Brief report

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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*, *CJJ81176-1367/1371*, *Tlp7*, *cj1321-cj1326*, *fucP*, *cj0178*, *cj0755|cfrA*, *ceuE*, *pldA*, *cstII*, *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

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Tipificación mediante secuencias multilocus de cepas humanas de *Campylobacter coli* en Granada (España)

RESUMEN

Introducción. Diferentes subtipos de *Campylobacter* spp. se han asociado con diarrea y la técnica de tipado mediante análisis de secuencias de múltiples locus (MLST) se ha empleado para la tipificación genética. En el presente trabajo, la técnica MLST se utilizó para analizar la diversidad genética de ocho cepas de *Campylobacter coli*.

Material y métodos. 19 marcadores genéticos fueron amplificados mediante el análisis MLST: *AnsB*, *DmsA*, *ggt*, *Cj1585c*, *CJJ81176-1367/1371*, *Tlp7*, *cj1321-cj1326*, *fucP*, *cj0178*, *cj0755|cfrA*, *ceuE*, *pldA*, *cstII*, *cstIII*. Después de comparar las secuencias obtenidas con la base de datos MLST para *Campylobacter*, se asignaron el número de los alelos, los secuenciotipos (STs) y los complejos clonales (CCs).

Resultados. Las 8 cepas de *C. coli* aisladas mostraron 4 STs diferentes pertenecientes a 2 CCs. Siete aislamientos pertenecieron al complejo clonal ST-828 y sólo un aislado perteneció al ST-21. Dos aislados pertenecieron al mismo paciente, pero fueron obtenidos en diferentes periodos de tiempo.

Conclusiones. La técnica MLST puede ser útil para la caracterización taxonómica de aislados de *C. coli*.

Palabras clave: *Campylobacter coli*; métodos de tipificación; análisis de secuencias; MLST; diarrea.

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 species[1]. 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 diarrhoea 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[4]. 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[6]. 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 19th, 2013 and October 21th, 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, BD, 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 (Selenito® 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]: *AnsB*, *DmsA*, *ggt* (g-glutamil transpeptidase), *Cj1585c* (oxidoreductase), *CJJ81176-1367/1371* (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 sequenciation was carried out in an ABI PRISM 3100 genetic Analyzer (Aplied 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].

Table 1 **Results for the eight samples analysed. Clonal complex and sequence type for the eight strains analysed are indicated. The samples with *, 56758 and 64611, were isolated from the same patient 23 days apart. Age of patients is indicated in the table.**

SAMPLE CODE	PATIENT AGE	CLONAL COMPLEX	SEQUENCE TYPE
56602	10 months	ST-21	1526
56628	10 years	ST-828	825
56758*	38 years	ST-828	825
57554	6 years	ST-828	825
64611*	38 years	ST-828	825
66537	2 years	ST-828	825
64642	5 months	ST-828	5755
70069	7 years	ST-828	464

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 humans[1]. These CCs, together with ST-353[10], and ST-443, ST-574[11] 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 system[12]. In USA, this ST-828 CC has been extensively found in chickens[13] and pigs[14]. A different study has shown that ST-21, ST-45 and ST828 CCs were the most prevalent CCs in ducks from South Korea[15]. The different CCs described above are commonly isolated in multiple animal species and shown extraordinarily rapid rates of zoonotic transfer leading to little or no phylogenetic association with specific host species[1]. Clonal complex shown differences in stress responses; this is probable the main reason for the presence of certain *Campylobacter* spp. subgroups in defined hosts, environments, and the spread over different transmission routes[4].

Risk factors for *Campylobacter* infection, include eating or preparing raw or undercooked meat, especially chicken meat, drinking unpasteurized milk or untreated water, contact with domestic animals, foreign travel and swimming in natural bodies of water[16]. Molecular epidemiological investigations are critical to understand the sources and routes of transmission. More than 95% of human infections are attributable to animals farmed for meat and poultry. In a study investigating 291 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 patients[17] .

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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