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The prevalence of major foodborne pathogens in ready-to-eat chicken meat samples sold in retail markets in Turkey and the molecular characterization of the recovered isolates

Seçil Abay [a, *], Reyhan Irkin [b], Fuat Aydin [a], Hamit Kaan Müştak [c], Kadir Serdar Diker [c]

[a] Erciyes University, Faculty of Veterinary Medicine, Department of Microbiology, Kayseri, Turkey
[b] Balikesir University, Engineering and Architecture Faculty, Food Engineering Department, Balikesir, Turkey
[c] Ankara University, Faculty of Veterinary Medicine, Department of Microbiology, Ankara, Turkey

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ABSTRACT

The aims of the present study were to evaluate the prevalence of *Arcobacter* spp., *Campylobacter* spp., *Listeria* spp., and *Salmonella* spp. in heat-processed ready-to-eat (RTE) chicken products manufactured by various companies using bacterial culture methods and to perform virulence gene analysis, serotyping, genotyping, and antibacterial susceptibility tests on the isolated strains. For this purpose, 50 packages of chicken convenience products were used as the study material. Phenotypic tests and a molecular method (Polymerase Chain Reaction, PCR) were used to identify the isolated bacteria. All samples examined were negative for *Arcobacter* spp., *Campylobacter* spp., and *Salmonella* species. *Listeria* species were isolated from 12 (24%) of the examined samples. Among the *Listeria* species isolated, 9 were identified as *L. monocytogenes*, 2 were identified as *L. innocua*, and one was identified as *L. welshimeri*. All isolates were susceptible to the antibiotics tested. A detailed molecular analysis of the *Listeria* spp. revealed that the examined food products posed a significant public health hazard. Considering the presence of different genotypes of *L. monocytogenes* in RTE food production facilities, all the steps of food production must be reviewed in terms of conformity with sanitation and hygiene rules, and necessary measures must be set in place.

1. Introduction

Campylobacter spp., *Listeria* spp. and *Salmonella* spp. are the most prevalent and serious foodborne pathogens (Mor-Mur & Yuste, 2010). Additionally, some infections originating from *Arcobacter* spp. in water and food (chicken, turkey, beef, sheep and pork) have been reported (Ho, Lipman, & Gaastra, 2006; Kayman et al., 2012; Patyal, Rathore, Mohan, Dhama, & Kumar, 2011). These bacteria are frequently found as commensals in the intestinal tracts of numerous animals, including poultry.

Arcobacter spp. and *Campylobacter* spp. are closely related genera in the family (Vandamme, 2000). They are Gram-negative, spiral-shaped, motile organisms and cause a variety of diseases in humans and animals. Chicken meats contaminated with *Campylobacter* spp. constitute the largest potential source of human infections by far (Butzler, 2004; Collado & Figueras, 2011).

Foodborne listeriosis in humans is rare but severe. *Listeria* species are found in soil, water, effluents, a large variety of foods, and humans and animal feces (Barbuddhe & Chakraborty, 2009). The genus *Listeria* currently includes 17 recognized species (*L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. marthii*, *L. innocua*, *L. grayi*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia* and *L. booriae*) (Orsi & Wiedmann, 2016). These bacteria are Gram-positive, facultative anaerobes that are motile at 10–25 °C, non-spore forming and are able to multiply even at high salt concentrations and in acidic conditions (McLauchlin, Catherine, & Christine, 2014). *Listeria* spp. can be endemic in food processing environments. Unlike most bacteria, *Listeria* can grow and multiply in some foods in the refrigerator. Therefore, their presence may be indicative of poor hygiene or cross-contamination, which is a possible source of *Listeria* contamination in processed meat (Nyenje, Odjadjare, Tanih, Green, & Ndip, 2012).

* Corresponding author.
E-mail addresses: ranaabay@hotmail.com, sabay@erciyes.edu.tr (S. Abay).

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More than 2500 *Salmonella* serovars are considered potential pathogens in animals and humans. *S. enterica* has 6 subspecies (*S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *Indica*). The principle sources of these microorganisms include poultry, eggs, raw meat and raw milk (Anonym, 2005).

Ready-to-eat (RTE) foods are consumed without further treatment, such as cooking, that would eliminate or reduce the microbial load. A variety of RTE foods, such as cooked meat and poultry, are commonly consumed; recently, the consumption of this type of food has rapidly increased due to their good taste and simple preparation. Therefore, the safety of these products has been a source of concern to consumers, especially in relation to their microbiological contamination (Yang et al., 2016). A variety of RTE foods originate from animals, including meat, poultry, seafood and dairy products. The microbiological risks to the consumer from these products have also increased (Hwang & Huang, 2010). Improperly heated-up and/or stored RTE-type meat products (especially poultry products) can be a cause of food poisoning by bacteria such as *Salmonella enteritidis*, *Listeria monocytogenes* and *Campylobacter jejuni* (Pietrzak, Cegielka, Fonberg-Broczek, & Ziarno, 2011). Ready-to-eat foods are important vehicle in the transport of *Listeria* spp. to humans. One study that investigated 384 food samples in Ethiopia reported a 25% prevalence of *L. monocytogenes,* among which some isolates were multi-drug resistant (penicillin, nalidixic acid, tetracycline and chloramphenicol), indicating the need for the application of hygienic practices in the food processing industries (Dhama et al., 2015). Ready-to-eat cooked chicken products can easily be contaminated with *L. monocytogenes* in the post-processing steps. The consumption of contaminated RTE cooked chicken foods results in severe health problems, including listeriosis, with a high death rate (Goh et al., 2014). Similarly, humans can be subjected to *Campylobacter* when consuming improperly processed poultry products. However, the most important route of food-borne disease is related to the consumption of foods that are cross-contaminated with *Campylobacter* during food preparation of a meal with poultry products (Signorini et al., 2013).

The common contamination of poultry products with *Arcobacter* has frequently been reported (Smet, Zutter, Hende, & Houf, 2010). Generally, *Arcobacter* spp. are found in foods of animal origin, such as chicken, pork, beef, lamb, and raw milk. The highest prevalence was shown for poultry, followed by pork and beef meat (Girbau, Guerra, Martinez-Malaxetxebarria, Alonso, & Astorga, 2015).

Although several studies showed that *Salmonella* spp. prevailed in RTE poultry products (Karadal, Ertas, Hizlisoy, Abay, & Al, 2013), there are no prevalent studies concerning *Arcobacter* spp., *Campylobacter* spp., and *Listeria* spp. contaminations in our country. Our aims were *i*: to determine the prevalence of *Arcobacter* spp., *Campylobacter* spp. and *Listeria* spp. in commercial RTE poultry products using culture methods, *ii*: to perform virulence gene analysis, serotyping, and genotyping and antibacterial susceptibility tests on the isolated strains.

2. Materials and methods

2.1. Ready-to-eat poultry meat samples

Various processed chicken meat samples were purchased from four local retail markets and poultry shops in the cities of Kayseri and Balıkesir, Turkey. A total of 50 samples, including meatballs (n = 10), nuggets (n = 8), sausages (n = 8), burgers (n = 8), Doner kebabs (n = 8) and kebabs (n = 8) (see Table 1 for details), were examined. The samples were kept cool and examined within 1 h of purchase.

2.2. Isolation and identification of Arcobacter spp.

Arcobacter spp. isolation was performed according to the method of Aydin, Gümüşsoy, Atabay, Içа, and Abay (2007).

2.3. Isolation and identification of Campylobacter spp.

For the isolation of *Campylobacter* spp. from the samples, Aydin et al. (2007)'s method was used; however, in the enrichment step, the samples were incubated at 37 °C. Isolates identified with the phenotypic tests as *Campylobacter* spp. were confirmed using molecular analysis (mPCR) (Wang et al., 2002).

2.4. Isolation and identification of Listeria spp.

Isolation and phenotypic identification of *Listeria* spp. from samples were performed according to the method of Abay, Aydin, and Sumerkan (2012). Isolates were confirmed at the species level by molecular methods, including *hly* gene-specific PCR (Pourjafar et al., 2010) and 16S rRNA sequence analysis (Lane, 1991).

2.5. Isolation and identification of Salmonella spp.

The procedure was performed according to ISO 6579–2002. Positive colonies (2–3) were confirmed by the API 20E kit (Biomerieux, France).

2.6. DNA extraction

DNA was extracted from each isolate using the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, 12224–250) following the manufacturer's instructions.

2.7. PCR amplification of the 16S rDNA gene

The universal primers 27F AGAGTTTGATCMTGGCTCAG and 1492R GGTTACCTTGTTACGACTT were used to amplify the 16S rDNA gene (Lane, 1991). Amplified products were resolved by 1.5% agarose (Prona) gel electrophoresis and visualized under a UV transilluminator (G:BOX Chemi XRQ, Syngene).

2.8. Sequencing and phylogenetic analysis

The amplified PCR products were purified using the QIAquick PCR Purification Kit (Qiagen), and the Big Dye Direct Cycle Sequencing Kit (Applied Biosystems) was used in the sequence analysis; both kits were used according to the manufacturer's instructions. After cycle sequencing, the amplicons were purified with Sephadex G-50 (Sigma-Aldrich) and sequenced on the Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems). All sequences were analyzed with the CLC Main Workbench 6 and compared with reference sequences in the National Center for Biotechnology Information website with BLASTN.

The evolutionary history was inferred using the neighbor-joining method (Saitou & Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was determined (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor, 1969) and were in the units of the number of base substitutions per site. The analysis involved 15 nucleotide sequences, including the 3 reference strains