*, *B. merdae* and *B. thetaiotaomicron*. These results are different from those reported by STARK et al.[22] in Sweden, who isolated 82 *B. fragilis* group strains from outpatients not treated with antimicrobials agents. In this study, *B. thetaiotaomicron* was the species most frequently isolated followed by *B. ovatus*, *B. uniformis*, *B. distasonis*, *B. vulgatus*, *B. fragilis* and *B. caccae*. In other region of Brazil in the period of 1981 to 1982, ALMEIDA & UZEDA[1] isolated 228 *B. fragilis* group strains from human intestinal microbiota and the results were similar to our findings.

B. fragilis group clindamycin resistance has been reported by several investigators. It has been observed considerable variation in the resistance rates in different surveys, ranging from low and moderate resistance (5%, CUCHURAL, et al.[7]; 6%, HORN et al.[15]; 13%, PANICHI et al.[21]) to high resistant rates (21%, BETRIU et al.[4]; 24%, LEE et al.[18]). The high resistance rate observed in our study can be associated to the wide and indiscriminate use of macrolides (erythromycin) and lincosamides (especially lincomycin in our region), since these drugs are not routinely administered to inpatients in the center studied.

The resistance rate to clindamycin for the *B. fragilis* group strains isolated from human intestinal microbiota was 19%. This resistance rate was very high and similar to the rate obtained by us in the study of clinical strains. This is an alarming finding, mainly when one considers the endogenous origin of *B. fragilis* group species or the possibility of transmission among patients in the same ward, especially with intraabdominal infections. It is noteworthy the clindamycin resistance rate of 37%, obtained in a study of susceptibility of 228 *B. fragilis* group strains isolated from human intestinal microbiota in Rio de Janeiro[1].

Resistance to cefoxitin was also present with a frequency of 10.9% for the *B. fragilis* group isolated from clinical specimens. Higher rates, ranging from 16 to 22% have been documented in other studies [11,25]. TURGEON et al.[26] in Canada, found that cefoxitin resistance at the Hôpital Saint-Luc had risen from 7.9% in 1987 to 20.7% in 1990, probably due to the wide use of this antibiotic in that medical center. Cefoxitin is one of the drugs of choice for treating patients with anaerobic infections in our hospital center. A prospective study should be done in order to see if the resistance rates for this drug is increasing in our Institution.

The resistance rate of cefoxitin for the *B. fragilis* group isolated from human intestinal microbiota was low (5.4%) and the difference in resistance rate for isolates from clinical specimens was statistically significant. Cefoxitin is commercialized in Brazil only for parenteral use. Consequently its use is limited and probably this is one of the reasons for this finding.

Although the number of strains tested was low, we observed susceptible patterns variation among the species of *B. fragilis* group. We found, as have other workers, that *B. fragilis* is often less resistant to some antimicrobial agents than the other species of the group. In view of the differences in susceptibility among the various species of the *B. fragilis* group, it has been recommended to speciate this group of organisms in order to better direct of modify empirical antibiotic therapy[7,11].

All the species of the *B. fragilis* group examined were susceptible to metronidazole and chloramphenicol, Metronidazole is an effective drug for anaerobic bacterial infections. It is a drug widely administered to patients in surgical wards, from where more than half of the microorganisms were isolated in this study. A few reports in the literature have described metronidazole resistance to *B. fragilis* group organisms[10,17].

Chloramphenicol use has declined in many countries (due to the toxicity of the drug, that causes bone marrow depression leading to blood disorders such as aplastic anemia). There are now very few indications for which chloramphenicol is the antibiotic of choice[8]. In the hospital center studied, chloramphenicol is not a drug used for treatment of patients in surgical wards.

The high resistance rates to penicillin and tetracycline for the strains isolated from clinical specimen and human intestinal microbiota were the ones expected and similar results are described in the literature[3,4,15,22].

Seven multidrug resistant strains, (i.e., strains resistant to more than 3 of the antimicrobial agents tested) were isolated from the 46 patients studied. Four of them were isolated from intraabdominal infections, one from a patient with pelvic inflammatory disease and the last two species were recovered from patients with soft tissue infections. Six of these multiresistant strains were identified as *B. thetaiotaomicron*. BORGAULT et al. (1992)[5] and APPLEMAN et al. (1991)[2] in a survey of *B. fragilis* group strains susceptibility in Cananda and in the United States respectively, found that *B. thetaiotaomicron* demonstrated higher resistance to the antimicrobial drugs tested than the other species of the group. Few reports are available in the literature about the pathogenicity and virulence mechanisms of *B. thetaiotaomicron*, although the frequency of isolation and antimicrobial resistance of this species have been described to occur in a high proportion of species within the group *B. fragilis*. The epidemiological aspects of nosocomial infections caused by this particular microorganism have not been reported yet.

In Brazil, a few institutions have been doing periodic antimicrobial susceptibility tests for anaerobic bacteria or even for *B. fragilis* group strains isolated from clinical specimens. The researches carried out in many countries and the results obtained in this study reaffirm the need to test periodically the antimicrobial susceptibility of this group of microorganisms in, at least, the main hospital centers of Brazil. The high and moderate rates of resistance to clindamycin and cefoxitin respectively, found among the species of the *B. fragilis* group tested should guide the clinical treatment of the patients in our institution. The follow-up of this study will also allow detection of emerging trends of resistance among these important microorganisms.

RESUMO

Epidemiologia e resistência a antimicrobianos de microorganismos do grupo *B. fragilis* isolados de espécime clínico e microbiota intestinal humana.

Alguns aspectos epidemiológicos e o perfil de sensibilidade a antimicrobianos de amostras do grupo *B. fragilis* isoladas de espécime clínico e microbiota intesti-

nal humana foram delineados neste estudo. As espécies do grupo *B. fragilis* foram isoladas de 46 (37%) de 124 espécimes clínicos, como segue: hemocultura (3) infecção intra-abdominal (27), abscesso cerebral (2), infecção de tecido mole (17), seios da face (3), aspirado pleural (9), abscesso pulmonar (3), ferida cirúrgica (22), doença inflamatória pélvica (22), otite média crônica (9) e diversos (7). Mais da metade destes microorganismos foram isolados de infecção intra-abdominal e infecção de tecido mole. Os antimicrobianos testados foram Penicilina G, Cefoxitina, Cloranfenicol, Metronidazol, Tetraciclina e Clindamicina. Todas as amostras estudadas apresentaram-se sensíveis ao Cloranfenicol e ao Metronidazol. Resistência à clindamicina e cefoxitina foi observada em 21.7 e 10.9% dos microrganismos para cada droga respectivamente. Variação na sensibilidade aos antimicrobianos entre as espécies do grupo foi detectada. O perfil de sensibilidade a antimicrobianos de amostras do grupo *B. fragilis* isoladas da microbiota intestinal de 37 indivíduos que não faziam uso de antimicrobianos nos últimos 30 dias antes do exame foi também estudado. Todas as amostras apresentaram-se sensíveis ao cloranfenicol e metronidazol sendo de 19.4% e 5.4% os percentuais de resistência à clindamicina e cefoxitina respectivamente. Poucas Instituições no Brasil, realizam periodicamente teste de sensibilidade a antrimicrobianos para amostras do grupo *B. fragilis* isoladas de espécimes clínicos. A variação no perfil de sensibilidade entre as espécies do grupo e o alto percentual de resistência à clindamicina e cefoxitina encontrados neste trabalho, reforçam a necessidade do isolamento, caracterização e monitoração do perfil de sensibilidade a antimicrobianos destes microrganismos, pelo menos nos Hospitais Universitários do país.

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