Macrolide resistance mechanisms and virulence factors in erythromycin-resistant Campylobacter species isolated from chicken and swine feces and carcasses

Suk-Kyung LIM1,*, Dong-Chan MOON1, Myung Hwa CHAE1, Hae Ji KIM2, Hyang-Mi NAM1, Su-Ran KIM1, Gun-Chan JANG1, Kichan LEE1, Suk-Chan JUNG1 and Hee-Soo LEE1

1)Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon-si, Gyeongsanbuk-do, Republic of Korea
2)Ministry of Food and Drug Safety, Republic of Korea

(Received 25 June 2016/Accepted 16 August 2016/Published online in J-STAGE 2 September 2016)

ABSTRACT. Resistance to antimicrobials was measured in 73 isolates of Campylobacter jejuni (C. jejuni) and 121 isolates of Campylobacter coli (C. coli) from chicken and swine feces and carcasses in Korea. Both bacterial species showed the highest resistance to (fluoro)quinolones (ciprofloxacin and nalidixic acid) out of the nine antimicrobials tested. Erythromycin resistance was much higher in C. coli (19.0%, 23/121) than in C. jejuni (6.8%, 5/73). The mutation in the 23S rRNA gene was primarily responsible for macrolide resistance in Campylobacter isolates. Several amino acid substitutions in the L4 and L22 ribosomal proteins may play a role in the mechanism of resistance, but the role requires further evaluation. A total of eight virulence genes were detected in 28 erythromycin-resistant isolates. Several amino acid substitutions in the L4 and L22 ribosomal proteins may play a role in the mechanism of resistance, but the role requires further evaluation. A total of eight virulence genes were detected in 28 erythromycin-resistant Campylobacter isolates. All C. jejuni isolates carried more than four such genes, while C. coli isolates carried fewer than three such genes. The high rate of resistance highlights the need to employ more critically important antimicrobials, such as fluoroquinolones and macrolides, in swine and poultry production, and to more carefully monitor antimicrobial resistance in Campylobacter isolates in food animals.

KEYWORDS: Campylobacter, macrolide resistance, virulence factor

doi: 10.1292/jvms.16-0307; J. Vet. Med. Sci. 78(12): 1791–1795, 2016

Campylobacteriosis is one of the most commonly reported gastrointestinal diseases worldwide [7]. Campylobacter spp., such as Campylobacter jejuni and Campylobacter coli, are usually normal intestinal flora in animals. Contamination of food products during processing is the main source of food poisoning in humans. Although campylobacteriosis is generally a self-limiting disease, antimicrobial treatment may be required for systemic Campylobacter infections, such as severe or long-lasting infections, in immune-deficient people or immunosuppressed patients [20].

Macrolides are one of only a few antimicrobials available to treat Campylobacter infection [21]. Macrolides, such as erythromycin and tylosin, are also widely used in animal industry [12]. The potential risk that macrolide-resistant Campylobacter spp. will be transmitted from animal products to humans has raised concerns that using macrolides in animals will compromise the treatment of human infections.

Two main mechanisms of macrolide resistance, ribosomal target modifications and active efflux, may be involved. High-level resistance is mainly caused by mutations at positions 2,058 and 2,059 (Escherichia coli numbering) of the 23S rRNA gene [2, 3, 11, 18]. In addition, several modifications in the ribosomal proteins L4 and L22, which are associated with macrolide resistance, have been reported in Campylobacter [2, 3, 11, 18]. The other resistance mechanism is mediated by the CmeABC efflux pump, which protects Campylobacter against erythromycin, tetracyclines, bile salts, detergents and dyes [2, 13].

Studies conducted in Korea [8, 9, 16] demonstrated a relatively high level of antimicrobial resistance in Campylobacter from animals and meats, compared with that in the European Union, Canada and United States [9]. Furthermore, outbreaks of food poisoning caused by Campylobacter have increased in Korea recently [16]. The choices for treating Campylobacter infections are limited, because there is a high level of resistance to (fluoro)quinolones among Campylobacter found in food animals and meats in Korea [8, 9]. Thus, macrolides are very important antimicrobials for treatment of Campylobacter infection in human in Korea. The aims of the present study are to examine antimicrobial resistance and to investigate the molecular mechanisms involved in macrolide resistance, focusing on region V of the 23S rRNA gene, the rplD (L4) and rplV (L22) genes, and to detect the presence of virulence factors in erythromycin-resistant C. jejuni and C. coli strains isolated from animals and carcasses in Korea.

MATERIALS AND METHODS

Bacteria collection: Campylobacter isolates were recovered from laboratories and centers participating in the Korean Veterinary Antimicrobial Resistance Monitoring System (KVARMS). We collected 194 Campylobacter spp. isolated from chicken and swine animal feces and carcasses in 2010: 73 C. jejuni from chicken feces (n=43) and chicken carcasses (n=30), and 121 C. coli from pig feces (n=46), pig carcasses (n=12), chicken feces (n=38) and chicken carcasses (n=25). Animal feces and carcass samples were...
A total of 194 Campylobacter isolates were collected from slaughterhouses in nine provinces. Campylobacter were isolated using Bolton broth (Thermo Scientific, Basingstoke, U.K.) and Campylobacter blood-free selective agar (Thermo Scientific) and confirmed by polymerase chain reaction (PCR) [5].

Antimicrobial resistance: Minimum inhibitory concentrations (MICs) for Campylobacter were determined by the broth dilution method using commercially available Sensititre® panel Campy (TREK Diagnostic Systems, West Sussex, U.K.) according to the manufacturer’s instructions. Briefly, the antimicrobials, azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin and tetracycline, were tested. The interpretation of MICs was carried out according to the National Antimicrobial Resistance Monitoring System (NARMS, 2011) [17]. C. jejuni ATCC 33560 was used as a quality control strain.

Analysis of the molecular mechanisms of macrolide resistance: Domain V of the 23S rRNA [3], L4 protein [2] and L22 protein [3] were amplified by the PCR. Amplified PCR products were purified, and the products were then directly sequenced at Macrogen (Seoul, Korea). DNA sequences of resistant and susceptible strains were compared with the sequence of the C. coli JV20 genome (GeneBank accession number NZ_ AEER01000024).

Detection of virulence genes: The presence of 12 Campylobacter virulence genes, flaA, flhA, cadF, docA, cdtA, cdtB, cdtC, ciaB, iam, wlaN, virB11 [4] and ceuE [1], in 194 Campylobacter spp. was detected by PCR as previously described work [1, 4].

RESULTS

Table 1. Antimicrobial resistance among Campylobacter jejuni and Campylobacter coli isolates from chicken and swine feces and carcasses

| Antimicrobials | Chicken feces (n=73) | Chicken carcasses (n=30) | Pig feces (n=46) | Pig carcasses (n=12) | Chicken feces (n=38) | Chicken carcasses (n=25) |
|---------------|----------------------|--------------------------|------------------|----------------------|----------------------|--------------------------|
|               | MIC50 | MIC90 | R (%) | MIC50 | MIC90 | R (%) | MIC50 | MIC90 | R (%) | MIC50 | MIC90 | R (%) | MIC50 | MIC90 | R (%) |
| Azithromycin  | 0.03  | 0.06  | 0 (0) | 0.03  | 0.06  | 0 (0) | 0.03  | 0.06  | 0 (0) | 0.03  | 0.06  | 0 (0) | 0.03  | 0.06  | 0 (0) |
| Ciprofloxacin | 8     | 32    | 35 (81.4) | 16    | 64    | 29 (96.7) | 16    | 32    | 39 (84.8) | 8     | 16    | 8 (66.7) | 8     | 16    | 8 (66.7) |
| Clindamycin  | 0.06  | 0.25  | 4 (9.3) | 0.13  | 16    | 3 (10)  | 0.5   | 16    | 8 (17.4)  | 0.5   | 64    | 3 (25.0) | 0.13  | 4    | 0 (0)  |
| Erythromycin | 0.25  | 0.5   | 3 (7.0) | 0.25  | 8     | 2 (6.7)  | 0.25  | 64    | 11 (23.9) | 0.5   | 64    | 3 (25.0) | 0.25  | 4    | 0 (0)  |
| Florfenicol  | 0.5   | 1     | 4 (9.3) | 1     | 64    | 4 (13.3) | 1     | 2     | 2 (4.3)  | 0.5   | 1     | 0 (0)   | 0.5   | 1     | 0 (0)  |
| Gentamicin   | 0.25  | 0.5   | 4 (9.3) | 0.25  | 32    | 14 (43.3)| 0.5   | 32    | 3 (25.0) | 0.5   | 0.5   | 0 (0)   | 0.5   | 32    | 7 (28.0) |
| Nalidixic acid| 64    | 64    | 35 (81.4) | 64    | 64    | 29 (96.7) | 64    | 64    | 39 (84.8) | 64    | 64    | 8 (66.7) | 64    | 64    | 38 (100) |
| Telithromycin| 0.25  | 1     | 0 (0)   | 0.5   | 4     | 0 (0)   | 0.5   | 4     | 0 (0)   | 1     | 8     | 0 (0)   | 0.5   | 4     | 0 (0)  |
| Tetracycline | 64    | 64    | 34 (79.1) | 64    | 64    | 26 (86.7) | 2     | 64    | 33 (71.7) | 32    | 64    | 9 (75.0) | 2     | 64    | 16 (42.1) |

MIC50 and MIC90, the lowest concentration to inhibit 50% and 90% of the isolates tested, respectively; R, resistant isolates (%).
| Isolate | Sample       | MIC (µg/ml) | Nucleotide/amino acid substitution | Virulence factor |
|---------|--------------|-------------|------------------------------------|-----------------|
|         |              | EM | AZI | TEL | 23S rRNA | L4 | L22 |
| C. jejuni | V01-10-A03-008-016 chicken feces | ≥64 | 32 | ≥8 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cadB, cadF, racR, cdtA, cdtC |
|         | V01-10-A03-008-017 chicken feces | 64 | 64 | 4 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cadB, cadF, racR, cdtA, cdtC |
|         | V09-CJ-02 chicken carcass | 32 | 2 | 4 | - | V196A | - | fluA, cdtB, cadF, racR, cdtA, cdtC |
|         | V06-CJ-04 chicken carcass | ≥64 | 32 | 8 | - | V196A | - | fluA, cdtB, cdtA, cdhC |
| C. coli | V01-10-A03-027-027 chicken feces | ≥64 | 32 | 8 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cdhF, ceeE |
|         | V01-10-A03-027-029 chicken feces | 64 | 64 | 4 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cadF, ceeE |
|         | V01-10-A03-027-031 chicken feces | 32 | ≥64 | 4 | A2075G | V196A | Q24R, S109A | fluA |
|         | V01-10-A03-027-026 chicken feces | ≥64 | ≥64 | 4 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cadF, ceeE |
|         | V01-10-A03-027-025 chicken feces | ≥64 | ≥64 | 8 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cadF |
|         | V01-10-A03-027-028 chicken feces | ≥64 | ≥64 | 8 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cadF |
|         | V06-10-S03-027-009 chicken carcass | 32 | 4 | 4 | - | V196A | - | cdf |
|         | V06-10-S03-027-015 chicken carcass | ≥64 | ≥64 | 4 | A2075G | V196A | - | - |
|         | A02-008-017 pig feces | ≥64 | 32 | ≥8 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA, cadF, ceeE |
|         | A02-008-024 pig feces | ≥64 | 64 | 0.12 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA |
|         | A02-008-006 pig feces | ≥64 | ≥64 | 4 | A2075G | M192I, V196A | I65V, A74G, S109T, E111A, T114A | fluA, virB11 |
|         | A02-008-009 pig feces | ≥64 | ≥64 | 8 | A2075G | M192I, V196A | I65V, A74G, S109T, E111A, T114A | fluA |
|         | A02-008-010 pig feces | ≥64 | ≥64 | 8 | A2075G | V176I, T177S, V184I, M192I, V196A | I65V, A74G, A103V, S109T, E111A, T114A | fluA, cadF |
|         | A02-008-018 pig feces | ≥64 | ≥64 | 8 | A2075G | V196A | Q24R, S109A | - |
|         | V14-10-S02-028-001 pig feces | ≥64 | ≥64 | ≥8 | A2075G | V196A | I65V, A74G, A103V, S109T, E111A, T114A | fluA, cadF |
|         | V01-10-A02-027-018 pig feces | ≥64 | ≥64 | ≥8 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | ceeE |
|         | V11-CC-01 pig carcass | ≥64 | 32 | 8 | A2075G | V196A | - | fluA |
|         | V02-CC-02 pig carcass | 64 | 64 | 8 | A2075G | V196A | - | fluA, cdtA, cdhC |
|         | V11-CC-03 pig carcass | ≥64 | ≥64 | 8 | A2075G | V196A | - | fluA |
|         | A02-008-013 pig feces | 32 | 64 | 8 | A2075G | V196A | I65V, A74G, S109T, E111A, T114A | fluA |

a) Abbreviations: EM, erythromycin; AZI, azithromycin, TEL, telithromycin. b) Position according to *Escherichia coli* numbering. c) Position of amino acids changes. DNA sequences of *rplD* and *rplV* genes coding L4 and L22 ribosomal proteins, respectively, were compared with the sequence in the *C. coli* JV20 genome.
resistant isolates (MIC 32 µg/ml). A comparison of the amino acid sequences of the ribosomal proteins L4 and L22 in the C. jejuni and C. coli strains with type strains revealed several different amino acid substitutions and a combination of such substitutions. Four amino acid substitutions in the L4 ribosomal protein and two in the L22 ribosomal protein were observed in C. jejuni with intermediate-level resistance to erythromycin (MIC 32 µg/ml): T91K (n=1), V176I (n=1), T177S (n=1) and V196A (n=4) in L4, and A73T (n=1) and S109A (n=1) in L22. In erythromycin-resistant C. coli (MIC ≥32 µg/ml), eight amino acid substitutions in the L4 ribosomal protein and seven in the L22 ribosomal protein were observed: V196A (n=23), M192I (n=8), V121A (n=5), P28S (n=2), V176 (n=2), T177 (n=2), V184 (n=2) and A140T (n=2) in L4, and I65V (n=18), A74G (n=18), S109T (n=18), E111A (n=18), T114A (n=18), Q24R (n=2) and S109A (n=2) in L22.

**Prevalence of virulence factors:** The erythromycin-resistant Campylobacter isolates were analyzed for the presence of 12 virulence markers that are associated with human invasion and infection. Distinguishing features separating C. jejuni and C. coli were observed. C. jejuni isolates possessed more virulence genes than did C. coli; all C. jejuni isolates carried four to six virulence genes, whereas C. coli isolates had zero to three such genes. Almost all C. jejuni and C. coli isolates possessed the flaA gene; however, three gene subunits, cdtA, cdtB, and cdtC, were found in 100% of C. jejuni isolates, but in none of the C. coli isolates. Furthermore, the cadF gene was more prevalent in samples from chickens [C. jejuni 80% (4/5) and C. coli 77.8% (7/9)] than in samples from pigs [C. coli 28.6% (4/14)], irrespective of the bacterial species.

**DISCUSSION**

In the present study, Campylobacter isolates from animals and carcasses that were tested against nine antimicrobials were most commonly resistant to ciprofloxacin and nalidixic acid (81.4–96.7% for C. jejuni and 66.7–100% for C. coli). We noted higher resistance to (fluoro) quinolones in C. jejuni isolates of pig origin than in samples from pigs C. coli was more prevalent in samples from chickens C. jejuni in none of the isolates. Furthermore, the C. coli gene cadF was found in 100% of C. jejuni isolates, but in none of the C. coli isolates. In the L22 ribosomal protein, we noted two amino acid substitutions at positions, at 28, 91, 140, 192, 176, 177, 184 and 181, respectively; Hungary 52.6% and 86.1%; Switzerland 41.1% and 40.7%; France 46.3% and 56.9%; and the Netherlands 10.9% and 67.3% [7]. Although most of the C. jejuni isolates were susceptible to erythromycin, C. coli isolates from pigs (23.9%) and pig carcasses (25.0%) showed a relatively high rate of resistance. This finding agreed with the results of other studies [7, 22]. Generally, resistance to macrolides was more prevalent in C. coli isolates of pig origin than in C. coli from chickens or C. jejuni from pigs or chickens [7, 22]. In Korea, fluoroquinolones (enrofloxacin) and macrolides (tylosin) are routinely given to chickens and pigs to prevent and treat enteric and respiratory diseases, respectively [12]. This practice, which is also followed by other countries, may favor the selection of resistant bacteria [6, 7].

In the present study, mutations in highly resistant strains were identified at position 2,075 in the 23S rRNA gene in Campylobacter spp. The primary mechanism of macrolide resistance was due to a single point mutation in the 23S rRNA gene, as previously reported by researches in Poland [2, 3, 18] and Korea [19]. Mutations in five C. jejuni and one C. coli showing intermediate-level resistance (MIC 32 µg/ml) were not identified at position 2,075 in the 23S rRNA gene. Thus, a further study on low-level resistance mechanisms, such as the CmeABC efflux pump and mutation of other ribosomal proteins, is required.

The 50S ribosomal subunit proteins L4 and L22, encoded for by the rplD and rplV genes, respectively, were characterized in erythromycin-resistant isolates [2, 18]. Amino acids spanning positions 63–74 are reported to be the most important target regions of the L4 protein [3]. Mutations within this region confer high-level macrolide resistance in various bacterial species [3]. In the present study, four and eight amino acid substitutions in the L4 ribosomal protein were identified in C. jejuni and C. coli, respectively. The mutations at positions, 196 and 121, were reported by previous studies [3, 18]; however, the present study is the first to report mutations at positions, at 28, 91, 140, 192, 176, 177, 184 and 841. In the L22 ribosomal protein, we noted two amino acid substitutions in C. jejuni and seven in C. coli, respectively. Although in the present study, erythromycin-resistant strains were not included for mutations, such amino acid substitutions were reported in both susceptible and resistant isolates in other studies [3, 11, 18]. Thus, these substitutions may have little direct involvement in erythromycin resistance in Campylobacter spp. So far, the significance of the amino acid substitutions in the ribosomal proteins L4 and L22 remains unknown and warrants further evaluation.

The presence of virulence factor genes in erythromycin-resistant Campylobacter isolates varied by bacteria species and source. The C. jejuni isolates carried more virulence genes than did the C. coli isolates that we tested. Although further studies on the relationship between virulence genes in bacteria and pathogenicity in the host are needed, our results may explain why C. jejuni is a more common cause of human infections (90–95%) than C. coli (5–10%) [20]. The most common virulence gene in both C. jejuni and C. coli was a flagellin-coding flaA gene. Motility expression via the flagella is essential for cell adhesion and invasion to achieve infection [10, 14, 20].

The second most common virulence gene in this study was cadF, which is responsible for adhesin, and the fibronectin-binding protein involved in invasion, influencing microfilament organization in host cells [1, 20]. Furthermore, this gene had a high prevalence in Campylobacter isolates in human cases and chicken carcasses [15]. In the present study, this gene in C. coli was more prevalent in isolates from chickens than those from pigs. Although the presence or absence of key genes in Campylobacter spp. cannot be used to predict the virulence of strains [4], further studies on virulence genes in C. coli from different origins are needed in order to develop effective intervention strategies to prevent transmission of resistant strains via the food chain.

We discovered a high rate of antimicrobial resistance in both C. jejuni and C. coli, with a mutation in the 23S rRNA gene mainly responsible for erythromycin resistance in Campylobacter isolates and more virulence genes in C.
jejuni than in C. coli. The effect of the amino acid substitutions in the L4 and L22 proteins on macrolide resistance and the relationship between the presence of virulence genes and pathogenicity require further evaluations. To prevent the transmission to humans of resistant and virulent Campylobacter spp. via the food chain, we urge more prudent use of critically important antimicrobials, such as fluoroquinolones and macrolides, in swine and poultry production, as well as constant monitoring of resistance among Campylobacter isolates in animals and animal products.

ACKNOWLEDGMENT. This work was supported by a grant from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural Affairs, Republic of Korea.

REFERENCES

1. Bang, D. D., Nielsen, E. M., Scheutz, F., Pedersen, K., Handberg, K. and Madsen, M. 2003. PCR detection of seven virulence and toxin genes of Campylobacter jejuni and Campylobacter coli isolates from Danish pigs and cattle and cytotoxophil distending toxin production of the isolates. J. Appl. Microbiol. 94: 1003–1014. [Medline] [CrossRef]

2. Caglierio, C., Mouline, C., Clochekeert, A. and Payot, S. 2006. Synergy between efflux pump CmeABC and modifications in ribosomal proteins L4 and L22 in conferring macrolide resistance in Campylobacter jejuni and Campylobacter coli. Antimicrob. Agents Chemother. 50: 3893–3896. [Medline] [CrossRef]

3. Corcoran, D., Quinn, T., Cotter, L. and Fanning, S. 2006. An investigation of the molecular mechanisms contributing to high-level erythromycin resistance in Campylobacter. Int. J. Antimicrob. Agents 27: 40–45. [Medline] [CrossRef]

4. Datta, S., Niwa, H. and Itoh, K. 2003. Prevalence of 11 pathogenic genes of Campylobacter jejuni by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. J. Med. Microbiol. 52: 345–348. [Medline] [CrossRef]

5. Denis, M., Soumet, C., Rivoal, K., Ermel, G., Blivet, D., Salvat, G. and Collin, P. 1999. Development of a m-PCR assay for simultaneous identification of Campylobacter jejuni and C. coli. Lett. Appl. Microbiol. 29: 406–410. [Medline] [CrossRef]

6. European Food Safety Authority 2014. Campylobacter. Available at: http://www.efsa.europa.eu/en/topics/topic/campylobacter.htm (accessed on 5 August 2014).

7. European Food Safety Authority and European Centre for Disease Prevention and Control 2015. EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. EFSA Journal 2015 13: 4036. 178 pp.

8. Kim, J. M., Hong, J., Bae, W., Koo, H. C., Kim, S. H. and Park, Y. H. 2010. Prevalence, antibiograms, and transferable tet(O) plasmid of Campylobacter jejuni and Campylobacter coli isolated from raw chicken, pork, and human clinical cases in Korea. J. Food Prot. 73: 1430–1437. [Medline]

9. Kim, H. J., Kim, J. H., Kim, Y. I., Choi, J. S., Park, M. Y., Nam, H. M., Jung, S. C., Kwon, J. W., Lee, C. H., Kim, Y. H., Ku, B. K. and Lee, Y. J. 2010. Prevalence and characterization of Campylobacter spp. isolated from domestic and imported poultry meat in Korea, 2004–2008. Foodborne Pathog. Dis. 7: 1203–1209. [Medline] [CrossRef]

10. Konkel, M. E., Monteville, M. R., Rivera-Amill, V. and Joens, L. A. 2001. The pathogenesis of Campylobacter jejuni-mediated enteritis. Curr. Issues Intest. Microbiol. 2: 55–71. [Medline]

11. Lehtopolku, M., Kotilainen, P., Haanperä-Hiikkinen, M., Nakari, U. M., Hänninen, M. L., Huovinen, P., Siitonen, A., Eerola, E., Jalava, J. and Hakanen, A. J. 2011. Ribosomal mutations as the main cause of macrolide resistance in Campylobacter jejuni and Campylobacter coli. Antimicrob. Agents Chemother. 55: 5939–5941. [Medline] [CrossRef]

12. Lim, S. K., Lee, J. E., Lee, H. S., Nam, H. M., Moon, D. C., Jang, G. C., Park, Y. J., Jung, Y. G., Jung, S. C. and Wee, S. H. 2014. Trends in antimicrobial sales for livestock and fisheries in Korea during 2003–2012. Korean J. Vet. Res. 54: 81–86. [CrossRef]

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

14. Malik-Kale, P., Raphael, B. H., Parker, C. T., Joens, L. A., Klena, J. D., Quiñones, B., Keesch, A. M. and Konkel, M. E. 2007. Characterization of genetically matched isolates of Campylobacter jejuni reveals that mutations in genes involved in flagellar biosynthesis alter the organism’s virulence potential. Appl. Environ. Microbiol. 73: 3123–3136. [Medline] [CrossRef]

15. Melo, R. T., nalevai, P. C., Mendonca, E. P., Borges, L. W., Fonseca, B. B., Beletti, M. E. and Rossi, D. A. 2013. Campylobacter jejuni strains isolated from chicken meat harbour several virulence factors and represent a potential risk to humans. Food Contr. 33: 227–231. [CrossRef]

16. Ministry of Food and Drug Safety 2015. Foodborne pathogens statistics. Available at: http://www.foodsafetykorea.go.kr/portal/healthyfoodlife/foodPoisoningStat.do (accessed on 30 December 2015) (In Korean).

17. National Antimicrobial Resistance Monitoring System Reports and Data. 2011. Available at: http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm (accessed on 2 February 2015).

18. Rożynek, E., Mačkiv, E., Kamińska, W., Tomczuk, K., Antos-Bielska, M., Dzierżanowska-Fangrat, K. and Korsak, D. 2013. Emergence of macrolide-resistant Campylobacter strains in chicken meat in Poland and the resistance mechanisms involved. Foodborne Pathog. Dis. 10: 655–660. [Medline] [CrossRef]

19. Shin, E. and Lee, Y. 2010. Characterization of erythromycin-resistant porcine isolates of Campylobacter coli. Microb. Drug Resist. 16: 231–239. [Medline] [CrossRef]

20. Thakur, S., Zhao, S., McDermott, P. F., Harbottle, H., Abbott, J., English, L., Gebreyes, W. A. and White, D. G. 2010. Antimicrobial resistance, virulence, and genotypic profile comparison of Campylobacter jejuni and Campylobacter coli isolated from humans and retail meats. Foodborne Pathog. Dis. 7: 835–844. [Medline] [CrossRef]

21. World Health Organization 2011. Critically Important Antimicrobials for Human Medicine 3rd Revision. Available at: http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf (accessed on 2 February 2015).

22. Zhao, S., Young, S. R., Tong, E., Abbott, J. W., Womack, N., Friedman, S. L. and McDermott, P. F. 2010. Antimicrobial resistance of Campylobacter isolates from retail meat in the United States between 2002 and 2007. Appl. Environ. Microbiol. 76: 7949–7956. [Medline] [CrossRef]