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(RESEARCH ARTICLE)

# Serotyping and antibiotic susceptibility pattern of *Salmonella* serovars from children diagnosed of typhoid fever in Lagos, Nigeria

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## Abstract

Typhoid fever is a major health problem in developing countries especially in Nigeria where this infectious disease is endemic, with more concern in children. This prospective study was conducted in 3 Primary Health Centres on children diagnosed clinically of typhoid fever between January, 2017 and December, 2019. The aim of this study was to determine the incidence of *Salmonella* serovars and antibiotic susceptibility pattern from faecal specimens of children diagnosed clinically based on symptoms of typhoid fever, while demographic information was recovered from their case notes and their mothers. The children were randomly selected having obtained informed consent from their mothers. Four hundred and twenty (420) stool specimens of children aged between 6 months and five years were cultured and serotyped using standard microbiological procedures for isolation and characterization of Salmonella and other enteric bacterial pathogens. The bacteria were isolated by using cultural methods on selective media using a series of biochemical tests and confirmed by using Microgen identification kits and API20E. Serological tests were carried out on suspected *Salmonella* and *Vibrio* isolates. Antibiotic susceptibility of the bacterial isolates was determined by using the Kirby-Bauer disc diffusion technique. Bacteriological analysis of the faecal specimens revealed a total of 381 bacterial isolates, among which were 180 (42.9%) Salmonellae. *Salmonella enteritidis* was the most frequently isolated serovar, 84 (22%), followed by *S. typhi*, 56 (14.7%), *S. paratyphi A,* 30(7.9%), and *S. typhimurium* 10 (2.6%). Other bacteria recovered from the children included *Shigella dysenteriae*, 64 (16.8), *Proteus mirabilis*, 60(15.8%), *Klebsiella oxytoca*, 15(3.9%), *Klebsiella ozaenae,* 12( 3.2%) and *Vibrio parahaemolyticus*, 10(2.6%) shows that the Salmonellae and other enteric bacteria showed sensitivity ranging from 70-96% for ampicillin, ciprofloxacin (83.3%-96.4%), gentamicin (56-97.5%), sparfloxacin (83.3-100%), ceftriaxone (83.3-96%) and cefuroxime (80-94%). They were however highly resistant to ampiclox, amoxicillin, and septrin which are over-the-counter drugs. The high incidence of multidrug resistance among Salmonellae and corresponding multidrug resistance among other enteric bacteria, thus posing problems of treatment failures are of great public health significance in a developing country like Nigeria.

**Keywords:** *Salmonella enteritidis*; *S. typhi*; *S paratyphi* A; Enteric bacterial pathogens; Salmonella serovars

## 1. Introduction

Enteric fever (typhoid fever) is a systemic infection caused by the human adapted bacterial pathogen, *Salmonella enterica* serotype typhi *(S. typhi),* which is a major cause of morbidity and mortality globally [1]. Typhoid fever is a life-threatening systemic febrile illness which still remains a global public health problem with an estimated 21.7 million

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illness and 217,000 deaths worldwide in year 2000 [2]. Paratyphoid fever has an estimated 5.4 million diseases outbreaks. The prevalence of typhoid fever is between 16-30 million cases per year especially in developing countries with a mortality rate of about 10% [3].

Human beings are the only natural host and reservoir of *S. typhi* and typhoid fever was linked to a chronic carrier of the aetiology, *S typhi*, Mary Malon who lived between1869-1938 [4]. Typhoid fever is endemic and resulted in epidemics in Nigeria where it has been associated with low socio-economic status and poor hygiene [5]. Typhoid fever is the most serious infectious disease that constitutes a threat to man globally, thus of global concern being a common and serious disease among both children and adults [3]. Like in most developing countries worldwide, typhoid fever has become a major public health challenge in Nigeria with an estimated annual incidence of 340 per 100,000 [6]. In Nigeria, enteric and paratyphoid fevers caused by *S. typhi* and *S. paratyphi* respectively are endemic and constitute a great socio-medical problem characterised by pyrexia of unknown origin, high morbidity and mortality [7]. Nigeria has been confronted perennially by the dual problems of diagnosis and treatment particularly among children.

In Nigeria, typhoid fever is often diagnosed on the clinical grounds or Widal serological test treatment is often done without certainty about the sensitivity of the causal agent(s), even when Widal test is bedevilled with the problem of low sensitivity and specificity with positive predictive value as low as 20% [8]. In addition to the above, the Widal serological test has the challenge of lack of baseline titre value which varies from place to place, perhaps because molecular diagnosis which provides definitive diagnosis of bacterial diseases is difficult to come by especially due to cost and lack of manpower [9].

*S. typhi* strains have been responsible for increasing resistance to a wide array of antimicrobial agents, especially the first line drugs [10, 11]. This has made reliable diagnosis and treatment difficult among children in developing country like Nigeria. The aim of this study was to determine the distribution of Salmonellae, and other enteric bacterial pathogens vis-à-vis their antimicrobial susceptibility pattern and assessment of clinical diagnosis of typhoid among children in the Lagos metropolis, Nigeria.

## 2. Material and methods

### 2.1. Study Population

Children between the ages of 6 months and five years clinically diagnosed as having typhoid fever in Lagos, Nigeria, were recruited for this study.

### 2.2. Sample Collection

The prospective study was conducted in three different Primary Health Centres in Lagos (Ejigbo, Isolo and Iyana Ejigbo) on 420 children attending the Health Centres that clinically diagnosed as having typhoid fever between January, 2017 and December, 2019. The clinical history and examination findings were recorded on standard forms before faecal specimens were collected.

A total of 420 stool samples were collected aseptically and screened in the present study. All the samples were collected using Cary Blair transport medium (Oxoid, UK) and were transported to the Microbiology laboratory, Lagos State University, Lagos, Nigeria or Molecular Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria, within three hours for further isolation and identification studies.

### 2.3. Isolation of *Salmonella*

Isolation of *Salmonella* was performed as recommended by FDA [12]. In brief 1 ml of the sample from the transport swab was inoculated into 9 ml of buffered peptone water (BPW) and selenite-cystein broth (SCB) (Oxoid, UK) and incubated at 37 ℃ for 18 h for pre-enrichment. Further, for selective enrichment, 0.1ml of the pre-enriched inoculum was transferred into 10ml of Rappaport-Vassiliadis broth (Oxoid, UK) and incubated at 42 ℃ for 24 h. After enrichment, a loopful (10µl) of the inoculum was then streaked on xylose lysine deoxycholate (XLD) agar (Oxoid, UK), MacConkey agar, and Salmonella-Shigella (SS) agar and were incubated at 37 ℃ for 24 h. The presumptive *Salmonella* colonies (4-5 colonies per plate) appearing slightly transparent red halo with a black centre surrounded by a pink-red zone on XLD agar, colonies appearing as low convex, pale green translucent 1-3mm in diameter on MacConkey agar, smooth and opaque or colourless with black centres on SS agar, were screened further for biochemical characterisation and Microgen GNA +B-ID test.

## 2.4. Identification of Salmonella

### *2.4.1. Biochemical characterization*

The presumptive colonies of Salmonella were further subjected to biochemical tests viz: triple sugar iron (TSI), ortho-nitrophenyl galactosidase (OnPG), urease broth, indole, methyl red, Voges-Proskauer and citrate test (IMViC) as per standard protocol described in Cheesbrough, 2006. For the characterization of other enteric bacteria. Any distinct colony was Gram-stained. Pure isolates were maintained on nutrient agar (NA) slants at 4 ℃ for further laboratory investigations. All media were prepared using instructions from the manufacturers. Discreet colonies showing greenish metallic sheen with black centres, yellow (sucrose fermenting) and blue green (non-sucrose fermenting) colonies were picked as presumptive *E. coli* 0157:H57, *Salmonella* spp. and *Vibrio* spp. respectively. The presumptive isolates were subjected to routine IMViC tests, oxidase test, string test among other tests. Isolates giving atypical responses for any of the above tests were examined further using MICROGEN GNA +B-ID test kit. The data obtained by the MICROGEN GNA + B-ID microwell strip was designed to generate a 4-digital code for Enterobacteriaceae and 9-digit octal code for Vibrionaceae which was used to interpret the result from the Microgen Identification System Software (MISS) [3]. MISS identified some other pathogenic Gram-negative bacterial species among which were *Acinetobacter baumannii, Citrobacter sakazakii, Citrobacter freundii, Enterobacter liquefaciens, Salmonella typhi, Salmonella arizonae, Salmonella pullorum, Hafnia alvei*, Non-0157:H7 *E. coli, Proteus mirabilis, Morganella morganii, Klebsiella oxytoca, Klebsiella ozaenae and Vibrio parahaemolyticus.*

## 2.5. Serological identification of Salmonellae

Tree (3) colonies of the overnight bacterial growth suspected to be Salmonella on NA at 37 ℃were suspended in 0.5ml physiological saline against suspension of standard O and H typhoid and paratyphoid antigens. A drop of antiserum and physiological saline (30µl) as a control was placed onto a clean glass slide partitioned into several parts. Development of high or four-fold rise in antibody titre for typhoid or paratyphoid was taken as indicative of *S. typhi* and *S. paratyphi* infections

### *2.5.1. Identification of E. coli serogroup 0157 using the M44 Microgen E. coli 0157*

A smooth suspension of the presumptive *E. coli* 0157:H7 grown on sorbitol MA agar at 37 ℃ for 24 h was prepared in two wells of an agglutination slide. The slide was rocked gently for 30 seconds and observed for agglutination. If there was no agglutination in either well, a drop of Microgen *E. coli* 0157:H7 test latex (M44a) was added to one well and one drop of the control latex (M44b) to the other. The slide was then rocked gently for 2 min. An obvious agglutination only in the well containing test latex indicated a positive result.

### *2.5.2. Serological Identification of Vibrio cholerae using Vibrio cholerae 01 and Vibrio cholerae 0139 Antisera*

For the detection of *Vibrio* species, specimens were plated on thiocitrate bile salt sucrose (TCBS) medium and enriched with BPW. Three (3) colonies of the overnight bacterial growth on NA at 37 ℃were suspended in 0.5ml physiological saline and the antigenic suspension. A drop of antiserum and physiological saline (30µl) as a control, were placed on a clean glass slide partitioned into several parts. The antigenic suspension was placed onto the serum and the physiological saline on the glass slide. The reagents were then mixed by tilting the glass slide back and forth for 1 min to see if there was agglutination. Only strong agglutination observed within 1 min in the reaction with each serum was regarded as positive. Delayed or weak agglutination was regarded as negative [12]

All the test organisms with negative polyvalent sera were re-tested by heating antigen suspensions as follows: Three colonies of the bacterial growth were suspended in 3 ml physiological saline and heated at 121 ℃for 15 min. The heated solution was then centrifuged at 900 rpm for 20 min. The supernatant was discarded and the precipitate was suspended with 0.5 ml of physiological saline and used as heated cell suspension. Only polyvalent sera that showed negative results with heated antigen suspension were identified as *Vibrio cholerae* 01, non-01, *Vibrio cholerae* 0139. The bacterial isolates were confirmed using API 20E.

## 2.6. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of all bacterial isolates was determined using the Kirby-Bauer disc agar diffusion technique [13]. Briefly, five pure colonies of each bacterial strain were inoculated into 2ml of sterile Mueller Hinton broth in Bijou bottle and incubated at 37$^{0}$c for 6 h. The turbidity was adjusted to match 0.5 McFarland turbidity. Sterile cotton tipped swab was rotated against the wall of the tube above the liquid level to remove excess inoculum. The inoculum was swabbed on the entire surface of a Mueller Hinton agar. The automatic disc dispenser was adjusted to dispense the antibiotic discs were applied on the surface of the agar and the plates incubated at 37 ℃ for 24 h. After

incubation, the organisms were classified as sensitive (S) and resistant (R) according to CLSI [14] guidelines, using a total of 12 antibiotics.

The antibiotic containing discs obtained from Oxoid, UK, consisted of the following: amoxicillin (AMC, 30µg), ampicillin (AMP, 10µg), Ampliclox (APC, 30µg), Augmentin (AUG, 30µg), Chloramphenicol (C, 10µg), Ciprofloxacin (CIP, 5µg), Erythromycin (ERY, 10µg), Gentamicin (GN,30 ), Perfloxacin (30µg), Sparfloxacin (SPA, 10µg), Septrin (SEP, 30µg), Streptomycin (SEP, 30µg), and Tarivid (TAR, 10µg).

### 2.7. Reference strains

The following standard strains were used as controls: *E. coli* ATCC35216 beta-lactamase producers and *K pneumoniae* ATCC 700603, Extended spectrum B- lactamase (ESBL). Others include *E. coli* ATCC strain 25922, *S. typhimirium* ATCC strain 14028, *S. enteritidis* ATCC strain 13076 and *Sh. dysenteriae* ATCC strain 29027 were obtained from NIMR, Yaba.

## 3. Results

The results obtained from faecal specimens of a total of 420 children indicated that 180 (42.9%) of these samples were positive for Salmonellae. *Salmonella enteritidis* was the most frequently isolated serovar, 84 (20%), followed by *Salmonella typhi*, 56 (13.3%), *Salmonella paratyphi* A, 30(7.1%) and *Salmonella typhimurium*, 10(5.6%). Other bacteria recovered from the children included *Proteus mirabilis* 60 (15.8%), *Morganella morganii*, 40 (10.5%) and *Shigella dysentenae,* 64 (16.8%) among others (Figure 1)

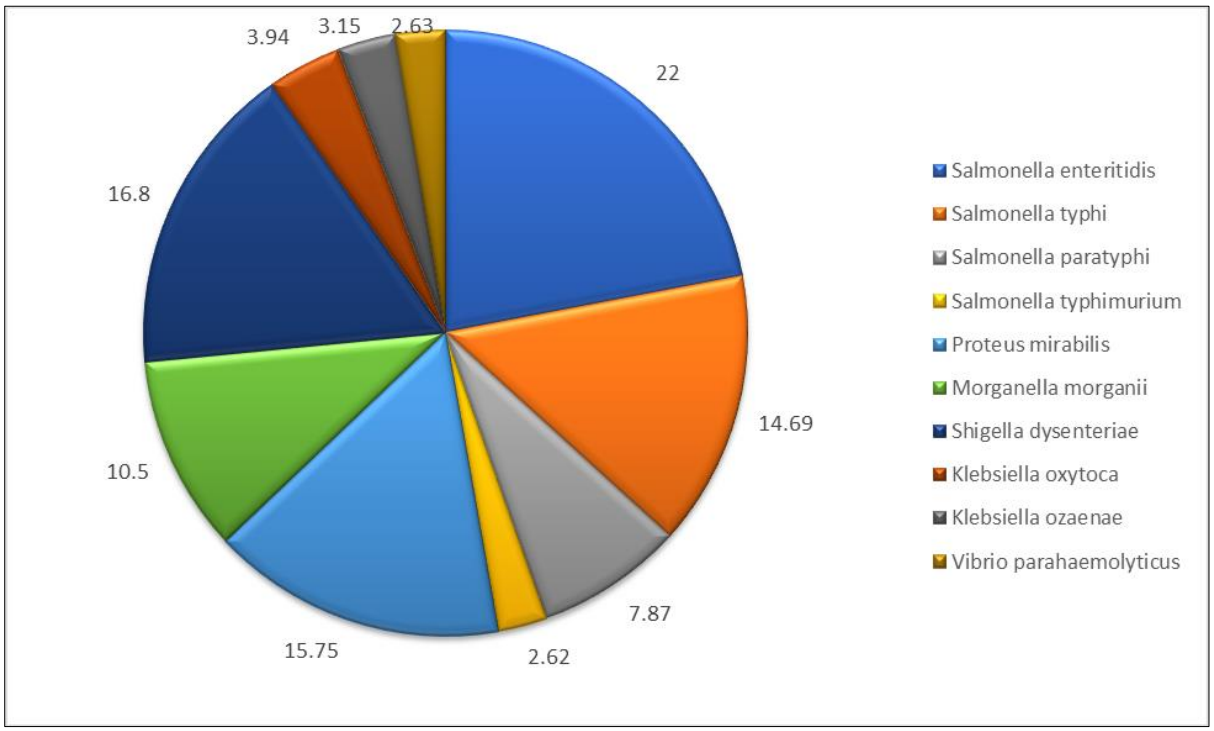


**Figure 1** Frequency distribution of bacteria among children (n=420)

Figure 2 shows that majority of the *Salmonella* isolates were recovered from children 0 to 18 months while a fewer *Salmonella* isolates were isolated from children within the 37 to 60 months age range ($p<0.005$).

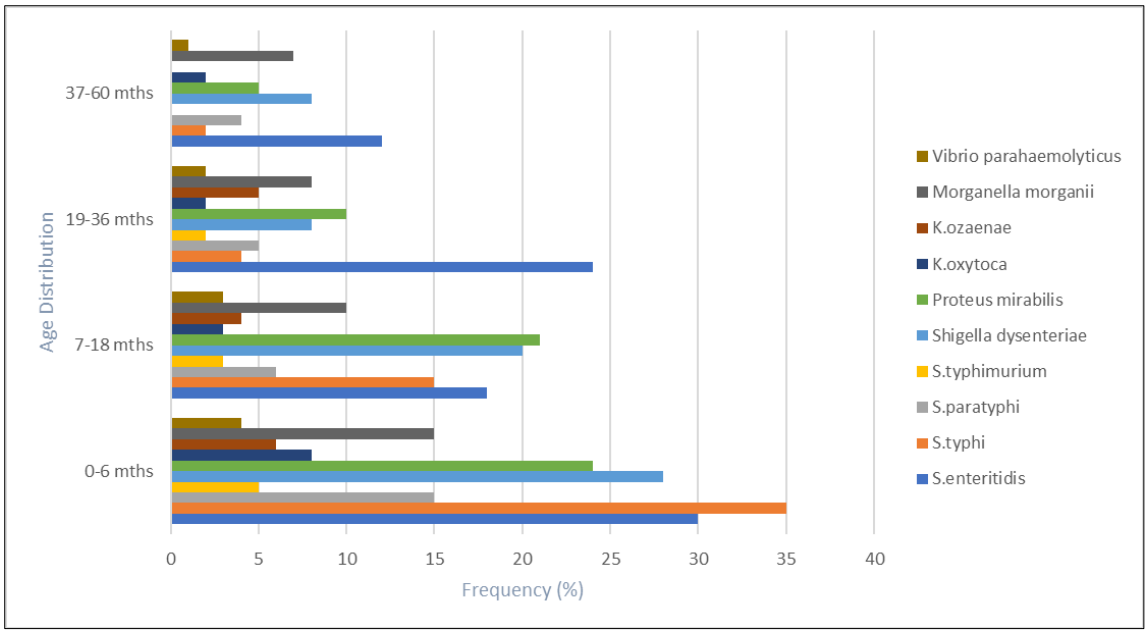


**Figure 2** Age distribution of bacteria pathogens from children

A study of the sex distribution of bacteria among the children showed that males had higher incidence of *Salmonella* serovars as well as other bacterial pathogens as shown in Figure 3 ($p<0.005$).

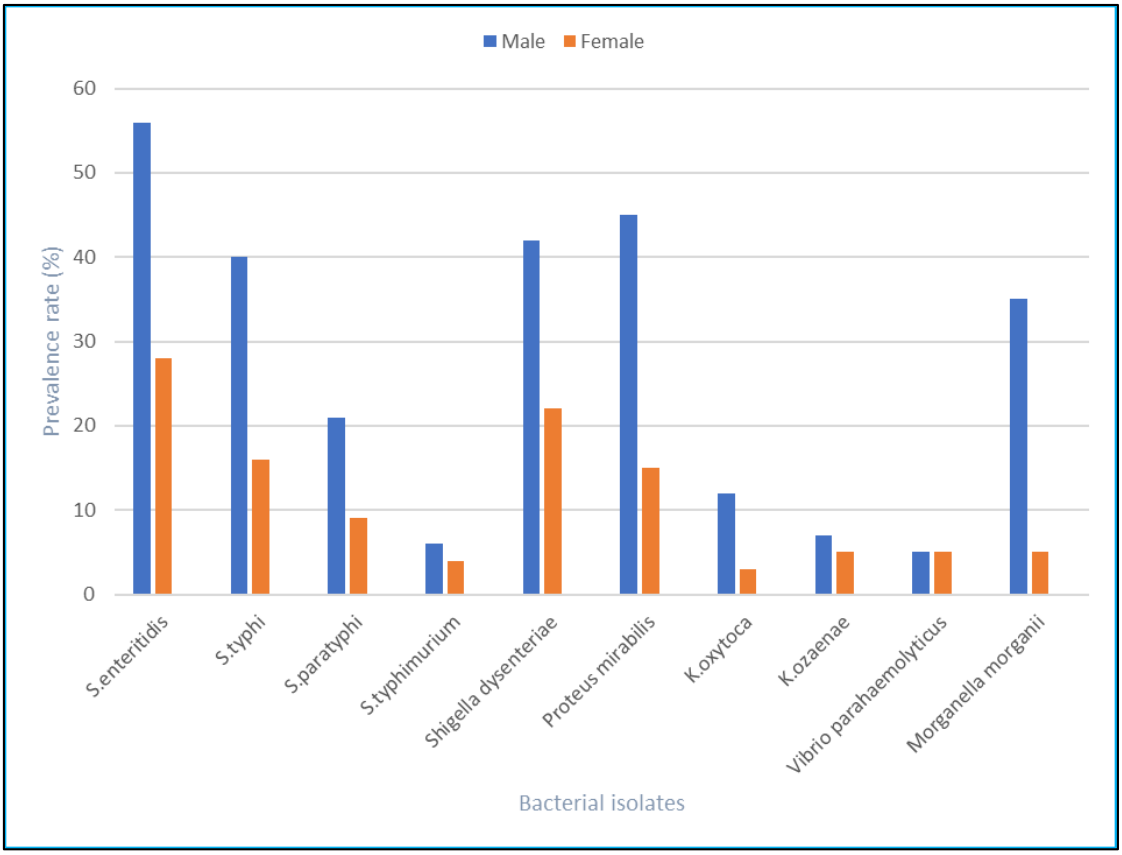


**Figure 3** Sex distribution of bacterial isolates (%) from children

Antibiotic susceptibility testing of bacterial isolates showed a higher degree of susceptibility of Salmonellae to ciprofloxacin, perfloxacin, and sparfloxacin and a higher resistance to amoxicillin, septrin, augmentin and chloramphenicol (Figure 4).

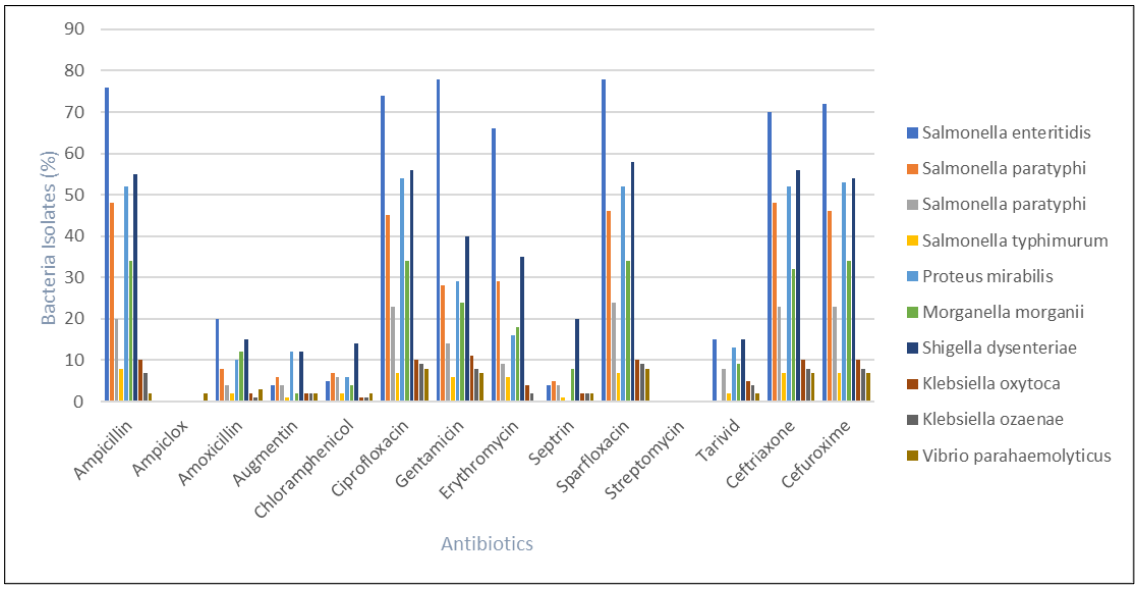


**Figure 4** Antibiotic susceptibility pattern of bacterial isolates from children

## 4. Discussion

From the 420 faecal specimens cultured, 381 yielded bacteria, 180 of which were *Salmonella* species representing an isolation rate of 90.7% and 47.2% respectively, which is similar to that of Dulthie and French [15] and Ekundayo and Enya, [16]. *S. typhi* constituted 31.1% and of *S paratyphi* A 16.7% recovered, a finding which accounts for the high incidence of typhoid and paratyphoid fevers as reported by Whitaker *et al*. [1], which showed a high occurrence typhoidal *Salmonella* in stool. This study showed how common typhoid fever was among the children which agrees with Saha [17], who had earlier reported that typhoid fever was common among the children India. Thus, typhoid fever should not be allowed to spread in the society in order to avoid its adverse public health consequences due to its high morbidity and mortality, as salmonellosis is likely to be rampant among the study population.

The sensitivity of stool culture is beloved to be lower than that of the blood culture which recommended as the gold standard for confirmation of the diagnosis of typhoid fever [16, 18]. Stool culture may not give accurate incidence rate, yet the results of this study suggest that stool culture can yield adequate number for monitoring antimicrobial profile of salmonella species from human. Blood culture is however more expensive and difficult and as well requires higher laboratory infrastructure and greater technical expertise [19]. Onile and Odugbemi] [20] reported more *S. typhi* from blood than stool and urine samples, with *S. typhi* accounting from as much as 54(90%) of all their isolates.

*Salmonella* infection is endemic and sometimes in epidemic proportion in tropical countries including Nigeria, having been reported sporadically in different parts of the country [20]. Clinical diagnosis of typhoid fever is done with reasonable certainty in classical form which is often confounded by certain endemic conditions [21]. Several typhoid fever outbreaks in Nigeria which are often characterized by high morbidity and mortality [5, 9]. Transmission of salmonellosis in Nigeria is mainly from food particularly of animal origin. Apart from *S. typhi*, other Salmonellae recovered in this study include *S. enteritidis* (22.1%), *S. paratyphi A* (7.9%) and *S. typhimurium* (2.6%).

In this study, there was a high isolation rate of Salmonellae which showed a high incidence of typhoid fever, paratyphoid, salmonellosis and enteric bacterial diseases in children. Similarly, high incidence of enteric bacterial pathogens along with Salmonellae underscores the significance of the prevalence of bacterial pathogens in children with its attendant public health hazards. These point to the fact that these children who were diagnosed of typhoid mainly on clinical presentation resulted in a high level of mis-diagnosis as much as 57.1% which automatically or inadvertently resulted in mis-treatment. It is evident from this study that many cases of gastroenteritis are clinically diagnosed wrongly as typhoid fever [5, 8, 9].

This study also revealed that *Salmonella* serovars can be easily be recovered from stool contrary to report by Onile and Odugbemi [20], a finding which underscores the significance of microscopy, culture, and sensitivity prior to treatment [5,14, 22]. Children suffering from self-limiting and non-microbial gastroenteritis are likely to as well be treated by clinician as typhoid fever, hence diseases associated with side effects of antimicrobial agents maybe common among these children consequent on the low diagnostic efficacy of clinical diagnosis of the disease.

Of the 180 *Salmonella* isolates, 123 (68.3%) were recovered from the males and 56 (31.7%) from the females. It was observed that the number of male children affected by salmonellosis were more than the females. According to Charles *et al* [23], males were affected with about two folds than females, a finding similar to that reported by FAO [24]. Ramyil *et al* [25] reported that males seem to be more affected with salmonellosis than females, a finding which is consistent with what was observed in this study. Males have been reported to have more prevalence of salmonellosis independent of age more than females from this study and other earlier studies [24, 25].

In this study, the antibiotic susceptibility profile of *Salmonella* isolates from faecal specimens of both males and females indicated that Salmonella strains were resistant to first line drugs such as chloramphenicol, ampliclox, amoxicillin, septrin and tarivid which are commonly used in the study area. A very high resistance was observed in the *Salmonella* strains as well as other bacterial isolates with all isolates tested showing a 100% resistance to ampliclox and streptomycin. These drugs are mostly over-the-counter drugs which are used by individuals without recommendation by physicians. The isolates showed a generally consistent pattern of resistance whereby the few restricted antimicrobial agent showed a high in vitro efficacy through they were susceptible to gentamicin, ciprofloxacin, sparfloxacin, cefuroxime and ceftriaxone. A similar finding had been reported by Moro [5] and Okiki and Moro [26], in which most of their *Salmonella* isolates showed high resistance to most commonly used antibiotics but were susceptible to the third generation cephalosporins and fourth generation fluoroquinolones. Wain *et al*. [27] and several other studies reported a similar pattern of antibiotics susceptibility, which agrees with the finding of this study may likely to be due to self-medication, non-completion of recommended doses or over-use of antibiotics [5. 14, 26]. This will greatly limit the treatment options for the isolates that have developed resistance to the first line drugs. It is interesting to note from this study that the isolates that showed multi-drug resistance were however highly susceptible to ciprofloxacin, sparfloxacin, cefuroxime and ceftriaxone [26, 28]. These antibiotics are not commonly used in the treatment of enteric bacterial infections, but in an environment where there is a high level of self-medication, and where self-medication is common, the organisms may likely acquire resistance to these antibiotics under antibiotic pressure or through horizontal transfer of resistance genes [5, 14, 25]. An increase in MDR was reported among Salmonellae in other countries including Iran, Ethiopia, and India but the result of this study, however revealed a comparatively higher proportion of multi-drug resistance characterised by generally high prevalence rate [14, 25, 26].

Based on the findings of this study, it is recommended that diagnosis of salmonellosis and other enteric bacterial diseases should not be diagnosed only on clinical signs and symptoms and this should be discouraged among clinicians. While microscopy, culture and sensitivity are necessary prior to treatment and compulsory incorporation of serology into laboratory, which could be subsidized by government. Furthermore, incorporation of molecular diagnosis into our healthcare delivery system is strongly recommended while taking cognizance especially of both of its advantages of high specificity and sensitivity.

## 5. Conclusion

A high incidence of salmonellosis in children was observed, in addition to other enteric bacterial infections. In addition, a high level of mis-diagnosis and mis-treatment of other bacterial diseases as typhoid fever, perhaps due to the overlap of signs and symptoms of these diseases was also observed. Multiple infections of enteric bacterial pathogens were also diagnosed and treated as typhoid fever by physicians, especially where laboratory facilities are not available. The bacterial isolates were sensitive to perfloxacin, sparfloxacin, ciprofloxacin and gentamicin. In contrast, a high resistance to ampiclox, ampicillin and septrin, which are mostly over-the-counter drugs, was recorded in the study. The high incidence of salmonellosis, mis-diagnosis and mis-treatment of typhoid fever is of great public health significance in a developing country like Nigeria.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors reported no conflict of interest in this work.

### *Statement of ethical approval*

Ethical approval was collected through the assistance of the Medical Officers of each Local Government Area where the Primary Health Centre is situated. Informed consent was obtained from the mothers of the children before the collection of faecal specimens in accordance with the Helsinki Declaration of 1926. Issues of confidentiality and anonymity were also maintained and complied with accordingly using codes in place of names.

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