*Original Research Article*

# Incidence of Multi-Drug Resistant among Diarrhoeagenic children 0-60 months with Non-Typhoidal *Salmonella* Organisms Isolated in Selected Hospitals of Bauchi State Metropolis


## ABSTRACT

**Background:** *Salmonella* non-typhoidal is associated with various gastrointestinal diseases with a significant increase in antibiotic resistance. Antimicrobial resistance is a global public health challenge, which has accelerated by the overuse of antibiotics, causes severe infections, complications, longer hospital stays and increased mortality in the study area. To screen and isolate multi-drug resistant non-typhoidal *Salmonella* from diarrhoeagenic children aged 0-60 months.

**Methodology:**A total of 222 stool samples were collected from symptomatic diarrhoeagenic children between August-December, 2019 in selected hospitals in Bauchi state metropolis. Samples were subjected to microbiological analysis and antibiotic resistance was determined by the Multiple Antibiotic Resistance (MAR) index.β-lactamase genes were detected by polymerase chain reaction (PCR). A total of 50 isolates of *Salmonella* obtained and 9 positive isolates of non-typhoidal was obtained respectively. Non-typhoidal *Salmonella* were discovered to be more prevalence among age between 48-60 months (37.50%), male children (62.50%), outpatients (62.50%) and more prevalence among patient attending Bauchi State Specialist Hospital (33.33%). **Result:**The results of antimicrobial susceptibility of the commonly used and prescribed antibiotics showed that, 87.5% of the non-typhoidal *Salmonella* were resistant to cloxacillin, followed by 75% to tetracycline and 75% to chloramphenicol. Multiple antibiotic resistance (MAR) index of the non-typhoidal *Salmonella* in this study shows that 62.5% have MAR index of ≥ 0.5, this indicate a significant level of misuse of these antibiotics. The result revealed a positive correlation and the relationship was statistically significant (0.005) at 1% level of significant. The polymerase chain reaction (PCR) detected *tet*A gene and *tet*B gene.

**Conclusion:**The study demonstrated a relatively high level of gene mediated antibiotic resistance to tetracycline and other antibiotics. The high prevalence and increased resistance especially among non-typhoidal *Salmonella* is a cause of concern and reiterates the need for extensive routine checks along with stool samples for better management of gastroenteritis.



**Keywords:** *Non-typhoidal Salmonella, Multi-Drug Resistance, PCR, tetA, tetB genes and Antibiotics*


## 1. INTRODUCTION

The prevalence rate of acute diarrhoea in Nigeria is about 18.8% [8]. Diarrhoea infections are associated with acute gastroenteritis, one of the most common alimentary diseases caused by the consumptions of contaminated water and foods especially meats [12]. A major cause of mortality and morbidity among children under five years of age [4, 5, 8].

Non-Typhoidal *Salmonella* infections, globally, a leading cause of acute bacterial gastroenteritis. *Salmonellae*are Gram negative, non-lactose fermenting and non-sporing bacteria, actively motile with the exception of *Salmonella pullorum-gallinarum.Salmonella typi* is the only capsulated *Sallmonella* belonging to the family Enterobacteriaceae [7]. Of clinical importance, there are two main groups, first group including members of the genus; causative agents of enteric fever

(typhoidal salmonellosis): *Salmonella typhi* and *Salmonella paratyphi*. Second groups including members of the genus that are involved as causative agents of food poisoning (Non-typhoidal salmonellosis): *Salmonella typhimurium* and more recently serotype DT 104. Other members are *Salmonella enteritidis, Salmonella heidelberg, Salmonella agona, Salmonella newport, Salmonella hadar, and Salmonella dublin*[3, 2].
Although there has been progress with improved water andsanitation since in the 1990s. Many people remain atrisk for typhoid fever by consumption of unsafe water and food.Earlier work suggested that risk for typhoid fever in Africa might be concentrated in urban areas and informal settlements [15].

Timely and appropriate antimicrobial therapy is needed to prevent complications and death from typhoid fever. However, antimicrobial resistance has been a growing problemin *Salmonella enteric*, compromising antimicrobialmanagement. Traditionally, ampicillin, chloramphenicol andtrimethoprim-sulfamethoxazole were the drugs of choice for the management of invasive *Salmonella* infections worldwide. However, the emergence of resistance to all of these agents termed multi-drug resistance (MDR) [9, 18, 13], lead to the adoptions of fluoroquinolones as drugs of choice. Fluoroquinolone-resistant *Salmonella typhi* is now widespread in Asia [13, 14] and present in some parts of Africa [13, 14]. In 2017 the WHO identified fluoroquinolone-resistant *Salmonella enterica* among the priority pathogens for research and development of new antimicrobial agents [17].

## 2. MATERIALS AND METHODS

**Sample Collections:** A total of 222 fecal samples were collected from children (0-60months) in clean, wide-mouthed containers, without disinfectant or detergent residue and tight-fitting leak-proof lids. The design of the study is both hospital and community based which allows for the collections, laboratory isolations, identifications and culturing of Salmonella species occurring in both symptomatic and asymptomatic infections among children 0-5 years

**Cultural Isolation and Identifications of Fecal Specimens**: Stool samples collected were inoculated within two hours of collections onto selective and differential media using a calibrated inoculating loop in the spread plate method, then incubated aerobically at 35°C for 18 to 24 hours. Samples were also inoculated into selenite cystine enrichment broth which favors the development of potentially pathogenic microorganisms and contains substances that inhibit the native flora [16] and incubated at 35°C for 8-12 hours before re-inoculation onto MacConkey (MAC) and *Salmonella-Shigella* (SS) agars.
**Biochemical Screening:** Identification of Salmonella species was done biochemically. *Salmonella* is citrate negative as such Simmon's citrate agar slopes remained as green in color. And blue color indicates a positive reaction [6].
**Inoculum preparation:** The inoculum was prepared by picking 3-5 parts of colonies of the test organism with a sterile wire loop, suspended in sterile peptone water and incubated up to 2 hours to allow organism reach log phase in growth. This was then diluted to match the turbidity standard (McFarland 0.5) which contain approximately $1.5x10^8$cfu/ml [10].
**Antibacterial Susceptibility Testing:** Susceptibility testing was performed by disc diffusion method using modified Kirby-Bauer sensitivity testing techniques [6]. The diameters of the zones of inhibition were measured to the nearest millimeters according to CLSI [6]. The antibiotic agents that were used include Co-trimoxazole, Gentamicin, Ceftriaxone, Augmentin

(Amoxicillin+Clavulanic acid), Chloramphenicol, Ciprofloxacin, Amoxicillin and Erythromycin etc.

**Determination of multiple antibiotic resistance (MAR) index:** Multiple Antibiotic Resistance (MAR) index was determined by the number of antibiotic(s) to which the organism is resistant divided by the total number of antibiotics tested [11].

**DNA extraction and genomic analysis**: Genomic DNA from representative non-typhoidal isolates was extracted using Gene Elute bacterial genomic DNA kit (Sigma-Aldrich). The extracted DNA was analyzed for tetA and tetB in polymerase chain reaction (PCR).

## 3. RESULTS AND DISCUSSION

The results shows the percentage distributions of *Salmonella* isolate according to hospitals in the study population. A total of 222 samples were collected with 102 samples from ATBUTH, 78.0 samples from Bauchi Specialist Hospital and 42.0 samples from Under 5 Hospital. ATBUTH shows 12(50.00) isolates are typhoidal *Salmonella* positive and 4(50.00) isolates are non-typhoidal *Salmonella* positive, Bauchi Specialist Hospital 8(33.33) isolates are typhoidal *Salmonella* positive and 3(37.50) isolates are non-typhoidal *Salmonella* positive and Under 5 Hospital 4(16.66) isolates are typhoidal *Salmonella* positive and 1(12.50) isolates are non-typhoidal *Salmonella* positive respectively as tabulated in Table 1.

The result thus revealed a negative correlation of -1.000 between hospital and number of positive isolate (typhoidal) and the relationship was statistically significant (0.000) at 1% level of significant. The result shows a negative correlation of -0.982 between hospital and number of positive isolate (non-typhoidal) and the relationship was statistically insignificant (0.121) at 1% level of significant as presented in statistical table 2.

**Table 1: Distributions of *Salmonella* isolate according to hospitals in the study population**

| **Hospitals** | **No. of Specimens Collected** | **No. (%) Typhoidal *Salmonella* Isolated** | **No. (%) Non-Typhoidal *Salmonella* Isolated** |
|---|---|---|---|
| ATBUTH | 102.0 | 12(50.00) | 4(50.00) |
| Bauchi Specialist Hospital | 78.0 | 8(33.33) | 3(37.50) |
| Under 5 Hospital | 42.0 | 4(16.66) | 1(12.50) |
| **Total** | 222.0 | **24(99.99)** | **8(100.0)** |

**Table 2: Relationship between hospital & bacterial isolates (Typhoidal and Non-typhoidal)**

| | | Hospital | No of positive isolate (Typhoidal) | No of positive isolate (Non-typhoidal) |
|---|---|---|---|---|
| Hospital | Pearson Correlation | 1 | -1.000** | -0.982 |

| | | | | |
|---|---|---|---|---|
| | Sig. (2-tailed) | | 0.000 | 0.121 |
| | N | 3 | 3 | 3 |
| No of positive isolate (Typhoidal) | Pearson Correlation | -1.000** | 1 | 0.982 |
| | Sig. (2-tailed) | 0.000 | | 0.121 |
| | N | 3 | 3 | 3 |
| No of positive isolate (Non-typhoidal) | Pearson Correlation | -0.982 | 0.982 | 1 |
| | Sig. (2-tailed) | 0.121 | 0.121 | |
| | N | 3 | 3 | 3 |

**WHERE: **= Correlation is significant at the 0.01 level (2-tailed). NS = Not Significant**

The results presented in Table 3 shows the percentage distributions of *Salmonella* isolates in the study populations according to patient demographic details. For ages, result shows the highest prevalence of 3(37.50) for non-typhoidal isolates among 48-60 whereas none was recorded for infants within the range of 0-6 months. For gender, the result shows the highest prevalence of 5(62.5) for non-typhoidal isolates among male child and for groups, the result shows the highest prevalence of 5(62.5) for non-typhoidal isolates among out-patients respectively according to previous studies on Infants and young children more susceptible to NTS infection compared to other age groups, making them a high-risk population [9]

Also, the result shows correlation on the relationship between age of patient in the study area and bacterial isolates. The result revealed a positive correlation of 0.942 between different age and number of positive isolate (typhoidal) and the relationship was statistically significant (0.005) at 1% level of significant. The result also shows a positive correlation of 0.932 between age group and number of positive isolate (non-typhoidal) and the relationship was statistically significant (0.007) at 1% level of significant. The result therefore means that there are more number of bacterial isolates (typhoidal) than in non-typhoidal. And also older aged patient are seen to be more susceptible to bacterial as presented in Table 4

**Table 3: Distribution of *Salmonella* isolates according to patient Demographic details**

| Patient Demographic Details | No. of Specimens Collected | No. (%) Positive Typhoidal *Salmonella* | No. (%) Positive Non-Typhoidal *Salmonella* Isolated |
|---|---|---|---|
| **Age (months)** | | | |
| 0-6 | 47 | 0(0.0) | 0(0.0) |
| 7-11 | 56 | 01(4.2) | 01(12.5) |
| 12-23 | 33 | 02(8.3) | 01(12.5) |
| 24-35 | 35 | 07(29.2) | 01(12.5) |

| | | | |
|---|---|---|---|
| 36-47 | 23 | 06(25.0) | 02(25.0) |
| 48-60 | 28 | 08(33.3) | 03(37.5) |
| **Gender** | | | |
| Male | 116 | 14(58.3) | 05(62.5) |
| Female | 106 | 10(41.7) | 03(37.5) |
| **Groups** | | | |
| In-patients | 89 | 16(66.7) | 03(37.5) |
| Outpatients | 133 | 8(33.3) | 05(62.5) |
| **Total** | **222** | **24(100.0)** | **8(100.0)** |

**Table 4: Relationship between Age (years) and bacterial isolates (Typhoidal and Non-typhoidal)**

| | | Age | No of positive isolate (Typhoidal) | No of positive isolate (Non-typhoidal) |
|---|---|---|---|---|
| Age | Pearson Correlation | 1 | 0.942** | 0.932** |
| | Sig. (2-tailed) | | 0.005 | 0.007 |
| | N | 6 | 6 | 6 |
| No of positive isolate (Typhoidal) | Pearson Correlation | 0.942** | 1 | 0.796 |
| | Sig. (2-tailed) | 0.005 | | 0.058 |
| | N | 6 | 6 | 6 |
| No of positive isolate (Non-typhoidal) | Pearson Correlation | 0.932** | 0.796 | 1 |
| | Sig. (2-tailed) | 0.007 | 0.058 | |
| | N | 6 | 6 | 6 |

**WHERE: **= Correlation is significant at the 0.01 level (2-tailed). NS = Not Significant**

Table 5 reveals the result of multiple antibiotic resistance index and patterns of resistance of non-typhoidal *Salmonella*.has the highest multiple antibiotic resistance with MAR index of 0.8 and isolate No 4 with the least with MAR index of 0.2.

**Table 5: Multiple antibiotic resistance (MAR) index and resistance patterns of Non Typhoidal *Salmonella* isolates in the study area**

| S/No. | Isolate No. | Resistance Pattern | MAR Index |
|---|---|---|---|
| **1** | 5 | C, SXT, CN, TT, CX, NM | 0.5 |
| **2** | 3 | E, C, SXT, AML, TT, DX | 0.5 |
| **3** | 1 | E, CIP, CN, TT, CX, NM | 0.5 |
| **4** | 9 | E, C, SXT, CIP, CN, CX, NM | 0.6 |
| **5** | 8 | E, C, AML, TT, CX, NM | 0.5 |
| **6** | 7 | E, C, AMC, SXT, CIP, AML, CN, DX, CX | 0.8 |
| **7** | 4 | TT, CX | 0.2 |
| **8** | 6 | C, AML, CN, TT, DX, CX | 0.5 |

***Key:*** *SXT=cotrimoxazole, CN=gentamycin, CIP=ciprofloxacin, AMC=amoxycillin/clavulanic acid, E=erythromycin, C=chloramphenicol, AML=amoxycillin, CRO=ceftriaxone, TT=tetracycline, DX=doxycycline, CX=cloxacillin, NM=neomycin, No. of antibiotics used=12*

The percentage of Multiple Antibiotics Resistance (MAR) index and the numbers of the non-typhoidal *Salmonella* isolates from selected hospitals in under 5 years populations in Bauchi is as shown in Table 6 reveals high MAR index of 0.5 with occurrence of 62.5%, indicate a significant level of misuse of these antibiotics. The bacterial isolate were sensitive to ceftriaxone, amoxicillin and clavulanic acid, ciprofloxacin, cotrimoxazole, amoxicillin and doxycycline. Isolate were resistance to cloxacillin, tetracycline, gentamycin, chloramphenicol agrees with the study of [1, 5]

**Table 6: Percentage occurrence of multiple antibiotic resistance index**

| MAR Index | No. of Non-Typhoidal Salmonella Isolates | Percentage Occurrence (%) |
|---|---|---|
| 0.2 | 01 | 12.5 |
| 0.5 | 05 | 62.5 |
| 0.6 | 01 | 12.5 |
| 0.8 | 01 | 12.5 |
| **Total** | **08** | **100.0** |

**Agarose Gel Electrophoresis of PCR product for tetA and tetB in non-typhoidal *Salmonella* isolates**

The result as shown in Figure 1 and 2 reveals the agarose gel electrophoresis of the PCR products of tetA and B genes in the non-typhoidal *Salmonella* isolate, the result further reveals that Optimized reaction mixture and cycling conditions for each gene revealed expected product size of 571 bp for fortetA and 494 for tetB genes respectively. The specificity of the primers was checked by using *Salmonella typhi* as positive control and *E. coli* ATCC25922 as negative control, all the isolates yielded expected products confirming both genus and serovars respectively. This shows a rise in resistance pattern confirming the alarming spread of resistance genes. In prior clinical surveys, the *tet*A gene was the most prevalent tetracycline resistance determinant identified, evidence shows that it has wide host range due to the ability to reside on highly mobile genetic elements that effectively transfer them among bacterial genera, according to previous work by [13]

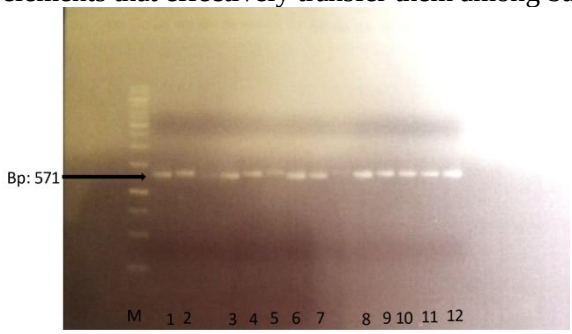


**Figure 1: Agarose Gel Electrophoresis of PCR product for tetA in non-typhoidal *Salmonella* isolates**
*Lane1: +ve control (Salmonella typhi), -ve control (E.coli), 3-12 are non-typhoidal isolates Bp: 571*

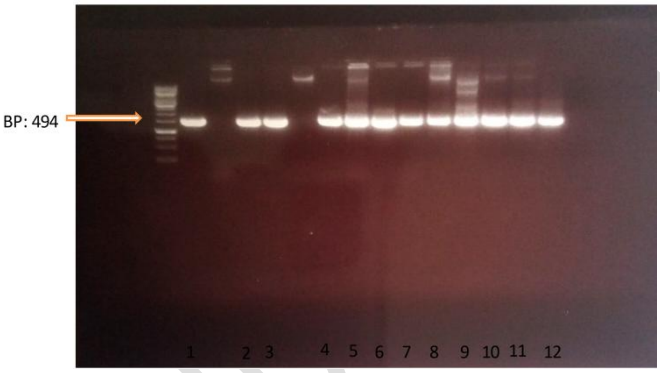


**Figure 2: Agarose Gel Electrophoresis of PCR product for tetB in non-typhoidal *Salmonella* isolates**
*Lane 1 & 2: +ve control, 3: -ve control, 4-12 non typhoidal isolates Bp: 494*

## 4. CONCLUSION

Based on the findings of this research work, it can be concluded that, only 8 (3.6%) non-typhoidal *Salmonella* isolates were recovered from 222 diarrrhoeagenic stool samples of children. The non-typhoidal *Salmonella* isolates were generally resistant to antibiotics used except ceftriaxone. The high carriage rate of *tet*A and *tet*B genes among the *Salmonella* isolates suggests that these genes are the major determinant of resistance to tetracycline in this environment.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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