

## ABSTRACT

**Background:** Thermophilic *Campylobacter* species are among the major etiologies of bacterial enteritis globally. This study aimed at assessing the antimicrobial resistance (AMR) profiles, virulence genes, and genetic diversity of thermophilic *Campylobacter* species isolated from a layer poultry farm in South Korea. One hundred fifty-three chicken feces were collected from two layer poultry farms in Gangneung, South Korea. The *Campylobacter* species were isolated by cultural techniques, while PCR and sequencing were used for species confirmation. Antimicrobial susceptibility testing for six antimicrobials [ciprofloxacin (CIP), nalidixic acid (NAL), sitafloxacin (SIT), erythromycin (ERY), tetracycline (TET), and gentamicin (GEN)] was carried out by broth microdilution. Three AMR and nine virulence genes were screened by PCR. Genotyping was performed by *flaA*-restriction fragment length polymorphism (RFLP) and multilocus sequence typing (MLST). Of the 153 samples, *Campylobacter* spp. were detected in 55 (35.9%), with *Campylobacter jejuni* and *Campylobacter coli* being 49 (89.1%) and six (10.9%), respectively. High-level resistance was observed for CIP (100%), NAL (100%), and TET (*C. jejuni*, 93.9%; *C. coli*: 83.3%). No resistance was observed for SIT. The missense mutation (C257T) in *gyrA* gene was confirmed by sequencing, while the *tet(O)* gene was similar to known sequences in GenBank. The rate of multidrug-resistant (MDR) strains was 8.2%, and they all belonged to *C. jejuni*. All *Campylobacter* isolates possessed five virulence genes (*cdtB*, *cstII*, *flaA*, *cadF*, and *dnaJ*), but none possessed *ggt*, while the rates for other genes (*csrA*, *ciaB*, and *pldA*) ranged between 33.3 and 95.9%. The *flaA*-RFLP yielded 26 *flaA* types (*C. jejuni*: 21 and *C. coli*: five), while the MLST showed 10 sequence types (STs) for *C. jejuni* and three STs for *C. coli*, with CC-607 (STs 3611) and CC-460 (ST-460) being predominant. Among the 10 STs of *C. jejuni*, three were newly assigned. The findings of this study highlight the increased resistance to quinolones and TET, the virulence potential, and the diverse genotypes among *Campylobacter* strains isolated from the layer poultry farm. Results: Out of 153 fecal samples, the detection rate of *Campylobacter* spp. was 35.9% (55), with *C. jejuni* and *C. coli* being 89.1% (49) and 10.9% (six), respectively. None of the 20 fecal samples from the second farm was positive for *Campylobacter*. Conclusion: This study highlights the role of layers as a reservoir of *Campylobacter* spp., harboring various AMR and virulence genes. The genotyping highlighted that *C. jejuni* isolates were more diverse than *C. coli* as analyzed by MLST. The MLST revealed that CC-607 (ST-3611) and CC-460 (ST-460) were the predominant ones, while three STs were newly assigned. ERY, GEN, and SIT need to be appropriately used to prevent or delay the increasing resistance in *Campylobacter* species. The isolates of this study may present a potential hazard to public health based on their AMR profiles, virulence genes, and genotyping data.

**Keywords:** *Campylobacter*, quinolones, antimicrobial resistance, *flaA* RFLP, multilocus sequence typing, poultry, Korea