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Serotyping Of Campylobacter Jejuni Strains Isolated From Hospitalized Patients

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ABSTRACT

Campylobacter jejuni is a major foodborne pathogen in developed and developing countries. It is recognized as one of the most commonly causes of bacterial gastroenteritis accompanied by abdominal pain, fever and vomiting. Rare postinfectious complications, associated with a preceding C. jejuni infection, consist mainly of neurological immunopathologic disorders. So far, there is no data about the distribution of Campylobacter serotypes in R. of Macedonia.

The Aim of the present study was to serotype C. jejuni strains isolated from hospitalized patients with gastroenteritis in order to establish preliminary data for the presence of particular serotypes in our patients.

Material and methods: In the study were included a total of 47 C. jejuni strains, isolated from the same number of patients during two different periods. 21 and 26 strains were isolated in 2010 and 2015, respectively. All the strains were isolated on Skirrow's selective medium in microaerophilic atmosphere for 48 hours and identified by their typical macromorphology, micromorphology, positive oxidase and hippurate hydrolysis rections. Serotyping was performed with a commercially available set of antisera (Campylobacter Antisera Set, Denka Seiken, Japan), based on Penner's heat-stable HS-serogroups.

Results: 44 (93.61%) out of 47 C. jejuni strains were serotyped and the rest of 3 (6.38 %) strains were characterized as non typable (NT). The most frequent C. jejuni serotype was HS : 2. It was followed by the HS : 4/13/16/43/50, HS : 1/44, HS : 5, HS : 3, HS : 15 and HS : 19. It has been not identified significant difference in the distribution of serotypes between the two periods of investigation.

Conclusion: Serotyping could be the main typing method for the characterization of C. jejuni isolates of clinical and epidemiological importance. This is the first report from our country concerning C. jejuni serotyping. We hope it will establish the basis for a continuous epidemiologic surveillance.

Keywords:- *Campylobacter jejuni*, gastroenteritis, serotyping, heat-stable antigens.

I. INTRODUCTION

Campylobacter is a microaerophilic, curved gram negative rod that is recognized as major foodborne pathogen. Campylobacteriosis is often a self-limiting disease, lasting 3-5 days, commonly associated with diarrhea, abdominal pain, fever and vomiting. But, severe cases including those resulting in dysentery, may require medical attention. Long-term complications like Guillain-Barre Syndrom (GBS) and reactive arthritis have been linked to campylobacter infection (1).

Many typing methods were developed for differing *Campylobacter* isolates (2). The most widely used phenotyping procedure is serotyping, based on the detection of heat-stabile antigens by a passive hemagglutination technique following Penner and Hennessy scheme (3). Recent data show that *Campylobacter* express lipooligosaccharides (LOS) and a capsule polysaccharide (CPS) instead of lipopolysaccharides (LPS) (4, 5, 6). The CPS is one of the few identified virulence factors in *Campylobacter* and the primary serodeterminant of the Penner scheme (**7**). Serotyping has a clinical importance providing a causative relation of certain serotypes in antecedent

Campylobacter infection and postinfectious campylobacter-related disorders (8). So far, the kind of *Campylobacter* serotypes causing gastroenteritis have not been surveyed in our country.

The aim of the present study was to establish reference data for the presence of *Campylobacter* serotypes in patients hospitalized at the Clinical Center "Mother Teresa" in Skopje and to investigate their distribution within two different periods of time.

II. MATERIALS AND METHODS

In the study participated 47 patients with acute gastroenteritis from 3 to 30 years of age, hospitalized at the Pediatric Clinic and the Clinic for infectious diseases, Clinical Center "Mother Teresa" in Skopje. Stool samples were collected and sent for bacteriological examination on the day of hospitalization, to the Institute for microbiology and parasitology, Medical faculty in Skopje.

Primary isolation of *Campylobacter* was done by inoculation the samples on Skirrow's selective medium and incubation of the plates at 42^0 C, for 48 hours, under microaerophilic atmosphere (Genbox, Biomerieux, France) containing 10 % CO_2, 5 % O_2, and 85 % N_2).

The identification of *Campylobacter* isolates was performed on the basis of their typical growth, microscopic examination, positive oxidase and hippurate hydrolysis rections. A total of 47 isolates were collected during two periods. 21 were collected during 2010 and 26 during 2015. All isolates were kept frozen in 20 % glycerol on - 20^0 C until the day of serological examination. They were freshly isolated on Columbia blood agar before the procedure of serotyping. Serotyping was performed with a commercially available set of antisera (*Campylobacter* Antisera Set, Denka Seiken, Japan), based on Penner's heat-stable HS-serogroups and containing 25 absorbed antisera against the following HS-serotypes: (1,44), 2, 3, (4,13,16,43,50), 5, (6,7), 8, 10, 11, 12, 15, 18, 19, 21, (23,36,53), 27, 31, 32, 37, 38, 41, 45, 52, 55, 57. Heat stable antigen extracts were prepared by the original Penner boiling-method (2). Bacteria were harvested from blood agar plates and suspended in saline (Mc Farland No. 6). Heat-stable antigens were extracted by boiling and fixed onto freshly prepared sheep erythrocytes. The sensitized erythrocytes were mixed with antisera in microtiter plates and examined for positive haemagglutination. A control serum included in the set was run for detecting any non-specific reaction. The absence of positive reaction of haemagglutination was characterized as not typed strain.

III. RESULTS

A total of 47 *Campylobacter* isolates belonged to *C. jejuni* species. 21 (44.68%) and 26 (55.32%) strains of *C. jejuni* were isolated in 2010 and 2015, respectively. 44 (93.61 %) out of 47 *C. jejuni* strains were serotyped and the rest of 3 (6.38 %) strains were characterized as non-typed strains (Table 1).

Table 1. Serotyped *Campylobacter jejuni* strains isolated in 2010 and 2015

Typed and nontyped *C. jejuni* strains	*C. jejuni* strains isolated in 2010 No. (%)	*C. jejuni* strains isolated in 2015 No. (%)	Total No. and % of *C. jejuni* strains isolated in both periods
Typed	19 (90.48)	25 (96.16)	44 (93.61)
Nontyped	2 (9.52)	1 (3.84)	3 (6.38)
Total No. and %	21 (44.68)	26 (55.32)	47 (100)

The most common *C. jejuni* serotype in both periods presented with 28.57 % in 2010 and with 34.61 % in 2015, was HS : 2.

It was followed by the HS : (4/13/16/43/50), HS : (1/44), HS:5, HS : 3, HS : 15 and HS : 19 serotypes presented with 23.80 %, 14.28 %, 9.52 %, 4.76 %, 4.76 %, 4.76 % in 2010 and 11.53 %, 11.53 %, 3.84 %, 3.84 %, 11.53 %, 7.69 % in 2015, respectively. Serotype HS : 37 was not detected in 2010, but in 2015 it was found in 11. 53 %. (Table 2).

There was no significant difference in the frequency of isolation of *C. jejuni* as well as in the distribution of *C. jejuni* serotypes between the two periods of investigation.

Table 2. *Campylobacter jejuni* serotypes in 2010 and 2015

Serotype	*C. jejuni* in 2010 No. and (%)	*C. jejuni* in 2015 No. and %	Total No. and % of *C. jejuni* in both periods
HS : 2	6 (28.57)	9 (34.61)	15 (31.91)
HS : (4/13/16/43/50)	5 (23.80)	3 (11.53)	8 (17.02)
HS : (1/44)	3 (14.28)	3 (11.53)	6 (12.76)
HS : 5	2 (9.52)	1 (3.84)	3 (6.38)
HS : 3	1 (4.76)	1 (3.84)	2 (4.25)
HS : 15	1 (4.76)	3 (11.53)	4 (8.51)
HS : 19	1 (4.76)	2 (7.69)	3 (6.38)
HS : 37	0	3 (11.53)	3 (6.38)
Not typed	2 (9.52)	1 (3.84)	3 (6.38)

IV. DISCUSSION AND CONCLUSION

The distribution pattern of *C. jejuni* serotypes did not show predominance of a certain serotype in *Campylobacter* associated gastroenteritis, which is compatible with the incidence of sporadic cases. *C. jejuni* serotypes: HS : 2, HS : 1/44 and HS : 4 (as a part of the complex antiserum HS : 4, 13, 16, 43, 50) detected in our study have been found as the 10 mostly common serotypes in several countries: UK, USA, Sweeden, Greece (9, 10). The same three *C. jejuni* serotypes were detected in 61.70 % of our *C. jejuni* isolates. The remaining 31.92 % of *C. jejuni* isolates belonged to the group of 5 serotypes: HS : 3, HS: 5, HS : 15, HS : 19 and HS : 37. The low percentage of every particular serotype of *C. jejuni* belonging to these group of 5 serotypes, might be a result of the total low number of *C. jejuni* isolates in our study. Our study also revealed that 6.38 % of *C. jejuni* isolates were non-typable. The reason for this might be the panel of the used antisera. Probably, a higher percentage of *C. jejuni* isolates would be typed with a larger set of antisera.

Serotyping could be the main typing method for the characterization of *C. jejuni* isolates of clinical and epidemiological importance. This is the first report for *C. jejuni* serotypes in R. of Macedonia, which could be the basis for a continuous epidemiological surveillance.

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