Bacteriological And Parasitological Study Of Acute Gastroenteritis Among Patients Visiting Sukraraj Tropical And Infectious Disease Hospital (STIDH), Teku, Kathmandu

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Abstract

BACKGROUND: Gastroenteritisis a major public-health problem in developing nations like Nepal, where communities are still ravaged by poverty, poor sanitation, poor personal hygiene, and poor water supplies.The objective of our study was to find the bacterial and parasitic agents responsible for causing gastroenteritis.

METHODOLOGY: A hospital based prospective study was carried out for 3 months in Bacteriology and Parasitology section of STIDH. Stool samples received in respective section from patients with gastroenteritis were included in the study. Standard Microbiological Guidelines were employed for collection and processing of samples, followed by Isolation, Identification and Antimicrobial Susceptibility Testing of bacterial isolates. Normal saline and Iodine preparation were done for microscopic examination of parasites.

RESULTS: Among 421 stool samples processed in Bacteriology section, 28(6.65%) showed bacterial growth with 15(53.57%) *Shigella flexneri*, 4(14.28%) *Shigella sonnei* and 9(32.14%) *Salmonella typhimurium*. Ceftriaxone and Nalidixic acid were found to be the most Sensitive and Resistant antibiotic for *Shigella* spp. and *Salmonella typhimurium.* Similarly, in parasitology section, microscopic observation of 648 stool samples showed 136(20.98%) cysts of *Entamoeba histolytica*, 3(0.46%) cysts of *Giardia lamblia*, 2(0.30%) trophozoites of *Giardia lamblia*, 1(0.15%) larva of *Strongyloides stercoralis*, 1(0.15%) ova of hookworm and 1(0.15%) ova of *Ascaris lumbricoides*.

CONCLUSION: The study revealed *Shigella* species as the predominant bacterial agent with *S. flexneri* being the major one. Hence, extensive study of shigellosis with greater emphasis on resistance pattern of different group of antibiotics is essential in such hospital. Similarly, parasitic

infections are found in significant amount. Hence, concerned hospital authorities and government official's needs to take strict action in contending such infections.

KEY WORDS: gastroenteritis, *Shigella flexneri*, Ceftriaxone, *Entamoeba histolytica*

Background

Gastroenteritis (also named as gastric flu, intestinal flu, stomach flu) is marked by inflammation of gastrointestinal tract which may be acute or chronic (Ingle et al., 2000). Symptoms include fever, diarrhea, stomachache, nausea, vomiting, abdominal pain and cramp (Kazemian et al., 2015).

The etiological agents of gastroenteritis are bacterial, viral and parasitic. The most common bacterial agents causing gastroenteritis are *Salmonella* species, *Shigella* species, *Escherichia coli* and *Campylobacter* species. A number of parasites can also cause gastroenteritis mostly *Giardia lamblia*, *Entamoeba histolytica, Cryptosporidium parvum* (Bhandari et al., 2009).

Gastroenteritis is one of the major causes of diarrhea and vomiting among children and adults (Khan et. al 2014). Poor sanitation, lack of safe drinking water and lower socio-economic status contribute important cause of morbidity and mortality, and are also the major factor of disease transmission in Nepal where children are more affected than adults (Rai et al., 2004).

Acute gastroenteritis causes 1.5 million visits to primary care providers each year and 220,000 hospital admissions for children under the age of 5 years, a staggering 10% of all the hospital admissions of children (Chow et al., 2010).

In this study, we have tried to observe the bacterial and parasitic agents responsible for causing gastroenteritis. Reflecting the current status of patients suffering from gastroenteritis and the emergence of antibiotic resistance among pathogens, the findings will also help the concerned authorities to combat such infections through necessary policies and regulations in the near future.

Methods and Materials

Study area and population

After acquiring an ethical approval from Institutional Review Committee (IRC) at Nobel College, Sinamangal, the study was conducted in bacteriology and parasitology section of the hospital from August 2017 to October 2017. A total of 421 stool samples were processed for culture in the bacteriology department and 648 stool samples were processed for routine microscopic examination in the parasitology section following standard microbiological guidelines.

Bacteriological procedure

The stool samples received were cultured onto MA, XLD, TCBS and kept for overnight incubation at 37°C.

Based upon colony morphology, gram staining, biochemical reactions (catalase, Oxidase, Methyl-Red, SIM, Urease, TSI, Citrate) organisms were identified. Serotyping was done for Salmonella and Shigella species using respective antisera (Denka Seiken Co). Non-Lactose Fermenting colonies on MA and XLD were suspected for Salmonella and Shigella species, then it was subcultured on Nutrient Agar for further investigation.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller- Hinton Agar plates by Modified Kirby- Bauer Disc Diffusion method following CLSI guidelines. Antibiotics like Ampicillin (10mcg), Ciprofloxacin (5mcg), Cotrimoxazole (25mcg), Cefexime (5mcg), Ceftriaxone (30mcg), Ofloxacin (5mcg), Gentamicin (10mcg), Nalidixic-Acid (30mcg), and Tetracycline (30mcg) were used for susceptibility testing of bacterial isolates.

Parasitology procedure

For saline wet mount, a small quantity (about 2mg) of faeces was mixed with few drops of saline on a clean grease-free slide and then cover slip was applied to avoid formation of air bubbles. The same procedure was followed to prepare the iodine wet amount. Commercially available Normal Saline and Lugol's Iodine was used for the procedure. Slides were observed under microscope at low power magnification─10X, 20X and 40X for the detection of cysts and trophozoites of protozoa, eggs and larvae of helminths and other parasites.

Results

Bacterial enteropathogens

Out of total 421 stool specimens examined, 28 (6.7%) were culture positive for the bacterial entero-pathogens. Among the 28 isolated samples, 19 were *Shigella* spp. and 9 were *Salmonella* spp.

Distribution of different species of *Shigella* spp. and *Salmonella* spp.

Among 19 *Shigella* species confirmed by serotyping, *S. flexneri* accounted for 15(78.95%), and *S. sonnei* 4(21.05%), which shows *Shigella flexneri* to be the most common species. Similarly, among 9 *Salmonella* spp., all were *Salmonella typhimurium* (Figure:1).

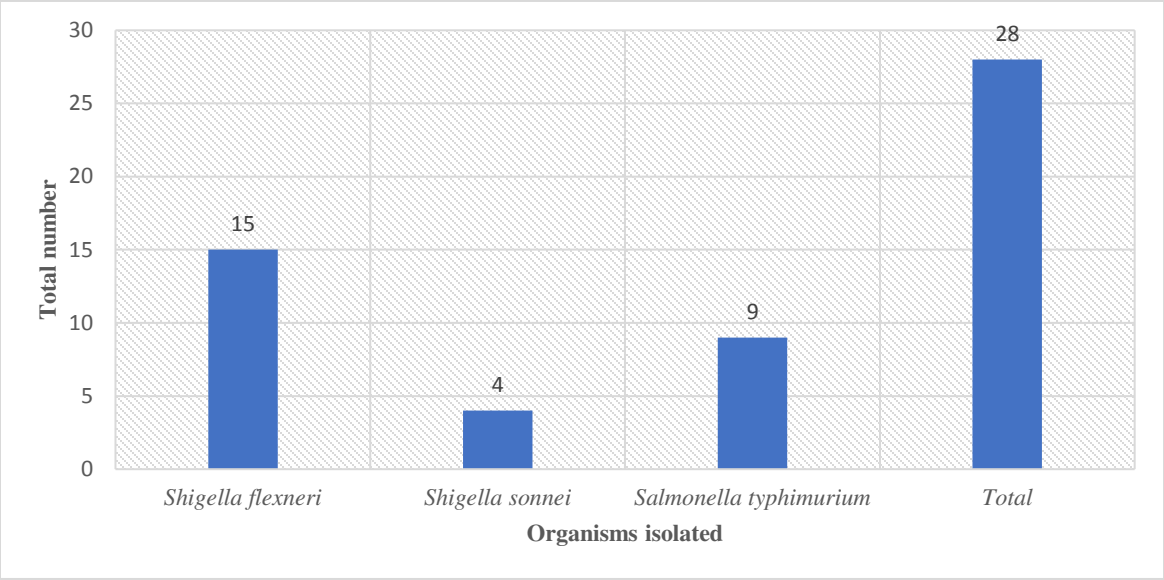

Figure 1: Distribution of different species of *Shigella* spp. and *Salmonella* spp.

Of the 28 culture positive samples, 11 (39.28%) were from the age group of 40-60 years, 9 from 20-40 years, 5 from 0-20 years and 2 from 60 and above age group illustrated in (Table 1).

Table 1: Age-wise distribution of bacterial isolates

Organisms	Age group			
	0-20	**20-40**	**40-60**	**Above 60**
Shigella flexneri	2	7	6	0
Shigella sonnei	2	1	0	1
Salmonella typhimurium	1	2	5	1
Total	5	9	11	2

Antibiotic susceptibility patterns of *Shigella* spp. and *Salmonella* spp.

Among *Shigella* spp., Ceftriaxone 18(94.73%) was found to be the most sensitive antibiotic followed by Cefixime 17 (89.47%). Cotrimoxazole 12(80%) and Nalidixic acid 12(80%) were the most resistant one. *Salmonella typhimurium* was 100% sensitive to Cefixime and Ceftriaxone, whereas 100% resistant to Cotrimoxazole and Nalidixic acid (Table 2).

Table 2: Antibiotic susceptibility patterns of *Shigella* spp. and *Salmonella* spp.

Antibiotics	*Shigella* spp. (n=19)				*Salmonella* spp. (n=9)	
	Shigella flexneri (n=15)		*Shigella sonnei* (n=4)		*Salmonella typhimurium* (n=9)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin/Amoxicillin	4(26.67)	11(73.33)	1(25)	3(75)	4 (44.44)	5(55.56)
Cefixime	13(86.67)	2(13.33)	4(100)	0	9 (100)	0
Ceftriaxone	14(93.33)	1(6.66)	4(100)	0	9 (100)	0
Ciprofloxacin	5(33.33)	10(66.67)	1(25)	3(75)	4 (44.44)	5(55.56)
Cotrimoxazole	3(20)	12(80)	0	4(100)	6(66.67)	3 (33.33)
Gentamicin	10(66.67)	5(33.33)	3(75)	1(25)	5(55.56)	4(44.44)
Nalidixic acid	3(20)	12(80)	0	4(100)	0	9(100)
Ofloxacin	8(53.33)	7(46.67)	0	4(100)	7(77.78)	2(22.22)
Tetracycline	8(53.33)	7(46.67)	0	4(100)	6(66.67)	3(33.33)

Parasitology

Out of total 648 stool specimens—344 were male and 304 were female—144 were found to be positive for parasites, with 69 of them being male and 75 being female.

Types of parasites observed in male and female

Out of 144 positive cases, 136 cyst of *E. histolytica* (65 male and 71 female), 3 cyst of *Giardia lamblia* (2 male and 1 female), 2 trophozoites of *Giardia lamblia* (1 male and 1 female), 1 larva of *Strongyloides stercoralis* in female, 1 ova of *Ascaris lumbricoides* in male and 1 ova of hookworm in female were observed (Table 3).

Table 3: Parasites observed in male and female

Type of parasites	Male	Female	Total number	%
Cyst of *Entamoeba histolytica*	65	71	136	94.4%
Cyst of *Giardia lamblia*	2	1	3	2.1%
Trophozoites of *Giardia lamblia*	1	1	2	1.4%
Ova of *Ascaris lumbricoides*	1	0	1	0.7%
Larvae of *Strongyloides stercoralis*	0	1	1	0.7%
Ova of hookworm	0	1	1	0.7%
Total	69	75	144	100%

Age wise distribution of parasitic infections

With 32.63% of its total patients found affected, the age group 20-30(32.63%) appeared to be most common victims of parasitic infection, whereas the age group 0-10 ranked second, with 22.22% of its total patients being infected. (Table 4).

Table 4: Age wise distribution of parasitic infections

Age group	Male	Female	Total	Percentage
0-10	15	17	32	22.22%
10-20	10	13	23	15.97%
20-30	25	22	47	**32.63%**
30-40	12	10	22	15.27%
40-50	3	8	11	7.63%
50 and above	4	5	9	6.25%
Total	69	75	144	100%

Discussion

Gastroenteritis still accounts for a significant proportion of mortality and morbidity especially in developing countries(Khan, Singh et al. 2014). The major symptoms of gastroenteritis is diarrhea, vomiting and abdominal cramps(Ingle, Hinge et al. 2000). It is one of the most important cause of death and major public health problem in developing countries.

The study was conducted from 15th August 2017 to 17th October 2017 among patients visiting Sukraraj Tropical and Infectious Disease Hospital. Keeping in view the threat of gastroenteritis in Nepal, the present study was carried out for isolation and identification of bacterial species responsible for gastroenteritis. Out of the total 421 cases enrolled for the study, 214 were female and 207 were male, with female to male ratio being 1.03:1. Of the total positive cases, most of the bacterial species were isolated from female aged 41-50 years. Here by our study shows that adults were affected more compared to children and elder people.

In this study, *Shigella* species isolated were 4.51% of total cases. The pattern of serotypes found in this study was 68.42 % S. *flexneri*, 31.58 % *S. sonnei* of the total enteric isolates. This pattern of serotype was analogous to (Dhital, Sherchand et al. 2017) . The predominance of *S. flexneri* may be due to highly infectious nature through the oral route; an ingestion of as few or ten organisms can cause an infection. The Incidence of *Shigella* was commonly seen highest in summer to monsoon.

Table 5: Isolation rate of Shigella species in various studies

Name of author/ Year	**Sample size**	**Isolation rate**
(Agtini, Soeharno et al. 2005)	16872	7.16%
(Wilson, Easow et al. 2006)	770	10.8%
(Shrestha, Malla et al. 2008)	340	18.6%
(Huruy, Kassu et al. 2011)	384	15.6%
(Kansakar, Baral et al. 2011)	877	4.6%
(Njunda, Assob et al. 2012)	223	4.5%
(Khan, Singh et al. 2013)	507	13.60%
(Shah, Sharma et al. 2013)	268	2.24%
(Khan, 2014 .52)	458	14.2%
(Dhital, Sherchand et al. 2017)	717	2.1%
Present study (2017)	421	4.51%

The present study shows that the isolation rate was 4.51% out of 421 samples. The isolation rate was similar to the studies done by Kansakar, Dhital, Shah Sharma, and Njunda, although Khan, Huruy, Agtini, Shrestha, Wilson, Khan Singh showed that the isolation rate was comparatively more.

Table 6: Commonly involved age group in different studies

Name of author / year	Age group
(Agtini, Soeharno et al. 2005)	0-1
(Bhattacharya, Khanal et al. 2005)	0-5
(Wilson, Easow et al. 2006)	1-10
(Shrestha, Malla et al. 2008)	21-30
(Kansakar, Baral et al. 2011)	20-29
(Njunda, Assob et al. 2012)	1-15
(Shah, Sharma et al. 2013)	1-10
(Khan, Singh et al. 2013)	1-10
(Khan, 2014 .52)	1-10
(Dhital, Sherchand et al. 2017)	0-5
Present study (2017)	31-40

The present study shows that Shigellosis was more common in age group 31-40. Whereas the studies conducted by Dhital, Khan, Shah, Sharma, Singh and Wilson showed that children between 1-10 age group were more susceptible to Shigellosis than adults.

Antibiotic Susceptibility Pattern of *Shigella* spp.

In this study, the third generation Cephalosporin were found to be more sensitive antibiotic than other antibiotic (95% of *Shigella* species were sensitive to Ceftriaxone and cefexime). However, 84% of isolates were resistant to Ampicillin, Nalidixic acid, Cotrimoxazole and 68% Ciprofloxacin resistant. Similar, kind of result have been shown in a study done by (Dhital, Sherchand et al. 2017). But the study carried by (Agtini, Soeharno et al. 2005) showed that the isolates were resistant to Ampicillin, Chloramphenicol and Tetracycline whereas susceptible to Nalidixic acid, Ciprofloxacin and Ceftriaxone. The study done by(Kansakar, Baral et al. 2011),(Shrestha, Malla

et al. 2008) also showed that all the Shigella isolates were susceptible to Ceftriaxone and resistant to Ciprofloxacin, Ampicillin, Chloramphenicol, Cotrimoxazole which is similar to our study. Likewise, the study of (Kahsay and Muthupandian 2016) showed that the isolates were highly resistant to Ampicillin but some strains were sensitive to Ciprofloxacin. The study conducted by (Tiruneh 2009) showed that all *Shigella* isolates were sensitive to Nalidixic acid and Ceftriaxone and *Shigella flexneri* serotype 1 showed highest resistance to Ciprofloxacin.

In this study, one of the isolates, *S. flexneri* was found to be ESBL-producer which accompanied ciprofloxacin resistance along with the third generation Cephalosporin. Similarly, ESBL has been reported in *Shigella* species from different parts of the world.

Antibiotic Susceptibility of *Salmonella serovar*:

Among the 421samples, 9(2.13%) of the isolates were *Salmonella typhimurium.*

Table 7: Isolation rate of Salmonella species in various studies

Name of author/ year	**Sample size**	**Isolation rate (%)**
(Shrestha, Malla et al. 2008)	340	8.82%
(Shah, Sharma et al. 2013)	268	3.37%
Present study 2017	421	2.13%

The present study shows that the isolation rate was 2.13% out of 421 samples. The isolation rate was similar to the studies done by Shah, Sharma whereas study done by Shrestha, Malla shows that the isolation rate was comparatively more.

Antibiotic susceptibility patterns of *Salmonella serovar*:

In this study, Salmonella serovar showed susceptibility to the third generation of Cephalosporin (Ceftriaxone), whereas Cotrimoxazole, Gentamicin, Tetracycline and Nalidixic Acid were resistant, which was similar to the study conducted by (Shrestha, Malla et al. 2008) .

Discussion of Parasitological study:

Roughly half of the world's population lives under the conditions that generate nutritional stress and parasitic disease with protozoan parasites or helminthes. The present study included a parasitological analysis of 648 stool samples with special attention to both intestinal protozoa and helminthes. This result showed females harbored larger proportion of intestinal parasitic infection in contrast to the males. In this study five different types of specific intestinal parasites were found:136 cyst of *Entamoeba histolytica,* three cyst of *Giardia lamblia* , a Larva of *Strongyloides stercoralis*, an ovum of *Ascaris lumbricoides*, two trophozoites of *Giardia lamblia* and an ovum of Hookworm .Protozoan positivity rate was significantly higher compared to helminthic parasites, the reason being cysts being able to survive in harsh conditions.

The overall prevalence of intestinal parasites in this study was found to be 22.22% which is similar to the study findings by (Chandrashekhar, Joshi et al. 2005) 21.3 % and (Shrestha, 2012 .48)21.04%.

Our study shows that 21% of the people visiting the Sukraraj Tropical and Infectious Disease Hospital were infected by the *Entamoeba histolytica.* The isolation rate was similar to the study done by (Magar, Rai et al. 2011).The isolation rate of cyst of Giardia lamblia was 0.5%, which is less(2.6%) than the study done by (Akuffo, Armah et al. 2017).

The infection rate of *Strongyloides stercoralis* 0.2% is slightly lower (1.6%.) than findings of (Widjana and Sutisna 2000).

Conclusion

The present study addresses some important issues about diarrheal infection and their most common bacterial agents at the Sukraraj Tropical and Infectious Disease Hospital. Through this study, we found that *S. flexneri* was an important etiological agent causing acute gastroenteritis. It is highly resistant to first-line drugs, only Ceftriaxone remains the drug of choice. However, there

was one case of *S. flexneri* which was resistant to ceftriaxone and was an ESBL producer. With the increase in improper and imprudent use of antibiotics the chances of multi drug resistant isolates being isolated are higher, hence an alarming situation warranting proper and prudent use of antimicrobial agents and its continuous monitoring.

Intestinal parasites are worldwide in distribution. Poverty, illiteracy, unsanitary living style, usual contact with soil and consumption of vegetables, fruits and water contaminated with infected feces, walking bare foot in fecal contaminated area may play leading role in increasing rates of parasites. Hence, there is a need for a comprehensive program to combat intestinal parasites in Nepal.

ABBREVIATIONS

APW-Alkaline Peptone Water

AST-Antimicrobial Susceptibility test

ATCC-American Type Culture Collection

CLSI- Clinical Laboratory Standard Institute

GIT-Gastrointestinal tract

MA-Mac-Conkey agar

MHA-Mueller Hinton agar

NPHL- National Public Health Laboratory

SIM-Sulphide Indole Motility

SOP-Standard Operating Procedure

SPSS-Statistical Package for Social Science

TCBS- Thiosulphate citrate bile salt sucrose agar

TSI-Triple Sugar Iron agar

XLD-Xylose Lysine Deoxycholate

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Ethics and consent to participate

The ethical approval for study was taken from Nobel Institutional Review Committee (IRC), National Ethical Guidelines for Health Research in Nepal. The issued letter of IRC can be presented on request.

Consent for publication

Not applicable

Availability of data and materials

Please contact author for data requests.

Competing interest

The authors declare that they have no competing interests.

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Authors contributions

PC reviewed the manuscript and approved the final version of the manuscript. SJ, NL developed and designed the research. BM, PS and NM analyzed and interpreted the data. SJ finalized and drafted the manuscript. All authors have read and approved the final manuscript.

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Figures

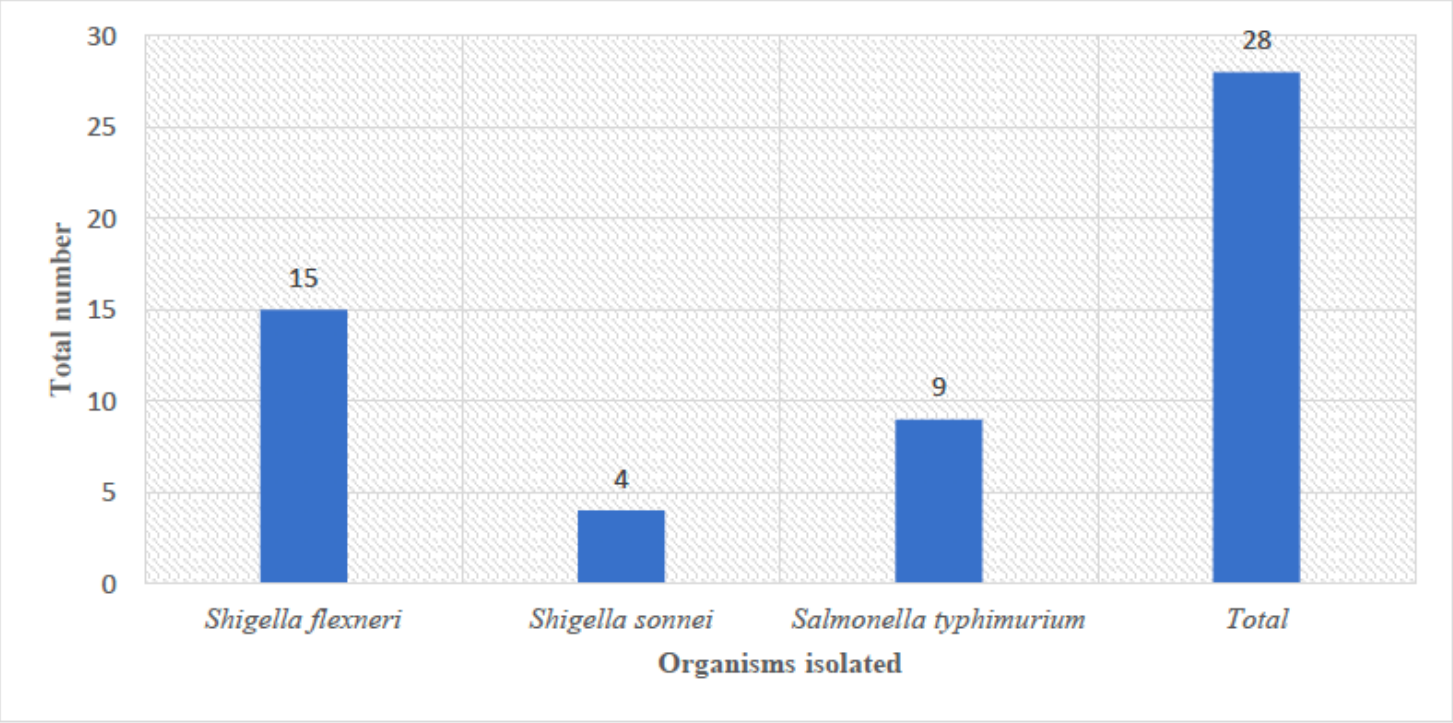

Figure 1

Distribution of different species of Shigella spp. and Salmonella spp.