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Prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella Species* among patients with diarrhea attending Gondar town health institutions, Northwest Ethiopia

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Abstract: Background: Shigellosis and salmonellosis are still global health problems, especially, in developing countries where poor sanitation, lack of clean water supply and proper sewage disposal system exist. The emergence of increased antimicrobial resistance of *Shigella* and *Salmonella* species are global challenges, particularly in developing countries like Ethiopia where increased misuse of antimicrobial agents by human beings occur. Objectives: To determine the prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella* isolates from patients with diarrhea attending the health institutions in Gondar town, Northwest Ethiopia. Materials and Methods: Health institutional based cross sectional study was carried out on diarrheic patients attending Gondar town health institutions, February 29, 2014 to May 20, 2014. Systematic random sampling technique was used and stool samples were collected from 372 study subjects. Samples were cultured onto MacConkey and *Salmonella-Shigella* agars and drug susceptibility patterns of the isolates were determined following standard bacteriological method. Data were coded and entered for statistical analysis using SPSS version 20. Data were presented using tables and chi-square. Result: Of the total of 372 stool cultures, 17(4.57%) *Shigella spp.* and 4(1.08%) *Salmonella spp.* were isolated. Most commonly isolated strains of *Shigella* were *S. flexneri* 11(64.7%) followed by *S. dysenteriae* 3(17.65%), *S. boydii* 2(11.77%) and *S. sonnei* 1(5.88%). *Shigella* isolates presented high resistance rate to ampicilin (94.1%), amoxicillin (88.2), tetracycline (88.2%) and *Salmonella species* were highly resistance to tetracycline (100%), amoxicillin (100%), and ampicillin (75%). However, all isolates of *Shigella* and *Salmonella* were 100% susceptible to ciprofloxacin and norfloxacin. Conclusion and recommendation: This study revealed that isolates of *Shigella* and *Salmonella* showed high rate of drug resistance to the commonly used antibiotics. However, all the isolates were susceptible to ciprofloxacin, and norfloxacin. Therefore, ciprofloxacin and norfloxacin can be used as drugs of choice for the treatment of *Shigellosis* and *Salmonellosis.*

Keywords: Antibiotic Susceptibility, Prevalence, *Salmonella* and *Shigella*

1. Introduction

The *Shigellae* are members of the enterobacteriaceae, non lactose fermenters, non motile, and non gas producers gram negative rods (1). There are four species of *Shigell*a, which includes *S. dysenteriae (group A), S. flexneri (group B), S. boydii (group C)*, and *S. sonnei (group D)* (1).

Shigellae are transferred from person to person usually by asymptomatic carriers and via contaminated food, flies, faeces, fingers, and water (1). To initiate infection, as few as 100 ingested *Shigella* organisms are enough. After the organisms enter the human body, they remain in the cytoplasm of the epithelial cells and spread laterally to

invade adjacent cells which result in the formation of abscesses and ulcerations with high concentration of neutrophils in the stools (1).

Shigellosis is only a human disease caused by the four species of genus *Shigella* and is characterized by increasing in frequency of stool motion and increase in number with the presence of blood, mucous and pus in the stool (1, 2).

Shigellosis is endemic throughout the world where, it is held responsible for some 165 million cases of severe dysentery. The majority of these cases occur in the developing countries. More than one million people are estimated to die from *Shigella* infection each year. Since the late 1960s, pandemic waves of *Shigella* dysentery had stricken Sub-Saharan Africa, Central America and East Asia (3).

In Africa, an estimate of 115 people die of diarrheal diseases every hour, mostly of shigellosis and salmonellosis which are linked to contaminated food and water due to poor sanitation and hygiene (4). In Ethiopia, one in every 17 Ethiopian children dies before the first birthday and one in every 11 children dies before the fifth birthday. According to the Ethiopian demographic and health survey (EDHS) report of 2011, 13 percent of the children under age five were reported to have had diarrhea, and 3% had diarrhea with blood in to the two-week periods before the survey (4). It is highest (18%) among children residing in households that drink water from unprotected wells. The prevalence of diarrhea in children residing in Amhara region was also found to be 13.5% (4).

Genus *Salmonella* are a member of enterobactericeae, non lactose fermenters, motile and gas producer gram negative rods (1). Transmission of these organisms are from person to person via fecally contaminated food, water or through direct fecal oral route (5, 6). They invade the mucosa of the small and large intestines and produce inflammation. Invasion of epithelial cells induces an inflammatory reaction which cause diarrhea due to salmonella (7). Salmonellosis is a disease caused by a large group of bacteria of the genus *Salmonella* (1).

Two hundred million to more than one billion cases of diarrhea result worldwide due *Salmonella* infections every year, leading to 3 million deaths (5).The highest incidence of infection is among the very young and elderly. Mortality is highest in children less than one year old. The increase susceptibility of this age group may be due to the fact that children less than 2 months old produce little hydrochloric acid, a natural barrier to many microorganisms (5).

The wide spread occurrence and distribution of *Salmonella* infection in Ethiopia is increased. It is due to contamination of water, food, and poor sewage disposal system. The very young, elderly, and immunocompromized individuals are particularly more susceptible to *Salmonella* infections at a lower infective dose than healthy adults (8).

Due to different factors in the last few decades, *Shigella* and *Salmonella* have become increasingly resistant to the most commonly used antimicrobials from time to time which results in some challenges in selecting the drug for therapeutic management (9, 10). The emergence of antibiotic resistant *Shigella* and *Salmonella* are serious problems in antimicrobial therapy globally. The incidence varies with the area of isolation of these strains. The progressive increase in antibiotics resistance among these pathogens in developing countries is also becoming a critical area of concern (7).

In developing countries like Ethiopia, resistance may be acquired mostly by selective pressure due to indiscriminate and misuse of antibiotics (11, 12). This leads for the emergence of resistance strains of *Shigella* and *Salmonella* which may be difficult for treatment and prevention (13, 14). In the 1940s, shigellosis was treated successfully with sulfa-drugs. In the 1950s, it was treated with tetracycline (5). In the 1970s ampicilin was the drug of choice for the treatment of bacillary dysentery (15). After the pathogen began to develop resistance to ampicilin the new drug trimethoprime-sulphamethoxazole (TMP-SXT) was used (16). Even if the drug of choice in that time was TMP-SXT, in the 1980s, the *Shigella* species started to develop resistance to the drug (17). Then the emergence of resistance strains continues with any new drugs.

Until about 1960, nearly all *Salmonellae* were sensitive to a wide range of antimicrobial agents but since 1962 emergence of resistance, frequently plasmid mediated, have appeared in *Salmonella* worldwide. The relative importance of antibiotic resistance, and the serotypes in which it occurs, differs from country to country (5).

Knowing the prevalence and the local patterns of antimicrobial susceptibility of *Shigella* and *Salmonella* species is important for reducing the burden of the diseases(7). Therefore, the aim of this study is to determine the prevalence and susceptibility patterns of *Shigella* and *Salmonella* isolates from patients with diarrhea attending the health institutions in Gondar town, Northwest Ethiopia.

2. Materials and Methods

The study was conducted in Gondar town health institutions from January 2014 to May 2014. Gondar is a capital city of North Gondar administration zone, in Amhara region, Northwest Ethiopia. The town has an estimate of > 300,000 total populations (18) with one referral hospital (University of Gondar Referral Hospital) and 8 health centers that include Woleka, Gondar, Gebrieal, Ginbot 20, Maraki, Azezo, Tseda, and Blajig Health Centers which are currently giving health service to the community. All the populations who were living in and around Gondar town were the source of population. The study populations were patients having diarrhea attending health institutions in Gondar town during the study period.

2.1. Inclusion Criteria and Exclusion Criteria

Inclusion criteria; all patients with diarrhea

Exclusion criteria; all patients with diarrhea who had been taken antibiotics treatment in the last 14 days

2.2. Sample size Determination and Sampling Technique

There are studies which were conducted in different regions related with this study. Taking prevalence from the previous study which was conducted in Gondar town, Ethiopia (16.9%) on diarrheal patients (7).Using 95 % of confidence interval with 4% of margin of error sample size was calculated as follows.

$$N=\frac{Z2\ P(1-p)}{W^2}$$

Where:
n = No of sample that will be included
$\frac{Z2}{2}$ = confidence level
P= prevalence from the previous study.
W= acceptable difference

$$n=\frac{(1.96)2\ x\ 0.169(1-.169)}{(.04)2}$$

n= 338
Adding the 10 % contingency 372
So, the sample size was 372

Systematic random sampling technique was used to select the study participants.

In the study area all the patients who were attended in one month before study was 539. The estimated number of patients in the data collection time (February 29 -May 20) was 1437.

To determine the k value. Population size (N) =1437, sample size=372

$$k=N/n=1437/372=3.86,\ K=4$$

Then every 4th cases of diarrheal patients were selected as study subjects.

The total sample size was allocated proportionally to the one hospital (University of Gondar Hospital) and to eight health centers based on the size of the patients with diarrhea. In UOG Hospital, around 302 patients with diarrhea; while there were about 88 patients in Wolka health center, 170 in Teda, 181 in Maraki, 223 in Azezo, 72 in Blajig, 90 in Gebrila, 122 in Ginbot 20, 189 in poly health centers in the year 2013/14 for 3 consecutive months. Therefore, about 76 study subjects were recruited from University of Gondar Hospital and the remaining were recruited from eight health centers in the study period. Systematic random sampling procedure was used to recruit for cases of all health institutions.

2.3. Operational definition

Diarrhea means passing loose, watery, muciod and bloody stools three or more times a day.

Stool samples from diarrheic patients were collected in a clean, dry, disinfectant-free suitable wide-necked container and immediately transferred to Selenite F broth as a transport medium and inoculate onto *Salmonella-Shigella* agar (Oxoid) and MacConkey (Oxoid), and incubated at 37^{0}C for 24 hours. After incubation, the plates were examined for growth and gram stain was done and further bacterial species were identified following standard biochemical test procedure. Biochemical tests performed were triple sugar iron agar, indole, urea, Simon's citrate agar, lysine iron agar, and motility tests. *Shigella species* were serogrouped by the slide agglutinations test using commercially available polyvalent O antisera for *S. dysenteriae*, S. *flexneri, S. boydii* and *S. Sonnei* (20).

Suspension of test organisms were prepared by picking pure colonies with a sterile wire loop suspended in sterile nutrientt broth and incubated for 2 hrs (20). The density of suspensions to be inoculated were determined by comparing with 0.5 McFarland standards. A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube and then spread evenly over the Muller Hinton agar plate. Susceptibility testing was performed on isolates using agar disc diffusion technique against ampicillin (10 µg), amoxicillin (10µg), tetracycline (30 µg), trimethoprime-sulphamethoxazole (30µg), gentamicin (10µg), kanamycin (25µg), nalidixic acid (30 µg), chloramphenicol (30µg), norfloxacin (10µg), ciprofloxacin (5µg)) and cefaclor (30 µg). The plates will be left at room temperature for 30 minutes for diffusion then incubated for 18-24 hours at 37^{0}C. After 18-24 hrs, the zone of growth of inhibition around each disc was measured in millimeters, using a metal caliper, and interpreted as sensitive; intermediate and resistance following the method of CLSI(20, 21).

The reliability of the study findings was guaranteed by implementing quality control (QC) measures through the whole process of the laboratory work. All materials, equipment, and procedures were adequately controlled. Culture media was tested for sterility and performance. Pre analytical, analytical, and post analytical stage of quality assurance those were incorporated in standard operating procedures (SOPs) of the Microbiology Laboratory at the University of Gondar was strictly followed. Standard reference strains of *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used to check the performance of culture medias, biochemical tests and antimicrobial discs (20, 21).

Data were checked for completeness and all responses to the questionnaire were coded against the original English version and analysis was made by using SPSS version 20 software. Data were analyzed by using tables and chi-square. Logistic regression was used for explanatory variables. P-values ≤ 0.05 were considered as statistically significant.

Ethical clearance was obtained from research and ethics committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Permissions were obtained from the concerned bodies of the Zonal Health office and selected health institutions. Informed consent was obtained from each study participants. For each confirmed cases, the responsible clinician of the patient was informed and treatment was started as per the guideline. For children

assent was obtained from their parents or guardians.

3. Results

A total of 372 study participants were included in this study, of which 180 (48.4 %) were males and 192 (51.6 %) were females. The ages of the study participants ranged from 1 to 84 years with a mean age of 21.18. Among the study participants 274 (73.7%) lived in urban areas. One hundred two (27.4%) diarrheic patients were from primary school and majority 147 (39.5%) of the diarrheal patients were under 18 years who are not eligible for legal labor working (Table 1).

Table 1. *Socio-demographic characteristics of diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014*

Characteristics	Number	Per cent
Sex		
Male	180	48.4
Female	192	51.6
Age in (years)		
<20	189	50.8
20-40	139	37.4
41-60	32	8.6
≥61	12	3.2
Educational status		
Under 5 years *	68	18.3
Illiterate	35	9.4
Wright and read	58	15.6
Primary school (1-8)	102	27.4
Secondary school (9-12)	74	19.9
College/University	35	9.4
Occupational status		
Government employees	33	8.9
Private employees	39	10.5
Non employees	99	26.6
Merchant	29	29.8
Farmer	25	6.7
Under 18 years **	147	39.5
Residence		
Urban	274	73.7
Rural	98	26.3

**Under 18 years-they are illegible for work, *under 5 years-they are illegible for school.

Table 2. *The frequency of Shigella and Salmonella species isolated from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014*

Bacterial isolates	Number (%)
Shigella speies	17 (80.95)
S.flexneri	11(64.7)
S.dysenteriae	3(17.65)
S.boydii	2(11.77)
S.sonnei	1(5.88)
Salmonella species	4(19.05)
Total	21 (100)

Of the 372 study participants with diarrheal diseases 17(4.57%) *Shigella spp.* and 4(1.08%) were *Salmonella spp* were isolated. Of the *Shigella spp* isolates, 11 (64.7%) were *S. flexneri,* the predominant isolates followed by *S. dysenteriae* 3(17.65%), *S. boydii* 2(11.77%) and *S. sonnei* 1(5.88%) (Table2).

3.1. Clinical Characteristics of Diarrheal Patients

Fever, abdominal pain, vomiting, headache were the major clinical symptoms of the patients in this study (Table 3). Of the total participants, abdominal pain was found to be 315(84.7%) of the study subjects.

Table 3. *Clinical characteristics of diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014*

Clinical characteristics	Number	per cent
Abdominal pain		
Yes	315	84.7
No	57	15.3
Vomiting		
Yes	164	44.1
No	208	55.9
Fever		
Yes	233	62.6
No	139	37.4
Headache		
Yes	214	57.5
No	158	42.5
Joint pain		
Yes	161	43.3
No	211	56.7

Of the 17(4.57%) *Shigella* isolates, bloody diarrhea was found in 6(35.32%) and mucoid diarrhea was 7(41.1%) whereas watery diarrhea was 3(17.6%) and 8(47.1%) had previous diarrhea.

3.2. Antimicrobial Susceptibility Pattern of Shigella and Salmonella Species

The antimicrobial susceptibility patterns of the *Shigella* and *Salmonella* isolates were presented in Table 4. Strains of *Shigella species* were resistant to ampicilin (94.1%), tetracycline (88.2%), amoxicillin (88.2%), but susceptible to norfloxacin (100%), and ciprofloxacin (100%). *Salmonella* isolate were also resistant to ampicilin (75%), amoxicillin (100%) and tetracycline (75%), but highly susceptible to norfloxacin (100%), chloramphenicol (100%) and ciprofloxacin (100%).

Table 4. *Antimicrobial susceptibility patterns of Shigella and Salmonella isolates from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, 2014.*

Bacterial isolates	Resistance Pattern to antimicrobial agents Number (%)										
	AMP	AMOX	TTC	CN	K	NA	CEF	SXT	C	NOR	CIP
Shigella(n=17)	16(94.1)	15(88.2)	15(88.2)	7(41.2)	7(41.2)	5(29.4)	11(64.7)	10(58.8)	3(17.6)	0(0)	0(0)
Salmonella(n=4)	3(75)	4(100)	3(75)	1(25)	1(25)	1(25)	4(100)	2(50)	0(0)	0(0)	0(0)
total 21	19(84.55)	19(94.1)	18(81.6)	8(33.1)	8(33.1)	6(27.2)	17(82.35)	12(54.4)	3(17.6)	0(0)	0(0)

Key: AMP-Ampicillin, Amox-Amoxacillin, TTC-Tetracycline, CN-Gentamicin, K-kanamycin, NA-Nalidixic acid, CEF-Cefaclor, SXT-Trimethoprime-Sulphamethoxazole, C-Chloramphenicol, NOR-Norfoloxacin, CIP-Ciprofloxacin.

3.3. Multiple Antimicrobial Resistance Patterns of Shigella and Salmonella Isolates

The results of multiple antimicrobial resistant patterns were presented in Table 5. Of the 17(4.57%) of the *Shigella* isolates 16(94.1%) showed multi-drug resistant, while one of them was sensitive to all antimicrobial agents tested. The *Shigella spp*. showed multi drug resistance up to 8 antibiotics and the *Salmonella spp*. showed up to 5 antibiotics.

Table 5. *Multidrug-resistance pattern of Shigella and Salmonella isolates from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014*

Bacterial isolates	Number of antibiotics resisted	Antibiotics tested	Total (%)
		AMP, AMOX, TTC, SXT	2(12.5%)
	R4	AMP, AMOX, TTC, CEF	2(12.5%)
		AMP, TTC, CF, NA	1(6.25%
		AMP, AMOX, TTC, CN,KAN	1(6.25%
	R5	AMP, AMOX, SXT, CF, NA	1(6.25%
		AMP, AMOX, CF, CN, NA	1(6.25%
Shigella species		AMP, AMOX, TTC, SXT, CEF, KAN	1(6.25%
		AMP, AMOX, TTC, SXT, CEF, NAL	1(6.25%
	R6	AMP, AMOX, TTC, SXT, CN, KAN	1(6.25%
		AMP, AMOX, TTC, SXT, CEF, CN	1(6.25%
		AMP, AMOX, TTC, CEF, KAN, NAL	1(6.25%
	R7	AMP, AMOX, TTC, SXT, CEF, CN, KAN	2 (12.5%)
	R8	Amp, Amox, TTC, SXT, CEF, CN, K, C	1(6.25%)
	R3	AMOX, CEF, AMP	1(25%)
Salmonella species	R4	AMOX, CEF, AMP, SXT	2(50%)
	R5	AMOX, CEf, AMP, CN, K	1(25%)

4. Discussion

In this study, the isolation rate (4.57%) of *Shigella spp.* was almost in line with studies from Indonesia (9.3%) (22) and Trinidad (8%) (23). This was lower than studies done in Tehran (14.5%) (24), Kenya:(16%) (25), (43.9%) (26). The low rate of isolation as observed in the present study may be due to differences in the method of sample collection, isolation identification. The rate of *Shigella* was also lower than previous studies in Ethiopia: Jimma (20.1%) (27), Hawasa (34.6%) (28) , and Gondar: (16.9%) (7), (15.6%) (12) but in agreement with Harar (6.7%) (29) and Gondar :7.4%(30), 7.5%(31). However, higher than study from Hawasa (0.4%) (32). This might be due to differences in awareness of the people about personal and environmental hygiene from the continuous health education made by the different health educators in the different health institutions against of shigellosis and salmonellosis.

In the current finding the isolation rate (1.076%) of *Salmonella spp.* was found to be lower than studies done in Ethiopia at different places: Harar (11.5%) (29), Jimma : (15.4%) (33), (6.2%) (19). However, it was consistent with the findings reported in Tanzania (0.8%) (34) and in Ethiopia: Hawasa: (0%) (32), (2.5%) (35), Gondar: (1.6%) (12), and (0%) (36). This might be due to the increasing awareness of the people about personal and environmental hygiene made by the health institutions and other partners.

Our findings showed that *S. flexneri* (64.7%) was the predominant serogroup in the present study which was found to be higher than the finding reported in Addis Ababa (54.0%) (13), and lower than the findings reported in Tanzania (90%) (34), Gondar (72.2%) (31) and in line with a report in Indonesia (63.2%) (22). However, the prevalence of *S .flexneri* at the same study area decreased from (72.2 %) in 2008 (31) to (64.7%) in the present study, whereas the prevalence of *S. dysentriae* increased from (10%) in 2008 (31) to (17.65%) in the present study. The current finding indicated that *S. Sonnei* (5.88 %) were the least frequently isolated spp. which was in line with the previous study conducted in the same study area 8.9%((31).

Majority of *Shigella* isolates were found in mucoid diarrhea 8(47.1%), not washing hands after defecation 12 (70.6%) and not washing hands before preparing food 13 (76.5%). However, it was not stastically significant associated with an increasing of infection with *Shigella* wich was in line with study done on childhood diarrhea in

sub-saharan africa (37).

The emergence of antibiotic resistance among *Shigella* and *Salmonella spp. isolates* are serious problems in antimicrobial therapy globally, especially in developing countries. The incidence varies with the area of isolation of these strains (7). When results of the antimicrobial susceptibility patterns of *Shigella* and S*almonella species* in this study compared with earlier reports from other parts of Ethiopia showed that there has been a change in resistance pattern to antibiotics use in Ethiopia. It showed a high degree of resistance to the commonly used antimicrobials. Similar observations were also reported in Indonesia(22), Iran(38), Tehran(24), and Ethiopia(13, 19, 29). According to this study, therefore: ampicillin, tetracycline, amoxicillin and SXT are no longer effective for the treatment of shigellosis and salmonellosis in the study area

More than 58% of *Shigella* isolates were resistance to amoxacillin, tetracycline, ampicillin, cefaclor, and 29-42% of the isolates were resistance to gentamicin, kanamycin, and nalidixic acid. This was much higher than the findings reported in the 1980s, and 1990s(37).The resistance to all other antibiotics in this revealed higher rate of resistance than the findings reported in the 1980s, 1990s and (37). This may be due to overuse of antimicrobials in the area.

In this study, the overall multiple drug resistance pattern of *Shigella* were 16(94.1%) of the isolates; this was higher than studies conducted in Ethiopia, Gondar: (87.8%) (30) and (68%) (36) and lower than study done in Jimma (100%) (19).

The present study showed that there was an increasing frequency of resistance of *Shigella* strains to nalidixic acid (58.8%) and gentamicin (41.2%) as compared with the previous reports in Ethiopia: nalidixic acid (2.7%) (13) and gentamicin (0%) (13) and nalidixic acid(0%) (31) and gentamicin (12.2%) (31).

We identified no resistance to chloramphenicol, norfloxacin and ciprofloxacin. Other studies also found that little resistance to chloramphenicol, norfloxacin and /or no resistance to ciprofloxacin at the same study area (31, 36).Thus, at the present time ciprofloxacin and norfloxacin are the best drugs of choice for the treatment of shigellosis and salmonellosis in the study area.

In conclusion, this study confirmed that *Shigella* and *Salmonella spp*. are the causative agents of diarrheal diseases. *S .flexneri* was the predominate *spp.* isolated followed by *S. dysenteriae*, *S .boydii, S. sonnei*. All *Salmonella spp.* were resistant against amoxicillin and Cefaclor whereas one strain of *Shigella* as susceptible to all antibiotics. Most of the *Shigella* and *Salmonella* isolates were resistance against ampicillin, amoxicillin, tetracycline, cotrimoxazole but all are susceptible to norfloxacin and ciprofloxacin. The *Shigella* and *Salmonella* species showed high level of multidrug resistance to the commonly used antibiotics.

Recommendation

It is recommended to establish an antimicrobial surveillance system for *Shigella* and *Salmonella* species in different regions of Ethiopia. *Shigella* and *Salmonella* strains were sensitive for Ciprofloxacin and norfloxacin than ampicilin, amoxicillin, SXT, tetracycline and chloramphenicol. Therefore, physicians and clinicians should use ciprofloxacin and norfloxacin as first line antibiotics for the treatment of shigellosis and salmonellosis. Avoid overuse of antibiotics to reduce the emergence of multi-drug resistance strains

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