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COMUNICACIONES

Isolation of thermotolerant species of *Campylobacter* from river water using two collection methods*

Aislamiento de especies termotolerantes de *Campylobacter* de aguas fluviales utilizando dos métodos de colecta.

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RESUMEN

Campylobacter jejuni y *C. coli* son agentes zoonóticos de infecciones intestinales que pueden contaminar cuerpos de agua. Varios brotes de gastroenteritis por *Campylobacter* asociados al consumo de agua han sido descritos. En este estudio se compara el rendimiento de los métodos de la tórula de Moore (MSM) y de filtración por membrana para el aislamiento de *Campylobacter* en 181 muestras de agua obtenidas del curso urbano del río Calle-Calle. En el 24.3% de las muestras obtenidas por MSM y en el 7.2% de las obtenidas por MFM fue posible aislar especies termotolerantes de *Campylobacter*, siendo *C. coli* aislado en mayor proporción que *C. jejuni.* Los resultados sugieren que MSM es un método eficiente para el aislamiento de estas bacterias a partir de agua.

Palabras claves: *Campylobacter jejuni*, *C. coli*, agua, tórula de Moore,técnica de filtración por membranas.

Key words: *Campylobacter jejuni*, *C. coli*, water, Moore swab, membrane filtration technique.

INTRODUCTION

The thermotolerant species of *Campylobacter*, *C. jejuni* and *C. coli* are considered as important agents of intestinal infection all over the world (Allos and Blaser, 1995; Fernández, 1992; Friedman *et al*., 2000). Many domestic and wild animals are known as natural reservoirs that, together with sewage, could contaminate water bodies. Both species can survive in water from several days to several weeks, depending on water temperature (Anderson *et al.,* 1997; Pickert and Bozenhart, 1985).

Several outbreaks of *Campylobacter* gastroenteritis associated with the consumption of contaminated water have been reported (Anderson *et al.,* 1997; Sacks *et al*., 1986; Skerjve and Brennhovd, 1992; Jacobs-Reitsma, 2000).

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Various methods have been described in order to determine the presence of enteropathogenic bacteria in water, being enrichment procedures and filtration through membranes the most frequently used (Bolton *et al.*, 1987; Mathewson *et al*., 1983). The Moore swab is a concentration method described in 1948 to isolate *Salmonella* and *Vibrio cholerae* from water (Barret *et al.,* 1980). However, little information is available in relation to the isolation of *Campylobacter* species from water bodies using this method. The Moore swab was used previously in a pilot study in our laboratory (Fernández *et al*., 1990).

In this study we compared the performance of the Moore swab (MSM) and the filtration through membrane (MFM) methods for the isolation of thermotolerant species of *Campylobacter* from water samples obtained from the Calle-Calle river, Valdivia, Chile (39º 47' Southern latitude, 73º 15' Western latitude).

MATERIAL AND METHODS

We obtained duplicated water samples (181 for MSM and 181 for MFM) from different points of the urban course of the Calle-Calle river (26 Km long, 297 m average wide), according to the following methods:

Moore Swab Method (MSM) (Fernández *et al*., 1990): consists of a piece of gauze of 70x22 cm, folded several times to form cylindrical rolls which were wrapped in thick paper and autoclaved. Each swab, attached to a 5 m long nylon string, was left for 24 hrs in the river. When removed, each swab was placed into a flask containing 125 ml of an enrichment broth with a formulation twice higher than the normal one, to compensate the dilution produced by the water absorbed by the gauze. This medium consisted of Brucella broth, FBP supplement (ferrous sulphate, sodium metabissulfite and sodium pyruvate 0.5 g/L of each), vancomycine (20 mg/L), polimyxin B (5.000 I.U./L), trimethoprim (10 mg/L), cephalotine (20 mg/L) and anphotericin (4 mg/L). The flasks were incubated at 42ºC for 48 hrs under microaerobic atmosphere, and 200 µl were seeded on Butzler selective medium incubated as described above.

Membrane Filtration Method (MFM) (Mathewson *et al*., 1983): Four litre of water were passed through a sterile 47 mm membrane filter (Millipore) with 0.45 µm pore size, using a vacuum pump. The filter was aseptically placed into a flask containing 100 ml of the enrichment broth described above. The rest of the procedure was similar to that used with the MSM.

Suspect *Campylobacter* colonies were seeded on blood agar in order to obtain pure culture and then, they were identified and biotyped by their morphological characteristics, susceptibility to cephalotine and nalidixic acid and by their capacity to produce hypuricase, desulfhydrase and DNAse (Fernández *et al*., 1990).

RESULTS AND DISCUSSION

As shown in table I, 24.3% of the samples were positive for *Campylobacter* using the MSM method whereas, with the MFM, only in 7.2% these micro-organisms were isolated, being this difference statistically significative (chi-square test). The higher isolation rate obtained with the MSM could be due to the fact that the swabs remain during 24 hrs into the water, thus allowing filtration of a great quantity of water and the capture of *Campylobacter* cells, as well as other micro-organisms, suspended in the water. The water sample volume used for the MFM was 4 L, an amount that compared with the MSM, could be insufficient to allow a higher isolation rate.

Contrariously to earlier reports (Bolton *et al*., 1987; Skerjve and Brennhovd, 1992), *C. coli* was most frequently isolated (77.3%) than *C. jejuni* (22.7%). Similar results were obtained with both methods (MASM and MFM). A higher isolation rate of *C. coli* from wastewater samples was observed by Höller (1988) and in river water samples by Fernández *et al*. (1990). Some authors suggested that *C. coli* could be more resistant than *C. jejuni* to environmental

Table I. Isolation rates of *Campylobacter* species using the Moore Swab and the Membrane Filtration Methods.
Aislamiento de especies de Campylobacter utilizando la tórula de Moore y el Método de Filtración por Membrana.

	POSITIVE SAMPLES		*C. coli*		*C. jejuni*	
METHOD	N/n	%	N/n	%	N/n	%
Moore Swab	44/181*	24.3	34/44	77.3	10/44	22.7
Membrane filtration	13/181*	7.2	10/13	76.9	3/13	23.1

N= number of positive samples; n= total number of samples; *p<0.01

conditions (Tresierra-Ayala *et al*., 1999). That could explain the differences observed in the isolation rates between both species. Currently, experimental studies are carried out in our laboratory in order to establish if *C. coli* is more resistant than *C. jejuni* to environmental conditions. Despite this difference, it is necessary to bear in mind that both species are considered as important agents of diarrheal disease, and contaminated water are recognised as an epidemiological source of outbreaks as well as sporadic cases of intestinal campylobacteriosis.

Our results show that the MSM is an efficient method to isolate *Campylobacter* species. Recently we start to use the MSM to isolate *Arcobacter* species from the same type of samples with good results (data not showed). Based on these results we propose the use of MSM as an adequate, easy and inexpensive method for the isolation of *Campylobacter* species from river water samples.

SUMMARY

Campylobacter jejuni and *C. coli* are zoonotic agents of intestinal infection that could contaminate water bodies. Several outbreaks of *Campylobacter* gastroenteritis associated with the consumption of contaminated water have been reported. Various methods have been described to determine the presence of these bacteria in water bodies. In this study the performance of the Moore swab (MSM) and the filtration through membrane methods (MFM) for the isolation of *Campylobacter* from 181 water samples obtained from the urban course of the Calle-Calle river water samples was compared. *Campylobacter* species were isolated in 24.3% of the samples obtained with MSM and in 7.2% of those processed by MFM thus, appearing MSM as an efficient method to isolate *Campylobacter* from river water samples.

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