

In vitro* susceptibility of *Arcobacter butzleri* to six antimicrobial drugs

Susceptibilidad *in vitro* de *Arcobacter butzleri* a seis drogas antimicrobianas

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RESUMEN

Se determinaron los patrones de susceptibilidad de 50 cepas de *A. butzleri* mediante el método del E-test. Ninguna cepa fue resistente a gentamicina y tetraciclina, pero, hubo cepas resistentes a eritromicina (2%) y ciprofloxacina (2%). Además, el 90 y el 98% de las cepas fueron resistentes a ampicilina y cloramfenicol respectivamente. Solamente dos de las 45 cepas ampicilina-resistentes fueron productoras de β -lactamasa.

Palabras clave: *Arcobacter butzleri*, susceptibilidad antimicrobiana, bacterias emergentes, E-test, β -lactamasa
Key words: *Arcobacter butzleri*, antibiotic susceptibility, emerging bacteria, E-test, β -lactamase.

INTRODUCTION

The genus *Arcobacter* belongs to the family *Campylobacteraceae*, class Proteobacteria, sub-class Gracillicutes and comprises four species *Arcobacter butzleri*, *A. cryaerophilus*, *A. nitrofigilis* and *A. skirrowii*, formerly known as aerotolerant *Campylobacter*-like organisms (Vandamme, 2000).

The first isolates were obtained by Ellis *et al.* (1977) from aborted bovine fetuses. Further studies related these microorganisms with mastitis and abortion in the bovine, ovine, equine and porcine species (Logan *et al.*, 1982; Vandamme 2000).

Of the four described species, only *A. cryaerophilus* and *A. butzleri* have been isolated from human beings, and are associated with bacteremia and diarrhea. *A. butzleri* has been isolated from patients with endocarditis, peritonitis and appendicitis. Both are considered as emerging foodborne pathogens that could be acquired by consuming mussels, poultry meat, offal and

contaminated water (Jacob *et al.*, 1998, Mansfield and Forsythe, 2000).

Some studies have been carried out in industrialized countries in order to establish the susceptibility of *Arcobacter* to several antimicrobial drugs. As the antibiotic resistance of *Arcobacter* strains from developing countries is not known, the aim of this study was to assess the susceptibility and resistance patterns of *A. butzleri* strains isolated in Southern Chile.

MATERIAL AND METHODS

A total of 50 strains of *A. butzleri* isolated from cattle (5), pelicans (8) duck feces (2) mussels (17), chicken livers for human consumption (8) and river water (10) were examined. All the strains were isolated using the enrichment medium of de Boer *et al.* (1996), incubated aerobically at 26°C for 48 h. They were then plated on the medium of Atabay and Corry (1998) and incubated as described above. All the strains were identified phenotypically using the standard tests (Vandamme, 2000) complemented with the APICAMPY system (bioMérieux).

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Susceptibility to ampicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin and tetracycline was assessed using the E-test method (AB Biodisk, Solna, Sweden). This method has been used previously in our laboratory for testing *Campylobacter jejuni* (Fernández *et al.*, 2000). In brief, several colonies of each strain, obtained from a fresh culture on a blood agar plate, were suspended in 5 ml of Mueller-Hinton broth to a turbidity equal to 0.5 MacFarland standard. The suspensions were inoculated with sterile swabs onto 150 mm diameter Mueller-Hinton agar plates supplemented with 5% sheep blood. The agar surfaces were allowed to dry, and six E-test strips were applied to each plate. Plates were incubated aerobically at 26°C for 48 h and inhibitory concentrations were read at the point where the elliptical zone of inhibition intersected the E-test strip. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and a *C. jejuni* isolate of known susceptibility/resistance were used as control strains. The susceptibility criteria were those defined for *C. jejuni* by the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 2003).

RESULTS AND DISCUSSION

All the ampicillin-resistant strains (45) were tested for β -lactamase using the chromogenic cephalosporin method (DIFCO Laboratories)

complemented with a disc diffusion test for detecting ampicillin-sulbactam susceptibility.

The results of the E-test are shown in table 1 and are expressed as the Minimal Inhibitory Concentrations 50 and 90 (MIC₅₀ and MIC₉₀), corresponding to the antibiotic concentration that inhibits at least the 50 or 90% of the strains respectively.

All the strains were susceptible to gentamicin and tetracycline. The lowest MIC₅₀ and MIC₉₀ values were obtained with gentamicin and ciprofloxacin. The latter, together with erythromycin, showed the lowest percentages of resistance (2%).

The highest MIC₅₀ and MIC₉₀ values were obtained with ampicillin and chloramphenicol which also showed high percentages of resistance (90 and 98% respectively).

All the strains were susceptible to gentamicin and tetracycline. With regard to erythromycin and ciprofloxacin we found that 98% of the strains were susceptible to both antibiotics with one strain resistant to erythromycin and another to ciprofloxacin. These results are similar to those reported by Atabay and Aydin (2001). They found that all 39 strains of *A. butzleri* studied by the disk diffusion method were susceptible to tetracycline, to the aminoglycoside tobramycin and to the quinolones danofloxacin and enrofloxacin. Harrass *et al.* (1998) reported that two out of 89 *A. butzleri* strains were resistant to tetracycline and another two showed intermediate resistance

TABLE 1. MIC₅₀, MIC₉₀ and percentages of resistance obtained for 50 strains of *A. butzleri*.

MIC₅₀, MIC₉₀ y porcentajes de resistencia obtenidos en 50 cepas de *A. butzleri*.

Antibiotics	Range (mcg/ml)	MIC ₅₀	MIC ₉₀	% Resistance
Gentamicin	0.064-0.5	0.125	0.38	0
Tetracycline	0.5-8	2	4	0
Erythromycin	0.25-8	1.5	3	2
Ciprofloxacin	0.012-3	0.023	0.094	2
Ampicillin	1-256	24	64	90
Chloramphenicol	3-256	64	192	98

to erythromycin, also using the disc diffusion method. Kiehlbauch *et al.* (1992), using the broth microdilution method, found that aminoglycosides, quinolones and tetracyclines were most active *in vitro* against 64 strains of *C. butzleri* (currently *A. butzleri*).

The other two antibiotics included in our study were ampicillin and chloramphenicol with five strains susceptible to the former and only one to the latter. These results are in agreement with those reported by other authors for ampicillin (Kiehlbauch *et al.*, 1992; Harrass *et al.*, 1998; Atabay and Aydin, 2001). However, some differences have been observed with relation to chloramphenicol. While Kiehlbauch *et al.* (1992) reported 100% resistance, Harrass *et al.* (1998) found 11.2% of strains to be resistant, but all the strains studied by Atabay and Aydin (2001) were susceptible to this antibiotic. Such differences could be due to local differences, probably related to the use of this antibiotic. In Chile, chloramphenicol is the drug of choice in the treatment of typhoid fever which, declining in incidence, remains endemic here (Fica *et al.*, 2001).

All the ampicillin resistant strains of *A. butzleri* (45) were tested for beta lactamase production. Table 2 shows the results obtained with the chromogenic cephalosporin test and the disc diffusion susceptibility test for ampicillin-sulbactam. Only two strains were able to produce β-lactamase, giving a positive chromogenic cephalosporin test. However, because the use

of this test to determine beta lactamase production in species of the genus *Arcobacter* has not been described before, we repeated these tests with a disc diffusion test, using ampicillin and ampicillin-sulbactam discs. Both strains were resistant to ampicillin but susceptible to the combination ampicillin-sulbactam. As sulbactam is a beta lactamase inhibitor we conclude that in these two strains the ampicillin resistance mechanism could be mediated by beta lactamase production.

Finally, we conclude that, in general, our strains are susceptible to gentamicin, tetracycline, erythromycin and ciprofloxacin. They have high resistance to ampicillin and chloramphenicol. Further studies should be conducted in order to establish the resistance mechanisms in *A. butzleri* as well as to explain the geographical differences observed in their susceptibility to these antimicrobial drugs.

SUMMARY

The susceptibility patterns of 50 *A. butzleri* strains to six antimicrobial agents were determined using the E-test method. No strain was found to be resistant to gentamicin and tetracycline, but two different strains (2%) were resistant to erythromycin and ciprofloxacin. Ninety and 98% of the strains were resistant to ampicillin and chloramphenicol, respectively. Only two of the 45 ampicillin resistant strains were able to produce β-lactamase.

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TABLE 2. β-Lactamase production and susceptibility to ampicillin-sulbactam in 45 *A. butzleri* ampicillin resistant strains.

Producción de β-lactamasa y susceptibilidad a ampicilina-sulbactam en 45 cepas de *A. butzleri* ampicilina resistentes.

Characteristic	n/N	%
β-lactamase +	2/45	4.5
β-lactamase -	43/45	95.5
Susceptible to ampicillin-sulbactam	2/45	4.5
Resistant to ampicillin-sulbactam	43/45	95.5

N: number of positive strains; n: number of ampicillin resistant strains.

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