MS.ID-IJPBS-5956
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2024) 14 (4): 119-126
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Finding of Multi Drug Resistance *Escherichia coli* as Noteworthy Emergent Environmental Contaminants

[1]Prutha N. Patel*, [2]Jignaben P. Naik and [1]Poonam B. Chauhan

[1]KBS Commerce & NATARAJ Professional Sciences College, Affiliated to VNSGU, Vapi, Gujarat, India
[2]Shri J. S. Bhakta & Shri K. M. Bhakta Arts, Shri A.N. Shah Science and Shri N. F. Shah Commerce College, Kholwad, Affiliated to VNSGU, Surat, Gujarat, India

Received: 22 Jul 2024/ Accepted: 6 Aug 2024 / Published online: 1 Oct 2024
***Corresponding Author Email:** prutha2210@gmail.com

Abstract

The global increase in antimicrobial resistance (AMR) poses an important threat, diminishing the efficiency of common antibiotics against prevalent bacterial infections. AMR, particularly multidrug resistance (MDR), has become a global issue, endangering human and animal health, food safety, and the environment. Furthermore, MDR *Escherichia coli* (*E. coli*) can colonise the intestine, displacing native commensal *E. coli* and become the dominant strain, which can easily spread between hosts. The large load of AMR in the environment can indirectly lead to increased AMR in humans through the food chain. The objective of this study was the characterisation of MDR *E. coli* from various environmental samples. Standard microbiological testing for *E. coli* isolation, identification, and antibiotic susceptibility was carried out. Out of 311 *E. coli* isolates, 155 were characterised as MDR *E. coli.* These findings are important for evaluating possible environmental pollution. *E. coli* is prevalent in environments with high AMR and MDR occurrence in a variety of fields, which provides the basis for future cooperative MDR control.

Keywords

Antibiotics Susceptibility Test (AST), Environment, *Escherichia coli,* multi-drug resistant (MDR)

*****

INTRODUCTION

Antimicrobial resistance (AMR) is a concern about world-wide health pertaining to the increasing resistance to infectious microorganism to commonly used antibiotics. Thus, among the present issues relating to the health of people, animals, and environments is the spread of AMR. The overuse of antibiotics leads to a significant increase in antibiotic resistant bacteria that are capable of subsequently transmit antibiotic resistance genes to people via food, animals, or agricultural products. Antibiotic resistance genes (ARG) are found in many different bacterial groups in the environment and are not limited to the clinic [1]. Generally, as a consequence of faecal contamination, pathogenic *Escherichia coli* (*E. coli*) can colonise different environments, such as soil and water [2]. Furthermore, certain pathotypes, most notably Shiga toxin-producing *E. coli* (STEC) have also been related to a number of foodborne disease outbreaks, which are still a leading cause of sickness in people globally. Bacterial contamination can arise from environmental, animal, or human sources at any point in the farm-to-fork food chain. Numerous causes, including the using of contaminated raw materials, inadequate sanitary conditions during food handling, and cross-contamination after processing from apparatus and the environment, may occur because of the pathogen's persistence. Commensal *E. coli* may be a major cause of AMR in the food chain in addition to

pathogenic strains [3]. *E. coli* is the third of the 12 antibiotic-resistant "priority pathogens" that the World Health Organisation has designated, is seeing an increase in the AMR burden [4]. Multidrug resistance (MDR) *E. coli* is a public health issue that has been linked to higher rates of morbidity and mortality worldwide because MDR infections restrict the options for antimicrobial treatments and complicate infection management [5]. Additionally, with its ability to acquire MDR genes, it is also a useful bio indicator for AMR tracking [6].

MATERIALS AND METHODS

In present study, various environmental samples were used to investigate antibiotic susceptibility patterns and find out MDR *E. coli*. Environmental sample collection and bacteriological analysis of *E. coli* isolated from food and water samples was done according to ICMR-Indian Council of Medical Research, Standard Operating Procedures, ICMR Foodborne Pathogen Survey and Research Network, 2024 [7].

Sample collection:

In present study, various environmental samples were collected from in and around Surat city and are categorised as food, water, and excreta.

Food: These types of samples are further categorised as based on plant and animal sources. Plant sources, like vegetable samples, were collected aseptically in sterile plastic bags to prevent cross-contaminaFtion. Obtained samples of ≈ 20–50 gram randomly from the local market of Surat. All samples were marked then sent to the laboratory. Vegetables such as green coriander, spinach, potatoes, spring onion, spring garlic, bottle gourd, brinjal, fenugreek, ginger, and carrots were collected. Animal sources like meat and milk samples were collected. A portion of ≈ 25 gram of meat and ≈ 250 gram of fish from the top centre and elsewhere of the sample was cut using a sterile knife and collected aseptically and put into a wide-mouth jar. Buffalo and cow milk samples were collected from milk dairy and cattle farms were first stirred or shaken, then collected by sterile utensil and transferred ≈ 20 mL into a sterile container.

Water: Water samples were further categorised as potable water and non-potable water and collected from different regions of Surat city. Potable water samples (lake, well, river, tap water), ≈20 mL taken in heat-sterilised glass bottle having small mouth with newly prepared sodium thiosulphate (1.8% w/v) to remove any chlorine residues (1.0 mL/1 lit). Prior to sample collection, the water was let to pass for 2-3 min. A sample was collected 30 cm below the surface, with the bottle's mouth facing the direction of running water. Non-potable water and sewage samples were collected from the different sewage treatment plants from inlet pipes in sterile containers in Surat city.

Excreta: Using sterile spatula ≈5-gram samples were collected in sterile containers from the top portion of a fresh faecal in order to prevent cross-contamination of environmental bacteria from ground [8].

All samples were collected in sterile containers appropriately marked with complete information of location, source, time, and date of collection and transported to the laboratory immediately with ice packs (4°C) to the microbiology lab for bacterial examination.

Sample processing:

Food: From 100-gram sample unit of vegetables, aseptically weighed 25 gram was mixed with 225 mL Phosphate Buffered Saline (PBS) (HIMEDIA, M1866) then swirled with a sterile glass rod for 20 min to thoroughly mix. Then incubated at 37º C for 24 hrs. From 100-gram meat sample, 25 grams crushed into fine pieces using a sterilized scissor and homogenised in 225 mL of peptone water (HIMEDIA, M028) through mixing. From each vegetable and meat sample 1 mL of the homogenate and 1 mL of milk sample were inoculated into 9 mL of modified EC broth (HIMEDIA, M1285) for enrichment.

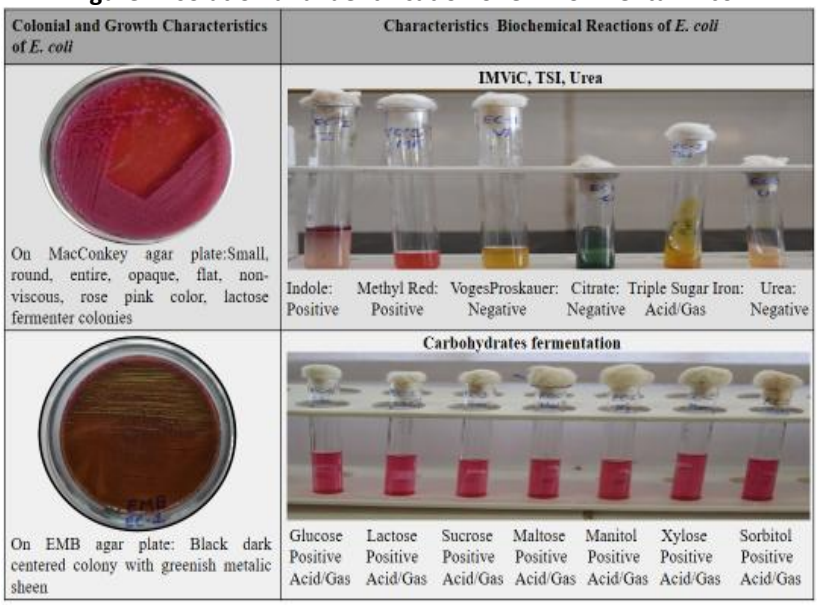
Water: one mL water sample was inoculated into 9 mL modified EC broth and incubated at 37°C for 18-24 hrs.

Excreta: One gram of faecal sample was inoculated in 9 mL modified EC broth and then incubated at 37°C for 18-24 hrs.

Isolation and Identification of *E. coli*:

One loopful of EC-broth culture was streaked on MacConkey agar (HIMEDIA, M081) plates. Streaked plates were kept in incubator at 37ºC for 18–24 hrs under aerobic conditions. Once incubation, plates were observed for *E. coli* identification. A well-isolated, rose-pink lactose-fermenting colony with typical growth and colonial features on MacConkey agar was suspected as *E. coli* and selected for standard bacteriological examination viz., Gram staining, motility test, growth characteristics on highly selective medium Eosin Methylene Blue (EMB) agar (HIMEDIA, M317) for *E. coli*, and standard biochemical tests such as the IMViC reactions (I-Indole production test, M-Methyl red test, Vi-Voges-proskauer test, C- Citrate utilization test), Urease production, and carbohydrates fermentation tests [9]. A colony with standard *E. coli* characteristics as shown in figure I was phenotypically confirmed and identified as *E. coli*.

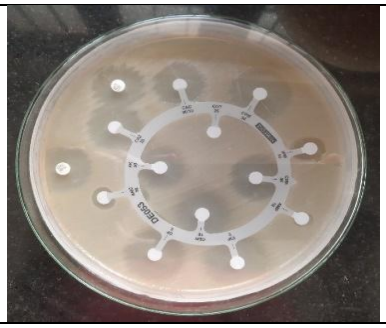
Figure I: Isolation and identification of environmental *E. coli*



Detection of MDR isolates:

Susceptibility testing to antimicrobial agents for each isolate of *E. coli* was carried out by the standard Kirby Bauer's disc diffusion methods in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. *E. coli* isolates inoculum of 0.5 Macfarland standard (HIMEDIA, R092A) density was set, then Mueller-Hinton agar (MHA) (HIMEDIA, M173) plates were inoculated by using a sterile cotton swab (HIMEDIA, PW005). Leave the inoculum to dry for 5 - 15 min. Dodeca Enterobacteriaceae-1 (HIMEDIA, DE053) and Dodeca Enterobacteriaceae-2 (HIMEDIA, DE054) a flat, inert circular ring with 12 discs, each measuring 6 mm in diameter that are coated with antibiotics facilitate the assessment of antibiotic susceptibility test (AST) of Enterobacteriaceae species were placed at the center of the plate by sterile forceps and incubated at 37°C for 18-24 hrs. Next day diameters of zone size showed in figure II were measured and recorded using a zone scale (HIMEDIA, PW096). Result was considered as sensitive, intermediate, or resistant based on the standard zone sizes for each antibiotic [11,12]. The susceptibility test was carried out using *E. coli* ATCC 25922 as the control.

Figure II: Antibiotic sensitivity patterns of environmental *E. coli* isolates

Sensitivity pattern on MHA agar plate:	**Resistance pattern on MHA agar plate:**	**MDR *E. coli* isolates**
		MDR refers to the inability to respond to at least one agent in three or more antimicrobial groups [13].
zone of inhibition around antibiotics indicates sensitivity of *E. coli* to antibiotics	No zone or reduced zone of inhibition around antibiotics indicate resistance against antibiotics	

RESULTS AND DISCUSSION

Sample type and isolation of *E. coli*:

Various environmental samples (≈410) from in and around Surat city were collected and processed for the selective isolation of *E. coli*. Different sources of isolates (Table I) identified as *E. coli* by the standard microbiological procedures are represented in figure

I. Total 311 *E. coli* isolates obtained as pure growth form were used in this study.

Table I: Environmental *E. coli* isolates distribution in sample source type

Environmental samples source	No. of Samples analyzed	No. of *E. coli* identified
Food	170	128
Water	110	74
Excreta	130	109
Total	**410**	**311**

In our study, 67% prevalence of *E. coli* obtained from water samples, which is near Kheirjou, Kheirjou, and Soltani; in their study, 56% *E. coli* isolated from water samples [14]. Further 75% *E. coli* were obtained from food samples in this research, whereas Hariri, 2022, and Ema *et al.,* 2022, in their studies, obtained 30% and 21% *E. coli* isolates from food samples, respectively [15,16].

Detection of MDR *E. coli*:

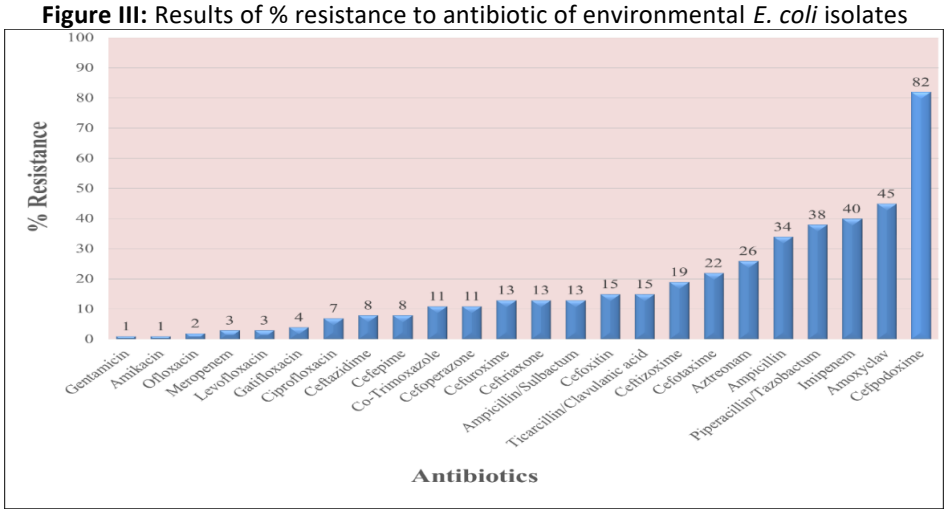
Each well-characterised isolate of *E. coli* was subjected to AST against a total of 24 antimicrobial agents, inclusive of different groups of antibiotics for MDR detection. The results of AST were as represented in Table II.

Table II: Results of Environmental *E. coli* isolates AST

ANTIBIOTIC CLASS	CLASSES NO.	ANTIBIOTICS (CODE) CONCENTRATION	TOTAL (%)		
			R	I	S
Penicillin	1	Ampicillin (AMP) 10 µg	34	14	52
Other	2	Co-Trimoxazole (COT) 25 µg	11	04	85
Monobactam	3	Aztreonam (AT) 75 µg	26	13	61
Amino-glycoside	4	Gentamicin (GEN) 10 µg	1	0	99
		Amikacin (AK) 30 µg	1	3	96
Quinolones	5	Ciprofloxacin (CIP) 5 µg	7	11	82
		Gatifloxacin (GAT) 5 µg	04	03	92
		Levofloxacin (LE) 5 µg	03	02	95
		Ofloxacin (OF) 5 µg	2	2	96
β-lactam/ β-lactam-inhibitor	6	Piperacillin/Tazobactam (PIT) 100/10 µg	38	34	28
		Ampicillin/Sulbactam (A/S) 10/10 µg	13	19	68
		Amoxyclav (AMC) 30 µg	45	24	31
		Ticarcillin/Clavulanic acid (TCC) 75/10 µg	15	21	64
Carbapenem	7	Meropenem (MRP) 10 µg	03	26	71
		Imipenem (IPM) 10 µg	40	13	47
Cephalosporin 2nd generation	8	Cefoxitin (CX) 30 µg	15	02	83
		Cefuroxime (CXM) 30 µg	12	07	81
Cephalosporin 3rd generation		Cefotaxime (CTX) 30 µg	22	27	51
		Ceftazidime (CAZ) 30 µg	08	9	83
		Ceftriaxone (CTR) 30 µg	13	11	76
		Cefoperazone (CPZ) 75 µg	11	17	72
		Ceftizoxime (CZX) 30 µg	19	16	65
Cephalosporin 4th generation		Cefpodoxime CPD 10 µg	82	11	7
		Cefepime (CPM) 30 µg	8	11	81

In this study, environmental *E. coli* isolates showed the highest sensitivity to Gentamycin (308/311) followed by Ofloxacin (299/311). Total 256/311 *E. coli* isolates show high rates of resistance against Cefpodoxime (82%) antibiotic, which is a third-generation cephalosporin. Additionally, many *E. coli* isolates (140/311) were resistant to Amoxyclav antibiotics. Resistance observed by environmental *E. coli* isolates against commonly used various antibiotics in this research is depicted in figure III.

Figure III: Results of % resistance to antibiotic of environmental *E. coli* isolates



Eight different groups or classes of antibiotics were utilized to check the AST of environmental *E. coli* isolates (Table III). Ten environmental *E. coli* showing resistance toward ≥6 classes of antibiotics and 155 environmental *E. coli* showing resistance toward ≥3 classes of antibiotics; these are MDR *E. coli* isolates. 4% did not exhibit any antibiotic resistance. These results suggest that *E. coli* might be a possible pollutant in the environment and MDR *E. coli* poses the risk to human healthiness that limits the treatment option.

This study found 50% of MDR environmental *E. coli* isolates was about similar with other studies, Iwu *et al*., (2022), according to their research 60% MDR *E. coli* isolated from non-clinical samples [17]. 27% and 20% MDR environmental *E. coli* were found by Bhowmik *et al*., (2023) and by Zhang *et al*., (2024) in their studies respectively [18,19].

Table III: Antibiotic Classes wise % resistance of *E. coli* isolates.

Antibiotic Classes	Antibiotic Classes wise % Resistance of *E. coli* isolates
Class 1	**15**
Classes 1 & 2	**31**
Classes 1, 2 & 3	**20**
Classes up to ≥ 3	**16**
Classes up to ≥ 4	**9**
Classes up to ≥ 5	**2**
Classes up to ≥ 6	**3**
Classes up to ≥ 7	**0**

MDR Environmental *E. coli* isolates (n=155 or 50%)

Total 155 MDR environmental *E. coli* isolates show different resistance patterns by number of resistant antimicrobial classes and source type wise, which is displayed in table IV. Large no. of *E. coli* (24/155) shows resistance pattern by 3 number of resistance antibiotic categories, i.e. Cephalosporin, B-lactam/B-lactam-inhibitor, Carbapenem. Total 20/155 MDR *E. coli* isolates show resistance pattern by 4 number of resistant antibiotic categories, i.e., β-lactam/ β-lactam-inhibitor, Cephalosporin, Carbapenem, and Monobactam. Followed by 11/155 MDR *E. coli* isolates show resistance pattern by 4 number of resistant antibiotic categories, i.e. Penicillin, Co-Trimoxazole, β-lactam/ β-lactam-inhibitor, and Cephalosporin. Many MDR *E. coli* isolates show resistance to four antimicrobial classes. But there are 8 such MDR *E. coli* isolates that show resistance by 7 number of antimicrobial classes. This noteworthy finding reveals that emergent of resistance *E. coli* strain against commonly used antibiotics found in environmental samples is a life-threatening issue. AMR *E. coli* present in the environment suggests possible risks to public health. Antibiotic resistance has been rising alarmingly, and the reasons for this are mutation processes, horizontal gene transfer (HGT) of resistance genes, and spontaneous genetic diversity. This mechanism allows pathogens to acquire resistance genes from environmental bacteria.

Table: IV Distribution of resistant pattern of environmental *E. coli* isolates by number of resistant antimicrobial classes and source type

***E. coli* isolates resistance to no. of classes for MDR (≥3 classes): Resistance Pattern**	**Sources of *E. coli***	**No. of MDR *E. coli***	**Total MDR *E. coli***
3: Cephalosporin, B-lactam/B-lactam-inhibitor, Monobactam	Water (WEC)	3	7
	Food (FEC)	1	
	Excreta (EEC)	3	
3: Penicillin, B-lactam/B-lactam-inhibitor, Cephalosporin,	Water (WEC)	6	16
	Food (FEC)	9	
	Excreta (EEC)	1	
3: Cephalosporin, B-lactam/B-lactam-inhibitor, Carbapenem	Water (WEC)	3	24
	Food (FEC)	13	
	Excreta (EEC)	8	
3: Penicillin, B-lactam/B-lactam-inhibitor, Co-Trimoxazole	Food (FEC)	2	5
	Excreta (EEC)	3	
3: Carbapenem, Cephalosporin, Monobactam	Water (WEC)	2	2
3: Quinolones, B-lactam/B-lactam-inhibitor, Cephalosporin,	Food (FEC)	2	2
3: B-lactam/B-lactam-inhibitor, Cephalosporin, Co-Trimoxazole	Food (FEC)	3	3
3: Penicillin, Cephalosporin, Monobactam	Water (WEC)	2	2
3: Penicillin, B-lactam/B-lactam-inhibitor, Carbapenem	Excreta (EEC)	1	1
3: B-lactam/B-lactam-inhibitor, Carbapenem, Monobactam	Excreta (EEC)	1	1
4: B-lactam/B-lactam-inhibitor, Cephalosporin, Carbapenem, Monobactam	Water (WEC)	1	20
	Food (FEC)	6	
	Excreta (EEC)	13	
4: Penicillin, B-lactam/B-lactam-inhibitor, Cephalosporin, Carbapenem	Water (WEC)	2	9
	Food (FEC)	4	
	Excreta (EEC)	3	
4: Penicillin, Co-Trimoxazole, B-lactam/B-lactam-inhibitor, Cephalosporin	Water (WEC)	2	11
	Food (FEC)	9	
4: Quinolones, Carbapenem, B-lactam/ B-lactam-inhibitor, Cephalosporin	Excreta (EEC)	1	1
4: Penicillin, B-lactam/ B-lactam-inhibitor, Cephalosporin, Monobactam	Water (WEC)	4	7
	Food (FEC)	2	
	Excreta (EEC)	1	
4: Penicillin, Quinolones, B-lactam/B-lactam-inhibitor, Cephalosporin	Excreta (EEC)	1	1

4: Penicillin, Co-Trimoxazole, B-lactam/B-lactam-inhibitor, Carbapenem	Excreta (EEC)	1	1
4: Penicillin, Co-Trimoxazole, Carbapenem, Cephalosporin	Excreta (EEC)	1	1
5: Penicillin,Co-Trimoxazole,B-lactam/B-lactam-inhibitor,Cephalosporin,Monobactam	Water (WEC)	3	3
5: Penicillin,Co-Trimoxazole,B-lactam/B-lactam-inhibitor,Cephalosporin, Carbapenem	Water (WEC) Excreta (EEC)	2 4	6
5: Penicillin, B-lactam/ B-lactam-inhibitor, Cephalosporin, Carbapenem, Monobactam	Water (WEC) Food (FEC) Excreta (EEC)	3 3 4	10
5: Carbapenem, B-lactam/B-lactam-inhibitor, Monobactam, Quinolones, Cephalosporin	Food (FEC) Excreta (EEC)	1 1	2
5: Penicillin, Quinolones, Co-Trimoxazole, B-lactam/B-lactam-inhibitor, Cephalosporin	Food (FEC) Excreta (EEC)	2 2	4
5: Penicillin, Co-Trimoxazole, Cephalosporin, Carbapenem, Monobactam	Excreta (EEC)	1	1
6: Penicillin, Quinolones, Co-Trimoxazole, B-lactam/B-lactam-inhibitor, Cephalosporin, Monobactam	Water (WEC)	2	2
6: Penicillin, Co-Trimoxazole, B-lactam/B-lactam-inhibitor, Cephalosporin, Carbapenem, Monobactam	Excreta (EEC)	2	2
6: Penicillin, Quinolones, B-lactam/B-lactam-inhibitor, Cephalosporin, Carbapenem, Monobactam	Excreta (EEC)	1	1
7: Penicillin, Quinolones, Co-Trimoxazole, B-lactam/B-lactam-inhibitor, Cephalosporin, Carbapenem, Monobactam	Water (WEC) Food (FEC) Excreta (EEC)	1 3 4	8
7: Penicillin, Quinolones, Co-Trimoxazole, B-lactam/B-lactam-inhibitor, Cephalosporin, Amino-glycoside, Monobactam	Water (WEC) Food (FEC)	1 1	2

CONCLUSION

We here conclude that, current study investigated 311 environmental *E. coli* isolates and revealed that about 50% of them were MDR. According to our findings, the prevalence of MDR is concerning rising, which severely restricts the options for treating infections. Additionally, MDR increases the risk of horizontal gene transfer between pathogenic and non-pathogenic bacteria as well as environmental reservoirs for resistance genes. These results are important for understanding possible pollution of the environment. Antimicrobial resistance surveillance systems must be expanded, early detection and close monitoring of MDR bacterial strains are necessary, and technological solutions that can stop the rise of MDR microorganism and genes into the environment must be implemented. In order to lessen or completely eradicate the risk of harmful antibiotic-resistant bacteria coming from raw foods, it is imperative to encourage careful application of antibiotics in cattle as well as to implement safe food handling and cooking procedures. For understanding the evolutionary history of environmental *E. coli*, additional data is required, particularly genomic information. More investigation is necessary as environmental antibiotic contamination and resistance are still unclear. This could support the worldwide coordinated fight against AMR and aid in tracking the spread of AMR from non-clinical sources. Additionally, it might make it easier to find and create novel antibiotics.

ACKNOWLEDGEMENT

We are grateful to the government of Gujarat. SHODH-ScHeme of Developing High quality research provided funding for this research study.

REFERENCES

1. Boripun R, Saengsawang P, Intongead S, Narinthorn R, Wongtawan T, Nissapatorn V, de Lourdes Pereira M, Mitsuwan W. Molecular characterization and nucleotide substitution of antibiotic resistance genes in multidrug-resistant *Escherichia coli* isolated from environmental swine farms. *Emerg Contam.* 9:100249, (2023).
2. Mcauley C.M, McMillan K, Moore S.C, Fegan N, Fox E.M. Prevalence and characterization of foodborne pathogens from Australian dairy farm environments. *J Dairy Sci.* 97:7402-12, (2014).
3. Crippa C, De Cesare A, Lucchi A, Parisi A, Manfreda G, & Pasquali, F. Occurrence and genomic characterization of antimicrobial-resistant and potential pathogenic *Escherichia coli* from Italian artisanal food productions of animal origin. *Italian Journal of Food Safety*. *13*(2), 12205, (2024). doi:10.4081/ijfs.2024.12205
4. Denamur E, Clermont O, Bonacorsi S, Gordon D. The population genetics of pathogenic *Escherichia coli. Nat Rev Microbiol*. 19:37-54, (2021).
5. Kasanga M, Shempela D. M, Daka V, Mwikisa M. J, Sikalima J, Chanda D & Mudenda S. Antimicrobial resistance profiles of *Escherichia coli* isolated from clinical and environmental samples: findings and implications. *JAC-antimicrobial resistance*. *6*(2), (2024). dlae061. https://doi.org/10.1093/jacamr/dlae061
6. Ramos S, Silva V, Dapkevicius M.L.E, Caniça M, Tejedor-Junco M.T, Igrejas G, Poeta P. *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: health implications of extended spectrum β-lactamase (ESBL) production. *Animals.* 10:2239, (2020).
7. Standard Operating Procedures ICMR Foodborne pathogen survey and research Network. Indian council of medical research ICMR. Revised edition 2024.
8. Cooley M.B, Jay-Russell M, Atwill E.R, Carychao D, Nguyen K, *et al.* Development of a Robust Method for Isolation of Shiga Toxin-Positive *Escherichia coli* (STEC) from Fecal, Plant, Soil and Water Samples from a Leafy Greens Production Region in California. *PLoS ONE.* 8(6): e65716, (2013) doi: 10.1371/journal.pone.0065716
9. Collee J.G, Miles R.S, Watt B. Tests for identification of bacteria. Collee J.G, Fraser A.G, Marnion B.P, Sunmon A. (Eds). In: Mackie and McCartney Practical Medical Microbiology (14thed). (pp.131–49) Churchill Livingston: New York, (1996).
10. Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing; Clinical and Laboratory Standards Institute (CLSI); Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute, (2014).
11. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute (CLSI). M100S, 27th Ed., 37(1), Wayne, PA: Clinical and Laboratory Standards Institute, (2017).
12. Bauer A. W, Kirby W. M. M, Sherris J. C, & Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 36:493-496, (1996).
13. Magiorakos A.P, Srinivasan A, Carey R.B, Carmeli Y, Falagas M.E, & Giske C.G. Multidrug-resistant, extensively drug-resistant and pan drug resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. *18*(3):268-81, (2012).
14. Kheirjou S, Kheirjou R, Soltani S. Characterization of *Escherichia coli* Isolated from the Water Supplies in Meshkin Shahr, Iran. *J Bacteriol Vaccin Res*. *2*(1): 1006, (2020).
15. Hariri S. Detection of *Escherichia coli* in food samples using culture and polymerase chain reaction methods. *Cureus*. 14:32808. doi: 10.7759/cureus.32808, (2022).
16. Ema F.A, Shanta R.N, Rahman M.Z, Islam M.A, Khatun M.M. Isolation, identification, and antibiogram studies of *Escherichia coli* from ready-to-eat foods in Mymensingh, Bangladesh. *Vet World*. *15*(6):1497-1505, (2022). doi:10.14202/vetworld.2022.1497-1505
17. Iwu C.D, Kayode A.J, Igere B.E, & Okoh A.I. High Levels of Multi Drug Resistant *Escherichia coli* Pathovars in Preharvest Environmental Samples: A Ticking Time Bomb for Fresh Produce Related Disease Outbreak. *Front. Environ. Sci.* 10:858964, (2022). doi: 10.3389/fenvs.2022.858964
18. Bhowmik A, Shah S. M. T, Goswami S, Sirajee A. S, & Ahsan S. Predominance of Multidrug Resistant *Escherichia coli* of Environmental Phylotype in Different Environments of Dhaka, Bangladesh. *Tropical medicine and infectious disease.* *8*(4), 226, (2022). https://doi.org/10.3390/tropicalmed8040226
19. Zhang *et al*., High prevalence of multidrug-resistant *Escherichia coli* in retail aquatic products in China and the first report of mcr-1-positive extended-spectrum β-lactamase-producing *E. coli* ST2705 and ST10 in fish, *International Journal of Food Microbiology.* 408:110449 (2024).