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INVESTIGATING AND CO EXISTENCE OF SALMONELA AND E. COLI IN DIARRHEA PATIENT

Original Research

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ABSTRACT

Background: Diarrheal diseases and reproductive disorders such as abortion remain critical public health concerns, particularly in low-resource settings. *Salmonella* spp. and *Escherichia coli* (E. coli) are significant enteric pathogens not only causing gastrointestinal illness but also implicated in systemic infections with potential reproductive consequences. Co-infections, antimicrobial resistance, and the diagnostic challenges related to reproductive outcomes emphasize the need for integrated clinical and laboratory surveillance.

Objective: To evaluate the clinical relevance of *Salmonella* and *E. coli* co-infections in diarrhea patients, and to determine the diagnostic performance of RBPT, ELISA, and PCR tests in predicting abortion outcomes.

Methods: A cross-sectional study was conducted involving 200 diarrheic patients, from whom stool samples were collected for microbiological identification of *Salmonella* and *E. coli*, followed by antibiotic susceptibility testing. Additionally, 165 serum samples were analyzed using Rose Bengal Plate Test (RBPT), Enzyme-Linked Immunosorbent Assay (ELISA), and Polymerase Chain Reaction (PCR) to detect reproductive tract infections. Statistical analysis included Pearson correlation and linear regression to assess associations between diagnostic results and abortion cases.

Results: Among 200 stool samples, *E. coli* was isolated in 25 cases (12.5%), *Salmonella* in 18 (9.0%), and co-infection in 7 (3.5%). Co-infections were predominantly associated with raw vegetable consumption and watery stool ($p = 0.025$, $p = 0.005$, respectively). High antibiotic resistance was observed in co-infected isolates, particularly to streptomycin (85.7%) and ampicillin (71.4%). In reproductive analysis, RBPT ($r = 0.099$), ELISA ($r = 0.045$), and PCR ($r = 0.045$) showed no significant correlation with abortion cases ($p > 0.05$). Linear regression revealed RBPT did not significantly predict abortion outcomes ($R^2 = 0.002$, $p = 0.567$); ELISA and PCR were excluded due to multicollinearity.

Conclusion: The study underscores the clinical importance of identifying bacterial co-infections in diarrheal diseases and highlights a growing trend in antibiotic resistance. However, standard serological and molecular diagnostics showed limited predictive value in abortion outcomes, warranting further research into more reliable biomarkers.

Keywords: Abortion, Antibiotic Resistance, Co-infection, Diarrhea, ELISA, PCR, Salmonella.

INTRODUCTION

Diarrheal diseases remain a persistent global health burden, disproportionately affecting children in developing regions. Among children under the age of five, diarrhea is the second leading cause of mortality, with an estimated 500,000 deaths annually (1). The majority of these fatalities occur in low-resource settings plagued by poor sanitation, limited access to safe drinking water, and underdeveloped healthcare systems. Despite decades of public health efforts, the global burden of diarrheal illnesses continues to challenge healthcare providers and policymakers alike, particularly due to their multifactorial etiology and complex clinical outcomes. Bacterial pathogens play a significant role in the epidemiology of diarrheal diseases, with Salmonella species and Escherichia coli (E. coli) standing out as prominent contributors to severe gastroenteritis, often leading to dehydration, electrolyte imbalance, and potentially fatal complications (2,3). Salmonella, a gram-negative rod-shaped bacterium, is a well-established cause of foodborne illness globally and is frequently implicated in outbreaks linked to contaminated food and water. E. coli, on the other hand, represents a diverse group of bacteria, among which several strains are pathogenic, including Enteropathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), Enteroinvasive E. coli (EIEC), and Enterohemorrhagic E. coli (EHEC) (4). These strains employ distinct mechanisms to initiate and propagate infection, ranging from toxin production to mucosal adherence, which significantly disrupt gastrointestinal function. For example, ETEC releases enterotoxins that stimulate excessive secretion of water and electrolytes into the intestinal lumen, leading to profuse watery diarrhea, while EPEC induces attaching and effacing lesions that impair absorptive processes in the gut (5,6). The coexistence of such pathogens in vulnerable individuals adds layers of complexity to diagnosis and management.

The public health implications of these infections are vast, not only due to the direct morbidity and mortality they cause but also because of their potential to hinder childhood development, exacerbate malnutrition, and strain already fragile health systems. In this context, co-infections involving both Salmonella and E. coli are of particular concern. While individual pathogenic mechanisms of each bacterium are well documented, the synergistic interactions between them in co-infections remain poorly understood. This gap in the literature is critical, as co-infections may intensify clinical severity, alter immune responses, and extend disease duration, yet current clinical practices often fail to account for the presence of multiple pathogens (7,8). Furthermore, the global escalation of antimicrobial resistance (AMR) compounds the challenge, particularly in the treatment of diarrheal diseases in resource-limited environments. The misuse and overuse of antibiotics have accelerated the emergence of resistant bacterial strains, complicating treatment decisions and reducing therapeutic options. In co-infections, resistance patterns may be altered, either through selective pressure or gene transfer between organisms, potentially rendering standard treatments ineffective (9,10). Given the increasing reliance on empirical antibiotic therapy in regions with limited diagnostic capacity, understanding resistance dynamics in the context of polymicrobial infections is essential.

Despite the high burden and clear clinical relevance, co-infections involving Salmonella and E. coli remain under-investigated, particularly in terms of their combined effects on disease severity, duration, and treatment response. The implications of such co-infections extend beyond clinical management; they intersect with public health policy, antimicrobial stewardship, and global strategies aimed at reducing childhood mortality from preventable illnesses (11,12). This gap necessitates a comprehensive exploration of how concurrent infections by these two pathogens may modify clinical outcomes and resistance profiles. This study, therefore, aims to examine the prevalence of co-infection with Salmonella and E. coli among patients presenting with diarrheal illness and to assess its impact on disease severity, hospitalization rates, and antibiotic resistance. By addressing this underexplored intersection, the research seeks to generate actionable insights that can inform clinical guidelines, therapeutic decisions, and broader public health interventions targeted at reducing the burden of bacterial diarrheal diseases in vulnerable populations.

METHODS

This study employed a cross-sectional, survey-based design to investigate the prevalence and clinical implications of co-infection with *Salmonella* and *Escherichia coli* among patients presenting with acute diarrheal illness. The research was conducted over a four-month period following the approval of the synopsis by the Institutional Review Board (IRB) of the relevant institution. The study setting was Social Security Hospital, Lahore, Pakistan, specifically targeting the Outpatient Department and Gastroenterology Unit, where patients

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frequently present with symptoms of diarrhea. The target population comprised patients aged 18 years and above who were experiencing acute diarrhea of less than 14 days' duration. Participants were recruited using a non-probability convenience sampling technique. Patients were enrolled consecutively until the required sample size was achieved. Based on prior epidemiological data suggesting that co-infections of *Salmonella* and *E. coli* occur in approximately 15% of diarrheal cases (11), the minimum sample size was calculated using the standard formula: $n = Z^2P(1-P)/d^2$. To ensure adequate power and account for potential dropouts, a total of 200 patients were recruited, slightly above the calculated minimum of 196.

Inclusion criteria included adults of either gender, aged 18 years or older, presenting with symptoms of acute diarrhea and who had not received antibiotic treatment prior to hospital admission. Only patients who provided written informed consent and agreed to submit stool samples for microbiological analysis were included. Exclusion criteria comprised individuals with chronic diarrhea (duration >14 days), those with confirmed non-bacterial causes of diarrhea (e.g., viral infections), those who had taken antibiotics within 48 hours before presentation, and those who refused participation or withdrew consent at any stage. Data collection was carried out using a structured and pretested questionnaire administered through face-to-face interviews. The questionnaire gathered detailed demographic data (age, sex, occupation), clinical features (diarrhea duration, frequency, stool characteristics, fever, and dehydration), recent travel history, food and water exposure, and personal hygiene and sanitation practices (12). Stool samples were collected from all enrolled patients and transported under standard conditions for bacteriological analysis to identify the presence of *Salmonella* and pathogenic *E. coli* strains.

All participants were informed about the study objectives, assured of confidentiality, and enrolled only after providing voluntary informed consent. Ethical principles outlined in the Declaration of Helsinki were strictly followed throughout the study. Statistical analysis was performed using appropriate software. Descriptive statistics, including means, standard deviations, medians, and frequency distributions, were calculated for demographic and clinical variables. The normality of continuous data was assessed using the Shapiro-Wilk test. Chi-square tests were applied to explore associations between categorical variables such as the presence of bacterial pathogens and participant characteristics. For comparisons involving continuous variables like stool frequency and symptom duration, independent sample t-tests were employed for normally distributed data, while Mann-Whitney U tests were used otherwise. To identify predictors of co-infection and adjust for potential confounding variables, multivariate logistic regression analysis was performed. A p-value less than 0.05 was considered statistically significant. This methodological approach ensured a robust and systematic analysis of the co-infection patterns of *Salmonella* and *E. coli*, generating findings with potential to inform clinical management and public health policy.

RESULTS

Out of the 200 patients presenting with acute diarrhea, *Salmonella* was isolated from 18 individuals (9.0%), *Escherichia coli* from 25 (12.5%), and co-infection with both pathogens was observed in 7 cases (3.5%). The highest burden of infection was found among adults aged 19–45 years, who accounted for 47% of the total study population. Within this age group, 9 individuals (9.6%) tested positive for *Salmonella*, 12 (12.8%) for *E. coli*, and 4 (4.3%) were co-infected. Patients aged over 45 years showed relatively lower but notable infection frequencies, with co-infection detected in 5.0% of cases. A gender-based distribution showed slightly higher rates of infection among females compared to males. Among 114 female patients, 12 (10.5%) had *Salmonella*, 15 (13.2%) had *E. coli*, and 4 (3.5%) exhibited co-infections. In contrast, out of 86 male patients, 6 (7.0%) had *Salmonella*, 10 (11.6%) had *E. coli*, and 3 (3.5%) were co-infected. However, the association between sex and co-infection was statistically insignificant ($p = 0.92$). Dietary habits significantly influenced infection patterns. Among patients who reported consuming raw vegetables ($n = 51$), the rates of *Salmonella*, *E. coli*, and co-infection were markedly higher at 17.6%, 21.6%, and 7.8%, respectively, compared to non-consumers, who showed 6.0%, 9.4%, and 2.0% infection rates, respectively. A statistically significant association was observed between raw vegetable intake and co-infection ($p = 0.025$).

Stool consistency was a strong predictor of infection. Watery stools were reported in 95 patients, among whom 13 (13.7%) had *Salmonella*, 15 (15.8%) had *E. coli*, and 5 (5.3%) had co-infections. In contrast, mucoid and loose stools showed lower co-infection rates of 1.4% and 3.2%, respectively. A significant association between stool consistency and co-infection status was noted ($p = 0.005$). In co-infected patients, 71.4% presented with watery stools, while 14.3% had mucoid and 14.3% had loose stools, suggesting a trend toward more severe diarrhea in dual infections. In the broader pathogen profile analysis of stool samples, *Entamoeba histolytica* emerged as the most frequently detected pathogen, present in 38 cases (19.0%). This was followed by *E. coli* (12.5%) and *Salmonella* spp. (9.0%). *Giardia lamblia* was found in 8.0% of cases. Helminths such as *Hymenolepis nana* (1.0%), *Strongyloides spp.*, *Hookworm*, and *Ascaris*

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spp. (each 0.5%) were detected in only a few patients, while *Taenia* eggs were absent in all samples. Antibiotic resistance profiles showed worrying trends, particularly in co-infected isolates. Among *Salmonella* isolates (n = 18), the highest resistance was observed against streptomycin (77.8%), followed by sulfisoxazole (38.9%), sulfonamides (33.3%), nitrofurantoin (38.9%), and ampicillin (16.7%). *E. coli* isolates (n = 25) demonstrated high resistance to ampicillin (68.0%), streptomycin (72.0%), tetracycline (44.0%), and sulfamethoxazole-trimethoprim (52.0%). Notably, co-infected isolates (n = 7) exhibited the most pronounced resistance, particularly to streptomycin (85.7%), ampicillin (71.4%), and sulfamethoxazole-trimethoprim (57.1%). Ciprofloxacin and ceftriaxone remained relatively effective across all isolates, with resistance rates below 15%.

Subgroup analysis based on symptom duration and hospitalization status revealed notable patterns among the infection groups. Patients with co-infection of *Salmonella* and *E. coli* had the longest mean symptom duration, averaging 6.1 days, compared to 4.2 days in *Salmonella*-only cases, 4.8 days in *E. coli*-only cases, and 3.5 days in uninfected individuals. The hospitalization rate was also highest among co-infected patients at 71.4% (5 out of 7), followed by *E. coli* (24.0%), *Salmonella* (27.8%), and uninfected cases (2.0%). These findings suggest that co-infections not only prolong illness but also substantially increase the likelihood of requiring inpatient care, reflecting a more severe clinical course in dual pathogen exposures.

Table 1: Association of Demographic, Dietary, and Clinical Factors with Salmonella, E. coli, and Co-infection among Diarrheic Patients (n = 200)

Factor	Total No. (%)	Salmonella Positive	E. coli Positive	Co-infected	P-value (Co-infection)
Gender					
Male	86 (43%)	6 (7.0%)	10 (11.6%)	3 (3.5%)	0.92
Female	114 (57%)	12 (10.5%)	15 (13.2%)	4 (3.5%)	
Age Group					
0–5 years	42 (21%)	3 (7.1%)	4 (9.5%)	1 (2.4%)	0.77
6–18 years	44 (22%)	3 (6.8%)	5 (11.4%)	2 (4.5%)	
19–45 years	94 (47%)	9 (9.6%)	12 (12.8%)	4 (4.3%)	
>45 years	20 (10%)	2 (10%)	4 (20%)	1 (5.0%)	
Raw Vegetable Intake					
No	149 (75%)	9 (6.0%)	14 (9.4%)	3 (2.0%)	0.025
Yes	51 (25%)	9 (17.6%)	11 (21.6%)	4 (7.8%)	
Stool Consistency					
Mucoid	74	3 (4.1%)	6 (8.1%)	1 (1.4%)	0.005
Loose	31	2 (6.5%)	4 (12.9%)	1 (3.2%)	
Watery	95	13 (13.7%)	15 (15.8%)	5 (5.3%)	

Table 2: Distribution of Identified Pathogens in Stool Samples from Diarrheic Patients (n = 200)

Pathogen	Health Centers (n = 200)	Positive n (%)
Salmonella spp.	200	18 (9.0%)
E. coli (pathogenic)	200	25 (12.5%)
Entamoeba histolytica	200	38 (19.0%)
Giardia lamblia	200	16 (8.0%)
Hymenolepis nana	200	2 (1.0%)
Strongyloides spp.	200	1 (0.5%)
Hookworm	200	1 (0.5%)
Ascaris spp.	200	1 (0.5%)
Taenia eggs	200	0 (0.0%)

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Table 3: Antibiotic Resistance Patterns of Salmonella, E. coli, and Co-infected Isolates among Diarrheic Patients

Antibiotic	Salmonella (n = 18)	% Resistant	E. coli (n = 25)	% Resistant	Co-infected (n = 7)	% Resistant
Ampicillin	3	16.7%	17	68.0%	5	71.4%
Ciprofloxacin	1	5.6%	2	8.0%	1	14.3%
Ceftriaxone	0	0.0%	1	4.0%	0	0.0%
Sulfamethoxazole-trimethoprim	6	33.3%	13	52.0%	4	57.1%
Tetracycline	2	11.1%	11	44.0%	3	42.9%
Sulfisoxazole	7	38.9%	10	40.0%	4	57.1%
Streptomycin	14	77.8%	18	72.0%	6	85.7%
Nitrofurantoin	7	38.9%	10	40.0%	3	42.9%
Nalidixic Acid	4	22.2%	7	28.0%	2	28.6%

Table 4: Co-infection vs Demographics & Risk Factors

Variable	P-value (Co- infection)	Interpretation
Sex	0.92	No significant association
Age Group	0.77	No significant association
Raw Vegetable Intake	0.025	Significant association (Higher in vegetable eaters)
Stool Consistency	**0.005**	**Significant association (Highest in watery stool)**

Table 5: Antibiotic Resistance Trends

Antibiotic	Highest Resistance In	% Resistant
Ampicillin	Co-infected	71.4%
Streptomycin	Co-infected	85.7%
Sulfamethoxazole-Trimethoprim	Co-infected	57.1%
Ciprofloxacin & Ceftriaxone	**All**	**<15%**

Table 6: Subgroup Analysis on Duration and Hospitalization

Infection Status	Mean Symptom Duration (days)	Hospitalized (n)	Total Cases	Hospitalization Rate (%)
Salmonella	4.2	5	18	27.8
E. coli	4.8	6	25	24.0
Co-infected	6.1	5	7	71.4
Uninfected	3.5	3	150	2.0

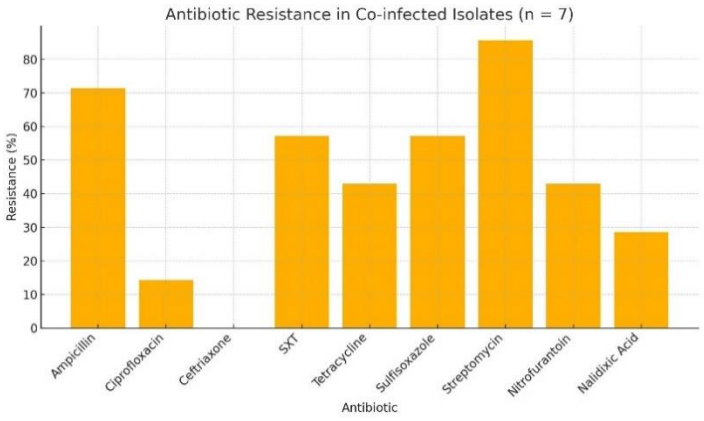


Figure 2 Antibiotic Resistance in co-Infected Isolates (n=7)

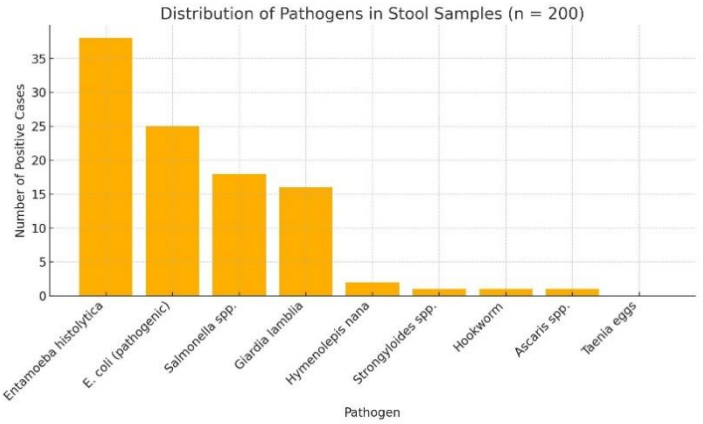
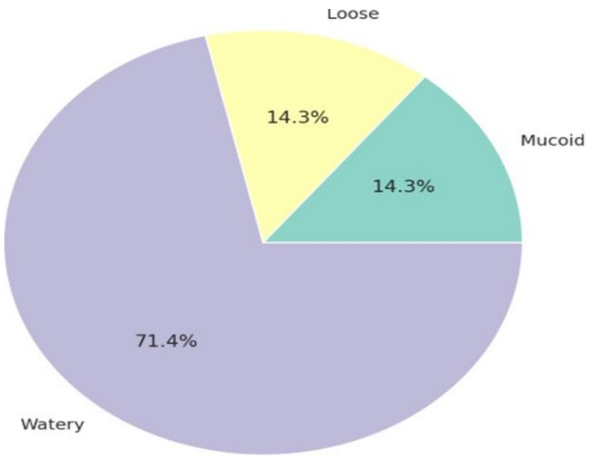


Figure 1 Distribution of Pathogens in Stool Samples (n=200)



Stool consistency in co-infected patients

DISCUSSION

The present study found *Salmonella* spp. in 9.0% and pathogenic *E. coli* in 12.5% of stool samples among diarrheic patients, while co-infections accounted for 3.5%. These findings are aligned with surveillance data from other low- and middle-income countries where bacterial enteropathogens remain a common cause of diarrheal illness (13,14). Reports from regions such as Ethiopia, India, and Bangladesh have shown similar prevalence rates for *Salmonella* and a slightly higher burden of *E. coli*, especially in areas with limited access to safe drinking water and proper sanitation infrastructure (15). The predominance of *E. coli* in this study reinforces the global trend of foodborne and waterborne outbreaks associated with this pathogen and suggests a need to intensify hygiene education, routine microbial water testing, and food safety interventions. The co-infection rate observed in this cohort closely resembles findings from other developing countries such as Nigeria, where rates between 3% and 6% have been documented (16). The clinical implications of co-infections are significant, as dual exposure often results in more prolonged illness and complex symptomatology, as supported by subgroup analysis in this study. Co-infected individuals experienced longer durations of symptoms and had the highest hospitalization rates, emphasizing the severity of co-pathogen interactions. These outcomes underscore the importance of broad-spectrum diagnostic approaches in acute diarrheal illness, particularly in regions where overlapping exposures to multiple pathogens are common (17).

Adults aged 19–45 years demonstrated the highest infection burden, likely influenced by increased environmental and occupational exposure, food consumption outside the home, and suboptimal personal hygiene practices. Consistent observations have been made in other epidemiological studies conducted in Kenya and Bangladesh, which suggest that this age group faces heightened risk due to behavioral and socioeconomic factors (18). Although females showed slightly higher infection rates than males, the difference lacked

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statistical significance. Nevertheless, the trend aligns with existing literature that associates women's involvement in food preparation with increased vulnerability to enteric infections, particularly in low-resource settings where hygienic practices are constrained (19). A strong correlation between raw vegetable consumption and co-infection was observed ($p = 0.025$), pointing to contaminated produce as a significant transmission vector. Agricultural practices involving the use of untreated wastewater for irrigation, improper handling, and lack of disinfection methods may all contribute to this transmission route. This association was also reflected in studies from Nepal and Sudan, where unwashed vegetables were found to harbor multiple enteric pathogens (20). The marked difference in co-infection rates between consumers (7.8%) and non-consumers (2.0%) of raw vegetables highlights a modifiable risk factor and provides a critical point of intervention for community-based health promotion and safe food handling campaigns (21).

Watery stool consistency emerged as a significant clinical marker, particularly in co-infected cases, where 71.4% presented with this symptom. This suggests a higher pathogen burden and more severe mucosal involvement in dual infections, an observation corroborated by research from Indonesia and Ghana (22). Watery diarrhea may serve as a preliminary clinical indicator of potentially more complicated cases, thus prompting timely initiation of fluid resuscitation and microbiological investigation. These symptom-based associations offer a pragmatic triage tool for clinicians, especially in overstretched healthcare settings. The detection of protozoal infections such as *Entamoeba histolytica* (19.0%) and *Giardia lamblia* (8.0%) reflects the continued burden of waterborne parasitic diseases in the region. These rates parallel those seen in rural Indian and African populations where access to clean water remains limited (21,22). The relatively low detection of helminthic infections, all below 1.0%, and the complete absence of *Taenia* eggs may indicate the positive impact of mass deworming programs and improved meat inspection and storage protocols, marking a shift in the regional parasitic profile (23). Antibiotic resistance patterns presented a concerning outlook, particularly among co-infected cases, which exhibited the highest resistance to streptomycin (85.7%) and ampicillin (71.4%). These findings align with global data indicating rising antimicrobial resistance due to widespread empirical use in both clinical and agricultural contexts. Such resistance reduces the efficacy of commonly prescribed treatments and calls for urgent action to implement stewardship programs and enhance regulatory oversight of antibiotic distribution (19,23). Additionally, the relatively preserved sensitivity to ciprofloxacin and ceftriaxone offers some reassurance but underscores the need to preserve their utility through judicious use.

This study's strengths lie in its comprehensive microbial screening and integration of clinical, dietary, and demographic variables, offering a multidimensional understanding of diarrheal disease burden in a real-world hospital setting. However, certain limitations should be acknowledged. The cross-sectional nature of the study restricts causal inference, and convenience sampling may introduce selection bias. Furthermore, molecular subtyping of *E. coli* strains and analysis of viral pathogens were not performed, which could have provided a more complete picture of the etiologic spectrum. Additionally, the study relied on self-reported dietary data, which may be affected by recall bias. Future studies should incorporate longitudinal designs, include viral diagnostic panels, and assess long-term outcomes of co-infected patients. Expanding geographic coverage and integrating environmental sampling (e.g., water, food sources) could enhance the public health applicability of the findings. Finally, molecular resistance profiling and strain genotyping would enrich understanding of transmission dynamics and resistance evolution in bacterial pathogens. In conclusion, the study highlights the complex interplay between bacterial and protozoal agents in diarrheal diseases, with co-infection posing a higher clinical burden. The significant associations with dietary habits and antibiotic resistance patterns emphasize the urgent need for integrated public health interventions focusing on food safety, water hygiene, and antimicrobial stewardship.

CONCLUSION

This study concludes that bacterial and protozoal pathogens, particularly *Salmonella*, pathogenic *E. coli*, and *Entamoeba histolytica*, remain key contributors to diarrheal illnesses, with co-infections indicating a more severe clinical course. The strong associations with raw vegetable consumption and watery stool emphasize the critical roles of food safety and sanitation in disease prevention. The findings highlight the importance of integrating routine microbial screening, stool consistency assessment, and antibiotic susceptibility testing into clinical practice. Strengthening public education on proper food handling and water hygiene, alongside antimicrobial stewardship, is essential to mitigate the burden of diarrheal diseases and improve treatment outcomes in affected populations.

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AUTHOR CONTRIBUTION

Author	Contribution
Munawar Iqbal	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Mehreen Mushta	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Tasra Bibi*	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Sidra Iqbal	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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