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 and AV325306. 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.

Funding
Funding for this study was received from the School of Molecular Bioscience, The University of Sydney and NHMRC Project Grant APP1026189. M. H. was supported by a University of Sydney Postgraduate Research Award.

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

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J Antimicrob Chemother 2013
doi:10.1093/jac/dkt242
Advance Access publication 20 June 2013

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

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Keywords: thermophilic Campylobacter, chickens, turkeys, antimicrobial resistance, horizontal gene transfer

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
(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 Sköld4 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, erythromycin, tilmicosin, tylosin, lincosamides, clindamycin, tetracyclines, sulfamethoxazole + trimethoprim, and phenicols. Table 1.

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 | 100.0 | 0.0 |
| cefotaxime (30 μg) | 27.0 | 9.0 | 64.0 | 14.0 | 7.0 | 79.0 |
| cefotiofur (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 | 100.0 | 0.0 |
| 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 | 0.0 | 0.0 | 0.0 |

S, susceptible; I, intermediate; R, resistant.
template DNA was prepared as previously described. 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′-IBFO) and intI2 genes (5′-FAM-CGC GAT CCA GCC TGA CCT CTT CAC TGC GAT CGC G-3′-IBFO), 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 antibiotics can be attributed to intrinsic or acquired mechanisms. Acquired mechanisms of AMR involving mutations in genes targeted by the antibacterial, such as fluoroquinolones and macrolides, are the most frequently reported in Campylobacter spp. On the other hand, the transfer of resistance determinants targeted by the antimicrobial, such as fluoroquinolones and macrolides, are the most frequently reported in Campylobacter spp. of human and animal origin. Emerg Infect Dis 2000; 6: 50–5.

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