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Aetiological, seasonal and antibiotic susceptibility patterns of diarrhoeal diseases in Bhutan (2016–2022): a retrospective study of surveillance data

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ABSTRACT

Objectives This study aimed to identify the aetiological spectrum, seasonal distribution and antimicrobial resistance patterns of diarrhoeal diseases in Bhutan.

Study design and setting The study used a cross-sectional, retrospective analysis of secondary data gathered through a passive, hospital-based sentinel surveillance for diarrhoeal disease across 12 hospitals, representing Bhutan's demographically diverse regions.

Participants A total of 3429 participants' data of all age groups who presented with diarrhoea at sentinel hospitals between 1 January 1 2016 and 31 December 2022 were analysed.

Results Diarrhoeagenic *Escherichia coli* (DEC), *Shigella*, *Salmonella* and *Aeromonas* spp. were predominant bacterial pathogens, while *Rotavirus, Astrovirus* and *Norovirus* were the leading viral pathogens. Coinfections were observed in 195 cases. Children under nine were significantly affected than the other age groups. Seasonal trends revealed that bacterial pathogen incidence peaked during the summer/monsoon season, viral pathogens were more common in winter and spring, and parasites persisted year-round. Among the antibiotics tested, gentamicin, chloramphenicol, ceftriaxone and tetracycline exhibited high efficacy, with susceptibility rates of 93.4%, 87.2%, 81.5% and 69.5%, respectively. Conversely, high resistance rates were observed for amoxicillin (80.3%), ampicillin (77.4%) and nalidixic acid (69.5%). Multidrug resistance was prevalent, with β-lactamase production contributing to resistance rates of 80.7% to penicillin and 65.4% to fluoroquinolones groups. Cephalosporin resistance was also notable, with rates of 34.4% for cephalexin, 40.0% for cefazolin and 16.9% for ceftriaxone.

Conclusions DEC and *Rotavirus* were identified as the leading causes of diarrhoea, with significant resistance patterns observed in common bacterial isolates. These findings underscore the need for DEC screening in paediatric cases and emphasise the need for sustained antimicrobial resistance surveillance.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Leverages passive sentinel surveillance to assess the aetiology, seasonality and antimicrobial susceptibility of diarrhoeal diseases.
⇒ Provides a hospital-based, real-world perspective on diarrhoeal diseases.
⇒ Includes antimicrobial susceptibility, helping to understand resistance trends in enteric pathogens.
⇒ Provides potential under-representation of the community cases due to reliance on hospital-based surveillance.

BACKGROUND

Diarrhoeal disease is a major global health challenge, exerting a substantial burden on public health systems and disproportionately affecting vulnerable populations, particularly children. Despite advances in sanitation and increased public health awareness and prevention efforts, diarrhoeal diseases continue to be a leading cause of morbidity and mortality, predominantly stemming from contaminated food and water sources worldwide. Diarrhoeal disease accounts for nearly 1.7 billion cases annually, resulting in approximately 800 000 fatalities, with a disproportionate impact on children under five in developing regions such as South Asia and Sub-Saharan Africa. In these areas, inadequate sanitation, poor hygiene practices and limited access to clean water contribute to the persistence of these diseases. Viral and bacterial enteropathogens remain the primary causes of acute gastroenteritis, affecting populations in both developed and developing countries.[1 2]

While diarrhoeal disease manifests across all age groups, the aetiology and clinical trajectory vary depending on age and specific causative agents. Most studies focus predominantly on children under five, resulting in limited data on the aetiology and susceptibility trends among adults. Although diarrhoea accounts for only 2% of deaths in adults, it can still contribute to the spread of enteric infections to other vulnerable populations.[3] Furthermore, research on the aetiology of diarrhoeal disease in the

Bhutanese population is limited. A deeper understanding of pathogen aetiology, seasonal patterns and antimicrobial susceptibility is essential for the development of targeted preventive measures and effective antimicrobial therapies.

In Bhutan, diarrhoea is the leading cause of morbidity and the second most frequently reported disease after respiratory infections, presenting an ongoing public health challenge. Bhutan, a low-middle-income country in South Asia situated in the Eastern Himalayas between China and India, has made considerable advancements in healthcare and sanitation. Nevertheless, in 2022, there were 28 179 reported cases of diarrhoea, including 23 272 cases of acute watery diarrhoea and 2112 cases of acute bloody diarrhoea. Children under 9 years were particularly affected with 4232 cases and 2 mortalities reported that year.[4 5] Although diarrhoea-associated mortality remains relatively low, disease morbidity has not shown a significant decline. Rapid detection of pathogenic organisms is crucial for timely patient management and identifying potential outbreak sources. Additionally, the increased resistance to commonly used antimicrobials highlights the need for a comprehensive understanding of local factors contributing to these resistance patterns.

Since 2016, the Enteric, Zoonotic and Vector-borne Disease Laboratory within the Royal Center for Disease Control (RCDC) has implemented a hospital-based sentinel surveillance system to monitor diarrhoeal diseases. This system, relying on data from sentinel hospitals, focuses on patients presenting with diarrhoea. Over 6 years, surveillance efforts have aimed to unravel the prevalence and seasonal distribution of major agents responsible for diarrhoeal disease. This study determined the aetiology, seasonal patterns and antimicrobial resistance of pathogens in individuals who sought medical attention and were admitted to these sentinel sites from 2016 to 2022.

METHODS

Bhutan Diarrhoeal Surveillance and Information System (BDSIS)

The National Diarrhoeal Disease Surveillance programme, based at the RCDC, operates as a passive hospital-based sentinel surveillance system, which facilitates the rapid identification of pathogens causing diarrhoea. This programme ensures timely patient management and aids in identifying potential outbreak sources. It provides crucial data for evidence-based decision-making and effective disease control strategies, ultimately improving health outcomes. This study uses previously collected stool specimens and associated data from 12 strategically selected sentinel hospitals: one national referral hospital, two regional referral hospitals and nine district hospitals, as illustrated in figure 1. These hospitals served as critical nodes for a comprehensive data collection network,

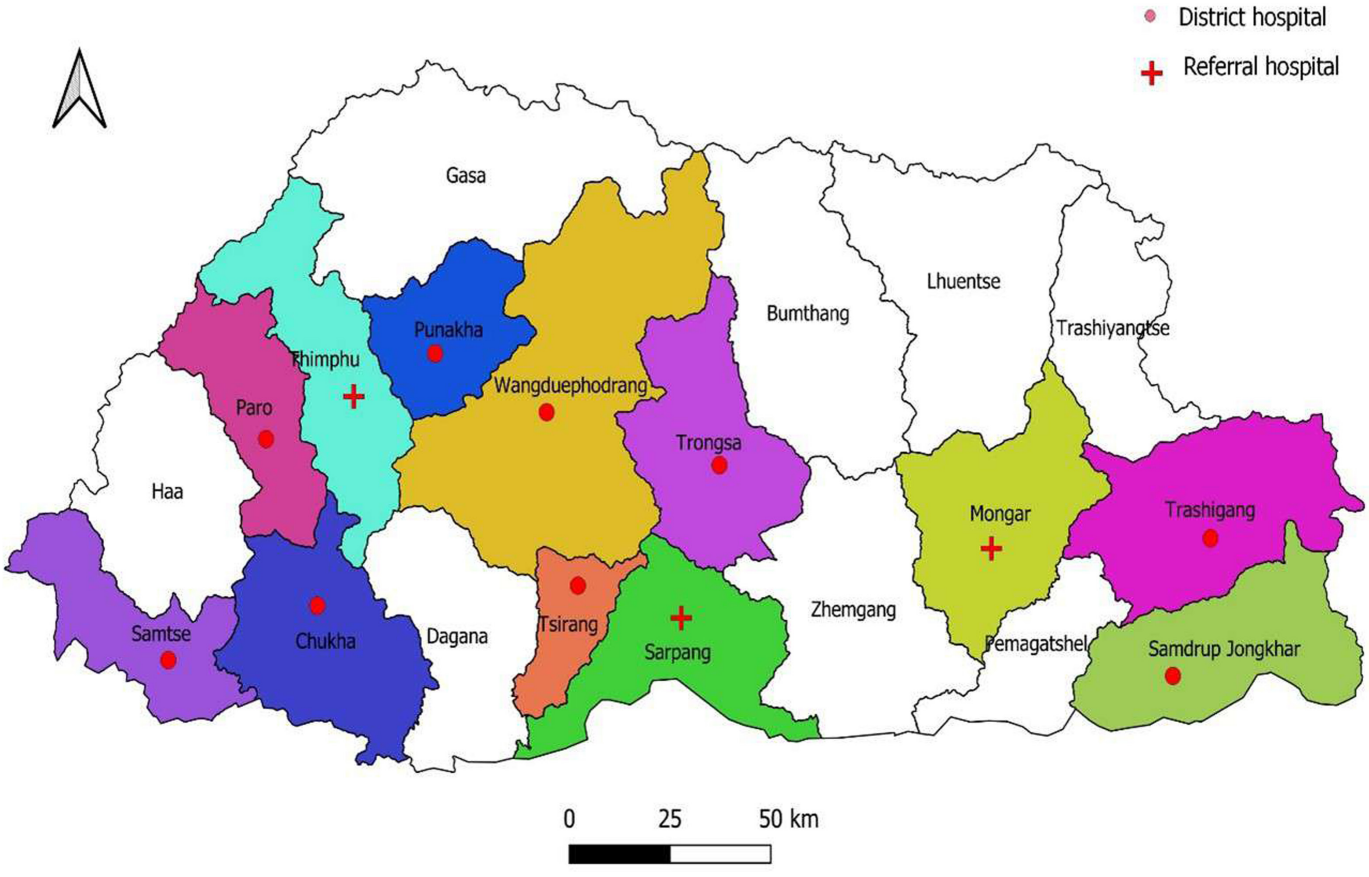

Figure 1 Diarrhoea surveillance sentinel site.

focusing on individuals with diarrhoeal diseases. The RCDC epidemiology unit, EZVDL, and sentinel hospitals collaborate to maintain and share surveillance data through the in-house-developed BDSIS real-time database system.[4]

Participants presenting with diarrhoea, defined as three or more loose stools in 24 hours, were documented using a standardised Case Investigation Form (CIF). Cases linked epidemiologically to outbreaks were excluded from this analysis.[6]

Patient and public involvement

Patients and the public were not directly involved in the research process in a formalised or participatory manner. The study relies on secondary data from a hospital-based sentinel diarrhoeal surveillance programme, where the data were collected as part of routine healthcare or surveillance efforts.

Sample preparation and laboratory methods

Fresh stool samples were used to detect the causative agents. Patients who had taken antibiotics prior to sample collection were excluded. Samples from the district hospital without microbiology capacity were delivered to the microbiology referral laboratory in Carry-Blair transport media and cryovials maintaining the cold chain following triple packaging and International Air Transport Association) regulations.[7]

All specimens were tested for a comprehensive panel of enteric pathogens, including eight bacterial species: *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Aeromonas* spp., *Enterococcus* spp., *Plesiomonas* spp. and Diarrhoeagenic *Escherichia coli* (DEC), which includes *Enteropathogenic E. coli* (EPEC), *Enterotoxigenic E. coli* (ETEC), *Enterohemorrhagic E. coli* (EHEC), *Enteroaggregative E. coli* (EAEC) and *Enteroinvasive E. coli* (EIEC). Viral pathogens screened included *Norovirus, Rotavirus, Astrovirus* and *Adenovirus,* and two parasitic pathogens, *Giardia lamblia* and *Cryptosporidium*, were also examined. Standard microbiological procedures and techniques were employed throughout the testing.

ELISA was used for viral detection, and multiplex PCR assays were employed to pathotype DEC, allowing for the simultaneous detection of multiple *E. coli* strains. Serum agglutination tests were used for subtyping *Shigella* and *Salmonella* spp. On-site microscopic examinations of stool samples were performed to identify intestinal ova and parasites, ensuring a thorough assessment of potential pathogens.

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion method, adhering to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI).[8] Bacterial isolates were tested against a comprehensive panel of antibiotics, including amoxicillin (AMX 30 µg), ampicillin (AMP 10 µg), cephazolin (CZO 30 µg), cephalexin (LEX 30 µg), chloramphenicol (CHL 30 µg), ciprofloxacin (CIP 5 µg), ceftriaxone (CRO 30 µg), nalidixic acid (NAL 30 µg), trimethoprim-sulfamethoxazole (SXT 25 µg), gentamicin (GEN 10 µg) and tetracycline (TCY 30 µg).

Susceptibility was interpreted based on the CLSI-defined breakpoints, categorising results as susceptible, intermediate or resistant. Multidrug resistance (MDR) was identified as resistance to at least one antimicrobial agent in three or more different antimicrobial classes, namely β-lactam or β-lactamase inhibitors (penicillin and cephalosporins groups), providing a detailed understanding of the resistance patterns observed in the bacterial isolates.[9–11] Coinfection was defined as the presence of two or more pathogens in a single sample, indicating simultaneous infections by multiple pathogens. This approach ensures a comprehensive assessment of both susceptibility and the presence of various pathogens, facilitating more accurate diagnosis and treatment planning.

Data management and statistical analysis

Demographic and laboratory data were systematically extracted from the BDSIS which facilitated the structured and consistent collection of clinical and laboratory information for each patient. The integration with BDSIS enabled seamless data management from extraction to analysis, supporting a comprehensive retrospective review of the data. Incomplete or missing data were flagged and excluded from specific analyses where appropriate.[4]

Statistical analysis was conducted using the STATA statistical package (V.13.1, StataCorp LP). Descriptive statistics, including frequencies and percentages, were used to summarise demographic characteristics (age, sex), pathogen prevalence, seasonal variations, coinfections and antimicrobial resistance patterns. Stratified analysis was performed to assess pathogen incidence and resistance patterns by age group and season. Two-tailed p values <0.05 were considered statistically significant.

Seasons were defined based on their climatic characteristics, dividing the calendar year into four distinct categories: winter (December to February), characterised by colder temperatures; spring (March to May), marked by blossoming flora; summer (June to September), associated with warmer temperatures and monsoon rains; and autumn (October and November), characterised by milder temperatures and changing foliage.[12] The seasonality of diarrhoeal pathogens was assessed by calculating the monthly incidence rates and identifying peaks for bacterial and viral infections relative to the seasonal changes. The term ‘suspicious food’ refers to self-reported dietary items by individuals that healthcare professionals deemed potentially linked to the onset of diarrhoea.

Quality control procedures

To ensure the reliability and accuracy of the data and results throughout the study, the stool samples were transported to the referral laboratory under cold chain conditions, following strict packaging protocols to maintain the cold chain and minimise the risk of sample degradation. A standardised CIF was used and the data were collected by the trained clinicians and laboratory officials across

the 12 sentinel hospitals involved in the study, ensuring consistent documentation of participant data.

Laboratory testing protocols followed established microbiological standards, where all participating laboratories were trained to reduce variability. For antibiotic susceptibility testing, the Kirby-Bauer disc diffusion method was employed, using *E. coli* (ATCC 25922) as a quality control reference to ensure accuracy. A comprehensive range of diagnostic methods was implemented, including ELISA for viral detection, multiplex PCR assays for pathotyping DEC and serum agglutination tests for subtyping *Shigella* and *Salmonella*, which enhanced both sensitivity and specificity in pathogen detection.

Demographic and laboratory data were systematically extracted from the BDSIS, facilitating structured data collection. Any incomplete or missing data were flagged and excluded from analyses as appropriate. Data analysis was performed using the STATA statistical package, applying descriptive statistics to assess variations in pathogen incidence and resistance patterns by age group and season.

RESULTS

Sociodemographic characteristics

A total of 3429 subjects were enrolled since the initiation of the surveillance in 2016. Among these, 1810 (53.0%) were males and 1619 (47.0%) females. Participants' age spectrum spanned from 1 month to 91 years, with a mean age of 20.4 years. A significant proportion, 1765 (51.5%), were under 9 years old, primarily categorised as dependents or students. Most cases, 2888 (84.2%), received treatment in the outpatient department, while 541 (16.0%) required admission. The sample characteristics predominantly constituted loose stool 2472 (72.0%), while 918 (27.0%) presented with watery stool and 39 (1.0%) had bloody stools. Mucus was positive in 595 (23.0%) samples, and blood was detected in 140 (6.0%) cases, as detailed in table 1. Among all cases, 4.0% (143/3429) of diarrhoea incidents were linked with the consumption of suspected junk food, and 1.5% (53/3429) had a history of travel within or outside the country prior to the onset of illness.

Aetiology of enteric bacteria and seasonality

Enteric pathogens, including *E. coli,* were identified in 3099 samples, representing a positive rate of 90.4% from a total of 3429 stool samples analysed. Bacterial agents accounted for 550 (16.0%), viral for 490 (14.3%), parasitic for 174 (5.1%) and 195 (5.7%) coinfections. Diarrhoeal disease was predominantly caused by enteric bacteria such as DEC, *Shigella, Salmonella* and *Aeromonas* spp., with positive rates of 8.4%, 4.3%, 3.0% and 2.0%, respectively. *Rotavirus* emerged as the predominant enteric virus pathogen, with a rate of 9.5% (239/2513). *Norovirus* and *Astrovirus* followed as the second aetiological agents, accounting for 5.2% (94/1804) and 4.9% (112/2292) of samples, respectively. Among parasites, *G. lamblia* and *Cryptosporidium* exhibited positive rates of

Table 1 Sociodemographic and clinical characteristics of the sample (n=3429)

Characteristics	n (%)	P value
Gender		
Male	1810 (53.0)	<0.001
Female	1619 (47.0)	
Age (years)		
0–9	1765 (51.5)	<0.001
10–19	285 (8.3)	
20–29	370 (10.8)	
30–39	320 (9.3)	
40–49	200 (5.8)	
50–59	191 (5.6)	
60 and above	298 (8.7)	
Visit status		
IPD	541 (16.0)	
OPD	2888 (84.0)	
Occupation		
Dependent	1126 (32.8)	
Student	561 (16.4)	
Farmer	482 (14.1)	
Housewife	253 (7.4)	
Public sector	332 (9.7)	
Civil servant	125 (3.6)	
Others*	550 (16.0)	
Consistency		
Loose	2472 (72.0)	
Watery	918 (27.0)	
Bloody	39 (1.0)	
Colour		
Black	71 (2.0)	
Brown	824 (24.0)	
Clay	22 (1.0)	
Green	353 (10.0)	
Red	67 (2.0)	
Yellow	1249 (36.0)	
Other	38 (1.0)	
Missing*	805 (23.0)	
Mucus		
Positive	595 (23.0)	
Negative	2029 (77.0)	
Missing*	805 (23.0)	
Blood		
Yes	140 (6.0)	
No	2283 (94.0)	
Missing*	805 (23.0)	

*Missing data.
IPD, inpatient department; OPD, outpatient department.

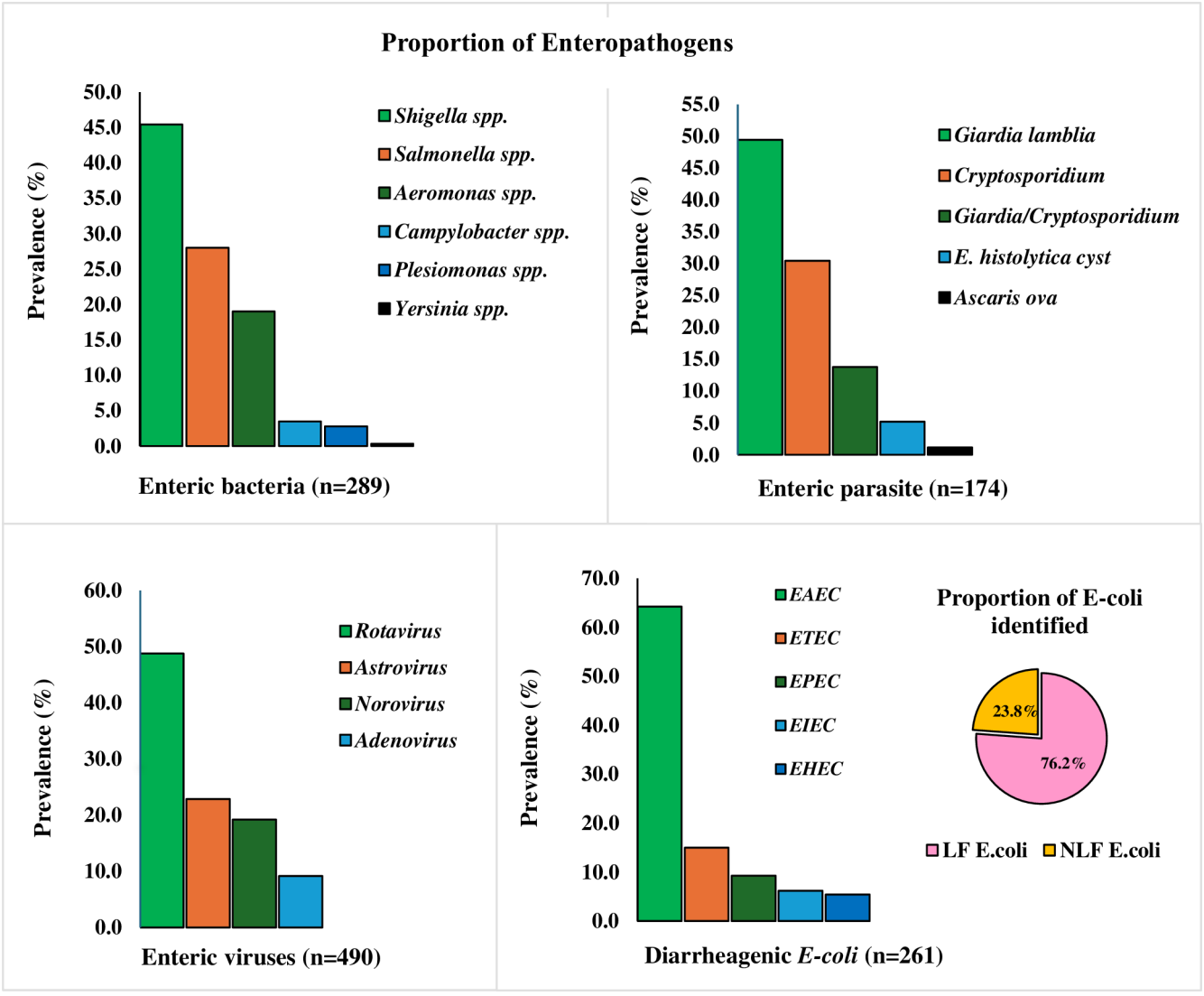

Figure 2 Aetiology of enteric pathogens (n=1214). *E. histolytica*, *Entamoeba histolytica*; EAEC, *Enteroaggregative Escherichia coli*; EHEC, *Enterohemorrhagic Escherichia coli*; EIEC, *Enteroinvasive Escherichia coli*; EPEC, *Enteropathogenic Escherichia coli*; ETEC, *Enterotoxigenic Escherichia coli*; LF *E. coli*, *Lactose fermenter Escherichia coli*; NLF *E. coli*, *Non-lactose fermenter Escherichia coli*; Spp, species; STEC, *Shiga toxin-producing Escherichia coli*.

4.0% (86/2402) and 2.2% (53/2402), as illustrated in figure 2. The DEC type identified included 167 EAEC, 39 ETEC, 24 EPEC, 16 EIEC and 14 EHEC.

The most prevalent coinfections were virus-virus (*rotavirus–astrovirus*) and virus-bacteria (*rotavirus*–DEC), each occurring in 65 cases. Additionally, 13 cases of bacteria-parasite and 10 cases of virus-parasite and parasite-parasite coinfections were observed, as detailed in online supplemental table 1. Notably, a significant number of enteric pathogens (n=737) were detected in individuals under the age of 9 years (n=1765), with the agent distribution by age group illustrated in figure 3.

The examination of enteric pathogens across various months revealed a consistent presence of enteric bacteria causing diarrhoea throughout the year, with the highest prevalence of bacterial agents recorded during the spring and monsoon seasons. DEC, *Shigella* spp. and *Salmonella* spp. were more common in spring, summer and early fall compared with late fall and winter. *Aeromonas* spp. were notably more prevalent from late spring to the end of summer, indicating a seasonal trend. Enteric viruses were predominant from winter through early spring (January to March), while parasites were detected consistently across all seasons, with peaks during the warm and humid summer months (June and July) and the colder months (January). *Rotavirus* and *Astrovirus* had a longer peak period from January to July, whereas *Norovirus* exhibited a less distinct seasonality, clustering around winter and monsoon seasons. Bacterial pathogens were more frequent during the summer, accounting for 232/550 (42.2%) of all cases, while viral pathogens were more common in winter and spring, accounting for 281/490 (57.3%) of all cases. Although enteric pathogens were detected year-round, cases were significantly increased during the monsoon seasons, as illustrated in figure 4.

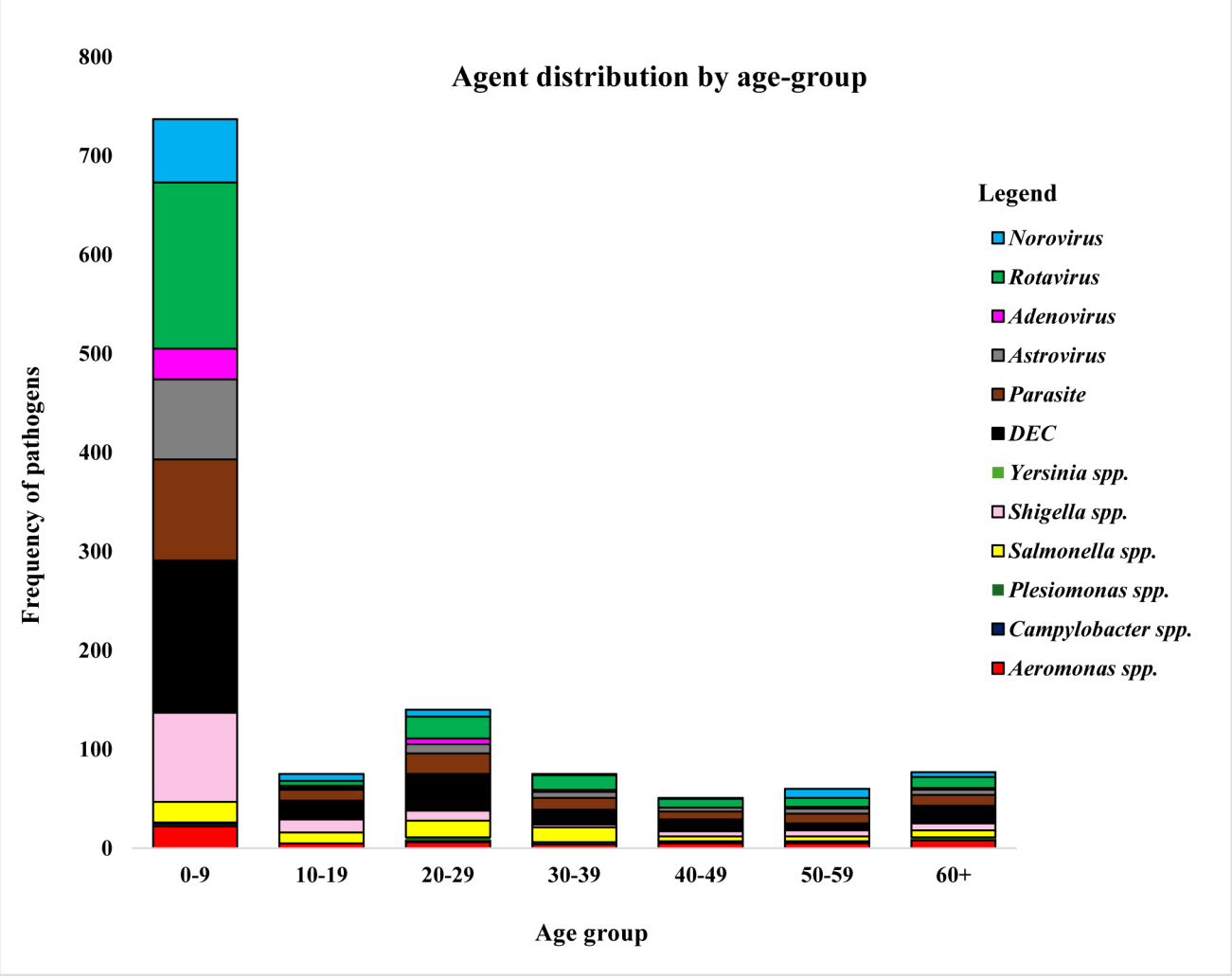

Figure 3 Agent distribution by age group. The number of participants in each age group is as follows: 0–9 years (n=344), 10–19 years (n=16), 20–29 years (n=44), 30–39 years (n=24), 40–49 years (n=10), 50–59 years (n=25) and 60+ years (n=23). DEC, diarrhoeagenic *Escherichia coli*; Spp, species.

Antibiotic susceptibility patterns

The susceptibility of isolated enteric bacterial pathogens was assessed against the 11 most commonly used antibiotics. GEN, CHL, CRO and TCY emerged as the most effective antibiotics, displaying overall pathogen susceptibility rates of 93.4%, 87.2%, 81.5% and 69.5%, respectively. Conversely, high resistance rates were observed for AMX, AMP and NAL, with prevalence rates of 80.3%, 77.4% and 69.5%, as illustrated in figure 5.

Shigella spp. exhibited notable susceptibility to various antibiotics, with high susceptibility observed for GEN (99.0%), CRO (84.3%), CHL (78.3%), CZO (77.6%) and LEX (56.7%) with similar patterns in *Salmonella*, *Aeromonas* and DEC. However, CIP demonstrated more than 50.0% intermediate results in DEC and 26.3% in other bacterial pathogens, indicating an emerging resistance to this antibiotic.

β-Lactamase production was observed in several isolates, predominantly within DEC, significantly contributing to their MDR profiles and indicating the presence of extended-spectrum β-lactamases (ESBLs). These isolates exhibited resistance to multiple classes of antibiotics, with 80.7% resistant to penicillin and 65.4% to fluoroquinolones. First-generation and third-generation cephalosporin resistance was also noted, with 34.4% LEX, 40.0% CZO and 16.9% CRO. Additionally, some isolates demonstrated resistance to non-β-lactam antibiotics, with 40.0% resistance to SXT and 33.9% to TCY. Detailed antimicrobial susceptibility patterns for the different bacterial species are presented in online supplemental figure 1A and 1B.

DISCUSSION

This surveillance study offers valuable insights into the epidemiology of diarrhoea-causing pathogens, identifying the most prevalent pathogens across all age groups, observing that children under nine are disproportionately affected, with males showing a slightly higher prevalence (57.0% compared with 43.0%). Statistical analysis revealed a strong correlation (p<0.001) between pathogen prevalence, gender and age group, underscoring a particularly

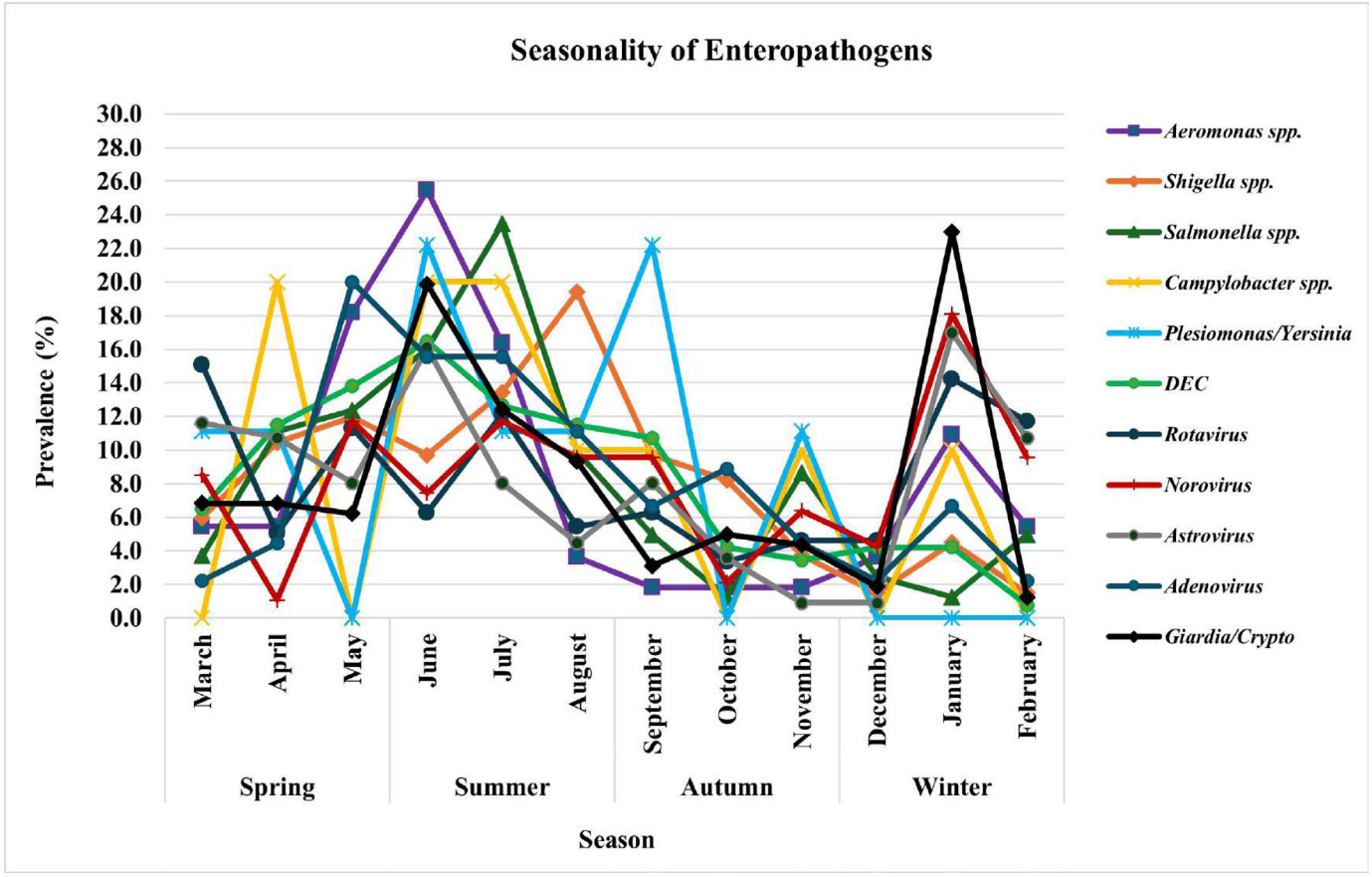

Figure 4 Seasonal prevalence of enteric pathogens. Crypto, *Cryptosporidium*; DEC, diarrhoeagenic *Escherichia coli;* Spp, species.

high risk among young children. This finding highlights the increased risk of diarrhoeal diseases among vulnerable groups, particularly males, and underscores the need for targeted public health interventions.[13–15]

The study also highlights distinct seasonal patterns in diarrhoeal disease, offering valuable data that could enhance the ability to predict and manage outbreaks. Additionally, the study provides crucial data on the antimicrobial susceptibility patterns of these pathogens, highlighting emerging resistance profiles that were not the focus of earlier studies, which concentrated on case-control data or hospitalised patients.[16–19] The findings have significant implications for public health, particularly in the context of managing diarrhoeal diseases and combating antimicrobial resistance in low-income countries like Bhutan. In such settings, diarrhoea remains a widespread issue caused by a growing range of enteric pathogens. Accurately determining the aetiology is essential for effective treatment and improving patient outcomes.[1]

Rotavirus and DEC collectively represent the primary causes of moderate-to-severe diarrhoea in low-income countries.[20] In our study, EAEC, *Shigella* spp., *Salmonella* spp. and *Rotavirus* were identified as the most prevalent pathogens associated with diarrhoeal disease, highlighting their significant role in the local burden of gastrointestinal infections. These pathogens exhibit similar and variable prevalence rates across different countries, as reported in the literature.[1 21–23] Notably, over 16.0% (550/3429) of stool samples in our study contained established diarrhoeagenic pathogens, with DEC showing a high prevalence, especially in cases of childhood diarrhoea, a significant health issue in developing nations. Our findings align with those from comparable studies.[24–26] Besides its prevalence in low-income settings, DEC is also emerging as antimicrobial-resistant enteropathogens in developed nations.[2] Previous studies have highlighted the emergence of DEC accounting for up to 25.0% of all diarrhoeal diseases in developing countries.[21 24] While our findings are consistent with this trend, contrasting data from a study in China identified *Norovirus* as the most common pathogen,[3] and research in Bangladesh highlighted *Campylobacter, Shigella* and *Vibrio cholera* as major bacterial pathogens linked to diarrhoea.[24 25] This variability emphasises the importance of continuous surveillance to track emerging pathogens and monitor seasonal trends specific to each region. The high prevalence of DEC and *Rotavirus* in Bhutan highlights the need for targeted public health measures, such as routine screening, especially in vulnerable paediatric populations. Identifying these pathogens enables more effective disease management strategies, including vaccination, improved sanitation and timely clinical interventions.

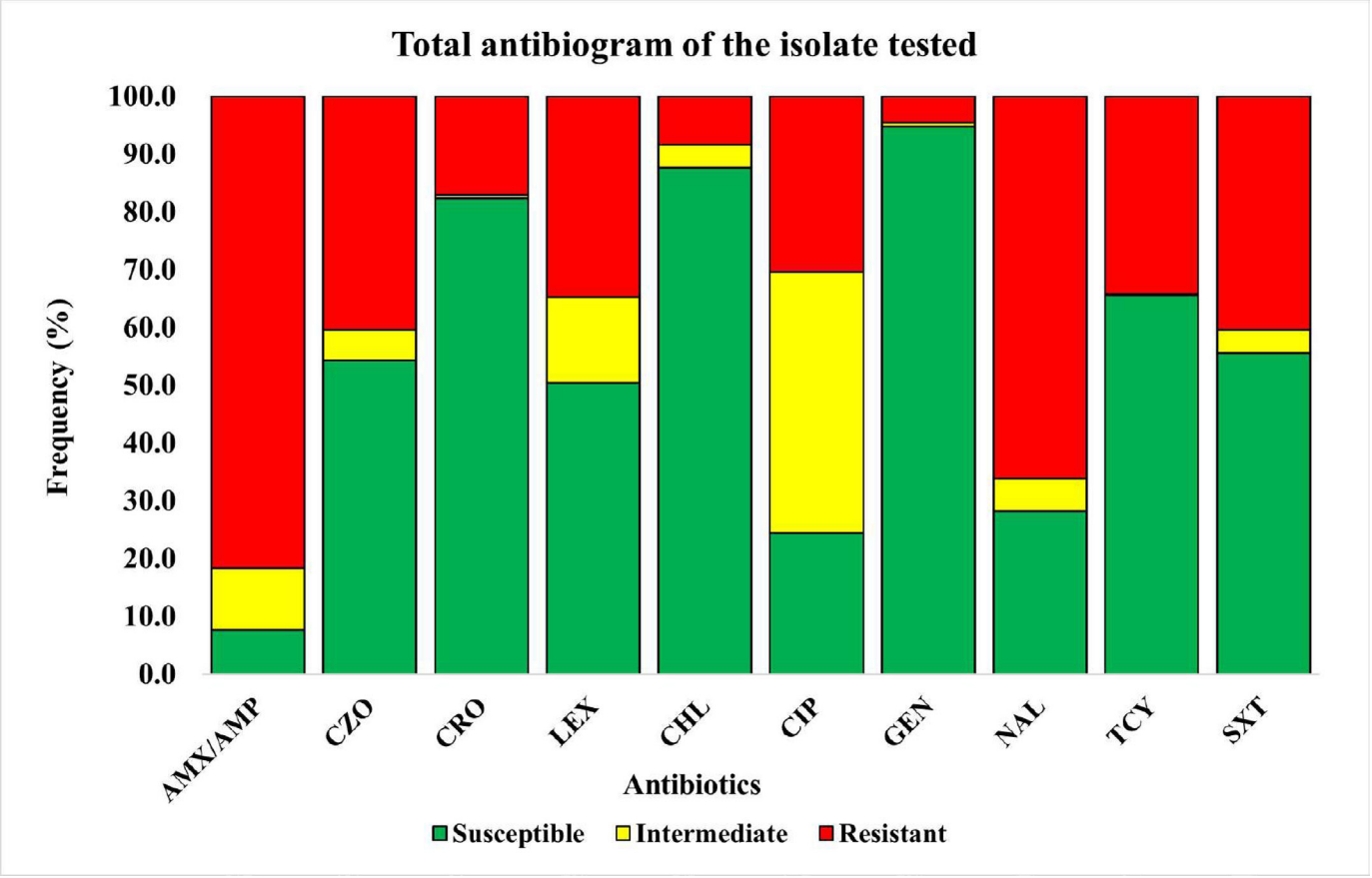

Figure 5 Overall antibiogram of the bacterial agents (n=556). AMX, amoxicillin; AMP, ampicillin; CZO, cephazolin; CRO, ceftriaxone; LEX, cephalexin; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; TCY, tetracycline; SXT, trimethoprim-sulfamethoxazole.

Rotavirus is associated with substantial hospitalisations and paediatric mortality as established in previous studies.[1 27] It emerged as the second most prevalent diarrhoeal pathogen in our study, which is consistent with prior research.[20] Despite being widely recognised as the primary cause of severe diarrhoea in children,[28 29] its burden and impact on adults have often been underestimated.[30] However, our findings challenge this perspective as 6.2% (71/1151) of the adults in our surveillance cohort tested positive for *Rotavirus*. This rate of infection in adults suggests that they may play a potential role in the transmission dynamics and propagation of *Rotavirus* outbreak, especially in households or areas with close contact between age groups, and requires further exploration as reported.[31] Our findings revealed numerous coinfections in 16.0% (195/1124) of the positive cases, with virus+bacteria and virus+virus combinations being the most common. Similar results have been observed in other studies that coinfections with enteric pathogens can intensify diarrhoeal symptoms, increasing the frequency of episodes and raising the risk of hospitalisation, and in severe cases, fatalities.[23 32] These findings underscore the need for vigilant monitoring and management of coinfections, as they pose a higher risk for severe disease outcomes.

The study also reveals distinct seasonal patterns varied among detected pathogens, with viral pathogens most prevalent during colder months, particularly peaking in January through March, while bacterial pathogens were more frequent in warmer months (spring and summer), and parasitic pathogens observed throughout the year with peaks in June, July and January. These patterns are generally consistent with previously documented seasonality,[1 27] though some studies report high *Rotavirus* incidence in dry and hot seasons.[1 33] Environmental factors such as temperature, precipitation and humidity likely contributed to these patterns by impacting exposure, host immunity and pathogenicity as reported elsewhere.[34] Public health strategies should therefore incorporate environmental and climate-related data to anticipate seasonal outbreaks and implement timely interventions. By leveraging this information, health systems can enhance outbreak preparedness and response efforts, ultimately reducing the burden of diarrhoeal diseases in the population.

The antimicrobial susceptibility analysis observed in our study reveals significant resistance among various enteric pathogens. *Shigella* spp. demonstrated high susceptibility to GEN, CRO, CHL and ceftazidime consistent with findings from previous studies.[35 36] Conversely, *Salmonella*

spp. exhibited high resistance to AMX, AMP and NAL, differing from earlier findings.[22 35 37] DEC and *Aeromonas* species showed resistance to AMP, LEX and CZO, which aligns with another research.[38] However, CRO, CHL and GEN were the most effective antibiotics in our findings, as similarly reported elsewhere.[22]

Fluoroquinolone resistance was noted in 50.0% of bacterial pathogens, demonstrating emerging resistance to this critical class of antibiotics. Overall, our report underscores the complex and diverse antimicrobial resistance patterns among enteric pathogens, with 80.7% of bacterial isolates exhibiting ESBL-producing MDR. This trend mirrors a global public health challenge, complicating treatment options, prolonging illness durations, escalating healthcare costs and increasing mortality rates.[39 40]

The study identified a low incidence of *Campylobacter* within the cohort, which may be attributable to methodological limitations as its detection often relies on culture-based methods, which are known for their limited sensitivity as previously documented by sources.[41 42] Inconsistencies in diagnostic practices across the 12 participating hospitals and potential detection inaccuracies likely influenced the detection rates.[40 41] Implementing active surveillance and adopting more advanced, sensitive diagnostic methods are essential to gain a more accurate understanding of *Campylobacter* prevalence.

Our study has limitations. Supply disruption during the COVID-19 pandemic impacted ELISA testing for enteric viruses, potentially underestimating their prevalence and inconsistencies in the denominator for analysis. Additionally, due to laboratory constraints, *Rotavirus* genotyping was not performed and reliance on hospital-based surveillance may have under-represented cases that did not seek medical attention.

CONCLUSIONS

Our study provides a valuable understanding of diarrhoeal diseases in Bhutan, emphasising the significant roles of DEC and *Rotavirus* as the primary contributors to diarrhoea. Children under 9 years of age are particularly vulnerable to bacterial, viral and parasitic infections, a susceptibility shared by the elderly. Social determinants of health, including poverty, limited access to clean water and living conditions, have a notable impact on infection rates, especially in children under nine in low-resource settings. Seasonal patterns were observed with viral pathogens peaking from winter to summer, bacterial pathogens more prevalent in spring and summer, and parasites persisting year-round. Research on coinfections and secondary infections remains crucial to understanding these complex dynamics.

The high prevalence of zoonotic pathogens such as *Salmonella* and *Aeromonas* in diarrhoeal cases suggests that contaminated food and water sources, as well as animal-human transmission, play a role in spreading these infections. Strengthening food safety measures, improving water sanitation and monitoring environmental reservoirs of enteric pathogens are essential to reduce the incidence of diarrhoea. Additionally, the significant presence of β-lactamase-producing MDR pathogens and emerging resistance to fluoroquinolones underline the need for coordinated interventions. Enhanced surveillance of diarrhoeal diseases and antimicrobial resistance is crucial for monitoring resistant enteric bacteria, vital for informing treatment guidelines. Adopting a One Health approach with integrated surveillance of ESBL-producing bacteria across human, animal and environmental health sectors could be instrumental in tackling this growing public health threat.

Our findings emphasise the importance of including DEC in routine screening protocols for childhood diarrhoea, given its rising trends in MDR as it evolves from a gut flora into a significant health threat. Continued monitoring of *Rotavirus* serotypes is necessary to track genotypic trends critical for effective disease management. Moreover, the integration of advanced molecular tools, such as next-generation sequencing, is vital for detecting novel antimicrobial resistance mechanisms and monitoring the emergence of MDR pathogens.

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