

Serogrouping and Randomly Amplified Polymorphic DNA Fingerprinting of *Campylobacter Jejuni*

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Abstract

Citation: Trajkovska-Dokic E, Stojkovska S, Icev K, Grozdanova A. Serogrouping and Randomly Amplified Polymorphic DNA Fingerprinting of *Campylobacter Jejuni*. Maced J Med Sci. 2011 Dec 15; 4(4):372-375. http://dx.doi.org/10.3889/MJMS.1957-5773.2011.0200.

Key words: *Campylobacter jejuni*; serogrouping; RAPD-PCR fingerprinting.

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Received: 21-Oct-2011; Accepted: 13-Nov-2011; Online first: 25-Nov-2011

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Competing Interests: The authors have declared that no competing interests exist.

Background: Thermophilic campylobacters are of worldwide significance in human and animal diseases. Sources of human infection remain mainly undetermined, but contaminated food, like poultry and raw milk, are widely regarded as important vehicles of infection. Accurate methods of strain identification, differentiation and typing are essential for diagnosing and epidemiological purposes.

Aim: The aim of our study was to determine the serologic and genetic diversity among the strains and to assess the discriminatory power of both typing methods in epidemiological studies.

Material and Methods: Within a period of six months were isolated 26 strains of *C. jejuni* from faecal samples of children with acute gastroenteritis. Heat-stable specific antigen of *C. jejuni* was used for serogrouping of the strains by the reaction of passive hemagglutination. Purified genomic DNA was obtained and used in RAPD-PCR reaction.

Results: Twenty one *C. jejuni* strains belonged to the following Penner's serogroups: 8 strains were group A, 13 strains were group O. The remaining 5 strains were non-typable. RAPD-PCR analysis of 26 strains of *Campylobacter jejuni* yielded multiple amplification products in all of them. None of the strains processed by this method was non-typable. According to the number and sizes of amplification bands, 4 different genotypes (*a*, *b*, *c*, *d*) of *C. jejuni* strains were distinguished within 26 investigated strains.

Conclusion: In this study we found that RAPD-PCR analysis provide better discrimination of *C. jejuni* strains than serogrouping by Penner's method. Each of the three Penner's antigenic groups comprised different genotypes. RAPD-PCR analysis of *C. jejuni* resulted in the generation of highly specific and reproducible DNA fingerprints that enable discrimination even between isolates of a single bacterial serogroup.

Introduction

Thermophilic campylobacters are of worldwide significance in human and animal diseases. *Campylobacter jejuni* in particular, is recognized as a major cause of acute bacterial enteritis in man in most developed countries [1, 2]. Sources of human infection remain mainly undetermined, but contaminated food, like poultry and raw milk, are widely regarded as important vehicles of infection [3].

Accurate methods of strain identification, differentiation and typing are essential for diagnosing enteritis caused by campylobacters as well as for detecting the source or the origin of the infection. Conventional phenotypic methods based on biotyping [4], serotyping [5] or phage typing [6] have been applied to campylobacters for over a decade. New molecular genotyping methods based on chromosomal DNA analysis are more stable and avoid dependence on

expressed and possibly variable phenotypic features. Plasmid profiling has not been developed as a typing method, because less than 50% of campylobacters carry plasmids [7]. Two molecular methods that provide precise and stable strain markers applicable to pure culture of campylobacters are ribosomal DNA (rDNA) gene profiles from Southern blot hybridization (ribotyping) and polymerase chain reaction (PCR) generated fingerprints from random primer sequences [8].

Ribotyping is of value in typing most bacterial pathogens including *Campylobacter* species [9], but is time-consuming and not well suited to routine use. The PCR has revolutionized molecular biology through the introduction of new genetic assays based on selective DNA amplification [10]. In 1990, a PCR method based on the amplification of random DNA fragments using a single primer of arbitrary sequence was described as a method for typing of different pathogenic microorganisms including isolates of the genus *Campylobacter*.

In the present study, we evaluated the application of *C. jejuni* serogrouping by Penner and RAPD fingerprinting with a 10-mer primer of arbitrary sequence, for detecting DNA polymorphisms in *C. jejuni* strains.

The aim of this study was to determine the serologic and genetic diversity among the strains and to assess the discriminatory power of both typing methods in epidemiological studies.



Figure 1: Colonial morphology of *Campylobacter*.

Material and Methods

Microorganisms and growth media

The primary isolation was done on blood agar base no. 2 (Oxoid CM271) supplemented with 5% defibrinated sheep blood (Oxoid SR51) and Butzler selective supplement (Oxoid SR85). Inoculated media were cultivated at 42°C, under microaerophilic conditions for 42 hours. Isolates were confirmed as *Campylobacter* by colonial (Fig. 1) and microscopic morphology, catalase and oxidase reactions. Differentiation of *C. jejuni* was done by positive reaction of hippurate hydrolysis (purple color) (Fig. 2).



Figure 2: Hippurate hydrolysis reaction for differentiation of *C. jejuni*.

Using this conventional method for isolation and differentiation of campylobacters, during a period of six months were isolated 26 strains of *C. jejuni* from faecal samples of children with acute gastroenteritis.

Serogrouping of C. jejuni by Penner

Heat-stable specific antigen of *C. jejuni* extracted by nitric acid was sensitized to the blood cells (DENKA SEIKEN, Japan). When the sensitized cells were mixed with the antiserum, specific reaction of passive hemagglutination was observed. All 26 isolates of *Campylobacter* were biotyped by the reactions for hippurat hydrolysis, H₂S production and DNA hydrolysis.

RAPD-PCR analysis

Purified genomic DNA was obtained in 200 µl suspension of bacterial cells by boiling for 10 min and centrifuging for 5 min. 10 µl of each dilution was used in a 40 µl PCR reaction volume by addition of 10 mM Tris-HCl pH 8.8; 2.5 mM MgCl₂, 200 mM of each deoxynucleotide triphosphate, 0.2 mM of the primer OPA-11 with sequence 5'-CAATCGCCGT-3', 1U Taq

DNA Polymerase and sterile distilled water. The solutions were overlaid with 100 ml of paraffin oil and cycled through the following temperature profile: an initial denaturation step at 94°C for 1 min, 45 cycles of 94°C for 1 min (denaturation), 36°C for 1 min (annealing), and 72°C for 2 min (DNA chain extension), and final elongation step at 72°C for 5 min. Incubation was performed in a thermocycler (JR Instrumentation, UK). The amplified DNA products were electrophoresed on 1.8 % agarose gels and stained in ethidium bromide solution. A 100 bp ladder was used as a marker for the PCR products.

Results

Twenty one *C. jejuni* strains belonged to the following Penner's serogroups: 8 strains were group A, 13 strains were group O. The remaining 5 strains were non-typable.

Table 1: Biotyping of *Campylobacter jejuni*.

Test	Biotypes of <i>Campylobacter jejuni</i>			
	I	II	III	IV
Hippurate hydrolysis	+	+	+	+
H ₂ S test	-	-	+	+
DNA hydrolysis	-	+	-	+

Biotyping of 26 *Campylobacter* isolates revealed four different biotypes (I, II, III and IV) (Table 1). 19, 4, 2 and 1 strains belonged to biotype I, II, III and IV respectively. All the strains from the Penner serogroup A and 11 strains from the Penner serogroup O were biotype I.

RAPD analysis of 26 isolates of *Campylobacter jejuni* yielded multiple amplification products in all of

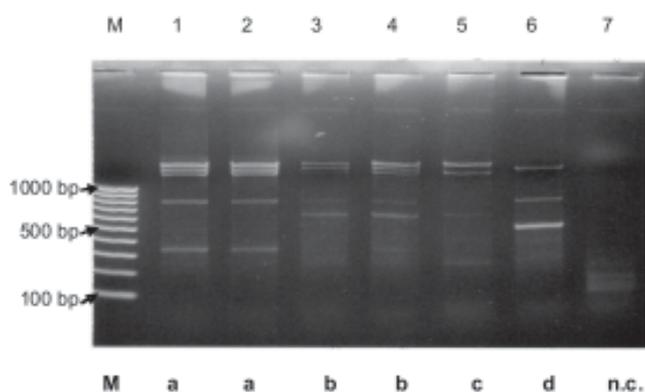


Figure 3: RAPD fingerprints of *Campylobacter jejuni*. M - Marker; Lines: 1 and 2 - genotype a; Lines: 3 and 4 - genotype b; Line: 5 - genotype c; Line: 6 - genotype d; Line: 7 - negative control (n.c.).

them. None of the strains processed by this method was non-typable. The resultant fingerprints comprised between 1 and 7 bands with sizes between 0.3 and 1.5 kb. The majority of strains had characteristic amplification bands of 1.5, 1.4, 1.3, 0.8, 0.7, 0.6 and 0.3 kb. According to the number and sizes of amplification bands, 4 different genotypes (a, b, c, d) of *C. jejuni* strains were distinguished within 26 investigated strains. Control assays in which cell suspensions were replaced by plain distilled water yielded no detectable amplified product. Representative example is shown in Figure 3.

Discussion

In this study we found that RAPD-PCR analysis provide better discrimination than biotyping and serogrouping by Penner's method. Each of the three Penner's antigenic groups comprised different genotypes. RAPD fingerprinting proved to have an excellent discrimination ability, confirming the higher degree of variation among *C. jejuni* strains. A simple procedure in which bacteria were boiled and the lysate was directly introduced in the PCR vessel enabled reproducible typing of serologically non-typeable strains. RAPD fingerprinting of *Campylobacter jejuni* is well suited for epidemiological studies.

RAPD-PCR process resulted in the generation of highly specific and reproducible DNA fingerprints that enable discrimination even between isolates of a single bacterial serotype. Apparently, minimal genetic differences can be determined through this relatively simple technique. For differentiation of *Campylobacter jejuni* strains, RAPD-PCR analysis has been applied successfully and may replace biotyping and serotyping tests. Such data are needed in the epidemiological surveillance for investigating the origin of the infection, and the distribution of particular bacterial types in different environments. This procedure may be especially time and cost effective, when in a single specimen are required the detection and typing of multiple infectious agents.

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