



### Investigación original

## Antimicrobial resistance of clinical isolates of anaerobic bacteria from a regional hospital in Costa Rica

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#### Resumen:

Se aislaron 31 cepas de bacterias anaerobias a partir de 28 muestras clínicas de un hospital regional de Costa Rica, recolectadas durante un período de 12 meses (en el año 2006). Se identificaron y se determinó la susceptibilidad a los antimicrobianos a todos los aislamientos utilizando 3 técnicas: el método de referencia de dilución en agar, el sistema ATB-ANA (bioMérieux) y E-test (AB Biodisk). El 19 de los aislamientos fueron identificados como del grupo *Bacteroides fragilis*, 6 como *Eggerthella lenta*. 3 como *Propionibacterium* y 3 como *Clostridium*. Todas las cepas del grupo *B. fragilis* fueron sensibles a metronidazol (con concentraciones inhibitorias mínimas, CMI, de 0.25 a 2 µg/ml) y resistentes a clindamicina (CM de 4 a >128 µg/ml). Algunas cepas de las estudiadas mostraron CMI altas como: penicilina 512 µg/ml, cefotaxime 256 µg/ml, cefolotina 128 µg/ml y clindamicina 128 µg/ml). Pocos bacilos Gram positivos fueron resistentes a los antibióticos ensayados, sin embargo, dos cepas mostraron CMI a metronidazol >128 µg/ml. La CMI considerablemente elevadas a algunos antibióticos y la alta resistencia a la clindamicina en aislamientos del grupo *B. fragilis* son datos muy importantes para el uso terapéutico que se le da principalmente a la clindamicina en infecciones por anaerobios. Los resultados del ATB-ANA discrepan con los obtenidos por el método de referencia y el E-test, por lo que recomendamos el uso de estas dos últimas metodologías de manera rutinaria en laboratorios clínicos para las pruebas de sensibilidad a los antibióticos en bacterias anaerobias.

**Palabras claves:** Resistencia a antibióticos, bacterias anaeróbicas.

**Abstract:**

From 28 clinical samples of a regional hospital in Costa Rica, 31 anaerobic bacterial strains were isolated through 12 months (year 2006). Anaerobic susceptibility test were performed to all isolates, using the reference agar dilution method, ATB-ANA system (bioMérieux), and E-test strips (AB Biodisk). Nineteen strains were identified as *Bacteroides fragilis* group, 6 as *Eggerthella lenta*, 3 as *Propionibacterium* sp. and 3 as *Clostridium* sp. All of the 19 strains of *B. fragilis* were metronidazole sensible (MIC of 0.25 a 2 µg/ml) and clindamycin resistant (MIC of 4 µg/ml to >128 µg/ml); some strains showed high MICs: penicillin 512 µg/ml, cefotaxime 256 µg/ml, cephalotin 128 µg/ml and clindamycin 128 µg/ml). Very few of the Gram-positive rods were resistant to the antibiotics tested; however two strains showed MICs to metronidazole >128 µg/ml. The high MICs to some antibiotics and the high resistance to clindamycin in the *B. fragilis* group strains are relevant findings considering their wide therapeutic use in Costa Rica. Several results obtained by the use of ATB-ANA were discrepant with those obtained when using agar dilution and E-test methods. On this basis, we recommend the use of E-test for routine testing and the agar dilution reference method for more accurate evaluations.

**Key word:** antibiotics resistance, anaerobic bacteria Delirium.

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## INTRODUCTION:

Anaerobic bacteria are important human pathogens and their antibiotic resistance has increased in the last decades [1-4]. In Costa Rica, the regional hospitals do not perform Antimicrobial Susceptibility Test (AST) to anaerobic isolates regularly, due to the complexity of the method. Hence empirical therapies are used as treatments in these infections [1,2]. However, the emergence of resistance to antibiotics that are 62 commonly used as first-line therapies and the variations in susceptibility patterns in different countries, geographic areas and even among hospitals in the same area, have shown the need to carry out the AST more often [2-5,7,8].

The AST method recommended by the Clinical Laboratories Standards Institute (CLSI) is the agar dilution method, which should be performed to a group of strains isolated in a certain period [1]. Nonetheless, given the need to identify individual susceptibility in some isolates, many laboratories have shifted to the use of simpler methods such as the ATB-ANA (bioMérieux) and the E-test (AB Biodisk), the latter approved by the U.S. Federal Drug Administration [2,7,8].

## Materials and methods

From 28 clinical samples of a regional hospital in Costa Rica, (Monseñor Sanabria Hospital, Puntarenas, Costa Rica), 31 anaerobic bacteria strains were isolated (25 strains of intra-abdominal origin and 6 of other origins), during a period of 12 months. AST was performed to all isolates, using the reference

agar dilution method for testing [9] their susceptibility to antimicrobials that are most commonly utilized in clinical practice, namely: penicillin (Sigma-Aldrich), cefotaxime (Karnataka Antibiotics and Pharmaceuticals Ltda.), chloramphenicol (Sigma-Aldrich), cephalotin (Vitalis), metronidazole (Sigma-Aldrich), clindamycin (Sigma-Aldrich), and imipenem (Merck Co.). In addition, the AST using the ATB-ANA system (bioMérieux), as well as metronidazole and clindamycin susceptibility using the E-test strips (AB Biodisk) were performed, following the recommendations of the manufacturers.

## Results and discussion

Nineteen strains were identified as belonging to the *Bacteroides fragilis* group, all of them being of intra-abdominal origin. On the other hand, 12 Gram-positive strains were identified as *Eggerthella lenta* (6 isolates), *Propionibacterium* sp. (3 isolates) and *Clostridium* sp. (3 isolates). Most of the isolates belonging to the *B. fragilis* group (84%) showed resistance to penicillin as expected, since frequently the resistance to penicillins among all members of this group is high (the common mechanism of resistance to penicillin is chromosomally encoded functional cephalosporinase) [2]. Fortunately, carbapenems (such as imipenem) and chloramphenicol resistance remains rare in all *B. fragilis* group strains worldwide [2]; in accordance, in this study, all these isolates were sensitive to

chloramphenicol and imipenem (Table 1). All of the 19 strains of *B. fragilis* were classified as clindamycin resistant by the agar dilution method, showing minimum inhibitory concentrations (MIC) within the range of 4 µg/ml to >128 µg/ml. However, only 14 and 17 strains were considered resistant when using ATB-ANA and E-test methods, respectively (Table 1). The resistance results that were obtained by the three methods (ranging from 75 to 100%) are higher than those reported in other studies, ranging from 10% to 70% [2,4].

A considerable resistance to cefotaxime in 14 isolates, and to cephalotin in 17 isolates belonging to *B. fragilis* group was also observed, showing MICs ranging from 32 to 256 µg/ml. This finding emphasizes that first and third generation cephalosporins may show a poor in vitro activity against *B. fragilis* group [2]. Furthermore, these MIC results are important, since these two antibiotics are used regularly in patients of this Costa Rican hospital.

All isolates of *B. fragilis* group were susceptible to metronidazole by the agar dilution and E-test methods, as reported in most geographic regions [1,2,4]. Again, these results were not identical when using the ATB-ANA method (Table 1). In addition, one *B. stercoris* strain (HMS-44) showed high MICs to four antibiotics (penicillin 512 µg/ml, cefotaxime 256 µg/ml, cephalotin 128 µg/ml and clindamycin 128 µg/ml) and two *B. thetaiotaomicron* strains (HMS-7C1 and HMS-43) showed high MICs to three antibiotics (penicillin 128 and 512 µg/ml, respectively and cephalotin 128 µg/ml,

clindamycin 128 µg/ml, for both strains). On the other hand, very few of the Gram-positive rods were resistant to all four antibiotics tested (Table 1). However, one *Eggerthella lenta* (HMS-42A) and one *Propionibacterium acnes* strains (HMS-28) showed high MICs to metronidazole (>128 µg/ml for both cases). One *C. clostridioforme* isolate (HMS-5B) was resistant to penicillin (MIC 16 µg/ml) and clindamycin (MIC 8 µg/ml).

### Conclusions

This study is one of the first reports of antimicrobial resistance for anaerobic bacteria using standard methods in Costa Rica. The high MICs to some antibiotics, the multiresistance of some strains, and the high resistance to clindamycin in the *B. fragilis* group strains are relevant findings of this survey, considering the wide therapeutic use of these agents in the Costa Rican health system. In addition, the present results confirm the geographical variations in susceptibility patterns of anaerobic bacteria, highlighting the need for an adequate laboratory surveillance of this important microbiological parameter.

Systematic AST evaluations in clinical isolates of anaerobic bacteria at the level of regional hospitals, as illustrated by the present study, should be of high benefit to recognize rapid changes in susceptibility patterns in a given hospital at a given time. This would contribute to the successful treatment of infections caused by anaerobic bacteria.

Of note, several results obtained by the use of ATB-ANA were discrepant with

Table 1. Antimicrobial susceptibility tests to anaerobic bacterial isolates

Group	Antimicrobial	Agar dilution	ATB-ANA	E-test		
		MIC Range (µg/ml)	n	n-R <sup>a</sup>	MIC Range (µg/ml)	n
<i>B. fragilis</i> group (n=19)	Penicillin	1 — 4	3	18	—	—
		8 — 512 <sup>b</sup>	16			
	Cefotaxime	8 — 16	5	—	—	—
		32 — 256 <sup>b</sup>	14			
	Cephalothin	4 — 16	2	—	—	—
		32 — 256 <sup>b</sup>	17			
	Chloramphenicol	2 — 8	19	0	—	—
Metronidazole	0.25 — 2	19	4	0.38 — 3	0	
Clindamycin	4 — 16 <sup>b</sup>	7	14	2 — 3	2	
	32 — >128 <sup>b</sup>	12				4 — >256 <sup>b</sup>
Imipenem	<0.125 — 4	19	0	—	—	
Gram-Positive Rods (n= 12)	Penicillin	<1 — 2	10	6	—	—
		4 — 16 <sup>b</sup>	2			
	Chloramphenicol	<4 — 8	12	0	—	—
	Metronidazole	<4 — 8	9	5	0.094 — 8	8
		16 — 128 <sup>b</sup>	3		16 — >256 <sup>b</sup>	4
Clindamycin	0.125 — 2	10	4	0.032 — 2	10	
	4 — 8 <sup>b</sup>	2		4 — 12 <sup>b</sup>	2	

<sup>a</sup> Number of strains catalogued as resistant.

<sup>b</sup> MIC's ranges of resistance.

those obtained when using agar dilution and E-test methods, as also described in other studies [10]. On this basis, it would seem important to recommend the use of

E-test for routine testing in the diagnostic clinical laboratory, and the agar dilution reference method for more accurate evaluations.

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susceptibilities of clinically