SHORT COMMUNICATION

Antimicrobial Susceptibility of *Campylobacter jejuni* subsp. *jejuni* Assessed by E-test and Double Dilution Agar Method in Southern Chile

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The susceptibility patterns of 108 Campylobacter jejuni *subsp.* jejuni *clinical strains, to six antimicrobial agents was determined by using the E-test and the double dilution agar methods. Using both metods, no strain was found to be resistant to ciprofloxacin, erythromycin and gentamicin, but two (1.8%) were resistant to tetracycline and all to aztreonam. Seven (6.5%) strains were resistant to ampicillin by the E-test and five (4.6%) by the double dilution agar method and by both meyhods. No great discrepancies were observed between both methods.*

Key words: *Campylobacter jejuni* subsp. *jejuni* - antimicrobial susceptibility - E-test - double dilution agar test

Campylobacter jejuni subsp. *jejuni* is one of the most frequent causes of infectious diarrhea in both, developed and developing countries (Fernández 1992, Allos & Blaser 1995). Although *C. jejuni* subsp. *jejuni* diarrhea is a self-limited disease, in some clinical instances it is necessary the prescription of antibiotics, being erythromycin the drug of choice (Allos & Blaser 1995). For several years however, higher levels of resistance to this drug, as well as to others, such as tetracycline and fluoroquinolones, have been reported in several countries (Taylor & Courvalin 1988).

The information on bacteriological, biological, pathogenical, clinical and epidemiological aspects of *C. jejuni* subsp. *jejuni* generated in South America is vast and valuable (Fernández 1992). However, the susceptibility patterns of this bacterium have not been well defined in this geographical region.

The aim of this study was to determine the susceptibility patterns of *C. jejuni* subsp. *jejuni* clinical strains, to six antimicrobial agents, in Southern Chile by using two quantitative methods.

A total of 108 *C. jejuni* subsp. *jejuni* strains submitted in this study, had been isolated and identified from pediatric diarrheic stools specimens using standard procedures between March 1996 and December 1997. Strains were stored at -35ºC in freezing medium until analyzed. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* and *E. coli* ATCC 25922 were used as control strains.

The antimicrobial susceptibility patterns of the strains under study were determined by means of the E-test and the double dilution agar method (Baker et al. 1991).

To perform the E-test, several colonies of each strain, obtained from a fresh culture in blood agar plate, were suspended in 5 ml of Mueller-Hinton broth to achieve a turbidity equal to the 0.5 Mac Farland standard. The suspensions were inoculated with sterile swabs onto 150 mm diameter Mueller-Hinton 5% sheep blood agar plates and after the agar surfaces were allowed to dry, six E-test strips were applied on each plate. Plates were incubated at 37ºC for 48 h under microaerobic conditions and inhibitory concentrations were read at the point where the elliptical zone of inhibition intersected the E-test strip.

For the agar dilution method, Mueller-Hinton agar plates containing serial twofold dilutions of each antimicrobial agent from 0.125 to 128 µg/ ml were prepared. Inocula of the strains were prepared diluting 1:10 each bacterial suspension made in Mueller-Hinton broth with a turbidity equal to the 0.5 Mac Farland standard. Plates were inoculated with a multipoint replicator delivering spots of 4

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µl of the diluted bacterial suspensions, starting from the lowest to the highest concentration of each antimicrobial drug. At the beginning of each series a Mueller-Hinton agar plate without antibiotics was inoculated as a viability control of the strains under study. The inoculated plates were incubated under the same conditions described above. For this method, minimal inhibitory concentrations (MICs) were defined as the lowest antibiotic concentrations yielding no growth.

The antimicrobial agents tested in this study were erythromycin, tetracycline, gentamicin, ciprofloxacin, ampicillin and aztreonam. The susceptibility criteria were those defined by the National Committee for Clinical Laboratory Standards (National Comimttee for Clinical Laboratory Standards 1995).

Table shows the MICs of the six antimicrobial agents tested. Using both metods, no strain was found to be resistant to ciprofloxacin, erythromycin and gentamicin, but all were resistant to aztreonam showing MICs ≥256 µg/ml. The MICs for ampicillin ranged from 0.064-48 µg/ml and from ≤0.125-32 µg/ml with the E-test and the double dilution agar method respectively. Seven (6.5%) strains were resistant to ampicillin by the E-test method, whereas five (4.6%) presented this characteristic with the double dilution agar method. We believe that these results are not in great discrepancy with both methods since they only differ in one step dilution, being MIC_{90} the same for E-test and agar dilution method (8 µg/ml). Similar observations were reported previously by Baker et al. (1991) for other bacteria and by Baker (1992) for *C. jejuni*.

The highest MIC found for tetracycline was 32 µg/ml and the same two strains (1.8%) were resistant to tetracycline by both methods. With the exception of two strains that appeared to be resistent to ampicillin with the E-test and susceptible with the double dilution agar method, no other major discrepancies were observed between both methods. The MICs obtained by both methods were very similar.

In this study, no strain of *C. jejuni* subsp. *jejuni* was found to be resistant to erythromycin, the antibiotic of choice to treat *Campylobacter* enteritis, and to ciprofloxacin and gentamicin, antimicrobial agents indicated to treat acute diarrhea and extraintestinal infections due to *Campylobacter* respectively (Allos & Blaser 1995), by either methods. In a previous study, using *C. jejuni* subsp. *jejuni* strains isolated from hens, we did not find resistance to gentamicin and erythromycin (Tejero et al. 1996). On the other hand, using the disk difussion method employed routinely in our laboratory as a screening method, we have isolated through various years only three erythromycin-resistant strains (unpublished data). With these results, we can speculate that erythromicyn resistance is not a common phenomenon observed in *C. jejuni* subsp. *jejuni* strains isolated in Southern Chile. However, we can not rule out that it could be an emerging problem in the future, as it occurred in other countries (Taylor & Courvalin 1988). With regard to ciprofloxacin, we have previously isolated *C. jejuni* subsp. *jejuni* resistant strains from domestic animals recognized as reservoir and as source of infection for human beings. These strains showed concomitant resistance to other quinolones (Fernández et al. 1996). This suggests that in the future we could isolate, from animals and from human beings, *C. jejuni* subsp. *jejuni* strains showing resistance to ciprofloxacin and other quinolones. Resitance to quinolones in *Campylobacter* has been increasingly reported since the past decade (Gootz & Martin 1991, Gaudreau & Gilbert 1998).

All our strains were highly resistant to aztreonam. This was reported previously in *C.*

TABLE

Susceptibility (minimal inhibitory concentrations) of 108 *Campylobacter jejuni* subsp. *jejuni* clinical strains to six antimicrobial agents by using the E-test and the double dilution agar method

	MIC (µg/ml)							
	E-test				Double dilution agar method			
Antibiotic	Range	50%	90%	% resistant	Range	50%	90%	% resistant
Ampicillin	0.064-48	1.5	8.0	6.5	≤0.125-32	1.0	8.0	4.6
Aztreonam	≥256	≥256	≥256	100	≥256	≥256	≥256	100
Ciprofloxacin	0.032-0.25	0.064	0.125	0	≤0.125	≤0.125	≤0.125	0
Erythromycin	0.064-2	0.19	0.5	0	≤0.125-2	0.125	0.5	0
Gentamicin	0.064-1.5	0.38	0.75	0	≤0.125-1	0.5	1.0	0
Tetracycline	0.023-32	0.094	0.38	1.8	≤0.125-32	0.125	0.125	1.8

jejuni strains by Goossens et al. (1985) and by our group (Tejero et al. 1996) in *C. jejuni* subsp. *jejuni* and *C. coli* strains isolated from hens. Bearing in mind the high resistance to aztreonam observed in *C. jejuni* subsp. *jejuni* and the high activity of this antimicrobial drug against several enteropathogens, we have successfuly tested a new selective antimicrobial mixture that includes aztreonam, to isolate *C. jejuni* subsp. *jejuni* and *C. coli* from different sources (manusc. in prep.).

We have previously found a low percentage (2.3%) of tetracycline resistance in strains isolated from hens (Tejero et al. 1996), and in the present study only two (1.8%) out of the 108 clinical strains tested were resistant to this antibiotic. So, based on the evidence mentioned above, we can conclude that in this geographical region tetracycline is not yet a problem as it is in other countries (Taylor & Courvalin 1988, Gaudreau & Gilbert 1998, Li et al. 1998).

Using the E- test we found seven strains (6.5%) resistant to ampicillin. Five of them (4.6%) were also resistant by the double diluition agar method. Since we have not isolated ampicillin-resistant strains (Tejero et al. 1996) before, these are the first documented data about ampicillin resistance in *C. jejuni* subsp. *jejuni* in our region. Probably this could be an emerging problem that could require the establishment of a laboratory surveillance system to assess its real magnitude.

In agreement with the reports of Baker et al. (1991*)* and Funke et al. (1993), no great discrepancies were observed between both methods. This, and the fact that the E-test is technically simple and needs no special equipment, it could be become a reliable method for susceptibility testing in *C. jejuni* subsp. *jejuni.*

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