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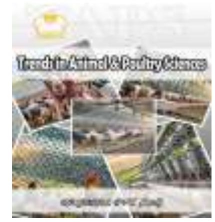

Prevalence, Antibiotic Resistance, and Antibacterial Activity of Herbal Extracts Against *Escherichia coli* from Food and Waterborne Sources in Multan, Pakistan

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Abstract

Escherichia coli is a major foodborne pathogen and indicator of fecal contamination, posing significant public health risks, particularly in developing countries. The study investigated the prevalence, antibiotic resistance profiles, and antibacterial potential of selected herbal extracts against E. coli in District Multan, Pakistan. A total of 100 food and water samples, including poultry, beef, dairy, fruits, vegetables, herbs, and tap water, were collected. The isolation and identification of E. coli were performed using standard microbiological techniques, including selective culturing and biochemical confirmation. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method against commonly used antibiotics, and MAR indices were calculated. Antibacterial activity of various herbal extracts was evaluated using the disc diffusion method. Results revealed high E. coli prevalence in tap water (60%), poultry (46.7%), beef (40%), and vegetables (40%), indicating critical points of contamination in the food chain. Antimicrobial susceptibility testing showed very high resistance to penicillin (81.8%) and amoxicillin (72.7%), high resistance to tetracycline (68.2%), azithromycin (65.9%), and erythromycin (61.4%), and lower resistance to ciprofloxacin (22.7%) and chloramphenicol (25%), reflecting substantial antibiotic selection pressure. MAR indices further confirmed high-risk contamination sources, particularly in β-lactam and tetracycline-exposed environments. Among the herbal extracts, clove (17.5 ± 0.5 mm), cinnamon (16.8 ± 0.7 mm), and Nigella sativa (15.3 ± 0.6 mm) exhibited the strongest antibacterial activity. These findings highlight the urgent need for improved food hygiene practices, rational antibiotic use, and the exploration of alternative antimicrobial strategies to mitigate the growing threat of foodborne antimicrobial resistance.

Introduction

Foodborne diseases continue to pose a major global public health challenge, particularly in low and middle-income countries where food safety systems and regulatory enforcement are often inadequate. Among bacterial foodborne pathogens, *Escherichia coli* is of particular importance due to its widespread occurrence in food and its capacity to cause serious human illness. While many *E. coli* strains exist as harmless commensals in the intestinal tract of humans and animals, pathogenic variants are responsible for a wide range of clinical conditions, including diarrhea,

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hemorrhagic colitis, urinary tract infections, septicemia, and hemolytic uremic syndrome (Kaper et al., 2004; Croxen et al., 2013).

The presence of *E. coli* in food is commonly used as an indicator of fecal contamination and reflects poor hygienic practices during food production, handling, processing, and storage. Raw and minimally processed foods such as milk, meat, fresh vegetables, and ready-to-eat street foods are particularly vulnerable to contamination, especially in informal food markets (Newell et al., 2010). According to the World Health Organization, foodborne diseases affect approximately 600 million people annually worldwide, resulting in an estimated 420,000 deaths, with bacterial pathogens contributing substantially to this burden (WHO, 2015). Developing countries experience a disproportionate impact due to unsafe water supplies, inadequate sanitation, and limited food safety surveillance.

Pathogenic *E. coli* strains are classified into several pathotypes based on their virulence mechanisms and disease manifestations, including enterotoxigenic (*ETEC*), enteropathogenic (*EPEC*), enterohemorrhagic (*EHEC*), enteroaggregative (*EAEC*), and Shiga toxin-producing *E. coli* (*STEC*) (Nataro and Kaper, 1998). These strains acquire virulence genes through mobile genetic elements such as plasmids, bacteriophages, and pathogenicity islands, enabling rapid evolution and adaptation (Allocati et al., 2013). Foodborne transmission remains one of the most significant routes for human exposure to pathogenic *E. coli*, particularly through contaminated animal-derived foods.

Antimicrobial resistance (AMR) has emerged as a critical threat to global health, food security, and sustainable development. The extensive use of antibiotics in human medicine, veterinary practice, and livestock production has accelerated the selection and dissemination of resistant bacteria (Van Boeckel et al., 2015). *Escherichia coli* plays a key role in the epidemiology of AMR as both a foodborne pathogen and a reservoir of resistance genes that can be transferred to other bacteria via horizontal gene transfer (Poirel et al., 2018).

The presence of antimicrobial resistant *E. coli* in the food chain represents a direct risk to consumers and complicates the treatment of human infections. Resistance to commonly used antibiotics, including penicillins, tetracyclines, macrolides, and sulfonamides, has been widely reported in *E. coli* isolated from food products (Olaimat et al., 2018). Multidrug-resistant strains are increasingly detected in food and environmental samples, reflecting intense antibiotic exposure to food producing animals. The Multiple Antibiotic Resistance (MAR) index is frequently used to assess contamination risk and antibiotic usage pressure associated with bacterial isolates (Krumperman, 1983).

In Pakistan, food safety and antimicrobial resistance are growing concerns due to rapid urbanization, expansion of informal food markets, and insufficient enforcement of food hygiene regulations. Antibiotics are commonly used in livestock and poultry production for therapeutic and non-therapeutic purposes, often without adequate veterinary supervision (Sohaib et al., 2017). Consequently, resistant bacteria and antibiotic residues frequently enter the food chain. Several studies have reported high prevalence and alarming resistance patterns of *E. coli* in raw milk, meat, poultry, vegetables, and street foods in different regions of Pakistan (Hussain et al., 2015, Bilal et al., 2021). However, region-specific data from southern Punjab, particularly District Multan, remains limited.

In parallel with concerns regarding antimicrobial resistance, interest has increased in plant-derived antimicrobial agents as potential alternatives or adjuncts to conventional antibiotics. Medicinal plants and culinary herbs such as clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), and *Nigella sativa* possess bioactive compounds with demonstrated antibacterial activity against *E. coli* and other foodborne pathogens (Cowan, 1999, Burt, 2004).

The present study aimed to determine the prevalence of *Escherichia coli* in commonly consumed food items in District Multan, Pakistan, evaluate their antimicrobial resistance profiles and MAR indices, and assess the antibacterial activity of selected herbal agents. The findings are expected to provide region-specific evidence to support food safety interventions, rational antibiotic use, and mitigation of public health risks.

Materials and Methods

Sample collection

A cross-sectional laboratory-based study was conducted in District Multan, Punjab, Pakistan, a major urban and agricultural region characterized by extensive informal food markets. A total of 100 samples including bakery products, dairy, poultry, beef, fruits, vegetables, herbs (ginger powder, rosemary leaves, turmeric powder, white onion powder, yellow mustard seeds), and tap water were collected randomly from multiple locations across District Multan, Pakistan. All samples were collected using standard hygienic procedures, placed in sterile containers, and transported promptly to the Microbiology Laboratory, Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan.

Isolation and Identification of *Escherichia coli*

The collected samples were homogenized and pre-enriched in buffered peptone water, followed by selective culturing on MacConkey agar and eosin methylene blue (EMB) agar. Presumptive colonies exhibiting characteristic morphology were subjected to Gram staining and biochemical confirmation using indole, methyl red, Voges-Proskauer, and citrate utilization tests (Quinn et al., 2011).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility of confirmed *Escherichia coli* isolates was determined using the modified Kirby-Bauer disc diffusion method against tetracycline (30 µg), penicillin (10 IU), ciprofloxacin (5 µg), amoxicillin-clavulanic acid (Augmentin; 20/10 µg), neomycin (30 µg), erythromycin (15 µg), kanamycin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), amoxicillin (25 µg), and azithromycin (15 µg).

Multiple Antibiotic Resistance (MAR) Index

The Multiple Antibiotic Resistance (MAR) index for each *Escherichia coli* isolate was calculated according to the method described by Krumperman (1983) to evaluate the extent of antibiotic exposure and to infer potential contamination from high-risk sources.

Antibacterial Activity of Herbal Agents

Furthermore, the antibiotic potential of selected domestic herbal extracts was evaluated using the agar diffusion method, including ginger powder, rosemary leaves, turmeric powder, onion powder, cinnamon powder, moringa powder, *Nigella sativa*, neem, cloves, and yellow mustard seeds (Burt, 2004). The zones of inhibition were measured in millimeters.

Statistical Analysis

Data was analyzed using SPSS software (version 20; IBM Corp., Armonk, NY, USA). Descriptive statistics were used to determine the prevalence and antibiotic susceptibility of *Escherichia coli*. Associations between categorical variables were evaluated using the chi-square (χ^2) test, and logistic regression was applied to assess potential risk factors. Statistical significance was set at $P < 0.05$.

Results

Prevalence of *Escherichia coli*

Escherichia coli isolates were confirmed using selective and differential media. Colonies exhibiting a green metallic sheen on eosin methylene blue agar and pink, lactose-fermenting colonies on MacConkey agar were considered confirmatory for *E. coli*. The prevalence of *E. coli* varies among food and water categories. The tap water showed the highest contamination rate (60%), followed by poultry (46.7%), beef (40%), and vegetables (40%), Table 1.

Antibiotic Resistance of *E. coli* Isolates

The antimicrobial susceptibility testing revealed high resistance rates to penicillin (81.81%), amoxicillin (72.72%), and tetracycline (68.18%). Moderate resistance was detected for erythromycin (61.36%), azithromycin (65.90%), and streptomycin (43.18%). Comparatively lower resistance levels were recorded for ciprofloxacin (22.72%), chloramphenicol (25 %), and kanamycin (29.54%), Table 2.

Fig. 1. Colony morphology of *Escherichia coli* observed on MacConkey agar.

Multiple Antibiotic Resistance (MAR) Index

The MAR indices of *E. coli* isolates varied across antibiotics Table 3. The high MAR indices were observed for penicillin (0.82), amoxicillin (0.73), tetracycline (0.68), azithromycin (0.66), and erythromycin (0.61). Intermediate MAR indices were noted for augmentin (0.34), streptomycin (0.43), kanamycin (0.30), and neomycin (0.39). Lower MAR indices were observed for ciprofloxacin (0.23) and chloramphenicol (0.25).

Antibacterial Activity of Herbal Extracts

The antibacterial activity of herbal extracts against *E. coli* exhibited considerable variation, (Fig. 2). Clove extract demonstrated the largest zone of inhibition (17.5 ± 0.5 mm), followed by cinnamon (16.8 ± 0.7 mm) and *Nigella sativa* (15.3 ± 0.6 mm). Moderate activity was observed for neem (13.9 ± 0.4 mm), moringa (14.1 ± 0.5 mm), ginger (13.2 ± 0.5 mm), yellow mustard (12.3 ± 0.5 mm), and turmeric (11.4 ± 0.4 mm), while onion (10.6 ± 0.3 mm) and rosemary leaves (9.5 ± 0.6 mm) displayed only mild antibacterial activity.

Discussion

The detection of *Escherichia coli* across a wide range of food items and water samples highlights persistent hygiene and sanitation challenges within the food production and distribution chain in District Multan, Pakistan. The occurrence of *E. coli* in tap water strongly suggests fecal contamination, which is commonly associated with inadequate water treatment systems, aging infrastructure, and cross-contamination from sewage sources. Similar observations have been reported from other regions of Pakistan and low- and middle-income countries, where unsafe drinking water remains a major contributor to foodborne and waterborne illnesses (Jalali et al., 2020, Tariq et al., 2023). Contamination of poultry, meat, and fresh produce further reflects poor handling practices, improper slaughtering conditions, and the use of contaminated water for irrigation and washing, all of which are well-recognized risk factors for *E. coli* transmission (Imdad et al., 2018, Saeed et al., 2019).

The antimicrobial resistance profile of the isolates indicates substantial selective pressure exerted by commonly used antibiotics. High resistance to β-lactam antibiotics and tetracyclines is consistent with their extensive and often indiscriminate use in veterinary medicine, livestock production, and human healthcare. These antibiotics are frequently administered for therapeutic, prophylactic, and growth-promotion purposes, creating an environment conducive to the emergence and persistence of resistant strains (McEwen & Collignon, 2018, Van et al., 2020). The comparatively lower resistance observed against fluoroquinolones and chloramphenicol suggests limited or more regulated use of these agents; however, the presence of resistance even to these antibiotics is concerning and reflects the global trend of diminishing antimicrobial effectiveness (WHO, 2021).

The elevated Multiple Antibiotic Resistance (MAR) indices observed among the isolates provide further evidence that these bacteria likely originated from high-risk environments with frequent antibiotic exposure. MAR indices exceeding the recommended threshold indicate contamination sources associated with intensive antimicrobial usage, such as commercial livestock farms and densely populated human settlements (Krumperman, 1983). Similar MAR patterns have been documented in foodborne *E. coli* isolates from South Asia, reinforcing the need for stricter antimicrobial stewardship and surveillance in both agricultural and public health sectors (Nhung et al., 2017, Zhang et al., 2019).

The observed antibacterial activity of selected herbal extracts supports the growing body of evidence advocating plant-derived compounds as potential complementary antimicrobial agents. The strong inhibitory effects of clove, cinnamon, and *Nigella sativa* are consistent with previous studies attributing their efficacy to bioactive constituents such as eugenol, cinnamaldehyde, and thymoquinone, which disrupt bacterial cell membranes and metabolic pathways (Chaieb et al., 2007, Friedman et al., 2002). While other extracts demonstrated moderate to mild activity, their cumulative effects highlight the antimicrobial potential of traditional medicinal plants. Although herbal extracts cannot replace conventional antibiotics, they may serve as alternative or adjunct options, particularly in food preservation and in mitigating the spread of resistant bacteria (Alzohairy, 2016, Singh et al., 2019).

From a public health perspective, the coexistence of food contamination and antimicrobial resistance poses a significant threat to consumer safety. The presence of multidrug-resistant *E. coli* in commonly consumed foods and drinking water increases the risk of difficult-to-treat infections and facilitates the transfer of resistance genes within the microbial community. These findings align with the One Health framework, emphasizing the interconnectedness of human, animal, and environmental health and the urgent need for integrated control strategies (WHO, 2021).

Overall, the study underscores the necessity for improved water sanitation, enhanced food hygiene practices, rational antibiotic use, and continuous surveillance of antimicrobial resistance. Strengthening regulatory frameworks, educating food handlers and farmers, and promoting alternative antimicrobial approaches are critical steps toward reducing the burden of foodborne *E. coli* and curbing the spread of antibiotic resistance.

Conclusion

This study confirms the widespread occurrence of *Escherichia coli* in food and drinking water, indicating deficiencies in sanitation, food hygiene, and water quality management. The presence of multidrug-resistant isolates with elevated Multiple Antibiotic Resistance (MAR) indices reflects substantial antibiotic selection pressure associated with inappropriate antimicrobial use in human and animal sectors. The observed antibacterial activity of selected herbal extracts highlights their potential as complementary antimicrobial agents. Future research should prioritize molecular characterization of resistance determinants and further evaluation of plant-based antimicrobials for controlling foodborne pathogens.

Author Contributions

AA designed the study. MTS, NA, IH and TI collected the samples. BK conducted lab experiments. MN, and MZ collected the data. RK, and SM performed statistical analysis. BK drafted the manuscript, and AA revised and finalized the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical statement

All animal handling procedures were approved by the Ethics Committee of the Department of Pathobiology & Biomedical Sciences, Faculty of Veterinary & Animal Sciences, MNS-University of Agriculture, Multan, Pakistan.

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Table 1: Prevalence of *E. coli* in food samples

Category	Samples Tested	Positive Samples	Prevalence (%)
Bakery Products	10	2	20%
Dairy	15	5	33.3%
Poultry	15	7	46.7%
Beef	10	4	40%
Fruits	10	3	30%
Vegetables	10	4	40%
Tap water	20	12	60%
Herbs	10	3	30%

Table 2. Patterns of antibiotic resistance among *Escherichia coli* isolates

Antibiotic	Dosage (µg)	Resistant Isolates (n)	Resistance (%)
Tetracycline	30	30	68.18%
Penicillin	10	36	81.81%
Ciprofloxacin	5	10	22.72%
Augmentin	20/10	15	34.09%
Neomycin	30	17	38.63%
Erythromycin	15	27	61.36%
Kanamycin	30	13	29.54%
Streptomycin	10	19	43.18%
Chloramphenicol	30	11	25.00%
Amoxicillin	25	32	72.72%
Azithromycin	15	29	65.90%

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Table 3. Antibiotic resistance profile of *Escherichia coli* isolates

Antibiotic	No. of Resistant Isolates (a)	Total Isolates Tested (b)	MAR Index (a/b)	Resistance Level
Tetracycline	30	44	0.68	High
Penicillin	36	44	0.82	Very High
Ciprofloxacin	10	44	0.23	Low
Augmentin	15	44	0.34	Moderate
Neomycin	17	44	0.39	Moderate
Erythromycin	27	44	0.61	High
Kanamycin	13	44	0.30	Moderate
Streptomycin	19	44	0.43	Moderate
Chloramphenicol	11	44	0.25	Low
Amoxicillin	32	44	0.73	Very High
Azithromycin	29	44	0.66	High

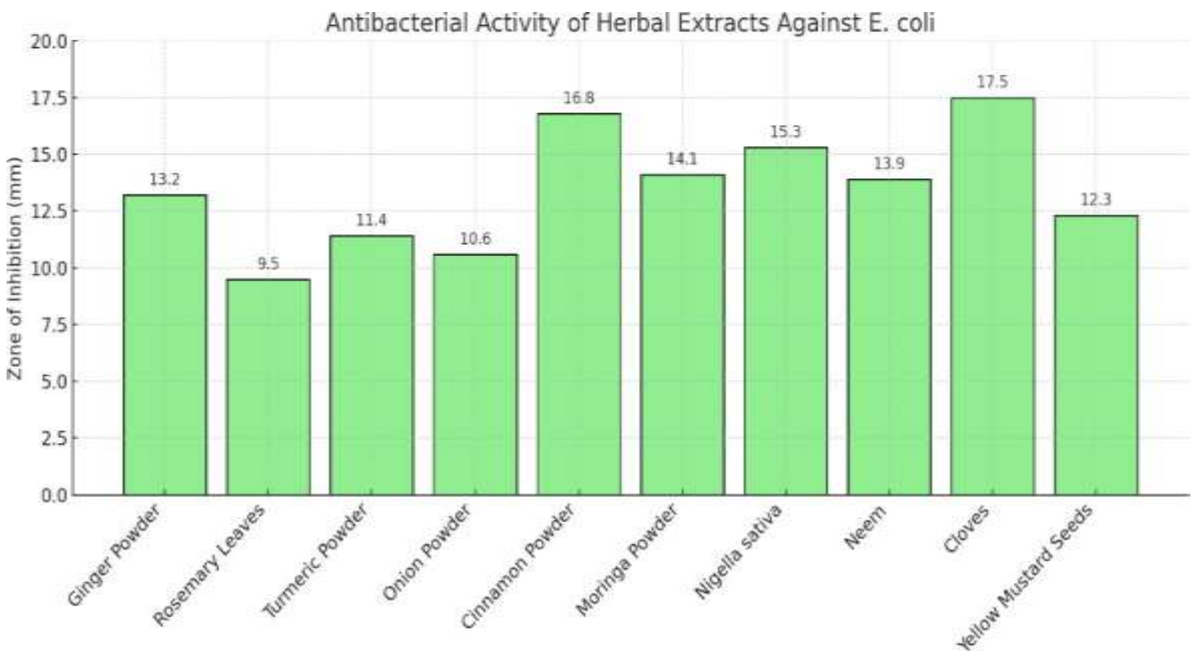

Fig. 2. Antibacterial activity of selected herbal extracts against *Escherichia coli*.