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Antibiogram of *Salmonella* isolates from clinical specimens in Umuahia, Abia State, Nigeria

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Abstract **Original Research Article**

A study was carried out to determine the antibiogram of *Salmonella* isolates from clinical specimens in Umuahia, Abia state. One hundred and thirty clinical specimens of blood and stool were asceptically collected, cultured, characterized and identified using standard microbiological methods. Molecular characterization using I6S rRNA was done to identify and characterize *Salmonella* isolate. Kirby–Bauer disc diffusion method using the protocol of the clinical and laboratory standard institute was carried out to determine the susceptibility profile of the isolates to ten different antimicrobials. Isolates that were resistant to at least three or four antibiotics were further subjected to plasmid profiling and curing of the resistant *Salmonella* isolate. Plasmid curing with sodium dodecyl sulphate (SDS), post-curing susceptibility testing was performed using agarose gel electrophoresis. Eighty-five isolates of *Salmonella* were recovered from two different clinical sources. Susceptibility profile of the uncured isolates to ten different antimicrobials revealed that the highest resistance percentage was against Ampicillin (73%), Vancomycin (66%), Nitrofurantoin (61%), Levofloxacin (54%), Gentamicin (51%), with moderate resistance to Ciprofloxacin (49%), Imipenem (41%) and lowest resistance rate to Ceftazidime (33%), Ofloxacin (34%) and Cefuroxime (39%). Then after plamid curing of the *Salmonella* isolates, the highest resistance percentage was against Ampicillin (72%), Vancomycin (62%), Nitrofurantoin (59%), Levofloxacin (51%), Gentamicin (51%), with moderate resistance to Ciprofloxacin (42%), Imipenem (41%) and lowest resistance rate to Ceftazidime (32%), Ofloxacin (32%), and Cefuroxime (38%). After plasmid curing, no significant difference ($p>0.05$) in the level of resistance was observed. The findings demonstrate widespread multidrug resistance of *Salmonella* in clinical samples. This definitely will create public health challenges and requires the need to innovate better antimicrobial treatment in our clinical and health settings.

Keywords: Antibiogram, Kirby-Bauer disc diffusion, *Salmonella* and Clinical specimens.

INTRODUCTION

The bacterium known as *Salmonella* is a gram negative, rod shaped organism which belong to the family Enterobacteriaceae. *Salmonella* is the aetiological agent of the disease known as Salmonellosis (Grunberg, 2022). It affects both human beings and animals. It is of public health and medical importance. It also causes a variety of infections like, gastroenteritis, meningitis, urinary tract infections, septicaemia and pneumonia (Elbadawi, 2017; Grunberg, 2022).

Salmonella as a genus is known to cause diarrhea and can lead to typhoid fever infection. The mode of transmission of this infection is through consuming underprepared beef, pork poultry and dairy products that has not been pasteurized. Another means of transmission may be through contamination of food handlers who do not observe appropriate hygiene and sanitation control methods, another way can be when food and water are contaminated through feaces (Bush and Maria 2025; Emmanuel and Emmanuel 2018).

After infection, the incubation period is generally between 12 hours to 48 hours after the bacteria have gained access into the intestine. The symptoms include fever, nausea, abdominal pains, watery diarrhea as well as vomiting. This infection can be asymptomatic or it can be symptomatic. Bacteremia can occur when the bacteria gain access to the bloodstream, also abscesses can occur as a result of pus collection. It can affect the joints, bones, lungs, arteries and urinary tracts (Bush and Maria, 2025).

Research indicates the existence of resistance multidrug strains of *Salmonella* (Ashneal *et al*., 2023), which has become a leading problem in the medical industry, which calls for further investigation and production of new antibiotics. The widespread and often indiscriminate use of antimicrobials in human medicine, has contributed significantly to the emergence of antibiotic *Salmonella* strains that are resistant. Of particular interest, is the rising resistance to β-lactam antibiotics and other critical drug classes which are usually recommended to treat severe infections? Antibiotic resistance in *Salmonella* is often caused by the presence of plasmids. Plasmid-mediated resistance is responsible for the dissemination of multidrug resistance in bacteria, especially in environments like hospitals where antibiotic pressure is high. (Partridge *et al*., 2018). The aim of this study is to evaluate the antimicrobial resistance profiles of *Salmonella* isolates from clinical sources in Umuahia, Abia State, Nigeria, and to also determine plasmids ability in causing resistance patterns in the various antibiotics.

MATERIALS AND METHODS

Types of Samples and collection

Blood specimens were aseptically collected from patients suspected of having typhoid fever. Patients suspected of having Salmonellosis were asked to provide stool samples. Blood and stool samples were collected from patients who consulted two Clinics in Umuahia Abia state, Nigeria. All participants were told of the research study and they willingly gave their informed consent before the collection and submission of blood and stool specimens from different patients. The clinics approved the collection of the clinical specimens. The patients recruited were both male and female between ages 18-50 years of age.

Isolation and characterization of *Salmonella* isolates

MacConkey, and Eosin Salmonella agar were used for primary isolation of *Salmonella* after enrichment with brain heart infusion (BHI) and selenite faeces broth. The specimens were inoculated within 24 hours of collection into enrichment media and incubated at 37^0C and was observed after for 18 hours -24 hours and plated on *Salmonella-Shigella* agar for primary isolation. Colonial growth was observed. Colonial growths on the plates were subcultured by streak plate method on nutrient agar slant.

Biochemical test for *Salmonella*

The isolates were confirmed using Gram stain reaction as well as (Indole test, Methyl Red test, Voges-Proskauer tests, Citrate tests) IMViC

tests for *Salmonella* classification. (Cheesbrough, 2003).

I6S rRNA gene sequencing methods to characterize isolate.

A zymo research bacteria DNA mini prep extraction kit was used. Agarose gel electrophoresis was done to qualitatively estimate the genomic DNA. The estimated DNA was extracted, amplified using the amplification protocol of Polymerase chain reaction. Nimagen brilliant dye terminator cycle sequencing kit was used to sequence the fragment DNA according to the instruction of the manufacturer (Platt, *et al*., 2007).

Antibiotics

The antibiotics used for this study are as follows: Ampicillin (25µg), Imipenem (10µg), Ceftazidime (30µg), Cefuroxime (30µg), Ciprofloxacin (30µg), Gentamicin (10µg), Levofloxacin (5µg), Ofloxacin (30µg), Nitrofurantoin (30µg), Vancomycin (20µg). These antibiotics belong to several different classes, primarily the beta-lactams (which include penicillins, cephalosporins, and carbapenems), fluoroquinolones, aminoglycosid es, nitrofuran derivative and glycopeptide. The breakpoints in µg/ml for resistant isolates according to Clinical and Laboratory Standards institutes (CLSI) (2019) are Ampicillin (25µg) ≤ 16, Imipenem (10µg) ≤ 19, Ceftazidime (30µg) ≤ 17, Cefuroxime (30µg) ≤ 14, Ciprofloxacin (30µg) ≤ 15, Gentamicin (10µg) ≤ 12, Levofloxacin (5µg) ≤ 13, Ofloxacin (30µg) ≤ 16, Nitrofurantoin (30µg) ≤ 14, Vancomycin (20µg) ≤ 12 (CLSI, 2015).

Antimicrobial Susceptibility Test

This test was done by Kirby-Bauer disk agar diffusion method, media used was Mueller Hinton agar. It was done according to the protocol of Clinical Laboratory Standard Institute (CLSI, 2015). Pure colonies from a 24 hour nutrient agar plate were suspended in a test tube containing normal saline to achieve turbidity similar to 0.5MacFarland turbidity Standard. A sterile wire loop was dipped in the bacterial suspension and used to inoculate the surface of already prepared Mueller Hinton agar plate. The plates were homogeneously rotated to maintain confluent growth of the bacterium. Flamed forceps were used to place the disks on inoculated plates. The inoculated plates were in an inverted position for 16-18 hours at 37^0C. A transparent metric ruler was used to measure the zone of inhibition and designated as resistant or susceptible according to already established breakpoints.

Standard. A cotton swab was dipped into the bacterial suspension and used to inoculate the entire surface of a Mueller Hinton agar plate. The plates were homogeneously rotated to ensure confluent growth of the bacterium. The antimicrobial susceptibility disks were placed on the surface of the inoculated plates with flame sterilized forceps. The inoculated plates were incubated in an inverted position for 16-18 hours at 37^0C. The dia A meter of the zone of inhibition produced by the antibiotic disks was measured to the nearest millimetre (mm) using a transparent metric ruler and designated as resistant or susceptible according to already established breakpoints.

Plasmid profiling of resistant isolate

The protocol of (Abraham, *et al.,* 2019) was used in testing for plasmid profiling of resistant isolates. Agarose gel powder of 1.6g was weighed and diluted in 200ml of 1xTAE buffer in a microwavable flask. Complete dissolution of the mixture was achieved by microwaving for 3 minutes. The mixture was allowed to cool at a temperature of 50 °C. Staining was done using ten (10) µL of EZ vision DNA stain to increase visibility, which was then poured into a gel tray and left to solidify at room temperature for 20-30 minutes.

Plasmid curing of resistant isolates

In the plasmid curing process, ten isolates showed resistance to more than three antibiotics. These isolates were treated with a sub-inhibitory concentration (10%) of sodium dodecyl sulphate (SDS). The method by Ehiaghe *et al*. (2013), was adopted were 9ml of nutrient broth was inoculated with an overnight culture broth. Then, 1ml of 10% SDS was added. The mixture was incubated for 48 hours at 37°C. After the first

incubation, fresh batch of nutrient broth was prepared, and then 1ml of the previously treated culture was added. This mixture was incubated again for 24 hours at 37°C. The resultant culture was then stored in the refrigerator to be used later.

Sample loading and Agarose gel run.

Extracted plasmid samples were mixed with loading dye, the resultant mixture was loaded onto an agarose gel. The gel placed on the electrophoresis unit was covered with 1xTAE buffer. A molecular weight ladder was loaded on the first lane to serve as a reference for estimation different fragment sizes of DNA. Plasmid DNA samples that were purified were loaded onto the remaining wells of the gel. Electrophoresis was done at 100V for 80 minutes. Purified plasmid DNA fragments were visualized under UV Trans-illumination so as to identify the bands harboring the plasmid (Ehiagh *et al.,* 2013).

Antimicrobial sensitivity test after plasmid curing

Isolates that have been cured were subjected to further antimicrobial testing so as to determine whether the resistant plasmid agent was removed successfully. The bacterial colonies from the cured isolates were inoculated into nutrient broth, then incubated at 37°C for 24 hours. 0.5 McFarland standard for turbidity was achieved. Bacterial colonies were streaked onto muller Hinton agar plates. Antimicrobial disc were placed onto the streaked plates with sterilized forceps. The plates were dried for 5 minutes then incubated at 37°C for 24 hours. A transparent metric ruler was used to measure the zone of inhibition and designated resistant or susceptible according to already established breakpoints.

Statistical Analysis.

Statistical analysis used was the percentage occurrence and the significance level at 0.05.

RESULTS

Total of 85 isolates of *Salmonella* were identified and characterized from One hundred and thirty clinical specimens of blood and stool which were analysed by standard microbiological methods as well as biochemical tests as shown in Table 1. The biochemical characteristic like Indole test, Methyl Red test, Voges-Proskauer test and Citrate test was performed and is seen in Table 2

Table 3 shows molecular characterization of isolates of *Salmonella* by16S rRNA gene sequencing method which revealed *Salmonella enterica* as the characterized organism.

A total of hundred and thirty study participants made up of 58 (45 %) males and 72 (55%) females were used for this study. The demographic features of the informed patients showed that equal number of specimen being sixty-five specimens was collected from both males and female participants. Female specimen (48%) yielding higher number of *Salmonella* isolates more than male (37%) specimen. Isolates of *Salmonella* recovered from blood and stool specimens according to gender and age group as shown in Table 4.

Table 1: Cultural characteristics of Isolates of *Salmonella*.

Sample number	Gram stain	Colour	Shape arrangement	Surface type	Arrangement of colony	Organism
1	Negative	pink	Rod	Smooth edge	Single or paired	*Salmonella*

Table 2 : Biochemical tests performed for *Salmonella* (Indole test, Methyl Red test, Voges-Proskauer test and Citrate test).

Key

- Negative
+ Positive

Biochemical tests	Indole test	Methyl Red test	Voges-Proskauer test	Citrate test	Organism
Isolate	-	+	-	+	*Salmonella*

Table 3: Molecular characterizations of *Salmonella* isolates.

S/N	Sequence ID	Percentage	NCBI Match	Isolate
1	NC003197.2	98	*Salmonella typhiAE006468*	*Salmonella enterica*

Table 4: Demographic features of informed patients and isolation of *Salmonella* from blood and stool specimens.

Features	Number (%) of blood specimen	Number (%) of stool specimen	Number yielding *Salmonella* Isolates
Sex			
Male	30 (46)	26 (40)	37
Female	35 (54)	39 (60)	48
Total	65	65	85
Age Groups (Years)			
18-25	14	18	27
26-33	27	29	33
34-41	15	12	14
42-50	9	6	11

The *Salmonella* isolates were observed for resistance pattern to ten antimicrobial agents. The results are shown in table 5 and table 6. Then resistant isolates were further subjected to plasmid profiling and plasmid curing. Their resistance level to the ten antimicrobial agents were shown in tables 9 and Table 10

The susceptibility of the isolates to ten (10) different antibiotics was examined by Kirby–Bauer disc diffusion test. Among different classes of antibiotics, the highest resistance percentage was against Ampicillin (73%), Vancomycin (66%), Nitrofurantoin (61%), Levofloxacin (54%), Gentamicin (51%), with moderate resistance to Ciprofloxacin (49%), Imipenem (41%) and lowest resistance rate to Ceftazidime (33%), Ofloxacin (34%), Cefuroxime (39%). After plasmid curing, the highest resistance percentage was against Ampicillin (72%), Vancomycin (62%), Nitrofurantoin (59%), Levofloxacin (51%), Gentamicin (51%), with moderate resistance to Ciprofloxacin (42%), Imipenem (41%) and lowest resistance rate to Ceftazidime (32%), Ofloxacin (32%), and Cefuroxime (38%). Then after plamid curing of the *Salmonella* isolates, there was slight positive change in the resistance percentage against the antibiotics. After plasmid curing, no significant difference ($p>0.05$) in the level of resistance was observed. The plasmid profiling for the ten resistant *Salmonella* isolates initially revealed the presence of plasmids which ranged between 5kbp to 9kbp. This molecular weight was for the plasmid before curing. Then after curing, the molecular weight of the plasmid changed slightly being between 5kbp to 8kbp. The slight change was observed in the blood specimen. There was also no significant difference ($p>0.05$) in the molecular weight for the plasmids.

The Plasmid profile of cured and uncured isolates and the resistance of plasmid before curing and resistance of plasmid after curing are shown in table 7.

TABLE 8 shows the molecular weight of Plasmid Profile of cured plasmid and molecular weight of uncured plasmids.

Tables 9 and 10 shows the antimicrobial susceptibility analysis of resistant cured isolates from blood specimen as well as the antimicrobial susceptibility analysis of resistant cured isolates from stool specimen.

TABLE 5: Antimicrobial resistant profile of *salmonella* from blood specimens.

ANTIBIOTICS	Number (%) *Resistant* Isolates		
		Salmonella (n=85)	
Ampicillin (25µg)		62(73)	
Ceftazidime (30µg)		28(33)	
Cefuroxime (30µg)		33(39)	
Ciprofloxacin (5µg)		42(49)	
Gentamicin (10µg)		38(45)	
Levofloxacin (5µg)		46(54)	
Imipenem (10µg)		36(42)	
Ofloxacin (30µg)		29(34)	
Nitrofurantoin (30µg)		52(61)	
Vancomycin (20µg)		56(66)	

TABLE 6: Antimicrobial resistant profile of *salmonella* isolates from stool specimens.

ANTIBIOTICS	No(%) of *Resistant* Isolates	
		Salmonella (n=85)
Ampicillin (25µg)		52(61)
Ceftazidime (30µg)		26(30)
Cefuroxime (30µg)		28(33)
Ciprofloxacin (5µg)		40(47)
Gentamicin (10µg)		35(41)
Levofloxacin (5µg)		41(48)
Imipenem (10µg)		32(38)
Ofloxacin (30µg)		28(33)
Nitrofurantoin (30µg)		46(54)
Vancomycin (20µg)		48(56)

TABLE 7: Gel electrophoresis showing profile of plasmid for both cured and uncured isolates.

Salmonella	Resistant Profile Before Curing	Resistant Profile After Curing	Probable Organism
1.Blood	5	4	*Salmonella*
2.Stool	5	5	*Salmonella*

TABLE 8: Gel Electrophoresis for Molecular weight of Profile of plasmid of cured and uncured Isolates.

Gel Lane	Molecular Weight (Kbp) of Uncured Plasmid	Molecular Weight (Kbp) of Cured Plasmid	Probable Organism	Sample
1	5-9	5-8	*Salmonella*	Blood
2.	5-9	5-9	*Salmonella*	Stool

TABLE 9: Antibiotic susceptibility analysis of resistant cured isolates from blood specimen.

ANTIBIOTICS	No(%) of Resistant Isolates		
		Salmonella (n=85)	
Ampicillin (25µg)		61(72)	
Ceftazidime (30µg)		27(32)	
Cefuroxime (30µg)		32(38)	
Ciprofloxacin (5µg)		36(42)	
Gentamicin (10µg)		43(51)	
Levofloxacin (5µg)		43(51)	
Imipenem (10µg)		35(41)	
Ofloxacin (30µg)		27(32)	
Nitrofurantoin (30µg)		50(59)	
Vancomycin (20µg)		53(62)	

TABLE 10**:** Antibiotic susceptibility analysis of resistant cured isolates from stool specimen.

ANTIBIOTICS	No(%) of Resistant Isolates		
		Salmonella (n=85)	
Ampicillin (25µg)		50(59)	
Ceftazidime (30µg)		24(28)	
Cefuroxime (30µg)		26(31)	
Ciprofloxacin (5µg)		40(47)	
Gentamicin (10µg)		37(49)	
Levofloxacin (5µg)		45(47)	
Imipenem (10µg)		31(36)	
Ofloxacin (30µg)		28(33)	
Nitrofurantoin (30µg)		45(53)	
Vancomycin (20µg)		46(54)	

Discussion

Clinical specimens of one hundred and thirty blood and stool specimens cultured identified and characterized, yielded 85 *Salmonella* isolates representing isolation rate of 65%. This agrees with the work of (Ekundayo, 2021) that had an isolation rate of 53%. This work shows resistance of the clinical isolates to commonly used antibiotics. The resistance of the isolates in blood specimen were higher than the resistance of isolates in stool specimen. The susceptibility pattern of the isolates to various antibiotics showed that the highest resistance percentage was against Ampicillin (72%), with moderate resistance to Ciprofloxacin (49%), Imipenem (41%) and lowest resistance rate to Ceftazidime

(33%). This agreed with the work of (Okere, *et al*., 2025) that highest comparative resistance percentage was against Ampicillin (96%), the moderate percentage was seen among Ciprofloxacin (52%) and the lowest resistance percentage was in Gentamicin (34%) followed by Levofloxacin (33%). According to World Health Organization, (WHO, 2019), the resistance level of *Salmonella* isolates to Ampicillin, cefotaxime, Ceftazedime and Meropenem describe these antibiotics as "critically important" antibiotics.

Profiling of plasmids for *Salmonella* revealed molecular weight of between 5kbp to 9kbp. This shows a variation in the plasmid size. It also demonstrates diversity within the population of bacteria. There is similarity with the findings of (Okere *et al*., 2025 and Ekundayo, 2021) who reported different plasmid weights of *Salmonella* to be between 4kbp to 10kbp

This work showed slight modification as seen in the cured plasmid for both blood and stool specimen. They had molecular weight of 5kbp to 8 kbp, which was not significant. It implies that elimination of plasmid did not really show in their molecular weight of plasmid. The agreed with the works of (Okere *et al*., 2025 and Ekundayo, 2021) says that cured plasmid alone did not eliminate antibiotic resistance. According to (Ehiaghe *et al*., 2013; Okoye *et al*., 2022) their works reported that multidrug resistant antibiotics was not caused by plasmid content alone. This might suggest other genetic factors like chromosomal factors which may be responsible for resistant isolates.

CONCLUSION

Salmonella as an aetiological agent of Salmonellosis remains a major foodborne pathogen and as such pose as a risk to the health of the public. This study revealed the high rate of multi drug resistance *Salmonella.* These isolates showed varying resistance rates against different classes of antibiotics. In Nigeria, ciprofloxacin is commonly prescribed in typhodial and Non Typhoidal *Salmonella* infections however moderate resistance to this antibiotic is emerging. The presence of multidrug resistance *Salmonella* strains are critical issue in the health sector which may make treatment ineffective and inefficient. Therefore, to mitigate this ugly trend, there should be well equipped diagnostic facilities, strict adherence to physician prescription on the use of antibiotics as well as good infection management. Awareness of Salmonellosis infection should be created through social media platforms, radio and television jingles. Good personal hygiene and sanitary hygiene should also be encouraged so as to combat salmonellosis.

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