

ERIC-PCR Based Molecular Typing of *Escherichia coli* Recovered from Milk in Peshawar, Pakistan

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ABSTRACT

Milk is frequently contaminated with *Escherichia coli* that could cause infections in human through the food chain. This study aimed to investigate genetic diversity among *Escherichia coli* isolates recovered from milk samples collected from shops in Peshawar, Pakistan, using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR). A total of 300 milk samples were collected and processed for isolation of *E. coli*. Two hundred *E. coli* isolates were recovered from these milk samples that were confirmed through morphological and biochemical tests. All the isolates were subjected to ERIC-PCR which generated distinct banding pattern indicative of high genetic diversity. The data generated was subjected to further phylogenetic analysis. This analysis revealed 98 clusters of *E. coli* based on a similarity coefficient of $\geq 85\%$. Isolates from the same or adjoining areas were found to have high genetic similarity showing the clonal dissemination of these isolates. In contrast, isolates from different areas showed a substantially high genetic diversity that suggests multiple routes of milk contamination. The main contributing factors to this multi-source contamination could be inadequate hygiene, inefficient equipment cleaning, suboptimal milk handling and suboptimum storage conditions. These findings highlight the need for development of proper sanitary conditions and quality assurance measures for production and distribution of milk to reduce *E. coli* contamination and to mitigate the resulting public health hazard. The study also highlights the usability of ERIC-PCR for estimating genetic diversity in *E. coli* of milk origin. This method could supplement microbiological and surveillance studies for devising better control and management measures especially in low income countries.

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FK performed samples collection, experimental work, data analysis and manuscript writing. KA conceived the idea, contributed to experimental work, conducted analysis, contributed to manuscript writing and proofreading. MJU and SH helped in data analysis and manuscript writing. All the authors approved the final version of the manuscript.

Key words

ERIC-PCR, *E. coli*, Genetic diversity, Milk, Public health, Contamination

INTRODUCTION

Milk is an important source of food that contains essential nutrients and is globally used to meet daily nutritional requirements. Raw milk directly coming out from the udder of healthy animals is considered safe for human use because it contains fewer microorganisms. However, it can be instantly contaminated through transfer of bacteria from animal feces, workers, surroundings (air, soil, milking equipment, water), dirty exterior surfaces of udders, etc. (Deddefo *et al.*, 2023). The quality of products across the dairy chain is determined by the initial microbial load of the raw milk at the farm level. Thus, it is important to follow proper hygiene practices during handling of milk

at the farm level (Chimuti *et al.*, 2024). One contaminant of major importance is *Escherichia coli* that is frequently associated with foodborne diseases.

E. coli is common inhabitant of warm blooded animals including human lower intestine. It is released into the environment through feces that could easily contaminate water and food. It is also frequently used as an indicator to assess the quality of water and food (Jang *et al.*, 2017). Many strains of *E. coli* are harmless, but some, such as Shiga toxin-producing *E. coli* (STEC), could cause serious diseases. The main source of *E. coli* infections in humans with this bacterium is contaminated food such as beef, raw milk, vegetables, and fruits. Ruminants are natural reservoirs for STEC strain. Contamination of milk with this strain of *E. coli* is frequent. *E. coli* is also a common inhabitant of soil, water, and animal bedding (Mesele *et al.*, 2023). The vicinity of anus to the udder is another source of milk contamination with *E. coli* (Calahorrano-Moreno *et al.*, 2022). *E. coli* is also one of the causes of mastitis that is a devastating disease in dairy cattle. It easily spreads from diseased animals to healthy animals through milking equipment and workers (Goulart and Mellata, 2022). These are potential sources of milk contamination at the farm level. Other factors involved in milk contamination

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include inadequate temperature treatment and improper packaging, storage, and transportation (Berhe *et al.*, 2020). Once milk is contaminated at any level of the chain, it poses serious health risks to consumers. Most bacteria can be killed by heating raw milk, but toxins are heat resistant and pose a risk to end users (Owusu-Kwarteng *et al.*, 2020). Thus, it is important to avoid contamination of milk with bacteria at each level of processing.

To reduce bacterial contamination in food, it is important to trace the main sources of contamination of *E. coli*. One efficient and reliable way to perform this tracing is to analyze bacterial genomes to group them on the basis of ecological niches. The bacterial genome contains repetitive sequences that could be accessed to determine genetic similarity and variability among bacterial strains. The frequency and location of these repeats varies among bacterial strains. This variation is the basis for determining clonal relatedness among isolates (Subirana and Messeguer, 2020). Various molecular methods that utilize these sequences to identify the sources of microbial contamination are available. Among these, the molecular technique of Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) is simple, easy to perform, less time-consuming and inexpensive (Ranjbar *et al.*, 2016). Enterobacterial repetitive intergenic consensus (ERIC) repeats are imperfect palindromes, 122-127 bp in size and their copy numbers varies among bacterial strains (Bakhshi *et al.*, 2018). They were first identified in *E. coli*, *Salmonella*, and other Enterobacteriaceae members (Bakhshi *et al.*, 2018). The difference in their numbers among different bacterial isolates is used as a tool for genetic screening (Otokunefor *et al.*, 2020).

In the present study, ERIC-PCR was used to estimate genetic variability among *E. coli* isolates recovered from milk in Peshawar, Pakistan. The study attempted to figure out the sources of milk contaminating *E. coli* in the region by determining genetic relatedness among the *E. coli* isolates from various locations. This information is crucial for devising proper strategies for ensuring milk safety for consumers and equipping the health sector to better respond to future outbreaks of *E. coli* infections.

MATERIALS AND METHODS

Sampling

A total of 300 milk samples (30 ml) were collected from different retail centers in Peshawar, Pakistan. Sterile falcon tubes were used to collect the samples. The samples were immediately transported to the laboratory at Center of Biotechnology and Microbiology, University of Peshawar and were processed directly or refrigerated until future

analysis.

Isolation and identification of *E. coli*

For isolation of *E. coli*, each milk sample was diluted in peptone buffered water up to 10^{-5} . Each diluted sample was inoculated onto MacConkey agar media plate using spread plate method. Plates were incubated at 37°C for 24 h. After incubation, colonies producing characteristic metallic sheen color were considered as presumptive *E. coli* (Younis *et al.*, 2021). All isolates were subsequently analyzed through microscopic and biochemical tests. Which included Gram staining, catalase, oxidase, methyl red, Voges-Proskauer, citrate utilization, triple sugar iron and indole tests (Megersa and Abdelhamid, 2019).

DNA isolation

All *E. coli* isolates were processed for genomic DNA extraction using the GeneJet Genomic (Catalog No. FERK0721) Bacterial DNA extraction Kit. Gel electrophoresis was afterwards performed to verify the quality of extracted DNA. DNA was stored at -20 °C for subsequent use.

ERIC-PCR

A total of 200 recovered *E. coli* isolates were subjected to Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) fingerprinting using reported primers (Versalovic *et al.*, 1991). Reaction mixture (20 µL) consisted of molecular grade water (13 µL), Master Mix (4 µL, Solis BioDyne, Estonia, Catalogue No. 04-12-00115, containing Taq polymerase, reaction buffer, dNTPs and $MgCl_2$), reverse (5'AAGTAAGTGACTGGGGTGAGCG3') and forward (5'ATGTAAGCTCCTGGGGATTAC3') primers (1 µL each, primer) and template DNA (1 µL). Amplification conditions were: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturing at 90 °C for 30 seconds, annealing at 40 °C for 1 min, extension at 72 °C for 1 min and a final denaturing at 72 °C for 8 min. Products of PCR were resolved using a 1.5% agarose gel and 100 bp ladder (Solis BioDyne, Catalogue No. 07-11-00050). After electrophoresis results were analyzed through gel documentation system. All easily observable and reproducible bands (also termed as Alleles) in each well were recorded according to the molecular sizes. This data resulting for the gel electrophoresis banding pattern was used for further statistical analysis.

Statistical and bioinformatics analysis

To estimate genetic diversity, the ERIC-PCR profiles for all isolates were subjected to statistical and bioinformatics analyses to generate bivariate data and

calculate genetic distances among the isolates. For this purpose, presence of an allele (band) was marked as 1 and absence of the the allele was represented as 0. To calculate genetic distances, the formula $D_{xy} = 1 - \{(N_{xy}) / (N_x + N_y - N_{xy})\}$ of the Nei and Li was applied (Nei *et al.*, 1979). where N_x = no. for alleles in the X genotype and N_y = no. alleles for the Y genotype and N_{xy} = no. of common alleles in both genotypes and D_{xy} = dissimilarity distance between the two genotypes. Genetic distance data were used to develop a phylogenetic tree and estimate clonal relationships among the isolates based on similarity coefficients $\geq 85\%$. For this purpose, the Molecular Evolutionary Genetics Analysis software (MEGA11) and POPGENE-32 software were used (Waters *et al.*, 2012).

RESULTS

Figure 1 illustrates the band patterns produced by the ERIC-PCR. These bands represent enterobacterial repetitive intergenic consensus (ERIC) repeats whose copy numbers vary among different isolates. This variation provides the basis for estimation of genetic diversity among the isolates. A total of 24 bands were generated that ranged in size from 100 bp to 3000 bp. Only clearly visible and reproducible bands were considered for genetic distances estimations. The maximum number of bands observed was seven in three isolates, whereas the minimum was one, observed in ten strains. The phylogenetic tree for the *E. coli* isolates is shown in Figure 2. All the two hundred isolates were grouped into 98 clusters based on a similarity coefficient of $\geq 85\%$. Each clusters had two isolates, except C56, C40 which had three isolates and C63 which had four isolates. Clonal relationships according to location are summarized in Table I. Generally, isolates from the same location or nearby locations were genetically more similar. In contrast, isolates from geographically distant locations were genetically less related. The findings represent the clonal spread of *E. coli* in milk.

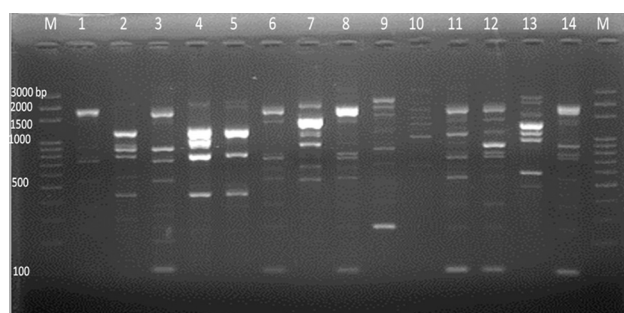


Fig. 1. ERIC-PCR products. Lane M = DNA ladder, Lane 1-14= *E. coli* samples.

Table I. Location-based distribution of *E. coli* clusters.

Location of sample collected	Clusters	<i>E. coli</i> isolates
Shaheen camp, Tehkal, Arbab Road, Abdra road and University town	C78	1, 2
	C79	3, 4
	C64	5, 6
	C81	7, 8
	C82	9, 10
	C70	11, 12
	C66	13, 14
	C67	15, 16
	C74	17, 18
	C75	19, 20
	C71	21, 22
	C73	23, 24
Board Bazar, Danishabad, Nemat Mahal and Tajabad	C11	25, 26
	C12	27, 28
	C76	41, 42
	C77	43, 44
	C5	45, 46
Saddar and Nouthia	C43	47, 48
	C44	49, 50
	C10	51, 52
	C46	53, 54
	C95	29, 30
Gulberg 1, 2 and 3	C93	31, 32
	C94	33, 34
	C97	35, 36
	C98	37, 38
	C96	39, 40
Ring Road	C47	55, 56
	C45	57, 58
	C80	59, 60
	C83	61, 62
	C84	63, 64
Nasir Bagh Road (Police Colony, Wazir Bagh, Regi, Askari Colony, Malkhander)	C85	65, 66
	C87	67, 68
	C88	69, 70
	C89	71, 72
	C90	73, 74
	C86	75, 76
	C49	101, 102
	C38	103, 104
	C32	105, 106

Table continues on next page.....

Location of sample collected	Clusters	Isolates name
Kohat Road, Hazar Khwani, Jameel Chowk	C33	107, 108
	C36	109, 110
	C34	111, 112
	C35	113, 114
	C22	77, 78
	C23	79, 80
	C24	81, 82
	C37	83, 84
	C13	85, 86
	C14	87, 88
Karkhano Market	C50	89, 90
Chamkani	C51	91, 92
	C39	93, 94
Hayat Abad	C52	95, 96
	C53	97, 98
Ashraf Road, Nishtarabad and Firdaus	C48	99, 100
	C63	191, 192, 197, 198
	C62	193, 194
	C61	195, 196
	C65	199, 200
Forest Bazar and Palosi	C1	138, 139
	C18	134, 135
	C19	136, 137
	C1	140, 141
	C2	142, 143
Warsak Road, Shahi baala	C25	144, 145
	C26	146, 147
	C3	148, 149
	C56	127, 128, 129
	C55	130, 131
Badbair	C54	132, 133
	C91	115, 116
	C92	117, 118
	C6	119, 120
	C7	121, 122
	C8	123, 124
	C9	125, 126
	C30	181, 182
	C31	183, 184
	C27	185, 186
	C28	187, 188

Table continues on next column.....

Location of sample collected	Clusters	Isolates name
Qisa Khwani, Ghanta Ghar, Khyber Bazar	C60	189, 190
	C68	161, 162
	C69	163, 164
	C15	165, 166
	C16	167, 168
Dalazak road	C58	169, 170
	C59	171, 172
	C57	173, 174
Gulbahar	C20	175, 176
	C21	177, 178
	C29	179, 180
	C4	150, 151
	C41	152, 153
	C42	154, 155
	C40	157, 158, 156
	C72	159, 160

DISCUSSION

ERIC-PCR has been utilized to assess genetic diversity of *E. coli* isolated from various sources. In the present study, 200 *E. coli* isolates from 300 milk samples collected from shops across Peshawar City were analyzed using ERIC-PCR. A total of 98 clusters were identified. High genetic similarity was observed among isolates collected from same areas, while high genetic diversity was observed among isolates from different areas. Samples collected from interconnected and proximate areas were grouped in the same clusters, and cluster C64 contained two isolates from different but adjacent areas, namely Tehkal and Shaheen camps. These two areas are in proximity and the milk supply to Shaheen camp is predominantly from vendors in the Tehkal area. This suggests the clonal spread of *E. coli* in these areas. Similar observations were made in other areas. The samples collected across the Nasir Bagh Road were clustered together. These results indicate that the supply of milk across these areas could be identical. The conditions of the shops, handling procedures, and supply chain processes seem to share notable similarities. Cluster C63 comprises four strains, isolated from samples collected from Hayatabad. Cluster C56 contained three isolates from different but adjacent areas. It was observed that samples from geographically distant areas did not cluster together indicating the circulation of diverse strains in the region. These findings suggest a multiple source contamination of milk samples across the region and clonal dissemination within adjacent areas.

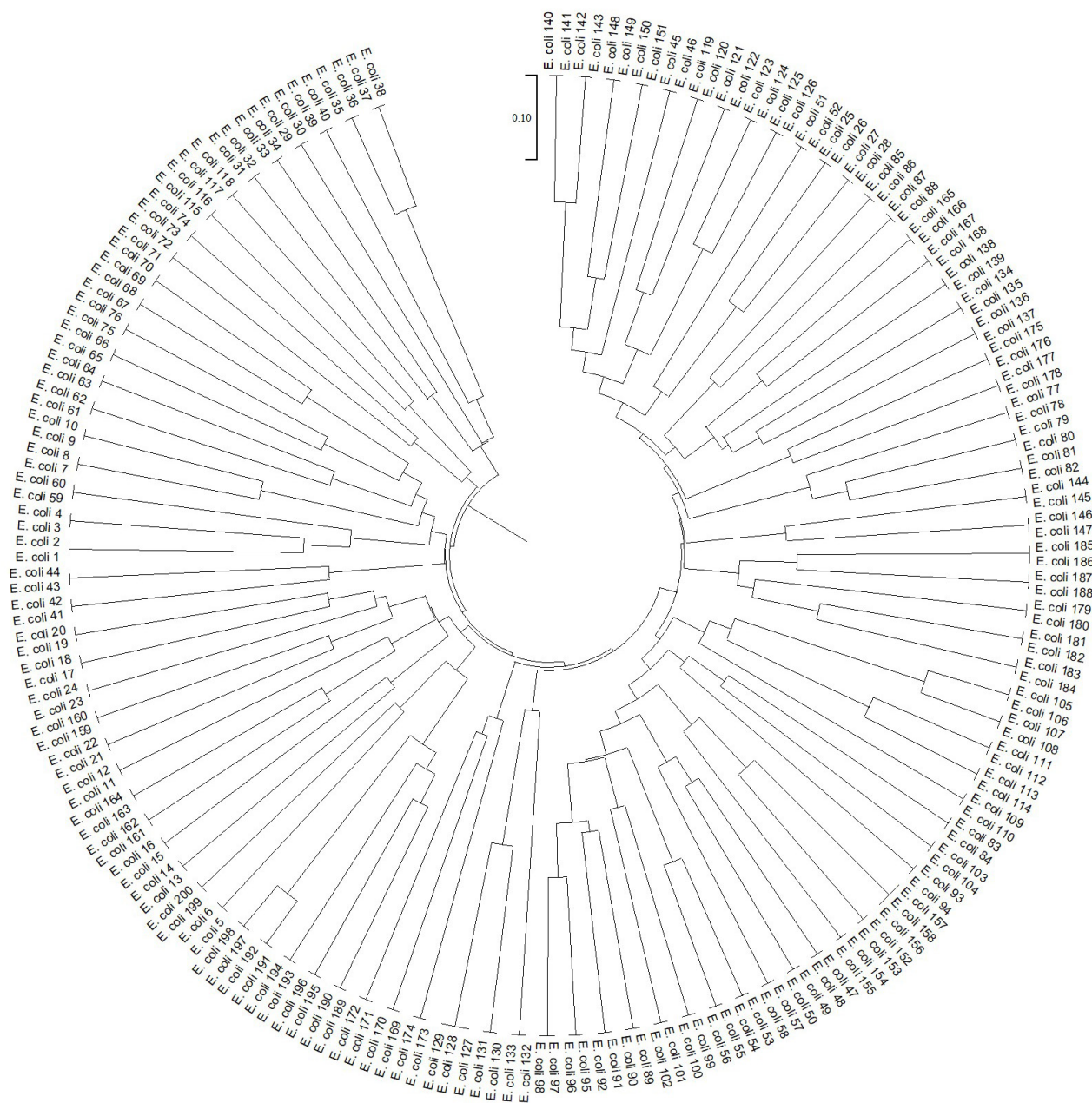


Fig. 2. Phylogenetic tree: The tree was generated using the MEGA 11 software. The isolates were clustered into groups based on genetic similarities.

The results of this study are consistent with the findings of several other studies. A study on pasteurized milk samples reported 90 clusters of *E. coli* with one clone circulating in multiple locations (Oltamari *et al.*, 2014). This study also reported clusters that contained *E. coli* isolated from different areas suggesting common contamination sources and failure of the pasteurization process. Hoffmann *et al.* (2014) reported high genotype

variability among eighty-seven *E. coli* isolates from pasteurized milk indicating the potential application of ERIC in epidemiological studies. They speculated the existence of multiple routes of milk contamination leading to high genetic diversity. In contrast to the findings of this study, a study from Egypt indicated the absence of endemic strains as high genetic diversity was observed among *E. coli* isolated from dairy farms including milk

samples (Awadallah *et al.*, 2016).

Apart from milk samples, ERIC-PCR has also been utilized to assess genetic diversity among *E. coli* isolates from various food, environmental, and clinical samples. Yar *et al.* (2022) employed this method to determine genetic diversity among *E. coli* isolates from animals (milk, feces), environment (waste water, soil), and human. They observed high genetic diversity among isolates from the environment. The results also revealed that strains from different sources were grouped into single clusters indicating the possible dissemination of these strains from animals and the environment to human and vice versa. A high genetic heterogeneity among *E. coli* isolates of fish origin was reported in another study that was attributed to multiple strains circulating in the area (Alttai *et al.*, 2023). An ERIC-PCR analysis of *E. coli* from frozen shrimp, beef and human showed similarities among the isolates from these sources and it was speculated that the food *E. coli* could act as human pathogens based on common ancestry (Alsultan and Alhadi, 2022). Several studies have also demonstrated the usefulness of ERIC-PCR for estimating genotype variability among bacterial strains from other sources (Ying *et al.*, 2015). ERIC-PCR has been extensively utilized in clinical settings to control and prevent the spread of infections, identify the source of infection, and develop strategies for improved disease management. This method has been employed for numerous pathogenic bacteria in clinical samples i.e. *Klebsiella*, *E. coli*, *Salmonella* spp. *Acinetobacter baumannii* etc (Secundo de Souza *et al.*, 2015; Jena *et al.*, 2017; Aljindan *et al.*, 2018; Sedighi *et al.*, 2020; Movahedi *et al.*, 2021).

The findings of the study provides insights into the genetic diversity, clonal relatedness and patterns of dissemination of milk contaminating *E. coli* isolates in the region. High genetic diversity among isolates from different areas shows the high adaptability nature of these isolates that could also contribute towards possession of diverse virulence factors, antimicrobial resistance mechanisms and other characteristics for flourishing in diverse environments. Whole genome sequencing studies could further elaborate these characteristic of the isolates. The findings are important with respect to public health as *E. coli* are known to cause various infections in human and their spread through milk highlight the need for proper hygienic practices during all stages of milk supply to the consumers. For the safety of consumers such studies should be routinely carried out to monitor the presence of bacterial contaminants in milk.

CONCLUSION

ERIC-PCR was found to be useful in estimating the

genetic heterogeneity among *E. coli* isolates recovered from milk that could supplement routine epidemiological studies. The results indicate that many strains of milk contaminating *E. coli* are circulating in the region. These could be pathogenic in nature posing serious threat to human. High genetic diversity of *E. coli* isolates points to a multi-source contamination of milk along the supply chain. In order to guarantee milk safety, deliberate steps should be taken to enforce hygienic practices during milk handling from production to the consumer.

DECLARATIONS

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Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Aljindan, R., Alsamman, K. and Elhadi, N., 2018. ERIC-PCR genotyping of *Acinetobacter baumannii* isolated from different clinical specimens. *Saudi J. Med. Med. Sci.*, **6**: 13-17. https://doi.org/10.4103/sjmms.sjmms_138_16
- Alsultan, A. and Elhadi, N., 2022. Evaluation of ERIC-PCR method for determining genetic diversity among *Escherichia coli* isolated from human and retail imported frozen shrimp and beef. *Int. J. Fd. Contam.*, **9**: 12. <https://doi.org/10.1186/s40550-022-00098-1>
- Alttai, N.A., Al-Sanjary, R.A. and Sheet, O.H., 2023. Genetic diversity of *Escherichia coli* harboring virulence gene Stx1 and Stx2 isolated from common carp fish in Nineveh Governorate using ERIC-PCR. *Iraqi J. Vet. Sci.*, **37**: 701-705. <https://doi.org/10.33899/ijvs.2023.137140.2642>
- Awadallah, M.A., Ahmed, H.A., Merwad, A.M. and Selim, M.A., 2016. Occurrence, genotyping, shiga toxin genes and associated risk factors of *E. coli* isolated from dairy farms, handlers and

- milk consumers. *Vet. J.*, **217**: 83-88. <https://doi.org/10.1016/j.tvjl.2016.09.014>
- Bakhshi, B., Afshari, N. and Fallah, F., 2018. Enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis as a reliable evidence for suspected *Shigella* spp. outbreaks. *Braz. J. Microbiol.*, **49**: 529-533. <https://doi.org/10.1016/j.bjm.2017.01.014>
- Berhe, G., Wasihun, A.G., Kassaye, E. and Gebreselasie, K., 2020. Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia. *BMC Publ. Hlth.*, **20**: 1-8. <https://doi.org/10.1186/s12889-020-09016-6>
- Calahorrano-Moreno, M.B., Ordoñez-Bailon, J.J., Baquerizo-Crespo, R.J., Dueñas-Rivadeneira, A.A., Montenegro, M.C.B. and Rodríguez-Díaz, J.M., 2022. Contaminants in the cow's milk we consume? Pasteurization and other technologies in the elimination of contaminants. *F1000 Res.*, **11**. <https://doi.org/10.12688/f1000research.108779.1>
- Chimuti, S., Mugadza, D.T., Ntuli, V. and Njage, P.M.K., 2024. Contribution of farm-level Hygiene and Handling Practices to Microbial Safety Profiles in the Informal Dairy Sector in Zimbabwe. *J. Fd. Prot.*, **87**: 100313. <https://doi.org/10.1016/j.jfp.2024.100313>
- Deddefo, A., Mamo, G., Asfaw, M. and Amenu, K., 2023. Factors affecting the microbiological quality and contamination of farm bulk milk by *Staphylococcus aureus* in dairy farms in Asella, Ethiopia. *BMC Microbial.*, **23**: 65. <https://doi.org/10.1186/s12866-022-02746-0>
- Goulart, D.B. and Mellata, M., 2022. *Escherichia coli* mastitis in dairy cattle: etiology, diagnosis, and treatment challenges. *Front. Microbiol.*, **13**: 928346. <https://doi.org/10.3389/fmicb.2022.928346>
- Hoffmann, S.A., Pieretti, G.G., Fiorini, A., Patussi, E.V., Cardoso, R.F. and Mikcha, J.M.G., 2014. Shiga-toxin genes and genetic diversity of *Escherichia coli* isolated from pasteurized cow milk in Brazil. *J. Fd. Sci.*, **79**: M1175-M1180. <https://doi.org/10.1111/1750-3841.12477>
- Jang, J., Hur, H.G., Sadowsky, M.J., Byappanahalli, M.N., Yan, T. and Ishii, S., 2017. Environmental *Escherichia coli*: Ecology and public health implications: A review. *J. appl. Microbiol.*, **123**: 570-581. <https://doi.org/10.1111/jam.13468>
- Jena, J., Debata, N.K., Sahoo, R.K., Gaur, M. and Subudhi, E., 2017. Genetic diversity study of various β -lactamase-producing multidrug-resistant *Escherichia coli* isolates from a tertiary care hospital using ERIC-PCR. *Indian J. med. Res.*, **146**(Suppl 1): S23-S29. https://doi.org/10.4103/ijmr.IJMR_575_16
- Megersa, R., Mathewos, M. and Fesseha, H., 2019. Isolation and identification of *Escherichia coli* from dairy cow raw milk in Bishoftu town, Central Ethiopia. *Arch. Vet. Anim. Sci.*, **1**: 1-7
- Mesele, F., Leta, S., Amenu, K. and Abunna, F., 2023. Occurrence of *Escherichia coli* O157: H7 in lactating cows and dairy farm environment and the antimicrobial susceptibility pattern at Adami Tulu Jido Kombolcha District, Ethiopia. *BMC Vet. Res.*, **19**: 6. <https://doi.org/10.1186/s12917-023-03565-9>
- Movahedi, M., Zarei, O., Hazhirkamal, M., Karami, P., Shokoohizadeh, L. and Taheri, M., 2021. Molecular typing of *Escherichia coli* strains isolated from urinary tract infection by ERIC-PCR. *Gene Rep.*, **23**: 101058. <https://doi.org/10.1016/j.genrep.2021.101058>
- Nei, M. and Li, W.H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. natl. Acad. Sci.*, **76**: 5269-5273. <https://doi.org/10.1073/pnas.76.10.5269>
- Oltamari, K., Cardoso, R.F., Patussi, E.V., Santos, A.C.B. and Mikcha, J.M.G., 2014. Genetic heterogeneity of *Escherichia coli* isolated from pasteurized milk in State of Paraná, Brazil. *Braz. J. Pharm. Sci.*, **50**: 337-343. <https://doi.org/10.1590/S1984-82502014000200013>
- Otokunefor, K., Ogugbue, C.J. and Fajoyomi, B.U., 2020. Enterobacterial repetitive intergenic consensus (ERIC) as a tool for genetic characterisation of bacterial isolates in Nigeria. *Niger. J. Biotechnol.*, **37**: 122-128. <https://doi.org/10.4314/njb.v37i1.13>
- Owusu-Kwarteng, J., Akabanda, F., Agyei, D. and Jespersen, L., 2020. Microbial safety of milk production and fermented dairy products in Africa. *Microorganisms*, **8**: 752. <https://doi.org/10.3390/microorganisms8050752>
- Ranjbar, R., Tabatabaee, A., Behzadi, P. and Kheiri, R., 2016. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) genotyping of *Escherichia coli* strains isolated from different animal stool specimens. *Iran. J. Pathol.*, **12**: 25. <https://doi.org/10.30699/ijp.2017.21506>
- Secundo de Souza, A.I., Freitas Neto, O.C.D., Batista, D.F.A., Estupinan, A.L.D.P.C., Almeida, A.M.D., Barrow, P.A. and Berchieri, A., 2015. ERIC-PCR genotyping of field isolates of *Salmonella enterica* subsp. *enterica* serovar *Gallinarum* biovars

- Gallinarum and Pullorum. *Avian Pathol.*, **44**: 475-479. <https://doi.org/10.1080/03079457.2015.1086975>
- Sedighi, P., Zarei, O., Karimi, K., Taheri, M., Karami, P. and Shokoohizadeh, L., 2020. Molecular typing of *Klebsiella pneumoniae* clinical isolates by Enterobacterial repetitive intergenic consensus polymerase chain reaction. *Int. J. Microbiol.*, **2020**: 8894727. <https://doi.org/10.1155/2020/8894727>
- Subirana, J.A. and Messeguer, X., 2020. Unique features of tandem repeats in bacteria. *J. Bact.*, **202**: 10-1128. <https://doi.org/10.1128/JB.00229-20>
- Versalovic, J., Koeuth, T. and Lupski, R., 1991. Distribution of repetitive DNA sequences in eubacteria and application to finger printing of bacterial genomes. *Nucl. Acids Res.*, **19**: 6823-6831. <https://doi.org/10.1093/nar/19.24.6823>
- Waters, V., Zlosnik, J.E., Yau, Y.C., Speert, D.P., Aaron, S.D. and Guttman, D.S., 2012. Comparison of three typing methods for *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. *Eur. J. Clin. Microbiol. Infect. Dis.*, **31**: 3341-3350. <https://doi.org/10.1007/s10096-012-1701-z>
- Yar, A., Choudary, M.A., Rehman, A., Hussain, A., Elahi, A., Rehman, F. and Khan, Z., 2022. Genetic diversity and virulence profiling of multi-drug resistant *Escherichia coli* of human, animal, and environmental origins. *Antibiotics*, **11**: 1061. <https://doi.org/10.3390/antibiotics11081061>
- Ying, C., Li, Y., Wang, Y., Zheng, B. and Yang, C., 2015. Investigation of the molecular epidemiology of *Acinetobacter baumannii* isolated from patients and environmental contamination. *J. Antibiot.*, **68**: 562-567. <https://doi.org/10.1038/ja.2015.30>
- Younis, W., Hassan, S. and Mohamed, H.M., 2021. Molecular characterization of *Escherichia coli* isolated from milk samples with regard to virulence factors and antibiotic resistance. *Vet. World*, **14**: 2410. <https://doi.org/10.14202/vetworld.2021.2410-2418>