OPEN ACCESS

Scientific Letters

Research Article

Small-Scale Testing of Poultry Feces to Enumerate the Risk of Environmental Contamination and Foodborne Illnesses

Muneeba Malick [1,2,3,*] and Fasiha Saeed [1,4]

[1] Jinnah University for Women, Karachi 74600, Pakistan; fasihasaeed37@gmail.com (FS)
[2] United Medical & Dental College, Karachi, Pakistan
[3] Otsuka Pakistan Limited, HUB, Balochistan, Pakistan
[4] Dr. Panjwani Centre for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

* Correspondence: muneebamicro@gmail.com

Abstract: Live poultry stocks from local grocery markets are a potent reservoir of environmental contamination and foodborne illness. Bacteria present in poultry feces pose a potential health risk to humans through cross-contamination, which may lead to the spread of infectious diseases. The present study specifically focused on bacteria associated with poultry feces. We aimed to conduct microbiological analysis to isolate and identify microbial load present in poultry fecal samples that could lead to environmental contamination and associated risks of foodborne illnesses. Isolation from fecal samples was performed through standard culture techniques, and differential and biochemical analyses were performed as isolation and identification methods. Twenty fecal samples were collected, and the findings of this study were as follows: *Staph spp*. 100%, *E. coli spp*. 25%, *Serratia spp*. 10%, *Salmonella spp.* 15% and *Citrobacter spp*. 20%. The findings of this study provided insight into the microbial association with live poultry stocks in grocery markets and their potential wellness concerns.

Keywords: poultry feces; environmental contamination; foodborne illnesses; microorganisms

Received: 14 February 2026; Accepted: 14 May 2026; Published: 03 June 2026

Introduction

The poultry industry plays a vital role in the development of the agricultural economy across the globe. In particular, over the last decade, there has been a noticeable increase in the consumption of broiler meat across the country. Due to high demand, live poultry stocks have become a staple of the grocery market in Karachi city. This poses an elevated risk of environmental and consumer impact through the supply chain. In addition, the microorganisms associated with poultry are of major concern as they pose a substantial risk of illnesses to the public because of the spread of foodborne infections and environmental contamination. These microorganisms can move around the environment, which could lead to constant contempt for biosecurity measures. The large numbers of bacterial counts present in live poultry stock, such as in poultry feces and in the air around poultry cages, may act as a potential source in the spread of infections. It is therefore important to understand the distribution and prevalence of different bacterial strains in poultry feces, given that many of these strains are known to cause serious human infections. Previous research findings reveal an association of various microorganisms with poultry, including *Staph spp.*, *E. coli spp.*, *Salmonella spp.*, *Serratia spp.*, and *Citrobacter spp.* [1–3].

Staphylococci are facultative anaerobic, Gram-positive organisms that appear singly, in pairs, and in irregular clusters. *Staphylococci* are widely prevalent in the poultry environment, as they are present in chicken intestinal flora and are desiccation-tolerant. All *Staph spp.* produce catalase when interacting with hydrogen peroxide, and coagulase, which clots plasma. By producing heat-stable enterotoxins, *Staph spp.* cause toxic shock syndrome (TSS) [4]. These enterotoxins are known as superantigens, which cause immunosuppression and nonspecific T-cell proliferation. Poultry meat can become contaminated during

slaughter if it comes into contact with the intestines. Fresh fruits and vegetables may become contaminated by reservoirs, resulting in the spread of infection if consumed without proper washing [5,6]. *Escherichia coli* typically resides in poultry as intestinal normal flora. *E. coli* exhibits a strong habitat-specific adaptation mechanism. It remains viable for multiple weeks in poultry feces due to strong environmental persistence and transmits genetic material horizontally by conjugation, which initiates gene encoding. The intake of food contaminated with *E. coli* is a leading cause of foodborne infection [7]. It colonizes the intestinal tract under anaerobic conditions, while in a facultative environment, it can adapt to extra-intestinal sites. Intestinal infections are primarily characterized by severe diarrheal disease caused by pathogenic strains of *E. coli*, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC). In contrast, extra-intestinal infections include urinary tract infections, neonatal meningitis, and septicemia, which are linked to extra-intestinal pathogenic *E. coli* (ExPEC). The widely acknowledged transmission of *E. coli* from poultry feces to humans occurs through both direct and indirect pathways, frequently resulting in infection [8,9].

Salmonella is known to use citrate as the sole source of carbon [10]. Although *Salmonella spp.* do not significantly replicate in the natural environment, they can survive under suitable conditions. Human infections are typically associated with *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella Typhi. Salmonella spp.* are responsible for a range of foodborne illnesses; for instance, *S. typhimurium* is frequently associated with gastroenteritis, causing diarrhea and abdominal cramps [11,12], whereas *S. enteritidis* can lead to food poisoning, particularly in individuals with compromised immune systems [13]. In contrast, *S. Typhi*, which is highly adapted to humans and is not considered a natural pathogen among poultry, is the causative organism of typhoid fever. Poultry commonly acquire *Salmonella*, which can inhabit the intestinal tract, from environmental sources. These bacterial strains generally do not cause any clinical complications in poultry. However, they can be shed asymptomatically through feces [14]. Consequently, *Salmonella spp.* may contaminate poultry products and enter the food chain, posing a potential risk of foodborne infections to public health [15].

Genus *Serratia* includes facultative anaerobes, rod-shaped, Gram-negative organisms with peritrichous flagella, is ubiquitous, and has minimal nutritional requirements [16]. *Serratia marcescens* is a commonly known species in the genus that produces chitinase enzyme. Chitin is added to poultry feed as a prebiotic to prevent the colonization of pathogens (such as *E. coli and Salmonella*), being hydrolyzed by chitinase[17]. *Serratia marsecens* have been sporadically associated with the risk of foodborne diseases in humans, generally causing opportunistic infections in individuals with compromised immune systems [18].

Citrobacter are facultative anaerobic, Gram-negative bacteria, motile by peritrichous flagella, abundant in food, soil, and water [19]. They are rarely known to cause foodborne diseases through poor maintenance of the food chain. Yet, they have a significant potential to infect poultry, which does not have natural resistance to *Citrobacter*. Furthermore, diarrhea and respiratory illness can be caused by *Citrobacter spp.* while in a human host, and are occasionally known to cause opportunistic infections [20].

Materials and Methods

Sample size and collection

Fresh poultry fecal samples were collected from five different live poultry retail shops between July 2014 and November 2014. Sampling was performed systematically, with one sample collected each day from a different shop, in a rotating sequence, until samples from all five shops had been obtained. Upon completing one cycle of five samples, the same sampling procedure was repeated throughout the study period.

All collected samples were processed on the same day of collection to ensure sample freshness and minimize potential microbial changes during storage. Samples were labeled sequentially as N1, N2, N3, and so forth for proper identification and traceability.

Bulk samples were collected from each sampling site, and a representative portion of 5 g was weighed from each bulk sample using a calibrated weighing balance for subsequent microbiological analysis.

Methods

Fecal samples, 5 g in weight, were dissolved in 10 mL of PBS in a test tube and shaken gently to mix. The mixture was left undisturbed until the fecal particles settled down, and 0.1 mL of the supernatant was pipetted out and streaked on different bacteriological agar media plates, including Eosin Methylene Blue **(**EMB), MacConkey, Tryptic Soy Agar (TSA), and Brain Heart Infusion (BHI) agar with 6.5% NaCl, for bacterial isolation, and incubated at 37°C for 24 hours. Gram staining was performed after 24 hours of incubation, followed by biochemical tests, including the oxidase test using freshly prepared 1% tetramethyl-*p*-phenylenediamine, and the catalase test using hydrogen peroxide, to detect the presence of oxidase and catalase enzymes, respectively. Additionally, slants of Triple Sugar Iron (TSI) were inoculated by stabbing and streaking, whereas citrate utilization slants were streaked to assess the bacteria's ability to use citrate as a carbon source. The slant tubes were incubated again at 37°C for 24

hours and, on the following day, observations and analyses were made based on color changes and reactions to interpret the results and identify the bacterial species [21].

Results

To identify the bacterial species, 20 fecal samples were collected from different poultry shops. All the samples were examined for Gram's reaction, cultural characteristics, and biochemical tests for the identification of different bacterial species. *Staph spp.* was predominantly found in each sample. By using the formula:

$$\text{Percentage of isolated species} = \text{Observed Species/Total Samples} \times 100$$

20 out of 20 fecal samples showed 100% positive results for *Staph spp.*, 25% for *E. coli spp.*, 10% for *Serratia spp.*, 15% for *Salmonella spp,* and 20% for *Citrobacter spp.* (Fig. 1).

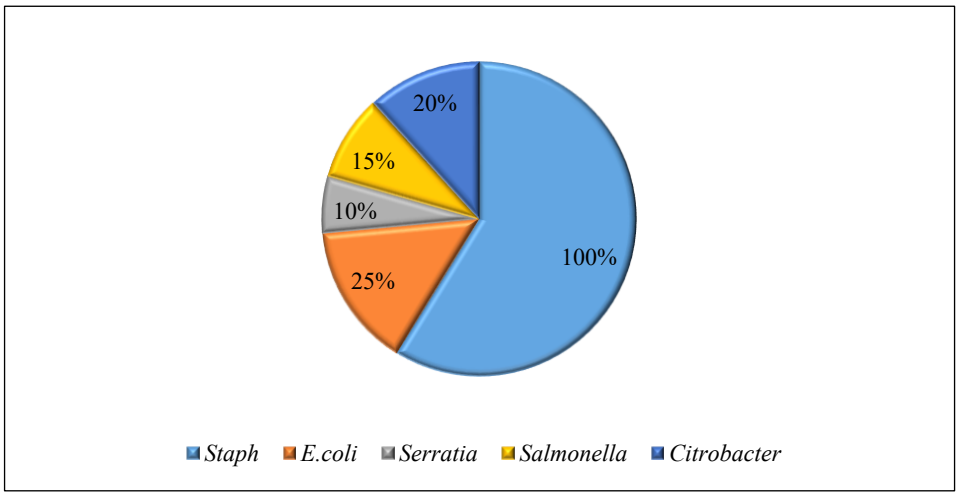

Figure 1. Percentage of isolated bacterial species from fecal poultry samples.

Differential culturing and biochemical tests were performed to identify bacterial species (Tables 1, 2, and 3).

Table 1. Colonial characteristics and Gram reaction of isolated microorganisms.

S. No.	Sample number	TSA & Gram Reaction	BHI Agar with 6.5% NaCl & Gram Reaction	MacConkey Agar & Gram Reaction	EMB Agar & Gram Reaction
1	N = 1, 2, 3, 4, 5 & 6	Convex with entire margin Gram +ve	Convex, Opaque, Circular, Smooth Gram +ve	No growth	No growth
2	N = 9 & 10	Convex with entire margin Gram +ve	Convex, Opaque, Circular, Smooth Gram +ve	Non-lactose fermenter, Pinpointed Gram -ve	Purplish colonies Gram -ve
3	N = 11 & 12	Convex with entire margin Gram +ve	Convex, Opaque, Circular, Smooth Gram +ve	Lactose fermenter, Pinpointed Gram -ve	Clear colonies Gram -ve
4	N = 7, 8, 13, 14 & 15	Convex with entire margin Gram +ve	Convex, Opaque, Circular, Smooth Gram +ve	Lactose fermenter, Pinpointed Gram -ve	Green metallic sheen Gram -ve
5	N = 18	Convex with entire margin Gram +ve	Convex, Opaque, Circular, Smooth Gram +ve	Lactose fermenter, Pinpointed Gram -ve	Light pink to colorless Gram -ve
6	N = 16, 17, 19 & 20	Convex with entire margin Gram +ve	Convex, Opaque, Circular, Smooth Gram +ve	Lactose fermenter, Pinpointed Gram -ve	Violet to black Gram -ve

BHI: Brain Heart Infusion (BHI) Agar; EMB: Eosin Methylene Blue Agar; N: sample number; TSA: Tryptic Soy Agar; +ve = positive; -ve = negative.

The first six samples had no growth on differential agar media.
All twenty samples were staph-positive, confirmed by biochemical tests (Table 2).

Table 2. Confirmatory tests of Gram-positive cocci.

S. No.	Sample number	Catalase Test	Oxidase Test	Confirmed Organism
1	N = 1 to 20	+ve	-ve	*Staph*

N: sample number; +ve = positive; -ve = negative.

The Gram-negative organisms were confirmed on the TSI slant (Table 3).

Table 3. Biochemical tests for the identification of Gram-negative microorganisms.

S. No.	Sample Number	TSI Agar				Citrate Agar	Catalase Test	Oxidase Test	Confirmed Organism
		Slant	Butt	Gas	H_2S				
1	N = 7, 8, 13, 14 & 15	A	A	-ve	-ve	-ve	+ve	-ve	*E. coli*
2	N = 9 & 10	A	A	-ve	-ve	+ve	+ve	-ve	*Serratia*
3	N = 11, 12 & 18	K	A	-ve	+ve	+ve	+ve	-ve	*Salmonella*
4	N = 16, 17, 19 & 20	K	A	+ve	+ve	+ve	+ve	-ve	*Citrobacter*

N: sample number; TSI: Triple Sugar Iron Agar; Slant/butt: Acidic (A)/Alkaline (K); +ve = positive; -ve = negative.

Discussion

Poultry-associated microorganisms contribute to the spread of foodborne infections, causing multiple illnesses and death events. The increased presence of live poultry flocks at local grocery markets questions biosecurity and has implications for management policies. Additionally, it has been reported that placing live flocks of poultry in proximity to other consumable food items enhances the transmission of poultry-associated enteric pathogens via environmental reservoirs [22–24]. *Staph spp.*, *E. coli spp.*, *Citrobacter spp.*, *Serratia spp.*, *and Salmonella spp.* were identified as poultry-associated microorganisms in this research study.

E. coli is a very well-known enteric organism and is related to various outbreaks worldwide. In the last 20 years, *E. coli* has been the primary causative agent of multiple outbreaks of foodborne illness [9]. Based on the specificity of its virulence factor, *E. coli* has been divided into different strains, from which, in 1982, the verotoxigenic *E. coli* (VTEC) or shigatoxigenic *E. coli* O157 were associated with the spread of intense diarrhea with rectal bleeding in the North American continent [25]. Three years later, another outbreak of VTEC was reported in Europe. It is also estimated that VTEC O157 causes approximately 73,000 cases of infection and 250 deaths yearly in the United States [8]. Further studies established that *E. coli* acquired the shiga toxin-producing gene from *Shigella spp.* via the horizontal process of conjugation.

S. aureus has been significantly linked to nosocomial and community-acquired infection and has emerged as a zoonotic issue due to the increasing incidence of foodborne illness. This research study has shown 100% *S. aureus* positive results. The Netherlands reported a prevalence of 11.9% methicillin-resistant *Staphylococcus aureus* (MRSA*)* in poultry meat. Another case-control study conducted in Denmark and the Netherlands documented that people who work on farms are directly in contact with *Staph spp.*, which puts them at high risk of acquiring associated infections. The risk of coming into contact with poultry-originating *Staph spp.* requires sufficient risk assessment [23,26]. It has also been documented that poultry and poultry meat are responsible for the spread of *Salmonella* infection. Reports confirmed that, in developing countries, transmission of foodborne illness by *Salmonella* species, including typhoid fever, is due to contaminated food supply, while isolated strains of *Citrobacter* and *Serratia* from poultry fecal samples are known to cause fatal opportunistic infections of the respiratory and urinary tracts in immunocompromised hosts [15,18,19].

This study concludes that live poultry stock should not be accommodated in grocery markets due to the risk of infections and foodborne illness among individuals. Poultry meat must be washed with clean water for bacterial shedding and must not be raw-cooked to kill bacteria and bacterial toxins. These isolated microorganisms are capable of contaminating fresh fruits, vegetables, and surfaces, which may result in the spread of fatal outbreaks of foodborne illness. In addition, poultry feces have also been used as manure to boost soil fertility and increase crop cultivation, which requires a microbiologically safe procedure before transport to prevent the spread of pathogenic strains in the environment. The outcome of this study shows that a specific, reachable location should be assigned for live poultry stock to eliminate the risk of environmental contamination and foodborne illness. Alongside this, consumers and the general public should be made aware of the health risks and hygiene issues associated with poultry.

Acknowledgments

This study was supported and funded by the Department of Microbiology, Jinnah University for Women. The authors are thankful to Muhammad Haque Nawaz, Sultana Khatoon, Aman ul Hassan Shaikh, Moosa Bin Aman, Prof. Dr. Sayyada Ghufrana Nadeem, and Ms. Farkhanda Afaque for their technical assistance and provision of facilities.

Author Contributions

MM and FS contributed to the design of the study. MM was responsible for sample collection, reagent preparation, experiment performance, and writing the first draft of the manuscript. FS arranged the funding, drafted the results, and commented on previous versions of the manuscript. Both authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

References

1. Nasrin, M.S.; Islam, M.J.; Nazir, K.H.M.N.H.; Choudhury, K.A.; Rahman, M.T. Identification of Bacteria and Determination of Their Load in Adult Layer and Its Environment. *Journal of the Bangladesh Society for Agricultural Science and Technology* **2007**, 69–72.
2. Bano, R.; Shah, H.; Sharif, M.; Akhtar, W. *An Overview of the Pakistan Poultry Sector*; 2009;
3. Chang, H.-S. Analysis of the Philippine Chicken Industry: Commercial versus Backyard Sectors. *Asian Journal of Agriculture and Development* **2007**, *4*, 41–56, doi:10.37801/ajad2007.4.1.4.
4. Mahami, T.; Togby-Tetteh, W.; Kottoh, D.I.; Amoakoah-Twum, L.; Gasu, E.; Annan, S.N.Y.; Larbi, D.; Adjei, I.; Adu-Gyamfi, A. Microbial Food Safety Risk to Humans Associated with Poultry Feed: The Role of Irradiation. *Int. J. Food Sci.* **2019**, *2019*, 1–7, doi:10.1155/2019/6915736.
5. Kadariya, J.; Smith, T.C.; Thapaliya, D. Staphylococcus Aureus and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. *Biomed Res. Int.* **2014**, *2014*, 827965, doi:10.1155/2014/827965.
6. University of Washington Environmental Health and Safety *Zoonotic Disease Prevention When Working with Poultry*;
7. Todar, K. Pathogenic E. Coli. In *Textbook of Bacteriology*; Department of Bacteriology, University of Wisconsin–Madison: Madison, WI, USA, 2008.
8. Food-Borne Diseases: E. Coli 0157:H7 Available online: https://www.infoplease.com/math-science/health/diseases/food-borne-diseases-e-coli-0157h7 (accessed on 16 January 2026).
9. Bélanger, L.; Garenaux, A.; Harel, J.; Boulianne, M.; Nadeau, E.; Dozois, C.M. Escherichia Coli from Animal Reservoirs as a Potential Source of Human Extraintestinal Pathogenic E. Coli. *FEMS Immunol. Med. Microbiol.* **2011**, *62*, 1–10, doi:10.1111/J.1574-695X.2011.00797.X.
10. Kabir, S.M.L. Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns. *Int. J. Environ. Res. Public Health* **2010**, *7*, 89–114, doi:10.3390/ijerph7010089.
11. Cabral, J.P.S. Water Microbiology. Bacterial Pathogens and Water. *Int. J. Environ. Res. Public Health* **2010**, *7*, 3657–3703, doi:10.3390/ijerph7103657.
12. Petkov, D.; Collins-Emerson, J.; French, N. *Characterisation of Salmonella (MAF Technical Paper No. 2011/67)*; Ministry of Agriculture and Forestry, 2010;
13. Rouger, A.; Tresse, O.; Zagorec, M. Bacterial Contaminants of Poultry Meat: Sources, Species, and Dynamics. *Microorganisms* **2017**, *5*, 50, doi:10.3390/microorganisms5030050.
14. Sharma, S.; Kaur, S.; Naguib, M.; Bragg, A.; Schneider, A.; Kulkarni, R.R.; Nazmi, A.; Abdelaziz, K. Major Foodborne Bacterial Pathogens in Poultry: Implications for Human Health and the Poultry Industry and Probiotic Mitigation Strategies. *Microorganisms* **2025**, *13*, 2363, doi:10.3390/microorganisms13102363.
15. Shaji, S.; Selvaraj, R.K.; Shanmugasundaram, R. Salmonella Infection in Poultry: A Review on the Pathogen and Control Strategies. *Microorganisms* **2023**, *11*, 2814, doi:10.3390/microorganisms11112814.

16. Młynarczyk, A.; Młynarczyk, G.; Pupek, J.; Bilewska, A.; Kawecki, D.; Łuczak, M.; Gozdowska, J.; Durlik, M.; Pączek, L.; Chmura, A.; et al. Serratia Marcescens Isolated in 2005 From Clinical Specimens From Patients With Diminished Immunity. *Transplant. Proc.* **2007**, *39*, 2879–2882, doi:10.1016/j.transproceed.2007.08.046.
17. David, M.D.; Weller, T.M.A.; Lambert, P.; Fraise, A.P. An Outbreak of Serratia Marcescens on the Neonatal Unit: A Tale of Two Clones. *Journal of Hospital Infection* **2006**, *63*, 27–33, doi:10.1016/j.jhin.2005.11.006.
18. Mahata, M.E.; Lestari, I.; Dharma, A.; Ryanto H., I.; Armenia; Rizal, Y. Use of Serratia Marcescens for Feed Processing: Broiler Performance and Pathogenicity Assay. *Int. J. Poult. Sci.* **2012**, *11*, 424–426, doi:10.3923/ijps.2012.424.426.
19. Guentzel, M.N. Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus. In *Medical Microbiology*; Baron, S., Ed.; University of Texas Medical Branch at Galveston, 1996 ISBN 0963117211.
20. Oakley, B.B.; Morales, C.A.; Line, J.; Berrang, M.E.; Meinersmann, R.J.; Tillman, G.E.; Wise, M.G.; Siragusa, G.R.; Hiett, K.L.; Seal, B.S. The Poultry-Associated Microbiome: Network Analysis and Farm-to-Fork Characterizations. *PLoS One* **2013**, *8*, e57190, doi:10.1371/journal.pone.0057190.
21. Cain, D.; Hanks, H.; Weis, M.; Bottoms, C.; Lawson, J. Microbiology Laboratory Manual Biol 2421L Available online: https://pt.scribd.com/document/257221438/Microbiology-Lab-Manual-Revised-Spring-2013.
22. Newell, D.G.; Koopmans, M.; Verhoef, L.; Duizer, E.; Aidara-Kane, A.; Sprong, H.; Opsteegh, M.; Langelaar, M.; Threfall, J.; Scheutz, F.; et al. Food-Borne Diseases — The Challenges of 20 Years Ago Still Persist While New Ones Continue to Emerge. *Int. J. Food Microbiol.* **2010**, *139*, S3–S15, doi:10.1016/j.ijfoodmicro.2010.01.021.
23. European Food Safety Authority (EFSA) Assessment of the Public Health Significance of Meticillin Resistant Staphylococcus Aureus (MRSA) in Animals and Foods. *EFSA Journal* **2009**, *7*, 1–73, doi:10.2903/j.efsa.2009.993.
24. Mgbeahuruike, A.C.; Agina, O.A.; Anyanwu, M.U.; Emejuo, N.T.; Ekere, S.O.; Ugwu, P.C.; Uju, C.N.; Andong, F.A. Microbial Contamination of Poultry Feed and the Effects on Birds' Performance. *Animal Research International* **2023**, *20*, 4834–4861, https://www.ajol.info/index.php/ari/article/view/247059.
25. Woodward, D.L.; Clark, C.G.; Caldeira, R.A.; Ahmed, R.; Rodgers, F.G. Verotoxigenic Escherichia Coli (Vtec): A Major Public Health Threat in Canada. *Canadian Journal of Infectious Diseases and Medical Microbiology* **2002**, *13*, 321–330, doi:10.1155/2002/383840.
26. Kolchyk, O. V.; Borovuk, I. V.; Buzun, A.I.; Illarionova, T. V.; Zazharska, N.M. Microorganisms' Growth Inhibition in Poultry Meat Using Bacillus Spp. *World's Veterinary Journal* **2024**, *14*, 424–434, doi:10.54203/scil.2024.wvj50.