

# Role of the plasmid-encoded *tet(O)* gene in tetracycline-resistant clinical isolates of *Campylobacter jejuni* and *Campylobacter coli*

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The prevalence of tetracycline resistance, tetracycline MICs and *tet(O)* gene localization were investigated in 83 *Campylobacter* isolates from patients suffering from acute gastroenteritis in Germany. Combined biochemical and molecular markers identified 74 isolates (89 %) as *Campylobacter jejuni*, including seven atypical isolates that failed to hydrolyse hippurate, and nine isolates (11 %) as *Campylobacter coli*. Tetracycline resistance was detected in six out of nine *Campylobacter coli* isolates (67 %) and 13 out of 74 *C. jejuni* isolates (18 %). Low-level tetracycline resistance was observed for *C. coli* (MIC 16 µg ml<sup>-1</sup> for all strains), whereas *C. jejuni* showed high-level resistance (MIC >256 µg ml<sup>-1</sup> for all strains). Both low- and high-level tetracycline resistance was associated with the presence of the *tet(O)* gene. In *C. jejuni*, *tet(O)* was plasmid-encoded in 54 % of tetracycline-resistant isolates, whereas in *C. coli*, *tet(O)* appeared to be located on the chromosome.

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## INTRODUCTION

Over the last few decades, *Campylobacter jejuni* and *Campylobacter coli* have emerged as important food-borne pathogens and are of major public health concern. Both species cause gastrointestinal infections as well as post-infection manifestations, such as Guillain–Barré syndrome and reactive arthritis (Schmidt-Ott *et al.*, 2006). Annually, approximately 60 000 cases of *Campylobacter* enteritis are reported in Germany (RKI, 2006). *Campylobacter* enteritis is a zoonotic disease, and poultry, cattle and pigs can be the source of infection (Mead *et al.*, 1999). Antibiotic supplementation in animal feed constitutes more than half of the total antimicrobial use worldwide (Wegener *et al.*, 1999). Transmission of antimicrobial resistance via the food chain can occur from food animals to humans (Pezzotti *et al.*, 2003; Putnam *et al.*, 2003). In the past few years, increased antibiotic resistance has been reported in *C. jejuni*, particularly tetracycline resistance (Gibreel *et al.*, 2004). In *Campylobacter* species, high-level tetracycline resistance is usually associated with the *tet(O)* gene carried on transmissible plasmids (Taylor & Courvalin, 1988). The Tet(O) protein belongs to the class of ribosomal protection proteins that confer resistance by dislodging tetracycline from its primary binding site on the ribosome (Connell *et al.*, 2003). Previously, it has been shown that 38.5 % of *C. jejuni*

isolates in Germany are resistant to tetracycline (Wagner *et al.*, 2003). However, the role of the plasmid-encoded *tet(O)* gene in tetracycline-resistant clinical isolates of *Campylobacter* in Germany has not been well described. Precise species differentiation is an important prerequisite for such investigations. We therefore combined biochemical and molecular markers for precise differentiation of clinical isolates of *Campylobacter* to determine their prevalence, with a focus on plasmid-encoded *tet(O)*-mediated tetracycline resistance in German clinical isolates.

## METHODS

**Campylobacter strain collection.** Eighty-three isolates of *Campylobacter* were collected from patients suffering from gastroenteritis in the university hospital of Göttingen. Faecal specimens from these 83 patients were examined for the presence of common faecal pathogens. During sample collection, no outbreak of campylobacteriosis was reported in the area.

**Media and growth conditions.** Prior to tetracycline MIC determination, *Campylobacter* isolates were cultured on Columbia agar base (Merck) supplemented with 5 % sheep blood, polymyxin B (2.5 IU ml<sup>-1</sup>), trimethoprim (5 µg ml<sup>-1</sup>) and vancomycin (10 µg ml<sup>-1</sup>), and incubated at 42 °C under microaerophilic conditions (5 % O<sub>2</sub>, 10 % CO<sub>2</sub>, 85 % N<sub>2</sub>) for 48 h. *Campylobacter* isolates were biochemically differentiated at the species level by Gram staining, oxidase and catalase activities, hippurate hydrolysis, hydrogen sulfide production and susceptibility to nalidixic acid using a commercially available species differentiation kit (API Campy; bioMérieux).

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**Determination of antibiotic resistance and tetracycline MICs.**

*Campylobacter* isolates were initially tested for resistance to ampicillin, ciprofloxacin, erythromycin, gentamicin and tetracycline using a disc-diffusion method (Gaudreau & Gilbert, 1997). The MIC of tetracycline was subsequently determined by an agar dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines (NCCLS, 1997; CLSI, 2006). Mueller–Hinton blood agar supplemented with tetracycline concentrations of 4, 8, 16, 32, 64, 128 and 256 µg ml<sup>-1</sup> was inoculated with 1 µl brain–heart infusion broth containing 1 × 10<sup>7</sup>–2 × 10<sup>7</sup> bacteria and incubated at 37 °C under microaerophilic conditions for 40–48 h. Two *C. jejuni* tetracycline-resistant strains and one *C. jejuni* strain known to be tetracycline susceptible (MIC 4 µg ml<sup>-1</sup>) were used as controls. Each experiment was conducted in triplicate. The lowest concentration of the antimicrobial agent that produced no visible growth was considered to be the MIC for the relative isolate. The CLSI has previously described testing conditions for *Campylobacter* spp., but did not define interpretive breakpoints (CLSI, 2006). Therefore, in agreement with a previously published study (Gaudreau & Gilbert, 1997), we considered isolates having a tetracycline MIC of ≤4 µg ml<sup>-1</sup> to be sensitive to tetracycline.

**Genomic DNA preparation.** Genomic DNA was prepared by a previously described CTAB (hexadecyltrimethyl ammonium bromide) genomic DNA isolation method (Colegio *et al.*, 2001). A lawn culture of *Campylobacter* grown overnight on Columbia agar base was flooded with physiological 0.9% NaCl and pelleted at room temperature. The pellet was resuspended in 564 µl TE buffer (pH 7.4), with 30 µl 10% SDS and 6 µl proteinase K (10 mg ml<sup>-1</sup>; Qiagen) and incubated for 1 h at 37 °C. Subsequently, 100 µl 5 M NaCl and 80 µl CTAB/NaCl solution were added, followed by a 1 h incubation at 65 °C. Genomic DNA was extracted with chloroform : isoamyl alcohol (1 : 24), ethanol precipitated, resuspended in 50 µl sterile water and stored at -20 °C.

**PCR and Southern blot analysis of the *hipO* gene.** A previously established PCR method was used to detect the *hipO* gene (Linton *et al.*, 1997). The presence of the *hipO* gene in isolates lacking biochemical hippurate hydrolase activity was confirmed by Southern blot analysis. For Southern blot analysis of the *hipO* gene, a PCR product labelled with digoxigenin-11-UTP (Roche Diagnostics) was used as a probe and hybridized with the *Bgl*II-digested genomic DNA of *Campylobacter* isolates. The DNA was blotted on a nitrocellulose membrane (Optitran BA-S 85; Schleicher & Schuell). Solutions and conditions were used according to a standard protocol (Sambrook & Russell, 2001). Hybridization was performed at 42 °C for 18 h. Washing of membranes was carried out twice at 37 °C in 2 × SSC, 0.5% SDS for 15 min and twice at 65 °C in 0.1 × SSC, 0.5% SDS for 30 min. Digoxigenin was detected with specific peroxidase-labelled antibodies using an enhanced chemiluminescence analysis system

(Amersham Pharmacia Biotech) according to the recommendations of the supplier. Hippurate hydrolase-negative isolates were confirmed as *C. coli* by PCR. Genomic DNA was isolated by the CTAB method and a previously reported primer pair (CC18F, 5'-GGTATGATTTCTACAAAGCGA-3'; CC519R, 5'-ATAAAAGACTATCGTCGCGTG-3') was used to amplify the expected 583 bp fragment of the aspartokinase (*aspA*) gene (Linton *et al.*, 1997).

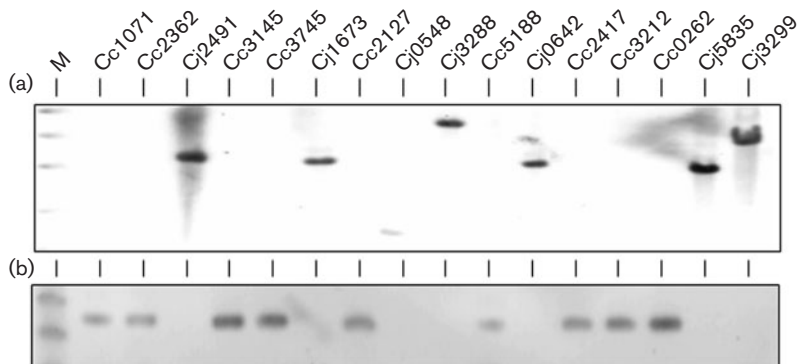
**Plasmid preparation from *C. coli* isolates.** Plasmid DNA from *Campylobacter* isolates was purified from an overnight culture on blood agar using mini Qiagen columns as recommended by the manufacturer. The plasmids were designated 'pCj' or 'pCc' for *C. jejuni* or *C. coli*, respectively, following species differentiation. Restriction digestion of plasmid DNA was performed using *Hind*III, *Bgl*II, *Pst*I and *Acc*I (New England Biolabs) and analysed on 1.2% agarose gels with TAE buffer (0.04 M Tris/acetate, 0.001 M EDTA).

**Detection and localization of the *tet(O)* gene in *C. coli* isolates.**

The presence of the *tet(O)* gene in tetracycline-resistant *C. coli* isolates was confirmed by a *tet(O)*-specific PCR (Schmidt-Ott *et al.*, 2005). Southern blot analysis was performed to determine the localization of the *tet(O)* gene. DNA probes were generated and labelled with digoxigenin-11-dUTP by PCR using the above-mentioned primer pair. Plasmid pCjA13 carrying the *tet(O)* gene was used as a template for this PCR (Schmidt-Ott *et al.*, 2005). The generated probes were hybridized as described above with the *Hind*III-digested *C. coli* plasmids. DNA was then blotted onto Optitran BA-S 85 nitrocellulose membranes and detection of the *tet(O)* gene was performed with a specific peroxidase-labelled antibody using an enhanced chemiluminescence analysis system.

**RESULTS AND DISCUSSION****Species identification of thermophilic *Campylobacter* strains**

Precise species identification of *Campylobacter* is an important prerequisite for epidemiological and resistance studies. Eighty-three thermophilic *Campylobacter* isolates were identified and tested for the presence of the *hipO* gene using phenotypic and molecular methods. On the basis of their hippurate hydrolase activity, 67 isolates (81%) were identified as *C. jejuni*. Sixteen *Campylobacter* isolates (19%) did not show any hippurate hydrolase activity. However, in seven of these hippurate hydrolase-negative isolates, the *hipO* gene was detected by probing genomic DNA with a digoxigenin-11-dUTP-labelled *hipO* probe (Fig. 1a). These



**Fig. 1.** Genotypic species differentiation of hippurate hydrolase-negative thermophilic *Campylobacter* strains. (a) *Bgl*II-digested genomic DNA probed with a digoxigenin-labelled *hipO* probe. (b) *C. coli*-specific PCR amplification of genomic DNA.

isolates were identified as atypical isolates of *C. jejuni* and the remaining nine hippurate hydrolase-negative isolates were confirmed as *C. coli* (Fig. 1b) using a specific PCR (Linton *et al.*, 1997).

### Antibiotic resistance in *C. jejuni* and *C. coli*

Tetracyclines have been used as an alternative choice in the treatment of *C. jejuni* and *C. coli* enteritis. Large geographical variation in the susceptibility patterns of *C. jejuni* and *C. coli* to tetracycline has been observed. The rate of resistance in Denmark ranges from 0 to 11% (Aarestrup *et al.*, 1997), in Spain it is 25% (Gomez-Garcés *et al.*, 1995) and in the USA it is 48% (Nachamkin, 1994). After precise identification at the species level, tetracycline MICs were determined for 19 *Campylobacter* isolates that were identified as tetracycline resistant by the disc-diffusion test. Tetracycline MICs ranged from 16 to >256 µg ml<sup>-1</sup>. High-level tetracycline resistance was found in *C. jejuni*, whereas in *C. coli* isolates, tetracycline resistance was significantly lower. For the 13 *C. jejuni* strains, the MIC was determined as >256 µg ml<sup>-1</sup>, whilst the six *C. coli* isolates had an MIC of 16 µg ml<sup>-1</sup>. The frequency of tetracycline resistance was significantly higher ( $P < 0.001$ ,  $\chi^2$  test) in *C. coli* (67%) than in *C. jejuni* (18%).

*C. coli* is frequently found in pigs (Moore & Madden, 1998), and it is known that the regular use of antimicrobial agents for therapeutic purposes and growth promotion can

play a role in the prevalence of antimicrobial-resistant strains of *C. coli* in pigs (Harvey *et al.*, 1999; Payot *et al.*, 2001). Therefore, a higher frequency of tetracycline resistance in clinical isolates of *C. coli* might be linked to the use of related antibiotics in the food chain.

### Plasmid prevalence and tetracycline resistance

Bacterial resistance to tetracycline commonly arises through one of four identified mechanisms: efflux of tetracycline, modification of tetracycline, ribosomal protection or mutation of the 16S rRNA (Burdett, 1991; Ross *et al.*, 1998; Schnappinger & Hillen, 1996). However, plasmid-mediated *tet(O)*-encoded tetracycline resistance is reported quite frequently in *Campylobacter* spp. (Manavathu *et al.*, 1988; Lee *et al.*, 1994). Plasmids bearing the *tet(O)* determinant have also been isolated from other bacteria, such as *Enterococcus faecalis* and *Streptococcus* spp., and the plasmids were shown to have similar sizes and restriction profiles to those isolated from *C. jejuni* and *C. coli* (Zilhao *et al.*, 1988). Previously, the isolation rate of plasmids from *Campylobacter* species has been reported to be quite variable, ranging from 44 to 91% for clinical and poultry isolates (Gaudreau & Gilbert, 1998). In this study, approximately 23% ( $n=19$ ) of *Campylobacter* isolates harboured plasmids, ranging in size from 5 to 66 kb. Significant differences among plasmids were detected in both species of *Campylobacter*: 19% ( $n=14$ ) of the *C. jejuni*

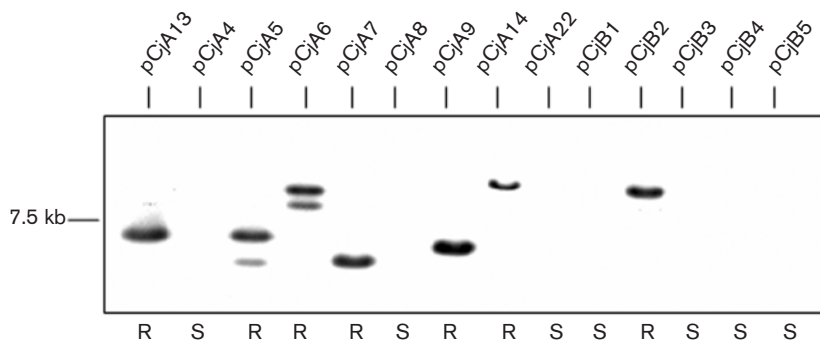
**Table 1.** Characterization of tetracycline-resistant *Campylobacter* isolates

The method applied in this study allowed the detection of plasmids of 1–66 kb.

Isolate*	<i>hipO</i>	<i>aspA</i> †	Detectable plasmids	Tet <sup>R</sup> (MIC, µg ml <sup>-1</sup> )	<i>tet(O)</i>	
					PCR	Hybridization
CjA5	+	ND	+	R (>256)	+	Plasmid
CjB2	+	ND	+	R (>256)	+	Plasmid
CjA9	+	ND	+	R (>256)	+	Plasmid
CjA13	+	ND	+	R (>256)	+	Plasmid
CjA14	+	ND	+	R (>256)	+	Plasmid
CjA6	+	ND	+	R (>256)	+	Plasmid
CjA7	+	ND	+	R (>256)	+	Plasmid
CjA15	+	ND	–	R (>256)	+	Genomic
CjB19	+	ND	–	R (>256)	+	Genomic
CjA27	+	ND	–	R (>256)	+	Genomic
CjB8	+	ND	–	R (>256)	+	Genomic
CjC2	+	ND	–	R (>256)	+	Genomic
Cj3288	+	–	–	R (>256)	+	Genomic
Cc1071	–	+	+	R (16)	+	Genomic
Cc3745	–	+	+	R (16)	+	Genomic
Cc2127	–	+	+	R (16)	+	Genomic
Cc3145	–	+	–	R (16)	+	Genomic
Cc3212	–	+	–	R (16)	+	Genomic
Cc0262	–	+	–	R (16)	+	Genomic

\*Cj, *C. jejuni*; Cc, *C. coli*.

†Aspartokinase gene of *C. coli*; ND, data not available.



**Fig. 2.** Correlation of tetracycline resistance of *C. jejuni* isolates with the presence of the *tet(O)* gene. *Bgl*II-digested *C. jejuni* plasmid DNA was probed with a digoxigenin-labelled *tet(O)* gene probe. For pCjA5 and pCjA6, two bands are visible, which is probably due to incomplete digestion of the plasmid DNA.

isolates and 56% ( $n=5$ ) of *C. coli* isolates harboured plasmids. Instead of the 33–66 kb plasmids found in *C. jejuni*, *C. coli* isolates harboured plasmids of 5–9 kb. Fifty per cent ( $n=7$ ) of plasmid-harboring *C. jejuni* and 60% ( $n=3$ ) of the plasmid-positive *C. coli* isolates were resistant to tetracycline (Table 1). To determine the localization of the *tet(O)* gene, plasmid DNA from *C. jejuni* and *C. coli* isolates was probed with digoxigenin-11-dUTP-labelled *tet(O)* (Fig. 2). Our results revealed that 54% ( $n=7$ ) of the tetracycline-resistant *C. jejuni* isolates carried the *tet(O)* gene on their plasmids. Surprisingly, in *C. coli* none of the plasmids carried the *tet(O)* gene. Amplification of the *tet(O)* gene from genomic DNA of tetracycline-resistant *C. coli* isolates indicated a chromosomal localization of the *tet(O)* gene. However, considering the limitation of the alkaline lysis method for plasmid isolation, the presence of low-copy-number plasmids larger than 70 kb cannot be totally excluded. It has been suggested previously that recombination events between plasmids and the chromosome, or integration of a plasmid, might occur, which could explain chromosomally mediated tetracycline resistance in these isolates (Boosinger *et al.*, 1990). It is also known that illegitimate recombination can cause integration of a heterologous plasmid in *C. coli* (Richardson & Park, 1997) and this would ultimately lead to a higher frequency of chromosomally mediated tetracycline resistance in *C. coli*. We previously confirmed conjugation in two isolates having plasmids of 40.5 kb (pCjA9) and 41.9 kb (pCjA13) (Schmidt-Ott *et al.*, 2005). In this study, Southern blot analysis showed that tetracycline resistance in these isolates was *tet(O)*-encoded and plasmid-mediated, which ultimately confirmed conjugation transfer of *tet(O)* in these *C. jejuni* isolates.

In conclusion, resistance against tetracycline in *C. jejuni* and *C. coli* isolates was associated with the *tet(O)* gene in all cases and there was a strong correlation between tetracycline resistance and plasmid carriage in *C. jejuni* isolates. Although all plasmid-containing isolates of *C. coli* were resistant to tetracycline, none of the *C. coli* isolates carried the *tet(O)* gene on their plasmid. Instead, the *tet(O)* gene appeared to be chromosomally encoded in all tetracycline-resistant *C. coli* isolates.

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## REFERENCES

- Aarestrup, F. M., Nielsen, E. M., Madsen, M. & Engberg, J. (1997). Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, pigs, cattle, and broilers in Denmark. *Antimicrob Agents Chemother* **41**, 2244–2250.
- Boosinger, T. R., Blevins, W. T., Heron, T. V. & Sunter, J. L. (1990). Plasmid profiles of six species of *Campylobacter* from human beings, swine and sheep. *Am J Vet Res* **51**, 718–722.
- Burdett, V. (1991). Purification and characterization of Tet(M), a protein that renders ribosomes resistant to tetracycline. *J Biol Chem* **266**, 2872–2877.
- CLSI (2006). *Performance Standards for Antimicrobial Susceptibility Testing*. Supplement M100–S16. Wayne, PA: Clinical and Laboratory Standards Institute.
- Colegio, O. R., Griffin, T. J., IV, Grindley, N. D. F. & Galán, J. E. (2001). In vitro transposition system for efficient generation of random mutants of *Campylobacter jejuni*. *J Bacteriol* **183**, 2384–2388.
- Connell, S. R., Trieber, C. A., Dinos, G. P., Einfeldt, E., Taylor, D. E. & Nierhaus, K. H. (2003). Mechanism of Tet(O)-mediated tetracycline resistance. *EMBO J* **22**, 945–953.
- Gaudreau, C. & Gilbert, H. (1997). Comparison of disk diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. *J Antimicrob Chemother* **39**, 707–712.
- Gaudreau, C. & Gilbert, H. (1998). Antimicrobial resistance of clinical isolates of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. *Antimicrob Agents Chemother* **42**, 2106–2108.
- Gibreel, A., Tracz, D. M., Nonaka, L., Ngo, T. M., Connell, S. R. & Taylor, D. E. (2004). Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to *tet(O)*-mediated tetracycline resistance. *Antimicrob Agents Chemother* **48**, 3442–3450.
- Gomez-Garces, J. L., Cogollo, R. & Alos, J. I. (1995). Susceptibilities of fluoroquinolone-resistant isolates of *Campylobacter jejuni* to 11 oral antimicrobial agents. *Antimicrob Agents Chemother* **39**, 542–544.
- Harvey, R. B., Young, C. R., Ziprin, R. L., Hume, M. E., Genovese, K. J., Anderson, R. C., Droleskey, R. E., Stanker, L. H. & Nisbet, D. J. (1999). Prevalence of *Campylobacter* spp. isolated from the intestinal

- tract of pigs raised in an integrated swine production system. *J Am Vet Med Assoc* **215**, 1601–1604.
- Lee, C. Y., Tai, C. L. & Lin, S. C. (1994).** Occurrence of plasmids and tetracycline resistance among *Campylobacter jejuni* and *Campylobacter coli* isolated from whole market chickens and clinical samples. *Int J Food Microbiol* **24**, 161–170.
- Linton, D., Lawson, A. J., Owen, R. J. & Stanley, J. (1997).** PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. *J Clin Microbiol* **35**, 2568–2572.
- Manavathu, E. K., Hiratsuka, K. & Taylor, D. E. (1988).** Nucleotide sequence analysis and expression of a tetracycline-resistance gene from *Campylobacter jejuni*. *Gene* **62**, 17–26.
- Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. & Tauxe, R. V. (1999).** Food-related illness and death in the United States. *Emerg Infect Dis* **5**, 607–625.
- Moore, J. E. & Madden, R. H. (1998).** Occurrence of thermophilic *Campylobacter* spp. in porcine liver in Northern Ireland. *J Food Prot* **61**, 409–413.
- Nachamkin, I. (1994).** Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* to ciprofloxacin, erythromycin and tetracycline from 1982 to 1992. *Med Microbiol Lett* **3**, 300–305.
- NCCLS (1997).** *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 4th edn. Approved Standard M7-A4. Wayne, PA: National Committee for Clinical Laboratory Standards.
- Payot, S., Dridi, S., Laroche, M., Federighi, M. & Magras, C. (2001).** Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in France. *Vet Microbiol* **101**, 91–99.
- Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M. & Perin, R. (2003).** Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in Northeastern Italy. *Int J Food Microbiol* **82**, 281–287.
- Putnam, S. D., Frenck, R. W., Riddle, M. S., El-Gendy, A., Taha, N. N., Pittner, B. T., Abu-Elyazeed, R., Wierzba, T. F., Rao, M. R. & other authors (2003).** Antimicrobial susceptibility trends in *Campylobacter jejuni* and *Campylobacter coli* isolated from a rural Egyptian pediatric population with diarrhea. *Diagn Microbiol Infect Dis* **47**, 601–608.
- Richardson, P. T. & Park, S. F. (1997).** Integration of heterologous plasmid DNA into multiple sites on the genome of *Campylobacter coli* following natural transformation. *J Bacteriol* **179**, 1809–1812.
- RKI (2006).** Ausgewählte Zoonosen im Jahr 2005: durch Lebensmittel übertragbare bakterielle gastrointestinale Infektionen. *Epidemiologisches Bulletin* **41**, 351–356.
- Ross, J. I., Eady, E. A., Cove, J. H. & Cunliffe, W. J. (1998).** 16S rRNA mutation associated with tetracycline resistance in a Gram-positive bacterium. *Antimicrob Agents Chemother* **42**, 1702–1705.
- Sambrook, J. & Russell, D. (2001).** *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schmidt-Ott, R., Pohl, S., Burghard, S., Weig, M. & Groß, U. (2005).** Identification and characterization of a major subgroup of conjugative *Campylobacter jejuni* plasmids. *J Infect* **50**, 12–21.
- Schmidt-Ott, R., Schmidt, H., Feldmann, S., Brass, F., Krone, B. & Groß, U. (2006).** Improved serological diagnosis stresses the major role of *Campylobacter jejuni* in triggering Guillain-Barré syndrome. *Clin Vacc Immunol* **13**, 779–783.
- Schnappinger, D. & Hillen, W. (1996).** Tetracyclines: antibiotic action, uptake, and resistance mechanisms. *Arch Microbiol* **165**, 359–369.
- Taylor, D. E. & Courvalin, P. (1988).** Mechanisms of antibiotic resistance in *Campylobacter* species. *Antimicrob Agents Chemother* **32**, 1107–1112.
- Wagner, J., Jabbusch, M. & Eisenblätter, M. (2003).** Susceptibilities of *Campylobacter jejuni* isolates from Germany to ciprofloxacin, moxifloxacin, erythromycin, clindamycin, and tetracycline. *Antimicrob Agents Chemother* **47**, 2358–2361.
- Wegener, H. C., Aarestrup, F. M., Bogø Jensen, L., Hammerum, A. M. & Bager, F. (1999).** Use of antimicrobial growth promoters in food animals and *Enterococcus faecium* resistance to therapeutic antimicrobial drugs in Europe. *Emerg Infect Dis* **5**, 329–335.
- Zilhao, R., Papadopoulou, B. & Courvalin, P. (1988).** Occurrence of the *Campylobacter* resistance gene *tet(O)* in *Enterococcus* and *Streptococcus* spp. *Antimicrob Agents Chemother* **32**, 1793–1796.