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Occurrence of Antibiotic Resistant *Salmonella* and *Shigella* in Diarrheal cases Resulting from a Common Source Consumption of Contaminated Water

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Abstract

Antimicrobial resistance of Salmonella and Shigella remains a significant public health concern globally, particularly in developing nations like Nigeria. This study aimed to assess the prevalence and antibiotic susceptibility of Salmonella and Shigella strains in diarrheal cases linked to the consumption of contaminated water from a shared source in Jimeta-Yola metropolis, Adamawa State. A total of 78 stool samples were obtained for culture using standard methods, with 37 (47.4%) displaying growth of Salmonella and Shigella strains. Through appropriate biochemical tests, the isolates were identified as Salmonella Typhi (35.1%), Salmonella Typhimurium (8.1%), and Shigella species (56.8%), with Shigella exhibiting the highest occurrence. Antibiotic susceptibility testing was conducted using ten selected antibiotics: Ampicillin, Ceftriaxone, Cephalexin, Chloramphenicol, Ciprofloxacin, Co-trimoxazole, Gentamicin, Nitrofurantoin, Ofloxacin, and Tetracycline. Salmonella Typhi isolates showed 76.9% susceptibility to Gentamicin and Ceftriaxone, 61.5% susceptibility to Ofloxacin, 53.8% susceptibility to Chloramphenicol, Ciprofloxacin, Nitrofurantoin, 38.5% susceptibility to Tetracycline, 30.8% susceptibility to Co-trimoxazole, and 23.1% susceptibility to Cephalexin. Shigella species exhibited 95.2% susceptibility to Ofloxacin, 90.5% susceptibility to Gentamicin, 85.7% susceptibility to Ciprofloxacin, 66.7% susceptibility to Ceftriaxone, 57.1% susceptibility to Chloramphenicol, 52.4% susceptibility to Nitrofurantoin, 33.3% susceptibility to Tetracycline, 28.6% susceptibility to Co-trimoxazole, 19% susceptibility to Cephalexin, and 4.8% susceptibility to Ampicillin. Meanwhile, Salmonella Typhimurium displayed 66.7% susceptibility to Ofloxacin, 100% susceptibility to Gentamicin, Chloramphenicol, Nitrofurantoin, Ciprofloxacin, and 33.3% susceptibility to Ampicillin, Cephalexin, Tetracycline, Co-trimoxazole, and Ceftriaxone. The study highlights the persistent high prevalence of Salmonella and Shigella infections and the emergence of complete resistance to Ampicillin, Cephalexin, and Co-trimoxazole. However, Gentamicin and Ciprofloxacin are identified as effective treatment options for infections caused by these pathogens. Therefore, ensuring the availability of safe water sources, promoting hygienic practices, treating drinking water at the point of use (POU) such as boiling, and continuous monitoring are crucial in controlling the dissemination of these organisms.

Keywords: Antibiotic resistance, Occurrence, Diarrhea, Pathogens, Contaminated water

INTRODUCTION

One of the highly-rated threats to public health in the 21st century is the incidence of antimicrobial resistance, which has been accelerated in developing countries like Nigeria due to factors such as poverty, laboratory diagnostic challenges, inappropriate use of antibiotics, unrestricted access, and breaches in the quality of drugs (Ovia *et al.,* 2023). The emergence of antibiotic-resistant *Salmonella* and *Shigella* poses a significant threat to delivering reliable therapies, which is a major concern for high-, middle-, and low-income countries. Antibiotic resistance refers to the decreased sensitivity or complete insensitivity of microbes to a variety of drugs that are meant to cause cell death or inhibit their growth (Nadeem *et al.,* 2020). Despite intensified efforts by governments at all levels to combat these organisms, their infections remain persistent due to antibiotic resistance. The tremendous therapeutic advantage afforded by antibiotics is being threatened by the emergence of these increasingly resistant strains of microbes. Contamination of drinking water also facilitates the transmission of gastrointestinal diseases such as diarrhea to human populations, which remains a persistent issue in developing countries where the quality of water supplies has been compromised due to unsustainable demographic growth and breaches in the integrity of distribution systems (Agbabiaka *et al.,* 2021).

Salmonella and *Shigella* cause mild to severe intestinal tract infections. *Salmonella* can lead to self-limited gastroenteritis or more severe systemic typhoid fever, while *Shigella* species typically remain in the human intestinal tract, causing diarrhea and bacillary dysentery, often transmitted through the fecal-oral route (WHO, 2003). Diarrhea is characterized by abnormal stool consistency, including watery, bloody, or loose stools occurring more than twice a day (WHO 2017). It ranks among the top three causes of childhood mortality in Sub-Saharan Africa, notably Nigeria, with over one billion cases globally resulting in 3 million deaths annually, predominantly from non-typhoidal *Salmonella* and *Shigella* infections, accounting for 99% of the 200 million diarrheal cases and over 650,000 deaths in developing countries, particularly affecting children and young adults (Tesfahun *et al.,* 2016). Legese *et al.* (2020) also noted severe post-diarrheal complications such as inflammatory bowel diseases, irritable bowel syndrome, arthritis, sepsis, myocarditis, peritonitis, and Reiter's syndrome in individuals previously infected with *Salmonella* and *Shigella*. These infections are widespread in developing nations with inadequate access to clean water, improper waste and sewage disposal, and poor hygienic practices among the population (Abebe *et al.,* 2018). Effective prevention in high morbidity areas heavily relies on proper hygiene practices and sanitation. Additionally, continuous epidemiological surveillance is crucial for combating antimicrobial resistance in *Salmonella* and *Shigella* infections, aligning with the UN's Sustainable development goal (SDG) 3 on ensuring good health and well-being. Given the escalating cases of antibiotic resistance in *Salmonella* and *Shigella* infections globally, it is imperative to conduct comprehensive studies in this region to assess the prevalence and dominance of these resistant strains. This study aims to determine the occurrence of antibiotic-resistant *Salmonella* and *Shigella* species in diarrheal cases associated with the consumption of contaminated water from a shared source in the Jimeta-Yola metropolis of Adamawa state.

MATERIALS AND METHODS

Study Area

The study area was Jimeta-Yola Metropolis, Adamawa State. Stool samples were collected from patients attending the Federal Medical Centre Yola. Jimeta is the commercial city in Adamawa State and is located at the center of the state. The area was selected for the study due to its high population density and the diverse group of people residing in the area.

Sample collection

Stool samples were collected from 78 patients who reported experiencing diarrhea after consuming contaminated water sold within the metropolis. The samples were collected in wide-mouthed sterile plastic containers with screw caps, and a corresponding water sample was appropriately labeled for bacterial culture to identify *Salmonella* and *Shigella* species. All samples were securely packed and transported to the microbiology laboratory at Modibbo Adama University of Technology (MAUTECH) Yola in cool boxes for isolation, identification, and antimicrobial susceptibility testing.

Isolation of bacteria

For the isolation of bacteria, a loopful of each stool sample was emulsified in normal saline and then inoculated directly onto pre-prepared plates of Deoxycholate Citrate Agar (DCA) and *Salmonella-Shigella* media. A sterile wire loop was used to make a pool first and then streak in a zigzag fashion while sterilizing the loop at intervals. Additionally, a loopful of the water sample was plated on both Deoxycholate Citrate Agar (DCA) and *Salmonella-Shigella* media to confirm the source of these organisms. The inoculated plates were incubated at 37°C for 24 hours. Subsequently, the isolated non-lactose fermenting colonies on the plates were screened and confirmed through appropriate biochemical tests (Adekunle *et al.,* 2015).

Biochemical Tests/ Identification of bacteria

Salmonella and *Shigella* species were identified through standard biochemical tests and identification charts for enteric organisms. *Salmonella* Typhi isolates were additionally confirmed by a slide agglutination test using *Salmonella* Typhi typing antisera (Cheesebrough, 2006).

Antibiotic susceptibility testing of the isolates

The disc diffusion susceptibility method was performed to test the susceptibility of *Salmonella* and *Shigella* isolates to antibiotics using standard procedures. Firstly, the inoculum was prepared by introducing a clear discrete colony of the test organism into peptone water and incubated for 6 hours at 37°C. Subsequently, the resultant turbidity of the broth was adjusted to 0.5 McFarland standard of turbidity. A sterile wire loop was then used to spread the suspension of the organism all over the entire surface of Mueller Hinton medium and allowed to absorb for 3 minutes. A single disc containing a specific concentration of each antimicrobial agent was firmly placed onto the inoculated surface with the aid of an applicator stick, and the plates were incubated inverted at 37°C for 24 hours.

After the 24 hours of incubation, we observed the plates for clear zones of inhibition, which were then measured in mm using a straight line rule. The diameter of the zone was read using an interpreting chart for zone sizes, and the findings were recorded as susceptible, intermediate, and resistant (CLSI, 2016). The antibiotics used included Ampicillin 25µg, Tetracycline 25µg, Chloramphenicol 30µg, Co-trimoxazole 25µg, Cephalexin 15µg, Nitrofurantoin 200µg, Ceftriaxone 30µg, Gentamicin 10µg, Ofloxacin 10µg, and Ciprofloxacin 10µg.

RESULTS

Isolation of Bacteria

Out of the 78 stool samples collected, 37 samples showed the growth of *Salmonella* and *Shigella* species. Among these, 21 (56.8%) were identified as *Shigella* species, 13 (35.1%) as *Salmonella enterica* Typhi, and 3 (8.1%) as *Salmonella enterica* Typhimurium, as depicted in Figure 1. In terms of gender distribution, 23 (62.2%) of the patients were males, while 14 (37.8%) were females, as illustrated in Figure 2. The mean age of the patients from whom either *Salmonella* or *Shigella* were isolated was 19 years, with an age range of 4 to 63 years, as shown in Figure 3.

Biochemical Tests/ Identification of bacteria

The colonial morphology of the *Salmonella* isolates was smooth, colorless colonies with or without blackening around them, indicating non-lactose fermentation. The *Shigella* isolates also displayed colorless colonies without blackening, consistent with non-lactose fermentation. Results from the Kligler iron agar (KIA) test showed that *Shigella* produced a red slope and yellow butt without gas or hydrogen sulfide (H2S) production. On the other hand, *Salmonella enterica* Typhi exhibited a red slope and yellow butt with a small amount of H_2S, while *Salmonella enterica* Typhimurium displayed a red slope and significant blackening of the entire butt due to high levels of H_2S production, as illustrated in Plate 1 and Table 1.

Antibiotic susceptibility testing of the isolates

Both *Shigella* and *Salmonella* isolates exhibited higher resistance than susceptibility to the tested antibiotics. The most significant resistance was observed for Ampicillin and Cephalexin, with *Salmonella* Typhi, *Salmonella* Typhimurium, and *Shigella* isolates showing 100%, 76.9%, and 95.2% resistance, respectively. In contrast, the highest susceptibility was seen with Ofloxacin and Gentamicin, with *Salmonella enterica* Typhi, *Salmonella enterica* Typhimurium, and *Shigella* isolates displaying 61.5%, 66.7%, and 95.2% susceptibility, respectively, as detailed in Table 2.

DISCUSSION

The findings of this study indicate the presence of *Salmonella* and *Shigella* in the analyzed stool samples. Out of the 78 samples, 37 (47.4%) showed the growth of these pathogens, with *Shigella* species being more prevalent than *Salmonella* species. This highlights the continued high prevalence of these pathogens in the metropolis, particularly in waterborne diseases originating from contaminated water sources, as both *Salmonella* and *Shigella* were identified in the tested water sample. These results align with previous reports by Abebe *et al.* (2018) and Dessale *et al.* (2023) which also noted a high prevalence of these organisms. The study also found that 62.2% of the isolates were from male patients, while 37.8% were from females, suggesting no gender association with *Salmonella* and *Shigella* infections. The age range of patients varied from 4 to 63 years, indicating that these infections are not limited to a specific age group. However, the study revealed that children between 0 to 9 years old were most susceptible to these infections, possibly due to unhygienic habits or poor sanitation practices among children.

Antibiotic resistance in *Salmonella* and *Shigella* is prevalent in developing countries, largely due to poor hygiene, lack of clean drinking water, and consumption of contaminated foods. Out of the 37 clinical isolates characterized, 33 (89.2%) were found to be multi-drug resistant strains, showing resistance to three or more commonly used antibiotics. This high rate of resistance may stem from the extensive use of these drugs as the primary treatment for infectious diarrhea and typhoid fever, or from issues like self-medication, under-dosage, or overuse of antibiotics. Among the *Salmonella enterica* Typhi isolates, resistance rates of 100% for Ampicillin, 76.9% for Cephalexin, and 61.5% for Tetracycline were observed. For *Salmonella enterica* Typhimurium, an overall resistance of 66.7% was noted for Ampicillin, Cephalexin, Tetracycline, Ceftriaxone, and Co-trimoxazole. Regarding *Shigella* species, an overall resistance of 95.2%, 81%, and 71.4% to Ampicillin, Cephalexin, and Co-trimoxazole, respectively, was identified. The study reveals a general resistance of the isolates to Ampicillin, Cephalexin, Co-trimoxazole, and Tetracycline, consistent with the findings of Diriba *et al.* (2020) who similarly reported resistance of *Salmonella* and *Shigella* to these antibiotics.

Table 1: Biochemical features of the *Salmonella* and *Shigella* species isolated

S/No	KIA Medium				Motility test	Citrate test	Isolates
	Slope	Butt	H_2S	Gas			
1	R	Y	-	-	-	-	*Shigella*
2	R	Y	-	-	-	-	*Shigella*
3	R	Y	+	-	+	-	S. Typhi
4	R	Y	-	-	-	-	*Shigella*
5	R	Y	-	-	-	-	*Shigella*
6	R	Y	-	-	-	-	*Shigella*
7	R	Y	-	-	-	-	*Shigella*
8	R	Y	-	-	-	-	*Shigella*
9	R	Y	+	-	+	-	S. Typhi
10	R	Y	-	-	-	-	*Shigella*
11	R	Y	+	-	+	-	S. Typhi
12	R	Y	-	-	-	-	*Shigella*
13	R	Y	-	-	-	-	*Shigella*
14	R	Y	-	-	-	-	*Shigella*
15	R	Y	$+^2$	+	+	+	S. Typhimurium
16	R	Y	$+^2$	+	+	+	S. Typhimurium
17	R	Y	+	-	+	-	S. Typhi
18	R	Y	+	-	+	-	S. Typhi
19	R	Y	+	-	+	-	S. Typhi
20	R	Y	+	-	+	-	S. Typhi
21	R	Y	-	-	-	-	*Shigella*
22	R	Y	+	-	+	-	S. Typhi
23	R	Y	-	-	-	-	*Shigella*
24	R	Y	-	-	-	-	*Shigella*
25	R	Y	$+^2$	+	+	+	S. Typhimurium
26	R	Y	-	-	-	-	*Shigella*
27	R	Y	+	-	+	-	S. Typhi
28	R	Y	-	-	-	-	*Shigella*
29	R	Y	+	-	+	-	S. Typhi
30	R	Y	-	-	-	-	*Shigella*
31	R	Y	+	-	+	-	S. Typhi
32	R	Y	-	-	-	-	*Shigella*
33	R	Y	+	-	+	-	S. Typhi
34	R	Y	-	-	-	-	*Shigella*
35	R	Y	+	-	+	-	S. Typhi
36	R	Y	-	-	-	-	*Shigella*
37	R	Y	-	-	-	-	*Shigella*

Key: R = Red slope; Y= Yellow butt; H_2S = Hydrogen sulphide blackening; - = Negative; + = Positive

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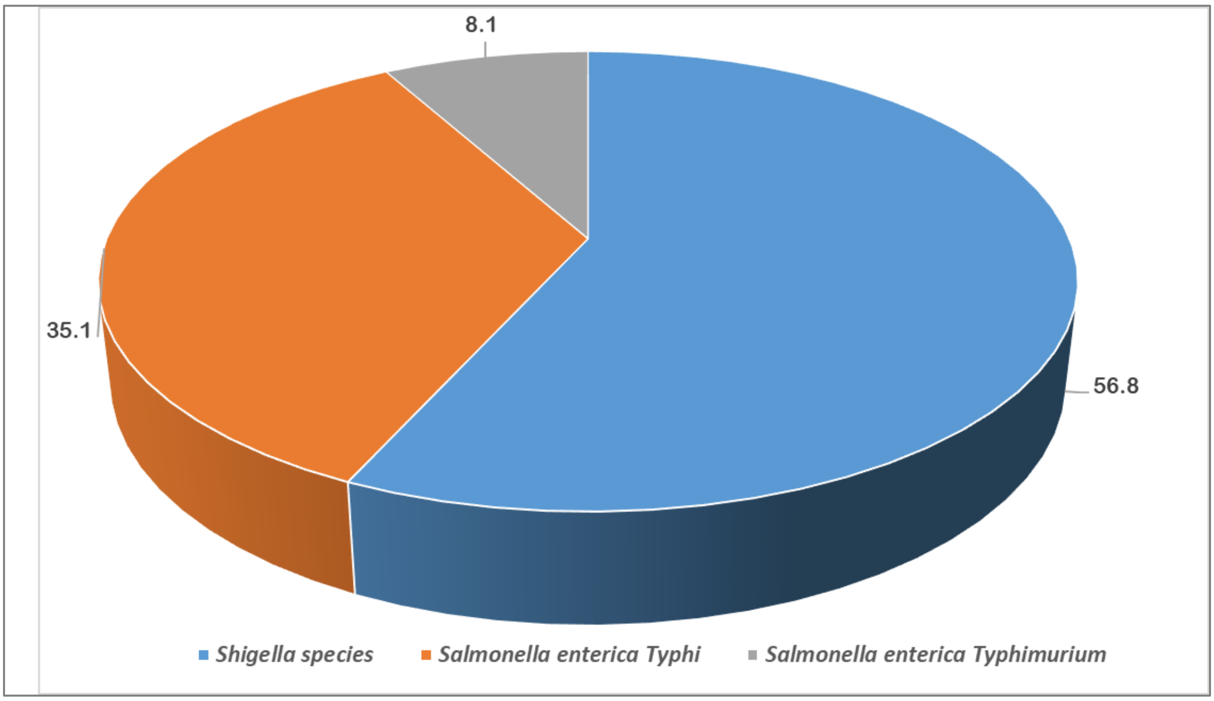

Figure 1: Percentage distribution of *Salmonella* and *Shigella* species

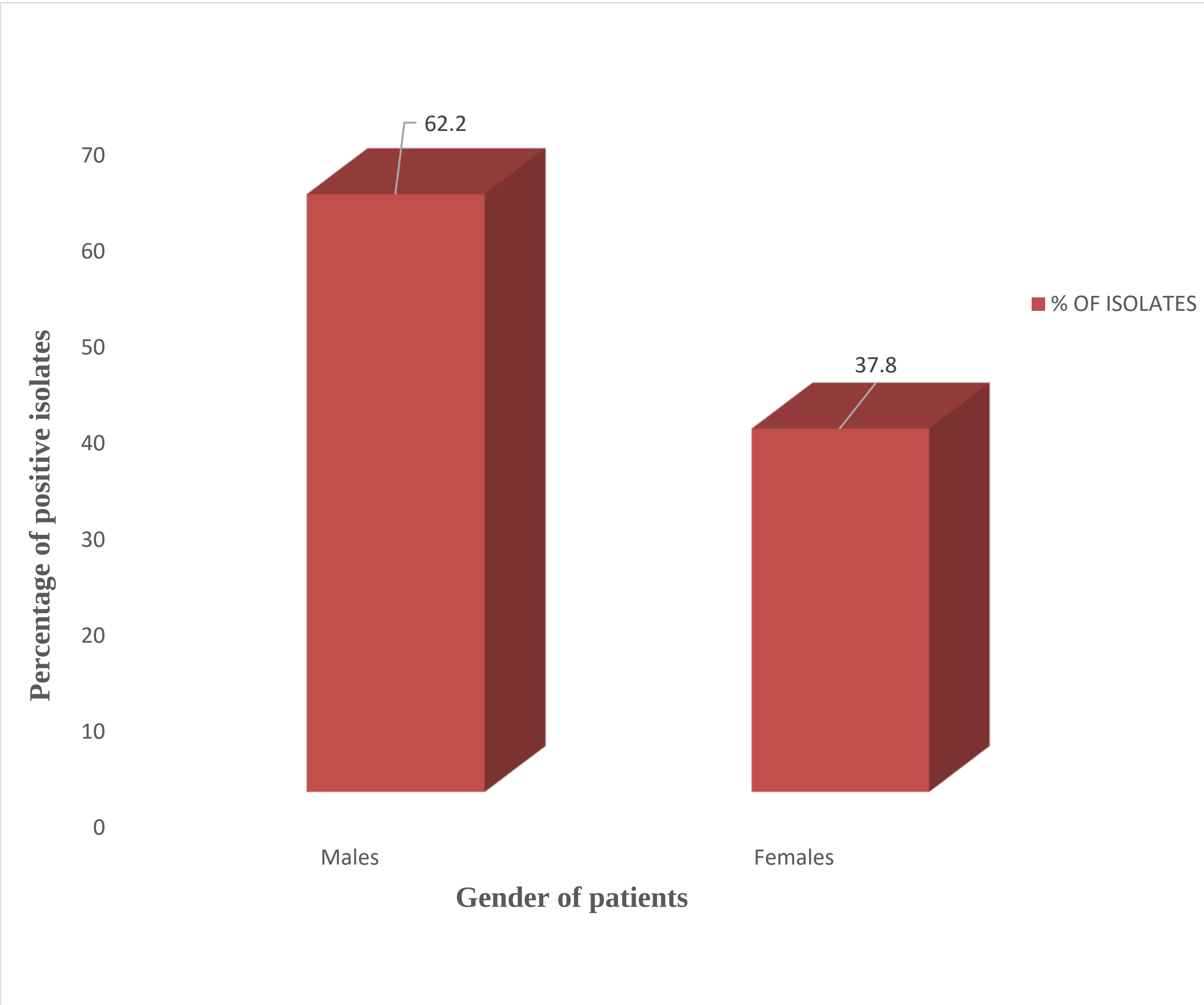

Figure 2: Distribution of the isolates based on gender

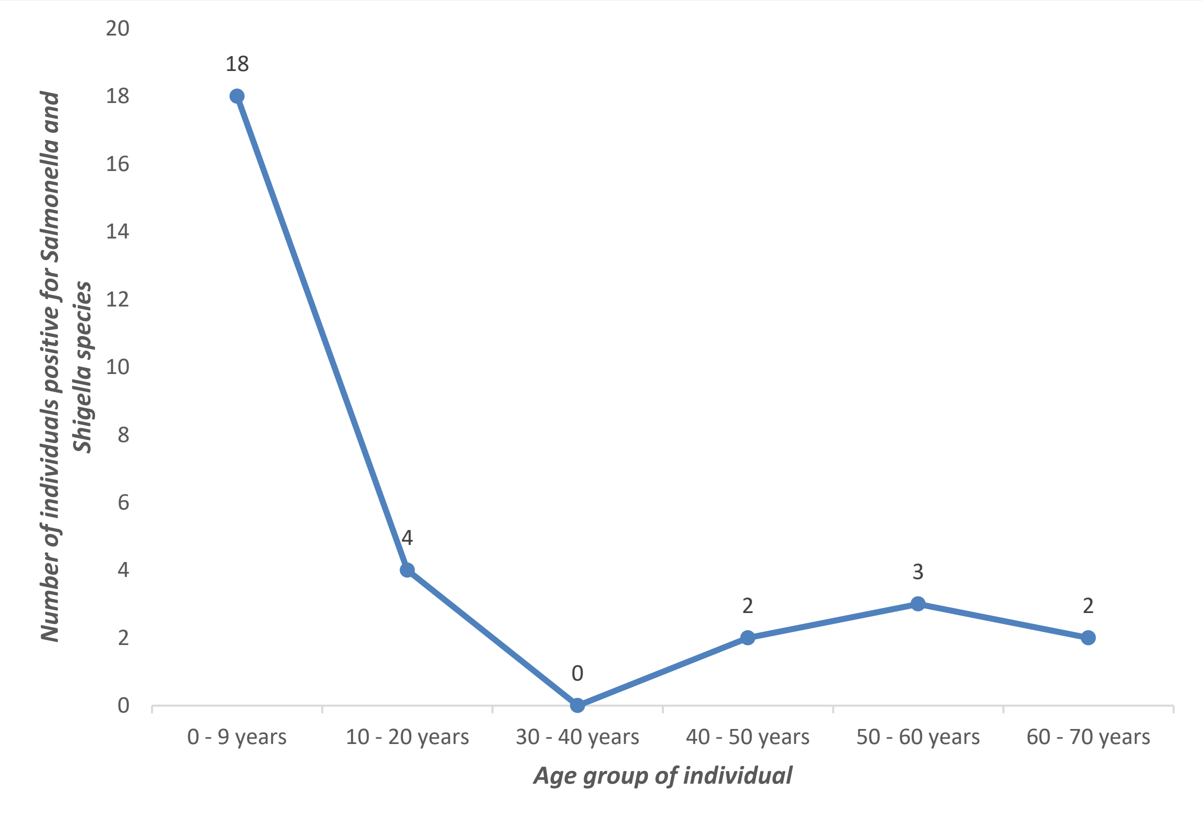

Figure 3: Age distribution of individuals positive for *Salmonella* and *Shigella* species

Plate 1: Tubes showing reaction of the isolates on Kligler iron agar

Table 2: Antibiotic susceptibility test of the isolates

Antibiotics	Organism isolated					
	Salmonella Typhi n = 13		*Salmonella* Typhimurium n =3		*Shigella spp.* n = 21	
	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Ampicillin 25µg	0 (0.0%)	13 (100%)	1 (33.3%)	2 (66.7%)	1 (4.8%)	20 (95.2%)
Ceftriaxone 30µg	10 (76.9%)	3 (23.1%)	1 (33.3%)	2 (66.7%)	14 (66.7%)	7 (33.3%)
Cephalexin 15µg	3 (23.1%)	10 (76.9%)	1 (33.3%)	2 (66.7%)	4 (19%)	17(81%)
Chloramphenicol 30µg	7 (53.8%)	6 (46.2%)	3 (100%)	0 (0.0%)	12 (57.1%)	9 (42.9%)
Ciprofloxacin 10µg	7 (53.8%)	6 (46.2%)	3 (100%)	0 (0.0%)	18 (85.7%)	3 (14.3%)
Co-trimoxazole 25µg	4 (30.8%)	9 (69.2%)	1 (33.3%)	2 (66.7%)	6 (28.6%)	15 (71.4%)
Gentamicin 10µg	10 (76.9%)	3 (23.1%)	3 (100%)	0 (0.0%)	19 (90.5%)	2 (9.5%)
Nitrofurantoin 200µg	7 (53.8%)	6 (46.2%)	3 (100%)	0 (0.0%)	11 (52.4%)	10 (47.6%)
Ofloxacin 10µg	8 (61.5%)	5 (38.5%)	2 (66.7%)	1 (33.3%)	20 (95.2%)	1 (4.8%)
Tetracycline 25µg	5 (38.5%)	8 (61.5%)	1 (33.3%)	2 (66.7%)	7 (33.3%)	14 (66.7%)

The *Salmonella enterica* Typhimurium isolates also displayed an overall susceptibility of 100% to Gentamicin, Ciprofloxacin, Chloramphenicol, and Nitrofurantoin. *Shigella* species showed an overall susceptibility of 95.2%, 90.5%, and 85.7% to Ofloxacin, Gentamicin, and Ciprofloxacin, respectively, while *Salmonella enterica* Typhi isolates exhibited an overall susceptibility of 76.9% to Gentamicin and Ceftriaxone. These findings also agree with those of Assefa and Girma (2019) and Tosisa *et al.* (2020), who reported susceptibility of *Salmonella* and *Shigella* to Gentamicin and Ciprofloxacin.

CONCLUSION

Findings from this study therefore indicate a *Salmonella* and *Shigella* incidence rate of 47.4% within the study area, with these organisms developing complete resistance to Ampicillin, Cephalexin, Co-trimoxazole, and Tetracycline. However, Gentamicin and Ciprofloxacin have proven to be the drugs of choice for the treatment of infections caused by these pathogens. Thus, the availability of potable water supplies, embracing hygienic practices, and continuous assessment would help curb the spread of these organisms. More importantly, the populace should also take charge of the quality of their drinking water by embracing point-of-use (POU) treatment methods such as boiling and disinfection.

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