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RESEARCH ARTICLE

Pathogenic *Escherichia coli* and *Salmonella* spp. in Chicken Carcass Rinses: Isolation and Genotyping by ERIC-PCR

Arife Ezgi Telli[1], Yusuf Biçer[1*], Nihat Telli[2], Candan Güngör[3], Gamze Turkal[1] and Nurhan Ertaş Onmaz[3]

[1]Selcuk University Faculty of Veterinary Medicine Department of Food Hygiene and Technology, 42130, Konya, Turkey
[2]Konya Technical University Vocational School of Technical Sciences Department of Food Processing, 42250, Konya, Turkey; [3]Erciyes University Faculty of Veterinary Medicine Department of Food Hygiene and Technology, 38280, Kayseri, Turkey
*Corresponding author: yusufbicer@selcuk.edu.tr

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A B S T R A C T

The present study aimed to determine the pathogenic *Escherichia coli* and *Salmonella* spp. and to investigate their phylogenetic relation by Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) in retail chicken samples. A total of 75 samples were processed for isolation of *E. coli* and *Salmonella* spp. by classical cultural methods and isolates were confirmed by the species-specific PCR. *Salmonella* spp. was detected in 21.3% and *E. coli* was detected in 74.6% of the chicken carcasses. *S.* Enteritidis and *S.* Typhimurium were not detected in chickens by duplex PCR-based assay. O157 based on serotyping and PCR, was not detected in any of the isolates. Besides, virulence and toxin genes were not detected in any of the *E. coli* isolates. According to ERIC patterns, the obtained ribotypes showed that all *Salmonella* spp. isolates presented large genetic diversity, whereas only two (3.5%) of *E. coli* isolates were genetically identical. Although virulent *E. coli*, and pathogenic serotypes of *Salmonella* spp. were not detected in our study, it is thought that their high incidence should be considered as an indicator of failure to comply with hygienic conditions and lack of sanitary practices especially in slaughterhouses.

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INTRODUCTION

Poultry meat is one of the most consumed and produced animal protein sources worldwide and is one of the substitutes for beef due to its low cost, great source of protein and low fat content (Katiyo *et al.,* 2020). In addition, it can pose a potential risk in terms of many foodborne bacterial pathogens during process, storage and consumption (Katiyo *et al.,* 2020). *Salmonella* spp. and *Escherichia coli* are recognized as the most common causes of foodborne illness in poultry and other foods of animal origin. (Rouger *et al.,* 2017; Koutsoumanis *et al.,* 2019). Worldwide, consumption of contaminated food is thought to cause approximately two million deaths per year. Non-typhoidal *Salmonella* spp. is the main cause of foodborne illness and in most cases, it is related to the consumption of animal products such as eggs, beef and poultry meat. (Le Hello, 2014; Kuang *et al.,* 2015). Antimicrobial resistance in bacteria, including *Salmonella* spp. is considered a major food safety concern and the spread of resistant strains along the food chain is to be handled for both veterinary medicine and public health (Little *et al.,* 2008; Maka *et al.,* 2015).

Although most *E. coli* strains are not pathogenic for human health, some are known to cause serious health problems. The widespread use of antibiotics in livestock, especially poultry, is the main cause of the spread of antibiotic-resistant bacteria (Mellata, 2013). Healthy poultry is considered the main reservoir of virulence genes and antibiotic resistance (Messaili *et al.,* 2019). Enterohemolysin (*hly*A), Shiga toxins (*stx*1 and *stx*2) and intimin (*eae*A) are the virulence factors in the pathogenesis of the diseases. In addition to inter-animal transmission, humans can be infected with antibiotic resistant bacteria through the contaminated foods (Graham *et al.,* 2008; Vincent *et al.,* 2010). For better investigation of the prevalence and to respond in food safety issues rapidly and effectively, it is crucial to determine the relationship between isolates using genotyping methods (Bakhshi *et al.,* 2018). Phenotypic

tests are time-consuming, costly, incapable of distinguishing strains in case high level of genetic similarity, and the results are inconvenient to interpret. PCR-based methods have been preferred to identify different DNA fingerprints because they are reliable, fast, sensitive and reproducible (Behzadi *et al.,* 2016). Among these methods, Enterobacterial recurrent intergenic consensus (ERIC)-PCR, is one the most commonly used depending on the target sequence. ERIC sequences are defined as extragenic consensus sequences within a genome which are located at different loci (Cristiani *et al.,* 2020). These sequences have been detected in the genome of *Enterobacteriaceae* members such as *E. coli* and, *Salmonella* spp. (Xu *et al.,* 2020). Repetitive sequences between 122-127 bp which are found in the *Enterobacteriaceae* family. These sequences and their differences in frequency of repetition are used as a molecular marker for genetic screening. Due to variation in the length and location of these repeats between different strains of the same species, one strain-specific fingerprint patterns are obtained on agarose gel (Sedighi *et al.,* 2020).

The aim of this study is to determine the presence of *Salmonella* spp. and *E. coli* in retailed whole chicken carcasses to investigate the virulence and toxin genes, and to genotype the isolates using ERIC-PCR method.

MATERIALS AND METHODS

Sample Collection: Whole chicken samples (n = 75) were collected from randomly selected butcher shops and supermarkets of Konya-Turkey between March to June in 2019. All the samples were brought to the laboratory under cold chain and analyzed within 2 hours.

Microbiological Analyzes: Whole chicken rinses were prepared according to the carcass rinse method recommended by ISO 6887-2 with slight modification. Accordingly, the collected chicken carcasses were placed in the stomacher bags and rinsed manually in 400ml Buffered Peptone Water (BPW) for 1 min and incubated at 35±2 ºC for 20 h to 24 h.

Salmonella spp. isolation was carried out according to the ISO 6579:2002 method with slight modification. Briefly, 0.1µL of pre-enriched culture was transferred into modified semisolid Rappaport Vassiliadis (MSRV) and incubated at 42±0.5ºC for 24 h. A loopful of selective enriched broth culture was streaked onto Xylose Lactose Tergitol 4 (XLT4, Merck 113919) agar supplemented with XLT4 selective supplement (Merck, 108981) and incubated at 37ºC for 24 h. Afterwards, up to five presumptive colonies were plated on Tyriptic Soy Agar (TSA) for further characterization using biochemical, PCR and ERIC-PCR analysis. Biochemical identification was performed using API20E (Biomeriux, France).

Determination of *E. coli* was performed according to the horizontal method of ISO 16649-3:2015 with slight modification. Accordingly, the pre-enriched chicken rinse samples in BPW were streaked onto Tryptone Bile X-glucuronide (TBX) agar and incubated at 44±1ºC for 22-24 h. Following the incubation, typically blue or greenish colonies were selected and subcultured onto TSA for further biochemical and molecular confirmation.

Confirmed *Salmonella* spp. and *E. coli* isolates were then stored in Brucella Broth (Liofilchem, 101917513) supplemented with 15% glycerol at -20ºC for further analysis.

Reference strains: *E. coli* O157 NCTC 12900, *S.* Enteritidis ATCC 13076 and *S.* Typhimurium ATCC 14028 were used as the positive control strains for isolation, identification and detection of virulence factor genes.

Molecular Analyses of the Isolates

DNA Isolation: Genomic DNA extraction was performed according to the method described by Pitcher *et al.* (1989). The DNA concentration and purity were measured using a nanodrop spectrophotometer (Titertek Berthold, Germany).

PCR analyses of isolates: *Salmonella* spp. and *E. coli* isolates were confirmed by *Inv*A and *pho*-A gene (Table 1) based classical PCR, respectively. The PCR master mix was prepared in 25µL volume containing 5X reaction buffer (My Taq, Bioline, including $MgSO_4$, dNTPs), 1 U Taq DNA polymerase and 0.5µL of each forward and reverse primers, nuclease free water and 5µL of template DNA. Thermal cycler conditions of *Salmonella* spp. and *E. coli* isolates were adjusted according to Paião *et al.* (2013) and Kong *et al.* (1999), respectively.

Detection of *flic*-C and *IE-1* Genes in *Salmonella* spp. Isolates: For duplex PCR analysis, primer pairs used were, *flic*-C, specific for *S.* Typhimurium described by Paião *et al.* (2013) and *IE*-1, specific for *S.* Enteritidis designed by Wang and Yeh (2002), amplifying fragments of 432 bp and 316 bp. PCR mix was prepared according to the above mentioned concentrations.

Detection of virulence genes in *E. coli* isolates: Detection of the *stx*1, *stx*2, *eae*A and *hly*A genes in *E. coli* isolates were performed using mPCR according to a study of Fratamico *et al.* (2000). Besides, the primer pairs for determination of *rbfO*157, *fliCh*7, *rfbO*111 and *wzx-wzy*O26 genes and the thermal cycler conditions were carried out according to the studies conducted by Maurer *et al.* (1999), Sarimehmetoglu *et al.* (2009), Paton and Paton (1998) and Durso *et al.* (2005), respectively. Agar gel electrophoresis of the amplified PCR products was performed in agarose gel (1%) containing ethidium bromide (5µg/ml) and visualized with a UV transilluminator.

ERIC-PCR: The ERIC-PCR patterns were obtained according to the method described by Houf *et al.* (2002). Briefly, the PCR mixture, consisted of 1xPCR buffer, 200 µM of dNTP mix, 4 mM of $MgCl_2$, 25 pmol of forward and reverse primers, 5 U Taq polymerase and 1 µL of template DNA and adjusted with nuclease free ddH_2O in a final volume of 50 µL. The ERIC-PCR conditions were as the following: 94°C for 5 min; 40 cycles of 94°C for 1 min, 25°C for 1 min, and 72°C for 2 min. The PCR products (5 µL) were subjected to electrophoresis on 2% gel in 1xTAE buffer at 120 V for 40 min. Then, the DNA

patterns were visualized by Gel Documentation System. The DNA profile was scored manually by assigning a value of 1 for band presence and a value of 0 for band absence on the gel photographs for prediction of similarity.

Based on electrophoresis band spectra, the similarity matrix was calculated using the Jaccard coefficient with an online program (DendroUPGMA; http://genomes.urv.es/UPGMA/). The visualization of the dendrogram was carried out from the obtained Newick format using the online iTOL (version 5) program (Letunic and Bork, 2019). The primer pairs used for PCR assay and amplified fragment sizes are shown in Table 1.

RESULTS

Salmonella spp. was detected in 16 (21.3%) and *E. coli* was detected in 56 (74.6%) of the chicken carcasses according to the conventional culture methods and confirmed using species specific PCR reaction. *S.* Enteritidis and *S.* Typhimurium were not detected in any of the chicken samples based on the IE-1 and *flic*-C genes based PCR reaction.

None of the isolates were positive for *E. coli* O157 based on serotyping and PCR. Besides, *E. coli* isolates were detected not to carry any of the virulence genes analyzed. Agarose gel electrophoresis of amplified products of *E. coli* and *Salmonella* spp. are shown in Fig. 1.

ERIC-PCR of 56 *E. coli* isolates from raw chicken samples produced 1-7 DNA fragments of different sizes (ranging in size between 140 and 2800 bp). ERIC-genotypic profiles of isolates were grouped into six major clusters (A-F) and subcluster from these clusters, except the A cluster. Clusters were divided into 5, 3, 2, 2, and 2 subclusters in cluster B (B1-B5), cluster C (C1-C3), cluster D (D1-D2), cluster E (E1-E2) and cluster F (F1-F2), respectively.

Fifteen (26.7%) of 56 isolates were found in cluster B followed by 11 (19.6%) in cluster C, 10 (17.8%) in clusters E and F, 6 (10.7%) in cluster D and 2 (3.5%) in cluster A. The remaining 2 (3.5%) isolates in the C cluster were determined singleton lineages. We assessed strains with a Jaccard coefficient of ≥ 0.76 as identical, and two isolates in cluster A were considered the same type (Fig 2).

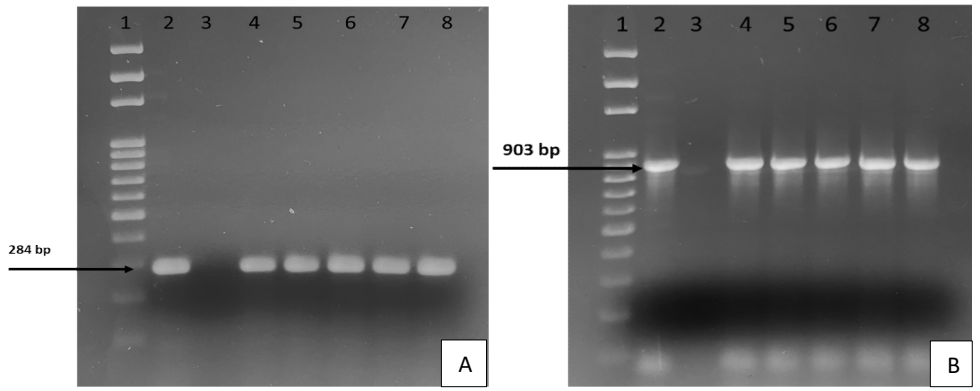

Fig. 1: Agar Gel Electrophoresis of *Salmonella* spp. and *E. coli* isolates. (A) 1:Ladder (100 bp), 2:Positive control strain for *inv*-A gene (*S.* Typhimurium ATCC 14028), 3: Negative control, 4-8: Positive isolates. (B) 1:Ladder (100 bp), 2:Positive control strain for *pho*-A gene (*E. coli* O 157 NCTC 12900), 3: Negative control, 4-8: Positive isolates.

Table 1: The primer pairs and amplified fragment size used in this study

Target gene	Primer	Sequence (5'-3')	Product size (bp)	Reference
invA	*inv-A* F	GTGAAATTATCGCCACGTTCGGGCAA	284	Rahn *et al.,* 1992
	inv-A R	TCATCGCACCGTCAAAGGAACC		
IE-1	*IE-1* F	AGTGCCATACTT TTAATGAC	316	Wang and Yeh, 2002
	IE-1 R	ACTATGTCGATACGGTGGG		
Flic-C	*Flic-C* F	CCCGCTTACAGGTGGACTAC	432	Paião *et al.,* 2013
	Flic-C R	AGCGGGTTTTCGGTGGTTGT		
pho-A	pho-A F	GTGACAAAAGCCCGGACACCATAAATGCCT	903	Kong *et al.,* 1999
	pho-A R	TACACTGTCATTACGTTGCGGATTTGGCGT		
fliCh7	FLICH7-F	GCGCTGTCGAGTTCTATCGAGC	625	Sarimehmetoglu *et al.,* 2009
	FLICH7-R	CAACGGTGACTTTATCGCCATTCC		
rbfO157	PF8	CGTGATGATGTTGAGTTG	420	Maurer *et al.,* 1999
	PR8	AGATTGGTTGGCATTACTG		
wzx-wzy O26	wzx-wzyO26F	AAATTAGAAGCGCGTTCATC	596	Durso *et al.,* 2005
	wzx-wzy O26R	CCCAGCAAGCCAATTATGACT		
rfbO111	O111F	TAGAGAAATTATCAAGTTAGTTCC	406	Paton and Paton, 1998
	O111R	ATAGTTATGAACATCTTGTTTAGC		
Stx 1	SLTI-F	TGTAACTGGAAAGGTGGAGTATACA	210	Fratamico *et al.,* 2000
	SLTI-R	GCTATTCTGAGTCAACGAAAAATAAC		
Stx 2	SLTII-F	GTTTTTCTTCGGTATCCTATTCC	484	Fratamico *et al.,* 2000
	SLTI-R	GATGCATCTCTGGTCATTGTATTAC		
eaeA	AE22	ATTACCATCCACACAGACGGT	397	Fratamico *et al.,* 2000
	AE20-2	ACAGCGTGGTTGGATCAACCT		
hlyA	MFSI-F	ACGATGTGGTTTATTCTGGA	166	Fratamico *et al.,* 2000
	MFSI-R	CTTCACGTCACCATACATAT		
ERIC	1R	ATGTAAGCTCCTGGGGATTCAC	-	Houf *et al.,* 2002
	2	AAGTAAGTGACTGGGGTGAGCG		

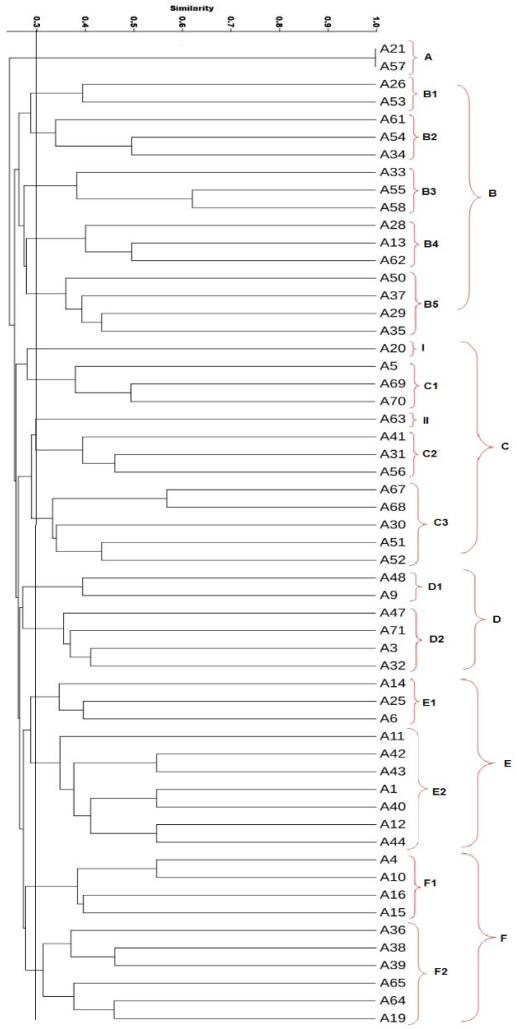

Fig. 2: Dendrogram of the *E. coli* isolates analyzed according to the ERIC-PCR.

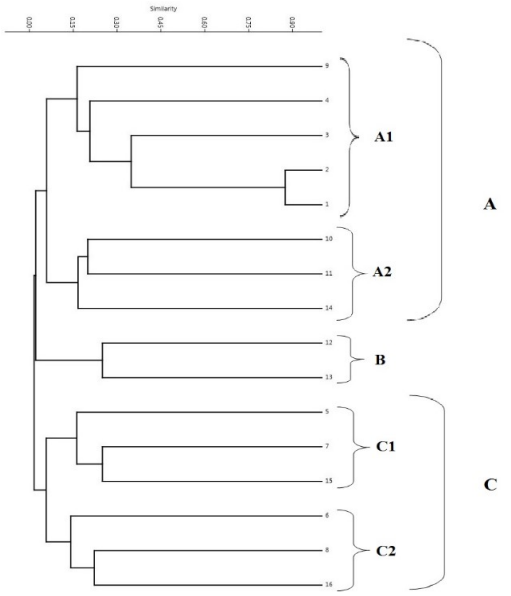

Fig. 3: Dendrogram of the *Salmonella* spp. isolates analyzed according to the ERIC-PCR.

As regard to the *Salmonella* spp. isolates, the Jaccard similarity coefficient of ERIC-PCR in this analysis was found to be 0.9082 with 100% reproducibility. There were 3-8 bands with molecular weight ranging from 100 bp to 900 bp generated by ERIC primers. ERIC-PCR also exhibited three main clusters (A-C). Briefly, Cluster A and Cluster C were further divided into two subclusters (Fig. 3). Eight (50%), two (12.5%) and six (37.5%) of 16 isolates were in cluster A, cluster B, cluster C, respectively. It was considered strains with a Jaccard coefficient ≥ 0.9082 as identical, accordingly, none of the isolates had identical profiles and they exhibited large genetic diversity.

DISCUSSION

The results showed that 74.6% of chicken carcasses were found positive for *E. coli*. These results were higher than the study of Hizlisoy *et al.* (2017) with a contamination rate of 15% conducted in a slaughterhouse with poultry and poultry originated samples in Kayseri. Also, a previous study conducted by Asensi *et al.* (2009) reported that 10% of the chicken carcass samples were found contaminated with *E. coli*. Our results were significantly lower than the findings of Vural *et al.* (2006) who isolated *E. coli* from 100% of chicken carcass rinses. Similar to our results, Yulistiani and Praseptiangga (2019) detected *E. coli* in 77.5% of chicken meat samples collected from traditional markets. *E. coli* O157 based on serotyping and PCR, was not detected in any of the isolates. Similarly, Hajian *et al.* (2011) reported none of the chicken samples were positive for *E. coli* O157:H7 and Apan *et al.* (2018) did not detect *E. coli* O157:H7 in any of the samples including human stool samples, animal feed and beef carcasses. However, Karadal *et al.* (2019) have detected *E. coli* O157:H7 in stuffed mussel and cheese halva with a prevalence rate of 0.95% out of 240 ready to eat food. Our study showed that, *E. coli* isolates were not detected to carry any of the virulence genes analyzed. However, Hizlisoy *et al.* (2017) found that 77% of the *E. coli* isolates obtained from chicken products had only the *eaeA* gene.

ERIC-PCR has been used as a highly sensitive genomic fingerprinting tool to detect and differentiate of *E. coli* strains from a variety of sources (De Moura *et al.,* 2001; Mohapatra *et al.,* 2007; Ardakani and Ranjbar, 2016). It was noted that the ERIC-PCR profiles of the *E. coli* isolates had high genetic diversity, which suggested that there were different sources of contamination in the sampling environments. Furthermore, Soltani *et al.* (2012) identified 65 different strains of avian *E. coli* from 95 samples with bands from 232 to 2690 bp on gel electrophoresis, confirming that ERIC-PCR is a distinguishing technique for genotyping among inexpensive and simple molecular methods.

In the present study, *Salmonella* spp. was detected in 21.3% of the samples. Our carcass contamination rate was lower than the 49.9% prevalence reported by Wang *et al.* (2014a) and higher than 16% and 2% prevalence stated by Vural *et al.* (2006) and Adesiji *et al.* (2011), respectively. *S.* Enteritidis and *S.* Typhimurium were not determined in any of the samples based on the *IE* and *flic*-C genes based

PCR reaction in the present study. Unlike our current study, Paião *et al.* (2013) reported that both serotypes (12% and 3%, respectively) were detected using the same primer pairs in broiler chicken samples in Brazil. In a similar study *S.* Enteritidis and *S.* Typhimurium were also detected using different primer pairs targeting *Sdf*I and *Flic*-C genes, respectively.

According to the ERIC-PCR results, none of the *Salmonella* spp. isolates had identical profiles and exhibited great genetic diversity, indicating that the sources of contamination of the samples might be dissimilar. There are also various studies that ERIC-PCR is used as a genomic fingerprinting tool to discriminate *Salmonella* spp. from a variety of food sources and found to harbour a diverse variety of *Salmonella* spp. genotypes. (Kumar *et al.,* 2008; Fendri *et al.,* 2013; Wang *et al.,* 2014b). Besides, ERIC-PCR may have some limitations on strain differentiation for *Salmonella* spp., including *S. dublin*, *S. abortusequi*, and *S. bareilly* because of their C2 profiles and is generally recommended to be supplemented by serovar differentiation method such as 16S-23S gap region polymorphism (Jensen *et al.,* 1993).

The presence of *E. coli* and *Salmonella* spp. in analyzed samples may result from the fecal contamination from the rupture of the intestines and insufficient sanitation conditions such as using unclean equipment, contaminated water, high humidity in the operation area, hands of personnel in manually processing systems and airborne contamination during slaughtering. (Shah and Korejo, 2012; Balakrishnan *et al.,* 2018).

Conclusion: Although virulent *E. coli* was not detected in our study, it is thought that its high incidence should be considered as an indicator of failure to comply with hygienic conditions and lack of sanitary practices especially during slaughter, and deficiencies in control of fecal contamination. In the same way of not detection of pathogenic serotypes of *Salmonella* spp., it should be still taken seriously in terms of the need to take the mentioned hygienic measures and prevent fecal contamination. Furthermore, it is thought that utilization of ERIC-PCR for genotyping and to elucidate the genetic diversity for discrimination of *E. coli* and *Salmonella* spp. isolates could be an alternative method that is quick, effective and low cost.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contribution: AET: Conceptualization; formal analysis; writing-original draft; writing-review & editing YB: Conceptualization; formal analysis; writing original draft; writing-review & editing NT: Methodology; writing-original draft; writing-review & editing. CG: Formal analysis. GT: Formal analysis. NEO: Methodology; visualization; writing-review& editing.

REFERENCES

Adesiji YO, Alli OT, Adekanle MA *et al*., 2011. Prevalence of Arcobacter, Escherichia coli, Staphylococcus aureus and Salmonella species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. Sierra Leone J Biomed Res 3:8-12.

Apan TZ, Yildirim M, Başalan M, *et al.,* 2018. Prevalence of *E. coli* O157: H7 Isolated from Human and Animal Source in Kırıkkale Province. Kırıkkale Üni Tıp Derg 20:256-62.

Ardakani MA and Ranjbar R, 2016. Molecular typing of uropathogenic *E. coli* strains by the ERIC-PCR method. Electron Physician 8:2291-6.

Asensi GF, Dos Reis EMF, Del Aguila EM, *et al.,* 2009. Detection of *Escherichia coli* and Salmonella in chicken rinse carcasses. Br Food J 111:517-27.

Bakhshi B, Afshari N and Fallah F, 2018. Enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis as a reliable evidence for suspected *Shigella* spp. outbreaks. Braz J Microbiol 49:529–33.

Balakrishnan S, Sangeetha A and Dhanalakshmi M, 2018. Prevalence of Salmonella in chicken meat and its slaughtering place from local markets in Orathanadu, Thanjavur district, Tamil Nadu. J Entomol Zool Stud 6:2468-71.

Behzadi MA, Ziyaeyan M and Alborzi A, 2016. A diagnostic one-step real-time reverse transcription polymerase chain reaction method for accurate detection of influenza virus type A. Arch Med Sci 12:1286.

Cristiani M, Flores MJ, Brandi RJ, *et al.,* 2020. ERIC-PCR technique applied to monitoring and quantification of DNA damage during water disinfection process. J Photochem Photobiol B: Biol 202: 111699.

De Moura AC, Irino K and Vidotto MC, 2001. Genetic variability of avian *Escherichia coli* strains evaluated by enterobacterial repetitive intergenic consensus and repetitive extragenic palindromic polymerase chain reaction. Avian Dis 45:173-81.

Durso LM, Bono JL and Keen JE, 2005. Molecular serotyping of Escherichia coli O26: H11. Appl Environ Microbiol 71:4941-4.

Fendri I, Ben Hassena A, Grosset N, *et al.,* 2013. Genetic diversity of food-isolated Salmonella strains through pulsed field gel electrophoresis (PFGE) and enterobacterial repetitive intergenic consensus (ERIC-PCR). PLoS One 8:e81315.

Fratamico PM, Bagi LK and Pepe T, 2000. A multiplex polymerase chain reaction assay for rapid detection and identification of *Escherichia coli* O157: H7 in foods and bovine feces. J Food Prot 63:1032-7.

Graham JP, Leibler JH, Price LB, *et al.,* 2008. The animal-human interface and infectious disease in industrial food animal production: rethinking biosecurity and biocontainment. Public Health Rep 123:282-99.

Hajian S, Rahimi E and Mommtaz H, 2011. A 3-year study of *Escherichia coli* O157: H7 in cattle, camel, sheep, goat, chicken and beef minced meat. In: Proceedings of the International Conference on Food Engineering and Biotechnology (IPCBEE'11). IACSIT Press Singapore.

Hizlisoy H, Al S, Ertaş Onmaz N, *et al.,* 2017. Antimicrobial resistance profiles and virulence factors of *Escherichia coli* O157 collected from a poultry processing plant. Turk J Vet Anim Sci 41:65-71.

Houf K, De Zutter L, Van Hoof J, *et al.,* 2002. Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. Appl Environ Microbiol 68:2172-8.

International Standard Organitation 6887-2:2003. Microbiology of food and animal feeding stuffs-Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 2: Specific rules for the preparation of meat and meat products. International Organization for Standardization, Geneva.

International Standard Organitation 16649-3:2015. Microbiology of the chain – Horizontal method for the enumeration of Beta-Glucuronidase Positive *Escherichia coli* – Part3: Detection and most probable number tecnique using 5-bromo-4-Chloro-3- Indolyl-β-D-Glucuronide. International Organization for Standardization, Geneva.

International Standard Organitation 6579: 2002. Microbiology of food and animal feding stuffs. Horizontal method for the detection of Salmonella spp. International Organization for Standardization, Geneva.

Jensen MA, Webster JA and Straus N, 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Appl Environ Microbiol 59:945-52.

Karadal F, Ertaş Onmaz N, Hizlisoy H, *et al.,* 2019. Isolation, genotyping and antimicrobial susceptibility of pathogenic *Escherichia coli* serotypes in ready to eat foods. J Hell Vet Medical Soc 70:1661-8.

Katiyo W, De Kock HL, Coorey R, *et al.,* 2020. Sensory implications of chicken meat spoilage in relation to microbial and physicochemical characteristics during refrigerated storage. LWT-Food Sci Technol 128:109468.

Kong RYC, So CL, Law WF, *et al.,* 1999. A sensitive and versatile

multiplex PCR system for the rapid detection of enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) and enteropathogenic (EPEC) strains of *Escherichia coli*. Mar Pollut Bull 38:1207-15.

Koutsoumanis K, Allende A, Alvarez-Ordóñez A, *et al.,* 2019. Scientific Opinion on the Salmonella control in poultry flocks and its public health impact. EFSA Journal 17:5596.

Kuang X, Hao H, Dai M, *et al.,* 2015. Serotypes and antimicrobial susceptibility of Salmonella spp. isolated from farm animals in China. Front Microbiol 6:602.

Kumar R, Surendran PK and Thampuran N, 2008. Molecular fingerprinting of *Salmonella enterica* subsp. *enterica* Typhimurium and *Salmonella enterica* subsp. *enterica* Derby isolated from tropical seafood in South India. Mol Biotechnol 40:95-100.

Le Hello S, 2014. Salmonella: une bactérie multi-résistante aux antibiotiques dans nos assiettes. J Anti-Infect 16:192-8.

Letunic I and Bork P, 2019. Interactive Tree of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res 47(W1): W256-W259.

Little CL, Richardson JF, Owen RJ, *et al.,* 2008. *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: prevalence, characterization and antimicrobial resistance pattern, 2003–2005. Food Microbiol 25:538-43.

Maka L, Mackiw E, Sciezynska H, *et al.,* 2015. Occurence and antimicrobial resistance of Salmonella spp. isolated from food other than meat in Poland. Ann Agric Environ Med 22:403-8.

Maurer JJ, Schmidt D, Petrosko P, *et al.,* 1999. Development of primers to O-antigen biosynthesis genes for specific detection of *Escherichia coli* O157 by PCR. Appl Environ Microbiol 65:2954-60.

Mellata M, 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. Foodborne Pathog Dis 10:916-32.

Messaili C, Messai Y and Bakour R, 2019. Virulence gene profiles, antimicrobial resistance and phylogenetic groups of fecal *Escherichia coli* strains isolated from broiler chickens in Algeria. Vet Ital 55:35-46.

Mohapatra BR, Broersma K and Mazumder A, 2007. Comparison of five rep-PCR genomic fingerprinting methods for differentiation of fecal *Escherichia coli* from humans, poultry and wild birds. FEMS Microbiol Lett 277:98-106.

Paião FG, Arisitides LGA, Murate LS, *et al.,* 2013. Detection of *Salmonella* spp., *Salmonella* Enteritidis and Typhimurium in naturally infected broiler chickens by a multiplex PCR-based assay. Braz J Microbiol 44:37-42.

Paton AW and Paton JC, 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx 1, stx 2, eaeA, enterohemorrhagic *E. coli* hlyA, rfb O111, and rfb O157. J Clin Microbiol 36:598-602.

Pitcher DG, Saunders NA and Owen RJ, 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 8:151-6.

Rahn K, De Grandis SA, Clarke RC, *et al.,* 1992. Amplification of an invA gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol Cell Probes 6:271-9.

Rouger A, Tresse O and Zagorec M, 2017. Bacterial contaminants of poultry meat: Sources, species, and dynamics. Microorganisms 5:50.

Sarimehmetoglu B, Aksoy MH, Ayaz ND, *et al.,* 2009. Detection of *Escherichia coli* O157: H7 in ground beef using immunomagnetic separation and multiplex PCR. Food Control 20:357-61.

Sedighi P, Zarei O, Karimi K, *et al.,* 2020. Molecular typing of *Klebsiella pneumoniae* clinical isolates by enterobacterial repetitive intergenic consensus polymerase chain reaction. Int J Microbiol 2020:8894727.

Shah AH and Korejo NA, 2012. Antimicrobial resistance profile of *Salmonella* serovars isolated from chicken meat. J Vet Anim Sci 2: 40-6.

Soltani M, Peighambari SM, Askari Badouei M, *et al.,* 2012. Molecular typing of avian *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus sequences-polymerase chain reaction (ERIC-PCR). Iran J Vet Med 6:143-8.

Vincent C, Boerlin P, Daignault D, *et al.,* 2010. Food reservoir for Escherichia coli causing urinary tract infections. Emerg Infect Dis 16: 88-95.

Vural A, Erkan ME and Yesilmen S, 2006. Microbiological quality of retail chicken carcasses and their products in Turkey. Med Weter 62:1371-4.

Wang Y, Chen Q, Cui S, *et al.,* 2014a. Enumeration and characterization of *Salmonella* isolates from retail chicken carcasses in Beijing, China. Foodborne Pathog Dis 11:126-32.

Wang H, Shu R, Zhao Y, *et al.,* 2014b. Analysis of ERIC-PCR genomic polymorphism of *Salmonella* isolates from chicken slaughter line. Eur Food Res Technol 239:543-8.

Wang SJ and Yeh DB, 2002. Designing of polymerase chain reaction primers for the detection of *Salmonella* Enteritidis in foods and faecal samples. Lett Appl Microbiol 34:422-7.

Xu Z, Wang M, Zhou C, *et al.,* 2020. Prevalence and antimicrobial resistance of retail-meat-borne *Salmonella* in southern China during the years 2009–2016: The diversity of contamination and the resistance evolution of multidrug-resistant isolates. Int J Food Microbiol 333:108790.

Yulistiani R and Praseptiangga D, 2019. Occurrences of *Salmonella* spp. and *Escherichia coli* in chicken meat, intestinal contents and rinse water at slaughtering place from traditional market in Surabaya, Indonesia. IOP Conference Series: Materials Science and Engineering, 633:012007.