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Resistance of *Bacteroides* isolates recovered among clinical samples from a major Costa Rican hospital between 2000 and 2008 to ß-lactams, clindamycin, metronidazole, and chloramphenicol

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ABSTRACT

Objective. To assess the susceptibility of 100 isolates of *Bacteroides* spp. recovered in a major Costa Rican hospital between 2000 and 2008 to several ß-lactams, chloramphenicol, clindamycin and metronidazole.

Methods. Susceptibility to amoxicillin, amoxicillin with clavulanic acid, piperacillin, piperacillin with tazobactam, ticarcillin, ticarcillin with clavulanic acid, cefoxitin, cefotetan, imipenem, chloramphenicol, clindamycin, and metronidazole was determined with the ATB ANA® system. In addition, minimum inhibitory concentrations (MIC) of clindamycin and metronidazole were determined with the broth microdilution method because these drugs are the treatment of choice for anaerobic infections in Costa Rica. Reference strains ATCC® 25285 and ATCC® 29741 were employed as indicated.

Results. According to the ATB ANA® system, 93 isolates were resistant to at least one antibiotic. Resistance to β-lactams was common. By contrast, resistance to β-lactams supplemented with β-lactamase inhibitors was rare. All of the strains were inhibited by imipenem and chloramphenicol. By a broth microdilución test, resistance to clindamycin was 20%, with MIC ranging from 64 mg/L to 256 mg/L; all of the strains were susceptible to metronidazole.

Conclusions. The high MIC for clindamycin obtained for the majority of the resistant strains is highly suggestive of the presence of mechanisms of acquired resistance among the isolates, therefore surveillance studies are required to determine its efficacy. The low resistance to metronidazole observed underlines its value as a first-line drug. On the other hand, imipenem could be used to treat infections that do not respond well to metronidazole or clindamycin.

Keywords: antibiotic resistance, clindamycin, metronidazole, Costa Rica.

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Resistencia a los ß-lactámicos, clindamicina, metronidazol y cloranfenicol de aislamientos de *Bacteroides* recuperados en muestras clínicas de un hospital costarricense, entre el año 2000 y el 2008

RESUMEN

Objetivo. Determinar la sensibilidad a varios ß-lactámicos, cloranfenicol, clindamicina y metronidazol de 100 aislamientos de *Bacteroides* spp. obtenidos en uno de los principales hospitales de Costa Rica entre 2000 y 2008.

Métodos. Se utilizó el sistema ATB ANA® para determinar la sensibilidad a la amoxicilina, amoxicilina con ácido clavulánico, piperacilina, piperacilina con tazobactam, ticarcilina, ticarcilina con ácido clavulánico, cefoxitina, cefotetan, imipenem, cloranfenicol, clindamicina y metronidazol. Debido a la utilización en Costa Rica de clindamicina y metronidazol como tratamientos de elección para infecciones anaerobias, se determinó la concentración mínima inhibitoria (CMI) de ambas drogas con el método de microdilución en caldo. Las cepas ATCC 25285 y ATCC 29741 se utilizaron como referencia.

Resultados. De acuerdo con el sistema ATB ANA®, 93 aislamientos fueron resistentes al menos a un antibiótico. La resistencia a los β-lactámicos fue frecuente, mientras que la resistencia a β-lactámicos con inhibidores de β-lactamasa fue escasa. Todas las cepas se inhibieron con imipenem y cloranfenicol. Con el método de microdilución en caldo, la resistencia a clindamicina fue del 20%, con CMI de 64 mg/La 256 mg/L; todas las cepas fueron sensibles a metronidazol.

Conclusiones. La alta CMI de clindamicina de la mayoría de las cepas resistentes sugiere la presencia de mecanismos de resistencia adquiridos en los aislamientos, por lo que se requieren estudios de vigilancia epidemiológica para determinar su eficacia. La baja resistencia observadadel metronidazol destaca su valor como una droga de primera línea. Por otra parte, imipenem podría usarse para tratar infecciones que no responden bien a metronidazol o clindamicina.

Palabras claves: resistencia aantibióticos, clindamicina, metronidazol, Costa Rica.

INTRODUCTION

The genus *Bacteroides* comprises anaerobic bacteria of clinical interest[1-3]. This group of microorganisms has gained significance in recent times because of its frequent appearance in clinical samples[4-6], the difficulties associated with its therapeutic handling[1,7,8], and the increasing rate at which it develops resistance to antibiotics[1,6,7,9-11].

Infections by *Bacteroides* spp. are usually treated with ß-lactams with or without inhibitors of ß-lactamases[12], clindamycin[9], metronidazole[13] or less frequently with fluoroquinolones in combination with clindamycin or metronidazole[14,15]. Consequently, a decrease in the activity of these drugs, has been observed over on a global scale the last few years[9,10,12-14-19].

Bearing in mind that most Gram-negative anaerobes of clinical relevance in Costa Rica belong to the genus *Bacteroides* [20], and that anaerobes are treated empirically with clindamycin and metronidazole in this country, we assessed the resistance of 100 isolates of *Bacteroides* spp. recovered between 2000 and 2008 to several ß-lactams, clindamycin, metronidazole, and chloramphenicol and determined minimum inhibiting concentrations of clindamycin and metronidazole to evaluate its effectiveness

MATERIALS AND METHODS

Isolation and identification of the isolates

The 100 strains of *Bacteroides* analyzed corresponded to *B. fragilis* (n=34), *B. ovatus* (n=16), *B. thetaiotaomicron* (n=11), *B. uniformis* (n=10), *B. vulgatus* (n=4), *B. cacae* (n=2) and to *B. merdae* (n=3), *B. distasonis* (n=7), and *B. capillosus* (n=13), recently reclassified to *Parabacteroides merdae*, *P. distasonis* and *Pseudoflavonifractor capillosus*[21,22].

The clinical isolates, most of them from intraabdominal infections, were obtained between 2000 and 2008 in a major Costa Rican hospital. These bacteria were cultivated on Columbia 5% Blood Agar plates supplemented with vitamin K (1 mg/L) and hemin (5 mg/L) and identified with the API 20A® or Rapid ID 32A® systems (BioMérieux®).

Determination of antibiotic susceptibility profiles and minimum inhibitory concentrations

The susceptibility of the isolates to two concentrations of amoxicillin (AMO; 4 mg/L, 16 mg/L), two concentrations of amoxicillin with clavulanic acid (AMC; 8/4 mg/L, 16/4 mg/L), piperacillin (PIC; 64 mg/L), piperacillin with tazobactam (TZP; 64/4 mg/L), ticarcillin (TIC64; 64 mg/L), ticarcillin with clavulanic acid (TCC; 64/2 mg/L), cefoxitin (CXT; 32 mg/L), cefotetan (CTT; 32 mg/L), clindamycin (CLI; 4 mg/L), metronidazole (MTR; 16 mg/L), imipenem (IMI; 8 mg/L) and chloramphenicol (CMP; 16 mg/L) was determined with the ATB ANA® system according to the recommendations of the manufacturer (BioMérieux®). Bacteria catalogued by the ATB ANA® system as intermediate were considered resistant. Furthermore, multidrug resistance was defined as resistance to three or more families of antibiotics.

MIC of clindamycin and metronidazole were determined using the broth microdilution technique[23]. In this regard, bacteria growing in the presence of ≥8 mg/L of clindamycin or ≥32 mg/L of metronidazole were considered resistant[23]. The reference strains ATCC® 25285 and ATCC® 29741 were used as controls for both techniques.

RESULTS

Ninety-three of the 100 strains analyzed were resistant to one or more of the 12 antibiotics included in the ATB ANA® system. Most isolates exhibited resistance to 1 or 2 antibiotics (n=60) and fourteen were resistant to 5 or 6 antibiotics (table 1).

Resistance to ß-lactams without ß-lactamase inhibitors was common, particularly in the case of amoxicillin (n=91) (table 1). The ticarcillin and the piperacillin inhibited approximately one-third of the isolates (table 1). The activity of both was completely restored by the two ß-lactamase inhibitors included in the ATB-ANA strips (clavulanic acid and tazobactam). On the other hand, and despite the fact that both drugs are classified as second generation cephalosporins, the number of isolates resistant to cefotetan (n=33) was greater than the number of isolates resistant to cefoxitin (n=4) (table 1).

According to the ATB-ANA® system, all of the strains were inhibited by imipenem and chloramphenicol; in the case of clindamycin and metronidazole, drugs that are widely used in Costa Rica to control infections by anaerobic bacteria, 22 strains were resistant to the first and 3 to the second one (table 1). By contrast, the broth microdilution technique revealed resistance to clindamycin for 20 strains with MIC usually above 256 mg/L and a complete susceptibility to metronidazole.

DISCUSSION

Despite the fact that 14 strains proliferated in the presence of five or six antibiotics, we cannot speak of multidrug resistance because the majority of the antibiotics tested belong to the family of the β-lactams. This absence of multiresistant strains, in addition to being congruent with previous studies conducted in Costa Rica[24,25] and in other countries[26,27], is optimistic in its reference to the therapeutic management of infections by *Bacteroides* sp. in the hospital studied.

The frequency of detection of β-lactam-resistant strains in this study is similar to that reported by Snydman et al.[28], who detected low resistance to imipenem and to combinations of β-lactams with β-lactamase inhibitors, as well as a decrease in the geometric average of the MICs of imipenem, piperacillin-tazobactam and cefoxitin, for many species of *Bacteroides* in a sample of strains isolated between 1997 and 2004[28]. On the other hand, the low resistance to cefoxitin compared to cefotetan has already been documented, particularly for species that are not *B. fragilis*[18,29].

With the exception of some strains, bacteria from the group typically produce β-lactamases[30,31]. Our results are

Table 1 **Resistance profiles of 100 isolates of *Bacteroides* sp. recovered from clinical samples collected in a Costa Rican hospital between 2000 and 2008.**

	Antimicrobials*										
Resistant to	AMO4	AMO16	CTT	TIC	PIC	CLI	AMC	CXT	AMC 16	MTR	n
1 antibiotic	R										16
(n=41)	R	R									24
										R	1
2 antibiotics	R				R						1
(n=19)	R		R								5
	R	R	R								8
	R	R			R						1
	R	R				R					1
	R	R		R							2
	R	R								R	1
3 antibiotics	R	R	R	R							1
(n=13)	R	R	R				R				3
	R	R		R	R						8
	R	R			R					R	1
4 antibiotics			R		R	R		R			1
(n=6)	R	R		R	R	R					3
	R	R	R	R	R						1
	R	R	R	R		R					1
5 antibiotics	R	R	R	R	R	R					8
(n=11)	R	R	R	R	R			R			1
	R	R		R	R	R	R		R		1
	R	R	R	R	R		R		R		1
6 antibiotics	R	R	R	R	R	R	R	R			1
(n=3)	R	R	R	R	R	R	R		R		1
	R	R			R	R	R	R	R		1
Total of strains	91	69	33	29	29	22	5	4	4	3	100

*AMO: amoxicillin; CTT: cefotetan, TIC: ticarcillin, PIC: piperacillin, CLI: clindamycin, AMC: amoxicillin with clavulanic acid, CXT: cefoxitin, MTR: metronidazole.

congruent with the presence of the class 2e cephalosporinase CepA, whose activity is inhibited by sulbactam, clavulanic acid and tazobactam[32], or of the class A beta-lactamase CfxA that confers resistance to cefoxitin but not to imipenem[32]. Furthermore, the low frequency of detection of resistance to amoxicillin-clavulanic acid and imipenem suggests that our isolates do not have unusual membranes proteins[16] or metallo-β-lactamases[12,33], respectively.

The MIC for clindamycin obtained for the majority of the resistant strains is highly suggestive of the presence of mechanisms of acquired resistance among the isolates, that usually may be caused by covalent modifications of the 23S ribosomal RNA by N-methyltransferases[34] or by enzymatic inactivation of the antibiotic by O-nucleotidiltransferases[35]. Of these two mechanisms, modification by methylation is highly frequent due to the association of the genes with mobile elements such as plasmids and conjugative transposons[19,34,35]. Incongruences between ATB ANA and microdilution test have already been reported by Koru and Ozyurt[36] and could be due the use of lower breakpoints in the former. Otherwise, by the reference technique, the resistance to clindamycin was high and the resistance to metronidazole was low, as it has been reported in other countries. This situation must be followed closely because clindamycin and metronidazole are widely used in Costa Rica and in another countries for the treatment of infections by anaerobic bacteria[3,15,24,25,36,37]. The MIC for clindamycin obtained for the majority of the resistant strains is highly suggestive of the presence of mechanisms of acquired resistance among the isolates, therefore surveillance studies are required to determine its efficacy as a treatment drug; the observed low resistance to metronidazole underlines its value as a first-line drug. Our results highlight, in agreement to other investigations dealing with more than 1000 strains[15,28], the utility of imipenem in infections by species of the group that do not respond to the aforementioned first-line drugs. Although the strains were also highly susceptible to chloramphenicol, the systemic use is limited by its potential bone marrow toxicity.

This study is relevant because it reflects part of the epidemiological situation of antimicrobial resistance in anaerobic bacteria in a Latin American country, where information is scarce.

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