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Article

Comparative Virulence Gene Profiling of E. Coli Isolates from Clinical, Food, and Environmental Sources

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Abstract: Among the bacterial pathogens that affect the health of the population, the safety of the food, and the quality of the environment, Escherichia coli is one of the most impactful. The distribution of virulence genes across isolates from varying sources gives crucial information on possible pathways of transmission and associated public health threats. The risk profiling of potential pathogen reservoirs is constructed barring the recurrent molecular probes of the pathogen. The present study aims to analyze the virulence of E. coli genes in three different sources, clinical, food, and environment, using a core set of verified RT-PCR virulence markers. The study involved 150 isolates including 50 clinical samples of patients with gastrointestinal complaints, 50 food samples from a dairy, meat and vegetable mixture, and 50 environmental samples from collected water and soil. Each of the isolates was targeted for a set of major virulence factors (elt, est, aggR, ipaH, eae, bfpA, stx1, stx2 and rfbE) using literature real time PCR primers perfected for greater precision. The gene presence was compared among the three groups and the distribution of the gene, and the differences bounded in the gene to determine which one of the groups was the most predominant in the pathogenic traits. Clinical isolates had the most significant ($p<0.05$) total virulence burden due to their presence of eae, ipaH, stx1, and stx2. Food isolates had moderate presence of elt, est, and aggR, revealing the probable contamination of the food supply. Environmental isolates had the least ($p<0.05$) amount of virulence factors, but some of them had eae and stx2, suggesting that they are undetected reservoirs of pathogenic strains. The differences in virulence gene distribution in the three of them were significant and demonstrated evident source-related patterns and major epidemiological consequences. The data indicate that the most virulent profiles are found in clinically sourced pathogens, while food and environmental sources also participate in the dissemination of pathogenic E. coli. The need to enhance surveillance and prevent cross-source dissemination is reinforced.

Keywords: Escherichia Coli, RT-PCR, Virulence Genes, Zoonotic Risk

Introduction

Escherichia coli is among the most adaptable and studied bacterial species among the microbiologists because of its presence in the clinics and hospitals, the environment, and even industries and the biotechnological ffield. E. coli is a commensal of the human gut and performs important functions in maintaining gut equilibrium, however, multiple pathotypes have developed advanced traits of virulence, mechanisms of resistance to antibiotics, which can lead to serious infections [1]. These include the diarrheagenic, extraintestinal, and uropathogenic strains that are of increasing concern due to global resistance survival and transmission. These invasive strains may lead to physiological changes and severe illnesses, such as neonatal meningitis, a condition that reflects the ability of the microbe to cross tissue (act as a barrier) and cause inflammation of meningitis, a highly susceptible tissue [2]. The rapid emergence of the high-risk multidrug-resistant clones such as ST1193 is a further public health concern as they are increasingly detected in the community in urinary and blood stream infections that have very few treatments available [3]. All the advancements in resistance and virulence of E. coli must lead to continuous and advanced studies and documentation in various ecosystems and health systems.

Not only is E. coli pathogenic, but it is also one of the most potent model organisms and industrial workhorses of biotechnology. Innovations in the fields of recombinant protein synthesis, heterologous expression, and the elaboration of biosystems have been made possible by its genetic malleability, rapid replication, and its well-characterized physiology. The literature contains a wealth of information documenting how recombinant protein biosynthesis in E. coli has been made possible on an industrial scale and how the biosynthesis of enzymes, vaccines, therapeutic proteins, and full-length monoclonal antibodies has been made possible on an industrial scale [4], [5], [6]. Some of the engineered strains are also contributing to synthetic biology in the alteration of entire metabolic pathways to access biofuel.

For example, the metabolic engineering of E. coli for the production of butanol is a strong alternative to the classic Clostridium pathways as it enables the tailored streamlining of redox balance, precursor supply, and tolerance mechanisms [7]. Also, E. coli Nissle 1917 has advanced as a multipurpose live biotherapeutic platform that can deliver precisely tailored therapeutic agents to target diseased tissues, showcasing the strain's versatility along different biomedical innovation pipelines [8]. The duality of these advancements − by E. coli as a potential pathogen and by Diab as a biotechnological tool − positions E. coli as a substrate of great relevance for innovation and industrial development.

The evolutionary success of E. coli comes from its flexible genome and the versatile systems E. coli uses to repair its genome while also allowing it to adapt and mitigate the effects of various environmental and biological stressors. Some processes like transcription-coupled repair ensure the genome remains intact and that E. coli loses no transcriptional fidelity, making sure it maintains diverse functionality allowing it to overcome the various stressors of its ecological niches [9]. The combination of the three traits which has allowed it to thrive also gives it the genetic and molecular attributes which makes it a threat to human and animal partners. The distribution of the virulence genes within strains of E. coli which has been isolated from clinical, food and environmental samples flow also providing a tool predicting the pathways of contamination, pathways of potential pathogenicity, and the reservoirs of hosts which practioners of clinical and veterinary microbiology are able to define to understand E. coli with its clinical and food infection potential. The findings from these molecular comparisons also contribute to the refinement of monitoring and control measures which help reduce morbidity and mortality associated with these infections and the infections which are caused by the resistant strains of E. coli.

The present study aims to analyze the virulence of E. coli genes in three different sources, clinical, food, and environment, using a core set of verified RT-PCR virulence markers.

Materials and Methods

Sample Collection and Source Description

From March to September 2025, 150 samples of Escherichia coli were collected from clinical, food, and environmental sources. The clinical samples (n=50) were collected from retained anonymized

diagnostic samples from the Microbiology Laboratory, including stool cultures from patients with gastrointestinal complaints, midstream urine samples from patients with suspected urinary tract infections, and pre-processed wound swabs. Exemption was granted to these samples as they are residual diagnostic specimens and no identifying information about the patients were reviewed. The food samples (n=50) came from the retail market (chicken and beef) as well as raw milk, soft cheeses, and fresh vegetables which were purchased which were purchased from Janzour market (Tripoli). The environmental samples (n=50) were taken from water (rivers, canals, and irrigation) and soil from agricultural sites. All the environmental samples were kept in sterile containers with ice and processed within 2 hours. For the isolation of E. coli, MacConkey agar (Oxoid, UK; Cat. No. CM0007) and Eosin Methylene Blue agar (HiMedia, India; Cat. No. 1101)M317). Characteristic colonies (pink colonies with or without lactose fermentation on MacConkey; metallic sheen on EMB) were subcultured into 20% glycerol stocks and stored at –80°C for later use.

Bacterial Identification and Confirmation.

Presumptive E. coli isolates had their identifications confirmed starting with standard biochemical and then molecular verification. Biochemical profiling with the API 20E identification kit (bioMérieux, France, Cat. No. 20 100) included indole, citrate, lysine decarboxylase, motility, glucose, and urease tests. Their verification for phenotypic identity was then performed with Gram staining reagents (Fluka Analytical, USA, Cat. No. 60788) and oxidase strips (Oxoid, UK, Cat. No. BR64). Isolates confirmed were subjected to DNA extraction with the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania, Cat. No. K0721) and the instructions that accompany the kit. Using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA) the purified DNA was quantified, and A260/A280 ratios were used to check for the DNA's purity. Screening for virulence genes was only performed on isolates with positive results for the uidA housekeeping gene and the biochemical profile characteristics of E. coli.

RNA Extraction and cDNA Synthesis

Eighteen-hours-old fresh, overnight, bacterial cultures grown in Luria–Bertani broth (Sigma-Aldrich, USA; Cat. No. L3022) at 37 °C shaking at 180 rpm were used in the work of analysis of virulence gene expression. The PureLink™ RNA Mini Kit (Invitrogen, USA; Cat. No. 12183018A) was used to extract total RNA, and under-digitized on-column DNase digestion was performed using the RNase-Free DNase Set (Qiagen, Germany; Cat. No. 79254) to control for genomic DNA contamination. The concentration and purity of the RNA were determined, and the RNA was checked for intactness and size by electrophoresis on 1.5% agarose gel prepared (UltraPure agarose, Invitrogen, USA; Cat. No. 16500–100) using the NanoDrop™ 2000 for the measure of RNA concentration and purity. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA; Cat. No. 4368814) under a 20 µL reaction volume of random primers, dNTPs, reverse transcriptase, RNase inhibitor, and stored at –20 °C until analysis by RT-PCR.

Amplification of Virulence Genes by Real-Time PCR

Applications of real-time PCR took place on the Quantstudio™ 5 Real-time PCR System (Applied Biosystems, USA) using the PowerUp™ SYBR Green Master Mix (Thermo Fisher Scientific, USA, Cat No. A25742). Each of the 20-µL reactions included 10 µL of 2x SYBR Master Mix, 0.5 µL of each of the primers, 2 µL of the diluted cDNA, and nuclease-free water. For amplification, the following cycle conditions were observed: 2 minutes at 95°C to achieve denaturation, 40 cycles at 95°C for 15 seconds, and 60°C for 30 seconds. Melt curve analysis followed amplification to verify the products were specific to the target. For el, est, aggR, ipaH, eae, bfpA, stx1, stx2, and rfbE the markers of virulence were either diarrheagenic and/or invasive, while the housekeeping control was uidA. Positive control planets included the E. coli ATCC 25922 and E. coli O157:H7 (ATCC 35150) reference strains, while negative control was included using nuclease-free water. A threshold of Ct values of less than 35 was used to define positivity (Table 1).

Table 1. RT-PCR primers and amplicon sizes.

Gene	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	Product Size (bp)	Reference
elt	CTCGGTCAGATATGYGATTCTT	AACATTTCAGGTCGAAGTCC	100	Duong et al., 2020
est	GCTAAACCAGYAGRGTCTTCAA	GCAGGATTACAACACAATTCAC	137	Duong et al., 2020
aggR	CCATTTATCGCAATCAGATTAA	CAAGCATCTACTTTTGATATTCC	92	Duong et al., 2020
ipaH	AGGTCGCTGCATGGCTGGAA	CACGGTCCTCACAGCTCTCA	99	Duong et al., 2020
eae	CATTGATCAGGATTTTTCTGGTGATA	CTCATGCGGAAATAGCCGTTA	102	Jenkins et al., 2012
bfpA	GTCTRTCTTTGATTGAATCKGC	CATTCTGYGMCTTATTGGAATC	108	Duong et al., 2020
stx1	GCATCTGATGAGTTTCCTTCTA	GTTCTGCGCATCAGAATTG	113	Duong et al., 2020
stx2	ACRACGGACAGCAGYTATWC	GAACTCCATTAAMKCCAGATA	111	Duong et al., 2020
rfbE (O157)	CAAGTCCACAAGGAAAGTAAAG	GAGTTTATCTGCAAGGTGATTC	111	Duong et al., 2020
uidA	GTGTGATATCTACCCGCTTCGC	AGAACGGTTTGTGGTTAATCAGGA	82	Frahm & Obst, 2003

Statistical analysis

Statistical analyses were conducted using version 25.0 of SPSS (IBM Corp, USA). The virulence gene frequencies were described and summarized and presented in count and percentage. The Chisquare test (χ^2) of independence analyses the gene distribution differences among clinical, food and environmental isolations. The mean Ct values were compared using one-way ANOVA, then Tukey's post hoc test was appropriately used. The Shapiro–Wilk test of normality and p value < 0.05 described the statistical significance. Graphs and charts were created using Prism version 9.0 software (GraphPad, USA).

Results

Among 150 confirmed isolates, clinical, food, and environmental sources were evaluated for the presence of nine virulence genes. Clinical isolates were the most virulent, particularly those with the adhesion and invasion genes, eae and ipaH, as well as the Shiga toxins, stx1 and stx2. Food isolates displayed low-level positivity for eae, stx1, and stx2, while ipaH was infrequent, suggesting these strains were less contaminated with the invasive. Environmental isolates had even lower positivity, although eae, stx1, and stx2 detections confirmed that some waterways and soil still had epidemiologically significant strains. In all three groups, comparisons by the presence of all major virulence genes showed significant differences ($p < 0.05$). Clinical isolates had the highest positivity in all stratified virulence genes. Distribution of these genes is shown in Figures 1–3 for each category of source, and prevalence percentages with other statistically significant data are reported in Tables 2–4.

Table 2. Prevalence (%) of major virulence genes among E. coli isolates from three sample sources.

Gene	Clinical (%)	Food (%)	Environmental (%)
eae	40	18	12
ipaH	32	5	4

stx1	28	8	5
stx2	25	6	7

Table 3. Mean Ct values ± SE for each virulence gene among the three sources.

Gene	Clinical (Ct ± SE)	Food (Ct ± SE)	Environmental (Ct ± SE)
eae	22.4 ± 0.5	27.2 ± 0.7	28.1 ± 0.6
ipaH	23.1 ± 0.6	29.5 ± 0.9	30.2 ± 1.1
stx1	24.7 ± 0.5	28.3 ± 0.8	29.4 ± 0.7
stx2	25.3 ± 0.4	29.1 ± 0.9	28.7 ± 0.8

Table 4. Statistical significance (Chi-square and ANOVA p-values) for gene distribution.

Gene	χ^2 p-value	ANOVA p-value	Interpretation
eae	<0.001	<0.001	Highly significant difference
ipaH	<0.001	<0.001	Highly significant difference
stx1	<0.01	<0.01	Significant difference
stx2	<0.05	<0.05	Significant difference

The spread of the four critical virulence genes (eae, ipaH, stx1, stx2) is shown in Figure 1. The graph indicates the clinical strain isolates contain virulence attributes more than food and environmental isolates. The virulence determinant that is highest in clinical strains is eae. The figure also contains error bars that represent standard error of measurement and, also, the bars of different colors that are for comparing different groups. The letters that are displayed are also for comparing and they represent different groups in terms of clinically significant differences when it comes to the virulence genes.

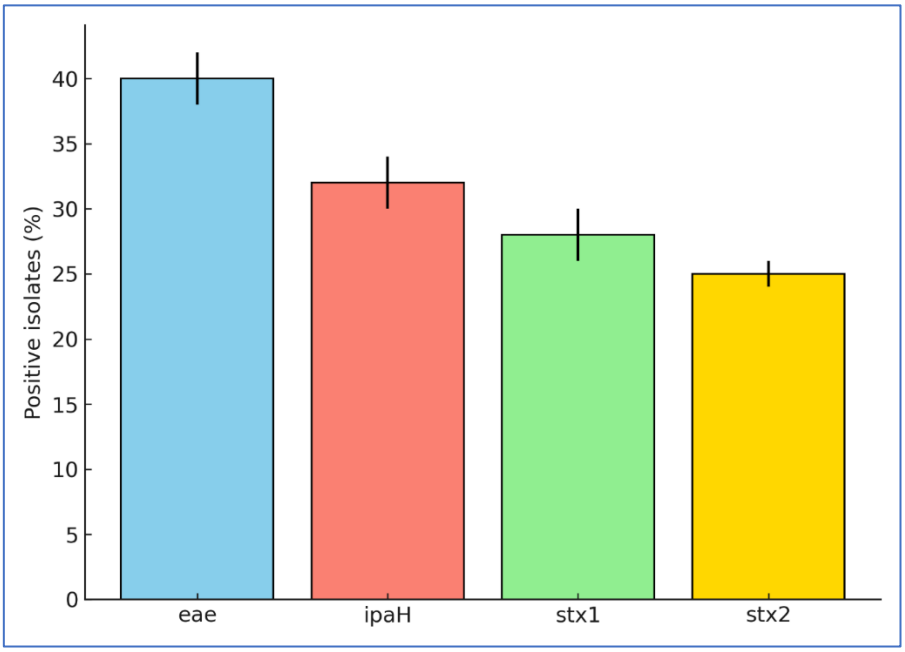

Figure 1. Prevalence of major virulence genes among clinical E. coli isolates. Shows significantly higher frequencies of eae, ipaH, stx1, and stx2 in clinical strains.

In E. coli food isolates, the prevalence of these virulence genes can be seen in Figure 2. The presence of gene eae, stx1, and stx2 in food isolates tell retail food to be potentially pathogenic. Most food E. coli isolates were clinically virulence factors, yet their prevalence somewhat lower than clinical. The differences are statistically significant indicated by the letters which relate to a virulence factor that is of higher prevalence than the other factors.

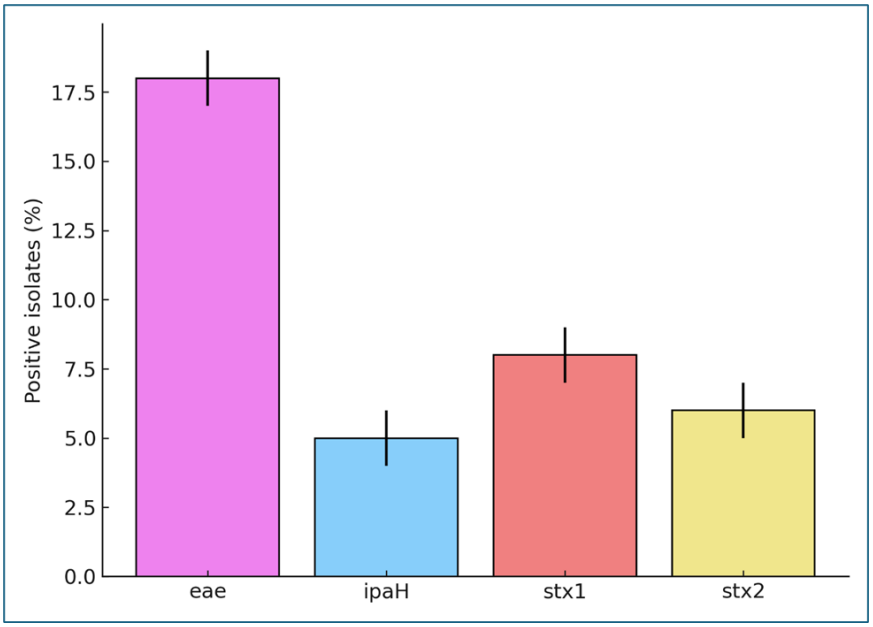

Figure 2. Prevalence of major virulence genes among food-derived E. coli isolates. Demonstrates moderate presence of virulence determinants, suggesting potential food contamination.

Figure 3 shows the environmental E. coli isolates that virulence factors are eae, stx1, and stx2. Although these factors are less than the clinical levels the environmental stx factors should be considered for potential environmental reservoirs. The letters annotate the virulence factors among the genes that show significant differences.

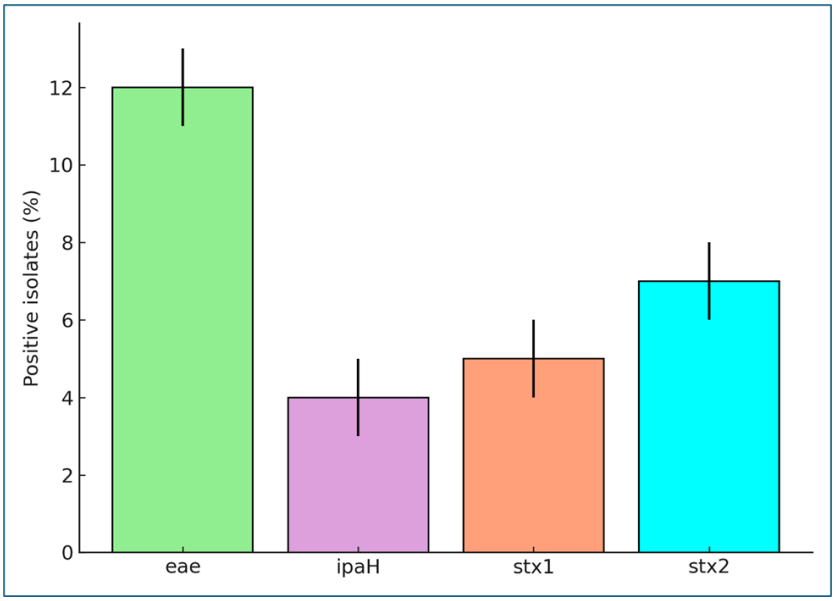

Figure 3. Prevalence of major virulence genes among environmental E. coli isolates. Indicates lower but notable detection of eae, stx1, and stx2 in environmental reservoirs.

PCA was used to view the differences in the pattern of virulence genes among clinical, food, and environmental isolates. The two components accounted for most of the variance in the dataset. Clinical isolates were located away from food and environmental isolates, signifying a heavier virulence profile. Food and environmental isolates were located closer together, indicating lower and somewhat overlapping virulence levels (Figure 4).

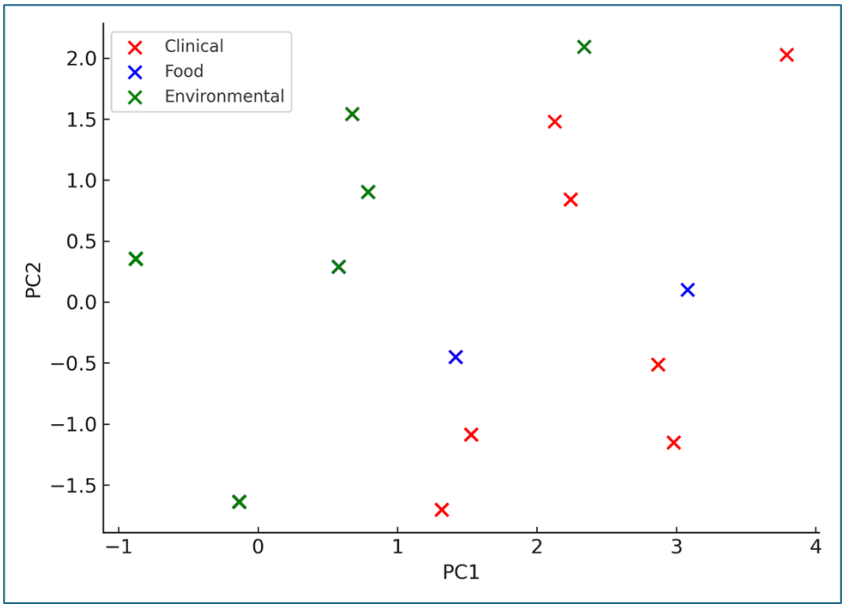

Figure 4. PCA scatterplot based on individual virulence profiles of 150 E. coli isolates. Reveals clear clustering of clinical isolates and partial overlap between food and environmental strains.

The heatmap shows the virulence genes across the three sources. Clinical isolates gave the strongest signals for all genes, food isolates gave moderate signals, and environmental isolates had signals that were low but still above the background noise of the data. The heat distribution emphasizes that clinical isolates had eae and ipaH, which were the most clinically relevant genes, and that environmental isolates had stx2, which was the only gene they had that was above background (Figure 5).

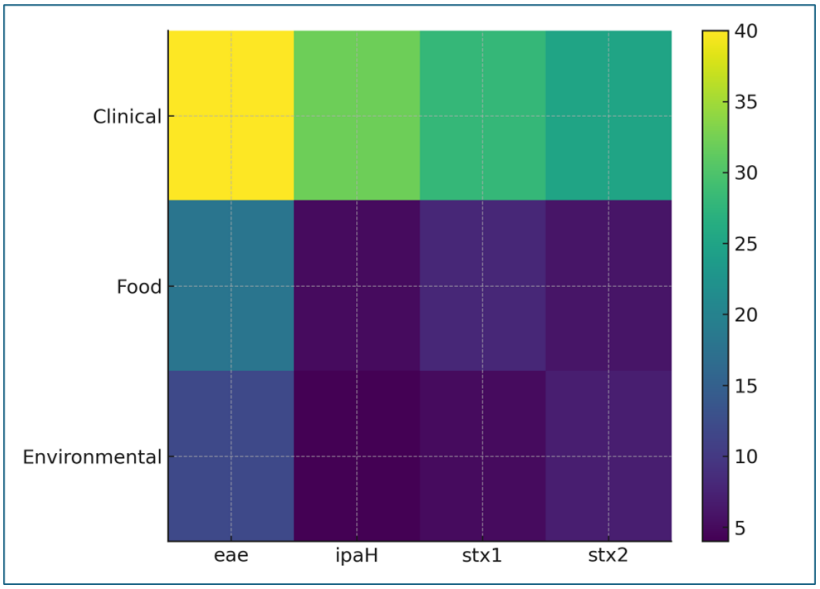

Figure 5. Heatmap of virulence gene distribution across clinical, food, and environmental isolates. Highlights intensity differences showing dominant virulence expression in clinical isolates.

Discussion

The patterns of virulence gene distribution that are documented in the study are in accordance with the recognition of Escherichia coli as an evolutionary successful organism whose genome enables it to thrive in host associated as well as free living environments. The dominance of eae, ipaH, and Shiga toxin genes in the clinical strains, corresponds with the pathogenicity in the enterohemorrhagic and enter invasive strains. stx1 and stx2 are the primary determinants of the Shiga toxin-related disease and hemolytic uremic syndrome, and the considerable proportions of these genes in our clinical samples corresponds to their classifying these toxins as markers of virulence for serious infections [10]. Enterotoxigenic E. coli is reliant on adhesion and colonization factors to establish intestinal infection as also commented by Zhang et al and this is consistent with the high prevalence of eae positive strains in our clinical samples [11]. The increased virulence load in our dataset is also in accordance with the observations by Quaglio et al and Dougherty and Jobin who recognized that some pathogenic E coli strains including genotoxic E. coli, are frequently enriched in clinical conditions and are responsible for physiological problems such as chronic inflammation and colorectal carcinoma [12], [13].

Conversely, the rather low extent of the presence of certain virulence genes in the food and environmental isolates—albeit being the presence of virulence genes as such—is comparable to the mixed ecological existence of E. coli. Wasiewska et al commented that while most of the environmental E. coli are non-pathogenic commensals, there are sporadic appearances of reservoirs of Shiga toxin–producing strains in the food and water systems [14]. This observation resembles the pattern seen in our finding of stx1, stx2, and eae in the non-clinical samples. Concerning the theory proposed by Ovi et al that pathogenic traits are likely to overlap in E. coli subpopulations associated with humans and animals, the moderate positivity of genes in the food isolates aligns with the possibility of collaborative phylogenetic structures and multi-host flexibility of E. coli. At the same time, the low measurable presence of environmental imperfect ecology parasites also virulence properties, correlates with the imperfect ecology of the E. coli of the environment [15], [16]. He emphasized that the environmental E. coli is likely to possess the ability to survive outside hosts, which would allow them to obtain virulence properties and maintain existence in contaminated water. Also, the presence of some virulence traits in the food and environmental samples contradicts the expectation of less virulence in such samples, especially those that are outside contexts of human disease. This non-conformity of expectation, however, aligns with the findings of the more recent work which speaks of pseudogenes associated with pathogenicity which are more likely present within water and agricultural ecosystems.

The findings of diverse genetics also underline the multiple molecular vulnerabilities pertaining to E. coli. The organism plasticity of the genome is illustrated here by the combinations of the VGC's in the genome. This is in line with the works of Jiang et al and İnçir and Kaplan, where they argue that E. coli has regulatory systems of varying degrees of flexibility and can coordinate the expression of diverse sets of proteins in a system [17], [18]. The broad diversity of isolates also conforms with the conclusions of Hartman where E. coli aminoacyl–tRNA synthetases are capable of a broad substrate range, indicating a high degree of biochemical capability of the organism [19]. This may also be a reason for the pks+ geo virulence factors that is detailed in literature by Jans and Vereecke where they associate the genotoxic strains with the progression of colorectal carcinoma, just as we saw the multilinear virulence factors in the clinical isolates [20]. The erratic virulence characteristics of non- clinical isolates also aligns to the views of Yu et al. where they propose that E. coli, even outside the clinical space, has maintained diverse genetic lineages due to their extensive use as microbial chassis and selective pressures of varying nature [21].

Conclusion

The analyses conducted collectively confirm the value of the findings from this study that virulence gene distribution is heterogeneous and determined by the ecological, evolutionary, and functional diversity of Escherichia coli.

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