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Detection of multidrug resistant *Salmonella typhi* in Sokoto metropolis

YM Zayyan [1*], SK Ahmad [2], ZR Maigandi [3], A Bello [4]
[1-3] Department of Microbiology, Sokoto State University, Sokoto State, Nigeria
[4] Department of Microbiology, Federal University Birnin Kebbi, Kebbi State, Nigeria

* Corresponding Author: **YM Zayyan**

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Abstract
Antibiotic resistance is a global health concern as it is one of the biggest threats to global health, food security and development today. Many different infectious diseases that were once easily treated with different brands of antibiotics are becoming more difficult to treat. The world Health Organization (WHO) estimated that around 700,000 people die yearly around the globe from diseases caused by bacteria that have developed antibiotic resistance and that if the current trend persists, the problem could kill 10 million people every year by 2050, making antimicrobial resistance more dangerous than diabetes, tuberculosis and HIV AIDS combined. Typhoid fever is prevalent in Nigeria and its taking a toll on the overall socioeconomic well-being of the populace. Data on antibiotic resistance pattern is very limited due to lack of comprehensive surveillance strategies The problem needs to be addressed urgently through research and development, as the country has one of the least comprehensive surveillance strategies for antibiotic resistance of all world regions alongside scarce infection prevention and control programs. This study aimed to detect multidrug resistant (MDR) salmonella typhi. A total of 150 patients blood samples from both males and females were collected and analysed. Salmonella isolates were confirmed and among them 5 were confirmed to be *Salmonella enterica* serovar *typhi* using biochemical and serological methods. Kirby Bauer disc diffusion antibiotics susceptibility testing was performed and it revealed the salmonella isolates exhibit multidrug resistance (MDR) with tetracycline showing the highest resistance (100%) followed by ampicillin and amoxicillin at 83.3% and 66.67% respectively. Lowest resistance was observed against ciprofloxacin and gentamicin while the isolates were moderately resistant to ofloxacin and doxycycline.

Keywords: Antibiotics, Resistance, Salmonella, Surveillance

Introduction
Antibiotics have been called as the single most important therapeutic discovery in the history of medicine, as their discovery led to the treatment of a variety of bacterial infections that were otherwise untreatable. They are a type of antimicrobials that are specifically designed to target bacterial infections. They work by inhibiting the growth of and/or kill a bacterium.
The introduction of antibiotics into medicine revolutionized the way infectious diseases were treated. Between 1945 and 1972, the average human life expectancy jumped by eight years, with antibiotics used to treat infections that were previously likely to kill patients. Today antibiotics are one of the most common classes of drugs used in medicine and make possible many of the complex surgeries that have become routine around the world (Microbiology Society, 2017).
The antibiotic revolution wasn't without a cost however, as the more antibiotics we use, the more resistant the bacteria become. Antibiotic resistance is the ability of bacteria to resist the effect of antibiotic that is designed to check them. It occurs when a bacterium changes or develops in some way that reduces or eliminates the efficacy of the antibiotics designed to cure or prevent the infections they cause.

Antibiotic resistance is a global health concern as it is one of the biggest threats to global health, food security and development today (WHO, 2020) [15]. It is rising dangerously to high levels in all parts of the world.

Already, 700,000 people die yearly around the globe from diseases caused by bacteria that have developed antibiotic resistance. The World Health Organization (WHO) estimates that if current trend persists, the problem could kill 10 million people every year by 2050, making antimicrobial resistance more dangerous than diabetes, tuberculosis and HIV aids combined. The problem will hit developing countries particularly hard (Scidev, 2019) [13].

About 200, 000 newborns die every year because they catch infections that simply do not respond to drugs. According to WHO around 40% of infections contracted by newborn babies resist available treatment. The vast majority of resulting deaths occur in developing counties such as Nigeria (Scidev, 2019) [13].

Typhoid fever cases and deaths occur among populations without access to drinkable water, adequate sanitation, and hygienic facilities primarily in south Asia and sub Saharan Africa (Crump and Mintz, 2010). In sub-Saharan Africa in particular, the rising threat of antibiotics resistance is making interventions at policy and medical level urgent. There is scarce data on antibiotic resistance in Sub-Saharan Africa that needs to be addressed urgently through research and development, as the region has the least comprehensive surveillance strategies for antibiotic resistance of all world regions alongside scarce infection prevention and control programs.

Salmonella species are intracellular pathogens (Jantsch *et al*., 2011). Salmonella enterica is the most frequently isolated gram negative pathogen in children older than 1 month. The incubation period is about 5 to 21 days (Kaur and Jain, 2012). An estimated 11 to 21 million infections occur each year worldwide (WHO, 2018) [16]. It causes typhoid fever which requires treatment with antibiotic, which is complicated by increasing resistance. Where resistance is uncommon, the treatment of choice is a fluoroquinolone such as ciprofloxacin, (Parry and Beeching, 2020, and Effa *et al*., 2011) [7]. There have been reports of resistance of salmonella species against antibiotics used beginning with the report of chloramphenicol resistance in 1972 to the report of Multi-drug Resistant Strains.

Enteric fever is prevalent in Nigeria but data on its resistance to antibiotics is very limited due to lack of comprehensive surveillance strategies. Nigeria, like many other tropical and developing countries has been described as endemic zone for typhoid fever by several researchers, (Oluyege *et al*., 2015) [11]. There was an incidence of typhoid fever outbreak in Sokoto State recently in which large numbers of people came down with the disease. Data collected from hospitals indicated that late diagnosis, late visit to the hospitals as well as excessive and unregulated use of antibiotics contributed in fueling the disease. Moreover, increase in large scale poultry farming in Sokoto, though good for the economy due to high demand for poultry products both within Sokoto and from the neighboring Niger Republic is also contributing to the increase in number of cases as poultry farms and products serve as some of the main source of the typhoid bacteria. Therefore, there is the need for research on the incidence of antibiotic resistant typhoid fever in Sokoto as it continues to be a public health concern. It mimics other illnesses such as malaria, yellow fever, dengue fever, rickettsial infection, Schistosomiasis, tuberculosis and acute HIV infection because it is relatively non-specific.

This study aimed at providing data on the prevalence antibiotic resistant *Salmonella typhi* isolated from outpatients within selected hospitals in Sokoto State.

Materials and Method

Study design, Period and Area

This research employed a cross-sectional sampling of patients/individuals, going for Widal test to selected hospitals in Sokoto state between April and November, 2021. The samples in this Study were bloodstream isolates of *Salmonella typhi*. The inclusion criteria in the study were patients who were going for Widal test and patients recommended for blood culture. Those have titre of 1: 160 were considered as having active infection and were regarded as positive for Widal test

Sample Collection

Venous blood samples were collected from patients with suspected cases of typhoid referred to hospitals from April 2021 to June. A total of 150 blood samples were collected from patients suspected of having typhoid fever according to presumptive diagnosis by a medical practitioner. 2ml of venous blood were collected aseptically and inoculated onto 18ml of Brain Heart Infusion Broth Agar and then incubated at 37ºC for 7 days (Stella *et al.,* 2011) [14].

Isolation of Salmonella Species

The inoculated tubes were examined daily for evidence of bacterial growth, including turbidity and haemolysis. The first sub- culture was done after 48 hours in Bismuth Sulfide Agar. Sub-culturing was done on the same medium till the 7th day. Brown and black colonies presumably Salmonella species were picked and characterized using standard methods.

Identification

Biochemical identification of Salmonella species was done using the appropriate presumptive identification protocols. Gram's stain, Catalase, citrate, MRVP, Kliger Iron Agar, Simmon Iron Medium Agar, Urea base agar, MacConkey agar and Simmons citrate agar were used to screen the isolates before serologic testing is performed (Cheesbrough, 2002).

Serological Testing

Serologic identification of Salmonella species was performed using Wellcolex colour Salmonella test kit. One suspected Salmonella colonies from the culture plate was carefully emulsified in approximately 200µl of sterile saline in a suspension tube. Holding the bottle vertically, re-suspended latex reagent 1 and 2 were dropped into a separate circle on a flat reaction card after shaking vigorously for few seconds. About 40µl of bacterial suspension was transferred to two of the reaction circles containing latex reagent 1 and 2 respectively and mixed. The card was placed on a suitable flatbed rotator and run at 150 ± 5 rpm for 2 minutes then switched off and observed for agglutination without removing the card rotator. Positive controls with the positive control reagents (green, blue and red control) was carried out alongside with the latex reagent 1 and 2 respectively without the inoculums. Results were interpreted according to the manufacturer guidelines for usage of the kit.

Determination of antibiotic susceptibility profile

The isolated *Salmonella typhi* clinical isolates were subjected to different classes of antibiotics ranging from both beta-lactamases, non-betalactamases, penicillins and cephalosporins including ampicillin, tetracycline, chloramphenicol, azithromycin, trimetoprim-sulfametoxazole, augmentin, and ceftriaxone using the Kirby Bauer disc diffusion method.

Statistical Analysis

All the work experiments were conducted in triplicates. All data obtained is expressed as percentages and standard deviation.

Results/Discussion

Demographic data

The demographic data of the sample population used in the researches (age and sex) was expressed in percentages and depicted on a pie chart. Of the 150 patients, 85 were adults while 65 were children, representing 56.67% and 43.33% respectively. Based on sex, 55 of the patients were male while 95 were females, representing 36.67 and 63.33% respectively. The data is presented in Table 1.

Table 1: Demographic data of the patients

Age	Category	Frequency	Percentage (%)
	Adults	85	56.67
	Children	65	43.33
SEX	Male	55	36.67
	Female	95	63.33

Isolation of Salmonella Species

Suspected *Salmonella typhi* bacterial colonies were isolated after culturing in bismuth sulfide agar after several days. The presumed Salmonella species were then subsequently characterized using standard methods.

Biochemical Characterization

Presumptive colonies of Salmonella grown on Bismuth sulfite agar were characterized biochemically. Grams stain was carried out to identify gram negative organisms. Indole tests, motility, citrate, MRVP, urease and H2S production tests were also performed. The results of the confirmatory biochemical test is shown in table 2.

Table 2: Result of Biochemical Test

S/N	Biochemical test	Positive	Percentage %	Negative	Percentage
1.	Gram Stain	0	0.00	7	100
2.	MR	7	100	0	0.00
3.	VP	0	0.00	7	100
4.	Indole	0	0.00	7	100
5.	Citrate	7	100	0	0.00
6.	TSI	7	100	0	0.00
7.	Catalase	7	100	0	0.00
8.	Motility	7	100	0	0.00

Serological Testing

The serological test for further confirmation of salmonella serotypes was carried out. Of the seven (7) isolates tested five (5) were positive for *Salmonella typhi* and the results are shown in below.

Table 3: Serological Test Result

ISOLATE	S. TYPHI	OTHERS
SS1	+	-
SS2	+	-
SS3	-	-
SS4	+	-
SS5	-	+
SS6	+	-
SS7	+	-

Antibiogram

Table four (4) shows the result of antibiotic sensitivity test. A few of the test isolated exhibited multiple antibiotic resistance.

Table 4: *Salmonella typhi* Antibiogram

Antibiotic Isolate	AMP	TET	AMX	CIP	GEN	OFL	DOX
SS1	R	R	S	S	S	R	R
SS2	R	R	R	S	S	S	S
SS3	S	R	R	S	S	S	R
SS4	R	R	R	S	R	S	R
SS5	R	R	R	S	R	R	S
SS6	R	R	S	R	S	R	R

Keys; R- resistant, S- sensitive

Statistical Analysis

All the data obtained from the study were collated and analysed. The prevalence of salmonella was analysed using descriptive statistics while the Chi squared test was used to determine the level o signifance of the research results. A p value of 0.2233 was obtained. A P value less than 0.05 (p-value <0.05) was considered statistically significant.

Discussion

Antibiotic resistance is a global health concern as it is one of the biggest threats to global health, food security and development today. Many different infectious diseases that were once easily treated with different brands of antibiotics are becoming more difficult to treat. According to the world health organization (who), already, 700,000 people die yearly around the globe from diseases caused by bacteria that have developed antibiotic resistance. The World WHO estimates that if current trend persists, the problem could kill 10 million people every year by 2050. Enteric fever is prevalent in Nigeria but data on its resistance to antibiotics is very limited due to lack of comprehensive surveillance strategies. Multidrug resistant salmonella has emerged as a major public health issue worldwide (Sobur *et al.*, 2019) and one of the classical examples of such public health issue is MDR *Salmonella enterica* serovar typhimirium which has been found severally to be resistant to several antibiotics, namely, ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. In the current study among the total samples analysed, 7 if the isolates were found to be positive for salmonella and of the 7, five wee positive for salmonella typhi serotype after a series of confirmatory biochemical and serological tests. In the current study, high rate of resistance were observed against some of the antibiotics tests like tetracycline (100%), ampicillin (83.3%) and amoxicillin (66.67%).

This is similar to previous studies by (Shanzida *et al.*, 2020).They are also similar to findings from a study by (Thobeka *et al.*, 2019) who also observed high rates of resistance against ampicillin, tetracycline, amoxicillin and ceftriaxone.

Conclusions

Result from the current study have shown how damaging the issue of antibiotic resistance could pose to the overall health the populace. The issue is especially important as the study area is one in which there is an increased active of poultry farming where there is frequent antibiotic use as well as widespread consumption of poorly processed poultry production which may explain the rise in the prevalence of multidrug resistant bacterial species.

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