**Prevalence and Antibiotic Susceptibility Pattern of *Salmonella* Species Isolated from Patients Attending Murtala Muhammad Specialist Hospital, Kano**

**ABSTRACT**

Typhoid fever is a disease of public health concern with high morbidity and mortality rates in developing countries like Nigeria. The treatment of typhoid fever is currently hamstrung by the emergence of *Salmonella* species strains resistant to antibiotics. Emergence of ESBL in MDR Salmonella species brings a new challenge of great concern in treatment of typhoid. This work was aimed at determining the prevalence and antibiotic susceptibility pattern of *Salmonella* species isolated from patients attending Murtala Muhammad Specialist Hospital, Kano. A total of 400 stool samples were collected from Murtala Muhammad Specialist Hospital, Kano and analysed for *Salmonella*. The isolates were identified phenotypically based on cultural, microscopic and biochemical characteristics. Antibiotic susceptibility test was conducted using Kirby-Bauer disc diffusion method according to CLSI guidelines of 2016. Multi-drug Resistant isolates detected were further screened for ESBL production. The results obtained showed that a total of 14 isolates were identified phenotypically. The antibiotic susceptibility testing of the isolates showed highest resistance to Ampicillin (57.4%), 50% resistance to Cefotaxime and Ciprofloxacin with high susceptibility to Azithromycin, Aztreonam and Tazobactam. Higher susceptibility to Azithromycin, Aztreonam and Tazobactam advocate for their use to treat typhoid fever.

Key words: Prevalence, Antibiotic susceptibility, *Salmonella,* Multi-drug Resistance

# **INTRODUCTION**

Typhoid fever poses a public health threat, recording high morbidity and mortality rates mainly in developing countries. Typhoid fever shows an annual global estimate of 20.6 million cases and 223,000 deaths. In sub-Saharan Africa, the incidence of typhoid fever is greater than 100 per 100,000 persons per year resulting in 33,490 deaths accounting for 26% of global typhoid deaths in Africa [(Gashaw & Jambo, 2022)](https://paperpile.com/c/E0Ault/awDl).

Typhoid fever is characterized by high fever, paradoxical bradycardia, and rose-colored rash out of which the most common complaints are headache, abdominal pain and diarrhea. Patient suffering from typhoid fever may develop symptoms like digestive hemorrhages, ileocaecal perforation, encephalitides, myocarditides, bacterial super infections, pancreatitis, intestinal perforation, bowel perforation, infarction abscess, hepatic dysfunction and hepatic abscess [(Gashaw & Jambo, 2022)](https://paperpile.com/c/E0Ault/awDl). It causes septicemia of digestive origin that can cross the placenta resulting in chorioameniotitis maternal fetal infection with Salmonella speciescan lead to miscarriage fetal death, neonatal infection as well as diverse maternal complication.

In the past, first line antibiotics for the treatment of typhoid included chloramphenicol, ampicillin, and trimethoprim-sulphamethoxazole. However, Multidrug resistant (MDR) *Salmonella* species, defined as strains resistant to these first-line antibiotics emerged in the late 1980s. This resulted in the use of fluoroquinolones and third generation cephalosporins as alternatives for treatment of MDR Salmonella species cases [(Marchello et al., 2020)](https://paperpile.com/c/E0Ault/OMIR).

Increased use of fluoroquinolones such as Ciprofloxacin for treatment has resulted in the emergence of strains resistant or with reduced susceptibility to this particular antibiotic [(Yan *et al*. 2016)](https://paperpile.com/c/OKPdzB/KoDQ). Other than the use of these antibiotics in the treatment of human cases, there have been many reports on the use of antibiotics in treatment of animal disease and as growth promoters in food derived from animals which similarly contribute to the occurrence and spread of antibiotic resistant bacteria [(Mahmoud *et al.,* 2016)](https://paperpile.com/c/OKPdzB/baMr).

Extended spectrum β-lactamases (ESBL) are enzymes that can hydrolyze oxyimino-beta lactams causing resistance to third generation cephalosporins, resulting in treatment failure and association with higher morbidity and mortality among immuno-compromised patients. Nosocomial infections caused by ESBL producing *Salmonella* species have been reported from Latin America, France, Senegal, Africa, Asia, and Europe. The emergence of ESBL in MDR *Salmonella* species constitutes a new challenge and has become a matter of concern especially in under developed countries. *Salmonella* species have been found to produce a wide variety of ESBL types including TEM, SHV, PER and CTXM enzyme [(Ahamed Riyaaz et al., 2018)](https://paperpile.com/c/E0Ault/rKnv). The study is therefore aimed to investigate the prevalence and antibiotic susceptibility pattern of Salmonella species isolated from patients attending Murtala Muhammad Specialist Hospital, Kano

# **MATERIALS AND METHODS**

# **Study Area**

The study was carried out at the Microbiology Laboratories of Murtala Muhammad Specialist Hospital, Kano and the Bayero University Kano. Murtala Muhammad Specialist Hospital is one of the oldest government owned hospitals in Kano State located in the ancient city of Kano on 12000’N8031’E. The hospital contains a wide range of departments, including Radiology, Laboratory Services, Pediatrics, Surgery, Internal Medicine, and Obstetrics & Gynecology. Additionally, it has an emergency room that is open twenty-four hours a day, seven days a week.

**Ethical Approval**

Ethical approval with the reference number MOH/Off/797/TI/1188 was obtained from Health Research Ethics Committee, Ministry of Health Kano State for permission to obtain samples from the study participants attending the Murtala Muhammad Specialist Hospital, Kano. The consent of the patients was asked to willingly participate in the research.

# **Sampling Procedure and Sample Size Determination**

The study participants were selected using multi-stage sampling. A total of 400 patients were selected using the Fisher formula (n = z2 p (1-p)$ ÷ $d2) and a prevalence of 50% was used reported by [Jung, (2014)](https://paperpile.com/c/E0Ault/fJ6k):

n = (1.962) (0.5) (1-0.5)$÷ $(0.05)2 = 384.16

At total of 384 samples was calculated but in order to cover for a non-compliance, a total number of samples for the study was rounded up to 400

# **Sample Collection**

A total of 400 stool samples were collected from patients who were positive for *Salmonella* species antigen according to their hospital record. Sample bottles were given to the patients for their stool samples. The samples collected were immediately inoculated in Salenite F broth. The process was repeated for a period of 4 weeks until 400 stool samples were collected.

# **Isolation and Identification**

The stool samples collected were enriched by aseptically inoculating them using a sterile wire-loop in to Selenite F broth (Smith *et al*., 2011) and incubated for 24hrs at 37oC in an incubator. The enriched bacterial cultures were aseptically inoculated with a sterile wire-loop onto *Salmonella-Shigaella* Agar and MacConkey Agar using streak plate method and incubated for 24hrs at 37oC in an incubator. The bacterial pure isolates obtained were preserved for identification and other tests. The pure bacterial isolates produced were identified based on morphological and biochemical characteristics that included TSI, urease, indole, motility, and citrate utilization tests (Lestari *et al.,* 2016).

# **Antibiotic Susceptibility Testing**

The antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method where commercially prepared antibiotics were used. The antibiotics were single disc antibiotics of Thermo Scientific Oxoid brands. The selected antibiotics comprises of Ampicillin (10µg), Azithromycin (15 *μ*g), Tazobactam (100 µg), Cefotaxime (30 µg), Aztreonam (15 µg), Ciprofloxacin (5µg) and Chloramphenicol (30 µg). The resulting clear zones were grouped into susceptible groups (S), intermediates (I) or resistant (R) based on the break-points provided by CLSI 2016.

*Salmonella* isolates in agar slants were subculture by inoculating into nutrient agar plates for the organisms to regain viability and incubated for 24hrs at 37oC. Pure cultures from the overnight culture were inoculated into sterile tubes with normal saline and a turbid suspension of the inoculum was formed. The prepared inoculum was standardized by comparing with prepared with BaSO4 solution equivalent to 0.5McFarland (1x108CFU/mL) turbidity.

The standardized cultures were inoculated into sterile Mueller Hinton agar (MHA) plates using spread plate method with a sterile swab cotton and allowed to stand for 5 minutes. The antibiotic-containing discs were placed on MHA culture plates, which had been dispersed with pure cultures, at a distance of 25-30 mm. Furthermore, the culture plates were incubated at 35ºC for 24hr [(Musa et al., 2025)](https://paperpile.com/c/E0Ault/gSBa). After incubation, the plates were examined for zones of inhibition and measured using ruler and interpreted. Isolates that are resistant to more than three (3) classes of antibiotics were considered multi-drug resistant.

# **Test for ESBL Production**

All the MDR *Salmonella* species isolates were screened for ESBL production using Ceftazidime (CAZ) and Cefotaxime (CTX) indicator discs. The isolates showing the zones of inhibition (ZoI) ≤ 22 mm and 27mm for CAZ and CTX respectively were further tested against combination with Amoxicillin/Clavulanic acid (AMC). The isolates showing an increase in ZoI by greater than or equal to 5mm when evaluated against combination with AMC were phenotypically considered as ESBL producers (Khalid *et al.,* 2011).

Double Disc Synergy Test (DDST) was performed by inoculating Mueller-Hinton (MH) agar with test isolates to give a semi confluent growth. Ceftazidime CAZ 30 µg and AMC 20 µg discs were placed 25 mm apart (center-to-center) following over-night incubation aerobically at 37°C. ESBL production was inferred when there is expanded zone of inhibition between the AMC and CAZ discs by 5mm (Khalid *et al.,* 2011).

**RESULTS AND** **DISCUSSION**

The result of the study shows that 14 (3.5%) of the studied stool samples had *Salmonella* species (Table 1). Patients aged 0-10years and 31-14years constitute the largest number of study subjects of 140 (35%) and 83 (20.75%) respectively. The least study patients were those aged 11-20years and 51 and above years. The highest number of *Salmonella* species isolates was detected from those aged 11-20years (0.75%).F Patients aged 41-50years and 50 and above years had the least with one isolate each (Table 1). The result of the study shows that males had the highest number *Salmonella* species isolates of 8 (2.0%) compared to females with 6 (1.5%) (Table 2).

Out of the 400 samples examined, 14 (3.50%) were found to be *Salmonella* species based on their phenotypic identification. This finding conforms to that of [Maharjan et al. (2021)](https://paperpile.com/c/E0Ault/O5NC) with a prevalence of 3.1%. Oluyege *et. al.,* (2015) got 3.49% in their findings conducted in Ekiti State University Teaching Hospital which approximately similar to the 3.50% found in this study. The 3.5% occurrence of *Salmonella* species in this study is found to be higher compared to the recent findings of typhoid fever in the same study area where [Mujahid *et al.* (2022)](https://paperpile.com/c/VScAxn/2B0S) reported 0.0% occurrence. The low growth rate in studied sample culture can be attributable to the practices of self-medication, which is more common in developing countries like Nigeria.

Age group 11-20 was observed to have the highest occurrence of *Salmonella* species. This finding also is not in conformity with that of Abdallah, (2019) where the highest occurrence was found among the children of age group 0-3. The prevalence may be because of their poor quality of drinking water, exposure to contaminated ready to eat food items available in open air cafeteria, reliance on fast food joints, lack of good hygienic practices, and common practice of consuming unwashed and partially cooked vegetables which possibly enhanced the spread of the infection. It was also discovered that the age group of 0-10years ranked second in the occurrence of *Salmonella* species despite the fact that highest stool samples 140 (35%) were collected from this age group. Of the 140 out of 400 samples examined in that age group, only 3 (0.75%) *Salmonella* species were isolated which is low considering the sample size analyzed. This may be an indication that typhoid fever in children under 5 years is low in the study area. Several studies were conducted to characterize bacteria associated with gastrointestinal infection and found *Salmonella* as one of the bacteria associated with diarrhea [(Yurist-Doutsch *et al.,* 2014)](https://paperpile.com/c/Iwg7ZU/PAn4).

Again, the same finding of Oluyege *et. al.,* (2015) identified high occurrence among the young people within the age group of 20-29 yrs. old, and this finding also reveals that highest occurrence among young teenage and adult people but within the age group of 11-20years. This difference in age group may be due to socio-economic and socio-cultural differences in northern and southern Nigeria. In Kano, at the age of 8-20, parents do send their children to school and market (or other training centers like mechanical workshops, tailoring center etc.) to learn how to read and write and other beneficial crafts. This practice may expose this group of people to the contaminated foods and drinks and be infected with *Salmonella.* Similarly, at that age group, there is very high number of *Almajiris* (students who migrated their homes in search of Islamic knowledge) who roam about searching for ready to eat foods not minding the hygienic nature of the food.

**Table 1: Prevalence of *Salmonella* Species Isolates among Different Age Groups**

|  |  |  |
| --- | --- | --- |
| **Age (in years)** | **No. Examined (%)** | **Prevalence of *Salmonella* Species N (%)** |
| **0-10** | 140 (35.00) | 3 (0.75) |
| **11-20** | 35 (8.75) | 5 (1.25) |
| **21-30** | 68 (17.00) | 2 (0.50) |
| **31-40** | 83 (20.75) | 2 (0.50) |
| **41-50** | 41 (10.25) | 1 (0.25) |
| **51 and above** | 33 (8.25) | 1 (0.25) |
| **TOTAL** | 400 (100.00) | 14 (3.50) |

**Table 2: Prevalence of *Salmonella* SpeciesIsolates by Gender**

|  |  |  |
| --- | --- | --- |
| Gender | No. Examined (%) | Prevalence of *Salmonella* speciesN (%) |
| Male | 189 (47.25) | 8 (2.00) |
| Female | 211 (52.75) | 6 (1.50) |
| Total | 400 (100.00) | 14 (3.50) |

The results showed that the *Salmonella* species isolates had higher resistance to Ampicillin (57.14%), Cefotaxime (50%) and Ciprofloxacin (50%). The *Salmonella* species isolates had high susceptibility to Aztreonam (85.71%), Tazobactam (78.57%) and Chloramphenicol (57.14%) (Table 3). The antibiotic susceptibility profile shows that seven 7 (50%) *Salmonella* species isolates are multi-drug resistant as they were resistant to more than two classes of antibiotics. Based on the Double Disc Synergy Test results, 3 isolates were found to produce ESBL phenotypically.

Based on the study, the most active antibiotics were Aztreonam,Tozobactam and Azithromycin. This may be due the ability of Tazobactam to inhibit the action of bacterial beta-lactamases, especially those belonging to the SHV-1 and TEM groups. Azithromycin susceptibility in Salmonella can be attributed to its ability to effectively penetrate bacterial cells and bind to the 50S ribosomal subunit, inhibiting protein synthesis. This action disrupts the bacteria's ability to produce essential proteins, ultimately leading to cell death.

This study also show that a high proportion of the isolates were resistant to the conventional first line antibiotic, ampicillin (57.14%), beta-lactam antibiotic. Chloramphenicol, ampicillin and cotrimoxazole were considered the first line antibiotics for the treatment of typhoid fever (Zaki and Karande, 2011). This result supported the findings of Mutai *et al.,* (2018) where high resistance to ampicillin (72%) was observed. In this study, penicillin resistance in bacteria would most likely be attributed to the production of beta-lactamase enzymes (also known as penicillinase). These enzymes are capable of inactivating the antibiotic penicillin by breaking down its beta-lactam ring, rendering it ineffective.

The result showed that there is increased susceptibility to Chloramphenicol and ampicillin (35.71% and 57.14% respectively) shown by the organisms compared to the findings of Mutai *et al.* where 72% resistance was recorded for both antibiotics (Mutai *et al.,* 2018). In recent years, the epidemiology and drug resistance profile of *Salmonella* strains causing enteric fever has changed. The shift towards re-emergence of susceptibility patterns towards former first-line antibiotics (eg, ampicillin, co-trimoxazole, and chloramphenicol) seen in many studies could be explored as an option for drug treatment if pathogens acquire resistance to newer oral drugs such as fluoroquinolones. Investigators of a north Indian study reported a statistically significant decrease in antimicrobial resistance to former first-line antibiotics among the 852 *Salmonella enterica* serotype Typhi isolates over a 12year period. They further reported that, in 2012, more than 95% *Salmonella* Typhi clinical isolates were susceptible towards each of these former first-line antibiotics [(Gupta & Gupta, 2016)](https://paperpile.com/c/E0Ault/Idn6).

The re-emergence of drug-susceptible strains might result from lack of antibiotic pressure since these drugs are not being used routinely. This possibility has also been suggested by Dutta and colleagues, who proposed that the re-emergence of susceptibility to these drugs might result from the emergence of de-novo susceptible strains or the loss of high-molecular-weight self-transmissible plasmids (Dutta *et al.,* 2005).

The result also shows that there is reduced susceptibility to Ciprofloxacin compared to some findings such as that of Mutai *et al.,* (2018) where only 5 out of 14 *Salmonella* species isolates were found to be susceptible which is approximately 35.71%. Recommendation to use fluoroquinolones for empirical treatment in place of first line antibiotics may have contributed largely to the emergence of fluoroquinolones resistance. Increased use of fluoroquinolones, such as Ciprofloxacin, for treatment has resulted in the emergence of strains resistant or with reduced susceptibility to this particular antibiotic (Yan *et al.,* 2016). Ciprofloxacin has been used as an alternative antibiotic in the treatment of MDR cases. However, with the currently reviewed breakpoints of Ciprofloxacin by CLSI, there has been a rather increase of isolates resistant or recording reduced sensitivity to this antibiotic. This study supported the finding of Mutai *et al.,* (2018) where MIC results of Ciprofloxacin showed that 13% of the isolates were resistant (Mutai *et al.,* 2018). Reduced susceptibility to Ciprofloxacin, poses a serious threat to the treatment failure of typhoid fever, especially in developing countries like Nigeria (Yan *et al.,* 2016). The result also showed that Cefotaxime has equal proportion of susceptibility and resistance (50%) on the isolates.

The result of the antibiotic susceptibility testing as well shows that some of the organisms were multi-drug resistant (MDR) as 7 (50%) of them were found to have resisted more than 2 classes of antibiotics. Based on the result of the Double Disc Synergy Test for the detection of ESBL isolates, 3 MDR isolates of *Salmonella* species were found to be ESBL producers. This resistance shown by ESBL producing isolates of *Salmonella* speciesmay be as a result of selective pressure imposed by the misuse of broad-spectrum antibiotics such as third-generation cephalosporins. This result is in line with the finding of [Ahamed](https://paperpile.com/c/E0Ault/rKnv) Riyaaz *et al.,* (2018) where *Salmonella* species ESBL producing isolates showed resistance to Ampicillin and cephalosporins.

**Table 3: Antibiotic Susceptibility Pattern of *Salmonella* species against some Antibiotics**

|  |  |  |
| --- | --- | --- |
| Antibiotics | Potency | Susceptibility Profile (N= 14) |
| **Susceptible****N (%)** | **Intermediate****N (%)** | **Resistance****N (%)** |
| Ampicillin | 10µg | 3 (21.43) | 3 (21.43) | 8 (57.14) |
| Tazobactam | 10 µg | 11 (78.57) | 1 (7.14) | 2 (14.29) |
| Cefotaxime | 30 µg | 7 (50) | 0 (0.0) | 7 (50) |
| Aztreonam | 15 µg | 12 (85.71) | 0 (0.0) | 2 (14.29) |
| Azithromycin | 15 μg | 11 (78.57) | 2 (14.29) | 1 (7.14) |
| Ciprofloxacin | 5µg | 5 (35.71) | 2 (14.29) | 7 (50) |
| Chloramphenicol | 30 µg | 8 (57.14) | 1 (7.14) | 5 (35.71) |

# **CONCLUSION**

The study identifies that the prevalence of *Salmonella* species in the study was 3.5%. The antibiotic susceptibility profile of *Salmonella* species shows 50% of them were MDR. Additionally, the isolates were found to be resistance to Ampicillin, Cefotaxime and Ciprofloxacin and highly susceptible to Aztreonam, Tazobactam and Azithromycin. The study as well shows that 21.43% of *Salmonella* species were ESBL producers. It is therefore recommended that good hygienic practices should be promoted to further reduce the occurrence of *Salmonella* species (typhoid fever) in the study area through various media and antibiotic susceptibility testing is highly recommended to be done before prescribing drugs for patients diagnosed with typhoid fever in order to reduce the rate of antibiotic resistance and indiscriminate use of [antibiotics](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/antibiotic)

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