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BRIEF COMMUNICATION

ANTIMICROBIAL RESISTANCE AND PLASMID DETECTION IN STRAINS OF THE *BACTEROIDES FRAGILIS* GROUP

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SUMMARY

Resistant populations of the **Bacteroides fragilis** group bacteria (two reference ones and two isolated from human and **Callithrix penicillata** marmoset) were obtained by the gradient plate technique, to clindamycin, penicillin G, metronidazole and mercuric chloride. All the four tested strains were originaly susceptible to the four antimicrobial drugs at the breakpoint used in this study. MICs determination for the four cultures gave constant values for each antimicrobial, on the several steps by the gradient plate technique. The intestinal human **B. fragilis** strains showed three DNA bands, that could be representative of only two plasmids in the closed covalently circular (CCC) form with molecular weights of approximately 25 and 2.5 Md. The results do not permit an association between the presence of plasmid in the human strain with the susceptibility to the studied drugs. The four strains were β-lactamase negative in the two methods used, and no particular chromosomal genetic resistance marker was demonstred. The resistance (MIC) observed, after contact with penicillin G and mercuric chloride, were two-fold in the four tested strains.

KEY WORDS: Bacteroides fragilis group; Antimicrobial resistance; Plasmid DNA.

INTRODUCTION

Species of the **Bacteroides fragilis** group are considered as the major component of the human intestinal microbiota, and they are the most frequently isolated bacteria from clinical specimens[1,6,10].

Bacteria of the **B. fragilis** group are highly sensitive to clindamycin and chloramphenicol; however unlike other anaerobic gram-negative bacteria they are usually resistant to several antimicrobial agents, including penicillins and aminoglycosides. Their resistance is considered as natural or intrinsic[17].

B. fragilis strains may produce several β-lactamases, but genetic markers have not yet been defined[17].

MANCINI & BEHME[5] reported the transfer of multiple resistance factors from **B. fragilis** to **E. coli.** Other genetic studies have shown that **Bacteroides** strains resistant to some antibiotics could act as recipients for a great number of epidemiologically significant plasmids[14,17].

Mercury and mercurial compounds have been used for many years as medicines and antiseptics and also in dentistry as an amalgam component, and in industry. These and other heavy metal compounds play an important role in the selection of resistant microorganisms to antibiotics and other antimicrobials[7,9].

Despite the considerable amount of literature on the production of β-lactamases by the **B. fragilis** group there is little information available about the genetic mechanisms of drug resistance in the **B. fragilis** group, particularly on those of chromosomal origin.

The objetives in this study were to obtain resistant "mutant" strains, to clindamycin, penicillin

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G, metronidazole, and mercuric chloride for bacteria of the **B. fragilis** group (1 strain isolated from man, 1 from **C. penicillata** marmoset, and 2 reference strains) using the gradient plate technique. In addition, the presence of plasmids in the tested strains and their correlation with the susceptibility to those drugs, were also investigated.

MATERIAL AND METHODS

Two intestinal strains isolated from healthy human and **C. penicillata** marmoset, and the reference strains **B. fragilis** ATCC 23745 and one **B. vulgatus** ATCC 8482, were studied.

The tested strains were isolated in **B. fragilis**-bile-esculin medium[4], incubated in anaerobic conditions. The strains were biochemically identified according to HOLDEMAN et al.[3] and SUTTER et al.[12].

The MICs for the four antimicrobials: chloride clindamycin (Rhodia Farma Ltda., São Paulo, Brazil), penicillin G (Fontoura-Wyeth S.A., São Paulo, Brazil), metronidazole (Rhodia S.A., São Paulo, Brazil), and mercuric chloride (Inlab,São Paulo,Brazil), were determined by the agar dilution method described by SUTTER et al.[11], using the BHI agar as growth medium. The antimicrobial concentrations used ranged from 0.25 to 128 ug/ml. The MIC was defined as the lower concentration of each antimicrobial agent capable of inhibiting macroscopically visible bacterial growth.

Strains were streaked with a Drigalski loop on agar plates with gradually increasing concentrations of each antimicrobial agent, by the gradient plate technique[13], using the BHI agar as the base medium. The inoculum was approximately 10^8 cells/ml, and plates were incubated under anaerobic conditions, at 37°C for 48h. After the incubation period, a loop of the confluent bacterial growth from the region containing higher concentration of drugs in each step were resuspended in 1.0 ml BHI broth and streaked immediately on plates containing higher concentration of these drugs. These suspensions were also used for the MIC determination in each step. The bacterial growth were transferred with a platinum loop from the highest concentrations of drugs in each gradient plate.

Initial concentrations of each drug used in the gradient plates were determined according to the initial MIC values observed for the four tested strains, respectively, human, non-human, and reference **B. fragilis** ATCC 23745 and **B. vulgatus** ATCC 8482 strains: Clindamycin, 0 - 0.25; 0 - 0.5; 0 - 1; 0 - 2; 0 - 4 ; and 0 - 8 ug/ml; penicillin G, 0 - 16; 0 - 24; 0 - 32; 0 - 48; and 0 - 64 ug/ml; metronidazole, 0 - 2; 0 - 4; 0 - 8; 0 - 12; and 0 - 16 ug/ml; mercuric chloride, 0 - 4; 0 - 8; 0 - 12; 0 - 16; 0 - 24; and 0 - 32 ug/ml.

The production of β-lactamase was determined by the iodometric[15] and chromogenic cephalosporin methods[16].

Plasmid DNA was extracted according to the method of RASCHTCHIAN et al.[8], modified by increasing the BHI broth volume from 20 ml to 50 ml, for the growth of the analysed strains. 50 ul of the DNA sample were submitted to vertical electrophoresis in 0.7% agarose gel. The gels were run at 100 V and 50 mA constant current for 4 to 5h, then stained in a solution of ethidium bromite (Sigma^R) in water (1 ug/ml) for 45 minutes. DNA bands were photographed over a shortwave ultraviolet transilluminator (Germetec), using a Zeiss-Kron camera, a Kodak Panatomic X film, and a red filter.

RESULTS

All the tested strains were originally sensitive to clindamycin, penicillin G, metronidazole, and mercuric chloride, at the breakpoint used for each drug (4,16,16 and 4 ug/ml, respectively). The four tested strains showed the same following MIC values: clindamycin, 0.25 ug/ml; penicillin G, 16 ug/ml; metronidazole, 2 ug/ml; and mercuric chloride, 4 ug/ml.

Resistant cultures from the four original strains were obtained for each drug, by using the gradient plate technique, in the different concentrations tested.

MIC determination for resistant "mutants" obtained from all the tested strains, gave constant values for each antimicrobial, on the four steps of selection, i.e., to clindamycin, 0.5 ug/ml, penicillin G , 32 ug/ml, metronidazole, 4 ug/ml, and mercuric chloride, 8 ug/ml. All the tested strains were β-lactamase negative in the two methods used.

From all four analysed strains only the intesti-

nal human **B. fragilis** strain (either original or the resistant ones with regard to metronidazole and mercuric chloride) showed three DNA bands, that seems to be representative of only two plasmids with molecular weights of approximately 25 and 2.5 Md (Fig. 1).

DISCUSSION

The profile of sensitivity to antimicrobial drugs of species of the **B. fragilis** group reported in the literature is very controversial. The resistance of members of this microbial group to tetracycline and clindamycin and particularly to penicillins and cephalosporins is well known. This fact has stimulated the studies on the transference of resistant markers within species of the **B. fragilis** group[5].

All the four tested strains were considered sensitive to the four antimicrobial drugs at the breakpoint used in this study. However, despite the net growth of the four tested strains in the highest drug concentrations in the gradient plates, their MIC values were constant in all the steps, i.e., twofold of that originally found before this treatment. Microbial growth in the gradient plates was confluent, although very dense at the lowest concentration of drug and less dense in the region of highest concentration.

In the **B. fragilis** strain of human origin, the presence of plasmid bands was observed after extraction and electrophoresis in agarose gel. The three DNA bands detected (Fig. 1) could be representative of only two plasmids in the closed covalently circular (CCC) form. These plasmids were detected in that human strain even after the four steps by the gradient plate technique. On lane 4 (**B. fragilis** of animal origin) it was noticed a weak band probably due to the lack of DNA in the region corresponding to a band of 2.5 Md of the human band (lane 5).

The results do not permit an association between the presence of plasmids neither in the human nor in the animal strains with the resistance to the studied drugs because the other two reference strains, in which plasmids were not detected, showed the same MIC values before and after treatment with drugs in the gradient plates.

Many bacteria are known to harbour these extrachromosomal DNA molecules that are capable of codifying a great variety of phenotipic characteristics, such as antimicrobial and heavy metal resistance, production of toxins, hemolysins, surface antigens and others. On the other hand, when their ability for codification of any phenotipic characteristic cannot be demonstrated the plasmids are in called "criptics"[2], as it seems to be the case for the data obtained.

Results showed that the MICs obtained initially for the four strains of different origin are their sensitivity range. However, after treatment by the gradient plates, the MICs were twofold the ini-

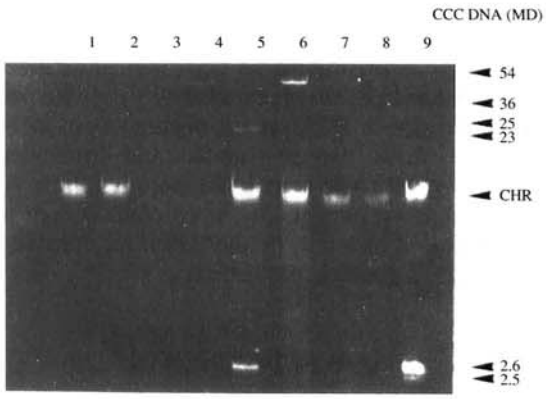

Figure 1. Agarose gel electrophoresis of plasmid DNA in strains of **B. fragilis.** Lane 1, **E. coli** K12 without plasmid; Lane 2, **B. fragilis** ATCC 23745; Lane 3, **B. vulgatus** ATCC 8482; Lane 4, animal strain of **B. fragilis**; Lane 5, human strain of **B. fragilis**; Lane 6, **E. coli** K12 with p307 (54 Md); Lane 7, **E. coli** K12 with RP4 (36 Md); Lane 8, **E. coli** K12 with sa (23 Md); Lane 9, **E. coli** K12 with pBR322 (2.6 Md).

tial values and thus the four strains showed resistance to penicillin and to mercuric chloride at the breakpoints used in this study. It is shown that even in the apparent absence of genetic resistance markers, the strains could show a detectable resistance after contact with some antimicrobial drugs that is yet to be defined, and that could be significant in therapeutic terms.

RESUMO

Resistência antimicrobiana e detecção de plasmídios em cepas do grupo *Bacteroides fragilis.*

Cepas resistentes de bactérias do grupo **Bacteroides fragilis** (2 isoladas de humano e de sagui **Callithrix penicillata** e 2 de referência) foram ob-

tidas pela técnica da placa gradiente para clindamicina, penicilina G, metronidazol e bicloreto de mercúrio. Todas as quatro cepas testadas foram originalmente sensíveis às quatro drogas antimicrobianas em relação ao ponto crítico usado neste estudo. A determinação das CIMs para as quatro cepas testadas mostrou valores constantes para cada antimicrobiano nas diversas concentrações usadas nas placas gradientes. A cepa **B. fragilis** de origem humana apresentou três bandas de ADN que devem corresponder a apenas dois plasmídios circulares covalentemente fechados (CCC), com pesos moleculares de aproximadamente 25 e 2.5 Md. Não se detectou a produção de β-lactamase em nenhuma das cepas nos dois métodos usados. Os resultados não permitem uma associação entre a presença de plasmídios na cepa humana com a susceptibilidade às drogas estudadas. As quatro cepas mostraram-se B-lactamase negativas nos dois métodos usados, não se demonstrando qualquer marcador genético de resistência cromossômica. A resistência observada (CIM), após contato com a penicilina G e bicloreto de mercúrio, foi duas vezes maior nas quatro cepas testadas.

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