2010-12-06

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Recommended Citation

Bullman, S., Corcoran, D., O'Leary, J., Lucey, B., Byrne, D. and Sleator, R.D. (2011). Campylobacter ureolyticus:an emerging gastrointestinal pathogen? FEMS Immunology & Medical Microbiology, 61(2), pp.228–230. https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1574-695X.2010.00760.x

SHORT COMMUNICATION

Campylobacter ureolyticus: an emerging gastrointestinal pathogen?

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Received 21 September 2010; revised 27 October 2010; accepted 1 November 2010. Final version published online 6 December 2010.

DOI:10.1111/j.1574-695X.2010.00760.x

Editor: Jacques Schrenzel

Keywords
Campylobacter ureolyticus; *Campylobacter*; gastroenteritis; pathogenic; PCR.

Abstract

A total of 7194 faecal samples collected over a 1-year period from patients presenting with diarrhoea were screened for *Campylobacter* spp. using EntericBio®, a multiplex-PCR system. Of 349 *Campylobacter*-positive samples, 23.8% were shown to be *Campylobacter ureolyticus*, using a combination of 16S rRNA gene analysis and highly specific primers targeting the HSP60 gene of this organism. This is, to the best of our knowledge, the first report of *C. ureolyticus* in the faeces of patients presenting with gastroenteritis and may suggest a role for this organism as an emerging enteric pathogen.

Campylobacter is the most common cause of bacterial enteritis worldwide, surpassing the other major bacterial enteric pathogens; *Salmonella* spp., *Shigella* spp. and *Escherichia coli* O157 (Tauxe, 2002; Glandis, 2007). In a recent study of enteric pathogen detection techniques, conducted at Cork University Hospital (CUH) in Ireland, O'Leary *et al.* (2009) compared conventional stool culture methods, using Preston agar [*Campylobacter* agar base (CM689; Oxoid, Basingstoke, UK) with supplement SR204E] under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2) at 42 °C, with the EntericBio® system (Serosep Ltd, Limerick, Ireland), a multiplex-PCR-based detection system for the rapid, simultaneous detection of *Campylobacter* spp., *Salmonella* spp., *Shigella* spp. and *E. coli* O157 at the molecular level. In this study, it was reported that 28.6% of the samples positive for *Campylobacter* spp., using the multiplex-PCR method, failed to grow on routine culture. Taking into account the inherent difficulty of *Campylobacter* spp. isolation, this observation, together with a clinical history of diarrhoea, suggests that traditional culture methods may fail to detect a significant proportion of genuine *Campylobacter* infections, a finding that, given the widespread dependence on culture-based diagnostic techniques, raises significant public health concerns. The initial focus of the current study was thus to investigate the discrepancy observed between culture- and molecular-based detection methods.

A retrospective analysis of faecal samples submitted to CUH in 2009 was performed to identify, to the species level, all *Campylobacter* strains detected using the EntericBio® system in a single calendar year. During this period, a total of 7194 patient faecal samples were processed, 349 (4.9%) of which were positive for *Campylobacter*. Uniplex *Campylobacter* spp.-specific PCR screening for six clinically significant enteropathogenic *Campylobacter* spp. (*Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter fetus*, *Campylobacter upsaliensis* and *Campylobacter hyointestinalis*) (Moore *et al.*, 2005), was initially conducted on each positive sample using primers described by Wang *et al.* (2002) and Yamazaki-Matsune *et al.* (2007).

Interestingly, 74 of the 349 samples (21.2%) yielded negative results for these *Campylobacter* spp.-specific PCR assays. *Campylobacter* genus-specific PCR, targeting the 16S rRNA gene region using the C412F and C1288R primer pair described by Linton *et al.* (1996), was subsequently performed on the 74 *Campylobacter* genus PCR-positive samples. This approach afforded further confirmation of the

presence of *Campylobacter* spp. in the sample, ruling out the possibility of false positives incorporated by the EntericBio® system. A representative sample of these *Campylobacter* genus-positive samples was sequenced using 16S rRNA gene-specific primers (C412F and C1288R) described by Linton *et al.* (1996). In each case, BLAST analysis revealed ≥99% identity (over the entire length of the sequence) to the *Campylobacter ureolyticus* type strain NCTC 10941T/DSM 20703. To investigate the prevalence of *C. ureolyticus* in the nonspeciated samples ($n = 74$), we developed a *C. ureolyticus* spp.-specific PCR primer set (CU-HSP60 F: 5′-GAA GTA AAA AGA GGA ATG GAT AAA GAA GC-3′ and CU-HSP60 R: 5′-CTT CAC CTT CAA TAT CCT CAG CAA TAA TTA AAA GA-3′), amplifying a 429-bp region of the heat shock protein (*hsp60*) gene of *C. ureolyticus*. The accession numbers for the HSP60 gene sequences of strains LMG 24746, R-37890, LMG 6451, LMG 24747, R-38115 and LMG 8448 are FN421436–FN421441, respectively. *In silico* analysis confirmed that the regions to which CU-HSP60 F and CU-HSP60 R bind are specifically targeted to *C. ureolyticus*, and at the annealing temperature used (61 °C), are highly unlikely to bind to any *Campylobacter* spp., or indeed any other enteric organisms in the National Centre for Biotechnology Information nonredundant database. The specificity of this assay was further assessed by wet laboratory analysis, whereby the DSMZ control strains *C. jejuni* ssp. *jejuni* DSM 4688, *C. coli* DSM 4689, *C. lari* ssp. *lari* DSM 11375, *C. fetus* ssp. *fetus* DSM 5361, *C. upsaliensis* DSM 5365 and *C. hyointestinalis* ssp. *hyointestinalis* DSM 19053 all tested negative for the *C. ureolyticus* spp.-specific PCR. Thus, the *hsp60* locus amplified by CU-HSP60R F and CU-HSP60 represents a specific biomarker for *C. ureolyticus*.

Targeting this specific biomarker, 71.6% ($n = 53$) of the nonspeciated samples were found to be positive for *C. ureolyticus*. This organism has been reclassified recently as *C. ureolyticus* from its previous classification as *Bacteroides ureolyticus*, on the basis of protein profiles and molecular analysis of conserved genes (Vandamme *et al.*, 2010). While the similarity between *C. ureolyticus* and members of the *Campylobacter* genus has been acknowledged on genotypic grounds (Paster & Dewhirst, 1988; Vandamme *et al.*, 1995), differences in protein metabolism and fatty acid composition have meant that, until now, it remained in the category of species '*incertae sedis*' (Vandamme *et al.*, 1995).

All 349 *Campylobacter*-positive samples were retrospectively screened using the *C. ureolyticus*-specific *hsp60* gene target. *Campylobacter ureolyticus* was detected in 83 samples, representing 23.8% of all *Campylobacter*-positive samples. Of these, 30 were found to exist as mixed isolates with other *Campylobacter* spp.

DNA extracted from the type strain for *C. ureolyticus* (NCTC 10941T/DSM 20703) (Vandamme *et al.*, 2010) tested positive for *Campylobacter* spp. when tested with the EntericBio® system. Routine culture, on the other hand, failed to detect the organism from samples that were PCR positive for *C. ureolyticus* only. Vandamme *et al.* (2010) have reported that *C. ureolyticus* is incapable of growing in the microaerobic atmosphere used in routine *Campylobacter* culture (5% O_2, 10% CO_2 and 85% N_2) with common agar bases unless hydrogen is supplied. The EntericBio® system thus displays significantly improved sensitivity over traditional culture methods for the detection of diverse species of *Campylobacter* or *Campylobacter*-like organisms. Furthermore, given that only 1.15% of the 7194 samples processed using the EntericBio® system were noted to contain *C. ureolyticus*, it seems unlikely that this organism exists as a common commensal of the gastrointestinal tract. Indeed, before this study, reports of *C. ureolyticus* have been rare, being isolated only from patients with superficial soft tissue or bone infections, nongynaelogical nonchlamydial urethritis, bacterial vaginosis and periodontal disease (Fontaine *et al.*, 1986; Duerden *et al.*, 1987, 1989; Mazuecos *et al.*, 1998). Moreover, it has rarely been the sole isolate from these sites of infection, with most findings reporting the presence of other organisms that may have been contributing to pathogenesis (Duerden *et al.*, 1982; Akhtar & Eley, 1992). Within the past decade, there has been a dearth of clinical studies investigating *C. ureolyticus*; indeed, the current study represents the first report identifying the presence of *C. ureolyticus* in the faeces of patients presenting with diarrhoeal illness. In total, we have identified 53 cases where *C. ureolyticus* was isolated from faecal samples in the absence of the other common bacterial enteric pathogens.

Interestingly, this organism is reported to be urease positive (Vandamme *et al.*, 2010), which is unusual for *Campylobacter* spp. Matsuda & Moore (2004) have reported the presence of urease-positive thermophilic *Campylobacter* (UPTC) in the natural environmental; however, they did not detect these organisms in any of the human faeces samples in their study. It would be of interest to investigate the similarity between the environmental UPTC isolates detected by Matsuda & Moore (2004) and the urease-positive *C. ureolyticus* that we have detected and hope to isolate from clinical samples.

A random subset ($n = 8$) of patients, presenting with diarrhoeal illness, in whom *C. ureolyticus* was the sole potential pathogen detected, was followed up clinically. Five patients (three females and two males) were ≥69 years of age, two, one female and one male, were 6 and 1 years of age, respectively, and one male was 52 years of age. All patients presented with abdominal cramping and reported symptoms lasting for a minimum of 5 days. Furthermore, within this subset, three patients were immunocompromised; two had diabetes mellitus; and one patient had an HIV-associated Burkitt's lymphoma.

The detection of this organism by the EntericBio® system further validates the molecular method for the identification of *Campylobacter* spp. Until such time as we have gained a greater understanding of the clinical impact of *C. ureolyticus* on human health, it is difficult to consider the true value of *C. ureolyticus* detection in diagnostic laboratories.

In conclusion, then, this is the first report to identify the presence of *C. ureolyticus* in the faeces of patients presenting with diarrhoeal illness, suggesting its possible role as a novel gastrointestinal pathogen. We are currently in the process of assessing the clinical significance of our findings and the implications that these results may have for patient health and infection control.

Acknowledgements

S.B. (NCTC 10941T) is supported by a scholarship from the Irish Research Council for Science, Engineering and Technology (RS/2009/1670). R.D.S. is an ESCMID Fellow. Funding was provided by Serosep Ltd. The authors declare that they have no competing interests.

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