Short communication

Evaluation of the Cape Town Protocol for the isolation of *Campylobacter* spp. from environmental waters

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Abstract

Campylobacter is recognised as one of the major causes of bacterial gastro-enteritis world-wide. In addition to poultry products, milk and water have also been implicated as possible sources of infection. Methods for the detection and isolation of this organism have been developed specifically for the medical field and select for *Campylobacter jejuni* and *C. coli*, excluding all other species of *Campylobacter*, whereas the filter-based Cape Town Protocol reportedly yields most *Campylobacter* spp. The Cape Town Protocol was evaluated for possible use in analysis of environmental water samples. It yielded only 0.1% of the total number of actively growing *C. jejuni* and *C. coli* cells, whereas the selective medium mCCDA yielded 10%. Analysis of 60 water samples yielded 221 putative *Campylobacter* isolates, but only four could be confirmed as *Arcobacter butzleri* and none as *Campylobacter*. Our results indicated that neither the Cape Town Protocol nor mCCDA can be used for the direct enumeration or isolation of *Campylobacter* spp. from environmental water samples.

Keywords: Campylobacter, Arcobacter, water-borne pathogens

Introduction

Campylobacter is recognised as one of the major causes of acute bacterial gastro-enteritis world-wide (Allos, 2001; Griffiths and Park, 1990; Lior, 1996). *Campylobacter jejuni*, *C. coli* and *C. lari* are amongst the species known to be pathogenic to humans, but *C. jejuni* is the most commonly isolated species from patients (Engberg et al., 2000; Stanley et al., 1998; Tauxe, 1997; Thomas et al., 1999). In a South African study, *Campylobacter* has been isolated from 22% of diarrhoeic stools, indicating that it is a common cause of diarrhoeal disease (Le Roux and Lastovica, 1998).

There are various routes of infection for *Campylobacter*-related illnesses, but poultry products still remain the primary source implicated in infections of humans, mainly because this organism forms part of the chicken's commensal intestinal microflora (Chan et al., 2001). In addition to poultry, unpasteurised milk and untreated surface water have also been implicated as possible sources of infection (Beumer et al., 1992; Jones et al., 1991; Koenraad et al., 1995). Recent reports by Obiri-Danso and Jones (1999a; 1999b) showed that surface water in the United Kingdom was regularly contaminated with *Campylobacter*, introduced by treated sewage or surface runoff, but that numbers decreased following extended exposure to UVB rays (Obiri-Danso et al., 2001). The role of water in the dissemination of *Campylobacter* therefore remains elusive.

Methods for the detection and isolation of this fastidious organism have been developed with specific application in the medical field. In most cases, selective culture media containing combinations of various different antibiotics, that reportedly inhibit the growth of competitor microflora, are used for the isolation and culturing of selected *Campylobacter* spp. Detection of *Campylobacter* spp. in environmental samples has been based strongly on molecular methods such as PCR (Kirk and Rowe, 1994; Koenraad et al., 1995; Lawson et al., 1998; Oyofo and Rollins, 1993; Waage et al., 1999) and nucleic acid hybridisation (Buswell et al., 1998). All of these methods have been designed to detect or isolate only the thermophilic *Campylobacter* spp., i.e. *C. jejuni*, *C. coli* and in some cases *C. lari*. The only method reported to yield isolates from most of the known members of the genus is based on the ability of *Campylobacter* to move through 0.6 µm pores in mixed ester membrane filters (Steele and McDermott, 1984). This method has been refined by Lastovica and co-workers at the Red Cross Children's Hospital in Cape Town, and is commonly referred to as the Cape Town Protocol. Since no selective agents are used in this method, it is possible to isolate virtually all of the known *Campylobacter* species (Le Roux and Lastovica, 1998). By making use of this method, extensive analysis of stool samples from infected South African patients has revealed that *C. jejuni* and *C. coli* collectively constitute 38% of species isolated, and that an array of other pathogenic *Campylobacter* spp. abound (Le Roux and Lastovica, 1998). A representative analysis of environmental samples for *Campylobacter* should therefore not be too stringent. The Cape Town Protocol represents the only current method for the isolation of all known *Campylobacter* spp.

The aim of the work reported here was to evaluate the Cape Town Protocol for the detection of *Campylobacter* spp. from environmental waters.

Materials and methods

Strains and culture media

Clinical strains of *C. jejuni* (MF 1217R) and *C. coli* (NCTC 11283) were obtained from the Red Cross Children's Hospital (Rondebosch, Cape Town). The *Campylobacter* strains were grown on blood agar base No. 2 (Oxoid CM271) supplemented with 10% (v/v)

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