Serotyping and PCR assay of Campylobacter jejuni/coli strains isolated from pigs, poultry and humans in the Czech Republic

I. Steinhauserová, J. Smola[1], H. Štegnerová
University of Veterinary and Pharmacological Sciences Brno,
Department of Meat Hygiene and Technology, [1]Department of Microbiology and Immunology
Palackeho 1-3, 612 42 Brno Czech Republic

Introduction

Thermophilic campylobacters are recognized as one of the most important bacteriological causes of gastroenteritis in man. Most of these outbreaks are caused by Campylobacter jejuni and in lesser degree by Campylobacter coli. Campylobacter spp. have been found as a normal commensals of intestinal microflora of many domestic animals especially poultry and pigs.

Aim

The aim of the study was to practise bio- and serotyping of isolated strains of Campylobacter jejuni and provide identificaton of representative strains using PCR.

Methods

In the course of years 1994-6 we isolated strains of Campylobacter spp. from domestic animals (pigs and poultry) and human patients. The isolates of Campylobacter spp. of animal origin were isolated from broiler chicken and pigs faeces. The human strains were obtained from patients in separate outbreaks of acute diarrhoea.

The samples of pigs´ and chickens´ intestinal content were randomly collected from rectum and large intestine. The isolates were placed in the biotyp scheme for Campylobacter jejuni and Campylobacter coli according to by Lior´s scheme with using 3 biochemical tests (hippurate hydrolysis, H_2S production and DNA hydrolysis). This scheme divided strains C. jejuni in 4 groups. Serotyping regarding the heat stable antigen was performed according to the procedure of Penner scheme. This scheme was used according to standard methods Manchester PHL, UK.

With chosen strains Campylobacter spp. we conducted identification by using PCR methods. Oligonucleotide primers were designed from the flagelin gene (*fla A* and *fla B*) sequences of C. jejuni previously described by Wegmuller at al. Sequences of the oligonucleotides are as follows: F03:5´-GCTCAAAGTGGTTCTTATGCNATGG-3´; CF02:5´-AAGCAAGAAGTGTTCCAAGTTT-3´ and CF04:5´-GCTGCGGAGTTCATTCTAAGACC-3´. The final PCR product was analyzed by agarose gel electoforesis and made visible by ethidium bromid staining.

Results and discussion

About 170 strains were collected from humans and animals. Campylobacter jejuni were detected in the most frequent occurence (92%) while C. coli were detected in 8 %. The strains isolated from poultry were identified mostly as C. jejuni, C. coli and C. lari, 90%, 8% and 2%, respectively. The pigs´ strains were identified as C. coli and C. jejuni, 55 % and 45 % respectively. The human strains were specified most frequently as biotyp I and some strains as biotyp II, 94 and 6 % respectively. The strains C. jejuni of animal origin were specified as biotyp I and II, 86 and 14 % respectively.

Over 80% of Campylobacter jejuni isolates were typable with Penner serotyping scheme. The results of serotyping are listed in Table 1. Ten different Penner serotypes were determined in the isolates. Penner serogroups 4, 1 and 10 prevailed in human strains, 36%, 25% and 16 % respectively, and serogroups 4, 23 and 1 in animal strains, 40, 22 and 20 % respectively. A PCR method was used to detect and identify Campylobacter jejuni and Campylobacter coli. Using PCR system consists of two primer oligonucleotides (CF 03 and CF 04) located at *fla A* and *fla B*. In this system a PCR fragment was yielded, expected to be 340 to 380 bp in length. The third primer CF 02 was used for confirmation of CF03-CF04 products. Using triple primer is suitable for separating C. jejuni, C. coli and C. lari by producing PCR fragment CF03-CF02 which is not detectable with C. lari.

Conclusion

We considered *fla A* and *fla B* to be very suitable primer for detection of C. jejuni and C. coli from food and clinical specimens. Using PCR method together with serotyping is an important presumption for clarifying some epidemiological circumstances.

This study was conducted under the support of grant agency CR 580/94/0830

Table 1
Overview of serotyping results of strains Campylobacter jejuni humans and animals origin

Origin of samples	Serovars	No. strains (%)
Human strains	4	43 (36)
	1	30 (25)
	9	19 (16)
	10	10 (8)
	2	9 (8)
	23	3 (3)
	50	1 (2)
	6	1 (2)
	11	1 (2)
	13	1 (2)
Animal strains	4	20 (40)
	23	11 (22)
	1	10 (20)
	10	5 (10)
	2	2 (3)
	23	2 (3)
	47	1 (2)