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# Prevalence and Antibiotic Susceptibility Pattern of *Salmonella enterica* Isolated from Apparently Healthy Students Screened for *Salmonella* Agglutinins

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## Abstract

Infections caused by *Salmonella enterica* remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease. This was a cross-sectional study conducted between October – December, 2021 in which the detection of *Salmonella enterica* by stool culture was carried out on sixty (60) apparently healthy students screened for *Salmonella* agglutinin by widal agglutination test. The susceptibility of the *Salmonella* isolates to commonly used antibiotics was carried out by disc diffusion method. The result showed that 52 (82.7%) subjects were found to be widal positive with at least one of the tested antigens. From these, 26 (86.7%) each were recorded among male and female students respectively. The most common *Salmonella* agglutinin detected in both male and female belong to the Paratyphi serovar. However, the percentage of the positive titres, 1:320 and 1:160 in both males (p=0.139). and females (p =0.382) were not significantly different. *Salmonella enterica* was isolated in 11(42.3%) males and 7(26.9%) females, with a combined prevalence of 18(34.6%). For male subjects, *Salmonella enterica* was more susceptible to ciprofloxacin (81.8%) and ofloxacin (81.8%) and least to nalidixic acid (45.5%). For female gender, *Salmonella enterica* was more susceptible to gentamycin, augmentin, ofloxacin, streptomycin, and cotrimoxazole with 71.4% susceptible rate each, but more resistant to nalidixic acid (85.7%) and ampicillin (71.4%). The result also showed that 11 of the *Salmonella enterica* exhibits multidrug resistance phenotype, accounting for the prevalence of 64.7%. From these, the MDR phenotype was more in female than male but with no statistical difference (P=0.569). The findings of this study established that *Salmonella* agglutinins are common among apparently healthy. It also shows the limitations of widal agglutination test, since *Salmonella* species could not be isolated in all the subjects whose sera were widal positive.

***Keywords***: *Salmonella, antibiotic, apparently healthy, agglutinins, students*

## Introduction

*Salmonella* infection remains a major public health concern worldwide, contributing to the economic burden of both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of disease (Crump *et al*., 2004). Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteraemia and enteric fever (Majowicz *et al*., 2010; Eng *et al*., 2015).

Typhoid fever is an acute systemic feverish illness caused by the bacterium *Salmonella enterica* serovar Typhi. *Salmonella enterica* serovars Paratyphi A, B, and C cause the clinically similar condition, paratyphoid fever (Kanungo *et al*., 2008). Collectively, both typhoid and paratyphoid fevers are known as enteric fevers. While both diseases share similar clinical features, paratyphoid fever tends to be less severe when compare with typhoid fever. Enteric fever is widespread in the tropical and sub-tropical countries, with its clinical effect

more severe on children and young adults. In developed countries however, enteric fever is common among returning traveller's (Barrett *et al*., 2013). In most developing countries, paratyphoid and typhoid fever is responsible for high rate of morbidity and mortality with an estimated annual global incidence of 5.4 and 21 million cases, with more than 2000 and 700, 000 deaths respectively. The morbidity and mortality rate were however reported to be on the increase due to poor sanitation and hygiene, unavailability of vaccines and a high cost of effective antimicrobials (Deksissa and Gebremedhin, 2019).

Human beings are the only reservoir host for enteric fever. The most important risk factors for the spread of this disease-causing agents are apparently healthy carriers, convalescent and chronic carriers which frequently shed the bacteria into the environment. Bacteria shed by these carriers contaminate food and water which further aid in the consistent spread of the organisms (Tula *et al*., 2018).

The gold standard for the diagnosis of typhoid fever is by stool culture on appropriate media. However, culturing is not routinely carried out in most medical laboratories due to limited bacteriological facilities, trained professionals and longer time required, especially in resources restrained environment (Deksissa and Gebremedhin, 2019). For these reasons, most health facilities in developing countries like Nigeria relied on Widal agglutination test for diagnosing enteric fever. This is because the test is simple, easy, readily available, inexpensive and relatively non-invasive; its diagnostic speed ensures prompt and adequate therapy for the disease. The most prominent drawback of Widal agglutination test is cross-reactions. This occurs when an antibody produced by non-typhoidal antigens reacts with typhoid specific –antigens. Despite the drawback, if properly harnessed and interpreted accurately, widal test still has significant diagnostic utility in providing presumptive diagnosis for typhoid fever (Tula *et al*., 2018).

Optimal antimicrobial treatment of patients with enteric fever depends on an understanding of local patterns of antimicrobial resistance. The emergence of antimicrobial resistance, especially the multidrug resistance (MDR) to ampicillin, chloramphenicol, and cotrimoxazole, is a major public health problem which further complicates the treatment and management of enteric fever (Deksissa and Gebremedhin, 2019).

Therefore, this study is aimed at isolating *Salmonella* enterica from apparently healthy individual tested for *Salmonella* agglutinin and also determining their susceptibility to commonly used antibiotics.

## Materials and Methods

### Study area

The study area was Federal Polytechnic Mubi located in Mubi metropolis, Adamawa State, Nigeria. The institution was established by decree no.33 of 1979 constitution. Mubi metropolis is a geographical area comprising of two local government area; Mubi North and Mubi south. The metropolis is located between latitude $10^0$ 05' and $10^0$ 30' N of the equator and between longitude $13^0$ 12' and $13^0$ 19'E of the Greenwich meridian. The two local government areas occupy a land area of 192,307km2 and support a total population 260,009 people (National population census 2006). The area shares boundary with Maiha L.G.A and Cameroon Republic in the East (Adebayo. 2004).

### Study Design

This was a cross-sectional study included 60 apparently healthy students (30 males and 30 females) who were randomly recruited for the study

### Inclusion and exclusion criteria

Students recruited into this study were apparently healthy, not on drugs, and have not visited medical facilities two month before consent was sought.

### Ethical consideration

Verbal informed consent of each participant was obtained prior to collection of blood samples. Ethical approval was also obtained from the Departmental research and seminar committee board. The individual laboratory result was kept confidential and given to the participant at the completion of the project.

**Collection of blood sample**
Blood sample from 60 apparently healthy students were collected randomly. The blood sample was allowed to clot and centrifuged to obtain serum. The serum collected were immediately used for detection of *Salmonella* agglutinin.

**Widal agglutination test**
SWE-CARE diagnostic febrile antigen kit (Spain) was used for the purpose of this test using the rapid slide screening procedure. The SWE-CARE diagnostic kit is suitable for both rapid slide and tube agglutination test against human sera for the detection of *Salmonella* agglutinins. The stained antigen suspensions are killed bacteria stained to enhance the reading of agglutination test. The blue stained antigens are specific to the somatic 'O' antigens while the red antigens are specific to the flagella (H) antigens. Positive and negative control from the kits was used throughout the experiment to authenticate all the results, especially where the agglutination was faint.

**Stool sample collection and handling**
Stool specimens were collected in sterile container and transported to Microbiology Laboratory, within an hour of collection.

**Isolation of *Salmonella enterica***
A loopful of stool sample was introduced into selenite F broth and was incubated at $37^0$C for 24 hours. Simultaneously a loopful of stool sample suspension was streaked on Salmonella-Shigella agar (SSA) and replicated on Deoxycholate citrate agar (DCA) and then incubated at $37^0$C for 24 hours. Cultures that are pale with hydrogen sulphide production on either SSA or DCA were taken for presumptive *Salmonella* species. The isolates were sub-cultured on nutrient agar slants and kept at refrigeration temperature for further use (Adikwu *et al*., 2021).

**Biochemical Tests**
After Gram-staining, the presumptive *Salmonella* isolates were further screened for gas and hydrogen sulphide production on triple sugar iron (TSI) agar, citrate utilisation, methyl red and Voges-Proskauer production (Qamar *et al*., 2020).

**Antibiotic susceptibility pattern of the *Salmonella enterica* isolates**
The antibiotic susceptibility test of the isolates was determined by the disc diffusion test according to the recommendations of Clinical Laboratory Standard Institute (CLSI). An inoculum of each isolate (approximately 1 x $10^8$ cfu/ml) was developed by using the 0.5 McFarland Standard and aseptically flooded on the surface of sterile Müller – Hinton Agar (MHA).

The antibiotic disc containing the following antibiotics were used: cotrimoxazole (30 µg), ceporex (30 µg), sparfloxacin (10 µg), ciprofloxacin (10 µg), ampicillin (30 µg), amoxicillin-clavulanic acid (30 µg), gentamycin (10 µg), perfloxacin (10 µg), ofloxacin (10 µg), nalidixic acid (30 µg), and streptomycin (30 µg). The antibiotic disc was aseptically placed in the seeded plates and incubated at $37^0$C for 18 – 24 hours. The zone diameter of each antibiotic disc was measured and interpreted using the criteria published by the Clinical Laboratory Standard Institute (CLSI, 2017).

**Statistical analysis**
Data obtained from the study was grouped into frequencies and percentages, and were analysed using non-parametric Mann-Whitney statistics. Values were considered significant when the p-values are $< 0.05$. All data were analysed using the statistical package for social sciences (SPSS) 20.0.

**Result**
From the tested 60 serum sampled, 52 (82.7%) were found to be Widal positive with at least one of the tested antigens (Table 1). From these, 26 (86.7%) each were recorded among male and female students respectively.

Table 2 showed the distribution of *Salmonella* agglutinin titres in 30 male subjects. The findings showed that only one serum was positive for *Salmonella* Paratyphi BH, and that serum had a titre of 1:320. Also, 13(81.3%) sera were positive for *Salmonella* Typhi H with titre of 1:320. For the titre of 1:160, the most common *Salmonella* agglutinin belongs to Paratyphi BO with 60% sera positive, while the least was *S*. Typhi H with 18.8% sera positive. Also, the percentage of the positive titres 1:320 and 1:160 in males were not significantly different (p=0.139).

Table 3 showed the distribution of *Salmonella* agglutinin titres in 30 female subjects. The findings showed that the most common *Salmonella* agglutinin belong to Typhi H (46.7%) and Paratyphi AO (43.3%), while the least belong to Paratyphi AH (20.0%). For titre of 1:320, the most common agglutinin belongs to Paratyphi CH (77.8%), while the least was Paratyphi CO (25.0%). Also, for titre 1:160, the most common agglutinin belongs to Paratyphi BO (72.7%), while the least was Paratyphi CH (22.2%). Also, the percentage of the positive titres 1:320 and 1:160 in females were not significantly different (p=0.382).

From the 52 subjects that were seropositive for *Salmonella* agglutinin and consented to give their stool; 26 (86.7%) each were from male and female subjects. From these, *Salmonella enterica* was isolated in 11(42.3%) males and 7(26.9%) females, with a combined prevalence of 18(34.6%) as shown in Table 4.

The susceptibility profile of *Salmonella enterica* for male gender was shown in Table 5. *Salmonella enterica* was more sensitive to ciprofloxacin (81.8%) and ofloxacin (81.8%) and least to nalidixic acid (45.5%). More so, *Salmonella enterica* in this study, was more resistant to ceporex (63.6%), and least to ciprofloxacin and ofloxacin with 18.2% each.

The susceptibility profile of *Salmonella enterica* for female gender was shown in Table 6. *Salmonella enterica* was more sensitive to gentamycin, augmentin, ofloxacin, streptomycin, and cotrimoxazole with 71.4% susceptible rate each. Whereas, the resistance pattern shows that *S. enterica* was more resistant to nalidixic acid (85.7%) and ampicillin (71.4%) as shown in Table 6.

The MDR status of the *Salmonella enterica* was shown in Table 7. The result showed that 11 of the *Salmonella enterica* exhibits Multidrug resistance phenotype, accounting for the prevalence of 64.7%. From these, the MDR phenotype was more in female than male but with no statistical difference (p=0.569).

**Table 1: Prevalence of Widal positive sera (Salmonella agglutinin titre) based on gender**

| Gender | No. (%) of sera tested | No. (%) of widal positive | No. (%) of widal negative |
|---|---|---|---|
| Male | 30(50%) | 26(86.7) | 4(13.33) |
| Female | 30(50%) | 26(86.7) | 4(13.33) |
| Total | 60(100%) | 52(86.7) | 8(13.33) |

**Table 2: Number (%) and distribution of sera with agglutinin end titre among male students**

| Salmonella antigen | No of sera tested | No (%) of widal positive sera | Titre 1:320[a] | Titre1:160[a] |
|---|---|---|---|---|
| S. Paratyphi A-O | 30 | 11(36.7) | 5(45.5) | 6(54.5) |
| S. Paratyphi B-O | 30 | 10(33.3) | 4(40.0) | 6(60.0) |
| S. Paratyphi C-O | 30 | 4(13.3) | 2(50.0) | 2(50.0) |
| S. Typhi O | 30 | 15(50.0) | 8(53.3) | 7(46.7) |
| S. Paratyphi A-H | 30 | 6(20.0) | 3(50.0) | 3(50.0) |
| S. Paratyphi B-H | 30 | 1(3.3) | 1(100) | 0 |
| S. Paratyphi C-H | 30 | 14(46.7) | 10(71.4) | 4(28.6) |
| S. Typhi H | 30 | 16(53.3) | 13(81.3) | 3(18.8) |

***Legend:*** *Values with the same superscripts from each parameter are not significantly different (P>0.05).*

**Table 3: Number (%) and distribution of sera with agglutinin end titre among female students**

| Salmonella antigen | No of sera tested | No (%) of widal positive sera | Titre 1:320[a] | Titre 1:160[a] |
|---|---|---|---|---|
| S. Paratyphi A-O | 30 | 13(43.3) | 6(46.2) | 7(53.8) |
| S. Paratyphi B-O | 30 | 11(36.7) | 3(27.3) | 8(72.7) |
| S. Paratyphi C-O | 30 | 8(26.7) | 2(25.0) | 6(75.0) |
| S. Typhi O | 30 | 12(40.0) | 4(33.3) | 8(63.7) |
| S. Paratyphi A-H | 30 | 6(20.0) | 2(33.3) | 4(66.7) |
| S. Paratyphi B-H | 30 | 8(26.7) | 5(62.5) | 3(37.5) |
| S. Paratyphi C-H | 30 | 9(30.0) | 7(77.8) | 2(22.2) |
| S. Typhi H | 30 | 14(46.7) | 8(57.1) | 6(42.9) |

***Legend:*** *Values with the same superscripts from each parameter are not significantly different (P>0.05),*

**Table 4: Number of stool positive for *Salmonella enterica* from widal positive subjects**

| Gender | No (%) of stool tested | No (%) of stool positive |
|---|---|---|
| Male | 26(86.7) | 11(42.3) |
| Male | 26(86.7) | 7(26.9) |
| Total | 52(86.7) | 18(34.6) |

**Table 5: Susceptibility pattern of *Salmonella enterica* isolated from stool of volunteered apparently healthy male students**

| Antibiotics | No (%) of isolates | No (%) sensitive | No (%) resistant |
|---|---|---|---|
| Gentamycin | 11(42.3) | 7(63.6) | 4(36.4) |
| Augmentin | 11(42.3) | 8(72.7) | 3(27.3) |
| Ciprofloxacin | 11(42.3) | 9(81.8) | 2(18.2) |
| Ofloxacin | 11(42.3) | 9(81.8) | 2(18.2) |
| Ceporex | 11(42.3) | 4(36.4) | 7(63.6) |
| Streptomycin | 11(42.3) | 8(72.7) | 3(27.3) |
| Cotrimoxazole | 11(42.3) | 7(63.6) | 4(36.4) |
| Ampicillin | 11(42.3) | 7(63.6) | 4(36.4) |
| Nalidixic acid | 11(42.3) | 5(45.5) | 6(54.5) |
| Pefloxacin | 11(42.3) | 8(72.7) | 3(27.3) |

**Table 6: Susceptibility pattern of *Salmonella enterica* isolated from stool of volunteered apparently healthy female students**

| Antibiotics | No (%) of isolates | No (%) sensitive | No (%) resistant |
|---|---|---|---|
| Gentamycin | 7(26.9) | 5(71.4) | 2(28.6) |
| Augmentin | 7(26.9) | 5(71.4) | 2(28.6) |
| Ciprofloxacin | 7(26.9) | 4(57.1) | 3(42.9) |
| Ofloxacin | 7(26.9) | 5(71.4) | 2(28.6) |
| Ceporex | 7(26.9) | 4(57.1) | 3(42.9) |
| Streptomycin | 7(26.9) | 5(71.4) | 2(28.6) |
| Cotrimoxazole | 7(26.9) | 5(71.4) | 2(28.6) |
| Ampicillin | 7(26.9) | 2(28.6) | 5(71.4) |
| Nalidixic acid | 7(26.9) | 1(14.3) | 6(85.7) |
| Pefloxacin | 7(26.9) | 4(57.1) | 3(42.9) |

**Table 7: Resistance profile of Salmonella isolates**

| SN | Gender | Resistance profile | MDR status |
|---|---|---|---|
| 1 | **Female** | CPX, SXT, S, PN, CEP, OFX, NA, PEF. | MDR |
| 2 | | CN, CPX, OFX, NA, PEF | MDR |
| 3 | | AU, SXT, PN, NA | MDR |
| 4 | | CN, AU, CPX, CEP | MDR |
| 5 | | PN, NA, PEF. | MDR |
| 6 | | NA, PN, S. | MDR |
| 7 | | NA, CEP | - |
| 8 | **Male** | PN, CEP, OFX, NA, PEF, CN, AU, CPX, SXT, S. | MDR |
| 9 | | SXT, S, PN, CEP, NA, CN, AU | MDR |
| 10 | | CN, AU, CPX, CEP, NA, SXT | MDR |
| 11 | | S, CN, CEP, NA, PEF. | MDR |
| 12 | | NA, CN, CEP, PN | MDR |
| 13 | | SXT, CEP | - |
| 14 | | CEP | - |
| 15 | | CEP | - |
| 16 | | PN | - |
| 17 | | NA | - |

**Key:**
*PN = ampicillin*
*CEP = ceporex*
*OFX = ofloxacin*
*NA = nalidixic acid*
*PEF = pefloxacin*
*CN = gentamycin*
*AU = augmentin*
*CPX = ciprofloxacin*
*SXT = cotrimoxazole*
*S = streptomycin*

**Discussion**

Though the preferred laboratory procedure for the diagnosis of enteric fever is culture based; however, most clinics and hospitals in developing countries do not have ready access to this method. Widal agglutination tests are widely used in many developing countries, including Nigeria, as an alternative laboratory procedure for diagnosis of enteric fever.

In this study, 82.7% of sera gave positive widal reactions. This indicates a high prevalence of typhoid/paratyphoid agglutinins in the sampled population. This observation was above the 60% prevalence rate reported in the same environment in 2017 (Tula *et al*., 2018).

A high prevalence of positive Widal reaction similar to the one of this study were also reported in various studies in Nigeria (Ibekwe *et al*., 2008; Okonko *et al*., 2010; Abalaka *et al*., 2013). The possible explanations for the high prevalence of *Salmonella* Typhi and Paratyphi titres among apparently healthy individuals may be that the bacilli are persistent in the human host at sub-clinical levels (Teddy *et al*., 2010; Tula *et al*., 2018). It may also be due to the repeated subclinical infections with either of *Escherichia*, *Shigella*, *Citrobacter* or *Proteus* species which shared common `O´ or `H´ antigens with *Salmonella* spp (Ezzi *et al*., 2014; Tula *et al*., 2018). Alternatively, other individuals may have developed tolerance to frequent exposure of small inoculum of *Salmonella* Typhi and Paratyphi (Teddy *et al*., 2010) which may lead to a high rate of asymptomatic infections. However, most of the subjects may not be having the active diseases. This was in agreement with the previous observation (Adeleke *et al*., 2006).

In this study, the widal positive reactions was same in both male and female subjects. This observation was contrary to previous report in the same study area that sera from males were more Widal positive than females (Tula *et al*., 2018). It was also contrary to the study conducted in Owerri, that reported higher prevalence rate among female than male (Ibegbulam *et al*., 2014).

The relatively high isolation of *Salmonella enterica* among apparently healthy male subjects with positive widal reaction in this study may be comparable to the report of previous studies (Beyene and Tasew, 2014; Aklilu *et al*., 2015; Mama and Alemu, 2016). Also, the low detection of *Salmonella enterica* among apparently healthy female subjects with positive widal reaction was similar to the report of previous study (Deksissa, and Gebremedhin, 2019). The differences in prevalence rate could be due to difference in cultural and hygienic practices.

In this study, *Salmonella* isolates showed relative high rate of resistance to a number of commonly used antimicrobial drugs such as ampicillin, ceporex, nalidixic acid, ciprofloxacin. This was similar to the report of previous study in Benue State (Adikwu *et al*., 2021) and in Ethiopia (Aklilu *et al*., 2015; Deksissa, and Gebremedhin, 2019). However, the susceptibility of *Salmonella* isolates in this study to most of the fluoroquinolone antibiotics and augmentin shows that these classes of antibiotics could be considered for treatment of enteric fever in the study area.

Multidrug resistance has been defined as resistance to at least one antibiotic in not less than 3 classes of antibiotics (Adikwu *et al*., 2021). This study reports a 64.7% multidrug resistance rate in *Salmonella enterica* isolates from apparently healthy students of Federal Polytechnic Mubi, Adamawa State. High rate of MDR in *Salmonella* Typhi similar to the one observed in this study was reported in Benue State (Adikwu *et al*., 2021), Warri, Delta State (Ehewarieme, 2011), and in Nepal (Poudel *et al*., 2014). Higher MDR in female than male subjects as shown in this study corroborates report from previous study in Benue State, Nigeria (Adikwu *et al*., 2021). However, earlier studies in countries other than Nigeria reported higher MDR in male than female subjects in their studies (Gupta *et al*., 2013; Mannan *et al*., 2014). Higher MDR in female than male as observed in this study could be attributed to female involvement in practices and activities that exposes them to sources of infections with MDR *Salmonella enterica* isolates. However, lack of significance difference between the male and female suggest that both genders are equally exposed to sources of infection with MDR *Salmonella*. More so, the high rate of MDR

*Salmonella enterica* in the study population could be attributed to inappropriate use of antibiotics occasioned by self-medication, ignorance, over the counter availability of antimicrobials, poverty, and wrong prescriptions from low-grade health professionals. The implication is that at any time the infectious dose of the typhoid and paratyphoid causing bacteria is high enough to cause significant infections or complications, the individuals concerned are likely to have a prolonged fever clearance time and high rates of treatment cost or failure (Crump *et al*., 2008). This is of grave consequences because studies have shown that death rate due to MDR *S*. Typhi is 21 times higher than individuals infected with non-antibiotic resistant strains (Kariuki *et al*., 2000; Helms *et al*., 2003; Adikwu *et al*., 2021).

## Conclusion

The findings of this study established that *Salmonella* agglutinins are common among apparently healthy individuals with variable Widal agglutination titre. The results also shows that *Salmonella enterica* was not isolated in some subjects with positive widal sera. As such, the result in this study depicts the limitations of widal test in the diagnosis of enteric fever. More so, the rate of multi-drug resistance observed in *Salmonella enterica* isolates of this study is alarming and constitute public health concern.

## Conflict of interest declaration

Authors have declared no conflict of interest

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