available in the GenBank non-redundant DNA database was examined. Two additional cases of ISAba1 associated with distinct *ampC* alleles were found in accession numbers EU604835[8] and AY325306.[3] In both of them, the IS was again in the same orientation and separated from the ATG initiation codon of *ampC* by 9 bp. The number of single nucleotide differences between the various *ampC* alleles is shown in Table 1.

Two ceftazidime and cefotaxime resistant isolates in our collection that did not belong to GC1 or GC2 were found to carry ISAba1 upstream of *ampC*, and these were also sequenced. D46 isolated in 2010 in Sydney, Australia was ST110 (Oxford scheme) and RBH2 isolated in 1999 in Brisbane, Australia was ST125 (Oxford scheme). Each contained a distinct *ampC* allele (Table 1) and ISAba1 was again appropriately oriented and 9 bp away from the *ampC* initiation codon (GenBank accession numbers KF030679 and KF030678).

The simplest explanation for the finding that ISAba1 was found in the same position and orientation relative to six different *ampC* alleles is that ISAba1 has repeatedly inserted at exactly the same position. Additional support for this conclusion comes from an examination of the sequences of ISAba1. A total of 10 single nucleotide polymorphisms, most of them near the ends of the IS, were found in various combinations when the six ISAba1 sequences were compared, and this suggests that different IS variants were inserted.

The currently unexplained site specificity could contribute to the importance of this mechanism of resistance to third-generation cephalosporins. A detailed examination of the location of ISAba1 upstream of the intrinsic *oxa-Ab* gene, which it also activates, may shed further light on this issue.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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Absence of class 1 and class 2 integrons among *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in Italy

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Sir,

Since their discovery in the late 1980s, integrons have been revealed to play a fundamental role in the horizontal gene transfer (HGT) of antimicrobial resistance (AMR) genes in bacteria, due to their frequent localization in mobile DNA elements, such as transposons and plasmids. Furthermore, integrons are considered to be involved in the transfer of multidrug resistance

Table 1. Results of antimicrobial susceptibility testing of *Campylobacter* isolates

Antimicrobial classes and drugs (disc content)	*C. jejuni* (%)			*C. coli* (%)		
	S	I	R	S	I	R
Aminoglycosides						
apramycin (15 μg)	100.0	0.0	0.0	100.0	0.0	0.0
gentamicin (10 μg)	98.0	0.0	2.0	96.0	0.0	4.0
streptomycin (10 μg)	99.0	0.0	1.0	82.0	0.0	18.0
Cephalosporins						
cefalotin (30 μg)	0.0	0.0	100.0	0.0	0.0	100.0
cefotaxime (30 μg)	27.0	9.0	64.0	14.0	7.0	79.0
ceftiofur (30 μg)	0.0	3.0	97.0	0.0	2.0	98.0
cefuroxime (30 μg)	0.0	0.0	100.0	0.0	0.0	100.0
Penicillins						
ampicillin (10 μg)	5.0	0.0	95.0	16.0	0.0	84.0
amoxicillin + clavulanic acid (30 μg)	85.6	12.5	1.9	46.4	46.4	7.2
Quinolones						
nalidixic acid (30 μg)	21.0	0.0	79.0	7.0	0.0	93.0
flumequine (30 μg)	8.0	0.0	92.0	7.0	0.0	93.0
enrofloxacin (5 μg)	13.0	17.0	70.0	9.0	14.0	77.0
ciprofloxacin (5 μg)	7.0	1.0	92.0	9.0	0.0	91.0
Macrolides						
erythromycin (15 μg)	90.0	2.0	8.0	52.0	0.0	48.0
tilmicosin (15 μg)	89.0	0.0	11.0	52.0	0.0	48.0
tylosin (30 μg)	90.0	1.0	9.0	52.0	0.0	48.0
Lincosamides						
clindamycin (2 μg)	89.0	3.0	8.0	52.0	0.0	48.0
Tetracyclines						
tetracycline (30 μg)	28.0	6.0	66.0	9.0	2.0	89.0
Potentiated sulphonamides						
sulfamethoxazole + trimethoprim (25 μg)	4.0	6.0	90.0	18.0	2.0	80.0
Phenicols						
chloramphenicol (30 μg)	100.0	0.0	0.0	100.0	0.0	0.0

S, susceptible; I, intermediate; R, resistant.

(MDR), because of their ability to cluster and express multiple resistance genes. Based on differences in the integrase gene (*intI*), diverse families of integrons have been identified to date, both in Gram-negative and, more recently, in Gram-positive bacteria.[1] However, little is still known about the presence of integrons and, more generally, about the mechanisms involved in HGT in the most common human food-borne pathogen, i.e. *Campylobacter* spp. Integron-like structures were first identified in *Campylobacter jejuni* human clinical isolates in 1998 by Gibreel and Sköld.[2] Soon after, Lucey *et al*.[3] and Gibreel and Sköld[4] confirmed the existence of integrons carrying *dfr* genes coding for trimethoprim resistance located on the chromosome of human- and animal-origin *C. jejuni* and *Campylobacter coli*. Subsequently, a number of class 1 integron-associated *aacA* and *aadA* gene cassettes, which code for resistance to aminoglycosides, have been found in *C. jejuni* and *C. coli* from humans, poultry and swine.[5–7] Conversely, no class 2 and 3 integrons have ever been detected in *Campylobacter*.[7,8]

In this study, a total of 362 *C. jejuni* and *C. coli* were analysed to detect the presence of class 1 and 2 integrons. The strains originated from various industrial poultry farms and flocks throughout Northern Italy collected between 2009 and 2010. Of these, 51 *C. jejuni* and 29 *C. coli* were isolated from broilers ($n=80$ strains), and 189 *C. jejuni* and 93 *C. coli* from meat turkeys ($n=282$ strains). A selection of 160 *Campylobacter* isolates (104 *C. jejuni* and 56 *C. coli*) was previously tested for antimicrobial susceptibility by the disc diffusion method. High resistance rates to quinolones and cephalosporins, ampicillin, sulfamethoxazole + trimethoprim and tetracycline were detected. Conversely, susceptibility prevailed to aminoglycosides, macrolides, chloramphenicol, amoxicillin +

clavulanic acid and clindamycin. See Table 1. For integron screening, template DNA was prepared as previously described.[9] Class 1 and 2 integrons were detected by real-time PCR assay using specific TaqMan and Molecular Beacon probes designed for *intI1* (5′-FAM-TGC CCG TTC CAT ACA GAA GC-3′-IBFQ) and *intI2* genes (5′-FAM-CGC GAT CCA GCC TGA CCT CTT CAC TGC GAT CGC G-3′-IBFQ), respectively. Real-time PCR assays were carried out in a final volume of 10 μL using a reaction mixture composed of 1× Kapa Probe Fast qPCR MasterMix (Kapa Biosystems, Woburn, MA, USA), 0.3 μM of each primer, 0.5 μM of the probes and 100 ng of DNA template. Amplification conditions were as follows: enzyme activation at 95°C for 3 min, followed by an amplification protocol of 45 cycles of denaturation at 95°C for 3 s and annealing–extension at 55°C for 30 s. Real-time PCR amplifications were performed in the LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Basel, Switzerland). Class 1 and class 2 integrons were not detected in any of the *C. jejuni* and *C. coli* strains analysed in this study.

In recent years MDR *Campylobacter* strains have been increasingly reported worldwide, which is now recognized as a major emerging public health concern. However, mechanisms and spread of AMR in *Campylobacter* are not totally clear. In particular, the extent to which the acquisition of resistance genes by HGT can play a role in the transmission and dissemination of AMR in *Campylobacter* is a matter of debate.[10,11] *Campylobacter* resistance to antimicrobials can be attributed to intrinsic or acquired mechanisms. Acquired mechanisms of AMR involving mutations in genes targeted by the antimicrobial, such as fluoroquinolones and macrolides, are the most frequently reported in *Campylobacter* spp. On the other hand, the transfer of resistance determinants borne on self-transmissible elements, such as plasmids, transposons and bacteriophages, seems to occur rarely among *Campylobacter* isolates or among *Campylobacter* and other unrelated bacteria. Unlike *Escherichia coli*, little evidence is available in the literature about the HGT of AMR genes in *Campylobacter* spp., and this concerns primarily the *tet*(O)-carrying plasmids encoding resistance to tetracyclines, the resistance to aminoglycosides mediated by plasmid-encoded *aphA* genes, and a few others.[10,11] HGT via transposon- or integron-associated AMR determinants not only has been rarely documented, but also has not been clearly defined.[2–8] To strengthen the assumption that HGT of AMR determinants is a rare event in *Campylobacter*, very recently Gardner and Olson[12] hypothesized that two 'bacterial immunity' systems, CRISP-Cas and restriction-modification enzymes, may act as barriers to genetic transfer.

Although evidence of class 1 integron-carrying *Campylobacter* exists, the presence of these genetic structures has been detected in a limited number of strains.[2–8] Furthermore, we underline that to the best of our knowledge no reports on the presence of integrons in the genomes of sequenced *C. jejuni* and *C. coli* strains can be found in the literature. In this study we found no class 1 and 2 integrons in a large collection of *C. jejuni* and *C. coli* isolated from poultry. Therefore, we can assume that the HGT mediated by integrons may not represent a significant mechanism for the dissemination of AMR determinants in *Campylobacter* organisms. Nevertheless, we emphasize the need to carry out further research to clarify the mechanisms of HGT among *Campylobacter* spp.

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Transparency declarations

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