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In-vitro antibiotic susceptibility profile of *Salmonella enterica* Serovar Typhi isolated from fecal specimens of humans in Umuahia metropolis, Abia State, Nigeria

Emmanuel O. Ekundayo[1, 2*] and Emmanuel Enya[2]

[1]Centre for Molecular Biosciences and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.
[2]Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

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Typhoid is routinely diagnosed in Nigeria on clinical grounds or based on Widal serological test result. This approach does not provide information on the antibiotic susceptibility of the bacterium; *Salmonella enterica* Serovar Typhi. This study was done to assess the antimicrobial susceptibility profile of *Salmonella* isolates in Umuahia. In this study, seventy-two (72) *Salmonella* isolates obtained from 135 fecal specimens were tested for their antibiotic susceptibility profile against 13 antimicrobial agents using the disk diffusion method according to the protocol of the Clinical and Laboratory Standards Institute (CLSI). Sixty-two (86.1%) of the isolates were resistant to Amoxicillin, fifty-two (72.2%) to Trimethoprim-sulfamethoxazole (Co-trimoxazole), forty-one (56.9%) to Chloramphenicol and fifty-two (72.2%) to Augmentin. Fifty-nine (81.9%) of the isolates were resistant to Tetracycline, 11(15.2%) were of intermediate susceptibility and only two (2.7%) were susceptible to the antibiotic. Thirty-five (48.6%) of the isolates were sensitive to Ciprofloxacin, forty-seven (65.2%) to ofloxacin and forty-three (59.7%) to gentamicin. the resistance profile of the isolates to cephalosporin antibiotics was as follows: cefuroxime (70.8%), ceftriaxone (68.0%) and ceftazidime (65.2%). Some of the isolates exhibited resistance to multiple antibiotics ranging from three or more of the antibacterial agents tested. The results obtained suggest that high proportion of S. *Typhi* strains circulating in the study area are resistant to multiple antibiotics and empirical treatment of typhoid fever without antibiotic susceptibility testing is not advisable in this setting.

Key words: *Salmonella typhi*, antimicrobial resistance, typhoid fever, antibiotic susceptibility profile, Umuahia

INTRODUCTION

Typhoid fever is a life-threatening systemic febrile illness caused by the bacterium *Salmonella enterica* Serovar Typhi (S. *Typhi*). Typhoid fever is a global public health problem with an estimated 21.7 million illnesses and

*Corresponding author. E-mail: ekundayo.emmanuel@mouau.edu.ng.

217,000 deaths worldwide in 2000 (Crump and Mintz, 2004). Antibiotic therapy has been the mainstay of management of typhoid fever but strains resistant to commonly used antibiotics have emerged and are spreading around the world (Zaki and Karande, 2011). Nigeria falls within the region of estimated high incidence of typhoid fever in Sub-Sahara Africa (Von Kalckreuth et al., 2016). The co-endemicity of typhoid fever along with other non-bacterial febrile infections like malaria and viral diseases makes direct clinical diagnosis difficult (Enabulele and Awunor, 2016).

For confirmation of diagnosis of typhoid fever and for effective antibiotic therapy, there is a need to isolate S. *Typhi* and carry out antibiotic susceptibility testing. Isolation of S. *Typhi* from culture of bone marrow aspirate is regarded as the gold standard for confirmation of typhoid fever but blood culture is preferred because obtaining blood specimen is easier and less invasive (Bhutta, 2006). Blood culture is slightly less sensitive than bone marrow aspirate culture (Tanyigna et al., 2001). Stool culture can be done as an alternative to blood culture. However, culture for isolation of S. *Typhi* is rarely done in low income countries due to high cost, lack of equipment and technical expertise. As a result, data on the prevalence and pattern of resistance to antimicrobials among strains of S. *Typhi* are limited in low income countries (Von Kalckreuth et al., 2016). Available data indicate that strains of S. *Typhi* resistant to multiple antibiotics have been isolated in some parts of Nigeria (Akinyemi et al., 2014).

In Nigeria, typhoid fever is typically diagnosed on clinical grounds or based on the results of Widal serological test and antibiotic treatment is done empirically with no certainty about the sensitivity of the infecting pathogen. Widal test has the problem of low sensitivity and specificity with positive predictive values (PPV) around 17-20% (Tanyigna et al., 2001; Enabulele and Awunor, 2016). In view of the low PPV of Widal test and the uncertainty associated with the antibiotic susceptibility profile of S. *Typhi* strains, many patients are likely to receive inappropriate antibiotic therapy in the absence of culture and antibiotic susceptibility testing.

Increasing rates of reduced susceptibility to ciprofloxacin commonly used in cases of suspected resistance to first line antimicrobials have been reported (Harrois et al., 2014; Lunguya et al., 2013; Kariuki et al., 2010, Akinyemi et al., 2007). Antibiotic susceptibility surveillance systems can provide insights into the areas where resistance is most prevalent and where the prevalence of resistance is increasing the fastest. Determination of antimicrobial susceptibility patterns not only helps shape successful treatment plans for individual patients but also assists with the development of public health policy for control of antimicrobial resistance. The aim of this study was to determine the antibiotic susceptibility profile of strains of *Salmonella* isolated from fecal specimens of patients in Umuahia.

MATERIALS AND METHODS

Specimen collection and inoculation

Fecal specimens were collected from patients attending four different clinics in Umuahia metropolis, the Capital of Abia State. Each of the patients that submitted specimens gave an informed consent. Only one fecal specimen was collected per participant. The management of each of the clinics gave approval for the collection of specimens. The specimens were inoculated within 24 h of collection into Selenite Faeces broth (Titan Biotech Ltd, India) for enrichment and incubated at 37°C for 18 - 24 h and subsequently plated on Salmonella-Shigella agar (Titan Biotech Ltd, India) for primary isolation. The culture plates were incubated aerobically at 37°C for 24 h and observed for growth through the formation of colonies. Colonies growing on the plates were purified by streak plate method on nutrient agar and subsequently maintained on nutrient agar slants.

Biochemical characterization of isolates

Standard microbiological techniques were performed for biochemical characterization of the isolates. Biochemical tests carried out according to standard procedures as described in Cheesbrough (2006), included Indole, Citrate, Urease, Methyl Red, Voges Proskauer and Triple Sugar Iron tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2015) on Mueller Hinton agar (Hardy Diagnostics, USA). Discrete colonies from a 24 h nutrient agar plates were suspended in sterile normal saline in a test tube to achieve a bacterial suspension equivalent to 0.5 McFarland Turbidity Standard**.** A cotton swab was dipped into the bacterial suspension and used to inoculate the entire surface of a Mueller Hinton agar plate, rotating the plate to ensure confluent growth of the bacterium. The antimicrobial susceptibility disks were placed on the surface of the inoculated plate with flame sterilized forceps. The plates were incubated in an inverted position for 16–18 h at 35-37°C. The diameter of the zone of inhibition produced by the antibiotic disks were measured to the nearest millimeter (mm) using a transparent ruler.

Data and statistical analysis

The criteria for categorizing the diameter of zones of inhibition into sensitive (S) or intermediate (I) or resistant (R) were based on the interpretive charts of the Clinical and Laboratory Standards Institute (CLSI, 2015). Proportions of males and females and resistant isolates were compared using the Pearson's approximation to the Chi-squared test. The significance level was tested at $\alpha = 0.05$.

RESULTS

Fecal specimens were collected from a total of 135 study participants, made of 59 (43.7%) males and 76 (56.3%) females. The demographic characteristics of the study participants and the number of *Salmonella* isolates recovered according to gender and age groups are shown in Table 1. Difference between the number of

Table 1. Demographic characteristics of study participants and isolation of *Salmonella* from fecal specimens.

Characteristics	No. (%) of fecal specimens	No. yielding *Salmonella* isolates	Isolation rate (%)
Sex			
Male	59 (43.70)	30	22.20
Female	76 (56.30)	42	31.10
Total	135 (100)	72	53.30
Age groups (years)			
10-20	14 (10.40)	9	6.67
21-30	63 (46.70)	39	28.9
31-40	30 (22.20)	12	8.99
41-50	15 (11.10)	9	6.67
51-60	11 (8.00)	3	2.22
>61	2 (1.50)	0	0.00

Table 2. Antimicrobial agents tested and criteria for assessment of susceptibility of *Salmonella* isolates.

Antimicrobial agents (code)[a]	Potency (µg)	Inhibition zone (mm) cut-off values*		
		(S)	(I)	(R)
Gentamicin (GEN)	10	≥15	13-14	≤12
Chloramphenicol (CHL)	30	≥18	13-17	≤11
Ofloxacin (OFL)	30	≥16	13-15	≤12
Augmentin (AUG)	30	≥18	14-17	≤13
Ciprofloxacin (CPR)	5	≥21	16-20	≤15
Amoxicillin (AMX)	25	≥18	14-15	≤16
Cefuroxime (CXM)	30	≥18	15-17	≤14
Cotrimoxazole (COT)	25	≥16	11-15	≤10
Ceftazidime (CAZ)	30	≥21	18-20	≤17
Nitrofurantoin (NIT)	100	≥17	15-16	≤14
Ceftriaxone (CRO)	30	>23	20-22	<19
Tetracycline (TET)	30	≥15	12-14	≤11
Streptomycin (STR)	25	≥15	12-14	≤11

*Interpretative break points according to Clinical Laboratory Standards Institute, CLSI (2015). **a**, Abtek ™ Biologicals, UK.

males and females that submitted fecal specimens was not significantly different ($p>0.05$). The difference between the number of *Salmonella* isolates recovered from males and females was however, significantly different ($p<0.01$). *Salmonella* was isolated from 53.3% of the total number of participants who submitted fecal specimens.

The antimicrobial agents tested and the interpretative criteria for assessment of the susceptibility of *Salmonella* based on the diameter of zones of inhibition of each antimicrobial agent are presented in Table 2. The antibiotic susceptibility profile showing the proportions of Sensitive, Intermediate and Resistant categories is presented in Figure 1. The isolates exhibited high resistance level to amoxicillin, augmentin, trimethoprim sulfamethoxazole (co-trimoxazole) tetracycline, chloramphenicol and the second and third generations cephalosporins. Sensitivity of the isolates to ofloxacin and gentamicin was moderate. Greater than 30% of the isolates have reduced sensitivity (intermediate category) to Chloramphenicol and Ciprofloxacin.

The isolates were assessed for resistance to multiple antimicrobial agents, from combination of three agents to seven. The results are shown in Table 3. Six patterns of resistance to combination of three antimicrobial agents were identified. About 52% of the isolates were resistant to combinations of three different types of antimicrobial agents and about 35% were resistant to combinations of four antimicrobial agents. The commonest multidrug resistant (MDR) phenotypes are amoxicillin, chloramphenicol and tetracycline (14 isolates), followed by amoxicillin, chloramphenicol, tetracycline and co-trimoxazole (12 isolates). The classical *Salmonella* MDR phenotype defined by resistance to amoxicillin,

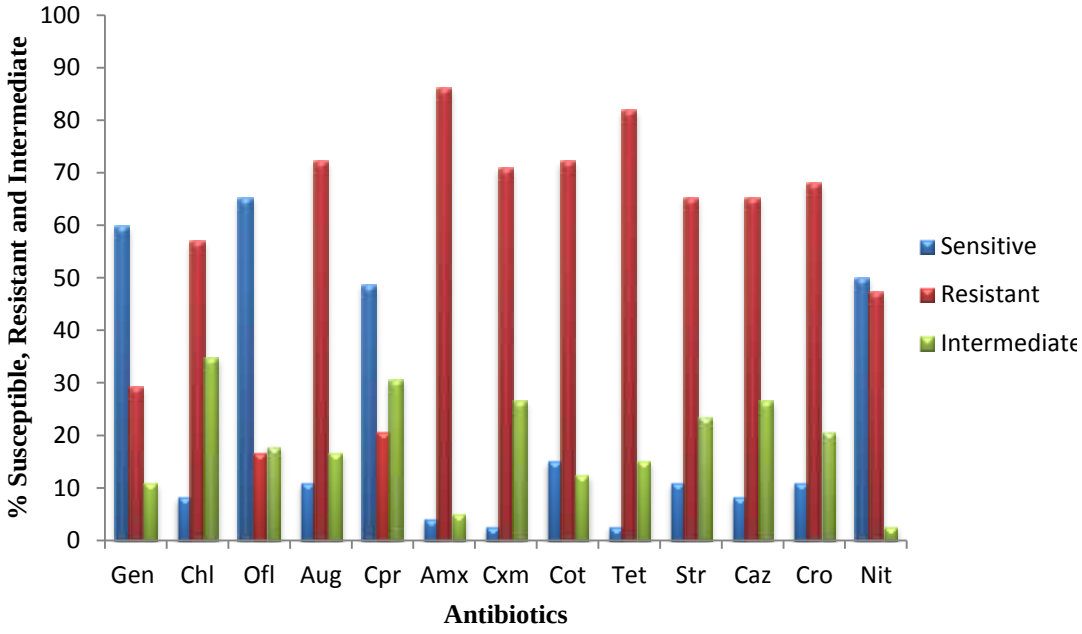

Figure 1. Antimicrobial susceptibility profile of salmonella isolates in Umuahia. Gentamicin (Gen), chloramphenicol (Chl), ofloxacin (Ofl), augmentin (Aug), ciprofloxacin (Cpr), amoxicillin (Amx), cefuroxime (Cxm), ceftriaxone (Ctx), tetracycline (Tet), streptomycin (Str), ceftazidime (Caz), cotrimoxazole (Crx), nitrofurantoin (Nit).

Table 3. Multiple antibiotic resistance profile of the isolates.

Antibiotics	No. of antibiotics	No. of antibiotic resistant isolates	Overall (%)
AXM, CHL, COT	3	9	
AMX, CHL, TET	3	14	
AMX, TET, GEN	3	1	38 (52.8)
AMX, TET, CPR	3	3	
AMX, CHL, CPR	3	2	
AMX, AUG, COT	3	9	
AMX, CHL, COT, TET	4	12	
AMX, AUG, GEN, CAZ	4	3	
AMX, AUG, OFL, COT	4	1	23 (31.9)
AMX, CHL, COT, CPR	4	4	
AMX, AUG, GEN, CAZ	4	2	
AMX, AUG, OFL, COT	4	1	
AMX, AUG, GEN, OFL, CHL	5	1	
AMX, TET, CRO, OFL, CHL	5	2	
AMX, CPR, CRO, OFL, NIT	5	1	4 (6.15)
AMX, CPR, COT, TET, NIT, GEN	6	1	
AMX, COT, CAZ, CRO, AUG, CPR	6	5	8 (12.30)
AMX, AUG, CRO, OFL, NIT, GEN	6	2	
AMX, AUG, CRO, CPR, NIT, STR, CHL	7	1	1 (1.53)
Number of MDR Patterns	**18**	**72**	**90.3%**

Gentamicin (GEN), chloramphenicol (CHL), ofloxacin (OFL), augmentin (AUG), ciprofloxacin (CPR), amoxillin (AMX), cefuroxime (CXM), ceftriaxone (CRO), tetracycline (TET), streptomycin (STR), ceftazidime (CAZ), cotrimoxazole (COT), nitrofurantoin (NIT).

chloramphenicol and co-trimoxazole was observed in nine isolates. The number of isolates resistant to amoxicillin, chloramphenicol and ciprofloxacin was relatively small (only two isolates).

DISCUSSION

From the 135 fecal specimens cultured, 72 yielded *Salmonella* organisms representing an isolation rate of 53.3%. This is similar to that of Duthie and French (1990) who reported an isolation rate of 59%. The sensitivity of stool culture is believed to be lower than that of blood culture which is recommended as the gold standard for confirmation of diagnosis of typhoid (Tanyigna et al., 2001; Ameya et al., 2017). However, blood culture is more difficult and requires more expensive laboratory infrastructure and greater technical expertise (Von Kalckreuth et al*.,* 2016). Although, stool culture may not give accurate incidence rate, the results of this study suggest that stool culture can yield adequate number of isolates for monitoring antimicrobial susceptibility profile of *Salmonella* from humans. In this study, we found a significant difference between number of isolates from male and female study participants. This is consistent with the findings of others (Ja'afar et al., 2013; Yaseen et al., 2017). The explanation for this is beyond the scope of the present work. It could be that the females are more susceptible to infection with *Salmonella* or more exposed to sources of infection. This requires further epidemiological study.

In this study, the antibiotic susceptibility profile of *Salmonella* isolates from fecal specimens of males and females indicate that strains of *Salmonella* resistant to first line drugs are common in the study area. However, we did not find strains that were completely resistant to all the antimicrobial agents. The isolates with multidrug resistant (MDR) phenotype were still highly susceptible to ciprofloxacin although considerable number of strains has developed resistance to ciprofloxacin without the MDR phenotype. The prevalence of isolates with reduced susceptibility to ciprofloxacin is also high. Resistance to the fluoroquinolones typically evolves in a stepwise fashion (Rahman et al., 2014). This means that prolonged and continuous abuse of this antibiotic could lead to full resistance to the antibiotic. This will greatly limit the treatment options for the isolates that have developed resistance to the first line drugs. The susceptibility of the isolates to ofloxacin and gentamicin stood at 65.2 and 59.7%, respectively. These antibacterial agents are not commonly used for treatment of typhoid but in an environment where self-medication is rampart and abuse of antibiotics is common, the organisms might have acquired resistance to these agents under antibiotic pressure or through horizontal transfer of resistance genes.

The results of this study indicate that *Salmonella* isolates from humans have developed resistance to common antibiotics such as amoxicillin, chloramphenicol, co-trimoxazole and this resistance has also spread to the fluoroquinolones. The decrease in the susceptibility of the *Salmonella* isolates to ofloxacin and ciprofloxacin has been reported in other parts of Nigeria (Akinyemi et al., 2014). Increased MDR has been reported among *Salmonella* isolates in many countries including Iran (Butaye et al., 2006) and Ethiopia (Zewdu and Cornelius, 2009). A previous study in Nigeria by Akinyemi et al. (2007) had reported a multi-drug resistance rate of 61.0%. The result of this study, however, revealed a comparatively high proportion of multidrug resistance with a prevalence rate of up to 90.3%.

The prevalence of multi-drug resistance reported in this study reveals a limitation of an effective treatment of human *Salmonella* infection with commonly used antibiotics. The antimicrobial susceptibility profile of *Salmonella* isolates from humans reported in this study suggests that effective treatment of typhoid fever cannot likely be achieved without antibiotic susceptibility testing of individual isolates. Therefore, diagnosis of typhoid fever by non-cultural methods and empirical antibiotic therapy without information on the antibiotic susceptibility of the causative agents are no longer appropriate. In addition, this study indicates a need for continuous monitoring of antibiotic susceptibility pattern of *Salmonella* isolates in Umuahia and the neighborhood.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Akinyemi KO, Bamiro BS, Coker AO (2007). Salmonellosis in Lagos, Nigeria: Incidence of *Plasmodium falciparum*-associated co-infection, patterns of antimicrobial resistance, and emergence of reduced susceptibility to fluoroquinolones. Journal of Health, Population and Nutrition 25(3):351-358.

Akinyemi KO, Iwalokun BA, Agboyinu JA, Ogunyemi O, Fasure AK (2014). Emergence of Third Generation Cephalosporin Resistance and Typing by Randomly Amplified Polymorphic DNA (RAPD) among Clinical *Salmonella* Isolates from Lagos, Nigeria. British Microbiology Research Journal 4(6): 668-677.

Ameya G, Atalel E, Kebede B, Yohanne B (2017). Comparative Study of Widal test against stool culture for typhoid fever suspected cases in Southern Ethiopia. Pathology and Laboratory Medicine International 9:1-7.

Bhutta ZA (2006). Current concepts in the diagnosis and treatment of typhoid fever. British Medical Journal 333:78-82.

Butaye P, Michael GB, Schwarz S, Barrett TJ, Brisabois A, White DG (2006). The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. Microbes and Infection 8:1891-1897.

Cheesbrough M (2006). District Laboratory Practice in Tropical Countries (Part 2). Cambridge University Press Edinburgh, UK. pp. 62-70.

CLSI (2015). Performance standards for Antimicrobial Susceptibility Testing Twenty Fifth Informational Supplement. CLSI document M100-S25 Wayne, PA: Clinical and Laboratory Standards Institute.

Crump JA, Mintz ED (2004). The global burden of typhoid fever. Bulletin of the World Health Organization 82(5):346-353.

Duthie R, French GL (1990). Comparison of methods for the diagnosis of typhoid fever. Journal of Clinical Pathology 43:863-865.

Enabulele O, Awunor SN (2016). Typhoid fever in a Tertiary Hospital in Nigeria: Another look at the widal agglutination test as a preferred option for diagnosis. Nigerian Medical Journal 57(3):145-149

Harrois D, Breurec S, Seck A, Delaune A, LeHello S, Pardosde la Gándara M (2014). Prevalence and characterization of extended-spectrum beta-lactamase producing clinical *Salmonella enterica* isolates in Dakar, Senegal, from 1999 to 2009. Clinical Microbiology and Infection 20:109-116.

Ja'afar NJ, Goay YX, Zaidi NFM, Low HC, Husain HM, Hamzah WM, Bhore SJ Balaram P, Ismail A, Phua KK (2013). Epidemiological analysis of typhoid fever in Kelantan from a retrieved registry Malaysian Journal of Microbiology 9(2):147-151.

Kariuki S, Revathi G, Kiiru J (2010). Typhoid in Kenya is associated with a dominant multidrug-resistant *Salmonella enterica* serovar Typhi haplotype that is also widespread in Southeast Asia. Journal of Clinical Microbiology 48:2171-76.

Lunguya O, Lejon V, Phoba MF, Bertrand S, Vanhoof R, Glupczynski Y, Verhaegen J, Muyembe-Tamfum JJ, Jacobs J (2013). Antimicrobial resistance in invasive non-typhoid *Salmonella* from the Democratic Republic of the Congo: emergence of decreased fluoroquinolone susceptibility and extended-spectrum beta lactamases. PLoS Neglected Tropical Diseases 7(3):e2103.

Rahman BA, Wasfy MO, Maksoud MA, Hanna N, Dueger E, House B (2014). Multi-drug resistance and reduced susceptibility to ciprofloxacin among Salmonella enterica serovar Typhi isolates from the Middle East and Central Asia. New Microbe and New Infections 2:88-92.

Tanyigna KB, Bello CS, Okeke N, Onwukeme KE (2001). Comparison of blood, bone marrow aspirate, stool and urine cultures in the diagnosis of enteric fever. Nigerian Journal of Medicine 10:21-24.

Von Kalckreuth V, Konings F, Aaby P, Adu-Sarkodie Y, Ali M, Aseffa A et al. (2016). The typhoid fever surveillance in Africa Programme (TSAP): Clinical, diagnostics, and epidemiological methodologies. Clinical Infectious Diseases 62(1): S9-S16.

Yaseen KM, Abdul J, Afshan S, Kifayat U, Ur RH, Osman A, Nasir H, Adeel A (2017). Endemic study of typhoid fever between male and female population of Ali Pur, Islamabad. BioMedicine and Surgery 1(3):105-108.

Zaki SA, Karande S (2011). Multidrug resistant typhoid fever. A review. The Journal of Infection in Developing Countries 5:324-337.

Zewdu E, Cornelius P (2009). Antimicrobial resistance of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. Tropical Animal Health and Production 41:241-249.