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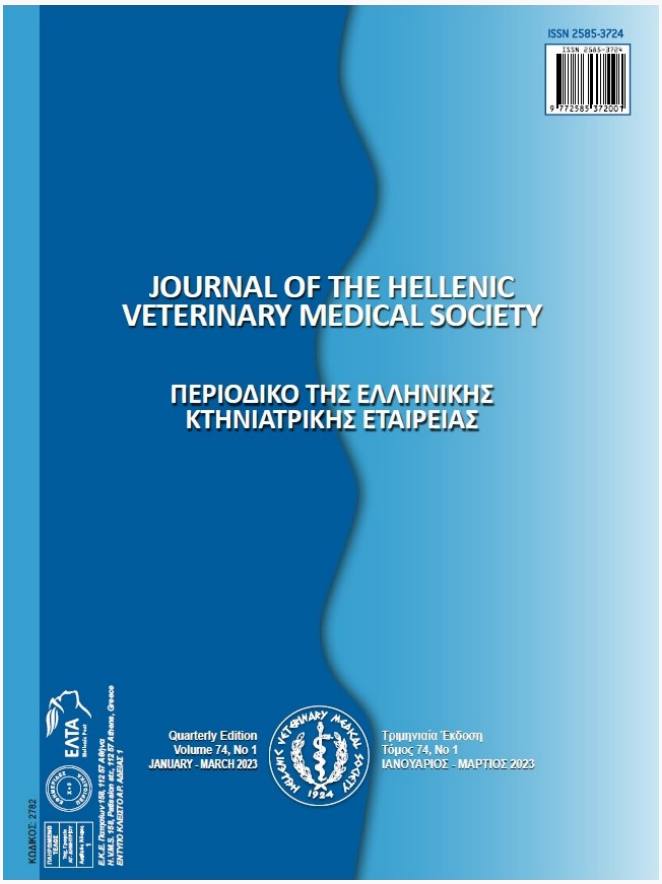

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Research article
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Fosfomycin Resistant *Enterobacterales* Isolated From Chicken Meat in Turkey

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ABSTRACT: This study was conducted to estimate the relative prevalence of fosfomycin resistant (FOS^r) Enterobacterales in raw chicken meat samples in Turkey. Samples (n=85) were enriched in non-selective media and transferred to MacConkey agar plates containing FOS and glucose-6-phosphate. As a result, FOS^{r}Enterobacterales isolates were detected by a selective method in 27% of raw chicken meat samples (n=23) and identified as Escherichia coli (21/26), Klebsiella oxytoca (2/26), Escherichia vulneris (1/26), Raoultella terrigena (1/26) and Kluyvera intermedia (1/26). PFGE analysis showed 16 different band patterns in Escherichia spp. isolates (n=22) based on the 85% similarity. The minimum inhibitory concentration for FOS against all isolates was determined to be ≥64 mg/L. In addition, the highest rate of resistance was determined for nalidixic acid (72.7%), ampicillin (68.2%), tetracycline (59.1%), trimethoprim-sulfamethoxazole (54.5%), and chloramphenicol (59.1%) among all Escherichia isolates. PCR screening and sequencing identified the presence of fosA4 and fosA3 genes in ten (47.6%) and seven (33.3%) E. coli isolates, respectively. The fosA3 gene has also appeared in K. intermedia, and R. terrigena isolates. Only two E. coli isolates were positive for the $bla_{CTX\text{-}M\text{-}55}$ gene, whereas the aac (6') -Ib-cr gene was identified in eight E. coli and one K. intermedia isolates. In addition, 19 different replicon types were determined by PCR-based plasmid replicon typing with IncFII (n=20) being the most common and followed by IncI1α (n=10), IncFIIS (n=8), and IncFIB (n=8). We report, to our knowledge, the first evidence on the presence of FOS^{r}Enterobacterales isolates in raw chicken meat samples in Turkey that might be an important reservoir for FOS^r organisms to humans.

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INTRODUCTION

The extensive use of β-lactam and quinolone antibiotics has led to resistance due to the significant selection pressure, leading to the use of older drugs such as fosfomycin (FOS). After its first introduction in 1969 (Hendlin et al., 1969), FOS, the class of phosphonic antibiotics, has recently gained increased popularity because of its ease of use, relative resilience to resistance, non-toxicity to mammals as well as its ability to diffuse in different systems and organs with low molecular (138 Da) structure (Dijkmans et al., 2017). It is a commonly prescribed antimicrobial agent, especially as oral medication, to treat urinary tract infections (UTIs) in humans in many countries (Benzerara et al., 2017; Falagas et al., 2019). FOS alone or in combination with other antibiotics has also been successfully used as an intravenous medication to treat severe infections caused by multidrug-resistant (MDR) and extensively drug resistant Gram-negative bacteria (Pontikis et al.,2014). This antibiotic is also classified among "*veterinary highly important antimicrobial agents,"* although its utilization has been allowed in few countries for over 40 years (Pérez et al.,2014; OIE, 2019).

FOS resistance (FOS^r) is an infrequently encountered phenomenon seen in <20% of clinical *Enterobacterales* isolates (Flamm et al.,2019; Sánchez-García et al.,2019).Previous studies, however, showed that increased utilization of FOS had altered the occurrence of FOS^r. For example, in Spain, (Oteo et al., 2010) reported that FOS^r was increased significantly from 4.4% in 2005 to 11.4% in 2009 among extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* isolates from UTIs in parallel with the increased (340%) community use of FOS between 1997 and 2008.

FOS inhibits MurA enzyme and disrupts peptidoglycan biosynthesis in the bacterial cell wall (Eschenburg et al., 2005).Different mechanisms have been described for resistance to FOS, including (i) MurA target modification, (ii) decreased permeability, and (iii) FOS-modifying enzymes (also termed as bacterial FOS^r proteins; FosA, FosB, FosC, FosX, FosK, FosD, FosE, FosI, FosL, FomA, and FomB) (Zurfluh et al.,2020; Chen et al., 2021).Notably, while the first two mechanisms are chromosomal, the latter has a mainly extrachromosomal nature and has been noted as the most crucial type because of its transmissible characteristics via horizontal gene transfer (Fillgrove et al.,2007; Castañeda-García et al.,2013).The *fosA* gene, encoding bacterial FOS^r proteins, was first identified in two different plasmids from the MDR strains of *Serratia marcescens* (Mendoza et al.,1980). In total, twelve molecular variants of this gene have been described in bacteria to date (Chen et al.,2019).A very high prevalence of the *fosA3* gene among FOS^r Enterobacterales isolates from human, animal pathogens, and food bacteria has been reported from Asian countries, but less commonly in other parts of the world (Zurfluh et al.,2020).

Recently, accumulating data indicates the presence of FOS^r bacteria globally from various environments, including humans and food, due to its spread through plasmid-mediated genes (Biggel et al., 2021; Chen et al., 2019; Cottell and Webber, 2019; Mueller et al., 2019). For example, in a study of fecal samples from 460 broiler chickens in Hong Kong, 7.4% exhibited FOS^r*E. coli* (Ho et al., 2013). However, the current epidemiological situation of FOS^r among *Enterobacterales* in Turkey is unknown. Therefore, the present study evaluated the presence and prevalence of FOS^r*Enterobacterales* in raw chicken meat samples commercialized for human consumption. We also determined the antimicrobial-resistant levels and resistance mechanisms of the obtained isolates.

MATERIALS AND METHODS

Sampling and isolation of fosfomycin resistant Enterobacterales

A total of 85 retailed chicken meat samples, including drumsticks (n=30), wings (n=45), and whole carcasses (n=10), were collected from different supermarkets during six months (January-June 2019) in Hatay and Kayseri provinces in Turkey. Samples were packaged products from eleven different companies, which have large-scale distribution throughout the whole country. Samples (25 gr) were enriched in 225 ml buffered peptone water (Merck, USA) at 37 °C for 18-20 h. After enrichment, 100 µl aliquot of broth was streaked onto MacConkey agar plates (Merck, USA) supplemented with FOS (32 mg/L) (Sigma) and glucose-6-phosphate (25 mg/L) (Sigma) and incubated at 37 °C for 18-20 h (Jiang et al., 2017). This selective medium was also tested with internal quality control strains including FOS^r *E. coli* isolates obtained previously from hospital sewage samples (not published) and *E. coli* ATCC 25922 strains.

Isolates were identified to the species level using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker,

Germany). *E. coli* isolates were subsequently confirmed by PCR targeting the universal stress protein (*uspA*) gene region (Chen and Griffiths, 1998).Genomic DNA (gDNA) was extracted using the Instagene DNA extraction kit (Bio-Rad, USA) according to the manufacturer's instructions. Determining of gDNA isolation efficiency and total gDNA quantities (ng/µl) were detected with Qubit 3 Fluorometric Quantitation (Thermo, USA). Obtained gDNA was stored at -20 °C until the analysis. All the primers pairs used in the current study are given in the Supplementary list. PCR amplifications were performed with Arktic™ Thermal Cycler (Thermo, USA). The gels stained with GelRed™ Nucleic Acid Gel Stain (Biotium, USA) were subjected to electrophoresis for 45 min at 120 V/cm and visualized under ChemiDoc XRS+ System (Bio-Rad, USA).

To determine the clonal affinity of *Escherichia* spp. (21 *E. coli* and one *E. vulnaris*) isolates obtained from poultry meats, PFGE analysis and the determination of the phylogenetic groups were performed. PFGE was conducted using the *Xba*I digestion enzyme to investigate the clonal relationships among the FOS resistant *Escherichia* spp. isolates according to the Pulsenet protocol (https://www.cdc.gov/pulsenet). The similarity was determined with the Dice coefficient with 1.3 % optimization and 1.1 % tolerance. The unweighted pair group method using arithmetic averages (UPGMA) was used to construct the dendrogram with BioNumerics software version 6.5 (Applied Maths, USA). Besides PFGE, all *E. coli* isolates were typed with PCR-based genetic markers, *chuA*, *yjaA,* and *TspE*4, as described by (Clermont et al., 2000).

Determination of antibiotic resistance profiles

The minimum inhibitory concentration (MIC; concentrations between 2-256 mg/L) for FOS was determined by the agar dilution method as described by (CLSI, 2018a; 2018b). The disk diffusion method was used to determine the resistance to panel of antibiotics; FOS (200 µg), LEV (5 µg), NOR (10 µg), NA (30 µg), CIP (5 µg), FOX (30 µg), CTX (5 µg), CAZ (10 µg), FEP (30 µg), ATM (30 µg), AMC (20/10 µg), AM (10 µg), TOB (10 µg), AK (30 µg), SXT (25 µg), TE (30 µg), C (30 µg) and IPM (10 µg). The results were interpreted according to the CLSI guidelines (CLSI, 2020), and for FOS, MIC results were interpreted according to the EUCAST guideline. *E. coli* ATCC 25922 strain was used as a control strain.

Determination of resistance genes and replicon types

Escherichia spp. isolates were screened for the presence of plasmid-mediated *fos* genes (*fosA*, *fosA3,* and *fosC*) (Ho et al., 2013), β-lactam determinants (bla_{CMY}, $bla_{CTX-M,}$ and bla_{SHV}) (Ahmed et al., 2007; Hasman et al., 2005; Leinberger et al., 2010), and plasmid-mediated quinolone resistance genes (PMQR; *aac (6') -Ib-cr, qnrA, qnrB, qnrC*, *qnrD,* and *qnrS*) (Cattoir et al., 2007; Cavaco et al., 2008, 2009).PCR positive amplicons were subjected to the Sanger sequencing (Medsantek Ltd. Co, İstanbul, Turkey), and the sequence data were analyzed using the BLAST programs at FASTA format (http://www.ncbi.nlm.nih.gov). In addition, the main plasmid incompatibility of isolates was typed using the PCR-based assay (PBRT kit, Diatheva, Italy).

Detection of virulence genes

The presence of *hlyA, fimH, iroN, kpsMT* K1, *kpsMT*II, *iutA, papAH, papC, papEF, papG* allele II, *papG* allele III, *papG* alleles II and III, stx_1, stx_2, and *univcnf*virulence genes was examined with PCR conditions reported by (Chapman et al., 2006).

RESULTS AND DISCUSSION

The past decade has seen a remarkable increase in the distribution of plasmid-mediated FOS^r in several Gram-negative bacteria isolated from humans, animals, and foods, which necessitated regular monitoring of foods of animal origins. We found that a considerably high number of chicken meat samples (27%; n=23) were contaminated with a variety of FOS^r*Enterobacterales* species, the most common being *E. coli* (80,7%; 21/26), followed by *Klebsiella oxytoca* (7.4%; 2/26), *Escherichia vulneris* (3.7%; 1/26), *Raoultella terrigena* (3.7%; 1/26) and *Kluyvera intermedia* (3.7%; 1/26) by MALDI-TOF MS. Additionally, the PCR results were also positive for the *uspA* gene in 21 *E. coli* isolates. This, as far as we know, is the first report in which FOS^r*Enterobacterales* were detected in chicken meat in Turkey. According to Ho et al., (2013), who tested fecal samples (n=2106) from cattle, pigs, chicken, cats, dogs, and wild rodents in Hong Kong, FOS^r*E. coli* was reported in 4.5% of tested animals, among which 7.4% of chickens were fecal carriers. Similarly, 114 *E. coli* isolates from chicken in China were investigated in another study, and 11 (9.6%) were resistant to FOS (Wang et al., 2017). It is currently unknown why the rate of contamination of FOS^r*Enterobacterales* was

high in chicken meat samples in Turkey. FOS^r were found to be present in strains of *E. coli* isolated from poultry in China, where this antibiotic agent has never been used (Ho et al., 2013; Yang et al., 2014), and this situation was speculated to have occurred due to the selective pressure imposed by previously used cephalosporin in poultry (Jiang et al., 2017). The high occurrence rate of FOS^r strains of *Enterobacterales* in raw chicken meat samples could also be attributed to cross-contamination in the raw chicken meat chain and the subsequent proliferation of these organisms. Finally, the use of selective methods to detect FOS^r isolates directly in the current study cannot be neglected in order to increase the isolation capacity.

In the PFGE analysis, 22 isolates of *Escherichia* spp. were divided into 21 different pulsotypes and 16 clusters based on the 85% similarity (Figure 1).It was determined that all isolates except two were in different pulsotypes, suggesting multiple sourcesfor these isolates into the poultry production chain. Similarly, 64 FOS^r*E. coli* isolates from chicken meat were distinct by PFGE and MLST methods (Jiang et al., 2017).Wang et al., (2017) also reported that a total of 29 different PFGE patterns were identified from 39 FOS^r*E. coli* isolates from various sources. In the current study, most *E. coli* isolates (57.1%) were found to be phylogroup A1 and followed by A0 (%28.5), B1 (9.5%), and D1 (4.7%) (Figure 1). In contrast to our finding, in China, phylogroup B1 accounted for 50% of the *E. coli* isolates, whereas phylogroup C and A accounted for 17.2% and 11%, respectively (Jiang et al., 2017).It was also shown that B2 (60%) and B1 (25.6%) were the predominant phylogroups, whereas the minority were A1 (7.7%) and D (7.7%) in China Wang et al., (2017),suggesting the geographical differences in phylogroup distribution.

Antibiotic susceptibility properties and minimum inhibitory concentrations (MICs) of *Escherichia* and non-*Escherichia* isolates obtained in the study are given in Figure 1 and Table 1, respectively. Our results showed that all the isolates had a MIC ≥64 mg/L using the agar dilution method. However, 11 isolates (42.3%) were found to be FOS susceptible using the disk diffusion method [zone diameter equal or greater than 22 mm: CLSI (2020)], clearly indicating the unsuitability of this method for the determination of the true FOS^r. This is consistent with previous reports (Kaase et al., 2014; Mojica et al., 2020). They found that among 107 carbapenemase-producing *Enterobacteriaceae* isolates, FOS^r (MIC ≥32 mg/L) were

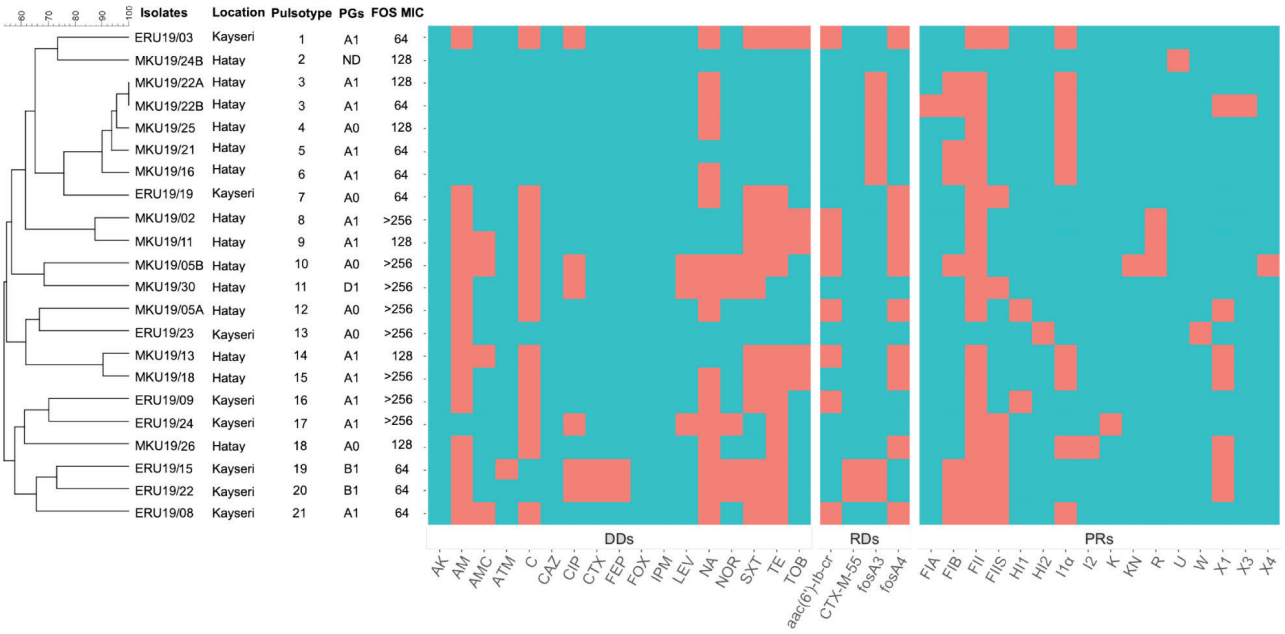

Figure 1. A dendrogram generated by PFGE analysis with *Xba*I restrictions and antibiotic resistance and virulence traits of fosfomycin resistant *Escherichia* spp. (n:21; *E. coli*, n=1; *E. vulneris*) isolates. PGs: Phylogroups; PRs: Plasmid replicons; RDs: Resistance determinants. DDs: Resistance profiles obtained from Disc Diffusions (LEV: Levofloxacin; NOR: Norfloxacin; NA: Nalidixic acid; CIP: Ciprofloxacin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; FEP: Cefepime; ATM: Aztreonam; AMC: Amoxicillin-Clavulanic Acid; AM: Ampicillin; TOB: Tobramycin; AK: Amikacin; SXT: Trimethoprim-Sulfamethoxazole; TE: Tetracycline; C: Chloramphenicol; IPM: Imipenem). Red cells indicate resistance in disc diffusion assay (DDs) and presence of replicon types (PRs) and resistant determinants (RDs).

Table 1. Summary of antibiotic resistance characteristics of FOS^r non-*Escherichia* isolates

Isolate ID	Species	Location (city)	FOS MIC (µg/ml)	DDs	PRs	RDs
MKU19/06	*Klebsiella oxytoca*	Hatay	>256	-	FIIK	*aac (6') -Ib-cr*
ERU19/27	*Klebsiella oxytoca*	Kayseri	>256	AM	-	-
MKU19/24A	*Raoultellaterrigena*	Hatay	>128	-	-	*fosA3*
ERU19/12	*Kluyvera intermedia*	Kayseri	>128	AM, AMC, FOX, TOB, SXT, TE, C	FIIK	*fosA3, aac (6') -Ib-cr*

FOS: Fosfomycin; PRs: Plasmid replicons; RDs: Resistance determinants;DDs: Resistance profiles obtained from disc diffusion assay

identified in 30 isolates, among which false-susceptible and false-resistant resulted from the disk diffusion method. The authors concluded that "*disk diffusion is not an appropriate method for fosfomycin susceptibility testing*."

In the current study, most isolates (57.7%) were resistant to three or more antimicrobial classes, and two isolates were sensitive to all antibiotics tested, rather than FOS. A recent study also reported by (Ho et al.,2013) showed that 64.4% of FOS^r*E. coli* isolates were MDR. Antimicrobial susceptibility assay revealed that all *Escherichia* isolates (n: 22) were sensitive to cefoxitin (FOX), amikacin (AK), imipenem (IPM), and ceftazidime (CAZ), and the highest rate of resistance was determined for nalidixic acid (NA; 72.7%; 16/22), ampicillin (AM; 68.2%; 15/22), tetracycline (TE; 59.1%; 13/22), trimethoprim-sulfamethoxazole (STX; 54.5%; 12/22) and chloramphenicol (C; 59.1%; 13/22). Low rates of resistance were detected against ciprofloxacin (CIP; 27.3%; 6/22), norfloxacin (NOR; 22.7%; 5/22), tobramycin (TOB; 22.7%; 5/22), amoxicillin/clavulanic acid (AMC; 18.2%; 4/22), levofloxacin (LEV; 13.6%; 3/22), cefotaxime (CTX; 9.1%; 2/22), cefepime (FEB; 9.1%; 2/22) and aztreonam (ATM; 4.5%; 1/22).Previous studies in Turkey also reported a high prevalence of TE and STX resistance among *E. coli* from chicken meat samples (Kürekci et al., 2019).A study in China investigated resistance patterns among the 39 FOS^r*E. coli* isolates from animalsand revealed high resistance (100%) to florfenicol, CTX, gentamicin, and TE (Wang et al.,2017).This is partly in contrast to our findings, in which resistance to β-lactam and aminoglycoside antibiotics was noted in a few isolates only.

The two *K. oxytoca* isolates obtained in this study were susceptible to all antibiotics examined, except that one isolate was resistant to AM. It has also been revealed that the *R. terrigena* isolate was susceptible to all antibiotics in the panel, but *K. intermedia* was found as resistant to FOX, AMC, AM, TOB, SXT, TE, and C.

To identify the genetic determinants responsible for FOS^r, conventional PCR analysis and sequencing were carried out. It was determined that ten (47.6%, 10/21) and seven (33.3%, 7/21) *E. coli* isolates carried *fosA4*, *fosA3* genes, respectively. It is worthwhile mentioning that the primer pair used for detection of the *fosA3* gene region also amplified the *fosA4* known asavariant of FosA3; FosA4 shares 94% amino acid identity with FosA3 (Nakamura et al., 2014).For this reason, sequence analysis must be performed to report the presence of *fosA3* and/or *fosA4*. Even though the occurrence and prevalence of these genes in the current study are not directly comparable with other studies, which mainly screened FOS resistance and related genes among ESBL producing isolates, the presence of the *fosA3* gene were commonly reported in FOS^r*E. coli* isolates from animal origins in many countries of Asia. For example, the results presented in a study carried by Ho et al., (2013) reported that 96% of FOS^r*E. coli* isolates from animals had the *fosA3* gene in China. Similarly, all FOS^r*K. pneumoniae* isolates (n=29) from clinical cases were found to be the *fosA3* gene-positive, whereas other FOS^r genes were not identified (Chen et al.,2019). However, somewhat in contradiction of this situation, the *fosA4* gene has been identified rarely. The occurrence of plasmid-mediated FOS^r traits has also been reported within Europe with less frequency. Additionally, none of the FOS^r genes (*fosA*, *fosA3*, and *fosC2*) tested were detected among FOS^r isolates of *E. coli* collected from human clinical samples in Turkey (Demirci-Duarte et al., 2020).A study by Mueller et al., (2019), analyzing the genetic mechanisms of FOS^r*E. coli* isolates (n=17) from community patients in Switzerland, showed that four isolates carried the *fosA3* gene while only one was positive for the *fosA4* gene. In another study conducted in France, the majority of FOS^r*E. coli* isolates (n=10) carried the *fosA3* gene (n=9) and

the *fosA5* gene (n=1) (Benzerara et al., 2017).

Previous studies showed that genes encoding FOS^r and β-lactam resistance, the bla_{CTX-M} genes, are often located on identical plasmids (Biggel et al., 2021; Benzerara et al.,2017; Ho et al.,2013; Mueller et al.,2019; Wang et al.,2017), suggesting that these mechanisms evolved together. Sato et al., (2013) showed that plasmids with varying replicon types, namely IncI1 and IncFII, contained *IS26* elements and the bla_{CTX-M} gene directly upstream and/or downstream of the *fosA3* gene associated with *E. coli* from humans and animals. In a study of eleven *E. coli* strains isolated from foods and wastewater samples in Switzerland, all appeared to have the *fosA3* gene and the bla_{CTX-M} gene (Biggel et al., 2021). Based on this data, some can infer, particularly in Asian countries, that ESBL producing genes appear to be an essential contributing factor to the observed increase in *fosA3* genes. However, we did not observe the previously described association between bla_{CTX-M} and *fosA3/4* genes since PCR screening and Sanger sequencing revealed that only two isolates were positive for the $bla_{CTX-M-55}$ ESBL gene. Of interest is also the high occurrence of the *aac (6') -Ib-cr* gene in *E. coli* isolates (n=8), whose association between the *fosA3* and *fosA4* genes deserves to be assessed.

It was determined that only one of the two *K. oxytoca* isolates harbored *aac (6') -Ib-cr*, while *K. intermedia* carried *aac (6') -Ib-cr* and *fosA3*, and *R. terrigena* isolate carried only *fosA3*. However, *E. vulneris* isolate did not possess any of the tested genes. In addition, none of the isolates were positive for *fosA, fosC2,*bla_{CMY}, bla_{SHV}, *qnrA*, *qnrC*, *qnrB-qnrS*, and *qnrD* gene regions screened in the study.

In the literature, a significant amount of genetic information has been obtained to explain FOS^r plasmids, which were reported to be approximately 70-140 kb in size (Wang et al.,2017).Previous analysis of genome sequences indicated the role of various incompatibility groups, including IncFII, IncFN, and B/O plasmids and *fosA3* determinant associated with FOS^r in the strains of *E. coli* (Ho et al., 2013).The presence of FOS^r genes, especially *fosA3*, on plasmids of different incompatibility groups constitutes an opportunity for the microbe to disseminate these adaptive genetic traits by horizontal gene transfer. In a recent investigation by Jiang et al., (2017), IncFII was found widely (17 isolates out of 33 transconjugative) distributed in 64 *fosA3* positive *E. coli* isolates. One recent study identified IncFII plasmid predominantly (18/39), IncN (12/39), and IncI1 (9/39) based on the RFLP results (Yang et al.,2014).In agreement with these studies, the IncFII plasmid carrying the *fosA3* gene was frequently identified among FOS^r*E. coli* isolates in France (Benzerara et al.,2017).In the current study, the results of PCR-based plasmid replicon typing exhibited that all isolates except two (*K. oxytoca* and *R. terrigena*) carried 19 different replicon types (Figure 1), and main replicon types were IncFII (n=20), IncI1α (n=10), IncFIIS (n=8) and IncFIB (n=8). Our experiments did not directly show which plasmid replicon types are *fosA3/4* carriers. Therefore, information concerning these plasmid replicons and the horizontal transfer of *fosA3* and *fosA4* genes to decipher the nature and impact of such plasmids on the epidemiology of the organism is necessary.

Aside from antimicrobial resistance genes, we also looked for virulence-related determinants among *Escherichia* spp. isolates in the current study. PCR approach, which was used to screen fifteen virulence traits, revealed that the *fimH* gene for the fimbria-mediated adherence was most abundant and found in 100% of *E. coli* isolates. This was followed by two siderophore synthesis genes, *iutA* and *iroN*, which had a percentage occurrence of 85.1% and 33.3%, respectively. In parallel with our findings,Kürekci et al., (2019) determined that 50 of 52 (96.1%), 50 of 31 (59.6%), and 50 of 26 (50%) of *E. coli* isolates from chicken samples were PCR positive for the *fimH*, *iutA*, and *iroN* genes. The current study determined that one isolate (3.7%) carried the *papEF* gene responsible for adhesion in screening the virulence gene presence in 21 FOS^r*E. coli* isolates. Detection of genes encoded in capsule synthesis, *KpsMTII* was detected in two (7.4%) isolates, but no *kpsMT* K1 was found, whereas papC, papG allele II, and papG allele II-III were found in low levels. In another study focused on the presence of *E. coli* and the determination of antibiotic resistance profiles in foods, it was similarly reported that all 14 *E. coli* isolates obtained from chicken meat carried *FimH* (100%), and two of them (14.3%) carried *iutA* and *iroN* (Van et al., 2008). Other genes (*papAH*, *papC*, and *papG* alleles II and III) related to adhesion examined in this study were not detected. None of these isolates were found to carry the *hlyA* gene associated with α-Hemolysin. Similarly, none of the isolates carried stx_1, stx_2 responsible for Shiga toxin production, and *univcnf*, the gene encoding the cytotoxic necrotizing factor 1.

In summary, to the best of our knowledge, we re-

port the first evidence of FOS^r*Enterobacterales* isolates in raw chicken meat samples in Turkey. These results provide critical insights into the potential role of foods of animal origin as an important reservoir of FOS^r organisms, especially *E. coli*, for humans. However, the current study had some limitations, including the modest sample size and generalizations of these findings; therefore, it should be viewed with caution. Hence, the occurrence and prevalence of FOS^r*Enterobacterales* must be studied among a much larger number of samples from foods of animal origins and human clinical samples. Further studies are also needed to sequence the plasmids to determine the molecular nature of the FOS^r phenotype in *E. coli* isolates owing to the severe impact of *E. coli* on human health.

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