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Diversity of *Campylobacter jejuni* and *Campylobacter coli* Genotypes from Human and Animal Sources from Rio de Janeiro, Brazil

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ABSTRACT

To compare the genotypes of *Campylobacter jejuni* and *Campylobacter coli* isolates of human and animal origin collected in Rio de Janeiro City, 30 *C. jejuni* and 35 *C. coli* isolates from animal sources ($n = 45$) and human patients with gastroenteritis ($n = 20$) were genotyped by PCR-based techniques, namely random amplified polymorphic DNA (RAPD-PCR) and enterobacterial repetitive intergenic consensus sequence (ERIC-PCR). RAPD-PCR identified 50 types and ERIC-PCR identified 22 genotypes, among the 65 *Campylobacter* isolates. Both PCR methods discriminated the *C. jejuni* and *C. coli* groups of isolates. Combining the results of both methods, no single genotype was shared between isolates from human and animal sources. Two groups of two *C. coli* isolates each with identical genotypes were found among poultry and pig isolates. A high level of genetic diversity observed among the *Campylobacter* isolates suggests lack of overlap between isolates from different sources.

Campylobacter jejuni and *Campylobacter coli* are recognized as important causative agents of acute human diarrheal diseases world-wide. However, the sources and transmission routes of human campylobacteriosis are not fully understood. Consuming and handling poultry meat is the most consistent risk factor, and other meat-producing animals and pet animals are suggested as potential sources for human infection (Steinbruecker et al., 2001; Zorman et al., 2006). This confusing epidemiological evidence is partly because of the sporadic nature of the disease, along with the organism's wide distribution, and high levels of genetic and antigenic diversity (Zorman et al., 2006). Several molecular typing methods have been used to support studies on the epidemiology of *Campylobacter* infections. Although pulsed-field gel electrophoresis and multilocus sequence typing are highly standardized (Zorman et al., 2006), polymerase chain reaction based methods, like arbitrarily primed PCR are also successfully applied for the discrimination of *C. jejuni* and *C. coli* strains (Zorman et al., 2006; Madden et al., 2007). In this study we describe a molecular epidemiological investigation using PCR-based DNA fingerprinting of *C. jejuni* and *C. coli* isolates from human enteritis and animal reservoirs.

The 65 *Campylobacter* isolates included in this study were collected over a 12-month period, in Rio de Janeiro City. They comprised 45 animal isolates, including eight *C. jejuni* and 16 *C. coli* strains from poultry carcasses from different flocks; 10 *C. coli* strains from slaughter pigs from different herds; six *C. jejuni* from a single sheep herd collected in one visit; three *C. jejuni* from dogs; one *C. jejuni* and one *C. coli* from *Rhesus* monkeys housed in caging. Twelve *C. jejuni* and eight *C. coli* strains from human patients with clinically confirmed gastroenteritis collected during the same period were also incorporated. All the *Campylobacter* isolates were recovered from stool samples, except those from rinsed poultry carcasses. *C. jejuni* ATCC 33291 and *C. coli* ATCC 43485 were included as control strains.

The *Campylobacter* strains were cultured on 5% Columbia blood agar plates (Oxoid Ltd.) in a microaerophilic atmosphere at 37 °C for 48 h. The strains were identified to the species level by biochemical tests for *Campylobacter* (Stern et al., 1992) and by the use of a multiplex PCR test (Harmon et al., 1997). For the molecular analysis, freshly cultured cells were inoculated in 10 mL of *Brucella* broth (Oxoid Ltd.) and incubated at 37 °C in a microaerophilic atmosphere for 24–48 h. From these cultures, 3 mL aliquots were centrifuged (13,000*g* for 1 min) and the pellets were used for DNA extraction according to Pitcher et al. (1989).

Random amplified polymorphic DNA (RAPD) typing was conducted using the method of Akopyanz et al. (1992) and ERIC-PCR was performed as described previously by Giesendorf et al. (1994). The profiles were analysed in duplicate to highlight the

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reproducibility of the described RAPD protocol as suggested by Hilton et al. (1997). Amplified products were analysed on agarose 2% gels, stained with ethidium bromide, and photographed under UV translumination. The ϕX-174 RF DNA-*Hae* III Digest (Amersham Pharmacia Biotech) and 1-kb DNA ladder (Gibco BRL) were used as molecular weight markers for RAPD-PCR and ERIC-PCR, respectively. All genotypes were analysed by using the Molecular Analyst Fingerprinting Plus software package version 1.12 (Bio-Rad). Gels were analysed and compared by using band matching with UPGMA (Unweighted Pair Group Method using Arithmetic Averages) clustering using the Dice coefficient, and 1.2% tolerance. Two dendrograms were constructed to assess genetic relationship among the isolates investigated (Figs. 1 and 2).

RAPD-PCR identified a total of 50 types among the 65 isolates, using a cut off of 90%, and 26 and 24 patterns were identified for *C. jejuni* (cluster I) and for *C. coli* (cluster II), respectively. Amplification reactions by ERIC-PCR identified 8 and 14 patterns among *C. coli* (cluster I- with two exceptions, H5 and PO7/11 identified as *C. jejuni*) and *C. jejuni* (cluster II- with one exception, H1103 identified as *C. coli*) isolates, respectively.

The results of this study showed extensive genomic diversity among the *C. jejuni* and *C. coli* isolates, most notably among *C. jejuni* as observed in both PCR typing methods. Combining the results of both methods, no identical pattern was identified among human isolates and no pattern observed for any animal isolate matched those of human origin. This significant genomic diversity is probably due to the lack of overlap of the different sources sampled. Earlier investigations also reported extreme heterogeneity of *Campylobacter* isolates (Madden et al., 2007; Ridley et al., 2008). Although *Campylobacter* spp. have a natural ability for transformation, genomic rearrangements most likely explain this increase in genetic diversity (Ridley et al., 2008). Reliable tracing of the source of *Campylobacter* strains by molecular typing is therefore very difficult. Besides the extreme heterogeneity of the isolates, mainly for *C. jejuni*, other reasons include contamination of meat samples with multiple strains and cross-contamination (Zorman et al., 2006). In this study, *C. jejuni* sheep isolates were cultured from rectal swabs collected from animals belonging to the same herd and no identical pattern was identified among them. However, two identical *C. coli* patterns from poultry carcasses purchased from

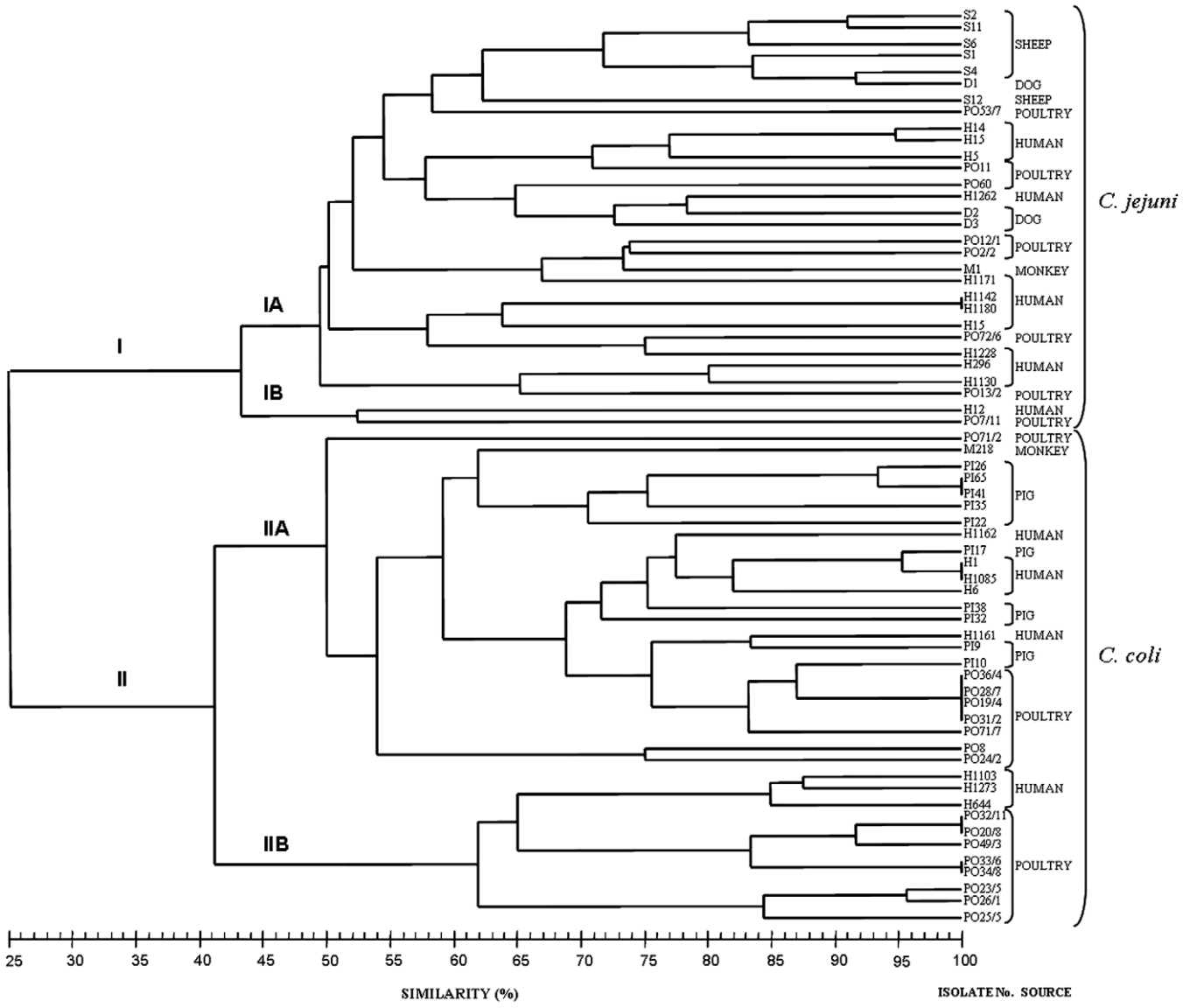

Fig. 1. Dendrogram of the cluster analysis of *C. jejuni* (cluster I) and *C. coli*(cluster II) isolates based on PCR/RAPD patterns from human (H), poultry (PO), pig (P), sheep (S), dog (D), and monkey (M) origin with their degree of relatedness.