Jundishapur J Microbiol. 2014 September; 7(9): e12129. DOI: 10.5812/jjm.12129

Published online 2014 September 01. **Research Article**

Tetracycline Resistance Genes in *Campylobacter jejuni* and *C. coli* Isolated From Poultry Carcasses

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Received: June 20, 2013; **Revised:** December 16, 2013; **Accepted:** December 25, 2013

Background: *Campylobacter* is one of the leading bacterial species causing foodborne illnesses in humans. Antimicrobial agents have been extensively used for treatment of *Campylobacter* infections; but in the recent years, both animal and human isolates of this bacterium have shown resistance to several antibiotics such as *tetracycline*.

Objectives: The aim of this study was to investigate the presence of genetic determinants of *tetracycline* resistance in *Campylobacter* spp. recovered from poultry carcasses in Shiraz, Iran.

Materials and Methods: Eighty-three thermophilic *Campylobacter* spp. Isolates were first identified based on multiplex polymerase chain reaction (PCR) and then screened for presence of *tetracycline* resistance genes (*tet* (A), *tet* (B), *tet* (O) and te (S)) by PCR.

Results: The overall prevalence of *Campylobacter jejuni* and *C. coli* among the examined isolates was 51.8% and 48.2%, respectively. *Tetracycline* resistance genes of *tet* (B) and *tet* (S) were not seen among these *Campylobacter* spp. Isolates, whereas the most common *tet* gene identified was *tet* (O), found in 83.1% (69/83) of all the isolates. The *tet* (O) gene sequence comparison between C. *jejuni* and *C. coli* showed 100% similarity and these sequences (JX853721and JX853722) were also identical to the homologous sequences of other strains of *Campylobacter* spp. existing in the GenBank databases. In addition, *tet* (A) was found in 18% (15/83) of *Campylobacter* spp. isolates. To our knowledge, this represents the first report of *tet* (A) in *Campylobacter* spp. There was 100% homology between the sequences of *tet* (A) from this study (JX891463 and JX891464) and the *tet* (A) sequences mentioned for other bacteria in the GenBank databases.

Conclusions: The high prevalence of *tet* (O) resistance gene along with new detection of *tet* (A) resistance gene in *Campylobacter* spp. isolated from poultry carcasses revealed an extensive *tetracycline* resistance among *Campylobacter* isolates from poultry in Iran. It emphasized the need for cautious use of *tetracycline* in poultry production to decrease the extension of *tetracycline*-resistant *Campylobacter* spp.

Keywords:Campylobacter; *Tetracycline* Resistance

1. Background

Campylobacter spp. has now emerged as one of the leading bacterial species, causing foodborne illnesses in humans around the world. Poultry products, especially poultry meat, are major sources of *Campylobacter* infection in humans (1). *Campylobacter* infections produce little or no clinical diseases in poultry (2), but poultry carcasses have frequently been contaminated in processing plants due to the high prevalence of *Campylobacter* in the intestinal tract of market-age poultry and can be transferred to human via poultry carcasses (3). For many years, *Campylobacter* was considered susceptible to various antimicrobial agents; but in the recent years, both animal and human isolates of this bacterium have shown resistance to several antibiotics such as fluoroquinolones and *tetracycline* (4). *Tetracycline* is a broad-spectrum antibiotic with inhibitory activity against Gram-positive and Gram-negative bacteria as well as some other atypical and non-infectious microorganisms via inhibition of protein synthesis in these harmful agents (5).

In recent years, *tetracycline* resistance has emerged among many pathogenic and nonpathogenic species of bacteria. This resistance is due to different *tetracycline* resistance (*tet*) genes (6). The efflux pump and ribosomal protection genes are the two most important mechanisms of *tetracycline* resistance in various genera of bacteria and acquisition of new *tetracycline* resistance genes is mostly associated with mobile components such as plasmids or transposons, which are often conjugative elements (6). These pathways of resistance genes acquisition are along with the selection pressure hypotheses, saying that *tetracycline* resistance genes can be exchanged by different bacteria in different ecosystems

and also between human and animals isolates of microorganisms (6). *Tetracycline* resistance in *Campylobacter* spp. is primarily mediated by a ribosomal protection protein (*tet*O), which is transferred as plasmid-encoded gene (7), or in the chromosome where it is not self-mobile (5). Another ribosomal protection protein (*tet*S) has a same characters and can be transferred as plasmid-encoded gene or be in the chromosome (5). The efflux genes, *tet* (A) and *tet* (B), code for an approximately 46-kDa membrane-bound efflux protein for membrane-associated proteins that export *tetracycline* from the cell (5).

2. Objectives

In recent years, polymerase chain reaction (PCR) assay has increasingly been applied for detection and identification of *tetracycline* resistance genes, but there was no report about the distribution of *tetracycline* resistance genes among *Campylobacter* isolates in Iran. Accordingly, in this study, we tried to determine the prevalence of *tet* (O) gene in C. *jejuni* and *C. coli,* isolated from broiler flocks in Shiraz, southern Iran. Along with *tet* (O), the prevalence of two common efflux protein genes (*tet*A and *tet*B) as well as a ribosomal protection protein (*tet*S) was investigated in these isolates. In our knowledge, this is one of the first evidences for presence of *tetracycline* resistance genes other than *tet* (O) in *Campylobacter* spp. isolates.

3. Materials and Methods

3.1. Samples

Samples were obtained from the Microbiology Department of Shiraz Veterinary School. These isolates were collected from 100 broiler flocks at the slaughterhouses of Shiraz county during August to September 2009 (8).

3.2. DNA Extraction

Eighty-three culture media containing *Campylobacter* spp. isolates were taken out from a 20°C refrigerator. The melted samples were cultured in an enriched broth media containing tryptic soy broth (TSB) (30 g/L), dextrose (2.5 g/L), sodium thioglycolate (0.5 g/L), rifampicin (10 mg/L), trimethoprim (10 mg/L), vancomycin (10 mg/L), ceftriaxone (10 mg/L), amphotericin-B (10 mg/L), incubated in a microaerophilic atmosphere (Anaerocult C, Merck) and at 37°C for hours, followed by incubation at 42°C for 44 hours (8). Unfortunately, due to the sensitive nature of this bacterium in frozen conditions (9), we could not retrieve the live bacteria. The DNA extraction was carried out directly on the culture media, using phenol-chloroform extraction (10).

3.3. Multiplex Polymerase Chain Reaction for Identification of Campylobacter Species

Multiplex PCR was used for simultaneous detection of *C. jejuni* and *C. coli*, with amplification of the *mapA* gene (11) and the *ceuE* gene, respectively (12). The primer sequence data and the annealing temperature of the three primers sets used for gene amplification are shown in Table 1.

Amplification reactions were performed in a 25-μL mixture, containing 1 U AmpliTaq polymerase (CinnaGen, Iran), 2 μL DNA template, 1 μL dNTPs (50 μM), 2.5 μL 10x PCR buffer (75 mM Tris-HCl, pH: 9.0, 2 mM $MgCl_2$, 50 mMKCl, 20 $mMNH_42SO_4$ (CinnaGen, Iran)) and 1 μL (25pmoL) of each primer (CinnaGen, Iran) (Table 1). The final reaction mixture volume was adjusted to 25 μL, using distilled deionized water. The amplification reactions were carried out using a DNA thermocycler (MJ mini, BioRad, USA) with the following program: initial denaturation at 94°C for five minutes, followed by 35 cycles of denaturation at 94°C for one minute, annealing (with temperatures shown in Table 1) for one minute, and extension at 72°C for one minute, and a final extension step at 72°C for 10 minutes.

The PCR products were determined by electrophoresis of 7 μL of each reaction product in a 1.5% (w/v) agarose gel in Tris-borate-ethylenediamine tetraacetic acid (EDTA) electrophoresis buffer and visualized under the UV light. The positive controls for C. *jejuni* (ATCC 33291) and *C. coli* (RTCC 2541) were provided by Mast International Co. (USA) and Razi Vaccine and Serum Research Institute (Tehran, Iran), respectively.

3.4. Tetracycline Resistance Gene Polymerase Chain Reaction

Four *tetracycline* resistance genes (*tet* (O), *tet* (A), *tet* (B) and *tet* (S)) were investigated among the *Campylobacter* isolates, using PCR. Genes, primers and annealing temperatures are given in Table 2. The PCR reaction (25 μL) was performed in 10 Mm Tris-HCl, pH = 8.3-8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 25 pmoL of forward and reverse primers (CinnaGen Inc., Tehran, Iran) for each *tet* gene stated in Table 2, and 1 U Taq DNA polymerase (Fermentas; Glen Burnie, Maryland), using 2 μL of the extracted DNA as template. The final volume of the reaction mixture was adjusted to 25 μL, using distilled deionized water. The similar amplification reactions and visual evaluation performed for *Campylobacter* species detection were used at this level. Only a different annealing temperature (56°C) was used for detection of *tetracycline* resistance genes. The positive controls for *tetracycline* resistance genes were obtained from the Aquatic Animal Health Unit, School of Veterinary Medicine, Shiraz University, Iran. *Aeromonas sobria* strain CW4 (JN806155), *Lactococcus garvieae* strain Ira.1s (JN998084), and *Pseudomonas putida* strain Fars 110 (JN937120) were used as positive controls for *tet* (M), *tet* (S), and *tet* (A), respectively.

3.5. Sequencing

The PCR products generated from different *tet* (O) and *tet* (A) genes in *C. coli* and C. *jejuni* were purified and sequenced (ABI 3730 capillary DNA analyzer; Applied Biosystems, Foster City, CA, USA) to assess the diversity of the

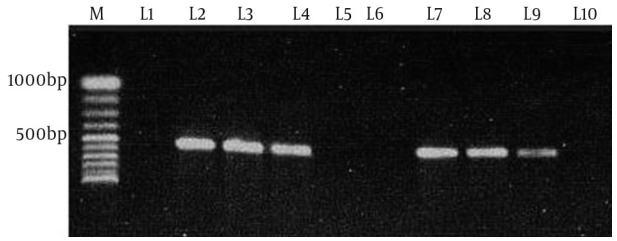
Table 1. Primer Sequences, Annealing Temperatures, and Product Sizes of the Primers Used For Genus- and Species-Specific Gene Amplifications

Gene	Primer Name	Primer Sequence (5'-3')	Annealing Temperature, °C	Product Size, bp
mapA	MapAF	CTATTTTATTTTTGAGTGCTTGTG	52°C	589
	MapAR	GCTTTATTTGCCATTTGTTTTATTA		
ceuE	ColF	AATTGAAAATTGCTCCAACTATG	52°C	482
	ColR	TGATTTTATTATTTGTAGCAGCG		

Table 2. Genes, Primers, and Annealing Temperatures Used for Detection of Tetracycline Resistance Genes

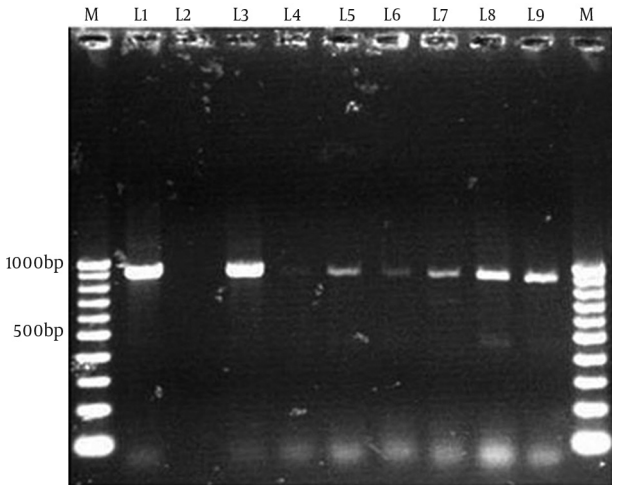
Target Gene	Sequence (5'-3')	Temperature,°C	Amplicon Size, bp	Reference
***Tet*(O)**	F: AACTTAGGCATTCTGGCTCAC	56°C	515	(28)
	R: TCCCACTGTTCCATATCGTCA			
***Tet*(A)**	F: GTGAAACCCAACATACCCC	56°C	888	(29)
	R: GAAGGCAAGCAGGATGTAG			
***Tet*(B)**	F: CCTTATCATGCCAGTCTTGC	56°C	774	(29)
	R: ACTGCCGTTTTTTCGCC			
***Tet*(S)**	CATAGACAAGCCGTTGACC	56°C	667	(28)

Figure 1. Polymerase Chain Reaction Amplification of the *tet* (O) Gene



M, 100bp DNA ladder (Vivantis, Malaysia); L1, negative control (distilled water); L2, *tet* (O) positive control (JX853721); L3, L4, L7, L8 and L9, *tet* (O) positive samples; L5, L6 and L10, *tet* (O) negative samples.

Figure 2. Polymerase Chain Reaction Amplification of the *tet* (A) Gene



M, 100bp DNA ladder (Vivantis, Malaysia); L1: *tet* (A) positive control (JX891463); L2, *tet* (A) negative control (distilled water); L3-L9, *tet* (A) positive samples.

genes and they were compared to the GenBank databases using the BLAST program maintained by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). In addition, multiple sequence alignments were made by the ClustalW method, using $MEGA_4$ software (13).

4. Results

The multiplex PCR results showed that 51.8% (43/83) and 48.2% (40/83) of the 83 thermophilic *Campylobacter* spp. isolates were C. *jejuni* and *C. coli*, respectively. In the screening of *tetracycline* resistance genes, 83.1% of *Campylobacter* spp. isolates (69/83) were positive for *tet* (O) (Figure 1). *tet* (O) was seen in 92.5% of *C. coli* isolates (37/40) and 74.4% of C. *jejuni* isolates (32/43). *tet* (A) was found in 18% of *Campylobacter* spp. isolates (15/83), including 7 (16.3%) *C. jejuni* and 8 (20%) *C. coli* (Figure 2). Nine (11%) *Campylobacter* spp. isolates were positive for both *tet* (O) and *tet* (A). *tet* (B) and *tet* (S) resistance genes were not seen in these *Campylobacter* spp. isolates.

Four amplified PCR products (*tet*O and *tet*A from both C. *jejuni* and *C. coli* sources) were sequenced and aligned with the other related *tet* gene sequences obtained from GenBank. The *tet* (O) genes from C. *jejuni* (JX853722) and *C. coli* (JX853721) detected in this study showed 100% homology with the sequences of *tet* (O) gene, previously reported for *Campylobacter* spp. The sequences of *tet* (A) genes for *C. coli* and *C. jejuni* have been deposited in the GenBank database under the accession numbers JX891463 and JX891464, respectively. *tet* (A) sequence was not mentioned previously for *Campylobacter* spp.; thus, the *tet* (A) sequences from this study was compared with the *tet* (A) gene of other bacteria instead of *Campylobacter*. In common with *tet* (O), there was 100% homology between the present Iranian *tet* (A) sequence and other *tet* (A) sequences mentioned in GenBank database.

5. Discussion

There is a significant concern about the increasing antibiotic resistance in *Campylobacter* spp. isolated from both humans and animals. In developing countries like Iran, most of the antimicrobial agents in human pharmacopeia are also used in poultry industry (14). Between these antimicrobial agents, *tetracycline* family is the most commonly used antibiotic in domestic animals farming, including poultry industry, because of its low cost, efficacy, and lack of side effects (5). *Tetracycline* has been extremely used in poultry industry of Iran for many years and mass application of this antibiotic for grow promotions and treatment aims has led to a high *tetracycline* resistance in different bacteria isolated from different poultry samples in Iran (14-17). *Campylobacter* spp. is one of the most important bacteria separated from poultry carcasses in Iran poultry slaughterhouses, with high resistance to different antimicrobial agents. Resistance to *tetracycline* has been one of the most common findings among antimicrobial susceptibility tests of *Campylobacter* isolates from poultry carcasses and meats in Iran (15-17). Moreover, there were some reports from Iran about the increasing *tetracycline* resistance in human *Campylobacter* isolates (18).

Due to the fastidious and slowly growing nature of *Campylobacter* spp., detection and species differentiation of this organism by biochemical tests is not reliable and leads to controversial results. These difficulties have led to introducing various DNA-based PCR methods for genus and species detection of *Campylobacter* spp. Among these methods, multiplex PCR proved to be accurate and simple to perform (19). In our study, multiplex PCR results showed that of 83 thermophilic *Campylobacter* spp. isolates, 51.8% were C. *jejuni* and 48.2% were *C. coli*. Among *tetracycline* resistance determinants investigated in these isolates, *tet* (O) was detected in 83.1% of *Campylobacter* spp. isolates. The high prevalence of *tet* (O) genes in these isolates represents high *tetracycline* resistance in *Campylobacter* spp. isolates obtained from poultry carcasses in Iran, because the *tet* (O) gene is the most commonly reported determinant conferring resistance to *tetracycline* in the *Campylobacter* genus, and in many previous study, this gene was detected in all tetracycline-resistant *Campylobacter* spp. isolates (20-23).

Previous studies via antimicrobial susceptibility testing in Iran have reported the prevalence rates of *tetracycline* resistance *of Campylobacter* spp.to be 70.6% and 78.3% in chicken carcasses of Shahrekord and Ahvaz, respectively (15, 16). High rates of *tetracycline* resistance in these studies might be due to this fact that *tetracycline* has been commonly used in the poultry industry of Iran as a therapeutic or prophylactic agent (16). Therapeutic and sub therapeutic uses of *tetracycline* for a long period may evolutionally generate *tetracycline*-resistant *Campylobacter* species and lead to widespread distribution of *tetracycline*-resistant *Campylobacter* in animal reservoirs (2).

Most of *C. coli* isolates harbored the *tet* (O) resistance gene, so that the presence of *tet* (O) gene in *C. coli* (92.5%) was higher than C. *jejuni* (74.4%). *C. coli* has demonstrated faster resistance increasing ability to larger number of antimicrobial agents, compared with C. *jejuni* (24). There was no evidence of *tet* (B) or *tet* (S) existence, but *tet* (A) was found in 18% of the *Campylobacter* spp. isolates. This is the first evidence for *tet* (A) presence in *Campylobacter* spp. isolates. *Tetracycline* resistance in *Campylobacter* spp. is primarily mediated by a ribosomal protection protein, *tet* (O); however, in recent years, with the finding of a novel transferable antibiotic resistance gene, *tet* (24), in *C. fetus*, this monopoly has been questioned (25). Between different *tetracycline* resistance genes, *tet* (A) has some special features, predisposing its spread to new genera. *Tet* (A) has a broad host range and is carried by various environmental genera (26). Furthermore, recent studies have demonstrated that *tet* (A) gene can be located on mobile elements such as plasmids and can be horizontally transferred among bacterial strains (27). Falsafi et al., (14) reported high prevalence (50%) of *tet* (A) resistance gene among *Escherichia coli* isolates from the Iranian poultry farms samples. Poultry *E. coli* strains with this high prevalence of *tet* (A) resistance gene can be a reservoir for this antimicrobial resistance gene and may play a role in distribution of this resistance gene to other bacteria such as *Campylobacter* spp. in poultry industry.

Sequencing of *tet* (O) and *tet* (A) showed 100% homogeneity with other related *tet* (O) and *tet* (A) sequences in GenBank. This homogeneity is a persist finding in recent years; for example, sequence analysis was performed for *tetracycline* resistance (Tcr) plasmids from C. *jejuni* by other groups and our sequences appeared to be highly conserved, in view of the fact that recently sequenced Tcr plasmids are identical with first Tcr plasmid isolated in the late 1970s (24). Presence of efflux genes such as *tet* (A) has not been demonstrated in previous studies in genus *Campylobacter* (24), but our study showed new evidence of *tet* (A) presence in this genus that may have a complementary rule in the appearance of high level resistance to *tetracycline* in *Campylobacter* spp.

High prevalence of *tet* (O) resistance gene along with new detection of *tet* (A) resistance gene in *Campylobacter* spp. isolated from poultry carcasses, revealed an extensive *tetracycline* resistance among *Campylobacter* isolates obtained from poultry in Iran. It emphasizes the need for cautious use of *tetracycline* in poultry production to decrease the extension of *tetracycline*-resistant *Campylobacter* spp.

Acknowledgements

There were no acknowledgments.

Authors' Contributions

For preparing this manuscript, all the authors contributed equally.

References

1. Humphrey T, O'Brien S, Madsen M. Campylobacters as zoonotic pathogens: a food production perspective. *Int J Food Microbiol.* 2007;**117**(3):237–57.
2. Luangtongkum T, Morishita TY, Ison AJ, Huang S, McDermott PF, Zhang Q. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of Campylobacter spp. in poultry. *Appl Environ Microbiol.* 2006;**72**(5):3600–7.
3. Jeffrey JS, Tonooka KH, Lozanot J. Prevalence of campylobacter spp. from skin, crop, and intestine of commercial broiler chicken carcasses at processing. *Poult Sci.* 2001;**80**(9):1390–2.
4. Iovine NM. Resistance mechanisms in Campylobacter jejuni. *Virulence.* 2013;**4**(3):230–40.
5. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev.* 2001;**65**(2):232–60.
6. Roberts MC. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett.* 2005;**245**(2):195–203.
7. Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, Taylor DE. 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.* 2004;**48**(9):3442–50.
8. Ansari-Lari M, Hosseinzadeh S, Shekarforoush SS, Abdollahi M, Berizi E. Prevalence and risk factors associated with campylobacter infections in broiler flocks in Shiraz, southern Iran. *Int J Food Microbiol.* 2011;**144**(3):475–9.
9. Murinda SE, Nguyen LT, Oliver SP. Problems in isolation of Campylobacter jejuni from frozen-stored raw milk and bovine fecal samples: genetic confirmation using multiplex PCR. *Foodborne Pathog Dis.* 2004;**1**(3):166–71.
10. Mayer MP. A new set of useful cloning and expression vectors derived from pBlueScript. *Gene.* 1995;**163**(1):41–6.
11. Stucki U, Frey J, Nicolet J, Burnens AP. Identification of Campylobacter jejuni on the basis of a species-specific gene that encodes a membrane protein. *J Clin Microbiol.* 1995;**33**(4):855–9.
12. Gonzalez I, Grant KA, Richardson PT, Park SF, Collins MD. Specific identification of the enteropathogens Campylobacter jejuni and Campylobacter coli by using a PCR test based on the ceuE gene encoding a putative virulence determinant. *J Clin Microbiol.* 1997;**35**(3):759–63.
13. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007;**24**(8):1596–9.
14. Falsafi T, Ebrahimi M, Asgarani E, Mirtorabi V. The pattern, association with multidrug-resistance and transferability of plasmid-mediated tetracycline resistance inEscherichia coli isolates from the poultry in Iran. *A Microbiol.* 2009;**59**(2):199–205.
15. Rahimi E, Ameri M. Antimicrobial resistance patterns of Campylobacter spp. isolated from raw chicken, turkey, quail, partridge, and ostrich meat in Iran. *Food Control.* 2011;**22**(8):1165–70.
16. Rahimi E, Momtaz H, Ameri M, Ghasemian-Safaei H, Ali-Kasemi M. Prevalence and antimicrobial resistance of Campylobacter species isolated from chicken carcasses during processing in Iran. *Poult Sci.* 2010;**89**(5):1015–20.
17. Taremi M, Mehdi Soltan Dallal M, Gachkar L, MoezArdalan S, Zolfagharian K, Reza Zali M. Prevalence and antimicrobial resistance of Campylobacter isolated from retail raw chicken and beef meat, Tehran, Iran. *Int J Food Microbiol.* 2006;**108**(3):401–3.
18. Hamidian M, Sanaei M, Azimi-Rad M, Tajbakhsh M, Dabiri H, Zali M. fla-typing, RAPD analysis, isolation rate and antimicrobial resistance profile of Campylobacter jejuni and Campylobacter coli of human origin collected from hospitals in Tehran, Iran. *Ann Microbiol.* 2010;**61**(2):315–21.
19. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, et al. Colony multiplex PCR assay for identification and differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. fetus. *J Clin Microbiol.* 2002;**40**(12):4744–7.
20. Mazi W, Senok A, Al-Mahmeed A, Arzese A, Bindayna K, Botta G. Trends in antibiotic sensitivity pattern and molecular detection of tet(O)-mediated tetracycline resistance in campylobacter jejuni isolates from human and poultry sources. *Jpn J Infect Dis.* 2008;**61**(1):82–4.
21. Piddock LJ, Griggs D, Johnson MM, Ricci V, Elviss NC, Williams LK, et al. Persistence of Campylobacter species, strain types, antibiotic resistance and mechanisms of tetracycline resistance in poultry flocks treated with chlortetracycline. *J Antimicrob Chemother.* 2008;**62**(2):303–15.
22. Pratt A, Korolik V. Tetracycline resistance of Australian Campylobacter jejuni and Campylobacter coli isolates. *J Antimicrob Chemother.* 2005;**55**(4):452–60.
23. Wardak S, Szych J, Zasada AA, Gierczynski R. Antibiotic resistance of Campylobacter jejuni and Campylobacter coli clinical isolates from Poland. *Antimicrob Agents Chemother.* 2007;**51**(3):1123–5.
24. Gebreyes WA, Thakur S, Morrow WE. Campylobacter coli: prevalence and antimicrobial resistance in antimicrobial-free (ABF) swine production systems. *J Antimicrob Chemother.* 2005;**56**(4):765–8.
25. Abril C, Brodard I, Perreten V. Two novel antibiotic resistance genes, tet(44) and ant(6)-Ib, are located within a transferable pathogenicity island in Campylobacter fetus subsp. fetus. *Antimicrob Agents Chemother.* 2010;**54**(7):3052–5.
26. Zhang XX, Zhang T, Fang HH. Antibiotic resistance genes in water environment. *Appl Microbiol Biotechnol.* 2009;**82**(3):397–414.
27. Szczepanowski R, Krahn I, Linke B, Goesmann A, Puhler A, Schluter A. Antibiotic multiresistance plasmid pRSB101 isolated from a wastewater treatment plant is related to plasmids residing in phytopathogenic bacteria and carries eight different resistance determinants including a multidrug transport system. *Microbiology.* 2004;**150**(Pt 11):3613–30.