CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Cork Institute of Technology

SWORD - South West Open Research Deposit

Articles

Biological Sciences

2000-2

Integronlike Structures in Campylobacter spp. of Human and Animal Origin

Brigid Lucey

D. Crowley

P. Moloney

B. Cryan

M. Daly

See next page for additional authors

Follow this and additional works at: https://sword.cit.ie/dptbiosciart

Part of the Bacteriology Commons, Bioinformatics Commons, Biology Commons, Environmental Microbiology and Microbial Ecology Commons, Epidemiology Commons, Food Microbiology Commons, Immunology and Infectious Disease Commons, Medical Sciences Commons, Nutrition Commons, Pathogenic Microbiology Commons, and the Toxicology Commons

Authors

Brigid Lucey, D. Crowley, P. Moloney, B. Cryan, M. Daly, F. O'Halloran, E. J. Threlfall, and S. Fanning

Integronlike Structures in *Campylobacter* spp. of Human and Animal Origin

Brigid Lucey,*† D. Crowley,* P. Moloney,‡ B. Cryan,† M. Daly,*
F. O'Halloran,* E.J. Threlfall,§ and S. Fanning*
*Cork Institute of Technology, Bishopstown, Cork, Ireland;
†Cork University Hospital, Cork, Ireland; ‡Cork Corporation
Veterinary Department, Cork, Ireland; and §PHLS Central Public
Health Laboratory, London, United Kingdom

Resistance to antimicrobial agents used to treat severe *Campylobacter* spp. gastroenteritis is increasing worldwide. We assessed the antimicrobial resistance patterns of *Campylobacter* spp. isolates of human and animal origin. More than half (n = 32) were resistant to sulphonamide, a feature known to be associated with the presence of integrons. Analysis of these integrons will further our understanding of *Campylobacter* spp. epidemiology.

Campylobacter spp. are isolated from animals and birds and from the environment, particularly surface water. Poultry have been implicated as a major source of sporadic infection (1). Thermophilic *Campylobacter* spp., particularly *Campylobacter jejuni* and *C. coli,* are recognized as one of the etiologic agents of acute diarrheal disease in humans worldwide (2,3). Antimicrobial chemotherapy is usually reserved for patients with advanced infection or patients prone to relapse. Erythromycin, fluoroquinolones, and tetracycline are the antimicrobial drugs of choice.

Bacterial resistance to antimicrobial agents, which is increasing worldwide, is frequently caused by the acquisition of new genes rather than by mutation (4,5). An efficient means of acquiring new genes is by mobile genetic elements such as resistance (R)-plasmids and transposons. Recently, a novel class of naturally occurring mobile genetic elements, integrons, have been described as vehicles for the acquisition of antimicrobial resistance genes (5). Horizontal and vertical transfer can occur readily, as shown by the widespread acquisition of these gene cassettes among the *Enterobacteriaceae* and *Pseudomonas* spp. Integrons comprise two conserved structural regions (5'CS and 3'CS) flanking an internal variable region containing one or more site-specific recombined gene cassettes. While most known cassette-associated genes located distal to the 5'CS region encode resistance to antimicrobial drugs, some cassettes may include one or more open reading frames whose product(s) and corresponding function(s) remain to be defined (5). In the 3'CS downstream of the gene cassette are two genes, one of which encodes resistance to quaternary ammonia compounds ($qacE\Delta1$), while the other is the sulphonamide resistance determinant ($sul1$). Antimicrobial resistance among *Campylobacter* spp. to drugs used in the treatment of human infection is increasing (6-8). This article reports the results of an investigation of a collection of Irish thermophilic *Campylobacter* spp. cultured from clinical cases of gastroenteritis and from porcine and poultry sources. We studied a representative sample of 55 isolates (47 *C. jejuni* and eight *C. coli* isolated between 1996 and 1998), cultured from intestinal tissue of animals at slaughter and from human fecal samples.

Antimicrobial agent susceptibility tests were performed by the agar diffusion method on IsoSensitest agar (Difco, Dublin, Ireland) with 5% horse blood (9). Cultures were prepared by inoculating colonies from a fresh, pure, 24-hour culture into sterile distilled water to give an inoculum turbidity equivalent to a 0.5 McFarland

Address for correspondence: Séamus Fanning, Molecular Diagnostics Unit, Cork Institute of Technology, Bishopstown, Cork, Ireland; fax: 353-21-545-343; e-mail: sfanning@cit.ie.

turbidity standard. The McFarland standard was prepared by adding 0.5 ml 0.048 M BaCl$_2$ to 99.5 ml 0.18 M H$_2$SO$_4$ with constant stirring. Samples were swabbed evenly onto agar plates and allowed to dry. Twelve antimicrobial agents were tested on disks. Antimicrobial drugs tested, together with their abbreviations and corresponding concentrations in parentheses, included ampicillin (Ap, 10 µg/disc), chloramphenicol (C, 10 µg/disc), ciprofloxacin (Cp, 5 µg/disc), colistin (Ct, 25 µg/disc), erythromycin (E, 5 µg/disc), gentamicin (G, 10 µg/disc), nalidixic acid (Na, 30 µg/disc), spectinomycin (Sp, 10 µg/disc), streptomycin (S, 25 µg/disc), sulphafurazole (Su, 100 µg/disc), tetracycline (T, 10 µg/disc), and trimethoprim (Tm, 1.25 µg/disc). The plates containing the antibiotic disks were incubated at 37°C under microaerophilic conditions for 24 hours. Inhibition zone sizes were recorded according to the guidelines of the National Committee for Clinical Laboratory Standards (10). Resistance profiles were further confirmed by E-Test (AB Biodisc, Solna, Sweden).

Briefly, 17% of all isolates were resistant to ampicillin, 3.8% to chloramphenicol, 1.9% to ciprofloxacin, 7.5% to colistin, 11.3% to erythromycin, 1.9% to gentamicin, 17% to nalidixic acid, 77.4% to spectinomycin, 20.8% to streptomycin, 62.3% to sulphonamide, and 24.5% to tetracycline. Many of the isolates tested (n = 42, 77%) were resistant to three or more antimicrobial agents with part of the R-type, including SSpTm among others. Two strains, *C. jejuni* CIT-H17 (R-type: ApCtENaSSpSuTTm) and *C. coli* CIT-V6 (R-type: CCpENaSSpTTm), were particularly resistant (Table 1); both were resistant to nalidixic acid, and the latter was also resistant to ciprofloxacin. In reviewing the R-types in the sample, the presence of sulphonamide resistance (in 62.3% of the sample) suggested that integron-like structures may exist in *Campylobacter* spp.

To test the latter hypothesis, genomic DNA was purified from all isolates (11). Using the oligonucleotide primers Int 1 F 5'-GGC ATC CAA GCA CGA AG-3'and Int 1 B 5'-AAG CAG ACT TGA CCT GA-3' designed to anneal to the 5'CS and 3'CS flanking regions (12) of integrons, we tested the *Campylobacter* spp. genome by polymerase chain reaction (PCR) for putative gene cassettes. *Escherichia coli* containing the characterized plasmids R100.1 and R751 (13) together with CIT-F 100, a *Salmonella enterica* serotype Typhimurium DT104 strain cultured from a contaminated food source (14), were included as controls. Gene cassettes of 1.0-kb and 800 bp, respectively, from *E. coli* (data not shown) and 1.0- and 1.1-kb (Figure 1a, lane 2),
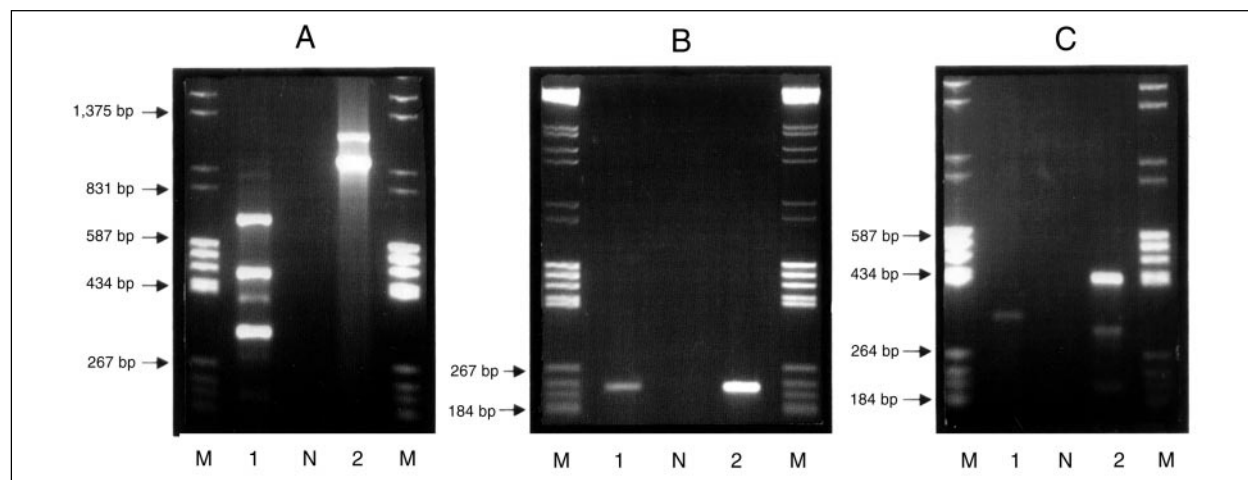

Figure 1. Agarose (1.5%) gel electrophoresis in 1x TAE buffer containing 0.5 µg/ml ethidium bromide. Purified genomic DNA was used as the template in a PCR reaction with the primers Int 1 F 5'-GGC ATC CAA GCA CGA AG-3' and Int 1 B 5'-AAG CAG ACT TGA CCT GA-3' (12). Lane M contains an equal mixture of molecular weight markers grades III and V (Boehringer Mannheim, Germany), Lane N is the negative containing all reaction components with the exception of template DNA, Lane 1, *Campylobacter coli* CIT-H 6 (IP-I); Lane 2, *Salmonella* Typhimurium DT104 CIT-F 100 (IP-C). As in A above except that the primers used were *qacE*?1 F 5'-AT GCA ATA GTT GGC GAA GT-3'and *qacE*?1 B 5'-CAA GCT TTT GCC CAT GAA GC-3' (13). As in A above using primers *sul1* F 5'-CTT CGA TGA GAG CCG GCG GC-3' and *sul1* B 5'-GCA AGG CGG AAA CCC GCG CC-3' (13).

from *Salmonella* Typhimurium were detected after amplification. These amplicon profiles were designated as integron pattern (IP)-groups A, B, and C, respectively (Table). After amplification and conventional agarose gel analysis of all *Campylobacter* spp. isolates in the study population, DNA amplicons of 230 bp to 1.47 kb were detected. A total of 22 gene cassette structures were identified (Figure 2). The most commonly occurring amplified gene cassette pattern was designated IP-group I, consisting of four DNA fragments of 350 bp to 700 bp (Figure 2, lane 1 and Figure 1a, lane 1). This gene cassette pattern was present in both poultry and clinically derived *C. jejuni*, accounting for 38% of strains. IP-group II (Figure 2, lane 2) accounted for 7% of all *C. jejuni* isolates only. The IP-groups III (Figure 2, lane 3), XIV (Figure 2, lane 14), XVI (Figure 2, lane 16), and XX1 (Figure 2, lane 21) each accounted for 6% of the collection, with IP-groups XIV and XVI being unique to *C. coli*. All other IP-groups (Table; Figure 2) were represented by single isolates. A 350-bp amplified DNA fragment was common to all isolates, with the exception of the poultry-derived *C. coli* CIT-P2 and a clinical isolate

Table. Isolates of *Campylobacter coli* and *C. jejuni* from which gene cassettelike structures were amplified

Isolate No.	Year[a]	R-type	IP-profile	Isolate No.	Year[a]	R-type	IP-profile
Campylobacter jejuni				*C. jejuni*, cont'd			
CIT-H6	1997	SpSuTm	I	CIT-H1	1996	SpSuTm	VIII
CIT-H7	1996	SpTm	I	CIT-P1	1997	SpSuTm	IX
CIT-H8	1997	ApSpTm	I	CIT-H2	1996	SpSuTm	X
CIT-H9	1997	SpTm	I	CIT-H3	1997	SuTTm	XI
CIT-H10	1997	Tm	I	CIT-H4	1996	SpSuTm	XII
CIT-H12	1997	NaSpTm	I	CIT-H5	1997	SuTm	XIII
CIT-H14	1997	SpTm	I	*C. coli*			
CIT-H15	1996	SpTm	I	CIT-P3	1996	EGSSpSuTm	XIV
CIT-H16	1997	SpTTm	I	CIT-V3	1998	ESpTTm	XIV
CIT-H22	1996	SpSuTm	I	CIT-V6	1998	CCpENaSSpTTm	XIV
CIT-H25	1997	SSpTm	I	CIT-V1	1998	SpSuTm	XV
CIT-H26	1997	ApSpTm	I	CIT-V4	1998	SSpTTm	XVI
CIT-P4	1997	TTm	I	CIT-V5	1998	ESSuTTm	XVI
CIT-P5	1997	SpSuTm	I	CIT-V2	1998	ESSpSuTTm	XVI
CIT-H30	1997	SuTTm	I	*C. jejuni*			
CIT-H31	1996	SpSuTm	I	CIT-H23	1996	SpSuTm	XVII
CIT-P10	1997	SpTm	I	CIT-H28	1997	CtTm	XVIII
CIT-P13	1996	NaTm	I	*C. coli*			
CIT-P14	1996	SuTTm	I	CIT-P2	1997	SpSuTm	XIX
CIT-P15	1996	SpSuTTm	I	*C. jejuni*			
CIT-P16	1996	ApSpSuTTm	I	CIT-H19	1997	SpSuTm	XX
CIT-H29	1997	SuTm	II	CIT-H17	1996	ApCtENaSSpSuTTm	XI
CIT-P7	1997	NaSuTm	II	CIT-H21	1997	SpSuTm	XXI
CIT-P8	1997	SpSuTm	II	CIT-H32	1996	SSpSuTm	XXI
CIT-P9	1997	/	II	CIT-H18	1997	ApCtSSpSuTm	XXII
CIT-P6	1997	SpSuTm	III	CIT-H20	1996	ApSpTm	XXII
CIT-P11	1996	NaSpTm	III	Control strains[b]			
CIT-P17	1997	ESpSuTm	III	*Escherichia coli*			
CIT-H11	1997	ApSpSuTm	IV	[R100.1]	/	/	A
CIT-H13	1997	ApCSpTm	IV	*E. coli*			
CIT-P12	1996	CtNaSpSuTTm	V	[R751]	/	/	B
CIT-H24	1997	ApNaTm	VI	*Salmonella* Typhimurium			
CIT-H27	1997	NaSSpSuTm	VII	CIT-F 100	1998	ACSSuT	C

[a]Year of isolation.
[b]*E. coli* and *Salmonella enterica* serotype Typhimurium control strains. The former carried plasmids R100.1 and R752, respectively, provided by D. Sandvang (13). *S.* Typhimurium DT104 [CIT-F 100] was previously characterized by M. Daly et al. (14).
H, hospital isolate; P, poultry isolate; V, veterinary isolate; /, not available or not determined. Antimicrobial agents: Ap, ampicillin; C, chloramphenicol; Cp, ciprofloxacin; Ct, colistin; E, erythromycin; G, gentamicin; Na, nalidixic acid; S, streptomycin; Sp, spectinomycin; Su, sulphafurazole; T, tetracycline; Tm, trimethoprim.
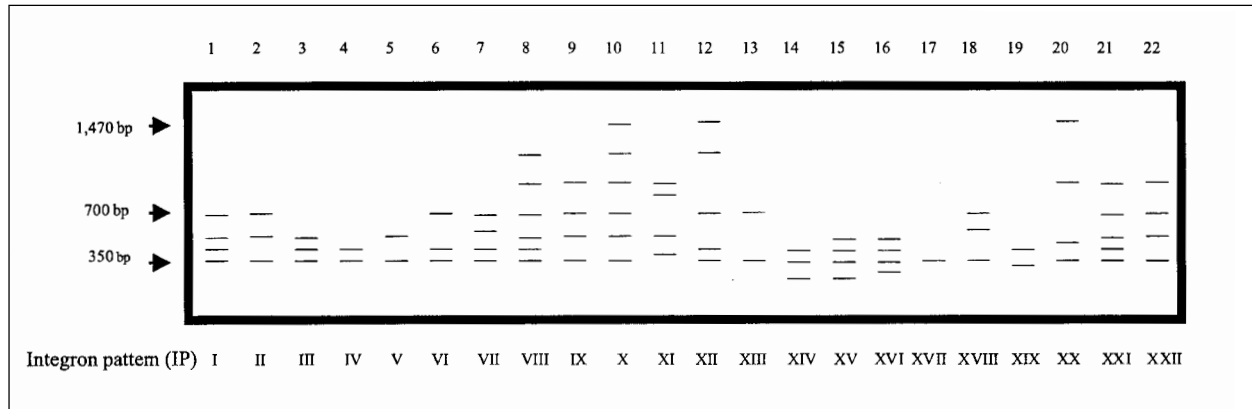
Figure 2. Schematic representation of all amplified gene cassettes from *Campylobacter* spp. in the study population. Roman numerals refer to the designated integron pattern (IP)-type assigned to each pattern.

C. jejuni CIT-H3. Amplicons of 230 and 250 bp were conserved among *C. coli* isolates only.

Three putative gene cassettes of 243, 388, and 466 bp were cloned after amplification by using the Int 1 F and Int 1 B primers (4,12) as described above. All were sequenced by automated methods. Sequencing data showed a short, imperfect inverted repeat element at the 3' end of the cloned fragments which represented the 59 base element (5'-GTTRR-3'). This is the target for site-specific recombination involved in the insertion and excision of gene cassettes (4,5,15). Isolates were also tested for the 5' CS encoded integrase (*int*) and the 3' CS encoded *qacE* 1 and *sul1* genes by PCR. A DNA fragment of 225 bp was detected after amplification and agarose gel analysis (Figure 1b, lane 1) using primers *qacE*?1 F 5'-ATC GCA ATA GTT GGC GAA GT-3', and *qacE*?1 B 5'-CAA GCT TTT GCC CAT GAA GC-3' (13). The latter fragment corresponded with a similar sized amplicon in *S*. Typhimurium (Figure 1b, lane 2). The 3'-CS region of integrons, known to contain a *sul*1 gene, was similarly tested with the primers *sul1* F 5'-CTT CGA TGA GAG CCG GCG GC-3' and *sul1* B 5'-GCA AGG CGG AAA CCC GCG CC-3' (13). When compared with an *S*. Typhimurium DT104 amplicon of 436 bp (Figure 1c, lane 2) (13), the *Campylobacter* spp. *sul*1-primer derived DNA fragment (Figure 1c, lane 1) appears smaller at approximately 360 bp. Nevertheless, the latter amplicon was consistently amplified from all *Campylobacter* spp. Smaller *sul*1 primer generated DNA fragments were also detected in *S*. Typhimurium after PCR and gel analysis (14).

These may derive from the partial *sul1* genes recently located in a 14-kb gene cluster on the chromosome of *S*. Typhimurium (16). On probing the *Campylobacter* spp. *sul1*-primer derived amplicon (Figure 1c, lane 1) with the digoxigenin-labeled 436 bp *S*. Typhimurium *sul1* DNA amplicon (Figure 1c, lane 2), no hybridizing signal was detected (data not shown). This result suggests that the *Campylobacter* spp. *sul1* gene is different when compared with *S*. Typhimurium. To investigate the 5'-CS region, primers (int1 F [Tn21]): 5'-GAA GAC GGC TGC ACT GAA CG-3' and int1 R [Tn*21*]: 5'-AAA ACC GCC ACT GCG CCG TTA-3') were designed to amplify a 1.2-kb DNA fragment from the integrase gene of Tn*21* and were tested against *Campylobacter* spp. and *S*. Typhimurium (as a control) (Table). The predicted amplicon was detected in the latter, together with two smaller amplimers of 270 bp and 450 bp. These latter PCR products (270 bp and 450 bp) were also identified in *Campylobacter* spp. (data not shown), consistent with a deleted form of a class 1 integrase gene in these isolates.

The DNA sequences from the amplified cassettes (of 243 bp, 388 bp, and 463 bp) above were also searched by using the BLAST search tool (17). GenBank accession numbers were assigned as follows: AF155357 (243-bp gene cassette); AF155356 (388-bp gene cassette), and AF152561 (463-bp gene cassette). The former amplicon contains two open reading frames. No corresponding sequences were identified in the database. The 388-bp amplicon also contained two open reading frames and did not match any sequences when subjected to a BLAST search of

the current databases. Finally, the larger 463-bp amplicon contained two incomplete open reading frames. BLAST searches using the latter sequence identified glycyl-tRNA synthetase from the genome of *Helicobacter pylori* matching 102 (85%) of 119 nucleotides. Further characterization of other gene cassettes is in progress, focusing on amplicons of 700 bp and greater.

Drug selection may promote recombinational events between *Campylobacter* spp., *Enterobacteriaceae* and other gram-negative organisms (15). A common habitat for these organisms is the human and animal gastrointestinal tract. Modern animal husbandry promotes the use of large animal housing facilities, thereby ensuring genetic interconnection between large populations of bacteria. *Campylobacter* spp. have a natural ability for transformation (18), and in shared animal reservoirs, interspecies transfer of DNA, including antimicrobial resistance encoding genes and other unrelated genes, may occur by strategies analogous to site-specific recombination (19,20). Our findings may indicate a novel mechanism by which unrelated DNA becomes incorporated into cells (21). Detailed characterization of these integronlike structures is an essential step in understanding the role(s) of these novel genetic elements. The existence of these structures may have interesting implications regarding the diversity of the *Campylobacter* spp. genome and the evolution of this species. Together with the corresponding DNA fingerprint profile (Lucey B., Fanning S., manuscript in preparation) the variation in genetic content and structure of these determinants may be used as a potential tool in elucidating the epidemiology of these pathogens (22,23).

Acknowledgments

Ms. Lucey, senior biomedical scientist, Molecular Diagnostics Unit, Cork's Institute of Technology, and Department of Medical Microbiology, Cork University Hospital, is completing a Masters thesis under the direction of Séamus Fanning. Her research interests include the molecular epidemiology of *Campylobacter* spp. and the genetic mechanisms underlying antimicrobial resistance in these organisms. She is a recipient of the Abbott Research Prize 1996 awarded to Medical Laboratory Scientists.

References

1. Stern NJ. Reservoirs for *Camplobacter jejuni* and approaches for intervention in poultry. In: Nachamkin I, Blaser MJ, Tompkins LS, editors. *Campylobacter jejuni*: current status and future trends. Washington: American Society for Microbiology; 1992. p. 49-60.
2. Skirrow MB. Diseases due to *Campylobacter, Helicobacter* and related bacteria. J Comp Pathol 1994;111;113-49.
3. Nachamkin I, Allos BM, Ho T. *Campylobacter* species and Guillain-Barré syndrome. Clin Microbiol Rev 1998;11:555-67.
4. Recchia GD, Hall RM. Gene cassettes: a new class of mobile element. Microbiology 1995;141:3015-27.
5. Hall RM, Collis CM. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Molecular Microbiol 1995;15:593-600.
6. Moore JE, Elisha BG. Workshop summary: session D. *Campylobacter* and *Helicobacter*: antibiotic resistance. In: Lastovica AJ, Newell DG, Lastovica EE, editors. *Campylobacter, Helicobacter* & related organisms, Institute of Child Health, University of Cape Town; 1998. p. 133-5.
7. Ruiz J, GoZi P, Marco F, Gallardo F, Mirelis B, De Anta TJ, et al. Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. Microbiol Immunol 1998;42:223-6.
8. Sjögren E, Kaijser B, Werner M. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* isolated in Sweden: a 10-year follow-up report. Antimicrob Agents Chemother 1992;36:2847-9.
9. Reina J, Ros MJ, Serra A. Susceptibilities to ten antimicrobial agents of 1,120 *Campylobacter* strains isolated from 1987 to 1993 from faeces of paediatric patients. Antimicrob Agents Chemother 1994;39:2910-20.
10. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. Vol. 1, p: 141-56 (1981). Approved standard. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. Mazurier S, Van de Giessen A, Heuvelman K, Wernars K. RAPD analysis of *Campylobacter* isolates: DNA fingerprinting without the need to purify DNA. Lett Appl Microbiol 1992;14:260-2.
12. Levesque C, Pichè L, Larose C, Roy P. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother 1995;39:185-91.
13. Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica typhimurium* DT104. FEMS Microbiol Lett 1998;160:37-41.
14. Daly M, Buckley J, Power E, O'Hare C, Cormican M, Cryan B, et al. Molecular characterization of Irish *Salmonella enterica* serotype Typhimurium: detection of class 1 integrons and assessment of genetic relationships by DNA fingerprinting. Appl Env Microbiol. In press, 2000.

15. Gibreel A, Sköld O. High-level resistance to trimethoprim in clinical isolates of *Campylobacter jejuni* by acquisition of foreign genes (*dfr1* and *dfr9*) expressing drug-insensitive dihydrofolate reductases. Antimicrob Agents Chemother 1998;42:3059-64.

16. Briggs C, Fratamico PM. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob Agents Chemother 1999;43:846-9.

17. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389-402.

18. Wang Y, Taylor DE. Natural transformation in *Campylobacter* species. J Bacteriol 1990;172:949-55.

19. Jackson CJ, Fox AJ, Jones DM, Wareing DRA, Hutchinson DN. Associations between heat-stable (O) and heat-labile (HL) serogroup antigens of *Campylobacter jejuni*: evidence for interstrain relationships within three O/HL serovars. J Clin Microbiol 1998;36:2223-8.

20. On SLW, Nielsen EM, Engberg J, Madsen M. Validity of *Sma*I-defined genotypes of *Campylobacter jejuni* examined by *Sal*I, *Kpn*I, and *Bam*HI polymorphisms: evidence of identical clones infecting humans, poultry, and cattle. Epidemiol Infect 1998;120:231-7.

21. Richardson PT, Park SF. Integration of heterologous plasmid DNA into multiple sites on the genome of *Campylobacter coli* following natural transformation. J Bacteriol 1997;179:1809-12.

22. Kokotovic B, On SLW. High-resolution genomic fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* by analysis of amplified fragment length polymorphisms. FEMS Microbiol Lett 1999;173:77-84.

23. Sallen B, Rajoharison A, Desvarenne S, Mabilat C. Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of *Enterobacteriaceae*. Microb Drug Resist 1995;1:195-202.