Antibiotic resistance genes in thermophilic *Campylobacter* spp. isolated from chicken and turkey meat

B. GLEISZ¹, D. SOFKA¹ and F. HILBERT¹*

Department of Veterinary Public Health, Institute of Meat Hygiene, Meat Technology and Food Science, Veterinary University Vienna, Veterinaerplatz 1, A-1210 Vienna, Austria

* Corresponding author: friederike.hilbert@vu-wien.ac.at

In a previous study antimicrobial resistant thermophilic *Campylobacter* have been isolated from chicken and turkey meat in Austria. In this study we analysed differences in the antimicrobial resistance profile of *Campylobacter coli* and *Campylobacter jejuni* and determined antibiotic resistance genes responsible for resistance to tetracycline, streptomycin, and ampicillin by PCR. In total 21% of the isolates were resistant to tetracycline and all isolates carried the *tetO* gene that was responsible for the tetracycline resistant phenotype. The *tetO* resistance gene was located on a plasmid or on the chromosome. Resistance to ampicillin was seen in 18% and to streptomycin in 11% of the isolates. No difference was seen between *Campylobacter coli* or *Campylobacter jejuni* isolates for tetracycline and streptomycin resistance; whereas, more *Campylobacter coli* isolates harboured resistances to quinolone and ampicillin.

Keywords: *Campylobacter* spp., resistance genes; PCR; poultry meat

**Introduction**

Worldwide the major reason of sporadic diarrhoeal disease caused by bacteria is *Campylobacter* (Nachamkin, 1995). As a foodborne disease, *Campylobacter* infection follows ingestion of undercooked food, mainly poultry. *Campylobacter jejuni* and *coli* are the main pathogenic species causing enteric symptoms in humans (80-90% by *Campylobacter jejuni* and 5-10% by *Campylobacter coli*) (Tam et al., 2003).

Main symptoms of human campylobacter infections are fever, abdominal pain and diarrhoea. In most cases infections are self-limiting and hence, in general antibiotic treatment of disease is not indicated. However, for severe cases, extra intestinal disease or when it concerns immune-compromised individuals such therapy is the only refuge (Thurm and Dinger, 1998). Consequently, macrolides (erythromycin) are considered the drug of choice whilst fluoroquinolones, gentamicin and tetracycline are recommended as alternatives (Oberhelman and Taylor, 2000, Nachamkin et al. 2000). *Campylobacter*, isolated from different sources, - including humans, animals and food, - have been reported to carry various types of antimicrobial resistances in industrialized and developing countries (NARMS, 2003; Nachamkin et al., 2000; DANMAP, 2004; Mayrhofer et al, 2004). Increasing resistance rates to quinolones have been reported (Talsma et al., 1998; Feierl et al., 1999, whereas NARMS reports a slight decrease in resistance from 1999 to 2003).

As meat and meat products are the major source of foodborne infections and the most important link between food-producing animals and humans we investigated antimicrobial resistance genes and transfer in *Campylobacter* isolates from poultry meat purchased in Austria from 2001-2005.

**Material and Methods**
**Bacterial strains and isolates:** In total 261 isolates of Austrian poultry meat, purchased from slaughter houses, supermarkets, butchers and street markets were subjected to antimicrobial resistance testing against tetracycline, ampicillin, nalidixic acid, ciprofloxacin, erythromycin, chloramphenicol, streptomycin and gentamicin. From these 261 isolates, 128 isolates were determined as resistant and were subjected to genetic analysis.

**PCR analysis:** Tetracycline, ampicillin, streptomycin, resistant isolates were subjected to PCR analysis of common antimicrobial resistance genes. Genes, primers and annealing temperature are given in table 1.

**Table 1 Resistance genes tested by PCR, primers and annealing temperature used**

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sequence (5'-3')</th>
<th>(T_a) (°C)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>tetO</td>
<td>AACTTAGGCATTTCTGGCTCAC TCCACTGTTCATATCGTCA</td>
<td>57</td>
<td>515</td>
<td>Ng et al., 2001</td>
</tr>
<tr>
<td>tetA</td>
<td>GCTACATCGTCTGGCCTTC CATAGATCGCCGTGAAGAGG</td>
<td>57</td>
<td>210</td>
<td>Ng et al., 2001</td>
</tr>
<tr>
<td>tetB</td>
<td>TTGGTTAGGGGCAAGTTTG TGAATGGGCAATAACCCG</td>
<td>57</td>
<td>659</td>
<td>Ng et al., 2001</td>
</tr>
<tr>
<td>tetC</td>
<td>CTTGAGACCCTCAACCCAG ATGGTCGTCATCTACCCG</td>
<td>58</td>
<td>418</td>
<td>Ng et al., 2001</td>
</tr>
<tr>
<td>bla(_{TEM})</td>
<td>CAGCGGTAAGATCGTTAGAT</td>
<td>55</td>
<td>642</td>
<td></td>
</tr>
<tr>
<td>bla(_{TEM2})</td>
<td>TGGCCGTTGCCGTTATCTAC</td>
<td>63</td>
<td>873</td>
<td></td>
</tr>
<tr>
<td>bla(_{TEM1})</td>
<td>TGGCTCGCAACTATGACTAC</td>
<td>63</td>
<td>418</td>
<td></td>
</tr>
<tr>
<td>str(_{A})</td>
<td>CCAATGCAGATAAGGGCAAG ATCAACTGGCAGGGAACAGG</td>
<td>65</td>
<td>580</td>
<td>Maidhof et al., 2002</td>
</tr>
<tr>
<td>add(_{A1})</td>
<td>GGGAAGTGATCGTCGCCGAG CTACATTGCGTCCTGCGCAG</td>
<td>61</td>
<td>554</td>
<td>Maidhof et al., 2002</td>
</tr>
</tbody>
</table>

**Plasmid analysis:** For analysis of resistance elements on plasmids the plasmids were extracted by a common plasmid isolation procedure (Quiagen midi prep). The plasmids were restricted with EcoRV and XbaI for further analysis.

**β-lactamase assay:** was performed according to Schassan, 1978.

**Southern hybridization:** A dot blot with total DNA of *Campylobacter* strains was hybridised using a commercial DNA-DNA hybridisation kit (Amersham) with the purified and labelled PCR fragment of tetO.

**Transfer of resistance plasmids:** Isolated *Campylobacter* plasmids were transformed into competent *E. coli* DH5α by chemical transformation.

**Results and discussion**

*Campylobacter jejuni* and *coli* isolated from poultry meat in Austria and antimicrobial resistance: We analyzed in total 261 isolates of thermophilic *Campylobacter* from poultry meat (from 498 meat samples, chicken meat: 243 and turkey meat: 256). Our poultry meat isolates were in 34% defined as *Campylobacter jejuni* and 63% *Campylobacter coli* (8 isolates could not be defined). These results are unusually compared to other studies describing more than 90% of the isolates from poultry meat as *Campylobacter jejuni*. However, we used standardized ISO procedures to isolate thermophilic *Campylobacter* from meat. Nevertheless, in some countries like Slovenia, Bosnia (Zorman et al., 2006), Hungary (Anonymous1, 2004) more isolates of *Campylobacter coli* can be isolated from poultry and poultry meat. Other studies conducted in Austria have found equal or less isolates of *Campylobacter coli* from poultry or poultry meat, too (Anonymous2, 2004).
From chicken meat 125 isolates of thermophilic *Campylobacter* were isolated of which 77 isolates were defined as *Campylobacter coli* and 42 isolates as *Campylobacter jejuni*. From turkey meat 136 isolates of thermophilic *Campylobacter* could be isolated of which 88 isolates were defined as *Campylobacter coli* and 46 isolates as *Campylobacter jejuni*.

All *Campylobacter* isolates were subjected to antimicrobial resistance testing against tetracycline, ampicillin, nalidixic acid, ciprofloxacin, erythromycin, chloramphenicol, streptomycin and gentamicin and results have been published by Mayrhofer et al., 2004. Quinolone resistance was found most often (almost in 41%), followed by resistance to tetracycline (21%), ampicillin (18%) and to streptomycin (11%).

Quinolone resistance was found more often in turkey meat isolates (50%) as in chicken meat isolates (34%). This might be due to the longer fattening period of turkeys compared to chickens and a possible higher frequency in the use of antimicrobial substances for therapy and prophylaxis (Ge et al., 2003).

A higher percentage of *Campylobacter coli* were found to be quinolone resistant (68%) compared to *Campylobacter jejuni* isolates (32%).

**Tetracycline resistance:** Next to quinolone resistance tetracycline resistance is reported most often in *Campylobacter* (Mayrhofer et al., 2004; Ge et al., 2003). In our study 21% of the chicken and turkey meat isolates were phenotypically resistant to tetracycline. Exactly equal strain numbers of the tetracycline resistant *Campylobacter* were determined as *Campylobacter jejuni* and *Campylobacter coli*. In 75% of the resistant strains the tetO gene could be amplified by PCR. Most other tetracycline resistant strains could be hybridized to the tetO gene using dot blot hybridization. Southern hybridization of the plasmid and chromosomal DNA to the tetO gene revealed that the resistance gene was either located on the plasmid or on the chromosome. Other resistance elements responsible for phenotypically tetracycline resistant *Campylobacter* still have to be determined.

These results are consistent with other studies analysing tetracycline resistance in *Campylobacter coli* and *Campylobacter jejuni* isolates (Pratt and Korolik, 2005; Schwartz et al., 1993; Lee et al., 1994)

**Ampicillin resistance** was found in 45 isolates of which 10 were determined as *Campylobacter jejuni*. PCR for common resistance genes for ampicillin resistance (bla\textsubscript{TEM-1}, bla\textsubscript{cmy-2} and bla\textsubscript{pse-1}) revealed that none of the isolates harbored one of these genes. Further analyses of crude cell extracts revealed that 26 of the 45 ampicillin resistant isolates had a high level β-lactamase activity against ampicillin (see figure 1)

![Figure 1: Typical plate for determining high level β-lactamase activity in the crude extract of *Campylobacter* against ampicillin.](image)

**Resistance to streptomycin** was seen in 27 isolates of which 14 isolates were found on turkey meat and 13 isolates on chicken meat. Eleven isolates of *Campylobacter jejuni* and 16 isolates of *Campylobacter coli* harboured a streptomycin resistant phenotype. Neither strA nor addA1 two genes that have previously been described to confer streptomycin resistance to *Campylobacter* (Nirdnoy et al., 2005) were responsible for the resistance phenotype in our isolates.

**Transfer of *Campylobacter* resistance plasmids** harbouring the tetO gene into competent *Escherichia coli* cells was not possible without genetic manipulation of the resistance plasmids. Additional we analysed tetracycline resistant *Escherichia coli* isolates found on the same poultry meat
sample where tetracycline resistant *Campylobacter* isolates were found, for a tetracycline resistance determinant in order to study if a transfer of tetracycline resistance determinants between those species occurs. In none of the *E. coli* isolates the tetO gene was responsible for the resistance phenotype and neither the tetA or tetB gene that was responsible for tetracycline resistance in *E. coli* was identified in *Campylobacter* (see figure 2).

![Figure 2](image_url)

Figure 2 Agarose gel analysis of a PCR for determining the existence of the tetO gene in tetracycline resistant *Campylobacter* and tetracycline resistant *Escherichia coli* isolated from the same poultry meat sample. None of the *E. coli* isolates harbored the tetO resistance gene while, all *Campylobacter* isolates carried the tetO gene.

**References**


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