Polymerase chain reaction-mediated DNA fingerprinting for epidemiological studies on *Campylobacter* spp.

B. A. J. GIESENDORF*†, H. GOOSSENS§, H. G. M. NIESTERS*‡, A. VAN BELKUM*, A. KOEKEN*,
H. P. ENDTZ‡, H. STEGEMAN† and W. G. V. QUINT*‡

* Diagnostic Centre SSDZ, Department of Molecular Biology, PO Box 5010, 2600 GA Delft, † State Institute for Quality Control of Agricultural Products, Department of Microbiology, PO Box 230, 6700 AE Wageningen, ‡ University Hospital Rotterdam-Dijkzigt, Department of Clinical Microbiology and Virology, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands and § Laboratory of Clinical Microbiology, University Hospital Antwerp, 2650 Edegem, Belgium

Summary. The applicability of polymerase chain reaction (PCR)-mediated DNA typing, with primers complementary to dispersed repetitive DNA sequences and arbitrarily chosen DNA motifs, to study the epidemiology of campylobacter infection was evaluated. With a single PCR reaction and simple gel electrophoresis, strain-specific DNA banding patterns were observed for *Campylobacter jejuni* and *C. upsaliensis*. DNA from multiple strains isolated during an outbreak of *C. jejuni* meningitis generated identical banding patterns and could be distinguished from randomly isolated strains. Strains from a community outbreak of *C. upsaliensis*, that were all identical by conventional typing methods, could be divided into two genetically different groups. This report illustrates that PCR fingerprinting can be successfully applied in epidemiological investigations of campylobacter infections.

Introduction

Since campylobacters were recognised as major human pathogens more than two decades ago, there have been significant improvements in bacterial isolation, taxonomy, pathogenesis, epidemiology and molecular biology.¹ Animals are the main reservoir for strains infecting man.² This emphasises the need for differentiation of isolates to define the possible role that non-human sources play in human infections. The most widely used methods for typing *Campylobacter* spp. include biotyping,^{3–5} serotyping^{6,7} and phage typing.^{8–10} Although a combination of these three typing methods offers additional discriminatory power,^{11,12} a considerable proportion of strains remain non-typable. Molecular techniques based on the detection of restriction fragment length polymorphisms (RFLP), pulsed-field gel electrophoresis (PFGE) and multilocus enzyme analysis enable subtyping of strains even within serotypes.¹³ However, these techniques are complex and unsuitable for routine application.

Recently, the polymerase chain reaction (PCR) has been used for the genetic typing of isolates of different micro-organisms.^{14–16} Besides the use of short, randomly chosen primers, described in a study of *C. jejuni* epidemiology in poultry broiler flocks,^{17,18} primers directed at repetitive DNA sequences can be of great value for the typing of micro-organisms.^{16,19–21} The presence of repetitive, extragenic palindromic

(REP) elements and enterobacterial repetitive intergenic consensus (ERIC) sequences has been demonstrated for many different bacterial species.²¹ Multiple functions have been proposed for these highly conserved, dispersed elements, including roles in transcription termination, mRNA stability and chromosomal domain organisation *in vivo*.²¹ Because no single function satisfactorily explains their DNA sequence conservation or ubiquitous distribution, the suggestion has been made that these elements represent “selfish” DNA sequences which are maintained and propagated through gene conversion.²²

In this report, the applicability of PCR-mediated DNA typing for various isolates of medically important *Campylobacter* spp. was investigated with the aid of REP, ERIC and arbitrary DNA motifs. Strains isolated during separate outbreaks of *C. jejuni* and *C. upsaliensis* infections were studied. The results were compared with those obtained from conventional typing methods.

Materials and methods

Bacterial strains

C. jejuni. The *C. jejuni* reference serotype strains used in this study are listed in table I. A second group of *C. jejuni* strains was isolated during a nosocomial outbreak of *C. jejuni* meningitis between 13 Jan. and 24 Feb., 1984 in Laon, France.²³ These strains are

Table I. *C. jejuni* reference strains used in this study

Biotype	Serovar	Strain	Source
I Lior's scheme			
<i>C. jejuni</i> I	LIO 1	134	man
<i>C. jejuni</i> II	LIO 2	195	man
<i>C. jejuni</i> I	LIO 4	1/NCTC11168	man
<i>C. jejuni</i> I	LIO 5	170	man
<i>C. jejuni</i> III	LIO 6	6	man
<i>C. jejuni</i> I	LIO 7	35	man
<i>C. jejuni</i> I	LIO 9	88	man
<i>C. jejuni</i> I	LIO 10	142	man
<i>C. jejuni</i> I	LIO 11	244	man
<i>C. jejuni</i> II	LIO 16	728	man
<i>C. jejuni</i> I	LIO 17	556	chicken
<i>C. jejuni</i> III	LIO 18	563	chicken
<i>C. jejuni</i> I	LIO 22	918/PC353	man
<i>C. jejuni</i> II	LIO 23	720	man
<i>C. jejuni</i> I	LIO 28	1180	man
<i>C. jejuni</i> III	LIO 30	1215/A1616	man
<i>C. jejuni</i> I	LIO 32	910 MK 192	man
<i>C. jejuni</i> I	LIO 33	1545	man
<i>C. jejuni</i> III	LIO 38	2418/Y 438	man
<i>C. jejuni</i> I	LIO 39	3238/AP14R81	man
<i>C. jejuni</i> II	LIO 49	3235/AP11R81	man
<i>C. jejuni</i> III	LIO 61	5778/AP39849	man
<i>C. jejuni</i> II	LIO 62	4452/CO 1084	man
<i>C. jejuni</i> II	LIO 68	7219/BC 4862	man
<i>C. jejuni</i> IV	LIO 74	7502/BC 4964	man
II Lauwer's scheme			
<i>C. jejuni</i>	LAU 1	A2	man
<i>C. jejuni</i>	LAU 16	Bal	Bangladesh
III Penner's scheme			
<i>C. jejuni</i> II	Pen T2	—	—
<i>C. jejuni</i> I	Pen T3	—	—
<i>C. jejuni</i> II	Pen T4	—	—
<i>C. jejuni</i> II	Pen T7	—	—
<i>C. jejuni</i> I	Pen T8	—	—
<i>C. jejuni</i> III	Pen T9	—	—

Table II. *C. jejuni* strains isolated during a nosocomial outbreak of *C. jejuni* meningitis

Baby no.	Strain no.	Source
1	1017	blood
	1021	CSF
	1023	stool
2	1025	blood
3	1026	blood
4	1027	stool
	1029	CSF
5	1031	blood
6	1034	blood
7	1035	blood

CSF, cerebrospinal fluid.

listed in table II and were biotyped⁴ and serotyped.^{7,24} Antibiotic susceptibility patterns were determined and plasmid and protein profiles were analysed.²⁵

C. upsaliensis. Two groups of strains were included for typing. The first group comprised 54 strains that were isolated during an outbreak of *C. upsaliensis* enteritis in four day-care centres in Brussels in 1991²⁶ and are listed in table III. A second group consisted of 30 strains (B531, B535, B542, B572, C222, C225, D391, D412, D424, D432, D457, E282, E292, E293,

Table III. *C. upsaliensis* strains isolated during an outbreak of *C. upsaliensis* enteritis in four day-care centres

Day-care centre	Strain no.	Date of isolation
A	3457	18 01 91
A	3461	29 01 91
A	3463	02 01 91
A	3464	04 02 91
A	3466	18 02 91
A	3473	07 03 91
B	3474	05 03 91
C	3494	28 04 91
C	3512	21 09 91
C	3515	05 10 91
C	3517	07 10 91
C	3518	07 10 91
C	3519	07 10 91
C	3520	07 10 91
C	3521	07 10 91
C	3522	07 10 91
C	3523	08 10 91
C	3524	09 10 91
C	3525	10 10 91
C	3538	15 10 91
C	3539	15 10 91
C	3544	16 10 91
C	3546	17 10 91
C	3548	18 10 91
C	3568	20 01 92
C	3569	20 01 92
C	3570	20 01 92
C	3571	20 01 92
C	3575	21 01 92
D	3513	17 09 91
D	3526	10 10 91
D	3527	10 10 91
D	3528	10 10 91
D	3529	10 10 91
D	3530	10 10 91
D	3531	10 10 91
D	3532	10 10 91
D	3533	10 10 91
D	3535	11 10 91
D	3536	11 10 91
D	3537	14 10 91
D	3541	15 10 91
D	3542	15 10 91
D	3543	16 10 91
D	3545	17 10 91
D	3552	24 10 91
D	3560	17 01 92
D	3561	17 01 92
D	3562	— 01 92
D	3572	— 01 92
D	3573	21 01 92
D	3574	21 01 91
D	3577	23 01 91
D	3578	23 01 91

E297, E321, H180, H184, H197, H204, K178, L194, L206, L221, M375, P97, R209, R219, S248, V295) isolated from the stools of patients during a 3-year survey of the prevalence of *C. upsaliensis* at St Pieter's Hospital, Brussels, Belgium.^{27,28} These strains were characterised by several procedures, namely SDS-PAGE of whole cell proteins, phenotypic and biochemical characterisation, antibiotic susceptibility testing and plasmid analysis.²⁷ All strains (B531–V295) were shown to be different by these typing methods.

Fig. 1. Comparison of PCR amplification of DNA extracted from a selected number of *C. jejuni* reference serotype strains (Lior serotypes as indicated). The DNA banding patterns shown were generated with a combination of primers ERIC-1R and ERIC-2. Marker, 1-kb DNA ladder.

Extraction of nucleic acids

Nucleic acids were extracted according to standard procedures.^{29,30} The bacterial pellet (100 μ l) was suspended in 150 μ l of GET buffer (50 mM glucose, 10 mM EDTA, 25 mM Tris-HCl, pH 8.0) and incubated for 5 min at room temperature with 10 μ l of lysozyme (125 mg/ml). After the addition of 12.5 μ l of proteinase K (1 mg/ml) and 12.5 μ l of sodium dodecyl sulphate (SDS) 10%, incubation was continued for 30 min at 37°C. Nucleic acids were extracted with phenol, phenol:chloroform:isoamylalcohol (25:24:1) and chloroform:isoamylalcohol (24:1), respectively. Nucleic acids were precipitated by adding 0.1 volume of 3M sodium acetate, pH 5.2, and 2.5 volume of ethanol 96%. After centrifugation, nucleic acid pellets were washed, dried and resuspended in double-distilled water. The nucleic acid concentration was estimated by ethidium bromide staining of samples run on an agarose 1% gel with known amounts of phage λ DNA as reference.

DNA amplification

REPIR-1, ERIC-1R and ERIC-2²¹ and an arbitrary primer 1026: 5'TACATTCGAGGACCCCTAAGT-G3',^{16,31} were evaluated with respect to their applicability in PCR-mediated genotyping studies. These primers were selected on the basis of the generation of polymorphic DNA banding patterns for different *C. jejuni* and *C. upsaliensis* strains.^{19,26} REP and ERIC primers are homologous to repetitive intergenic

sequences and amplify DNA fragments present between inserted copies of these sequences provided that the distance does not exceed 2 kb. Oligonucleotides were prepared on an ABI 386 DNA synthesiser by phosphoramidite technology. Oligonucleotides containing inosine were obtained from Pharmacia, Woerden, The Netherlands. PCR was performed as described previously¹⁹ and was identical in all experiments. PCR was performed in 100- μ l volumes containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, gelatin 0.01%, 200 μ M of each desoxynucleotide triphosphate and up to 100 ng of template DNA. Taq DNA polymerase (Sphaero Q, Leiden, The Netherlands) (0.625 unit) was included for each reaction and 35 reaction cycles (1 min at 94°C, 1 min at 25°C and 4 min at 74°C) were performed, preceded by an initial 5 min denaturation at 94°C and followed by a final elongation step of 10 min at 74°C. All PCR reactions were performed in a Biomed thermocycler (type 60). PCR products were separated on agarose 2% gels and stained with ethidium bromide.

Results

To investigate the applicability of PCR-mediated DNA typing, isolates collected during an outbreak of *C. jejuni* meningitis and an outbreak of *C. upsaliensis* enteritis were studied and compared to a number of reference strains.

To illustrate the polymorphism, fig. 1 shows DNA banding patterns from a selection of the *C. jejuni*

A

B

Fig. 2. A, Comparison of PCR amplification of DNA extracted from the 10 *C. jejuni* isolates from the meningitis outbreak, lanes 1017–1035, and a selection of serologically different *C. jejuni* strains (numbers as indicated on top). A combination of primers ERIC-2 and 1026 was used. Marker, 1-kb DNA ladder. B, Results of PCR amplification of DNA extracted from the 10 *C. jejuni* isolates from the meningitis outbreak, lanes 1017–1035, and a different set of serologically different *C. jejuni* strains, serotype (numbers as indicated on top), using primer REPIR-1. N, negative control; marker, 1-kb DNA ladder.

reference serotype strains. Although a resemblance in patterns can be observed for a number of strains (e.g., Lior 4 and Lior 5), all strains can be distinguished on the basis of the banding patterns generated with the combination of primers ERIC-1R and ERIC-2. In the case of Lior 4 and Lior 5, distinction is determined by the presence of additional fragments present for Lior 5: a 700-, a 350- and a 150-bp fragment. Primer combination ERIC-2 and 1026, and primer REPIR-1

generated similar results (data not shown). With respect to the outbreak of *C. jejuni* meningitis, chromosomal DNA of strains obtained from seven neonates with meningitis was analysed with PCR, and conventional typing was performed.

Figs. 2A and 2B show DNA fingerprints from the strains isolated during the meningitis outbreak, compared with banding patterns obtained from a selection of the 33 reference serotype strains. With primer set

Fig. 3. **A**, Results of PCR amplification of DNA extracted from a representative selection of strains isolated during the *C. upsaliensis* outbreak compared to a group of *C. upsaliensis* strains isolated during a 3-year survey, with primers ERIC-1R and ERIC-2. Lanes **B531–H180** contain a selected number of *C. upsaliensis* strains isolated from the stools of patients, unrelated to the outbreak. Lanes **A3463–D3573** contain strains isolated at day-care centre A, B, C or D as indicated. Marker, 1-kb DNA ladder; N, negative control. **B**, Comparison of PCR amplification of DNA extracted from the *C. upsaliensis* outbreak strains, lanes **A3463–D3533**, compared to *C. upsaliensis* strains isolated during a 3-year survey, lanes **B531–H180** as indicated. N, negative control; marker, 1-kb DNA ladder.

ERIC-2 and 1026 (fig. 2A), DNA products of sizes ranging from *c.* 2000 bp to 150 bp were generated. All outbreak-related strains had identical DNA banding

patterns and could be discriminated from the non-outbreak strains. Similar results were obtained with primer REPIR-1, as can be seen in fig. 2B. Outbreak

strain 1034 showed a less intense banding pattern which was, however, identical to the patterns from the other outbreak strains. This was confirmed by repeated testing. Primers ERIC-1R and ERIC-2 also generated identical banding patterns for all outbreak-related strains, and discriminated these from non-outbreak strains (data not shown).

PCR-mediated DNA typing was also used to study a community outbreak of *C. upsaliensis* enteritis in four day-care centres. Chromosomal DNA of 54 outbreak isolates was compared by PCR analysis with DNA of 30 non-outbreak strains of *C. upsaliensis*. With primer combinations ERIC-1R and ERIC-2, and ERIC-2 and 1026, outbreak strains could be divided into two groups. Figs. 3A and 3B show that the banding patterns obtained from the strains isolated at day-care centre A were identical, but different from the DNA patterns obtained from strains isolated from children of day-care centres B, C and D. DNA fingerprints from the latter group (B, C and D) were identical. Furthermore, *C. upsaliensis* outbreak-related strains (from all four day-care centres) could be clearly discriminated from all 30 non-outbreak strains by PCR. A selected number is shown in figs. 3A and B (B531–H180). Primer REPIR-1 could not be used successfully for PCR fingerprinting of *C. upsaliensis* (data not shown).

Discussion

The value of any typing procedure depends on the discriminatory power of the particular technique that is applied. This depends in turn on the characteristics being measured. Combining several typing schemes such as biotyping, serotyping and phage typing might facilitate epidemiological investigations¹¹ but would also complicate the methods and possibly require additional materials and time. Moreover, when conventional methods of typing *Campylobacter* spp. are used, a number of strains remain non-typable. Therefore, the value of PCR fingerprinting in delineating various characteristics was studied. The involvement of *Campylobacter* strains in epidemic spread, and whether new infectious episodes are due to persistence or re-infection, were investigated.

Characteristic DNA banding patterns, also known as DNA fingerprints, were obtained by the application of oligonucleotide PCR primers aimed at both dispersed repetitive DNA sequences and arbitrary DNA motifs.^{16, 21} Although several other primers were studied, ERIC-1R, ERIC-2 and 1026 proved to be the best choice for typing *C. jejuni*, but could not be used successfully for *C. upsaliensis*. The study of the meningitis outbreak showed that it was possible to discriminate between outbreak and non-outbreak strains with a single PCR assay followed by a direct gel electrophoresis. Conventional typing results also showed that all outbreak strains were identical. The outbreak strains were of biotype 1, serotype LAU7/

PEN 18 and showed identical antibiotic susceptibility patterns, and protein and plasmid profiles.²⁵ These results were in agreement with those obtained from PCR fingerprinting.

In the case of the *C. upsaliensis* outbreak, PCR fingerprinting provided additional information to that obtained with other typing methods. Although examination of the *C. upsaliensis* strains by plasmid analysis, antibiotic susceptibility testing, whole cell protein profile analysis, and phenotypic and biochemical testing^{26, 27} (H. Goossens, unpublished results) indicated that the *C. upsaliensis* outbreak was caused by one strain, PCR fingerprinting, with more than one primer combination, showed that strains isolated from children at day-care centre A could be distinguished from strains isolated at the other day-care centres. These PCR results were corroborated by a study of this outbreak in which RFLP was used in addition to conventional typing methods (J. Van den Plas and H. Goossens, unpublished results). However, RFLP analysis revealed that only one of the 16 enzyme-probe combinations could discriminate strains from day-care centre A from day-care centres B, C and D (data not shown), and not all enzyme-probe combinations could be used.

These results suggest that two independent strains may have been responsible for the *C. upsaliensis* infections in day-care centre A and day-care centres B, C and D, respectively. Another possibility is that during transmission of the infection from day-care centre A to the other day-care centres, or during culturing of the strains, some selection at the genetic level may have occurred. These potential genetic rearrangements during transmission or infection apparently have a more profound influence on the PCR fingerprint patterns generated with these primer combinations than on RFLP analysis.

Plasmid analysis showed an identical plasmid content for all these strains. Therefore, the differences observed with PCR fingerprinting and RFLP analysis cannot be explained by differences in plasmid DNA content.²⁶ Furthermore, because serotyping for *C. upsaliensis* has not yet been developed, it is possible that a future serotyping system for *C. upsaliensis* might differentiate the outbreak-related isolates on the basis of antigenic differences. However, to investigate the level of genetic difference between strains from day-care centre A and day-care centres B, C and D, more PCR primers, capable of discriminating between different *C. upsaliensis* strains, should be used.

Although clear correlations were observed between PCR fingerprinting and serotyping data (fig. 1),¹⁷ typing of *Campylobacter* strains requires more detailed epidemiological study of larger groups of strains. One improvement would be the development of an automated system for post-amplification analysis and documentation. This would enable the standardisation of the technique, and would allow a comparison of patterns derived from different agarose gels. However, the applicability of this simple and rapid technique in

epidemiological studies is clearly demonstrated by the results obtained in this study.

We thank H. Lior, J. Penner and S. Lauwers for providing the reference serotype strains, M. Henkens for technical support and M. Canning for correcting language use and spelling.

References

1. Penner JL. The genus *Campylobacter*: a decade of progress. *Clin Microbiol Rev* 1988; **1**: 157-172.
2. Blaser MJ, Taylor DN, Feldman RA. Epidemiology of *Campylobacter jejuni* infections. *Epidemiol Rev* 1983; **5**: 157-176.
3. Bolton FJ, Holt AV, Hutchinson DN. *Campylobacter* biotyping scheme of epidemiological value. *J Clin Pathol* 1984; **37**: 677-681.
4. Lior H. New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli* and "*Campylobacter lari*dis". *J Clin Microbiol* 1984; **20**: 636-640.
5. Skirrow MB, Benjamin J. Differentiation of enteropathogenic *Campylobacters*. *J Clin Pathol* 1980; **33**: 1122.
6. Lior H, Woodward DL, Edgar JA, Caproche LJ, Gill P. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. *J Clin Microbiol* 1982; **15**: 761-768.
7. Penner JL, Hennesy JN, Congi RV. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. *Eur J Clin Microbiol* 1983; **2**: 378-383.
8. Grajewski BA, Kusek JW, Gelfand HM. Development of a bacteriophage typing scheme for *Campylobacter jejuni* and *Campylobacter coli*. *J Clin Microbiol* 1985; **22**: 13-18.
9. Lior H, Patel A. Improved toluidine blue-DNA agar for detection of DNA hydrolysis by *Campylobacters*. *J Clin Microbiol* 1987; **25**: 2030-2031.
10. Salama SM, Bolton FJ, Hutchinson DN. Application of a new phage typing scheme to *Campylobacters* isolated during outbreaks. *Epidemiol Infect* 1990; **104**: 405-411.
11. Khakhria R, Lior H. Extended phage-typing scheme for *Campylobacter jejuni* and *Campylobacter coli*. *Epidemiol Infect* 1992; **108**: 403-414.
12. Lior H, Woodward D, Khakhria R. Characterization of *Campylobacter jejuni* outbreaks by 3 epidemiological typing markers. In: Kaijser B, Falsen E (eds) *Campylobacter* IV. Kungala, Sweden, Goterna. 1988: 103-104.
13. Patton CM, Wachsmuth IK, Evins GM *et al*. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. *J Clin Microbiol* 1991; **29**: 680-688.
14. Jayarao BM, Bassam BJ, Caetano-Anollés G, Gresshoff PM, Oliver SP. Subtyping of *Streptococcus uberis* by DNA amplification fingerprinting. *J Clin Microbiol* 1992; **30**: 1347-1350.
15. McMillin DE, Muldrow LL. Typing of toxic strains of *Clostridium difficile* using DNA fingerprints generated with arbitrary polymerase chain reaction primers. *FEMS Microbiol Lett* 1992; **92**: 5-10.
16. Van Belkum A, De Jonckheere J, Quint WGV. Genotyping *Naegleria* spp. and *Naegleria fowleri* isolates by interrepeat polymerase chain reaction. *J Clin Microbiol* 1992; **30**: 2595-2598.
17. Mazurier S, Van de Giessen A, Heuvelman K, Wernars K. RAPD analysis of *Campylobacter* isolates: fingerprinting without the need to purify DNA. *Lett Appl Microbiol* 1992; **14**: 260-262.
18. Van de Giessen A, Mazurier SI, Jacobs-Reitsma W *et al*. Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. *Appl Environ Microbiol* 1992; **58**: 1913-1917.
19. Giesendorf BAJ, Van Belkum A, Koeken A *et al*. Development of species-specific DNA probes for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* by polymerase chain reaction fingerprinting. *J Clin Microbiol* 1993; **31**: 1541-1546.
20. De Bruijn FJ. Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergenic consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. *Appl Environ Microbiol* 1992; **58**: 2180-2187.
21. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 1991; **19**: 6823-6831.
22. Higgins CF, McLaren RS, Newbury SF. Repetitive extragenic palindromic sequences, mRNA stability and gene expression: evolution by gene conversion? A review. *Gene* 1988; **72**: 3-14.
23. Goossens H, Kremp L, Boury R *et al*. Nosocomial outbreak of *Campylobacter jejuni* meningitis in newborn infants. *Lancet* 1986; **2**: 146-149.
24. Lauwers S, Vlaes L, Butzler JP. *Campylobacter* serotyping and epidemiology. *Lancet* 1981; **1**: 158.
25. Goossens H, Van den Borre C, Vlaes L *et al*. Comparison of epidemiological markers for the investigation of *Campylobacter* outbreaks. In: Kaijser B, Falsen E (eds) *Campylobacter* IV. Kungala, Sweden, Goterna. 1988: 271-272.
26. Goossens H, Hanicq P, N'Jufom S *et al*. Outbreak of *Campylobacter upsaliensis* in four day-care centers in Brussels, 92nd Annual Meeting of the American Society for Microbiology. C-105. 1992: 438.
27. Goossens H, Pot B, Vlaes L *et al*. Characterization and description of "*Campylobacter upsaliensis*" isolated from human feces. *J Clin Microbiol* 1990; **28**: 1039-1046.
28. Goossens H, Vlaes L, De Boeck M *et al*. Is "*Campylobacter upsaliensis*" an unrecognized cause of human diarrhoea? *Lancet* 1990; **335**: 584-586.
29. Giesendorf BAJ, Quint WGV, Henkens MHC, Stegeman H, Huf FA, Niesters HG. Rapid and sensitive detection of *Campylobacter* spp. in chicken products using the polymerase chain reaction. *Appl Environ Microbiol* 1992; **58**: 3804-3808.
30. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 1989.
31. Van Belkum A, Bax R, Peerbooms, Goossens WHF, van Leeuwen N, Quint WGV. Comparison of phage typing and DNA fingerprinting by polymerase chain reaction for discrimination of methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol* 1993; **31**: 798-803.