The occurrence of multi-drug-resistant (MDR) *Salmonella typhi* in Southern Benue, Nigeria

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**ABSTRACT**

Typhoid fever, whose causal agent is *Salmonella typhi* has high endemicity in developing countries. This study investigated the distribution of multidrug resistant *S. typhi* in Southern Benue, Nigeria. Stool samples were obtained from 583 (57.0%) male and 439 (43.0%) female patients presumptively diagnosed with typhoid fever in government hospitals within the study area. Isolation and identification of *S. typhi* followed standard cultural and biochemical procedures using a range of selective culture media. The *Salmonella typhi* isolates were subjected to antibiotic susceptibility test using the standard disc diffusion method (Kirby-Bauer), and results were interpreted using the criteria of the Clinical Laboratory Standards Institute. Chi-square and analysis of variance (ANOVA) were used to determine associations amongst variables were used to determine associations between variables at 95% confidence level. Isolates from Ohimini (57.1%, n=12) had the highest rate of multidrug-resistant strains, while Agatu (13.7%; n=7) had the least. Age groups >60, ≤10 and 21-30 exhibited high MDR rates of 50.0%, 32.8% and 30.8% respectively. Isolates from female patients, (33.3%) had higher MDR rates than their male (23.1%) counterparts. The results showed a statistically significant difference in the prevalence of MDR *S. typhi* in the various locations sampled. The rate of MDR *S. typhi* isolates demonstrated in this study is remarkable and of great concern.

**Keywords:** Occurrence, *Salmonella typhi*, Multi-drug, Resistance, Typhoid

**1. INTRODUCTION**

Multi-drug-resistant typhoid fever (MDRTF) is typhoid fever caused by *S. typhi* strains that are resistant to all the three first-line recommended drugs (chloramphenicol, ampicillin, and cotrimoxazole) used for its treatment¹,². Typhoid fever, otherwise known as enteric fever, is caused by *Salmonella typhi*, a Gram-negative, motile, non-spore forming, non-lactose forming, and facultative anaerobic, rod-shaped bacterium. The bacterium is solely a human pathogen with no known animal reservoir, and it is endemic in the world’s tropical and sub-tropical regions³. It has developed into a significant public health problem in developing countries of the world, including Nigeria, where it constitutes a significant disease of morbidity and mortality⁴. Its estimated global annual incidence is 540 per 100,000 and 17 million cases worldwide⁵. Factors such as residence in a crowded household, contaminated water and food as well as poverty favour the spread of typhoid fever⁴.

Treatment of typhoid fever is mainly by antibiotic therapy. However, the emergence of multdrug resistance (MDR) has become a significant threat to public health³,⁷. Previous researchers have reported occurrence of MDR *S. typhi* strains in recent years in different parts of Nigeria and around the world³,⁸-¹⁰. The high rate of occurrence reported by these researchers is remarkable and of great concern.
of Agriculture Makurdi for examination and microbiological analysis. Samples were collected between August 2016 and July 2017 at various health centres.

2.5. Isolation of Salmonella typhi

Each sample was first inoculated into Selenite broth base (Oxoid, CM 0395) and incubated for 24 hours at 37°C for pre-enrichment. Loopfuls of the broth were then streaked onto Salmonella Shigella agar (Oxoid, CM 0099) and Xylose Lysine Deoxycholate agar (Oxoid, CM 0469). Inoculated plates were then incubated at 37°C for 24 hours; suspected colonies were further sub-cultured onto Bismuth Sulfite agar (Oxoid, CM 0201) to obtain pure cultures.

2.6. Identification of the Isolates

Colonies were identified using their morphological and biochemical characteristics. The shape, colour and elevation of the bacterial colony were observed visually. Gram reaction, motility, catalase, indole, oxidase, citrate utilization and Triple sugar iron tests were performed\textsuperscript{15-16}.

2.7. Antimicrobial Susceptibility Test

Salmonella typhi isolates were subjected to antimicrobial susceptibility testing using the standard disc diffusion method. Results were interpreted using the criteria of the Clinical Laboratory Standards Institute\textsuperscript{17-18}. Overnight cultures of each S. typhi isolate were inoculated into a test tube containing 5 ml of phosphate-buffered saline (PBS). The turbidity of each inoculum was adjusted to 0.5 McFarland standard prepared by mixing given amounts of barium chloride and sulphuric acid.

The standardized culture was evenly spread over the entire surface of Mueller-Hinton agar (Oxoid, CM 0337) plates using a sterile swab stick. Sterile forceps was used to carefully pick and gently place the antibiotic discs of known concentrations on the dried, inoculated surface of the Mueller-Hinton agar plates. The discs were gently pressed onto the medium surface with sterilized forceps to ensure firm contact. The plates were incubated at 37°C for at least 18 hours.

The antibiotic impregnated discs (Oxoid Ltd) used were Amoxicillin/clavulanic (30μg), ceftazidime (30μg), ceftriaxone (30μg), amoxicillin (10μg), sulphamethoxazole/trimethoprim (25μg), chloramphenicol (30μg), ciprofloxacin (5μg), azithromycin (15μg), gentamycin (30μg) and imipenem (10μg). Diameters of zones of inhibition around the antibiotic disc were then measured to the nearest millimetre\textsuperscript{17-19}.

2.8. Data Analysis
IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA) was used to analyze the results obtained. Associations between variables were determined by Pearson’s chi-square test. The strength of the association between drug interaction and variables (location, age and gender) was also estimated using Pearson’s chi-square test. A P value less than or equal to 0.05 was interpreted to be statistically significant.

3. RESULTS

The distribution of multi-drug-resistant *S. typhi*, according to towns in the geographical zone was as shown in Figure 1. Isolates from Ohimini (57.1%; n=12) exhibited the highest rate of multidrug resistance within the geographical region, followed by Otukpo (39.4%; n=26). Agatu had the least rate of MDR isolates (13.7%; n=7). The differences in the occurrence rate of MDR strains in the various LGAs within the Benue South geographical zone was statistically significant ($\chi^2=27.459$, df=8, $p<0.05$).

Distribution of MDR *S. typhi* with respect to age was as presented in Figure 2. Age group >60, ≤10 and 21-30 years showed high MDR rates of 50.0%, 32.8% and 30.8% respectively. The difference in the distribution of MDR *S. typhi* with respect to age was not statistically significant ($\chi^2=8.591$, df=6, $p>0.05$).

Table 1 presents the distribution of MDR *S. typhi* with respect to gender. Isolates from female patients (33.3%) showed higher MDR rate than their male counterparts (23.1%). The differences observed was statistically significant ($\chi^2=5.662$, df=1, $p<0.05$).

![Figure 1. Occurrence of MDR S. typhi in Benue South geographical zone.](image1)

![Figure 2. Occurrence of MDR S. typhi with respect to the age of patients.](image2)
Table 1. Distribution of MDR *S. typhi* with respect to the gender of patients.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of MDR strains</th>
<th>Total number of isolates</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>64</td>
<td>273</td>
<td>23.1</td>
</tr>
<tr>
<td>Female</td>
<td>58</td>
<td>174</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>447</td>
<td>27.1</td>
</tr>
</tbody>
</table>

$\chi^2=5.662$, df=1, $p=0.000$ ($p<0.05$)

4. DISCUSSION

Multidrug resistance has been defined as resistance to at least one antibiotic in not less than 3 classes of antibiotics such as the quinolones, extended-spectrum cephalosporins, β-lactam/β-lactamase inhibitor combinations, aminoglycosides and carbapenems\(^\text{20}\). This study reports a 27.3% multidrug resistance rate in *S. typhi* isolates from patients in Benue South geographical zone. A higher occurrence rate of MDR in *S. typhi* isolates was reported in Warri, Nigeria\(^\text{21}\), and Nepal\(^\text{2}\). The high prevalence of MDR *S. typhi* in Ohimini LGA and Otukpo could be attributed to the population density and poor access to health care facilities in these areas. The effect is the indiscriminate use of antibiotics occasioned by wrong prescription, the lack of access to quality healthcare, and the inadequate presence of qualified health personnel. A strong link between such a phenomenon and the development of resistance by pathogens to antibiotics has been established\(^\text{22}\). A lot of these local inhabitants may resort to self-help and self-preservation of over the counter antibiotics when ill, thereby abusing the use of antibiotics. Horizontal transfer of genetic elements which bear resistance is also a known source of resistance in pathogenic bacteria, including species of Salmonella\(^\text{23}\). Other mechanisms by which resistance is developed by Salmonella are drug inactivation, alteration of antibiotic target sites, the activity of efflux pumps and reduced permeability of membranes\(^\text{24,25}\).

Isolates from patients who were 60 years or older (50%), and 10 years or younger (32.8%) harboured more MDR *S. typhi* isolates than the other age groups. This agrees with the findings of Gupta et al.\(^\text{26}\) and Mannan et al.\(^\text{27}\), though they reported higher rates in the paediatric age group. The reason for this observed differences is not clear to us. We believe that aside the earlier mentioned role of indiscriminate antibiotic use, the faecal-oral route of transmission could be responsible for the transfer of resistant *S. typhi*.

Results of the study showed that the number of MDR strains was higher in females (33.3%) than in males (23.1%). Earlier findings of Gupta et al.\(^\text{28}\) and Mannan et al.\(^\text{27}\) however, were at variance with this present finding. They reported higher MDR strain presence in males than females. Our observation reveals that women in the geographical area are more involved in activities that may expose them to street foods which could harbour pathogenic bacteria. While the men are predominantly farmers, the women engage more in trading and sales of farm produces. Exposure to street foods has been linked with a higher possibility of contracting typhoid fever and hence, the causal agent\(^\text{28}\). The rate of MDR demonstrated by *S. typhi* isolates especially in children and elderly patients in this study is remarkable and of great concern. Studies by Mandal et al.\(^\text{8}\), Kalu et al.\(^\text{29}\), Echewarieme et al.\(^\text{21}\) and Harriet and Nandita\(^\text{3}\) had reported an increased prevalence of multi-drug resistance around the globe. This finding suggests that most of the patients will not respond to treatment if placed on previously effective antibiotics. The implication is that patients are likely to have a prolonged fever clearance time and high rates of treatment failure\(^\text{11}\). The death rate of patients infected with antibiotic-resistant *S. typhi* is 21 times higher than individuals infected with non-antibiotic resistant strains\(^\text{30,31}\). In addition, resistance to antibiotics by bacteria results in increased treatment costs, a burden which would be too heavy for people in the rural areas to bear. The rate of poverty in some of the study areas occasioned by weak economic status of the inhabitants, in addition to poor health facilities, makes quality healthcare far fetched. The findings of this study therefore makes it important for relevant authorities to pay attention to providing adequate healthcare infrastructure and trained, qualified personnel for health centers in sub-urban societies. This can greatly mitigate arbitrary use of antibiotics and eventually reduce resistance to antibiotics in such societies.

5. CONCLUSION

Results of this study showed that a high number of the *S. typhi* isolates exhibited multi-drug resistance (MDR). Isolates from females had higher MDR strains than isolates from males. The difference observed in the distribution of MDR strains within the different age groups was not statistically significant. The rate of multi-drug resistance observed in *S. typhi* isolates in this study is remarkable and of great concern, considering the effect of the observed phenomenon on treatment outcome.

6. ACKNOWLEDGMENT

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Ethics approval
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