ORIGINAL ARTICLE

# Antimicrobial Resistance and Multilocus Sequence Types of Finnish *Campylobacter jejuni* Isolates from Multiple Sources

S. Olkkola[1], S. Nykäsenoja[2], S. Raulo[2], A.-K. Llarena[1], S. Kovanen[1], R. Kivistö[1], A.-L. Myllyniemi[2] and M.-L. Hänninen[1]

[1] Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland
[2] The Finnish Food Safety Authority Evira, Helsinki, Finland

## Impacts

- This study is the first from the Nordic countries combining antimicrobial resistance data with MLST types to find potential overlapping of resistant MLST types among sources and also provides an extensive assessment on the prevalence of antimicrobial resistance in *Campylobacter jejuni* from six domestic sources/hosts. These results can be used to evaluate the public health risks of acquisition of resistant *C. jejuni* when exposed to various sources/hosts.
- Results show that the proportion of resistant isolates was low for most sources. More than 80% of the bovine and broiler isolates were susceptible to the antimicrobials studied most probably due to the restrained use of antibiotics.
- A rare clonal complex ST-1034 CC previously associated with wild birds showed a high proportion of tetracycline-resistant isolates, most originating from the zoo and broilers with closely associated MLST types from these sources. This could suggest a common ancestor for these isolates, possibly originating from wild birds.

**Keywords:**
Antimicrobial resistance; campylobacter; human; broiler; bovine; environment; ciprofloxacin; tetracycline; streptomycin; multilocus sequence types

**Correspondence:**
S. Olkkola. Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, P.O. Box 66, FI-00014 Helsinki, Finland. Tel.: +358 50 941 57456; Fax: +358 9 191 57101;
E-mail: satu.olkkola@helsinki.fi

Received for publication January 9, 2015

doi: 10.1111/zph.12198

## Summary

Antimicrobial susceptibility was determined for 805 domestic *Campylobacter jejuni* isolates obtained from broilers ($n = 459$), bovines ($n = 120$), human patients ($n = 95$), natural waters ($n = 80$), wild birds ($n = 35$) and zoo animals/enclosures ($n = 16$) with known multilocus sequence types (MLST) for 450 isolates. The minimum inhibitory concentration (MIC) values for erythromycin, tetracycline, streptomycin, gentamicin and the quinolones ciprofloxacin and nalidixic acid were determined with the VetMIC method. MICs were compared with MLST types to find possible associations between sequence type and resistance. The proportions of resistant isolates were 5% (broilers), 6.3% (natural waters), 11.4% (wild birds), 11.6% (human patients), 16.7% (bovines) and 31.3% (zoo). The most common resistance among the human and bovine isolates was quinolone resistance alone while resistance to streptomycin alone was most often detected among the broiler isolates and tetracycline resistance was most commonly observed in the wild bird, water and zoo isolates. No or negligible resistance to erythromycin or gentamicin was detected. In all data, 12/26 of the tetracycline-resistant isolates were also resistant to streptomycin ($P < 0.001$) and the clonal complex (CC) ST-1034 CC showed a high proportion of 75% (9/12) of tetracycline-resistant isolates, most originating from the zoo and broilers with closely associated MLST types from these sources. No association between quinolone resistance and MLST type was seen. The low percentage of resistant isolates among the domestic *Campylobacter* infections is most probably due to the long-term controlled use of antimicrobials. However, the higher percentage of



 

tetracycline resistance observed among the zoo isolates could present a risk for zoo visitors of acquisition of resistant *C. jejuni*. The resistance pattern of tetracycline and streptomycin most often found in ST-1034 CC could indicate a common resistance acquisition mechanism commonly present in this CC. Overall, MLST typing was found to be a useful method in recognition of potential genetic lineages associated with resistance.

## Introduction

*Campylobacter jejuni* and *Campylobacter coli* are the most common causes of bacterial gastrointestinal disease in humans in the industrialized world. Campylobacteriosis is also the most commonly reported zoonosis in the European Union (EU) with more than 90% of infections caused by *C. jejuni* (EFSA, 2014a), and in Finland, a total of 4064 *Campylobacter* infections were notified in 2013 (National Institute of Health and Welfare (THL), 2014). Usually, campylobacteriosis is a self-limiting disease, but antimicrobial treatment, preferably with macrolides or fluoroquonolones, is indicated in severe or prolonged cases or in immunocompromised patients (Blaser and Engberg, 2008). The increasing resistance to antibiotics, particularly the high level of ciprofloxacin-resistant *Campylobacter* isolates in broilers, is a concern also in the EU (EFSA, 2014b). The use of antimicrobials in food-producing animals has been associated with the development of resistance in both *C. jejuni* and *C. coli* (McDermott et al., 2002; Luangtongkum et al., 2009; Juntunen et al., 2011).

Resistance to quinolones in *Campylobacter* is mediated by a single point mutation in the quinolone resistance determining region (QRDR) of the gene *gyrA* and also by the increased activity of the CmeABC efflux pump (Wang et al., 1993; Luo et al., 2003; Ge et al., 2005). Tetracycline resistance in *C. jejuni* and *C. coli* is mediated by the ribosomal protection protein TetO encoded by the *tet(O)* gene, located in either transferable plasmids or chromosomally, and the CmeABC efflux pump (Lin et al., 2002; Dasti et al., 2007). Also, aminoglycoside resistance can be mediated by plasmid-encoded modification enzymes, and a multidrug resistance plasmid harbouring resistance genes to tetracycline and aminoglycosides has been described in *C. jejuni* and *C. coli* (Nirdnoy et al., 2005; Chen et al., 2013).

There are only few published studies linking antimicrobial resistance and multilocus sequence typing (MLST) to explore potential connection and spreading of isolates between sources. Reports from Switzerland, Belgium and Korea show that quinolone and tetracycline resistance in *C. jejuni* and *C. coli* from various hosts are detected more often in certain less common genotypes than among the predominant types (Habib et al., 2009; Wirz et al., 2010; Kittl et al., 2013; Shin et al., 2013). However, another study from Switzerland found no association between particular sequence types (STs) of *C. jejuni* or *C. coli* derived from multiple sources and quinolone or macrolide resistance (Korczak et al., 2009). A study on *C. jejuni* and *C. coli* from retail poultry in the United Kingdom revealed that resistant isolates were distributed among rather distant genetic lineages, which indicated a widespread acquisition of resistance determinants. However, resistance to tetracycline and quinolones was not randomly distributed among different *C. jejuni* lineages which, according to the authors, indicated local spreading out of the resistant strains and could reflect the use of antimicrobials in poultry production (Wimalarathna et al., 2013).

The aim of this study was to determine the minimum inhibitory concentrations (MICs) of several antimicrobial agents for *C. jejuni* isolates from different sources and to find potential overlapping of resistant MLST types among those sources. Furthermore, we investigated whether resistance to selected antimicrobial classes would be more common in certain MLST types and whether resistance to different antimicrobial classes would be associated with each other.

## Materials and Methods

### Bacterial isolates

A total of 805 *C. jejuni* isolates included in the study are presented in Table 1. Of these, 226 domestic *C. jejuni* isolates originated from our collection (de Haan et al., 2013; Kovanen et al., 2014). The 27 water isolates from 2012 were obtained from rivers, lakes and the sea in southern Finland (unpublished data). In addition, MIC values of 579 broiler and bovine isolates obtained through the Finnish Veterinary Antimicrobial Resistance Monitoring Programme (FINRES-Vet) were included (Table 1) (Finnish Food Safety Authority Evira, 2011; Zoonoses centre, 2014). All isolates were stored at −70°C in skim milk and glycerol or Nutrient Broth No.2 (Oxoid, Basingstoke, Hampshire, England) and glycerol.

### MIC determination

The MIC values for erythromycin (ERY), tetracycline (TET), streptomycin (STR), gentamycin (GEN) and the quinolones ciprofloxacin (CIP), and nalidixic acid (NAL) were determined with the broth microdilution method

**Table 1.** The isolation years and sites of the *Campylobacter jejuni* isolates included in this study

| Source | solation years | Origin | *n* |
|---|---|---|---|
| Broilers | 2007–2012 | FINRES-Vet | 459 |
| Bovines | 2009, 2012 | FINRES-Vet | 120 |
| Human patients | 2012 | Central Finland (Kuopio, Mikkeli and Jyväskylä) | 95 |
| Wild birds | 2005, 2007, 2010 | Helsinki area | 35 |
| Natural waters | 2000–2001, 2005–2007, 2009, 2012 | River and lake and sea water from Southern Finland | 80 |
| Zoo | 2008 | Helsinki zoo animals/premises | 16 |

(VetMIC Camp; National Veterinary Institute, Uppsala, Sweden). Briefly, bacterial isolates were cultivated twice on nutrient agar with 5% blood and grown overnight after which cation-adjusted Mueller–Hinton Broth (Difco, Becton-Dickinson and Company, Sparks, NV, USA) with 5% lysed horse blood (Labema, Kerava, Finland) was inoculated with bacteria to reach a final inoculum of $10^6$ CFU/ml. The VetMIC panels were inoculated with this suspension and the panels were placed in a microaerobic atmosphere at 37°C for 48 h and read under a magnifying lens with a lamp. *Campylobacter jejuni* strain ATCC 33560 was used as a control. The isolates were classified as wild type (WT, also referred to as susceptible) or non-wild type (NWT, also referred to as resistant) according to the epidemiological cut-off values (ECOFFs, MIC > 4 mg/l for ERY and STR, MIC > 0.5 mg/l for CIP, MIC > 1 mg/l for TET, MIC > 2 mg/l for GEN and MIC > 16 mg/l for NAL) as determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org).

### MLST and detection of the *tet(O)* gene

All isolates from our collection and 224 of the broiler isolates were previously multilocus sequence typed using either PCR-based MLST or whole genome sequencing using the Illumina sequencing technology as described before (de Haan et al., 2013; Kovanen et al., 2014; Llarena et al., 2015). The bovine isolates were not typed.

Total genomic DNA of 10 *C. jejuni* isolates with TET MICs in the range of ≤0.5 mg/l to 8 mg/l originating from humans ($n$ = 1, TET MIC=4 mg/l), zoo ($n$ = 4, TET MIC=8 mg/l), wild birds ($n$ = 1, TET MIC ≤0.5 mg/l) and broilers ($n$ = 4, TET MIC 1–8 mg/l) was extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The presence of the *tet(O)* gene was screened for by PCR amplification of a 505-bp DNA fragment and the plasmid DNA of *C. jejuni* strain 81–176 used as a positive control (Fairchild et al., 2005). The PCR products were visualized on 1.5% agarose gel.

### Data analysis

The software SPSS for Windows ver. 21 (IBM Corp., Armonk, NY, USA) was used for data processing and statistical analyses. Fisher's exact test was used to test for statistically significant associations between resistance to different antimicrobial drugs and between resistance and MLST type. Statistical significance was set at $P < 0.05$.

The software ClonalFrame ver. 1.1 was used to generate a genealogy tree of all detected STs ($n$ = 106) based on the sequences of the seven housekeeping genes with settings of 100 000 iterations, 50 000 burn-in iterations and every 100th three sampled (Didelot and Falush, 2007). Three independent runs were conducted, and a consensus tree was constructed and exported as a Newick file to MEGA6 (Tamura et al., 2013) for display and labelled in CorelDraw X6.

## Results

The MIC distributions of the isolates ($n$ = 805) and the resistance patterns and MLST types observed are shown in Tables 2 and 3, respectively. The distribution of the isolates into clonal complexes (CCs) within source is shown in Fig. 1 and the ClonalFrame genealogy of all the STs and resistance profiles detected in each ST and source are shown in Fig. 2.

### Human patients

Overall, 11.6% of the human patient isolates had MICs above ECOFF for one or more antimicrobials studied, and the most common resistance trait observed was resistance to quinolones only (8.4%). The MLST types of the isolates have been described before and also shown in Fig 1 (Kovanen et al., 2014). The eight isolates resistant to quinolones belonged to five STs: ST-45 and ST-230 (ST-45 CC, $n$ = 4), ST-677 (ST-677 CC, $n$ = 2), ST-19 (ST-21 CC) and ST-1080 (unassigned) (Table 3). No statistically significant associations between resistance to quinolones and ST or CC were observed ($P > 0.05$).


 

**Table 2.** MIC values of 805 *C. jejuni* isolates included in this study. Vertical bars indicate epidemiological cut-off (ECOFF) values and the white area the range of concentrations tested. The numbers in the lowest concentration slots indicate isolates with MICs ≤ the lowest concentration tested, while numbers on the grey area indicate isolates with MICs above the highest concentration tested

| | | | MIC (mg/l) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | Agent | *n* | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | $MIC_{50}$ | $MIC_{90}$ | % Resistant |
| Human patients | CIP | 95 | | 84 | 3 | 2 | | 2 | 2 | 2 | | | | ≤0.25 | 0.5 | 8.4 |
| | ERY | 95 | | | | 93 | 1 | 1 | | | | | | ≤1 | ≤1 | 0 |
| | TET | 95 | | | 88 | 5 | 1 | 1 | | | | | | ≤0.5 | ≤0.5 | 2.1 |
| | STR | 95 | | | 5 | 38 | 43 | 7 | 1 | | | 1 | | 2 | 2 | 2.1 |
| | GEN | 95 | | 17 | 64 | 13 | 1 | | | | | | | 0.5 | 1 | 0 |
| | NAL | 95 | | | | | | 41 | 41 | 5 | 1 | 4 | 3 | 8 | 16 | 8.4 |
| Broilers | CIP | 459 | | 421 | 32 | | 1 | | 4 | 1 | | | | ≤0.25 | ≤0.25 | 1.3 |
| | ERY | 459 | | | | 410 | 45 | 4 | | | | | | ≤1 | 2 | 0 |
| | TET | 459 | | | 450 | 1 | 2 | 1 | 3 | 2 | | | | ≤0.5 | ≤0.5 | 1.7 |
| | STR | 459 | | | 7 | 114 | 292 | 31 | 5 | 1 | | 9 | | 2 | 4 | 3.3 |
| | GEN | 459 | | 58 | 231 | 162 | 7 | | | 1 | | | | 0.5 | 1 | 0.2 |
| | NAL | 459 | | | | 1 | 18 | 288 | 136 | 10 | | | 6 | 4 | 8 | 1.3 |
| Bovines | CIP | 120 | | 107 | 2 | | | 1 | 5 | 5 | | | | ≤0.25 | 0.5 | 9.2 |
| | ERY | 120 | | | | 120 | | | | | | | | ≤1 | ≤1 | 0 |
| | TET | 120 | | | 118 | | | | 1 | | 1 | | | ≤0.5 | ≤0.5 | 1.7 |
| | STR | 120 | | | 2 | 45 | 59 | 5 | | | | 9 | | 2 | 4 | 7.5 |
| | GEN | 120 | | 17 | 93 | 10 | | | | | | | | 0.5 | 0.5 | 0 |
| | NAL | 120 | | | | | 5 | 43 | 47 | 14 | | 1 | 10 | 8 | 16 | 9.2 |
| Naturalwaters | CIP | 80 | | 75 | 4 | | | | 1 | | | | | ≤0.25 | ≤0.25 | 1.3 |
| | ERY | 80 | | | | 80 | | | | | | | | ≤1 | ≤1 | 0 |
| | TET | 80 | | | 72 | 3 | 1 | | | | 4 | | | ≤0.5 | ≤0.5 | 6.3 |
| | STR | 80 | | | 4 | 50 | 22 | 3 | | 1 | | | | 1 | 2 | 1.3 |
| | GEN | 80 | | 21 | 55 | 3 | 1 | | | | | | | 0.5 | 0.5 | 0 |
| | NAL | 80 | | | | | | 33 | 40 | 6 | | | 1 | 8 | 8 | 1.3 |
| Wild Birds | CIP | 35 | | 33 | 2 | | | | | | | | | ≤0.25 | ≤0.25 | 0 |
| | ERY | 35 | | | | 35 | | | | | | | | ≤1 | ≤1 | 0 |
| | TET | 35 | | | 28 | 3 | | | 1 | 1 | 2 | | | ≤0.5 | 8 | 11.4 |
| | STR | 35 | | | 2 | 14 | 17 | 2 | | | | | | 2 | 2 | 0 |
| | GEN | 35 | | 6 | 25 | 4 | | | | | | | | 0.5 | 1 | 0 |
| | NAL | 35 | | | | | | 11 | 23 | 1 | | | | 8 | 8 | 0 |
| Zoo | CIP | 16 | | 13 | 1 | | | 2 | | | | | | ≤0.25 | 4 | 12.5 |
| | ERY | 16 | | | | 16 | | | | | | | | ≤1 | ≤1 | 0 |
| | TET | 16 | | | 11 | | | | 5 | | | | | ≤0.5 | 8 | 31.3 |
| | STR | 16 | | | | 9 | 3 | | | | | 4 | | 1 | >32 | 25 |
| | GEN | 16 | | 4 | 11 | 1 | | | | | | | | 0.5 | 0.5 | 0 |
| | NAL | 16 | | | | | | 11 | 2 | 1 | | | 2 | 4 | >64 | 12.5 |

MIC, minimum inhibitory concentration; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline; STR, streptomycin; GEN, gentamycin; NAL, nalidixic acid.

### Broilers

The lowest proportion of resistant isolates among all sources was detected in broilers (5%) and resistance to STR alone was the most frequently observed (2%). The 224 typed broiler isolates were attributed to 39 STs of which 197 isolates had 24 STs assigned to 10 CCs, and 27 isolates had 15 unassigned STs. The three typed TET-STR-resistant isolates had ST-4001 (ST-1034 CC), and the five typed STR-resistant isolates had several STs and CCs (Table 3). Two of the six quinolone-resistant broiler isolates were typed, and they both had ST-1721 (unassigned) (Table 3).

### Bovines

Of the bovine isolates, 16.7% had MICs above ECOFF to one or more antimicrobials studied. Quinolone resistance alone was the most common resistance observed (8.3%) followed by resistance to STR alone (6.7%). No MLST data for the bovine isolates were available.

### Natural waters

Tetracycline resistance was detected in 6.3% of the water isolates, and this covered all the resistant isolates including

**Table 3.** Resistance profiles and clonal complexes (CC) among the *Campylobacter jejuni* isolates

| Source (*n* res/*n* all) | Resistance profile (*n*) | CC (*n*) |
|---|---|---|
| Human patients (11/95) | TET (1) | ST-6591 (1) |
| | CIP-NAL (8) | 45 (4), 677 (2), 21 (1), ST-1080 (1) |
| | STR (1) | 677 (1) |
| | TET-STR (1) | ST-2068 |
| Broilers (23/459) | TET(3) | ND |
| | CIP-NAL (5) | ST-1721 (2), ND (3) |
| | STR (9) | 45 (3), 677 (1), 952 (1), ND (4) |
| | TET-STR (5) | 1034 (3), ND (2) |
| | CIP-NAL-STR-GEN (1) | ND |
| Bovines (20 /120) | CIP-NAL (10) | ND |
| | STR (8) | ND |
| | TET-STR (1) | ND |
| | TET-CIP-NAL (1) | ND |
| Water (5/80) | TET (4) | 21 (2), 1275 (1), 45 (1) |
| | TET-CIP-NAL-STR (1) | 45 (1) |
| Wild Birds (4/35) | TET (4) | 1275 (2), 1034 (2) |
| Zoo (5/16) | TET-STR (3) | 1034 (3) |
| | TET-CIP-NAL (1) | 21 (1) |
| | TET-CIP-NAL- STR (1) | 1034 (1) |

ND, not determined, CIP-NAL, ciprofloxacin-nalidixic acid; TET, tetracycline; STR, streptomycin; GEN, gentamicin.

one multidrug-resistant isolate (Table 3). The 80 isolates were attributed to 39 STs: 55 isolates belonged to 18 STs that were assigned to 11 CCs and 25 isolates belonged to 21 unassigned STs. The five TET-resistant isolates belonged to three STs assigned to three CCs: ST-50 (ST-21 CC, $n = 2$), ST-45 (ST-45 CC) and ST-1268 (ST-1275 CC), and the multiresistant isolate had ST-45 (ST-45 CC) (Table 3).

### Wild birds

Of the wild bird isolates, 11.4% had elevated MICs and all of these were resistant to TET. The 35 isolates were attributed into 21 STs with 22 isolates having 12 STs assigned to eight CCs, while 13 isolates had nine unassigned STs. The four TET-resistant isolates had ST-1268 (ST-1275 CC, $n = 2$), ST-5185 and ST-2611 (ST-1034 CC).

### Zoo

The highest proportion of resistant isolates (31.3%) came from the zoo and the most common resistance pattern detected among the zoo isolates was TET-STR (18.8%). The 16 isolates were attributed to nine STs with nine isolates belonging to four STs assigned to four CCs and seven isolates belonging to five unassigned STs. All isolates ($n = 4$) with resistance pattern TET-STR or TET-CIP-NAL-STR had ST-977 (ST-1034 CC), and an isolate resistant to TET and quinolones had ST-4579 (ST-21 CC) (Table 3). These five resistant isolates originated from different animal species or sites (great grey owl, peacock, red-necked wallaby, flamingo and swimming water of red-necked tortoise). There was a statistically significant association between resistance to TET and ST and CC ($P = 0.01$ and 0.001, respectively) with higher number of isolates than expected in ST-977 and ST-1034 CC. There was also a statistically significant association between resistance to STR and ST and CC ($P = 0.015$ and 0.002, respectively).

### All data

In the whole data, 26 isolates had NWT MICs for TET, and 14 (53.8%) of these were also resistant to other antimicrobial(s) with 12 resistant to STR ($P < 0.001$). Isolates resistant to both TET and STR were most frequently found from broilers ($n = 5$) and zoo ($n = 4$), but even when these sources were accounted for, an association was seen between resistance to TET and STR ($P = 0.009$).

Overall, ST-1034 CC and ST-1275 CC had more and ST-45 CC had less TET-resistant isolates than expected ($P < 0.001$): ST-1034 CC had a high proportion of 75% (9/12) of isolates resistant to TET, and all three isolates (derived from natural waters and wild birds) with STs attributed to ST-1275 CC were resistant to TET. The TET-resistant isolates from the zoo and broilers having ST-977 and ST-4001, respectively, have only one different allele among the seven MLST loci and cluster close to each other in the ClonalFrame tree. Furthermore, the wild bird-derived isolate having ST-5185 and showing resistance to TET alone appears to be closely associated with these STs as well (Fig. 2). Isolates resistant to quinolones were most commonly susceptible to the other antimicrobials studied with 5/28 (17.9%) isolates resistant to other antimicrobials


 

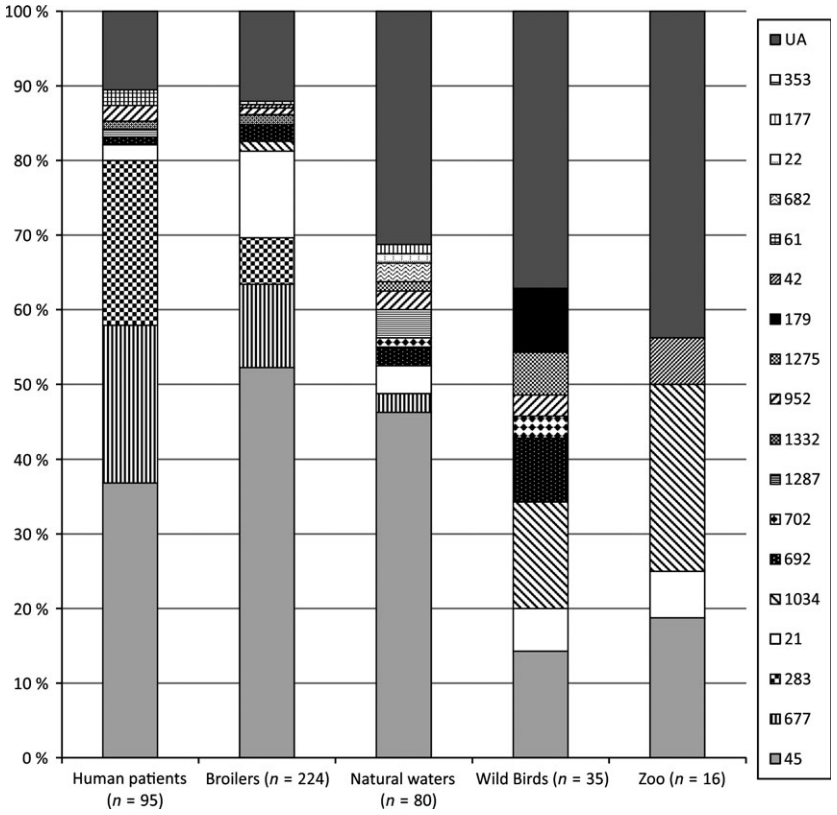


**Fig. 1.** Distribution of 450 *Campylobacter jejuni* isolates into clonal complexes.

as well, and four of these resistant to TET (P = 0.01). No association between MLST and quinolone resistance was seen ($P > 0.05$).

### Detection of the *tet(O)* gene

Ten isolates with TET MICs of 0.25–8 mg/l were screened for the presence of the *tet(O)* gene by PCR. These also included the abovementioned six typed TET-STR-resistant zoo and chicken isolates. All studied isolates with TET MICs of 1–8 mg/l ($n = 9$) harboured the gene.

## Discussion

The results presented here further extend the data obtained by the FINRES-Vet monitoring programme on broilers and bovines by the inclusion of MICs of additional isolates from various other sources and associating MIC data with MLST types. In our previous publications, we have extensively analysed population dynamics and molecular epidemiology of Finnish *C. jejuni* isolates obtained from human patients and on the other hand broilers, bovines and many other reservoirs which could be potential sources of human infection. These studies have shown that human patients, broilers and bovines often have the same predominant STs suggesting that these animals are important reservoirs of human domestically acquired infections (Karenlampi et al., 2007; Hakkinen et al., 2009; de Haan et al., 2010, 2013).

Previously, we have also found that human *C. jejuni* isolates of domestically acquired infections have a low level of resistance to ciprofloxacin and doxycycline compared to those acquired during travel abroad (Rautelin et al., 2003; Schonberg-Norio et al., 2006; Feodoroff et al., 2010). In this study, we found that quinolone resistance was the most common resistance trait detected among the domestically acquired isolates from humans with 8.4% of the isolates having elevated MICs for ciprofloxacin and nalidixic acid, while resistance to other antimicrobial groups was negligible. The ciprofloxacin $MIC_{90}$ value of 0.5 mg/l of the human isolates is similar to our previous publications, and therefore, there is no indication of increasing fluoroquinolone resistance rates in domestic *C. jejuni* from human patients (Rautelin et al., 2003; Schonberg-Norio et al., 2006).

The low proportion of resistant isolates found in broilers likely reflects the minimal use of fluoroquinolones and tetracyclines in poultry production in Finland. In some countries, these antimicrobials are widely used to treat poultry, especially for respiratory infections, leading to high level of resistance among *C. jejuni* and *C. coli*. For example, in Spain, 96.9% of broiler *C. jejuni* isolates were resistant to

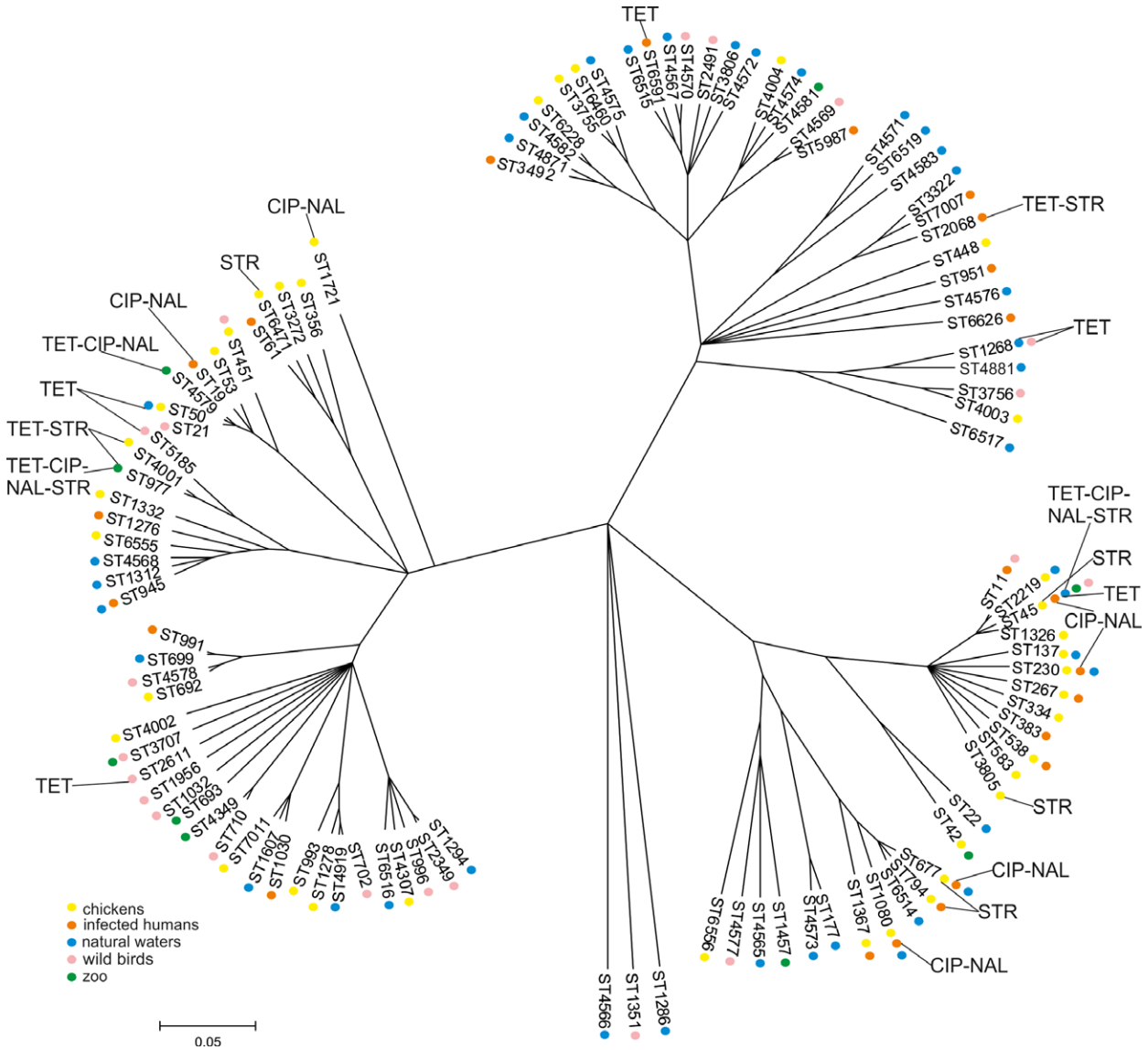


**Fig. 2.** ClonalFrame genealogy including all detected *Campylobacter jejuni* sequence types (STs) and resistance patterns observed in different sources.

ciprofloxacin and 90.6% to tetracyclines in 2012 (EFSA, 2014). Our results also indicate a history of low level usage of quinolones as resistance has been shown to persist for years after banning the use of these agents in poultry in the USA (Price et al., 2007) probably due to low fitness cost of quinolone resistance in *C. jejuni* (Luo et al., 2005). Multilocus sequence typing of the isolates indicated that the predominant CCs that colonize Finnish broilers, ST-45 CC, ST-677 CC, ST-21 CC and ST-283 CC covering more than 80% of all MLST types recovered during 2007 to 2012 were susceptible to quinolones and tetracycline. The only typed quinolone-resistant isolates had ST-1721 which is a rare ST with unknown reservoir and resistance to tetracycline was most often seen in a rare wild bird associated CC. This finding is in accordance with previously published studies from other countries, which showed that many of the quinolone and tetracycline-resistant isolates recovered from broilers or chicken meat were found to have uncommon STs and isolates attributed to ST-45 CC have been most often susceptible (Habib et al., 2009; Wirz et al., 2010).

Quinolone resistance was the most commonly detected resistance trait among the bovine isolates. There are currently four injectable fluoroquinolone containing drugs registered for cattle on sale in Finland. Whether the quinolone resistance could be due to increased use of


 

fluoroquinolones cannot be estimated as there are no data on the use of antimicrobials per animal species yet. The second most common resistance trait among bovine isolates was streptomycin resistance. The streptomycin resistance observed in both bovine and broiler isolates could indicate persistent resistance as streptomycin is not widely used for food-producing animals in Finland, and there is currently only one streptomycin containing drug accepted for veterinary use.

The highest percentage of resistance was found among the zoo isolates. This finding could be due to chance as there were only 16 isolates studied from this source with four resistant isolates having ST-977. It is also possible that there is more exposure to antimicrobials among the zoo animals or that the resistant isolates having the same ST have originated from a common source such as free-roaming birds in the zoo premises. The last hypothesis is supported by the fact that one of the animals carrying tetracycline- and streptomycin-resistant *C. jejuni* strain having ST-977 was a free-roaming peacock. The higher proportion of tetracycline resistance found among the zoo isolates could present a risk for visitors and workers of the zoo of acquisition of resistant *C. jejuni*.

Co-resistance to tetracycline and streptomycin was most often observed in certain broiler (ST-4001) and zoo isolates (ST-977) assigned to ST-1034 CC, a rare CC which has previously been associated with barnacle geese (Llarena et al., 2014). ST-977 and ST-4001 differ from each other only by one allele (two SNPs) among the seven housekeeping genes resulting in closely related STs. Furthermore, a tetracycline-resistant isolate from a wild bird that had ST-5185 (ST-1034 CC) was located in the same branch in the ClonalFrame genealogy. Even though the sequences of the seven MLST loci give only limited data on the genetic differences between strains, it can be hypothesized that these isolates could have originated from a common source such as migrating wild birds. Overall, ST-1034 CC had the largest number of isolates resistant to both tetracycline and streptomycin, and there was an association between these resistances. A similar association between resistance to tetracycline and streptomycin was also found in the EU surveillance data from 2012 (EFSA, 2014b) and could indicate a common resistance acquisition mechanism, for example a transferrable plasmid conferring both of these resistance traits. Resistance to aminoglycosides and tetracycline is often encoded in plasmids and a multidrug resistance conferring plasmid has been described in *C. jejuni* and *C. coli* (Nirdnoy et al., 2005; Chen et al., 2013). Furthermore, the *tet(O)* gene was detected in all the screened isolates with elevated MICs for TET which was contrary to the results from our previous study on *C. coli* from two Finnish swine farms where the *tet(O)* gene was not found in any of the included 300 *C. coli* isolates and tetracycline resistance did not develop in *C. coli* even after chlortetracycline treatment of the pigs (Juntunen et al., 2013). These results indicate differences between Finnish *C. jejuni* and pig-derived *C. coli* populations in their potential of becoming resistant to tetracycline.

We did not detect any associations between quinolone resistance and MLST types in all data or inside the groups (where relevant) and MICs above ECOFF for quinolones were observed also in isolates assigned to common CCs such as the ST-45 CC. In fact, isolates having ST-45 had several resistance patterns, and outside the broiler host quinolone resistance was observed in many common CCs especially among the human isolates. These findings differ from the reports from Switzerland and Korea where associations between certain uncommon MLST types and quinolone resistance were seen in *C. jejuni* isolated from humans whereas the isolates belonging to ST-45 CC were most often pan susceptible (Kittl et al., 2013; Shin et al., 2013). However, another study from Switzerland that also included human *C. jejuni* isolates found no association between MLST type and quinolone resistance (Korczak et al., 2009).

In conclusion, the proportion of resistant isolates was low for almost all sources. More than 80% of the bovine and broiler isolates had MICs below the ECOFFs for all the antimicrobials studied which most likely reflects the prudent use of antibiotics in food animal production. The uneven distribution of tetracycline and streptomycin-resistant isolates between CCs indicates more common presence of resistance determinants in certain genotypes. MLST typing was found to be a useful method in studying potential genetic lineages associated with resistance.

## Acknowledgements

Urszula Hirvi is aknowledged for her technical assistance. Satu Olkkola is a funded PhD student in the University of Helsinki, Doctoral Programme of Food Chain and Health and has also received funding from the Walter Ehrström Foundation. This publication utilized the Campylobacter MLST homepage (http://pubmlst.org/campylobacter/).

## Author Disclosure Statement

No competing financial interests exist.

## References

Blaser, M. J., and J. Engberg, 2008: Clinical aspects of *Campylobacter jejuni* and *Campylobacter coli* infections. In: Nachamkin, I., C. M. Szymanski, and M. J. Blaser (eds), Campylobacter, 3rd edn, pp. 99–121. ASM Press, Washington DC, USA.

Chen, Y., S. Mukherjee, M. Hoffmann, M. L. Kotewicz, S. Young, J. Abbott, Y. Luo, M. K. Davidson, M. Allard, P. McDermott, and S. Zhao, 2013: Whole-genome sequencing of gentamicin-resistant *Campylobacter coli* isolated from U.S. retail meats reveals novel plasmid-mediated aminoglycoside resistance genes. *Antimicrob. Agents Chemother.* 57, 5398–5405.

Dasti, J. I., U. Gross, S. Pohl, R. Lugert, M. Weig, and R. Schmidt-Ott, 2007: Role of the plasmid-encoded *tet(O)* gene in tetracycline-resistant clinical isolates of *Campylobacter jejuni* and *Campylobacter coli*. *J. Med. Microbiol.* 56, 833–837.

Didelot, X., and D. Falush, 2007: Inference of bacterial microevolution using multilocus sequence data. *Genetics* 175, 1251–1266.

EFSA, 2014a: The European Union Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. *EFSA J.* 12, 3547.

EFSA, 2014b: The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012. *EFSA J.* 12, 3590.

Fairchild, A. S., J. L. Smith, U. Idris, J. Lu, S. Sanchez, L. B. Purvis, C. Hofacre, and M. D. Lee, 2005: Effects of orally administered tetracycline on the intestinal community structure of chickens and on tet determinant carriage by commensal bacteria and *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 71, 5865–5872.

Feodoroff, B., P. Ellstrom, H. Hyytiainen, S. Sarna, M. L. Hanninen, and H. Rautelin, 2010: *Campylobacter jejuni* isolates in Finnish patients differ according to the origin of infection. *Gut Pathog.* 2, 22.

Finnish Food Safety Authority Evira, 2011: FINRES-Vet 2007-2009: Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents. Evira publications 1/2011. Available at: http://www.evira.fi/portal/en/about+evira/publications/?a=category&cid=28 (Accessed on 10 October 2014).

Ge, B., P. F. McDermott, D. G. White, and J. Meng, 2005: Role of efflux pumps and topoisomerase mutations in fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Antimicrob. Agents Chemother.* 49, 3347–3354.

de Haan, C. P., R. I. Kivisto, M. Hakkinen, J. Corander, and M. L. Hanninen, 2010: Multilocus sequence types of Finnish bovine *Campylobacter jejuni* isolates and their attribution to human infections. *BMC Microbiol.* 10, 200.

de Haan, C. P., K. Lampen, J. Corander, and M. L. Hanninen, 2013: Multilocus sequence types of environmental *Campylobacter jejuni* isolates and their similarities to those of human, poultry and bovine *C. jejuni* isolates. *Zoonoses Public Health* 60, 125–133.

Habib, I., W. G. Miller, M. Uyttendaele, K. Houf, and L. De Zutter, 2009: Clonal population structure and antimicrobial resistance of Campylobacter jejuni in chicken meat from Belgium. *Appl. Environ. Microbiol.* 75, 4264–4272.

Hakkinen, M., U. M. Nakari, and A. Siitonen, 2009: Chickens and cattle as sources of sporadic domestically acquired *Campylobacter jejuni* infections in Finland. *Appl. Environ. Microbiol.* 75, 5244–5249.

Juntunen, P., S. Olkkola, and M. L. Hanninen, 2011: Longitudinal on-farm study of the development of antimicrobial resistance in *Campylobacter coli* from pigs before and after danofloxacin and tylosin treatments. *Vet. Microbiol.* 150, 322–330.

Juntunen, P., T. Laurila, M. Heinonen, and M. L. Hanninen, 2013: Absence of tetracycline resistance in *Campylobacter coli* isolates from Finnish finishing pigs treated with chlortetracycline. *J. Appl. Microbiol.* 114, 974–981.

Karenlampi, R., H. Rautelin, D. Schonberg-Norio, L. Paulin, and M. L. Hanninen, 2007: Longitudinal study of Finnish *Campylobacter jejuni* and *C. coli* isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. *Appl. Environ. Microbiol.* 73, 148–155.

Kittl, S., G. Heckel, B. M. Korczak, and P. Kuhnert, 2013: Source attribution of human *Campylobacter* isolates by MLST and fla-typing and association of genotypes with quinolone resistance. *PLoS One* 8, e81796.

Korczak, B. M., M. Zurfluh, S. Emler, J. Kuhn-Oertli, and P. Kuhnert, 2009: Multiplex strategy for multilocus sequence typing, fla typing, and genetic determination of antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates collected in Switzerland. *J. Clin. Microbiol.* 47, 1996–2007.

Kovanen, S. M., R. I. Kivisto, M. Rossi, T. Schott, U. M. Karkkainen, T. Tuuminen, J. Uksila, H. Rautelin, and M. L. Hanninen, 2014: MLST and whole-genome MLST of human *Campylobacter jejuni* isolates from three districts during a seasonal peak in Finland. *J. Clin. Microbiol.* 12, 4147–4154.

Lin, J., L. O. Michel, and Q. Zhang, 2002: CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob. Agents Chemother.* 46, 2124–2131.

Llarena, A. K., C. P. Skarp-de Haan, M. Rossi, and M. L. Hanninen, 2014: Characterization of the *Campylobacter jejuni* Population in the Barnacle Geese Reservoir. *Zoonoses Public Health*. 62, 209–221.

Llarena, A. K., A. Huneau, M. Hakkinen, and M. L. Hanninen, 2015: Predominant *Campylobacter jejuni* sequence types persist in Finnish chicken production. *PLoS One* 10, e0116585.

Luangtongkum, T., B. Jeon, J. Han, P. Plummer, C. M. Logue, and Q. Zhang, 2009: Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. *Future Microbiol.* 4, 189–200.

Luo, N., O. Sahin, J. Lin, L. O. Michel, and Q. Zhang, 2003: *In vivo* selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. *Antimicrob. Agents Chemother.* 47, 390–394.

Luo, N., S. Pereira, O. Sahin, J. Lin, S. Huang, L. Michel, and Q. Zhang, 2005: Enhanced *in vivo* fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. *Proc. Natl. Acad. Sci. U S A* 102, 541–546.

McDermott, P. F., S. M. Bodeis, L. L. English, D. G. White, R. D. Walker, S. Zhao, S. Simjee, and D. D. Wagner, 2002: Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *J. Infect. Dis.* 185, 837–840.

National Institute of Health and Welfare (THL), 2014: Prevalence of *Campylobacters* in 2013 (in Finnish). Available at: http://www.thl.fi/fi/web/infektiotaudit/seuranta-ja-epidemiat/tartuntatautirekisteri/tartuntataudit-suomessa-vuosiraportit/tautien-esiintyvyys-2013/kampylobakteerin-esiintyvyys-2013 (Accessed on 10 October 2014).

Nirdnoy, W., C. J. Mason, and P. Guerry, 2005: Mosaic structure of a multiple-drug-resistant, conjugative plasmid from *Campylobacter jejuni*. *Antimicrob. Agents Chemother.* 49, 2454–2459.

Price, L. B., L. G. Lackey, R. Vailes, and E. Silbergeld, 2007: The persistence of fluoroquinolone-resistant *Campylobacter* in poultry production. *Environ. Health Perspect.* 115, 1035–1039.

Rautelin, H., A. Vierikko, M. L. Hanninen, and M. Vaara, 2003: Antimicrobial susceptibilities of *Campylobacter* strains isolated from Finnish subjects infected domestically or from those infected abroad. *Antimicrob. Agents Chemother.* 47, 102–105.

Schonberg-Norio, D., M. L. Hanninen, M. L. Katila, S. S. Kaukoranta, M. Koskela, E. Eerola, J. Uksila, S. Pajarre, and H. Rautelin, 2006: Activities of telithromycin, erythromycin, fluoroquinolones, and doxycycline against *Campylobacter* strains isolated from Finnish subjects. *Antimicrob. Agents Chemother.* 50, 1086–1088.

Shin, E., Y. Oh, M. Kim, J. Jung, and Y. Lee, 2013: Antimicrobial resistance patterns and corresponding multilocus sequence types of the *Campylobacter jejuni* isolates from human diarrheal samples. *Microb. Drug Resist.* 19, 110–116.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar, 2013: MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729.

Wang, Y., W. M. Huang, and D. E. Taylor, 1993: Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. *Antimicrob. Agents Chemother.* 37, 457–463.

Wimalarathna, H. M., J. F. Richardson, A. J. Lawson, R. Elson, R. Meldrum, C. L. Little, M. C. Maiden, N. D. McCarthy, and S. K. Sheppard, 2013: Widespread acquisition of antimicrobial resistance among *Campylobacter* isolates from UK retail poultry and evidence for clonal expansion of resistant lineages. *BMC Microbiol.* 13, 160.

Wirz, S. E., G. Overesch, P. Kuhnert, and B. M. Korczak, 2010: Genotype and antibiotic resistance analyses of *Campylobacter* isolates from ceca and carcasses of slaughtered broiler flocks. *Appl. Environ. Microbiol.* 76, 6377–6386.

Zoonoses centre, 2014: Antimicrobial resistance: Campylobacters (in Finnish). Available at: http://www.zoonoosikeskus.fi/portal/fi/mikrobilaakeresistenssi/zoonoosibakteerien_resistenssi/kampylobakteeri/ (Accessed on 10 October 2014).