Molecular characterization of class 1 integrons from Irish thermophilic 
Campylobacter spp.

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Objectives: In this study a large random collection (n = 378) of Irish thermophilic Campylobacter isolates 
were investigated for the presence of integrons, genetic elements associated with the dissemination of anti-
microbial resistance.

Methods: Purified genomic DNA from each isolate was analysed by PCR for the presence of class 1 inte-
grons. Four gene cassette-associated amplicons were completely characterized.

Results: Sixty-two of the isolates possessed a complete class 1 integron with a recombined gene cassette 
located within a 1.0 kb amplicon containing an aadA2 gene. This cassette was present in both Campylo-
bacter jejuni and Campylobacter coli isolates and following sequence analysis was shown to be similar to 
sequences recently reported in Salmonella enterica Hadar and on an 85 kb plasmid conferring quinolone 
resistance in Escherichia coli.

Conclusions: Aminoglycoside aadA2-encoding class 1 integrons were identified among unrelated Campylo-
bacter spp. Amino acid sequence comparisons revealed identical structures in both Salmonella and 
E. coli. The presence of class 1 integrons in Campylobacter spp. may be significant should these organisms 
enter the food chain and especially when antimicrobial treatment for severe infections is being considered.

Keywords: antimicrobial resistance, Campylobacter, gene cassettes, gene organization

Introduction

Campylobacter spp. are now regarded as the leading causative agents 
of acute diarrhoeal disease in humans worldwide, posing an increas-
ingly significant clinical challenge.1,2 Patients infected with Campy-
lobacter spp. have a higher mortality compared with controls.3 
Although the associated gastroenteritis is normally self-limiting, 
antimicrobial treatment is usually reserved for patients with severe 
and advanced infection and the drugs of choice often include erythro-
mycin, the fluoroquinolones or tetracycline.4 Intravenous aminoglyco-
side therapy may also be considered in more serious cases of 
Campylobacter infection, such as bacteraemia and other systemic 
infection(s).5 Several studies have recently signalled an increasing 
incidence of antimicrobial resistance among Campylobacter spp. 
isolates.6,7 Resistance to trimethoprim is intrinsic and increasing 
resistance trends for other agents including sulfonamides have been 
reported.8 Significantly, over the past decade there has been an 
increase in the number of quinolone-resistant and to a lesser extent 
macrolide-resistant strains reported, being identified from human infections.1,4,9

In general, bacterial populations respond to the threat of an anti-
microbial agent by eventually developing some type of resistance 
mechanism.10 The imposed selective pressure results in the develop-
ment of a corresponding resistance determinant that facilitates eva-
sion of the inhibitory substance.8 Horizontal transfer of such resistance 
determinants together with any genetic modification of pre-existing 
genes through point mutation or some other genetic event, are 
thought to be the main mechanisms contributing to bacterial resistance. 
Self-transmissible elements including plasmids, transposons and 
bacteriophage all facilitate the acquisition and subsequent dissemin-
ation of resistance determinants. In addition, integrons are now con-
sidered efficient vehicles for the transfer of resistance markers 
among unrelated bacterial populations.11 Integron structures are 
naturally occurring gene expression systems that can potentially

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capture and integrate one or more gene cassettes and convert them into functionally expressed genes. It is these gene cassettes that encode the resistance determinants to several antimicrobial agents.

Nine classes of integrons have been described to date and class 1 integrons are clinically significant. Briefly, the typical structure of a class 1 integron includes two conserved segments (CSs), denoted as 5'- and 3'-CSs, flanking a gene cassette. An intI1 gene encoding an integrase enzyme is located within the 5'-CS and this is responsible for the recombination of a gene cassette at a specific attI attachment site. Also within this region is a promoter which facilitates the efficient expression of any integrated gene cassette.

DNA isolation

Cultures were initially suspended in 1 mL 0.85% (w/v) NaCl and washed twice. DNase activity was inhibited using treatment with formaldehyde according to the method of Gibson et al. DNA extraction was performed according to Lind et al., and DNA concentrations were determined spectrophotometrically as described previously. The integrity of the purified template DNA was assessed by conventional agarose gel (1.5%, w/v) electrophoresis and DNA preparations were stored at 4°C.

Amplification of gene cassettes by PCR

Each isolate was analysed for the presence of gene cassettes associated with class 1 integron structures using a modified version of the PCR assay described by Lévesque et al. Briefly, for each isolate, 100 ng of purified template DNA was added to a reaction mixture which contained the following: 5 μL 10× PCR buffer [100 mM Tris–HCl pH 9.0, 500 mM KCl, 1% (v/v) Triton X-100], 2.5 mM MgCl₂, 0.2 mM each dNTP, 25 pmol each of the intI1 forward primer (5'-GGCATCCAGACGACGAGC-3') and intI1 reverse primer (5'-AACGACTTGACCTGAT-3'), 2.5 μU Taq DNA polymerase (Promega, Madison, WI) and sterile distilled water, which was added to a final volume of 50 μL. Thermal cycling reaction parameters included an initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min. A final extension at 72°C was carried out for 7 min and following this step all completed reactions were maintained at 4°C. Amplified DNA products were analysed by conventional agarose gel (1.5%, w/v) electrophoresis and each DNA sample was analysed in duplicate.

Results

In this collection 317/378 isolates were identified as C. jejuni, 55 as Campylobacter coli and six cultures were identified as mixed cultures of both species.

All isolates in the collection were analysed in duplicate by PCR for the presence of integrated gene cassettes. Several DNA amplicon profiles were identified after gel electrophoresis. These groups were broadly designated as integron pattern (IP) groups, IP-1–IP-4. The amplicon sizes within these groups ranged from 300 bp to larger DNA fragments of 1.4 kb (Figure 1a). Assignment to each group was defined based on the largest amplicon within the profile. IP-1 consisted of amplicons of ≤500 bp (data not shown), IP-2 consisted of amplicons of ≤700 bp. The remaining two groups, IP-3 and IP-4, contained amplicons of ≤1.1 and ≤1.4 kb, respectively (some of the IP-group composite profiles are shown in Figure 1a). Since the average size of a bacterial coding sequence is ~800 bp, amplified DNA fragments of ≥1.0 kb were investigated further, on the basis that these were more likely to contain a complete ORF corresponding to a potential gene. In total, the IP-3 and IP-4 groups were associated with 16.4% (62/378) of the Campylobacter spp. isolates in this collection. Of these, 54 of the 62 isolates were C. jejuni and the remaining isolates were identified as C. coli. In addition, 55 of these isolates were isolated from poultry and the remaining seven were isolated from humans. A 1.0 kb amplicon common to the IP-3 and IP-4 groups (Figure 1a) was identified and further characterized from four of the study isolates, three C. jejuni (CIT-325C, CIT-195C, CIT-134C) and one C. coli (CIT-181C). The characteristic conserved features associated with class 1 integrons were identified by PCR, in these four isolates. These included the 5′-CS-located integrase, and 3′-CS-located qacEΔ1 and sul1 genes (data not shown).

Materials and methods

Bacterial isolates

Three hundred and seventy-eight randomly collected Campylobacter spp. isolates were isolated from human and poultry sources during the year 2000. Isolates were subcultured onto Preston agar which consisted of Campylobacter agar base (Oxoid, Basingstoke, UK) containing modified selective supplement (Oxoid) and 5% (v/v) lysed horse blood (Oxoid). Subcultures were incubated at 42°C for 48 h in a microaerobic environment. All cultures were examined for purity by carbol fuschin staining, and species identification was performed using the hippurate hydrolysis test and species-specific PCR assays.
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Analysis of the DNA sequence of the 1.0 kb amplicon, from all four isolates, identified two ORFs of 39 and 789 bp. The 59-base element (be) core site necessary for recombination between gene cassettes and integrons was also noted. (A schematic illustration of two of these is shown in Figure 1(b) with the 59-be shown in the box and compared with the consensus sequence.) When the former ORF was compared with the current databases, BLAST searches identified perfectly matching sequences (Figure 1c) of unknown
Campylobacter spp. class 1 integrons

| CIT-325C | MRVATTVIESQSELYSIEHRELSTTLLAVHLGYSVAVGDLKPYSSDDLLTVAVKLD 60 |
| CIT-195C | MRVATTVIESQSELYSIEHRELSTTLLAVHLGYSVAVGDLKPYSSDDLLTVAVKLD 60 |
| CIT-101C | MRVATTVIESQSELYSIEHRELSTTLLAVHLGYSVAVGDLKPYSSDDLLTVAVKLD 60 |
| CIT-134C | MRVATTVIESQSELYSIEHRELSTTLLAVHLGYSVAVGDLKPYSSDDLLTVAVKLD 60 |
| S. Hadar | MRVATTVIESQSELYSIEHRELSTTLLAVHLGYSVAVGDLKPYSSDDLLTVAVKLD 60 |
| S. coli pHS1 | ----MTISIIESQSELYSIEHRELSTTLLAVHLGYSVAVGDLKPYSSDDLLTVAVKLD 56 |

function in two C. jejuni iso isolates along with a recent Salmonella enterica serovar Hadar isolate (accession number AY258269). This sequence was designated ORF-11. In the former, ORF-11 was located proximal to an aacA4-encoding gene in a class 1 integron and in S. enterica Hadar this sequence was similarly located on the proximal side of an aadA2-encoding gene.

BLAST searches with the larger ORF identified it as an aadA2-encoding aminoglycoside adenyltransferase, of 263 residues, which closely matched similar sequences in S. enterica Hadar and in an 11.6 kb In36 integron, on an 85 kb plasmid in Escherichia coli (Figure 1c).24 In each case resistance conferred by a gene cassette was consistent with phenotypic resistance as determined by susceptibility testing.4 Deduced amino acid sequences from all four aadA2-encoding genes from C. jejuni and C. coli were compared with each other using ClustalW.23 The alignment (Figure 2) showed that all of the Campylobacter isolates contained a similar AAD2 protein with a high level of amino acid identity (ranging from 98% to 100%) between the sequences. A small number of amino acid substitutions were identified as indicated in Figure 2 and these were particularly associated with two of the isolates, C. jejuni CIT-134C (five substitutions) and C. jejuni CIT-325C (three substitutions) (indicated in bold in Figure 2). When the Campylobacter spp. sequences were compared against those of S. enterica Hadar and the plasmid containing the aadA2-encoding gene in E. coli a similar amino acid identity was also identified.

**Discussion**

Treatment with antimicrobials is a risk factor for infection with organisms that are simultaneously resistant to several drugs and this may contribute to mortality.3 Horizontal gene transfer is a significant mechanism for disseminating antimicrobial resistance among bacterial populations. Integron structures can play a pivotal role and have been identified in several Gram-negative bacterial species including foodborne pathogens such as Salmonella spp., E. coli and Shigella spp.13,14,24 These genetic structures may contain several resistance markers with more than one gene cassette integrated between the conserved domains in a classical ‘head-to-tail’ arrangement. This feature has the potential to confer resistance to several antimicrobial agents simultaneously, including aminoglycosides, cephalosporins, the penicillins and trimethoprim. Until recently, integron structures were not identified in Campylobacter spp. and therefore potential for disseminating resistance by this mechanism was unknown. However, studies are now reporting the existence of these structures in Campylobacter spp. and therefore their role and contribution to antimicrobial resistance must be assessed.15–17

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**Figure 2.** ClustalW alignment of the deduced amino acid sequences of four AAD enzymes from unrelated Campylobacter spp. isolates along with S. enterica Hadar (AY258269) and an E. coli plasmid HSH1 localized24 aadA2-encoding sequence (AY259085). Amino acid substitutions are indicated in bold.
Several 

\textit{C. jejuni} genes, encoding resistance to streptomycin/spectino-

mycin, have been located within integrons as gene cassettes in several 

human and animal bacterial isolates.\textsuperscript{21,25} In fact these gene cassettes 

are common among class 1 integrons.\textsuperscript{11,13} Pinto-Alphandary et al.\textsuperscript{26} 

previously mapped aminoglycoside resistance-encoding genes to the 

chromosome of a number of \textit{Campylobacter} spp. isolates. These anti-

microbial agents are now seldom used therapeutically due to the high 

level of resistance reported among unrelated bacterial species. 

Surprisingly however, \textit{aad} gene cassettes remain prevalent within 

integrons despite the fact that the selective pressure associated with 

drug use is no longer a significant factor. White et al.\textsuperscript{27} suggested 

that this feature may indicate that even in the absence of selection by anti-

microbial agents normally used for therapeutic purposes, genes 

encoding resistance are not necessarily lost but can persist within 

bacterial populations. Therefore, integron screening and character-

ization of gene cassettes may be a useful approach to predict anti-

biotic resistance at the phenotypic level. 

In this study we reported the investigation of a large random 
collection of \textit{Campylobacter} spp. isolates for the presence of class 1 

integrons. Four unique amplification profiles were identified and 

amplicons of 1.0 kb were investigated in an attempt to identify any 
potential coding sequences. Sixty-two isolates were associated with 

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potential coding sequences. Sixty-two isolates were associated with 

this particular amplicon and this group consisted predominantly of 
\textit{C. jejuni} species isolated from poultry sources. Characterization of 

the 1.0 kb amplicon in four isolates independently identified highly 
similar \textit{aadA2}-encoding gene cassettes from \textit{C. jejuni} and \textit{C. coli}. This 
finding demonstrates that identical class 1 integron structures 

are present in different members of the same genus, suggesting that 
genetic exchange may have occurred in the gastrointestinal 
environment. Furthermore, \textit{S. enterica} Hadar and \textit{E. coli} were also found to 

contain identical gene cassettes, with \textit{aadA2}-encoding resistance 
determinants, suggesting that this gene cassette was transmitted 

between these microorganisms.\textsuperscript{7,10,11,13} Streptomycin resistance is 

prevalent in class 1 integrons found among poultry \textit{E. coli}.\textsuperscript{27} When 

increased resistance to human antimicrobials occurs in food animals, 

transmission via food or other routes is more likely.\textsuperscript{28} 

As the use of aminoglycoside therapy may be considered as a 
treatment option for some \textit{Campylobacter}-related infections our data 
suggest that the possibility now exists for treatment failure to occur 
due to these mobile elements. Furthermore, the presence of class 1 

integrons in several \textit{Campylobacter} isolates may in part offer an 

explanation for the high levels of resistance to sulphonamides 
frequently reported among these organisms.\textsuperscript{14} Increasing prevalence of 

macrolide and quinolone resistance is more usually attributed to 

specific mutations in chromosomally located genes although the 

future involvement of plasmid-encoded integrons cannot be ruled 

out.\textsuperscript{27} In conclusion, our findings highlight the possibility that 

integrons may be partly responsible for horizontal gene transfer as a 

potential vehicle for dissemination of MDR phenotypes among 

\textit{Campylobacter} spp. These findings may have further implications 

for future therapeutic strategies, leading to reduced drug efficacy 

and/or treatment failures in the case of MDR organisms, whose 

transmission through the food chain poses a real threat to public 

health. 

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