# 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 the most prevalent bacteria conveyed by using contaminated water and foods and are related to mild-to-severe diarrhea in humans. The present study aimed to consider the prevalence, antibiotic resistance profile, phototypes, and biofilm formation capacity of *E. coli* isolates retrieved from Olivier Salad and clinical samples. The current study was performed on 246 samples containing Olivier salad and stool samples collected in Tehran from 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 was 17% (7/41). The DEC phototypes obtained from the 41 isolates were as follows: enteropathogenic *E. coli* (EPEC): 4.8%, and enterotoxigenic *E. coli* (ETEC): 12.1%. Also, no 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 amongst the DEC strains, all strains exhibited resistance to at least one antibiotic. Isolates obtained from clinical samples had more biofilm formation capacity than food samples. Our finding evidenced the possibility of fecal contamination in foods of animal origin. Also, multi-drug resistances were found between DEC isolated from food that suggested animal-based foods would operate as the reservoir for multi-drug resistance bacteria. Therefore, the assessment of DEC strains obtained from food samples, as well diarrhea samples can improve food safety and prevent foodborne outbreaks.

**Keywords:** *Escherichia coli*; Antimicrobial resistance; Ready-to-eat foods, Biofilms; Diarrhea

## 1. Introduction

Diarrheagenic *Escherichia coli* (DEC) strains as the major reason for mild-to-severe diarrhea in humans (1) 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 related to contaminated food chains.

Foodborne diseases have become a major public health concern that causes more than 33 million illnesses and 420,000 fatalities annually worldwide (2, 3). The use of ready-to-eat (RTE) foods for instance Olivier salad is very common among the Iranian people due to its taste and easy preparation. Nevertheless, RTE foods could be contaminated in the preparation, transportation, storage, and sale stages. RTE foods are usually consumed and eaten cold without any extra heat treatment or washing.

Inappropriate conditions cause the growth of microorganisms in RTE foods including Olivier salad (4). In the preparation steps, 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 fungal spores in the air 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 the absence of hygiene factors in the processing and storage procedure (2).

Antibiotics have been administered in the handling of bacterial infections in humans and in veterinary medicine to decrease mortality and morbidity and the economic outcome of bacterial infections (6, 7). Consequently, the increasing use of antibiotics can be related to the increasing antibiotic resistance in 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 animals (9). When these antibiotics are used in low doses for a long time there may be a consequence of the selection and transmission of antibiotic-resistance genes to other microorganisms in the food chain (10). The development of multidrug-resistant (MDR) foodborne bacteria including *E. coli* caused public health concerns (11). Moreover, plant-founded foods, especially RTE foods, and salads have a vital character in antibiotic resistance spread 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 the 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 2022 to August 2022 at the Food Microbiology Research Center (FMRC), Tehran University of Medical Sciences, Tehran, Iran.

### 2.2. Clinical samples Collection

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

### 2.3. Food Samples Collection

Overall, 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 either 90 mL of lauryl sulfate broth double yielding a 1:10 sample dilution and placed at 37 °C for 24 h. 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 h. Then, gas-positive samples were injected into Peptone Water and were placed at 44°C for 48 h. Next, 10 µl of the indole-positive sample was streaked on MacConkey agar (MAC, Merck) and placed at 37°C for 24 h. 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 h. Confirmation was finished by a microbiology method as mentioned above. Afterward, identified *E. coli* strains were kept in skim milk, with 20% glycerol for further analysis.

### 2.6. Polymerase chain reaction (PCR) assay

Genomic DNA was obtained from a sweep of about five typical colonies of a portion of bacterial cultures by using the High Pure PCR Template Preparation kit (Roche, Germany) based on the instructions of the manufacturer. Assessment of the quantity and quality of each extracted DNA was evaluated by NanoDrop (Thermo Fisher Scientific; USA). The extracted DNA were surveyed by 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 were different for 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).

**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<br>R: ATTGTTTGCCTCCCTGCTGC | 187 | 58 | Denaturation: 94 °C, 30 s<br>Annealing: 58 °C, 30 s<br>Extension: 72°C, 60 s | (13) |
| EPEC | *bfp* | F: CAATGGTGCTTGCGCTTGCT<br>R: GCCGCTTTATCCAACCTGGT | 167 | 43 | Denaturation: 94 °C, 30 s<br>Annealing: 43 °C, 30 s<br>Extension: 72°C, 60 s | (14) |
| | *eae* | F: CATTATGGAACGGCAGAGGT<br>R: ATCTTCTGCGTACTGCGTTCA | 791 | 55 | Denaturation: 94 °C, 60 s<br>Annealing: 55 °C, 60 s<br>Extension: 72°C, 60 s | (14) |
| ETEC | *stx* | F: GAACGAAATAATTTATATGT<br>R: TTTGATTGTTACAGTCAT | 906 | 43 | Denaturation: 94 °C, 60 s<br>Annealing: 43 °C, 90 s<br>Extension: 72°C, 90 s | (14) |
| | *lt* | F: GCACACGGAGCTCCTCAGTC<br>R: TCCTTCATCCTTTCAATGGCTTT | 218 | 60 | Denaturation: 94 °C, 90 s<br>Annealing: 60 °C, 90 s<br>Extension: 72°C, 90 s | (14) |
| EAEC | *astA* | F: CCATCAACACAGTATATCCGA<br>R: GGTCGCGAGTGACGGCTTTGT | 111 | 55 | Denaturation: 95 °C, 30 s<br>Annealing: 55 °C, 30 s<br>Extension: 72°C, 30 s | (15) |
| EHEC | *eae* | F: CATTATGGAACGGCAGAGGT<br>R: ATCTTCTGCGTACTGCGTTCA | 791 | 55 | Denaturation: 94 °C, 60 s<br>Annealing: 55 °C, 60 s<br>Extension: 72°C, 60 s | (14) |
| | *stx* | F: GAACGAAATAATTTATATGT<br>R: TTTGATTGTTACAGTCAT | 906 | 43 | Denaturation: 94 °C, 60 s<br>Annealing: 43 °C, 90 s<br>Extension: 72°C, 90 s | (14) |
| | *ehxA* | F: CACACGGAGCTTATAATATTCTGTCA<br>R: AATGTTATCCCATTGACATCATTTGACT | 319 | 55 | Denaturation: 94 °C, 90 s<br>Annealing: 55 °C, 90 s<br>Extension: 72°C, 30 s | (14) |
| EIEC | *ipaH* | F: GCTGGAAAAACTCAGTGCCT<br>R: CAGTCCGTAAATTCATTCT | 425 | 56 | Denaturation: 94 °C, 60 s<br>Annealing: 56 °C, 120 s<br>Extension: 72°C, 60 s | (16) |

### 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, M100) guidelines (17). The 13 antibiotics (Mast, UK) tested were as follows: 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). Also, the reference strain (*E. coli* ATCC 25922) was considered as a control.

### 2.8. Biofilm formation

The 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. Then, non-adherent bacteria were removed, by twice washing with phosphate-buffered saline (PBS). Each well comprising biofilm was stained with 1% crystal violet and placed at 25 °C for 30 min and was then gently washed twice using PBS to remove the additional dye. Afterward, 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 comprising 1% glucose was considered as a negative control. Based on the adherence capacities, DEC strains were considered into four different groups (18).

### 2.9. Statistical Analysis

The differences among the DEC strains retrieved 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 a P value $< 0.05$.

## 3. Result

### 3.1. Identification of isolates

The isolates were confirmed by phenotypic as well as molecular analyses using PCR targeting the *uidA* gene. In total; 41 *E. coli* isolates were recognized which, 25 isolates were obtained from clinical samples (stool) versus 16 ones from food samples (Olivier salad) (Table 2).

**Table. 2.** Attendance of 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

### 3.2. DEC detection and isolation

Diarrheagenic *E. coli* pathotypes are characterized by possessing unique virulence factors. To identify them, a PCR assay was done. 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 recovered from the stool and Olivier salad samples, respectively (Table 2). The majority of the DEC strains obtained from the 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 retrieved from the diarrhea and food samples showed susceptibility to gentamicin and TMP-SXT (Fig. 1). In addition, all *E. coli* isolates isolated from Olivier salad samples were also susceptible to imipenem.

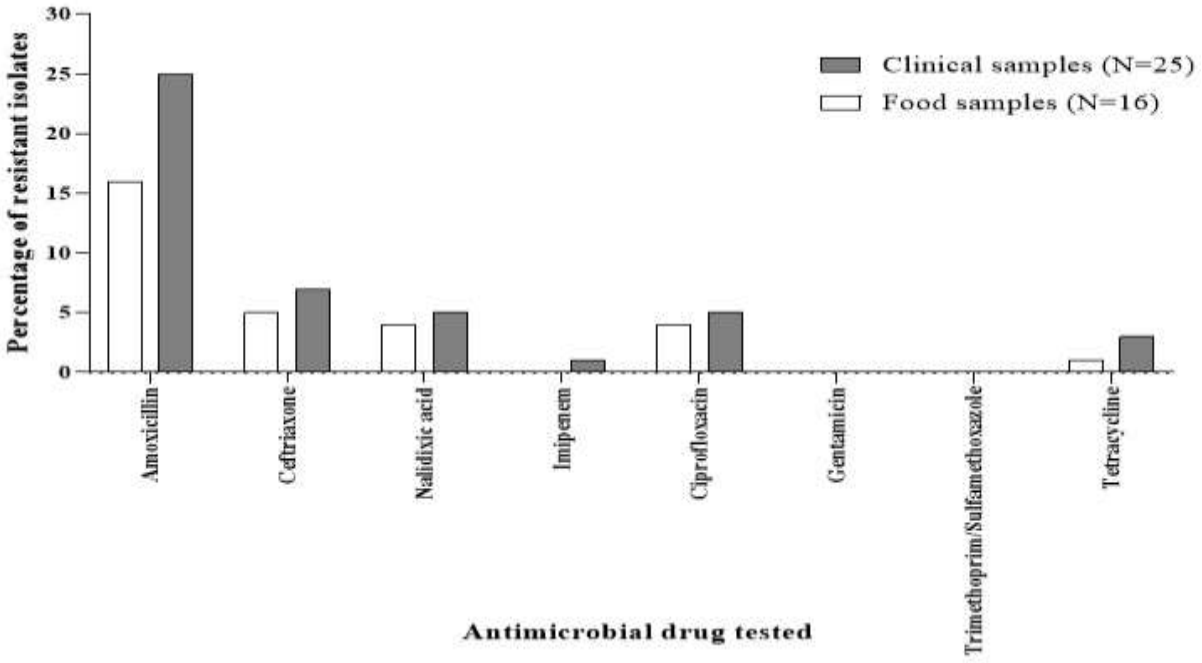


**Figure. 1.** Comparison of the resistance to antibiotics among *E. coli* isolates obtained from Olivier salad and clinical samples.

As shown in Table 3, in DEC groups, one ETEC strain isolated from Olivier salad samples was susceptible to all tested antibiotics except amoxicillin and ciprofloxacin. All DEC strains isolated from the clinical sample were resistant to amoxicillin. Also, among clinical isolates, ceftriaxone was the most common antimicrobial agent in resistance patterns with 50% and 100% of EPEC, and ETEC strains resistant to it, respectively. Moreover, 3 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. A substantial difference was noticed in the number of resistant isolates among clinical and food (Olivier salad) ($P$>0.05). Although this difference was not substantial amongst pathotypes.

**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 |

### 3.4. Biofilm formation

The *E. coli* isolates were considered to evaluate their biofilm-formation ability by a 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 (Fig. 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).

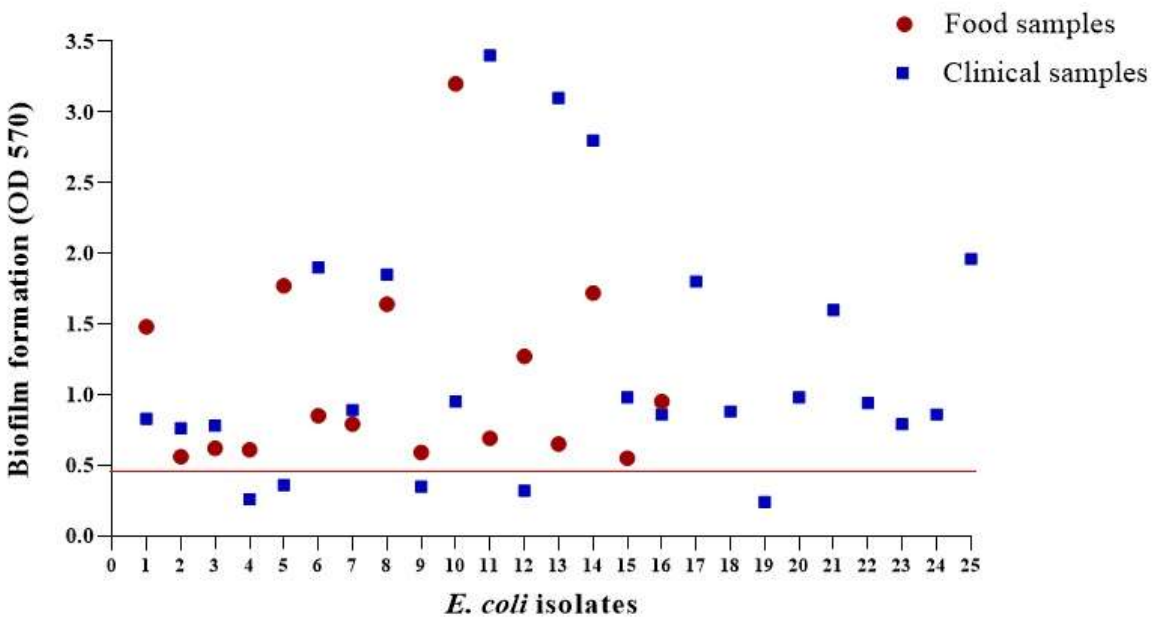


**Figure. 2.** Biofilm formation ability (OD570) of *E. coli* isolates obtained by the Microtiter plate method. OD cut-off (ODc) is a 0.49 (red line) which was used to differentiate between non, weak, moderate, and strong biofilm producer isolates.

**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.

## 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 vehicle 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 are diverse in 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, as well as to assess the possibility of foods being a carrier for the spread of DEC strains. Based on

the result obtained from a six-month-sampling, 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 (19). However, in the Fallah et al. study, the prevalence of *E. coli* in food samples was reported as 69% (20). The results of *E. coli* prevalence rates show that poor food hygiene practices and few standard activities have been applied in food preparation, and 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 who live 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 (21). 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 (22). 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 (20).

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 obtained from children (23). 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 (24). 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 (23, 25). However, in our study, the frequency of *E. coli* pathotypes shows a relative decrease compared to other studies, which is 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 with home care.

The results indicated that *E. coli* isolates were extremely resistant to amoxicillin as well as 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, permitting the development of MDR foodborne pathogens (20). 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 ascribed to the immethodical and unrestrained use of antibiotics particularly in developing countries in the last decades.

The study of biofilm formation ability showed that one *E. coli* isolate obtained from Olivier salad samples is a strong biofilm producer as well as has 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 resistance 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 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 showing susceptibility to the following three antibiotics, imipenem, gentamicin, and TMP-SMX. It is worth mentioning that the highest resistance rate was found for the antibiotics amoxicillin and ceftriaxone, so it is suggested to be more cautious in using these antibiotics. Also, a high number of bacteria from the Enterobacteriaceae family was 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 foods of animal 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 worth monitoring. 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.

**Author contributions**

Study concept and design: MM. Soltan Dallal, A Nasser, and S Yaslianifard. Drafting of the manuscript and Critical revision of the manuscript for important intellectual content: S Karimaei. Investigation: A Nasser Statistical analysis: S Karimaei. Study supervision: MM. Soltan Dallal.

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**Ethics approval statement**

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

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**Consent for publication**

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

**Data availability statement**

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

**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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**Figure legends**

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

**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.