Multilocus Sequence Typing analysis of human Campylobacter coli in Granada (Spain)

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ABSTRACT

Introduction. Different subtypes of Campylobacter spp. have been associated with diarrhoea and a Multilocus Sequence Typing (MLST) method has been performed for subtyping. In the present work, MLST was used to analyse the genetic diversity of eight strains of Campylobacter coli.

Material and methods. Nineteen genetic markers were amplified for MLST analysis: AnsB, DmsA, ggt, Cj1585c, CJ81176-1367/1371, Tlp7, cj1321-cj1326, fucP, cj0178, cj0755/cfrA, ceuE, pldA, cstll, cstIII. After comparing the obtained sequences with the Campylobacter MLST database, the allele numbers, sequence types (STs) and clonal complexes (CCs) were assigned.

Results. The 8 C. coli isolates yielded 4 different STs belonging to 2 CCs. Seven isolates belong to ST-828 clonal complex and only one isolate belong to ST-21. Two samples came from the same patient, but were isolated in two different periods of time.

Conclusions. MLST can be useful for taxonomic characterization of C. coli isolates.

Key words: Campylobacter coli; typing methods; Sequence Typing; MLST; diarrhoea

INTRODUCTION

The Gram-negative bacterium Campylobacter spp. is a zoonotic pathogen which may be part of the gut microbiota of a range of wild and domesticated mammal and bird species1. This bacterium is able to colonize the intestines of chicken, turkey and waterfowl and to be transmitted to human by faecal-oral...
route. Based on its frequency, *Campylobacter jejuni* is the most important causing diarrhea in humans whereas *Campylobacter coli* is the most resistant to antibiotics, although is much less frequent causing disease in humans\(^2\). *Campylobacter* infection in humans tends to be sporadic, and rarely manifests as outbreaks except when a single point source results in direct transmission to many people, for example, via contaminated drinking water\(^3\).

Different subtypes of *Campylobacter* spp. have been associated with different manifestations of disease such as diarrhoea, and several subtyping methods have been established during the past years\(^4\). In the last years sequence-based methods, such as Multilocus Sequence Typing (MLST)\(^5\) and the sequencing of the short variable region of the *flagellin* A gene are widely used\(^6\). MLST has shown some advantages over other molecular methods because includes transferability, standardized nomenclature, free access to the database and direct comparability of results between different studies\(^7\). In the present work, MLST was performed to analyse the genetic diversity of eight strains of *C. coli* isolated in Granada (Spain) in a short period of time.

### MATERIAL AND METHODS

**Clinical samples.** Eight faeces samples isolated in Granada (Spain) from seven patients with ages between 5 months and 38 years old were analysed for the presence of *Campylobacter* spp. All samples were cultured at the Microbiology Department of Virgen de las Nieves Hospital Complex, Granada, between August 19\(^{th}\), 2013 and October 21\(^{th}\), 2013. Samples were gathered in sterile containers with no transport media and delivered to our laboratory under refrigeration (4 °C), with a maximum delay of 2 hours before their processing. They were processed for coproculture immediately on their reception by culture in CampyBAP\(^{®}\) medium with 10% blood (Becton Dickinson, Franklin Lakes, NJ, USA) using a 30-µg cefoxitin disk (BD BBL\(^{®}\)) and incubated for 48h at 42°C in microaerophilic atmosphere (Campygen\(^{®}\), Oxoid, Basingstoke, UK). Suspicious colonies were identified by means of oxidase cytochrome tests (Difco, Detroit, MI, USA), Gram staining, and mass spectrometry using the Biotyper\(^{®}\) system (Bruker Daltonics, Coventry, UK). The samples were also seeded in the usual culture media for enteropathogens for 48 h (XLD\(^{®}\) agar [BD] at 37°C for recovery of *Salmonella*, *Shigella*, and *Plesiomonas*, and CIN\(^{®}\) agar [BD] at 30°C for recovery of *Yersinia* and *Aeromonas*) or 24h (Selentio\(^{®}\) broth [Difco] for recovery of *Salmonella*, followed by a subculture in Hektoen\(^{®}\) agar [BD] at 37 °C). Colonies suspected of being enteropathogenic were identified by means of the Biotyper\(^{®}\) system (Bruker Daltonics) and the usual biochemical tests (MicroScan; Siemens Healthcare, Rockville, MD, USA). Colonies identified as *Salmonella* were subjected to the agglutination test to determine the serogroup (Difco)\(^7\).

**DNA extraction and quantification.** DNA was extracted from chocolate agar plates cultures. One ml of PBS pH 7.5 was added to each plate. *Campylobacter* were resuspended with an inoculating loop. Resuspended bacteria in PBS were recovered and added to a 1.5 ml Eppendorf tube. The Eppendorf tubes were centrifuged for 10 min at 5000 g. Pellet were resuspended in 180 µl Buffer ATL (Qiagen, CA, USA) and DNA was extracted using QiAamp DNA Mini kit (Qiagen, CA, USA) following manufacturer instructions. DNA concentration and purity (A\(_{260}$/A\(_{280}\)) were measured using a Nanodrop (Thermo Scientific, USA).

**MLST.** Nineteen genetic markers were amplified for MLST analysis following Zautner et al. recommendation\(^8\): *AnkB*, *Dm-sA*, *ggt* (g-glutamil transpeptidase), *Cj1585c* (oxidoreductase), *Cj81176-1367/1371* (serin protease), *Tlp7*, *cj1321-cj1326*, *fucP*, *cj0178*, *cj0755/cfrA*, *ceuE*, *pldA*, *cstII*, *cstIII*. PCR products were purified using StrataPrep PCR Purification Kit (cat nº 400771, Agilent Technologies, CA, USA) and sequencing was carried out in an ABI PRISM 3100 genetic Analyzer (Applied Biosystem, CA, USA). After comparing the obtained sequences with the *Campylobacter* MLST database (http://pubmlst.org/ campylobacter), the allele numbers, sequence types (STs) and clonal complexes (CCs) were assigned\(^9\).

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<th>SAMPLE CODE</th>
<th>PATIENT AGE</th>
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### RESULTS

The 8 *C. coli* isolates yielded 4 different STs belonging to 2 CCs. Seven isolates belonged to ST-828 clonal complex and only one isolate belonged to ST-21. Two samples came from the same patient, but were isolated 23 days apart (table 1).

### DISCUSSION

Currently, phylogenetic methods like MLST are considered to be the standard typing methods for *Campylobacter* spp. isolates\(^5\). Many different host-adapted clonal complexes never or
only rarely cause disease in humans whereas others may be common human pathogens with different foodborne sources. In the present study one of the isolates belong to ST-21 and seven isolates to ST-828. This is consistent with previous reports by other authors as the ST-21, ST-45 and ST-828 are the most frequent CCs found in humans1. These CCs, together with ST-35310, and ST-443, ST-57411 are the most frequently isolated in poultry and cattle. A seasonal and geographical influence distribution for different STs have been described. In this sense, ST-828 is the most common CC found in sheep in Scotland although, it also has been found in swine production system12. In USA, this ST-828 CC has been extensively found in broiler farms in Andalusia, the prevalence of Campylobacter spp. in individual animals was 38.1% and the flock prevalence was 62.9%17. One of the limitations of our study is that we do not have information regarding the source of transmission of the isolates to the patients of our study. One possibility is that they could have been infected with C. coli isolates derived from cattle or poultry as these reservoirs are the most frequent in the geographical area of the patients17.

In this study, seven from eight isolates belong to the same clonal complex (ST-828), and five from those seven isolates belong to the same Sequence Type (825). This fact together with the short period of time when the samples were collected in Granada, allow us to think of a high probability of a common origin of this outbreak, at least for these 5 samples. In conclusion, this method based on nineteen genes MLST analysis, can be useful for taxonomic characterization of C. coli isolates.

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