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Original Article

Antimicrobial Susceptibility Patterns and Genetic Relatedness between Diarrheagenic *Escherichia coli* Pathotypes Isolated from Ready-to-Eat Olivier Salad and Clinical Samples

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ABSTRACT

Diarrheagenic *Escherichia coli* (DEC) strains are among the most prevalent bacteria transmitted through contaminated water and food, and are related to mild-to-severe diarrhea in humans**.** The present study aimed to evaluate the prevalence, antibiotic resistance profile, phototypes, and biofilm formation capacity of *E. coli* isolates obtained from Olivier Salad and clinical samples. A total of 246 samples—including Olivier salad and stool specimens—were collected in Tehran between March to August 2022. Microbiological and molecular diagnostic methods were used to detect DEC strains. Disk diffusion and biofilm formation methods were done to evaluate the antimicrobial resistance profile and biofilm formation capacity of the *E. coli* isolates. Overall, 16.6% (41/246) *of E. coli* isolates was attained from both Olivier Salad and clinical samples and the prevalence of DEC among these isolates was 17% (7/41). The identified DEC phototypes from the 41 isolates were as follows: enteropathogenic *E. coli* (EPEC, 4.8%), and enterotoxigenic *E. coli* (ETEC, 12.1%), while, no isolates of enteroaggregative *E. coli* (EAEC), enterinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EHEC) were found. The highest rate of resistance was found for amoxicillin (100%), and all DEC strains exhibited resistance to at least one antibiotic. Isolates obtained from clinical samples had more biofilm formation capacity than food samples. Our results evidenced the possibility of fecal contamination in animal-derived foods. Also, multi-drug resistances were found between DEC isolated from food that suggested animal-based foods would operate as the reservoir for multi-drug resistant bacteria. Monitoring DEC strains in both food and clinical samples is essential to improve food safety and prevent foodborne outbreaks.

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1. Introduction

Diarrheagenic *Escherichia coli* (DEC) strains as the major reason for mild-to-severe diarrhea in humans (1) and are classified into six pathogenic ,types including Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Enter invasive *E. coli* (EIEC), Shiga-like toxin-producing *E. coli* (STEC) and Diffusely adherent *E. coli* (DAEC) (2). DEC causes outbreaks that are mostly related to contaminated food chains.

Foodborne diseases have become a major public health concern, accounting for over 33 million illnesses and 420,000 deaths annually worldwide (2, 3). In Iran, ready-to-eat (RTE) foods such as Olivier salad are very common among the Iranian people due to their flavor and convenience. Nevertheless, RTE foods could be contaminated in the preparation, transportation, storage, and sale stages. RTE foods are usually consumed and eaten cold without additional heat treatment or washing.

Inappropriate conditions cause the growth of microorganisms in RTE foods ,including Olivier salad (4). During preparation, normal flora microorganisms such as *Staphylococcus aureus* may be transmitted to food (5). Vegetables are an additional main element in Olivier salad, which might be a source of *E. coli* and *Salmonella* transmission (2). Another bacterium is *Clostridium perfringens*, which causes food poisoning in industrial food products (1). Moreover, there are a lot of airborne fungal spores that can be transmitted to salads, thus leading to food decay and diseases in humans (1). Coliforms are often transmitted to salads over contaminated water by fecal waste or as a result of lack of hygiene factors during processing and storage procedure (2). Antibiotics are widely used to treat bacterial infections in humans and in veterinary medicine to decrease mortality , morbidity ,and the economic losses of bacterial infections (6, 7).

Consequently, the increasing use of antibiotics can be related to the increasing antibiotic resistance among foodborne pathogens (8). In the food industry, antibiotics are used to avoid diseases, promote the growth of farm animals, and increase feed efficacy in production livestock (9). When these antibiotics are used in low doses for a long time,it can lead to the selection and transmission of antibiotic-resistance genes to other microorganisms within the food chain (10). The development of multidrug-resistant (MDR) foodborne bacteria, including *E.* coli poses a serious public health concerns (11). Moreover, plant-founded foods, especially RTE foods, and salads have a vital role in the spread of antibiotic resistance and have become a critical problem. Previously, multi-drug resistant isolates and extended-spectrum beta-lactamase (ESBL) producing *E. coli* have been obtained from food items such as egg surfaces, meat, vegetable salad, raw fish, water, and milk (12).

To confirm broad food safety, research on pathogenic bacteria such as *E. coli* in food products should continue. *E. coli* infection treatment has become difficult because of rising incidence of MDR strains. Hence, the emergence of multidrug resistance strains of *E. coli* is a vital menace to public health (11). Thus, the present study evaluated the prevalence rate, pathotypes, biofilm formation, and antibiotic susceptibility profiles in *E. coli* strains retrieved from Olivier salad and clinical samples collected from Tehran Province (Iran).

2. Materials and Methods

2.1. Study Period And Location

This study was done from March to August 2022 at the Food Microbiology Research Center (FMRC), Tehran University of Medical Sciences, Iran.

2.2. Clinical samples Collection

A cross-sectional study was performed on diarrheic children <5 years, looking for diarrhea treatment at the Children's Medical Center in Tehran. A total of 123 diarrhea samples were collected in coded form and transferred in Cary–Blair medium under cold chain conditions to the microbiology laboratory.

2.3. Food Samples Collection

A total of 123 Olivier salad samples were aseptically received on a random basis from various markets of Tehran city between March to August 2022. The samples were immediately transported to suitable containers with ice and instantly moved to the food microbiology laboratory for further studies.

2.4. Isolation and Identification of *E. Coli* In Olivier Salad Samples

Ten grams of Olivier salad samples were added to 90 mL of lauryl sulfate broth double yielding a 1:10 sample dilution and placed at 37 °C for 24 hours. One milliliter of the diluted salad sample was added to *E. coli* broth (EC broth) (9 mL) with inverted Durham tubes and placed at 44°C for 24 hours. Then, gas-positive samples were injected into Peptone Water and placed at 44°C for 48 hours. Next, 10 µl of the indole-positive sample was

streaked on MacConkey agar (MAC, Merck) and placed at 37°C for 24 hours. Each probable *E. coli* colony on the MacConkey agar plate (pink to dark pink colonies) was selected and investigated through Gram-staining, sugar fermentation test, and traditional biochemical tests such as Oxidase, Motility, Citrate utilization, Indole production, Urease, Voges-Proskauer, Methyl red, and Lysine decarboxylase (2).

2.5. Isolation and Identification of *E. Coli* In Stool Samples

Isolate identification was done by inoculating onto MacConkey agar, and incubated at 37°C for 24 hours. Confirmation was finished by an above-mentioned microbiology method. Afterward, identified *E. coli* strains were stored in skim milk containing 20% glycerol for further analysis.

2.6. Polymerase chain reaction (PCR) assay

Genomic DNA was extracted from a sweep of five typical colonies of a portion of bacterial cultures using the High Pure PCR Template Preparation kit (Roche, Germany) according to the manufacturer's instructions . Assessment of the quantity and quality of each extracted DNA was evaluated by NanoDrop (Thermo Fisher Scientific; USA). The extracted DNA wasa surveyed using PCR assay with specific primers (Table 1).

PCRs were done in a 20µl final volume mixture comprising 10µl of Taq 2X master mix with 1.5mM MgCl2 (Ampliqon, Denmark), 2 µl of the template DNA, and a 10µM concentration of each primer. PCR conditions varied depending on each primer (Table 1) and then electrophoresis was done with 1.5% agarose gel TBE buffer at 90V. Finally, PCR products were visualized under UV light with gel documentation (Bio-rad, USA).

2.7. Antibiotic Susceptibility Test

The disc diffusion method was used to determine antibiotic susceptibility patterns based on the Clinical and Laboratory Standards Institute, (CLSI) guidelines(M100) (13). A total of 13 antibiotics (Mast, UK) were tested : ampicillin (10 µg), amoxicillin (20 µg), ciprofloxacin (5 µg), cefalotin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefalexin (30 µg), gentamicin (10 µg), kanamycin (30 µg), imipenem (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), and sulfamethoxazole-trimethoprim (TMP-SXT) (1/25 µg). *Escherichia coli* ATCC 25922 was used as control strain.

2.8. Biofilm Formation

Biofilm formation was assessed using crystal violet method. Briefly, overnight cultures of *E. coli* isolates were inoculated in trypticase-soy broth (TSB, Merck,) with 1% glucose (dilution of 1:10), and 200 µl of the suspension was inoculated in triplicate to flat-bottom microtiter plates and placed overnight at 37°C.

Non-adherent bacteria were removedby washing twice with phosphate-buffered saline (PBS). Each well containing biofilm was stained with 1% crystal violet and placed at 25°C for 30 minutes and then gently washed twice using PBS to remove the additional dye. By adding 200 µL of ethanol-acetone (80: 20, v/v) to each well, the dye bound to the attached cells was dissolved. The absorbance value was assessed at the wavelength of 570 nm (OD 570) by an ELISA reader. A sterile TSB medium with 1% glucose served as a negative control. Based on the adherence capacities, DEC strains were categorized into four different groups (14).

2.9. Statistical Analysis

The differences among the DEC strains collected from the two various sources were analyzed thru GraphPad Prism software 8 (GraphPad Software, Inc.) by applying a Chi-squared Test with Fisher's Exact Test. The significance level was considered at P value < 0.05.

3. Results

3.1. Identification of Isolates

All isolates were confirmed through phenotypic and molecular analyses using PCR targeting the *uidA* gene. A total of 41 *E. coli* isolates were recognized, comprising 25 isolates from clinical samples (stool) versus 16 ones from food samples (Olivier salad) (Table 2).

3.2. DEC detection and isolation

Diarrheagenic *E. coli* pathotypes are characterized by possessing unique virulence factors, which were identified in this study using PCR assays . The prevalence of DEC in 41 *E. coli* isolates was 17%. Overall, seven DEC strains were obtained from 246 samples (clinical and food). Overall, 24% (n=6) and 6.2% (n=1) DEC isolates were recovered from the stool and Olivier salad samples, respectively (Table 2). The majority of the DEC strains collected from 123 clinical samples related to ETEC (n=4) and EPEC (n=2), whereas only one DEC isolated from Olivier salad belonged to ETEC (Table 2).

3.3. Antibiotic Susceptibility

The antimicrobial resistance pattern of *E. coli* isolates is shown in Table 3. Briefly, the highest resistance frequency was detected for amoxicillin, which was 100% for both clinical and food samples. All *E. coli* isolates collected from the diarrhea and food samples showed susceptibility to gentamicin and TMP-SXT (Figure 1).

Table. 1. List of primers used in this study.

Target group	genes	sequence 5′→3′	Amplicon size (bp)	Annealing temperature	Reaction conditions	References
E. coli	*uid*A	F: ATGGAATTTCGCCGATTTTGC R: ATTGTTTGCCTCCCTGCTGC	187	58	Denaturation: 94 °C, 30 s Annealing: 58 °C, 30 s Extension: 72°C, 60 s	[38]
EPEC	*bfp*	F: CAATGGTGCTTGCGCTTGCT R: GCCGCTTTATCCAACCTGGT	167	43	Denaturation: 94 °C, 30 s Annealing: 43 °C, 30 s Extension: 72°C, 60 s	[39]
	eae	F: CATTATGGAACGGCAGAGGT R: ATCTTCTGCGTACTGCGTTCA	791	55	Denaturation: 94 °C, 60 s Annealing: 55 °C, 60 s Extension: 72°C, 60 s	[40]
ETEC	*stx*	F: GAACGAAATAATTTATATGT R: TTTGATTGTTACAGTCAT	906	43	Denaturation: 94 °C, 60 s Annealing: 43 °C, 90 s Extension: 72°C, 90 s	[41]
	lt	F: GCACACGGAGCTCCTCAGTC R: TCCTTCATCCTTTCAATGGCTTT	218	60	Denaturation: 94 °C, 90 s Annealing: 60 °C, 90 s Extension: 72°C, 90 s	[42]
EAEC	*astA*	F: CCATCAACACAGTATATCCGA R: GGTCGCGAGTGACGGCTTTGT	111	55	Denaturation: 95 °C, 30 s Annealing: 55 °C, 30 s Extension: 72°C, 30 s	[43]
EHEC	*eae*	F: CATTATGGAACGGCAGAGGT R: ATCTTCTGCGTACTGCGTTCA	791	55	Denaturation: 94 °C, 60 s Annealing: 55 °C, 60 s Extension: 72°C, 60 s	[40]
	stx	F: GAACGAAATAATTTATATGT R: TTTGATTGTTACAGTCAT	906	43	Denaturation: 94 °C, 60 s Annealing: 43 °C, 90 s Extension: 72°C, 90 s	[41]
	ehxA	F: CACACGGAGCTTATAATATTCTGTCA R: AATGTTATCCCATTGACATCATTTGACT	319	55	Denaturation: 94 °C, 90 s Annealing: 55 °C, 90 s Extension: 72°C, 30 s	[44]
EIEC	*ipaH*	F: GCTGGAAAAACTCAGTGCCT R: CAGTCCGTAAATTCATTCT	425	56	Denaturation: 94 °C, 60 s Annealing: 56 °C, 120 s Extension: 72°C, 60 s	[39]

Table. 2. Presence of *E. coli* and diarrheagenic *E. coli* in clinical and food samples.

Sample	*E. coli* (%)	DEC		
		Total (%) [a]	ETEC (%) [b]	EPEC (%) [b]
Olivier salad=123	16 (13)	1 (6)	1 (100)	0
Stool=123	25 (20)	6 (24)	4 (66.66)	2 (33.33)
Total N=246	41 (16.66)	7 (17)	5 (71.42)	2 (28.57)

a. Percentage based on the total *E. coli* detected in each sample.
b. Percentage based on the total diarrheagenic *E. coli* detected in each sample.

Table. 3. Antimicrobial resistance among the diarrheagenic *E. coli* strains.

	N (%)					
Antimicrobial agent	**Clinical**	**Olivier salad**	**Total *E. coli* isolates (N=41)**	**DEC strains N=7**	**Phenotype of resistance**	
					ETEC n=5	EPEC n=2
Aminoglycosides						
Gentamicin	0/25	0/16	0/41	0/7	0/5	0/2
β-Lactam						
Amoxicillin	25/25	16/16	41/41	7/7	5/5	2/2
Cephalosporins						
Ceftriaxone	7/25	5/16	14/41	5/7	4/5	1/2
Quinolones						
Ciprofloxacin	5/25	4/16	13/41	1/7	1/5	0/2
Nalidixic acid	5/25	4/16	9/41	6/7	4/5	2/2
Sulfonamides						
Co-trimoxazole (TMP-SMX)	0/25	0/16	0/41	0/7	0/5	0/2
Tetracyclines						
Tetracycline	3/25	1/16	4/41	0/7	0/5	0/2
Carbapenems						
Imipenem	1/25	0/16	1/41	0/7	0/5	0/2

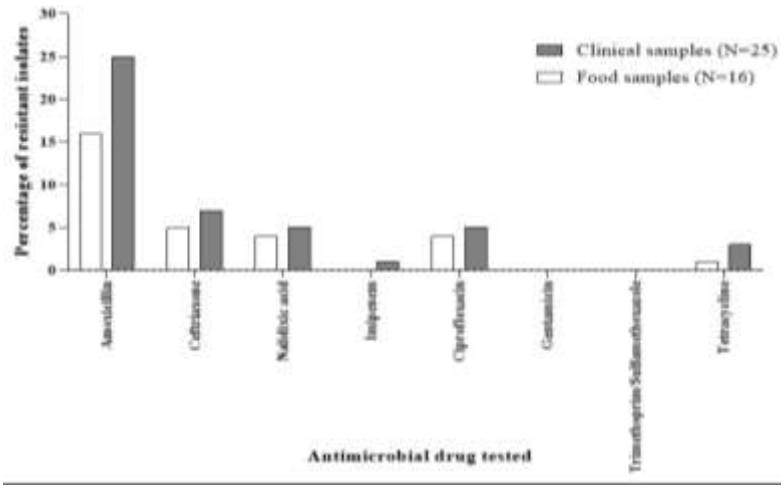

Figure. 1. Comparison of the resistance to antibiotics among *E. coli* isolates obtained from Olivier salad and clinical samples.

In addition, all *E. coli* isolates isolated from Olivier salad samples were also susceptible to imipenem.

As shown in Table 3, among the DEC groups, one ETEC strain isolated from Olivier salad samples was susceptible to all tested antibiotics except amoxicillin and ciprofloxacin. In contrast, all DEC strains isolated from the clinical sample were resistant to amoxicillin. Ceftriaxone was the most frequently resisted antibiotic among clinical isolates, with resistance observed in 100% of ETEC strains and 50% of EPEC strains . Moreover, 3 out of 4 (75%) ETEC and two EPEC strains obtained from clinical samples were resistant to nalidixic acid. A slight resistance rate (25%) was found for ciprofloxacin in the ETEC group. It is worth mentioning that both ETEC and EPEC strains were susceptible to gentamycin, imipenem, TMP-SXT, and tetracycline. Although a notable difference in the number of resistant isolates was observed between clinical and food-derived samples, this difference was not statistically significant among pathotypes($P > 0.05$).

3.3. Biofilm formation

The *E. coli* isolates were considered to evaluate their biofilm-formation ability using the microtiter plate method. In the study of biofilm formation in clinical isolates, it was found that three isolates can produce strong biofilm. Five and12 isolates were characterized to be moderate and weak biofilm producer, respectively. Finally, five isolates did not show the ability to form a biofilm (Figure 2). In *E. coli* isolate obtained from Olivier salad samples, only one isolate formed a strong biofilm. Finally, five and 10 isolates had moderate and weak capacity to form a biofilm, respectively. Overall, isolates obtained from clinical samples had more biofilm formation capacity than food samples. In DEC groups, all strains isolated from clinical samples were identified as weak or moderate biofilm producers except one strain with strong biofilm formation capacity (Table 4).

4. Discussion

Foodborne outbreaks are a major public health challenge and bacterial pathogens are the main cause of these diseases (1). The accessibility of RTE foods has a main role in city life if hygienic instructions are not followed during food preparation, food products can be a vector for the transmission of these pathogens and lead to a diverse variety of food-borne diseases (10).

Diarrheagenic *E. coli* is the main causative etiological agent of bacterial diarrhea worldwide. The amazing plasticity of the *E. coli* genome has permitted the development of pathotypes demonstrating specific antimicrobial resistance and virulence genes (2). Therefore, the occurrence of *E. coli* pathotypes and their antibiotic resistance vary among different regions. So, evaluating the antibiotic-resistant profile in *E. coli* pathotypes improves our understanding of antimicrobial resistance epidemiology. The current work was conducted to evaluate the spreading of *E. coli* pathotypes in food samples in contrast with clinical ones and to assess the the potential role of contaminated foods for the spread of DEC strains. Based on the result obtained from a six-monthsampling, of 123 food samples, 20% of contamination with *E. coli* was detected according to the identification of the *uidA* gene. This prevalence is similar to a study in the frequency of *E. coli* reported about 39.5% among RTE foods (15). However, in the Fallah et al. study, the prevalence of *E. coli* in food samples was reported to be 69% (16).

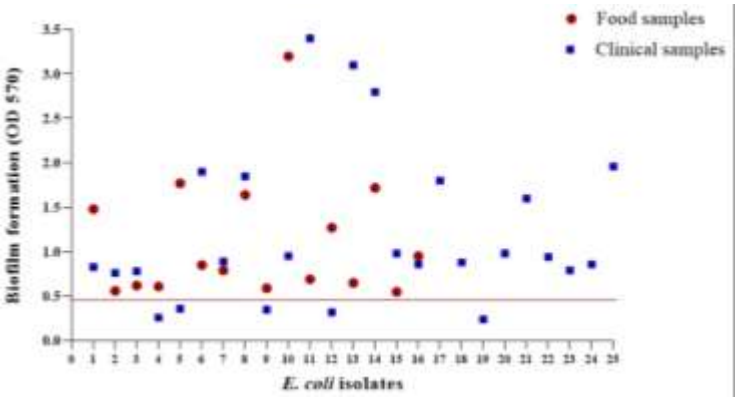

Figure. 2. Biofilm formation ability (OD570) of *E. coli* isolates obtained by the Microtiter plate method. OD cut-off (ODc) is a 0.49 (red line) which was used to differentiate between non, weak, moderate, and strong biofilm producer isolates.

Table 4. Characteristics of DEC strains recovered from food and human stool.

Source of *E. coli* isolate	Pathotype	Phenotype of resistance	No. Isolates in Each Pattern	Biofilm formation capacity
Olivier salad samples	ETEC	AMX- CIP	1	strong
Clinical samples	EPEC	AMX -NA	1	weak
Clinical samples	ETEC	AMX- CRO	1	weak
Clinical samples	EPEC	AMX- CRO-NA	1	moderate
Clinical samples	ETEC	AMX- CRO-NA	2	moderate
Clinical samples	ETEC	AMX- CRO-NA- CIP	1	strong

AMX, amoxicillin; CRO, ceftriaxone; NA, nalidixic acid; CIP, ciprofloxacin.

The results of *E. coli* prevalence rates show that poor food hygiene practices and few standard activities have been applied in food preparationand processing. On the other hand, in 123 stool samples obtained from children suffering from diarrhea, the frequency of *E. coli* isolates was 40%.

ETEC is the greatest common pathogen accountable for traveler´s diarrhea, which causes morbidity and mortality in children living in developing countries, along with in passengers traveling to these areas. In the previous study, the ETEC has been recognized as one of the main causes of diarrhea in children under five years old in Iran (17). The prevalence of ETEC in our food samples was 6.25%. It should be noted that the ETEC is often documented as a waterborne pathogen, rather than a foodborne one. Nevertheless, similar to the present outcomes, the ETEC isolation in food products has already been described in Colombia (18). According to the frequency of ETEC in the present study, it makes sense to consider that ETEC-contaminated RTE foods are the reason for diarrhea, especially in children. Nevertheless, none of the EIEC, EHEC, and EPEC pathotypes were isolated from 123 food samples assessed in the present work. Likewise, Fallah, et al. (2020) could not isolate any strains amongst 300 food samples (16).

Based on the results, 6 (24%) DEC isolates were found by PCR in clinical samples. In a study done by Hegde et al in India, a frequency rate of 26% DEC strains was reported from diarrheal samples collected from children (19). The DEC prevalence varies from area to area and even among different countries. Our data display the high frequency and low frequency of ETEC (16%) and EPEC (8%) in clinical isolates, respectively. Similar to the results of food samples, EIEC, EHEC, and EAEC strains were not isolated in clinical samples, while these pathotypes are regularly described as causes of diarrhea amongst children (20). In the current study, no EAEC, EIEC, and EHEC strains were isolated from clinical samples.

A low prevalence of EAEC and EIEC was formerly described in the studies of Ifeanyi et al, and Hegde et al, who recorded an occurrence of EAEC and EIEC in children with diarrhea at 2% and 1.5%, respectively (19, 21). However, in our study the frequency of *E. coli* pathotypes shows a relative decrease compared to other studies, probably due to the coincidence with the fifth peak of Corona disease in Iran. This coincidence caused a substantial reduction in the number of patients visiting the children's medical center, and parents prefer to take care of their children at home.

The results indicated that *E. coli* isolates were extremely resistant to amoxicillin and completely susceptible to gentamycin and TMP-SXT antibiotics. The development of antibiotic-resistant foodborne pathogens is the main concern for public health (11). An extensive diversity of antibiotics are currently administered globally to prevent and treat livestock diseases, and permit the development of MDR foodborne pathogens (16). The investigation of antibiotic resistance patterns discovered that all of the DEC strains showed resistance to at least one studied antibiotic. This alarming rate of antibiotic resistance may be attributed to the immethodical and unrestrained use of antibiotics, particularly in developing countries during the last decades.

The study of biofilm formation ability showed that one *E. coli* isolate collected from Olivier salad samples is a strong biofilm producer, with multi-drug resistance (MDR) to amoxicillin and ciprofloxacin. In addition, three *E. coli* isolates (12%) recovered from children's stool samples were strong biofilm producers as well as multi-drug resistant to the following antibiotics: TMP-SMX, amoxicillin, ciprofloxacin, nalidixic acid, and ceftriaxone. Among these strong biofilm producer isolates, there was a DEC strain that was resistant to amoxicillin, ceftriaxone, nalidixic acid, and ciprofloxacin.

Both clinical and food isolates have similar antibiotic resistance patterns which can suggest their transfer from food to humans or vice versa. As expected, the frequency of *E. coli* pathotypes in clinical samples was higher than those in food samples, and the same was true for antibiotic resistance. The resistance rate to antibiotics that are commonly used in clinical treatment was high, despite the isolates' susceptibility to the following three antibiotics: imipenem, gentamicin, and TMP-SMX. It is worth mentioning that the highest resistance rate was found for amoxicillin and ceftriaxone, so it is suggested to be more cautious in taking these antibiotics. Also, a high number of bacteria from the Enterobacteriaceae family were isolated from Olivier salad (data not shown), among which *E. coli* was an indicator bacterium. This level of contamination in food requires more monitoring and supervision of traditional food preparation.

Overall, the results showed great levels of fecal contamination in animal-derived foods origin, as well as contamination by DEC, particularly ETEC in Iran. It should be noted that ecological pollution with human sewage or contamination of human origin during the preparation process is the most possible source of pathotypes. Also, multi-drug resistances were found among DEC strains retrieved from RTE food that suggested food animals would operate as the reservoir for MDR bacteria. So, it is needed to study the health risks related to contamination with these MDR DEC, which could transfer the genes related to antibiotic resistance to other commensal inhabitants or pathogens of the human intestinal tract. The prevalence of ETEC and EPEC amongst human samples may reflect their prevalence in foods. The result that most DEC strains are resistant to > 3 antibiotics proposes that DEC is significant. The assessment of DEC strains in clinical samples, as well as in strains obtained from food products can improve food safety and prevent foodborne outbreaks.

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Authors' Contribution

Study concept and design: MM. S D, A. N, S Y.
Acquisition of data: A. N.
Analysis and interpretation of data: S. K.
Drafting of the manuscript: S. K.
Critical revision of the manuscript for important intellectual content: S. K.
Statistical analysis: S. K.
Administrative, technical, and material support: MM. SD.

Ethics

This study has the ethics code IR.TUMS.SPH.REC.1400.323.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data that support the findings of this study are available in the manuscript.

Consent for publication

The authors declare that they consent for publication of this study.

References

1. Soltan-Dallal MM, Karami-Talab M, Aminshahidi M, Arastehfar A, Fani F. Antimicrobial susceptibility patterns of Enteroaggregative E. coli, as the most common diarrheagenic E. coli, associated to gastroenteritis outbreaks in Iran. Archives of Pediatric Infectious Diseases. 2018;6(2)

2. Moeinirad M, Douraghi M, Foroushani AR, Sanikhani R, Dallal MMS. Molecular characterization and prevalence of virulence factor genes of Shiga toxin-producing Escherichia coli (STEC) isolated from diarrheic children. Gene Reports. 2021;25:101379

3. Soltan Dallal MM, Karimaei S, Hajighasem M, Hashemi SJ, Rahimi Foroushani A, Ghazi-Khansari M, et al. Evaluation of zinc oxide nanocomposite with Aloe vera gel for packaging of chicken fillet against Salmonella typhi and Salmonella para typhi A. Food Science & Nutrition. 2023;11(10):5882-9

4. Peidaei F, Ahari H, Anvar A, Ataei M. Nanotechnology in Food Packaging and Storage: A Review. Iranian Journal of Veterinary Medicine. 2021;15(2)

5. Karimaei S, Aghamir SMK, Pourmand MR. Comparative analysis of genes expression involved in type II toxin-antitoxin system in Staphylococcus aureus following persister cell formation. Molecular Biology Reports. 2024;51(1):324

6. Karimaei S, Mashhadi R, Mirzaei A, Deyhimfar R, Shabestari AN, Rahimnia R. Antibacterial and antibiofilm activities of nisin from Lactococcus lactis and alteration of the bacteria-induced pro-inflammatory responses on kidney and bladder tumor cell lines. Translational Research in Urology. 2022;4(1):47-53

7. Ashrafi F, Azari AA, Fozouni L. Prevalence and Antibiotic Resistance Pattern of Mannheima haemolytica and Pasteurella multocida Isolated from Cattle Lung Samples from an Industrial Abattoir: A Study from Northeastern Iran. Iranian Journal of Veterinary Medicine. 2022;16(4)

8. Hassani S, Moosavy MH, Gharajalar SN, Khatibi SA, Hajibemani A, Barabadi Z. High prevalence of antibiotic resistance in pathogenic foodborne bacteria isolated from bovine milk. Sci Rep. 2022;12(1):3878.https://doi.org/10.1038/s41598-022-07845-6.

9. Ema FA, Shanta RN, Rahman MZ, Islam MA, Khatun MM. Isolation, identification, and antibiogram studies of Escherichia coli from ready-to-eat foods in Mymensingh, Bangladesh. Vet World. 2022;15(6):1497-505.https://doi.org/10.14202/vetworld.2022.1497-1505.

10. Niroumand A, Razavizadeh SAT, Jamshidi A, Moghadam JA. A Survey on Drinking Water Contamination to Indicator Bacteria in Dairy Farms of Mashhad Suburb. Iranian Journal of Veterinary Medicine. 2020;14(3)

11. Rashid M, Kotwal SK, Malik M, Singh M. Prevalence, genetic profile of virulence determinants and multidrug resistance of Escherichia coli isolates from foods of animal origin. Veterinary World. 2013;6(3):139-42

12. Sivakumar M, Abass G, Vivekanandhan R, Anukampa, Singh DK, Bhilegaonkar K, et al. Extended-spectrum beta-lactamase (ESBL) producing and multidrug-resistant Escherichia coli in street foods: a public health concern. J Food Sci Technol. 2021;58(4):1247-61.https://doi.org/10.1007/s13197-020-04634-9.

13. Wayne P. Performance standards for antimicrobial susceptibility testing. Ninth informational supplement NCCLS document M100-S9 National committee for clinical laboratory standards. 2008:120-6

14. Karimaei S, Aghamir SMK, Foroushani AR, Pourmand MR. Antibiotic tolerance in biofilm persister cells of Staphylococcus aureus and expression of toxin-antitoxin system genes. Microbial Pathogenesis. 2021;159:105126
15. Zhang S, Wu Q, Zhang J, Lai Z, Zhu X. Prevalence, genetic diversity, and antibiotic resistance of enterotoxigenic Escherichia coli in retail ready-to-eat foods in China. Food Control. 2016;68:236-43
16. Fallah N, Ghaemi M, Ghazvini K, Rad M, Jamshidi A. Occurrence, pathotypes, and antimicrobial resistance profiles of diarrheagenic Escherichia coli strains in animal source food products from public markets in Mashhad, Iran. Food Control. 2021;121:107640
17. Alizade H, Teshnizi SH, Azad M, Shojae S, Gouklani H, Davoodian P, et al. An overview of diarrheagenic Escherichia coli in Iran: A systematic review and meta-analysis. Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences. 2019;24
18. Amézquita-Montes Z, Tamborski M, Kopsombut UG, Zhang C, Arzuza OS, Gómez-Duarte OG. Genetic Relatedness Among Escherichia coli Pathotypes Isolated from Food Products for Human Consumption in Cartagena, Colombia. Foodborne Pathog Dis. 2015;12(5):454-61.https://doi.org/10.1089/fpd.2014.1881.
19. Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic Escherichia coli by multiplex PCR. Indian Journal of Medical Microbiology. 2012;30(3):279-84
20. Santona S, Diaz N, Fiori PL, Francisco M, Sidat M, Cappuccinelli P, et al. Genotypic and phenotypic features of enteropathogenic Escherichia coli isolated in industrialized and developing countries. J Infect Dev Ctries. 2013;7(3):214-9.https://doi.org/10.3855/jidc.3054.
21. Ifeanyi CIC, Ikeneche NF, Bassey BE, Al-Gallas N, Aissa RB, Boudabous A. Diarrheagenic Escherichia coli pathotypes isolated from children with diarrhea in the Federal Capital Territory Abuja, Nigeria. The Journal of Infection in Developing Countries. 2015;9(02):165-74