

Antibiotic resistance pattern of *Salmonella* species isolated from Typhoid patients in Jigawa state, Nigeria

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Abstract

This study was designed to determine the antibiotic resistance pattern of *Salmonella* spp. isolated from clinical samples of symptomatic and asymptomatic typhoid patients, selected from some hospitals in Jigawa State, Nigeria. Four hundred bacterial isolates were recovered from these samples, were identified as; *Salmonella typhimurium*, *S. enteritidis*, *S. typhi*, *S. paratyphi* A, and *S. paratyphi* B using microgen biochemical system, according to the standard techniques. The antibiogram of all these *Salmonella* spp. were carried out using standard oxoid disc of different antibiotics. Double disc diffusion technique was employed to determine the isolates' capacity to produce β -lactamase. Results showed 5(1.25 %), 0(0.0 %), 0(0.0 %) of the samples collected were positive for extended spectrum β -lactamases (ESBL), AmpC β -lactamase, and Metallo β -lactamases (MBL), respectively. The antibiogram profile of the isolates showed that 100 % of these isolates are susceptible to Imipenem, only 3 % exhibited resistance to ceftriaxone, whereas 7 % demonstrated resistance to ciprofloxacin. To the best of our knowledge, this the first study of this kind in the Jigawa state, especially on these *Salmonella* spp. The study concluded that β -lactamases with the capacity of conferring multidrug resistance were recorded in *Salmonella* spp. isolated from patients in several hospitals of Jigawa State, Nigeria.

Keywords: Antibiotics, ESBL, Resistance, *Salmonella* spp.

1. Introduction

Antimicrobial resistance (AMR) is a complex issue of major global concern. Antimicrobial resistance take place when germs such as bacteria and fungi develop the ability to defeat the drugs designed to kill them. Infections caused by antibiotic resistance

microorganisms are difficult, and sometimes impossible to treat. In most cases, antibiotic resistant infections require extended hospital stays, additional follow-up, and the use of costly and toxic alternatives. [Hugo and Russel, \(2004\)](#) reported that resistance can

be achieved by horizontal acquisition of resistance genes, mobilized via insertion sequences, transposons and conjugative plasmids by recombination of foreign DNA into the chromosome, or by mutations in different chromosomal loci. Diseases caused by various members of the genus *Salmonella* are extremely important worldwide public health problems. The most important member of this genus is *S. typhi*, the causative agent of typhoid fever; however, there are more than 2500 serotypes of *Salmonellae*. Based on DNA hybridization studies, the genus *Salmonella* consists of two main species; *S. enterica* and *S. bongori*. [Apurba and Sandhya, \(2016\)](#) reported that most of the pathogenic typhoidal and non-typhoidal *Salmonella* serotypes are placed under species *enterica* and subspecies *enterica*. In Nigeria, seven hundred and forty seven isolates were recovered from Muhammad Abdullahi Wase Specialist Hospital, Kano, and were screened for ESBL's production. The results showed that 37 (9.25 %) of isolates were ESBL producers based on Double disc synergy test (DDST), while 20 (5.0 %) were positive using National Committee on Clinical Laboratory Standard (NCCLS) break points. Four species were identified as ESBLs producers including; *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli* and *Salmonella* spp. ([Yusha'u et al., 2007](#)). Moreover, [Lamini et al., \(2018\)](#) also reported the low prevalence of *S. typhi* beta lactamase producers among presumptive typhoid patients in Nassarawa state, Nigeria. However, they reported only 6 ESBL positives samples recovered from 450 stool samples of presumptive typhoid patients in this state, which indicated a prevalence of 1.33%. The objectives of this study were to determine the multi-drug resistance pattern of the *Salmonella* isolates, to screen and confirm ESBL, AmpC and Carbapenemase (MBL) production by the isolates using a phenotypic method.

2. Material and methods

2.1. Sampling

Stool samples were collected from patients of the General Hospitals Laboratories across Jigawa State, Nigeria, during the period of April, 2019- December,

2019. An Ethical clearance was obtained from Ethical Committee Jigawa state Ministry of Health, prior to commencement of this study.

2.2. Isolation and identification of the *Salmonella* spp.

The stool specimens were inoculated using sterile wire loop onto the surface of Salmonella-Shigella agar (SSA) (Titan biotech, India) plates, incubated at 37°C for 24 h, and observed for growth characteristics. The bacterial isolates were identified based on; Gram reaction, cultural characteristics and using Microgen (GN-ID B) agglutination kit, according to [Awong-Taylor et al., \(2007\)](#).

2.3. Antibacterial susceptibility testing

The antibiogram of the isolates were determined using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton (MH) agar, as described by the Clinical and Laboratory Standards Institute ([CLSI, 2012](#)) guidelines. A bacterial suspension was made from a 24 h culture of the isolate in saline, to match the 0.5 McFarland turbidity standards. The following antibiotic discs (Oxoid UK) were used with potencies; Ceftazidime (30 µg), Ceftriaxone (30 µg), Ampicillin (30 µg), Augmentin (Amoxicillin 20 µg and Clavulanic acid 10 µg), Gentamicin (10 µg), Imipenem (10µg), and Ciprofloxacin (30 µg), Chloramphenicol (30 µg). The MH agar plates were seeded with the tested bacteria, incubated at 37°C in an aerobic atmosphere for 24 h, after which the diameter of the zones of growth inhibition around the discs was measured with a calibrated ruler. A similar procedure was done using *E. coli* ATCC 25922 strain as negative control, obtained from isolates collections of Aminu Kano Teaching Hospital (AKTH), Nigeria.

2.4. Extended spectrum β-lactamase screening test

All isolates that were resistant to more than 3 antibiotics were subjected to beta lactamase screening tests according to [CLSI. \(2011\)](#) guidelines, in which Ceftazidime (10 µg) and Ceftriaxone (30 µg) discs were used. Isolates which expressed zone of inhibition

diameters on Mueller-Hinton agar medium with Ceftazidime zone < 22 mm, and Ceftriaxone zone < 23 mm, were subjected to confirmatory tests.

2.5. Confirmatory test

The Double-disk synergy test (DDST) was used as described by [CLSI. \(2005\)](#). A disk containing Cephalosporins antibiotics; Ceftriaxone (30µg), Ceftazidime (10 µg), were placed on the surface of seeded Mueller-Hinton agar 15 mm next to a disk of Augmentin (Amoxicillin 20 µg -Clavulanic acid 10 µg). A positive result is indicated when the inhibition zones around any of the cephalosporin disks are augmented, or there is 'keyhole' in the direction of the disk containing Clavulanic acid.

2.6. Detection for Plasmid AmpC β-lactamase production

The Cefoxitin-cloxacillin inhibition test was performed as described by [Tan *et al.*, \(2009\)](#). Briefly, the test isolates were seeded on MH agar plate, and then two 30 µg Cefoxitin discs (Oxoid, UK) were placed on the surface of the seeded plate. One of the Cefoxitin discs was supplemented with 200µg Cloxacillin. The plate was incubated at 37°C overnight. The inhibition zone diameter was measured. AmpC β-lactamase production was inferred if the inhibition zone of the Cefoxitin disc supplemented with Cloxacillin was ≥ 4 mm greater than that of Cefoxitin alone.

2.7. Detection of the inducible AmpC β-lactamase production

Screening for the inducible AmpC β-lactamase production was carried out using the Disc antagonism test, according [Sanders *et al.* \(1996\)](#). This was done by placing Cefoxitin disc (Oxoid, UK) at a distance of 20 mm from Ceftazidime (Oxoid, UK) on the surface of MH agar plate seeded with the test bacteria. Production of the inducible β-lactamase was recognized by having broad round end (blunting) of the Ceftazidime inhibition zone, adjacent to the cefoxitin disc.

2.8. Screening and confirmatory test for Carbapenemase production

The bacterial isolates were screened for Carbapenemase production according to [CLSI. \(2005\)](#) guidelines. In this method, Carbapenem antibiotics such as Meropenem disc (10 µg) (Oxoid, UK) was used. The antibiotic disc was placed on the surface of seeded MH agar plates using sterile forceps. The plates were incubated for 24 h at 37°C, after which zones of inhibitions were recorded. Isolates that showed a zone of inhibition ≤ 28 mm in diameter for meropenem were considered as suspected Carbapenemase producers, and were subjected to confirmatory test by the Modified Hodges Test (MHT).

2.9. Screening of Carbapenemase producers for metallo β-lactamases (MBLs)

EDTA-disc synergy assay was used as described by [Walsh *et al.*, \(2005\)](#); [CLSI. \(2005\)](#). A Carbapenemase producing isolate was streaked on the surface of the MH agar plate using a sterile wire loop. Two Meropenem discs, one containing EDTA and the other without EDTA, were placed on the surface of the MH agar at a distance of about 30 mm apart. The EDTA disc was prepared by adding 250 µl of neutral EDTA to Meropenem. The plates were incubated at 37°C. Zones of inhibition were recorded after 24 h of incubation. A control disc containing EDTA alone was used, to ensure that EDTA does not causes false-positive results by inhibiting the test isolate. Bacterial isolate that showed difference in the diameter of zone of inhibition by ≥ 4 mm on the Meropenem containing EDTA and Meropenem alone, was confirmed as MBLs producer.

2.10. Statistical analysis

The data obtained in this study were analyzed by means of the Microsoft excel 2007. The difference in antibiotic resistance between the *Salmonella* isolates was evaluated by means of the Chi-square test. *p*-value less than 0.05 is considered statistically significant.

3. Results and Discussion

3.1. Isolation and identification of the isolates

A total of 400 isolates were recovered and then identified as; *S. typhimurium*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B* and *S. enteritidis*, with the frequency of occurrence shown in Table 1.

3.2. Antibigram of the tested *Salmonella* spp.

Results of antibiogram of the tested isolates are shown in Table (2). They demonstrated that all the isolates are susceptible (100 %) to Imipenem. However, only 12(3.0 %) showed resistance to Ceftriaxone, and 7 % are resistance to Ciprofloxacin. Ampicillin expressed the highest resistance recorded in this study (3.75 %).

Table 1: Frequency of occurrence of the identified *Salmonella* isolates

Isolates	Number isolates	Percentage of occurrence (%)
<i>S. typhimurium</i>	119	29.75
<i>S. typhi</i>	82	20.5
<i>S. paratyphi A</i>	43	10.75
<i>S. enteritidis</i>	132	33
<i>S. paratyphi B</i>	24	6
Total	400	100

Table 2: Antibigram of the tested *Salmonella* spp.

Antibiotic	Resistance		Susceptibility	
	No. of isolates	Percentage (%)	No. of isolates	Percentage (%)
Gentamycin	31	7.75	369	92.25
Ciprofloxacin	28	7	372	93
Ceftriaxone	12	3	388	97
Imipenem	0	0	400	100
Chloramphenicol	76	14	344	86
Ceftazidime	38	9.5	362	90.5
Ampicillin	175	43.75	225	56.25
Augmentin	31	7.75	369	92.25
Cefoxitin	23	5.75	389	94.25

3.3. Production of ESBL, AmpC, MBL and Carbapenemase

Only 31 of the tested isolates (7.75%) are resistant to more than 3 different classes of antibiotics, whereas only 5 isolates (1.25 %) are confirmed phenotypically as ESBL positive (Table 3). Out of the 23 (5.75 %)

isolates which showed resistance to Cefoxitin, none (0 %) is an AmpC producer. Cefoxitin resistance in this type of AmpC negative isolates could be attributed to the decreased permeability of porins, as reported by [Clark, \(1996\)](#); [Lee *et al.*, \(2003\)](#). In addition, none of the isolates is Carbapenemase positive.

Table 3: The frequency of ESBL, AmpC, and MBL production by the recovered *Salmonella* spp.

Beta lactamases	No. of positive isolates	Number of confirmed positive isolates	Percentage of confirm positive isolates (%)
ESBL	10	5	50
AmpC	23	0	0
MBL	0	0	0

where; ESBL= Extended spectrum beta-lactamases, AmpC = AmpC beta-lactamase, MBL= Metallo- β -lactamases

3.4. Co-Production of ESBL, AmpC, MBL and Carbapenemase

The co-production of Carbapenemase, MBL, AmpC and ESBL is not observed among all the tested *Salmonella* isolates shown in Table (4). The low prevalence of ESBL, AmpC, MBL and Carbapenemase in Jigawa state compared to other states such as Kano state and Lagos state, could be attributed to the low population of the state, and less human activities. Moreover, the chances of introduction of new infections or genes are minimal, due to lower numbers of immigrations from other parts of Nigeria for commercial, educational, or tourism activities. These factors contributed to the incidences of ESBL ([EUCAST. 2017](#)).

The prevalence of ESBL depends on a several factors including; the species, geographic locality, hospital/ward, group of patients and type of infection; however, large variations have been reported in different studies ([EUCAST. 2017](#)). The prevalence of

ESBL (1.25 %) obtained in this study coincide with the previous findings of [Lamini *et al.*, \(2018\)](#), which also reported a prevalence of 1.33 % in a study conducted in Nassarawa state, Nigeria. In addition, the study conducted by [Yusha'u *et al.*, \(2010\)](#) at Murtala Muhammed specialist hospital Kano, Nigeria to determine the prevalence of ESBL among Enterobacteriaceae confirmed the occurrence of 2.1 % of ESBL-positive *Salmonella* spp. among the isolates using the disc replacement method (DRM), and 1.3 % occurrence of *Salmonella* spp. based on CLSI breakpoint. These results are in accordance with the current study. On the other hand, the prevalence of ESBL (12.5 %) reported by [Yusuf *et al.*, \(2013\)](#), disagree with the value obtained in this study.

Low prevalence of Carbapenemases resistance (0.5 %) in clinical isolates of ESBL producing *E. coli* in North central Nigeria was also recorded by [Onyedibe *et al.*, \(2018\)](#), where only 1 out of 220 clinical isolates *E. coli* was recorded as resistant to carbapenem.

Table 4: Frequency of ESBL, AmpC and MBL Co-production by the *Salmonella* spp.

Beta lactamases	No. of positive	No. of confirmed	Percentage occurrence (%)
ESBL	38	5	(1.25)
AmpC	23	0	0
MBL	0	0	0
ESBL + AmpC	0	0	0
ESBL +MBL	0	0	0
MBL +AmpC	0	0	0
ESBL +AmpC +MBL	0	0	0

where; ESBL= Extended spectrum β -lactamases, AmpC = AmpC β -lactamase, MBL= Metallo- β -lactamases

Current results of the AmpC recorded 0.0 % prevalence of AmpC in Kano and Northwest Nigeria. These findings are in agreement with similar studies of [Yusuf *et al* \(2013\)](#); [Yusuf *et al* \(2014\)](#), which were carried out in order to determine the prevalence, antibiotic susceptibility of AmpC and ESBL producing clinical isolates at these areas.

Conclusion

A prevalence of 1.25 %, 0.0 % and 0 % were recorded for ESBL, AmpC β -lactamase and MBL; respectively, among the recovered isolates of *Salmonella* spp. This study indicated that *Salmonella* isolates which produces these enzymes were more resistant to the various antibiotics. Therefore, prudent use of antibiotics in the hospitals and community settings is strongly advocated. However, the state has in place a system where all drugs used in the state health care facilities are procured from standard manufacturers whose product were certified as good and distributed by a body called Jigawa state medicare supply organization (JIMSO). This has indeed reduced the possibilities of spreading fake or substandard drugs which also contributed to antibiotics resistance.

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Conflict of interest

The authors declare no conflict of interests, as the study was not against the cultural or spiritual believe of the people of the Jigawa state.

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Ethical approval

An Ethical clearance was obtained from the Ethical Committee Jigawa state Ministry of Health, prior to commencement of this study.

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