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Trends of Salmonellosis and Its Resistance to Antimicrobials in the Buea Health District, Cameroon

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ABSTRACT

Salmonellosis accounts for a significant proportion of morbidity and mortality cases, particularly in low- and middle-income countries. Severe Salmonella infections frequently require antimicrobial therapy to facilitate the elimination of the infection. The threat to human health posed by antimicrobial-resistant pathogens is of growing concern to medical practice. The purpose of this study was to determine trends of salmonellosis as well as its antimicrobial resistance to provide the basis on which preventive and therapeutic guidelines for clinicians in the Buea Health district of Cameroon can be established. It was a retrospective descriptive analysis of stool cultures and their antimicrobial susceptibility test results from laboratory records of two health facilities from January 2012 to December 2017. Data was analysed with SPSS version 20 (IBM, Chicago, IL, USA). Out of the 385 participants, 105 were diagnosed with salmonellosis giving an overall prevalence of 27.3% (95% CI: 22.9 - 32.0). No significant decrease in the prevalence of salmonella infections was observed over the years (correction -0.627; p, 0.182). Salmonella isolates showed resistance to 14 of the 17 antimicrobial agents studied. All the Salmonella sp isolated were susceptible to gentamicin and at least 75% were susceptible to cloxacillin, ciprofloxacin, ofloxacin, and doxycycline. On the other hand, 100% resistance was seen in vancomycin and amoxicillin and at least 75% resistance to ceftazidime, minocycline, and azithromycin. 45.7% of the Salmonella isolates were resistant to multiple drugs. There was a decreasing trend of resistance of the Salmonella isolates to cefotaxime, pefloxacin, vancomycin, minocycline amoxicillin and azithromycin from 2012 to 2017. Nonetheless, only resistance to pefloxacin significantly reduced over the years (correlation = -0.918, p = 0.010) while resistance to doxycycline significantly increased over time (correlation = 0.813, P = 0.049).The findings of this study have further accentuated the growing concern about salmonellosis and its associated AMR resistance thereby underscoring the need for the rational application of antimicrobial and other necessary interventions that will help to control the menace of antimicrobial resistance. Accurate laboratory diagnosis, establishing policies on antimicrobial usages, as well as surveillance programs to monitor antimicrobial resistance are recommended.

Keywords: Salmonellosis; trend; antimicrobial resistance; Cameroon

INTRODUCTION

Over the past years, typhoid has remained a major global cause of morbidity and mortality, particularly in low- and middle-income countries is most common in areas that are overcrowded and have poor sanitation [1]. According to the World Health Organization (WHO) report of 2014, the global annual incidence of typhoid was approximately 21 million cases with approximately 222,000 annual typhoid-related deaths [2] Salmonella infections are usually attributed to consumption of contaminated food of animal origin such as eggs, chicken, pork, etc. Severe Salmonella infections usually require treatment with antimicrobials to aid in the elimination of the infection. A potential problem that has been developing for many decades is the development of antimicrobial resistance [3].

There have been increasing reports of antimicrobial resistance (AMR) in Salmonella species to a range of clinically important antimicrobial classes, particularly

fluoroquinolones and extended-spectrum cephalosporins [4]. Since 1996, the National Antimicrobial Resistance Monitoring System (NARMS) has identified increasing numbers of Salmonella isolates resistant to nine of the 17 antimicrobial agents tested: amoxicillin/clavulanate, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. These isolates also have decreased susceptibility to ceftriaxone, an antimicrobial used to treat serious infections in children [5].

Recent reports shows increasing levels of antimicrobial resistance in Cameroon [6-9]. This has been attributed to the inadequate means to properly carry susceptibility testing in most health facilities, circulation of fake drugs, and irrational use of un-prescribed drugs [9].The required standard for the diagnosis of Typhoid is not readily available in areas where the disease is endemic. Consequently, the diagnosis of Typhoid fever in Cameroon is predominantly done using the Widal test and typhoid rapid test kits. These have limitations because of their low sensitivity and/or specificity. As a result, there have been several cases of misdiagnosis leading to unnecessary exposure of patients to antimicrobials which contribute to increase the rates of antimicrobial resistance [10]. Despite some research that has been carried out in this field, there is still limited data on the trends of salmonellosis as well as its antimicrobial resistance in Cameroon. This study will therefore, provide a basis on which preventive and therapeutic guidelines for clinicians in the Buea Health district of Cameroon can be established.

Materials and Methods

Study Area

The study was conducted in the Buea Health District, which constitute one of the four health districts that make up the Fako Division in the South-West Province, Cameroon. The district has 21 health facility and 66 communities distributed across seven health areas, namely: Molyko, Muea, Buea Town, Bova, Bokwaongo, Tole and Buea Road health areas. The district is located on the eastern slope of Mt Cameroon with a total population of 133,092 [8]. Symptomatic and asymptomatic Salmonella infections has been reported in this area [11,13]. The role of food-animals serving as important reservoirs for Salmonella Typhimurium has been reported in cattle and pigs in Buea [12]. The irregular water supply in the Health District causes the inhabitants to consider alternative sources of water with uncertain quality and/or compromised hygienic practices thus, facilitating salmonella transmissions.

Study Design

This was a retrospective descriptive study that was conducted in two health facilities (St Albert Medical Centre, and Solidarity Clinic) in the Buea Health District, between January and February 2018. Individuals who did stool cultures and antimicrobial susceptibility testing in the laboratories of these health facilities from January 2012 to December 2017 were enrolled in the study. Data was retrieved from laboratory records using a structured checklist drawn from the laboratory registers.

Ethical Considerations

The Ethical clearance for this study was obtained from the Cameroon Baptist Convention Health Board Institutional review board (CBCHB IRB) (Re: IRB2017-23). The South West Regional Delegation of Public provided Administrative authorization while Permission to collect the data from the laboratory registers was obtained from the administration of the respective health facilities..

Data Collection

The data quality of laboratories doing stool culture and sensitive testing was assessed for availability, consistency and completeness as proposed by the DAMA UK Working Group on 'Data Quality Dimensions' [20] The the minimum score for any facility data to be acceptable for the study was set as 60% [8]. The laboratory capacity to perform culture and sensitivity testing was assessed by a modified WHO Antimicrobial Resistance Surveillance Questionnaire [19]. Information on culture test results (isolates and sensitivity results) and demographics such age and sex were collected with the use of a checklist. Of the four laboratories assessed two met both the minimum score for data quality and acceptable laboratory capacity for performing culture and sensitivity testing (had qualified personnel, required equipment and standard testing procedures)

Sample Collection and Processing

Between from January 2012 to December 2017, the two health facilities had collected stool samples in a

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sterile stool container with a spoon attached to the lid. Direct Saline/ Iodine Wet Mount were prepared and observed microscopically for diagnosis of intestinal parasites. To isolate Salmonella Species from the sample, about 1g of the stool was incubated in Selenite F broth at 37∘C for 24 hrs. In addition to that, a loop filled with stool sample was streaked on Salmonella Shigella agar (SSA) and incubated at 37∘C for 24 hrs. After 24hours, culture negative specimens on SSA were sub-cultured from Selenite F broth (an enrichment broth) to SSA plates to increase the recovery of the isolates. Salmonella growth on SSA produces colourless colonies with shiny black centres due to the production of hydrogen sulphite (Cheesbrough, 2010). All isolates were purified by sub-culturing on nutrient agar.

Pure single colony from SSA was spread on the centre of clean slide with the help of a wire loop and Gram stained. .The stained smear was observed under the microscope using the oil immersion objective. Morphology, appearance and Gram reaction of the isolates were determined.

Isolate with typical characteristics of Salmonella on the primary culture media were then sub cultured on Kliger Iron Agar (KIA) slant for further confirmation. A wire loop was used to pick up a colony, stab on the slant, streaked and incubated at 37oC for 24 hours On KIA. Isolates of salmonella were confirmed on KIA as described by Cheesbrough, 2010 were tested for antimicrobial Susceptibility.

Antimicrobial Susceptibility Test

The health facilities determined the susceptibility of each isolate to antimicrobial agents by the disc diffusion technique on Mueller Hinton agar methods [22]. Discs impregnated with the following antimicrobial agents were used: ceftazidime, cefuroxime, ceftriaxone, cefotaxime, coprofloxacin, ofloxacin, ciprofloxacin, perfloxacin, vancomcin, monocycline,Trimethoprim amoxicillin, chloramphenicol, azithromycin and doxycycline. The diameter of the zone inhibition was measured to the nearest millimetre and compared to the critical values of each antimicrobial disc. Based on the zone of inhibition the results were reported as sensitive, resistant or intermediate/moderately sensitive. Salmonella Typhi (ATCC 6539) was used as reference strain to ensure quality of results obtained. These data were entered into the laboratory registers from where they were retrieved for our retrospective descriptive study.

Data Analysis

Data was entered and analysed with SPSS version 20 (IBM, Chicago, IL, USA). Antimicrobial activity (resistance, moderate sensitivity and sensitive) was expressed as percentages. Differences in proportions were compared using the chi-square test. Pearson's correlation was used to study the trends of antimicrobial resistance over time at a statistical significance of level of $p < 0.05$.

RESULTS

Characteristics of the study population

A total of 385 participants were enrolled in the study aged 3 to 75years, with a mean age of 36years (SD 14years). With respect to age group, the age group ≤ 10 years was least represented 44(11.4%) while those aged > 40years were the most represented 148(38.4%). Of the 385 participants, 222 (57.7%) were male. Stool culture and wet mount (microscopy) revealed that 105(27.3%) [95% CI 22.9 – 32.0)] of the participants were infected with salmonella, 3(0.8%), [95% CI (0.2 – 2.3)] infected with Giardia lamblia while 44(11.4%) [95% CI (8.4 – 15.0)] were infected with Entamoeba Histolytica (Table 1)

Trends and Prevalence of salmonella infection in the study population

Out of the 385 patients enrolled, 105 were diagnosed of salmonellosis giving an overall prevalence 27.3(95% CI 22.9 - 32.0). None of the patients were co-infected with Giardia lamblia whereas, 13(29.5%) of those infected with Entamoeba histolytica were co-infected with salmonella (p, 0.001). No significant difference was found between the prevalence of salmonellosis and gender as well as with age group (p, 0.736 and p, 0.695 respectively). The highest prevalence of salmonellosis was found in 2014 (21.0) and the lowest in 2017 (6.7). This difference was statistically significant (p, 0.016) (see table 2). In a binary correction analysis, between the time in which the test was done and the prevalence of salmonella infection showed a reducing trend of the infection over time (correction -0.627). However, this observed trend was not statistically significant (p, 0.182)

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Prevalence of Salmonella resistance, intermediate resistance and susceptibility to the various antimicrobial agent used in the study

Salmonella isolates showed resistance to 14 of the 17 antimicrobial agents studied. All salmonella isolated were 100% susceptible to gentamicin while none was resistant to cloxacillin and for ceftriazone,intermediate activities were observed. At least a 60% susceptibility to ciprofloxacin, ofloxacin, and doxycycline was also observed. The salmonella isolates also showed at least 75% intermediate/moderate susceptibility to ceftrtriaxone, cefotaxime and trimethoprim. The isolates were found to be 100% resistant to vancomycin and amoxicillin while it was at least 75% resistant to ceftazidime, minocycline and azithromycin (Fig 1). All the 105 isolated salmonella isolates were resistant to at least one of the drug used while 48(45.7%) [95% CI (36.0 – 55.7)] were resistant to multiple drugs (Fig. 2)

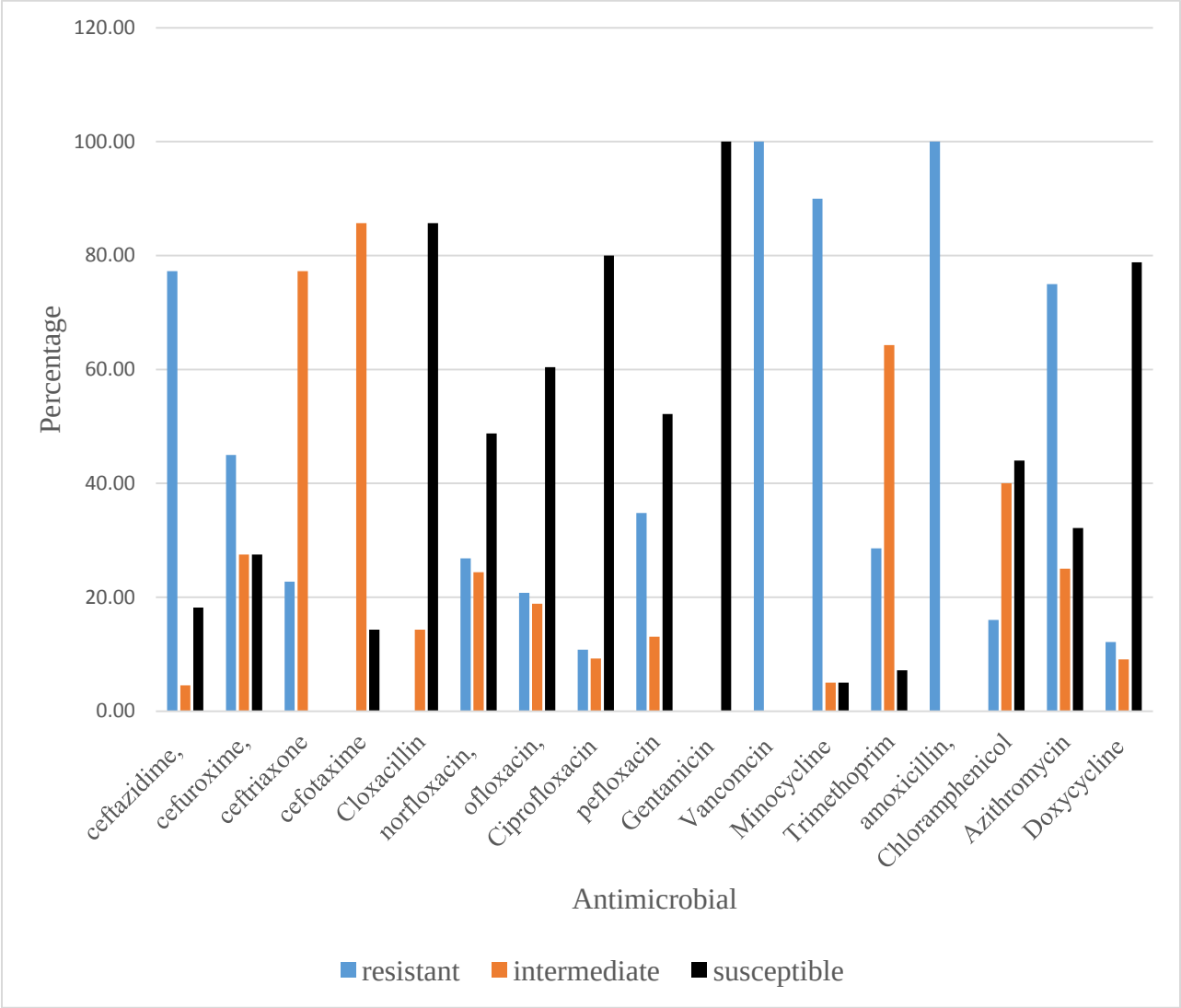

Figure 1: Prevalence of *salmonella* resistance, intermediate resistance and susceptibility to the various antimicrobials tested

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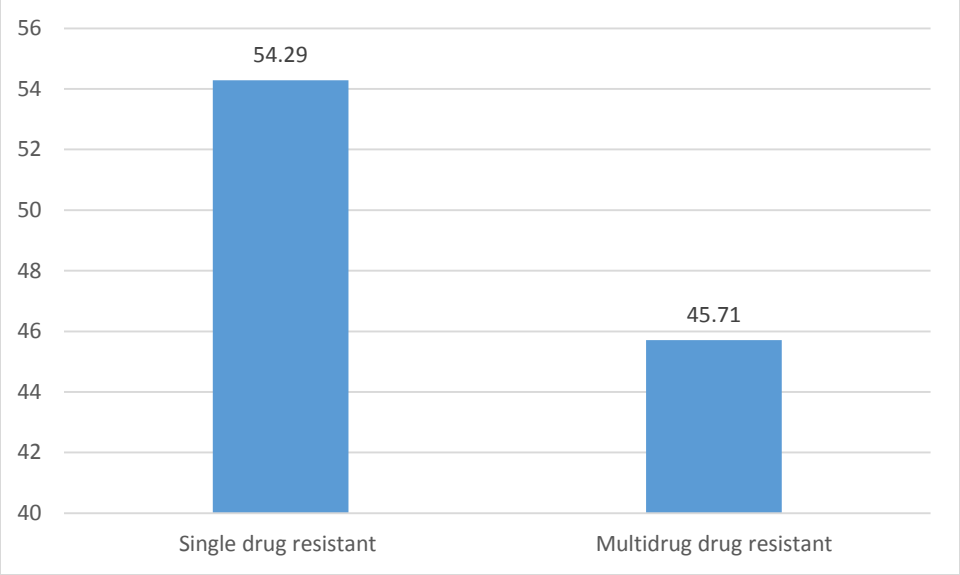

Figure 2: Proportion of Salmonella isolates resistant to multiple antimicrobials within the study population

The trend of Salmonella isolate resistance to antimicrobial over 6years

The resistance of salmonella isolates to antimicrobials showed a decreasing trend over the years (from 2012 to 2017) for cefotaxime, perfloxacin, vancomycin, minocycline, amoxicillin and azithromycin. Nonetheless, only resistance to perfloxacin was significantly reduced over the years (correlation = -0.918, p = 0.010). On the other hand resistance to ceftriaxone, cefuroxime, ceftazidime, norfloxacin, chloramphenicol, trimethoprim and doxycycline increased over time but only resistance to doxycycline significantly increased (correlation = 0.813, p = 0.049) (see table 3).

Discussion

Identifying the trends of both Salmonella infection and AMR of Salmonella isolates provides vital surveillance data to the scientific and medical communities to guide research, judicious antimicrobial use and selection of effective treatment plans [13]. The prevalence of salmonellosis was found to be 27.3% in this study. This was relatively low when compared to a prevalence of 39.7% reported in 2001 within the same Buea Health District [14]. The increased availability of pipe borne water which is much more controlled and treated is a possible reason for the reduced salmonella prevalence in humans within this Health District. However, the prevalence of 27.3% remains relatively high when compared to findings from two similar studies in Nigeria (17.2% and 17.9% respectively) [15,16]. This was further illustrated by the fact the reducing trend observed in salmonella infections over time in this study was not statistical significant even though the study was more focused on suspected cases of salmonellosis sent to the laboratory and not asymptomatic cases as previously reported.

In this study, the isolates were 100% susceptible to gentamycin and at least a 75% susceptibility level to ciprofloxacin, cloxacillin, ofloxacillin and doxycycline. Thesefindings agree with previous findings from this study area where average susceptibility rate of quinolones was 76.86% [11]. This indicates that despite the increasing AMR in quinolones as reported by other studies [17], quinolones still remained a preferable first line treatment for salmonellosis in this region.

They was also intermediate/moderate susceptibility of at least 75% noted in Ceftriaxone, cefotaxime and trimethoprim which are contrary to findings by Padma et al [18] in a similar study which indicated an increased susceptibility of 96% and 94% with cefotaxime and ceftriaxone respectively. This can be partially explained by the fact that, cephalosporins (particularly ceftriaxone) are the most commonly prescribed empiric antibiotics for comorbid malaria with bacterial infections, urinary tract infections, sepsis and gastroenteritis in this region [19], Besides, when it is not prescribed for the right purpose at the required dose, it may lead to resistance. Other

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antimicrobials to which the isolates were highly resistant were; vancomycin (100%) and amoxicillin (100%), ceftazidim, minocycline and azithromycin (at least 75% respectively). This observation was similar to findings reported by similar studies [20,21].

Multidrug resistance was one of the significant findings, with 45% of the isolates being resistant to more than one drug. This was synonymous to what has been reported elsewhere [22,23], indicating a progressive increase in multidrug resistance by the salmonella species. This calls for implementation of national surveillance systems of antimicrobial resistance and implementation of prudent antimicrobial usage, including revision of current national guidelines for antimicrobial usage due to the risk of resistance.

It has been reported that over the past years, there has been a progressive increase of AMR in Salmonella species to a range of clinically important antimicrobial [4]. The study indicates that Salmonella isolates resistance to antimicrobial agents such as ceftriaxone, cefuroxime, ceftazidime, norfloxacin, chloramphenicol, trimethoprim and doxycycline increases over time. These drugs are used on regular basis to treat a wide range of infections. Their constant usage is a possible reason for the increasing resistance over the years. A similar trend has been reported in Ethiopia [24] and in Cameroon [8]. Whereas, the salmonella isolate's resistance to cefotaxime, pefloxacin, vancomcin, minocycline amoxicillin and azithromycin indicated a decreasing trend over the years. Most of these do not seem to be the first choice of drugs for most prescribers. Thus, their usage is somehow minimised therefore limiting the chances of abusing them.

One potential limitation of this study was its retrospective nature, as it wasn't possible to appreciate the severity of all these infections clinically with signs and symptoms. However, considering that it was a hospital based study that examined culture and sensitivity test results of suspected clinical samples, it is assumed that the clinicians judged that the signs and symptoms of the participant were good enough to suspect salmonellosis. Further molecular characterization of the isolates with emphasis on resistant genes and mechanism of transmission of resistant gene could be an avenue for further research in identifying the mechanisms of antibiotic resistance.

Conclusion

This study identified the prevalence of salmonellosis to be 27.3 % which was associated with AMR in some commonly prescribe antimicrobials. The findings of this study has further accentuated the growing concern about salmonellosis and its associated AMR by underscoring the need for rational application of antimicrobial and other necessary interventions that will help control the menace of antimicrobial resistance. Accurate laboratory diagnosis, provision of potable water, public education etc are, therefore, recommended. Also, surveillance programs to monitor antimicrobial resistance trends and patterns in other parts of the country are also recommended.

Abbrevations

AMR: Antimicrobial Resistance

CBCHB IRB: Cameroon Baptist Convention Health Board Institutional review board

KIA: Kliger Iron Agar

MDR: Multi-Drug Resistance

SSA: *Salmonella Shigella* Agar

WHO: World Health Organization

Declarations

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Availability of data and materials

All the data collected that supports the results of this study has e been presented in the manuscript

Authors' contributions

NC and ETA designed the study, supervised data and manuscript write up. NC did the data analysis. AF, JAF were responsible for data collection and also

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review of final manuscript. PAJ was the main supervisor for the study. He review and corrected the study proposal and the final manuscript write up.

Ethics approval and consent to participate

The Ethical approval for this study was obtained from the Cameroon Baptist Convention Health Board Institutional review board (CBCHB IRB) (Re: IRB2017-23). The South West Regional Delegation of Public provided Administrative authorization while Permission to collect the data from the laboratory registers was obtained from the administration of the health facilities concerned.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Table 1: Characteristics of the study population

Variable	Category	Number enrolled (%) N =385
Gender	Male	222(57.7)
	Female	163 (42.3)
Parasitic infections	*Giardia lamblia*	3(0.8)
	Entamoeba histolytica	44(11.4)
Bacterial infections	*Salmonella*	105 (27.3)
Age group (years)	≤10	17(4.4)
	11-20	32(8.3)
	21-30	98(25.5)
	31-40	90(23.4)
	>40	148(38.4)

Table 2: Prevalence of salmonella infection with respect to Gender, age group, parasitic infection, and year

Variable	Category	Number enrolled	Positive for salmonella (%)	X2 P value
Gender	Male	222	62(27.9)	0.736
	Female	163	43(26.4)	
Co-infection Parasitic infections	**Giardia lamblia**	3	0(0)	**0.001**
	Entamoeba **histolytica**	44	13(29.5)	
Age group (years)	≤10	17	5(29.4)	0.695
	11-20	32	9(28.1)	
	21-30	98	24(24.5)	
	31-40	90	21(23.3)	

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	>40	148	46(31.1)	
Year	2012	92	18 (17.1)	**0.016**
	2013	86	20 (19.0)	
	2014	53	22 (21,0)	
	2015	81	20 (19.0)	
	2016	43	18 (17.1)	
	2017	29	7 (6.7)	
Total		385	105(27.3)	

Table 3: The trend of Salmonella isolate resistance antimicrobial drugs from 2012 to 2017

Drug	Correction value	P value
Ceftazidime,	0.478	0.337
Cefuroxime,	0.469	0.348
Ceftriaxone	0.445	0.377
Cefotaxime	-0.393	0.441
Norfloxacin,	0.344	0.504
Ofloxacin,	-0.283	0.587
Ciprofloxacin	0.226	0.666
Pefloxacin	-0.918	**0.010**
Vancomcin	-0.634	0.447
Minocycline	-0.120	0.724
Trimethoprim	0.245	0.640
Amoxicillin,	-0.386	0.449
Chloramphenicol	0.274	0.600
Azithromycin	-0.691	0.129
Doxycycline	0.813	0**.049**

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