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RESEARCH ARTICLE

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Prevalence and Antibiotic Susceptibility of *Salmonella* Species isolated from Patients attending Selected Hospitals in Zaria

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ABSTRACT

Salmonellosis is a major public health problem in developing countries and is associated with high morbidities and mortalities. The study aimed at isolating, characterizing and determining the antibiotic susceptibility of Salmonella species from stool samples of patients attending three hospitals in Zaria. A total of 219 stool samples were collected from suspected enteric fever patients. The samples were cultured and isolates characterized and antibiotic susceptibility pattern determined. Out of the 219 samples, 14 yielded positive for Salmonella accounting for a prevalence of 6.4%. With respect to gender and age, male and patients belonging to age group 0-9 were found to have the highest Salmonella infection with 64.3% and 43% respectively. The antibiotic susceptibility testing showed good susceptibility of Salmonella isolates to cefotaxime, ciprofloxacin, gentamicin and chloramphenicol, however the isolates demonstrated poor susceptibility to tetracycline, amoxicillin and cotrimoxazole. In addition, 64.3% of the Salmonella isolates were multidrug resistant. Antibiotic susceptibility test should be conducted for Salmonella isolates periodically to monitor development of resistance.

Key words: Prevalence, *Salmonella*, characterization, antibiotic, susceptibility.

INTRODUCTION

Salmonella remains one of the major pathogenic bacteria of humans as well as animals [1]. Its impact as an important public health problem is felt worldwide particularly in developing countries [2]. Salmonellosis is a disease caused by *Salmonella* species and species of *Salmonella* have been implicated as aetiologic agents of gastroenteritis, septicaemia and enteric fever. Symptoms range from mild self limiting diarrhoea, abdominal pain, headache, fever, chills, malaise, body aches, nausea and vomiting and sometimes reactive arthritis lasting from 1 to 7 days [3][4] to severe diarrhoea that requires hospitalization [5].

Most *Salmonella* infections are due to ingestion of contaminated foods such as milk, dairy products, poultry and poultry products, pork, beef, sea food, fresh fruits, juice and vegetables [6][7]. Humans get infected by ingesting these contaminated foods and water or coming in contact with faeces and or urine of infected persons and animals. The groups of people at higher risk for infection are children, infants, pregnant women, elderly people and immunocompromised persons. Stool serve as an important reservoir of *Salmonella* serovars and humans shed *Salmonella* mainly through the stool [8].

One of the most important steps towards controlling bacterial infections in the 21st century is the development and use of antibiotics [8]. However, the subsequent emergence and spread of antibiotic resistance in pathogenic

organisms have made many currently available antibiotics ineffective [9]. High rates of resistance of *Salmonella* serovars to conventional antibiotics such as ampicillin, chloramphenicol, tetracycline, cotrimoxazole (trimethoprim-sulfamethoxazole) and other newer antibiotics (quinolones and extended-spectrum cephalosporins) have been reported with increasing frequency in many areas of the world [10]. Infections caused by these resistant *Salmonella* and other resistant pathogens have resulted in significant morbidity and mortality and escalating health care cost worldwide [11][12]. The dynamics of the pathogen as well as the trend in antibiotic resistance pattern vary greatly from country to country and need to be subjected to regular study to ascertain the effectiveness of drug administration and response, which forms the basis for this study. Moreover, data obtained from the study will also provide knowledge on the burden of *Salmonella* infections in Zaria in addition to providing information on antibiotics most effective on *Salmonella*.

MATERIALS AND METHODS

Study Area and Population: The study was conducted in Zaria, a cosmopolitan suburb in Kaduna State and ancient city home to the Zaria Emirate. The residents are mainly Hausas with a great number of migrants from most part of the country owing to the various institutions located in the city. The combination of the indigent population, prominently an agrarian and animal rearing community as well as a mix with the people from other parts of the country that came to settle provides a blend that impacts of the infrastructure as well as lifestyle and sanitary conditions as evident in most communities. These scenarios often give rise to congestion and attendant ease of spread of pathogens such as *Salmonella* species.

Study Design: The study was a hospital based cross-sectional study in which three health care facilities were listed. Patients' selection was based on presentation of symptoms of enteritis and enteric fever, patients diagnosed with typhoid fever by the widal reaction, diarrhoea and gastroenteritis. The health facilities include: Hajiya Gambo Sawaba General Hospital, Major Ibrahim Babangida Abdullahi General Hospital and Saint Luke's Anglican Hospital. Ethical approval was obtained from the individual hospitals as well as informed consent from the participants before commencement of the studies.

Sample Size:
The sample size of the study was determined using a previous prevalence rate of 14.3% in Zaria [13].

$$N = \frac{Z^2 \, X \, P(1-P)}{d^2}$$

Where N = Number of samples to be collected
Z = Confidence level at 95% (standard value of 1.96)
d = Margin of error at 5% (standard value of 0.05)
P =Prevalence rate

Therefore; $$N = \frac{(1.96)^2 \, X \, 0.143 \;\; (1-0.143)}{0.05 \times 0.05}$$

$$= \frac{3.8416 \, X 0.143 \, (0.857 \;)}{0.0025}$$

$$= 188.3$$

From the above computation, number of samples to be collected is 188; however a total of 219 stool samples were collected for the study from patients in the selected hospitals.

Sample Collection and Analysis
A total of 219 stool samples were collected from patients of all age groups attending the three selected hospitals in Zaria into sterile stool sample containers and transported to the Laboratory in well insulated cold box. Each of the samples were first inoculated in selenite F broth at 37^{o}C for 6-8h, followed by subculturing on Bismuth Sulphite Agar for 24h at 37^{o}C. Presumptive *Salmonella* colonies were characterized using various biochemical tests which include: carbohydrate fermentation test (in triple sugar iron agar), citrate utilization test, indole, urease and motility

test. The isolates were confirmed using a commercial biochemical Characterization kit (Microgen GN-ID system). Antibiotic susceptibility was performed by Kirby-Bauer disk diffusion technique on Mueller Hinton agar as recommended by Clinical and Laboratory Standards Institute [14]. The antibiotics used were amoxicillin (10µg), cefotaxime (30µg), ciprofloxacin (5µg), chloramphenicol (30µg), cotrimoxazole (25µg), gentamicin (30µg) and tetracycline (30µg). The disc diffusion susceptibility tests were interpreted as resistant, intermediate susceptible and susceptible according to the guidelines provided by the Clinical and Laboratory Standards Institute. *Escherichia coli* ATCC 25922 was used as the control organism.

RESULTS

Out of the 219 samples, 14 *Salmonella* species were identified accounting for a prevalence of 6.4%. A prevalence of 43%, 36% and 21% were obtained for Hajiya Gambo Sawaba General Hospital, Major Ibrahim Babangida Abdullahi General Hospital and Saint Luke's Anglican Hospital respectively. In addition, with respect to gender and age group, male and patients belonging to the age group 0-9 were found to have the highest *Salmonella* infections with 64.3% and 43% respectively. All the *Salmonella* were sensitive to cefotaxime, ciprofloxacin and gentamicin except eight intermediate level isolates. Susceptibility of 78.6%, 71.4% and 64.3% was observed for ciprofloxacin and chloramphenicol, gentamicin and cefotaxime respectively. *Salmonella* isolates were 78.6%, 64.3% and 50% resistant to tetracycline, amoxicillin and cotrimoxazole respectively. A total of 64.3% of the *Salmonella* isolates were multidrug resistant.

Table 1 Prevalence of *Salmonella* species with respect to the study hospitals

Hospitals	Number examined	Positive cases	Percentage	P-value
HGSGH	73	6	43%	0.000*
MIBAGH	73	5	36%	
SLAH	73	3	21%	
Total	**219**	**14**	**100%**	

*Key: *Statistically significant; HGSGH - Hajiya Gambo Sawaba General Hospital; MIBAGH - Major Ibrahim Babangida Abdullahi General Hospital; SLAH - Saint Luke's Anglican Hospital*

Table 2 Prevalence of *Salmonella* species with respect to gender of patients

Gender	Number examined	Positive cases	Percentage	p-value
Male	120	9	64.3%	0.156*
Female	99	5	35.7%	
Total	**219**	**14**	**100**	

*Key: *Statistically not significant*

Table 3 Prevalence of *Salmonella* species with respect to age group

Age group	Number examined	Number of positive	Percentage	P-value
0-9	83	6	43%	0.000*
10-19	36	2	14	
20-29	25	1	7%	
30-39	17	1	7%	
40-49	21	1	7%	
50-59	23	1	7%	
60 & above	14	2	14%	
Total	**219**	**14**	**100**	

*Key: *Statistically significant*

Table 4 Antibiotic Susceptibility Pattern of *Salmonella* Isolates

Antibiotics (µg)	Percentage		
	Resistance	Intermediate susceptible	Susceptible
Amoxicillin (10)	64.3	7.1	28.6
Tetracycline (30)	78.6	0	21.4
Gentamicin (30)	0	28.6	71.4
Cotrimoxazole (25)	50	0	50
Cefotaxime (30)	0	35.7	64.3
Ciprofloxacin (5)	0	21.4	78.6
Chloramphenicol (30)	14.3	7.1	78.6

Table 5: Multidrug Resistance Patterns of *Salmonella* Isolates

No. of antibiotics	Resistance Pattern	No. of Isolates	Percentage
Two	AML, SXT	1	7.1
	AML, TE	2	14.3
Three	AML, TE, SXT	1	7.1
	AML, TE, SXT	3	21.4
Four	AML, TE, SXT, C	2	14.3
Total		9	64.3%

Key: AML-Amoxicillin; TE-Tetracycline; SXT-Cotrimoxazole; C-Chloramphenicol

DISCUSSION

From the study conducted, a prevalence of 6.4% was obtained for *Salmonella* species. However, our prevalence is lower when compared to the studies conducted in Kano (13.7%) [4], Addis Ababa (13.6%) [1] and Kenya (29%) [15]. However, our prevalence is higher than that obtained in Abuja (2.3%) [16]. The differences in the prevalence between this and earlier studies could be explained when factors such as differences in study time, hygienic, environmental and geographical variation are considered.

The patients in the age group 0-9 were found to have the highest *Salmonella* infection. This agrees with earlier findings of Abdullahi *et al*. [17] and Mengistu *et al*. [18] who reported higher infection rate in children. However our result is contrary to the findings of Ohalete *et al*., [19] who reported higher infection rate in middle aged persons. This could be due to the under developed immune system of children which makes them more prone to *Salmonella* infection as few cells are required to initiate infection. The low infective dose of bacilli needed to initiate infection makes exposed children easily infected [20].

Male were observed to have a higher infection rate than female. This conform to the findings of Ohalete *et al*., [19] and Ifeanyi *et al*. [16] who reported higher infection rate in male than in female. However, higher infection rate in female than in male have also been documented [18]. The differences in the isolation rate of *Salmonella* between the two genders could be due to differences in level of hygiene, awareness, occupation and behavioural factors.

All the *Salmonella* isolates were sensitive to Cefotaxime, Ciprofloxacin and Gentamicin except eight intermediate level isolates. However, contrary to our study, resistance of 2.5% was reported against Ciprofloxacin by Gordana *et al*. [21]. In addition, 94.73% and 55.6% resistance of *Salmonella* isolates against Cefotaxime have been observed by Sivakumar *et al*. [11] and Ifeanyi *et al*. [16] which contradicts our own findings. Moreover our observation is contrary to the results of Beyene *et al*. [22] who reported 74.3% resistance against Gentamicin.

Resistance of 64.3% was observed against Amoxicillin which is lower than 75% reported by Nesa *et al*. [8]. Resistance of 78.6% was recorded against Tetracycline which contradicts 33.3% and 6% reported by Addis *et al*. [1] and Wandili *et al*. [15] respectively. A lower level of resistance against Cotrimoxazole (31% and 37.5%) than that observed in the study (50%) has been reported by Abdullahi *et al*. [17] and Mengistu *et al*. [18] respectively. Resistance of 14.3% was observed against chloramphenicol which disagrees with the findings of Beyene *et al*. [22] who reported 81.4% resistance against Chloramphenicol. It was observed that 64.3% of *Salmonella* species were multidrug resistant in the study. This is contrary to the findings of Eman and Mohamed [23] and Addis *et al*. [1] who reported 100% and 83.3% multidrug resistance among *Salmonella* species respectively. In addition, the *Salmonella* isolates were resistant to 2-4 antibiotics which disagree with the findings of Eman and Mohamed [23] who reported multidrug resistance of *Salmonella* isolates to 4-5 antibiotics.

Due to the extensive use of antibiotics in both humans and animals, the normal flora of the intestine are disrupted resulting in the emergence of antibiotic resistant enteric pathogens including *Salmonella* and prolonged fecal shedding of these organisms into the environment [24]. Multidrug resistance is a cause for concern in both clinical and veterinary medicine because it limits the therapeutic options available for treatment.

The variation in the susceptibility and resistance level of *Salmonella* to different classes of antibiotics in this study and earlier studies could be due to differences in serovars from place to place. Antibiotic susceptibility patterns vary regionally and geographically and have been reported to change rapidly over time. The source of isolation and variability of strains among the same serovar may influence antibiotic susceptibility test. Although, antibiotics have been very effective in the treatment of in enteric bacterial infections and play a crucial role in reducing mortality, the

progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a cause for concern [18]. In such places, due to circumstances like poor or inadequate laboratory facilities, clinicians are made or forced to diagnose based on symptoms resulting to prescription of broad spectrum antibiotics which may lead to emergence of antibiotic resistant bacterial pathogens. Poor laboratory diagnosis in developing countries enforces physicians to symptomatic diagnosis and prescription of broad spectrum antibiotics that led to emergence of drug resistant bacterial pathogens [25].

CONCLUSION

A prevalence of 6.4% was obtained for *Salmonella* species. Male and children belonging to age group 0-9 were observed to have the highest *Salmonella* infection with 64.3%and 43% respectively. The *Salmonella* isolates were highly sensitive to cefotaxime, ciprofloxacin, gentamicin and chloramphenicol but resistant to amoxicillin, cotrimoxazole and tetracycline. A total of 64.3% of the *Salmonella* isolates were multidrug resistant. Public health awareness on personal, food and environmental hygiene should be developed to prevent or control *Salmonella* infections. In addition, antibiotic susceptibility test should be conducted regularly for *Salmonella* isolates to monitor development of resistance.

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