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Evaluation of ERIC-PCR method for determining genetic diversity among *Escherichia coli* isolated from human and retail imported frozen shrimp and beef

Afnan Alsultan[1] and Nasreldin Elhadi[1*]

Abstract

There is a global concern and increasing reports regarding foodborne disease infections associated with consuming contaminated vegetables, seafood, meat, and poultry products. Among foodborne bacterial pathogens globally, *Salmonella*, *Escherichia coli*, and *Shigella* were the most frequently implicated in causing food poisoning infections in children and adults. In Saudi Arabia, the consumption rates of imported fresh fruits, vegetables, seafood, and meat products are considered high. Therefore, the development of simple PCR based DNA fingerprinting methods is essential to track the source and route of microbial contamination among imported frozen meat and seafood products. A total of 38 *E. coli* strains were subtyped using ERIC1R, ERIC2, and a pair combination (ERIC1R + ERIC2) to generate genomic fingerprinting. The three Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR)-based primers were generated in 26, 24, and 16 different genotypes while using ERIC1R, ERIC2, and ERIC1R + ERIC2, respectively. The Discrimination Index values obtained by ERIC1R, ERIC2, and ERIC1R + ERIC2 were 0.976, 0.965, and 0.903, respectively. ERIC1R and ERIC2 primers had the best discriminatory ability and typeability value and proved suitable for investigating genetic analysis among the population of *E. coli* strains. At the same time, the ERIC1R + ERIC2 primer pair has average discriminatory power and typeability value for differentiating *E. coli* strains. These results suggest that subtyping using ERIC1R and ERIC2 primer is a more reliable and rapid typing strategy for *E. coli* strains.

Keywords Molecular typing, *Escherichia coli*, Epidemiologic analysis, ERIC-PCR

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Introduction

Escherichia coli is a Gram-negative rod-shaped bacterium, facultative anaerobic and classified as a member of the Enterobacteriaceae family. This bacterium is mainly of fecal origin and inhabits the digestive system of humans and animals and is usually released into environment and meanwhile found in municipal wastewater discharge effluent (Jang et al. 2017). In humans, *E. coli* are responsible for causing infections known as extraintestinal pathogenic *E. coli* (ExPEC) and intestinal pathogenic *E. coli* (IPEC). The ExPEC are important causes of septicemia, urinary tract infection, pneumonia, peritonitis, wound infection, pneumonia, and a leading cause of meningitis in neonates (Bonten et al. 2021; Leimbach et al. 2013; Pitout 2012; Russo and Johnson 2003). *E. coli* has been documented is the most common cause of severe invasive bacteremia and sepsis in high-income countries, and these infections are exceeding other leading bacteremia causing pathogens such as *Staphylococcus aureus* and *Streptococcus pnumoniae* in older ages populations (Poolman and Anderson 2018; Russo and Johnson 2003; Søgaard et al. 2011). The IPEC are pathotypes associated with intestinal infections and are also known as diarrheagenic *E. coli* (DEC).

Several molecular typing methods have been used to track the source and route of microbial contamination (Ferrari et al. 2017; Foley et al. 2004; Kabuki et al. 2004; Nakamura et al. 2021; Ramadan et al. 2020). These methods included the pulsed-field gel electrophoresis (PFGE), ribotyping, multi-locus variable-number tandem repeat analysis (MLVA), multilocus sequence typing (MLST) and, whole genome sequencing (WGS). However, choosing an appropriate bacterial genotyping method depends on their own advantages and limitations concerning the availability of instrumentation, speed, cost, strengths, sensitivity, and user-friendly databases (Chen and Perfect 2017; Ramadan 2022; Zou et al. 2013). For this reason, finding a simple PCR-based method such as enterobacterial repetitive intergenic consensus (ERIC) and have been widely used because it's easier, faster, and cheaper than PFGE or MLST for generating information about the genetic similarity of bacterial strains (Meacham et al. 2003; Souza et al. 2010).

This study focuses on a practical approach to evaluate and optimize ERIC-PCR typing for *E. coli* strains based on a widely recognized protocol by Versalovic et al. (1991), which improves the discriminatory power of the ERIC-PCR typing method. This study compared the genetic similarities of strains isolated from humans and strains isolated from frozen beef imported from India and frozen shrimp imported from China and Vietnam. The ERIC primers have been evaluated as a useful molecular typing method for determining the genetic relationship between strains of *E. coli* isolated and collected from different sources.

Materials and methods

Bacterial strains and cultivation

Thirty-eight strains of *E. coli* were used in this study, and their sources are described in Table 1. Among the 38 isolates of *E. coli* analyzed in this study, six of the collected strains were isolated from humans (Elhadi et al. 2020). Moreover, five, 13, and 14 strains were isolated from marketed retailed frozen beef imported from India and frozen shrimp imported from Vietnam and China, respectively. All strains isolated from frozen beef and shrimp were collected from our previous study on a microbiological examination of foodborne pathogens in imported frozen beef and shrimp in the Eastern Province of Saudi Arabia (unpublished data). All strains were reconfirmed to species-level identification by culturing on MacConkey agar (Oxoid, UK) and standard biochemical tests such as indole test and API 20E (BioMe'rieux, France) were used.

DNA extraction

All bacterial strains were sub-cultured and grown overnight on Tryptic soy agar (Oxoid, UK) plates. Before each DNA extraction, single colonies were picked from Tryptic soy agar (Oxoid, UK), inoculated into 5 ml of Luria Bertani (LB) broth, and incubated for 24 h at 37 °C. DNA was extracted with Wizard genomic DNA purification kit (Promega, USA). Steps of genomic DNA extraction from Gram-negative bacteria were performed according to the kit manufactural recommended protocol. All extracted DNA from all strains was kept and stored at -20°C for further use. The concentration of extracted DNA from all strains was measured and quantified by using the Quantus Fluorometer with the Quantifluor dsDNA system (Promega, USA). Steps of extracted DNA concentrations measurement were performed according to manufactural instructions of Quantus Fluorometer operating manual.

ERIC-PCR

E. coli strains were fingerprinted using ERIC-PCR with the following ERIC primers as described by Versalovic et al. (1991): ERIC1R primer (59-ATG TAA GCT CCT GGG GAT TCA C-39), ERIC2 (59-AAGTAAGT-GACTGGGGTGAGCG-39) and the combined ERIC 1R+ERIC 2 primer pair. All ERIC primers in this study were synthesized and purchased from Eurofins Genomics (Ebersberg, Germany). The PCR reaction mixture volume was 25 µl per reaction and consisted of 12.5 µl of GoTaq G2 Master Mixes (Promega, USA), 7.5 µl of nuclease-free water (Promega, USA), 2 µl of each ERIC primer, and 3 µl of 100 ng DNA template. ERIC-PCR amplification was performed in the Biorad T100 thermocycler (Biorad, USA) as described by Versalovic et al. (1991). Briefly, the initial denaturation at 95°C for 5 min, followed by 35 cycles of 10 min denaturation at 96°C,

Table 1 Genotypic patterns of unrelated *E. coli* strains by using Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) fingerprinting

Strain no.	Strain code	Isolation source	Country	Genotypes		
				ERIC-1R	ERIC-2	ERIC-1R+ERIC2
1	ECSV1	Imported frozen shrimp	Vietnam	A26	B24	C16
2	ECSV2	Imported frozen shrimp	Vietnam	A20	B23	C14
3	ECSV3	Imported frozen shrimp	Vietnam	A6	B18	C3
4	ECSV4	Imported frozen shrimp	Vietnam	A3	B2	C7
5	ECSC5	Imported frozen shrimp	China	A18	B17	C12
6	ECSC6	Imported frozen shrimp	China	A18	B17	C12
7	ECSC7	Imported frozen shrimp	China	A19	B17	C12
8	ECSC8	Imported frozen shrimp	China	A17	B18	NT*
9	ECSC9	Imported frozen shrimp	China	A18	B17	C12
10	ECSC12	Imported frozen shrimp	China	A19	B6	NT
11	ECSC13	Imported frozen shrimp	China	A19	B6	C12
12	ECSC14	Imported frozen shrimp	China	A1	B1	NT
13	ECSC15	Imported frozen shrimp	China	A12	B21	C16
14	ECSC16	Imported frozen shrimp	China	A18	B19	C15
15	ECSV17	Imported frozen shrimp	Vietnam	A11	B19	C11
16	ECSC18	Imported frozen shrimp	China	A8	B9	C15
17	ECSV19	Imported frozen shrimp	Vietnam	A16	B19	C12
18	ECSV20	Imported frozen shrimp	Vietnam	A15	B21	C16
19	ECSC21	Imported frozen shrimp	China	A23	B21	C13
20	ECSV22	Imported frozen shrimp	Vietnam	A21	B15	NT
21	ECSV23	Imported frozen shrimp	Vietnam	A21	B13	C15
22	ECSC24	Imported frozen shrimp	China	A25	B22	C15
23	ECSV25	Imported frozen shrimp	Vietnam	A25	B3	C12
24	ECSV27	Imported frozen shrimp	Vietnam	A21	B3	C14
25	ECSV28	Imported frozen shrimp	Vietnam	A24	B12	C12
26	ECSV29	Imported frozen shrimp	Vietnam	A7	B16	NT
27	ECSC30	Imported frozen shrimp	China	A5	B15	C10
28	ECSS31	Human	Saudi Arabia	A5	B5	C9
29	ECSS32	Human	Saudi Arabia	A14	B20	C8
30	ECSS33	Human	Saudi Arabia	A22	B14	C2
31	ECSS34	Human	Saudi Arabia	A4	B8	C1
32	ECSS35	Human	Saudi Arabia	A2	B15	C7
33	ECSS36	Human	Saudi Arabia	A9	B7	NT
34	ECBI37	Imported frozen Beef	India	A9	B4	C6
35	ECBI38	Imported frozen Beef	India	A3	B19	C3
36	ECBI40	Imported frozen Beef	India	A13	B11	C5
37	ECBI41	Imported frozen Beef	India	A13	B10	C5
38	ECBI42	Imported frozen Beef	India	A8	B15	C6
Number of types				26	24	16
D value★				0.976	0.965	0.903

*NT; non-typeable

★Simpson's index of diversity equation

annealing for 1 min at 52°C, and extension for 1 min at 72°C. The final extension step was carried out for 10 min at 72°C. Amplified products of ERIC1R, ERIC2, and combined ERIC1R+ERIC2 were separated with 1.5% agarose gel electrophoresis containing 1X TBE buffer (Promega, USA) at 80 volts for 120 min and 1 Kb GelPiolt plus ladders (Qiagen, Germany) and used as molecular markers. Electrophoresed agarose gel photography was captured using a Gel documentation system (G: Box, Syngene, UK).

Analysis of ERIC-PCR fingerprinting patterns

The pattern of DNA fingerprint bands generated by ERIC-PCR was analyzed using GelJ software (Heras et al. 2015). GelJ is a convenient software and appropriate for constructing phylogenetic trees and

dendrograms to analyze gel electrophoresis DNA and fingerprint bands. Clustering analysis was designed with GelJ software based on the Dice similarity coefficient, and the unweighted pair group method with arithmetic mean (UPGMA) to compare the discriminatory power of ERIC1R, ERIC2, and ERIC1R + ERIC2 primers.

Evaluation of ERIC-PCR as a typing tool

The discriminatory power of each fingerprinting among the 38 unrelated strains was calculated for ERIC1R, ERIC2, and ERIC1R + ERIC2 using the Simpson's index of diversity equation (Hunter and Gaston 1988):

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j(n_j - 1)$$

where D is the discriminatory power index, N is the total number of isolates, *s* is the total number of types, and *nj* is the number of strains belonging to the *j*th. Therefore, the discriminatory power index will be calculated from the distribution types of each isolate and based on the homogeneity and frequency distribution of isolate types. Thus, each isolate should have a different type (DI = 1), and typeable isolates will be grouped by achieving a DI of more than 0.90 for a reliable assessment of the clonal relatedness of isolates (Struelens 1996). The typeability of tested strains in this study was calculated according to the formula described by (Struelens 1996): Typeability (T) = T = N_t / N, N_t is the number of isolates designated a type and N is the number of isolates tested. The typeability of ERIC1R, ERIC2, and ERIC1R + ERIC2 was defined as the percentage of typeable isolates among the 38 unrelated strains.

Results and discussion

ERIC-PCR

Three ERIC primers (ERIC1R, ERIC2, and a combination of ERIC1R + ERIC2) were evaluated to investigate the genetic relationship among 38 strains of *E. coli* isolated from different sources and geographic origins, as shown in Table 1. The dendrogram of the obtained ERIC-PCR fingerprint pattern results was constructed using GelJ software version 2.0 to determine the strain's genetic relatedness. Several studies have used ERIC-PCR and other PCR-based typing methods to differentiate between clonally related and unrelated bacterial strains to investigate epidemic clones circulated in the population and responsible for outbreaks (Struelens 1998). The study was designed to assess the discriminatory power of ERIC primers and to find out the genetic linkage between *E. coli* strains isolated from humans and strains isolated from frozen beef imported from India and frozen shrimp imported from China and Vietnam, respectively.

ERIC1R-PCR fingerprint pattern analysis

Among the 38 strains of *E. coli* analyzed by ERIC1R primer were generated fingerprint bands ranging in size from 250 to 1400 bp, and each profile consisted of three to ten bands (Fig. 1-A). The DNA band patterns produced by ERIC1R primer were analyzed using the Dice coefficient. Numerical analysis of ERIC profiles revealed eight identical clusters (A-1 to A-8), and 26 genotypes (A1-26 A) were obtained, as presented in Table 1; Fig. 2. Of the 38 strains, 20 (52.6%) strains were grouped within these eight clusters, and the highest number of strains were seen in clusters A-5, A-6, and A-7. Other clusters from A-1 to A-4 and A-8 were composed of two strains each (Fig. 2). Among the eight clusters, cluster A-2 was composed of two identical strains (ECSC30 and ECSS31) isolated from frozen shrimp imported from China and a human strain isolated in the Eastern Province of Saudi Arabia.

On the other hand, 14 (36.8%) strains that showed a genetic similarity value greater than the 90% cut-off were considered genetically related (Fig. 2). Among these strains, the ECSC21 isolated from frozen shrimp imported from China and ECSS33 isolated from the human origin in Saudi Arabia shared 97% genetic similarity. Moreover, the ECSS32 strain of human origin shared 95% genetic similarity with two strains in cluster A-4 isolated from frozen beef imported from India (Fig. 2). Additionally, the ECSS34 strain of human origin showed 92% genetic similarity to two strains in cluster A-2. It is noteworthy that the ECSS36 of human origin shared 93% genetic similarity with the ECSI37 strain isolated from frozen beef imported from India (Fig. 2). Among the ERIC type patterns generated by ERIC1R primer, four strains (ESC14, ECSS35, ECSV3, and ECSV29) were found to be diverse, with single lineages and similarities below 90% and these strains were considered genetically unrelated as indicated by the dice coefficient (Fig. 2). Szczuka and Kaznowski (2004) indicated that strains with a similarity rate below 90% were considered genetically unrelated.

ERIC2-PCR fingerprint pattern analysis

The ERIC2 primer produced fingerprint bands ranging in size from 150 to 1200 bp, and each fingerprint profile consisted of five to ten bands (Fig. 1-B). Numerical analysis of ERIC profiles revealed that seven distinct clusters (B-1 to B-7) and 24 genotypes (B1 to B24) were resolved, as shown in Table 1; Fig. 3. Among the 38 strains analyzed using ERIC2 primer, 21 (55.3%) grouped within these seven clusters were identical and shared a 100% genetic similarity (Fig. 3). Moreover, 11 (28.9%) out of

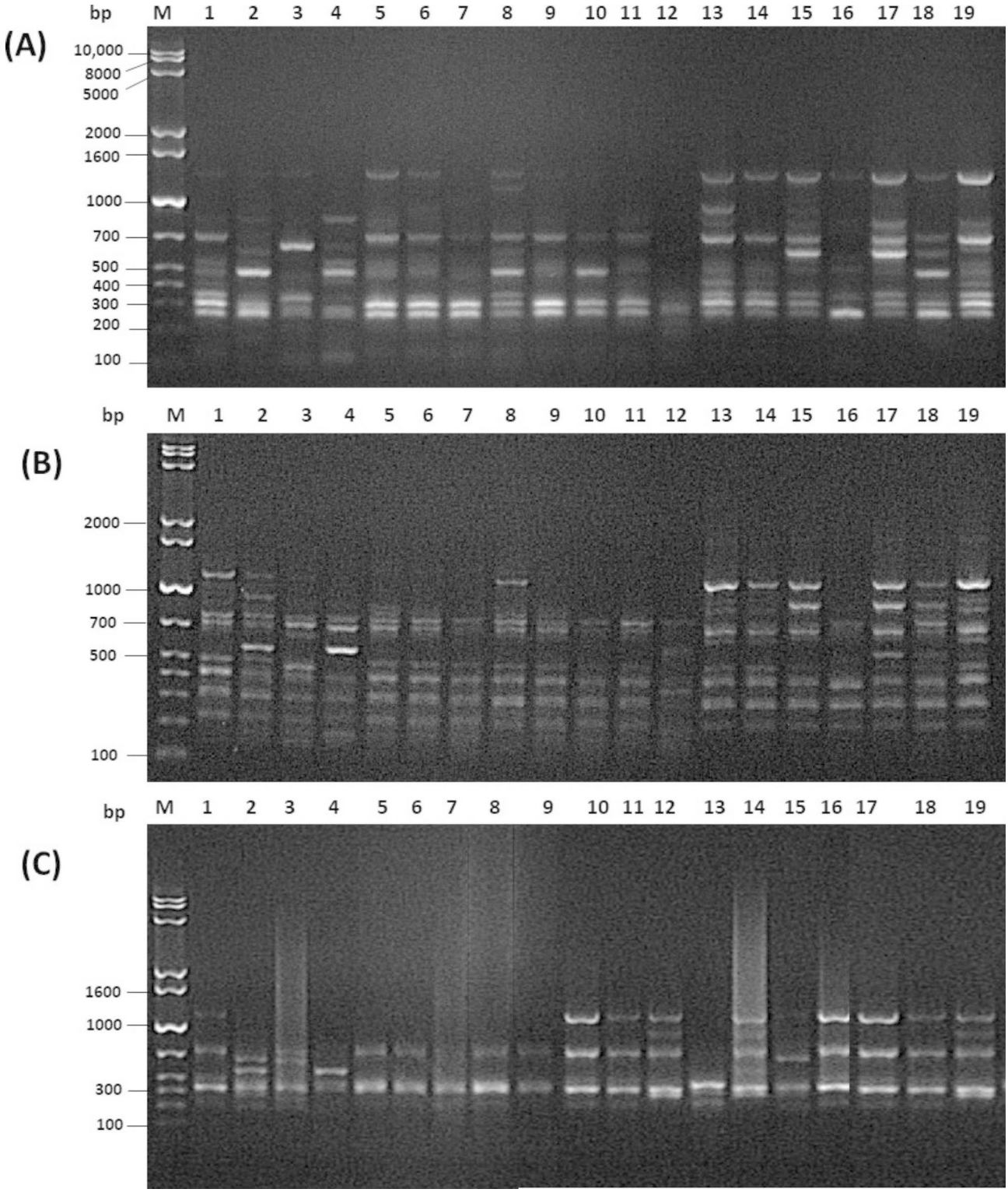

Fig. 1 Gel electrophoresis analysis of Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) fingerprinting of unrelated *E. coli* strains (A) with ERIC1R, (B) ERIC2, and (C) ERIC1R+ERIC primer

38 strains showed a genetic similarity greater than the 90% cut-off value, and six strains were revealed as single lineages below 90% genetic similarity and considered genetically unrelated (Fig. 3). These strains (ECSC14, ECSV4, ECBI37, ECSS36, ECSS34, and ECSC18) exhibited genetic similarity below 90% and were considered

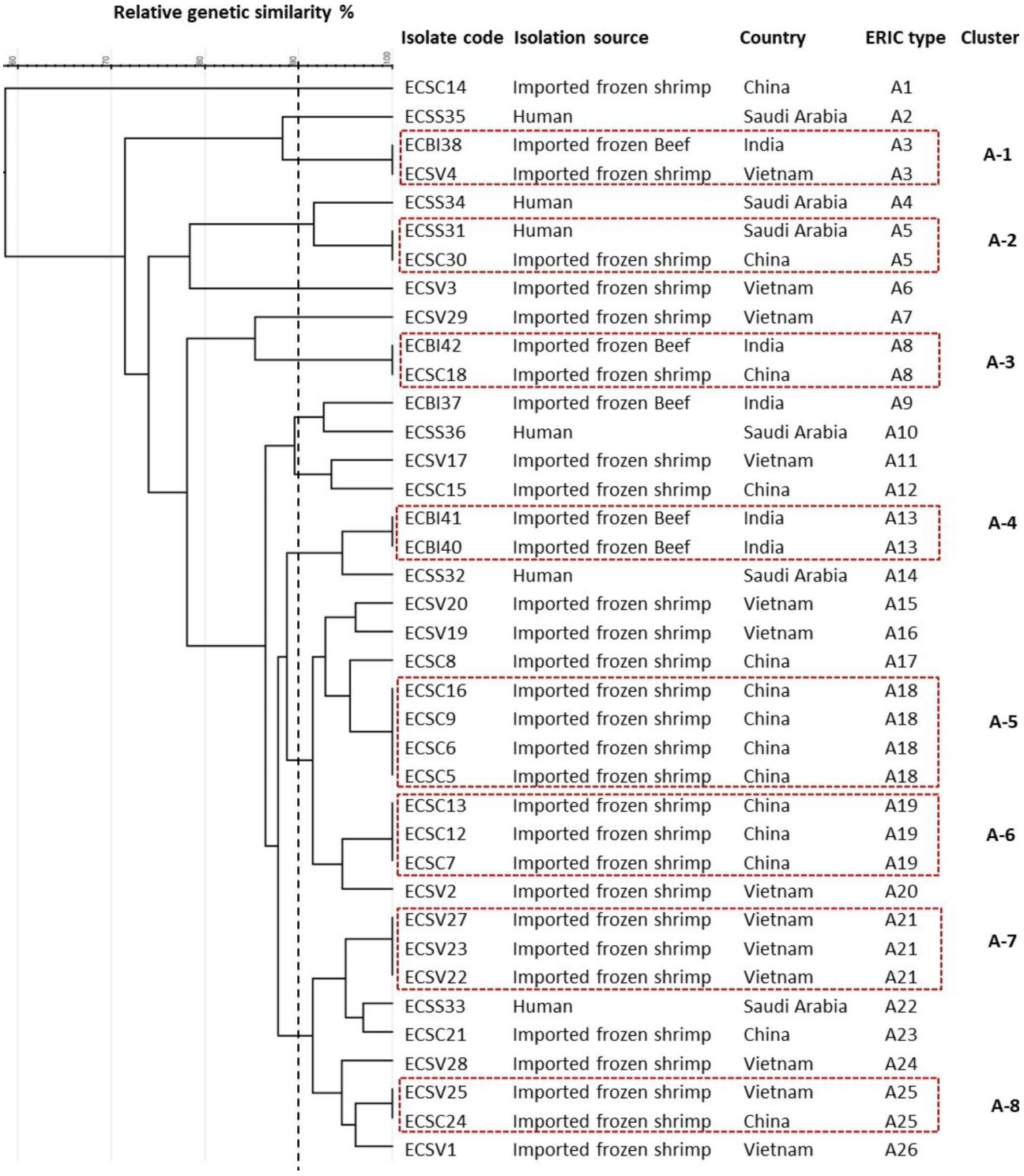

Fig. 2 Dendrogram generated from Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) fingerprinting using ERIC1R primer. The scale at the top of the dendrogram presents the genome similarity (%)

genetically unrelated (Fig. 3). The highest number of strains were grouped in clusters B-3, B-4, and B-5, while other clusters were composed of between two to three strains in each cluster (Fig. 3). Interestingly, cluster B-3 is composed of four identical strains and two of these strains (ECSV22 and ECSC30) were isolated from frozen shrimp imported from Vietnam and China, and other strains ECBI42 and ECSS35 were isolated from beef imported from India and humans in Eastern Province of Saudi Arabia (Fig. 3).

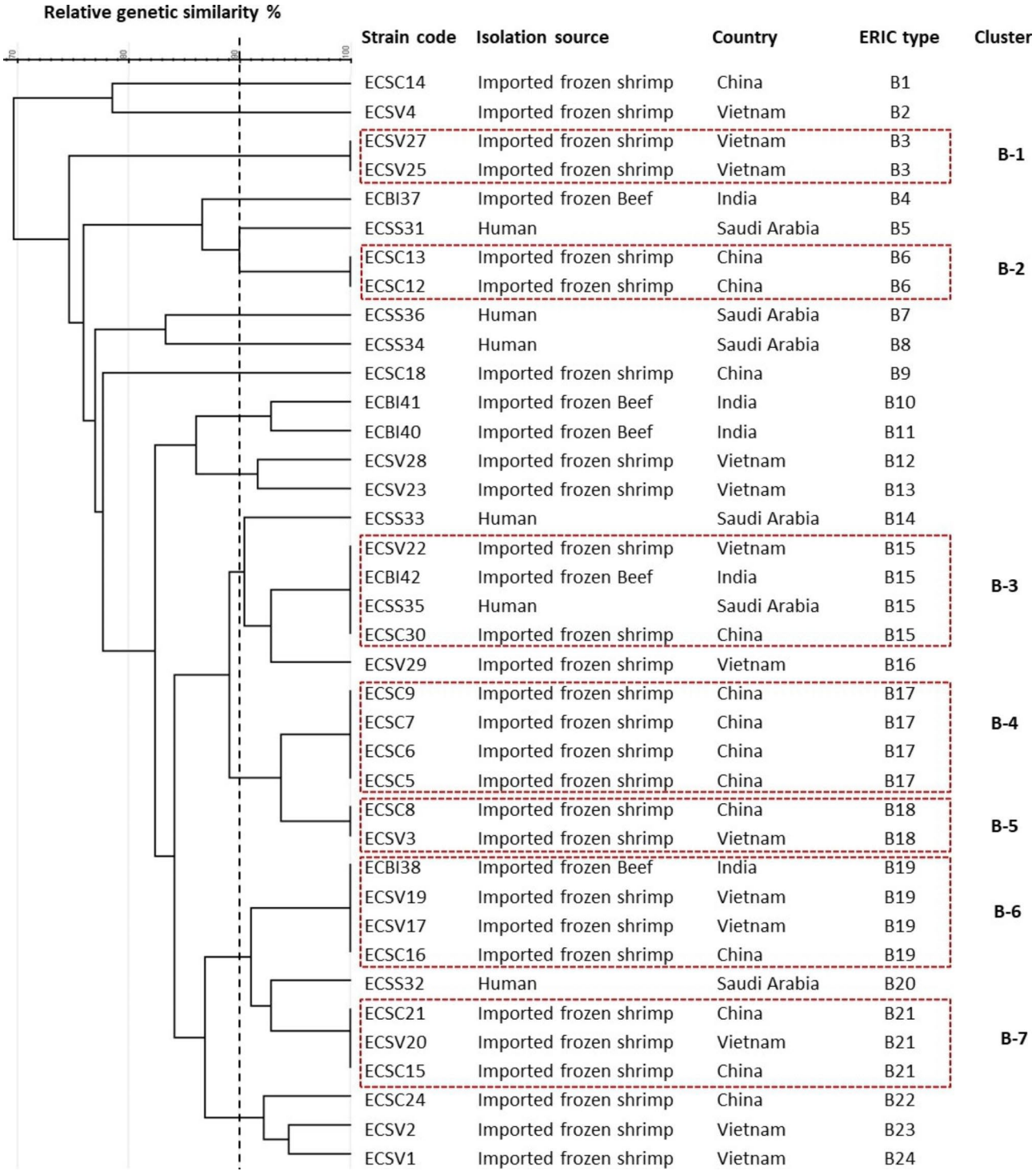

Fig. 3 Dendrogram generated from Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) fingerprinting using ERIC2 primer. The scale at the top of the dendrogram presents the genome similarity (%)

ERIC1R + ERIC2-PCR fingerprint pattern analysis

Concerning the combination of ERIC1R + ERIC2 primer, two to five bands were obtained for each fingerprint and sizes ranging between 200 and 1200 bp (Fig. 1-C). Among the 38 strains, six strains were not typeable by ERIC1R + ERIC2 primer (Table 1). The ERIC1R + ERIC2 primer revealed 16 genotypes (C1 to C16) and six clusters (C-1 to C-6) among the 32 genotyped strains, as shown in Fig. 4. Of the 32 strains, 22 (57.9%) were grouped within these six clusters, and

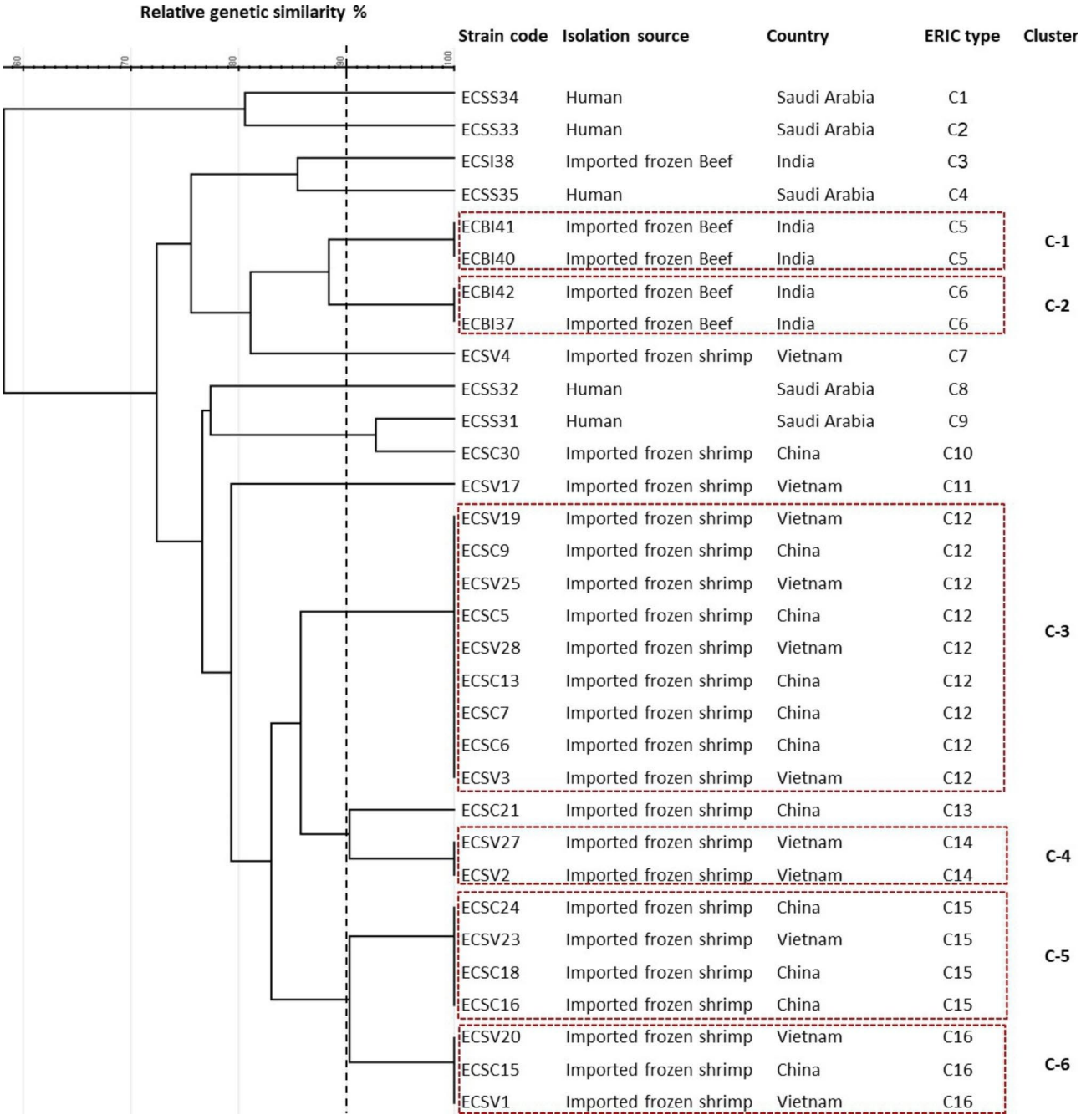

Fig. 4 Dendrogram generated from Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) fingerprinting using the ERIC1R+ERIC2 primer combination

the highest number of strains were composed in cluster C-3 (Fig. 4). Eighteen strains of *E. coli* isolated from frozen shrimp imported from China and Vietnam were grouped in identical clusters (C-3, C-4, C-5, and C-6) and showed 100% genetic similarities. Moreover, all strains isolated from frozen beef imported from India were grouped in separated clusters (C-1 and C-2), and except for strain, ECSI38 exhibited a single lineage cluster below 90% genetic similarity (Fig. 4). Among the five strains of human origin genotyped by ERIC1R+ERIC2 primer, only one strain, ECSS31, shows 93% genetic similarity to strain ECSC30 isolated from frozen shrimp imported from China. The other four strains of human origin exhibited single lineages and revealed genetic similarity below 90% and were considered genetically unrelated (Fig. 4).

Table 2 Evaluation of the Enterobacterial Repetitive Intergenic Consensus (ERIC) primers for typing 38 unrelated strains of *E. coli* isolated from different sources

ERIC primer	Number of clusters	Number of ERIC type	Discriminatory power	Typeability (%):
			$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j(n_j - 1)$	$T = N_t/N$
ERIC1R	8	26	0.976	100
ERIC2	7	24	0.965	100
ERIC1R + ERIC2	6	16	0.903	84

Discriminatory power analysis based on ERIC-PCR primers

The calculated discrimination indexes obtained by ERIC-1R, ERIC-2, and the ERIC-1R + ERIC-2 combination were 0.976, 0.965, and 0.903, respectively (Table 2). The Discrimination Index obtained by all evaluated ERIC primers was considered acceptable and above the confidence value (Szczuka and Kaznowski 2004). Among the three evaluated ERIC primers, the best discriminatory ability for epidemiological concordance was obtained by ERIC-1R (DI = 0.976) and ERIC-2 (DI = 0.965), and both primers revealed a 100% typeability value (Table 2). Among the three investigated ERIC primers, the least typeability of 84% was obtained by the ERIC1R + ERIC2 primer pair (Table 2). Our results suggest that the ERIC1R and ERIC2 primer are effective and suitable in differentiating genetic relatedness of *E. coli* isolated from different sources, and ERIC-PCR should be adopted as a molecular typing technique for tracking monitoring transmission during pathogenic *E. coli* outbreaks. In this study, the ERIC1R and ERIC2 primers had the best discriminatory ability and typeability value and proved to be suitable for epidemiological investigation and genetic analysis among the population of *E. coli* strains. A similar study conducted elsewhere evaluated ERIC-PCR primers as PCR-based tools to discriminate against different bacterial strains and concluded that the ERIC2 primer set had shown a more reliable ability to discriminate against the analyzed strains (Zara et al. 2006). In this study, the ERIC1R + ERIC2 primer combination had a discriminatory power value of D = 0.903 for differentiating *E. coli* strains (Table 2). However, several published articles used a combination of ERIC1R + ERIC2 primer as recommended in previously published studies elsewhere (Versalovic et al. 1991; Woods et al. 1993). Therefore, our obtained results in this study suggesting the use of ERIC1R and ERIC2 primer without combination will generate a more discriminative value index than the combination of the two primers. Furthermore, ERIC primers should be optimized to identify which primer will give an excellent typeability and discriminatory power, as were claimed in some published articles using the ERIC primer without specifying the exact primer used in ERIC-PCR protocol.

Phylogenetic analysis based on ERIC-PCR primers

Construction of phylogenetic trees based on ERIC-PCR fingerprint patterns generated by ERIC1R, ERIC-2, and ERIC1R + ERIC2 primers revealed a total of eight, seven, and six identical unique clusters with 100% similarity index (Figs. 2 and 3, and 4). However, the ECSS31 strain was isolated from humans and was found to be identical to the ECSC30 strain isolated from frozen shrimp imported from China, as shown in Cluster A-2 (Fig. 2). Similarly, a phylogenetic tree based on ERIC-PCR fingerprint patterns generated by ERIC-2 primer revealed three strains (ECSV22, ECSC30, and ECBI42) isolated from frozen shrimp and beef imported from Vietnam, China, and India, respectively, showed identical relatedness to human strain (ECSS35), as shown in Cluster B-3 (Fig. 3). A phylogenetic tree generated by a combination of ERIC1R + ERIC2 primer revealed the ECSS31 strain isolated from humans shared 92% genetic similarity with the ECSC30 strain isolated from frozen shrimp imported from China (Fig. 4). However, identical genetic linkage of these strains isolated from imported frozen beef and shrimp to human strains (ECSS31 and ECSS35) associated with gastroenteritis in the Eastern Province of Saudi Arabia indicates contamination of imported frozen beef and shrimp as a possible vehicle source of infection. Our study also revealed a strong genetic relationship between *E. coli* strains isolated from frozen shrimp imported from China formed identical group clones (Clusters: C-3, C-5, and C-6) with strains isolated from frozen shrimp imported from Vietnam (Fig. 4). Furthermore, we identified *E. coli* strain ECBI38 isolated from frozen beef imported from India had identical genetic similarity (Cluster: B-6) with strains isolated from frozen shrimp imported from China and Vietnam (Fig. 3). Therefore, genetic identical similarity patterns of these clones of *E. coli* strains proved that these strains are circulated and exist in these areas and might be spread through imported frozen beef and shrimp, lacking adequate hygiene during handling, storage, transportation, and commercialization the point of sale. A similar study conducted elsewhere demonstrated that contaminated frozen seafood and shrimp might play an important role in disseminating virulent and multi-drug resistant enterobacteria strains. Also, the study found that *E. coli* isolates

harbored major virulence genes and their contamination levels are beyond the recommended limits (Barbosa et al. 2016; Dib et al. 2018).

Our study also revealed that four out of six *E. coli* strains recovered from humans in the Eastern Province of Saudi Arabia were different clones, and only two strains (ECSS31 and ECSS35) were grouped in identical clone clusters with strains isolated from frozen shrimp imported from China as indicated in cluster: A-2 (Fig. 2). Also, the ECSS35 strain formed an identical clone with *E. coli* strains isolated from frozen beef and shrimp imported from India, China, and Vietnam, as indicated in cluster: B-3 (Fig. 3). *E. coli* comprise a diverse group of bacteria with pathogenic variants, pathovars responsible for causing morbidity and mortality. These pathogen have low infectious doses and are transmitted through food and water, causing major public health problems (Croxen et al. 2013). Food or water might be contaminated with the feces of infected humans and animals, and this contamination plays a significant role in the transmission of *E. coli*. Contamination of meat and seafood products often occurs during the processing and packing (García et al. 2010). In addition, the use of manures from animals as fertilizer can contaminate food and irrigation water (Croxen et al. 2013; García et al. 2010). Another area of interest we found in our study was the determination of genetic similarity among strains isolated from frozen shrimp imported from China and Vietnam; these were grouped in identical clone clusters. However, the present study revealed the coexistence of this circulated identical clone of *E. coli* in these geographical areas.

Over the last two decades, several authors have used ERIC-PCR as a rapid molecular typing method for determining genetic relationships among several bacterial species isolated from food, environmental, and human samples. In this study, ERIC-PCR demonstrated excellent discrimination among unrelated *E. coli* strains, and this method is a useful DNA fingerprinting technique for distinguishing *E. coli* isolated from different sources (Ateba and Mbewe 2014; Dorneles et al. 2014; Fendri et al. 2013; Gautam et al. 2022; Igwaran and Okoh 2020; Pakbin et al. 2021; de Sa Guimaraes et al. 2011; Szczuka and Kaznowski 2004). Our results indicate that imported frozen beef and shrimp products might become an important source of enteric pathogenic *E. coli* in Saudi Arabia. The use of ERIC-PCR typing provides information on the genetic relatedness of *E. coli* strains isolated from humans to *E. coli* strains isolated from imported frozen beef and shrimp in the Eastern Province of Saudi Arabia. However, our findings indicate that the use of ERIC1R, ERIC2, and a combination of ERIC1R+ERIC2 is effective in finding genetic relatedness among strains of *E. coli* isolated from human and retail imported frozen shrimp and beef sources and facilitating a better understanding of the transmission route and tracking the origin of pathogenic *E. coli* in Saudi Arabia.

The identical overlap of two strains (ECSS31 and ECSS35) isolated from humans and strains of *E. coli* strains isolated from frozen beef and shrimp imported from India, China, and Vietnam highlights the importance of these sources for human enteric pathogenic *E. coli* in Saudi Arabia. The ERIC-PCR results demonstrated a clear source of contamination that may occur in the harvest area of shrimp and handling or storage of meat and shrimp. However, there is an urgent need for monitoring programs, and rigorous molecular studies are required to track the exact source of contaminants of enteric pathogenic *E. coli* and other pathogens in imported frozen beef, shrimps, and other frozen seafood and meat products. Consequently, accurate control measures by food authorities must be implemented to warranty a safe quality of imported frozen seafood and meat products for adequate consumer protection.

Conclusion

The three ERIC primers evaluated in this study were varied in their abilities to discriminate *E. coil* strains isolated from different sources (human, frozen beef, and frozen shrimp). Based on the discriminatory power values of fingerprinting of three ERIC primers, the ERIC1R and ERIC2 primers had the best discriminatory ability and typeability value and proved to be suitable for investigating genetic analysis among the population of *E. coli* strains. The evaluation of ERIC-PCR method in this study proved ERIC-PCR to be rapid, easy to perform, cost-effective, and could be implemented as a molecular typing method to discriminate enteric pathogenic *E. coli* from other strains of *E. coli* isolated from food and environmental sources.

Abbreviations

ExPEC	extraintestinal pathogenic *E.* coli
IPEC	intestinal pathogenic *E. coli*
EPEC	enteropathogenic *Escherichia coli*
ETEC	enterotoxigenic *Escherichia coli*
EAEC	enteroaggregative *Escherichia coli*
EHEC	enterohemorrhagic *Escherichia coli*
EIEC	enteroinvasive *Escherichia coli*
DAEC	diffusely adherent *Escherichia coli*
GEMS	Global Enteric Multicenter Study
GBD	Global Burden of Diseases
ERIC-PCR	enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction
LB	Luria Bertani broth
UPGMA	unweighted pair group method with arithmetic average mean
DI	discriminatory power index
T	Typeability
WHO	World Health Organization
FERG	Foodborne Disease Burden Epidemiology Reference Group
IRB	The Institutional Review Board

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Authors contribution
All authors made significant contributions to the manuscript and agree to its publication. NE conceived and designed the study. AA performed laboratory experiments. All authors analyzed the data, draft the manuscript, and approved the final manuscript.

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Data Availability
All data generated during this study are included in the manuscript.

Declarations

Ethical approval and Consent to participate
This study was approved by the Institutional Review Board (IRB) of Imam Abdulrahman Bin Faisal University (IRB approval number: IRB-2022-03-170).

Consent for publication
The data presented in this manuscript does not contain any individual's personal data.

Competing interests
All authors state that they have no competing interests related in this study.

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