

# Occurrence of extended-spectrum and AmpC $\beta$ -lactamases in multiple drug resistant *Salmonella* isolates from clinical samples in Lagos, Nigeria

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**Purpose:** *Salmonella* spp. are important foodborne pathogens exhibiting increasing resistance to antimicrobial drugs. Resistance to broad-spectrum  $\beta$ -lactams, mediated by extended-spectrum  $\beta$ -lactamase (ESBL) and AmpC  $\beta$ -lactamase enzymes is fast spreading and has had negative impacts on the clinical outcomes, particularly on third-generation cephalosporins. This study investigated the carriage of AmpC gene among multidrug-resistant *Salmonella* spp. from Lagos, Nigeria.

**Methods:** Forty *Salmonella* spp. from clinical samples (*S. typhi* = 13; *S. typhimurium* = 10; *S. enteritidis* = 8; *S. choleraesuis* = 5; *S. paratyphi* = 4) were subjected to in vitro susceptibility test by disk diffusion methods. Isolates that were resistant to cefoxitin and third-generation cephalosporins were screened for ESBL (Double Disk Synergy Test Method) and AmpC enzyme (AmpC disk test) production. Detection of AmpC *fox* gene was carried out by polymerase chain reaction.

**Results:** Thirty-two (80%) of the *Salmonella* isolates were cefoxitin resistant. Plasmid-mediated AmpC  $\beta$ -lactamase and ESBL enzymes were recorded in 10/40 (25%) and 16/40 (40%) of the *Salmonella* isolates, respectively. Specifically, 16/40 (40%) of the *Salmonella* isolates possessed 380 bp AmpC *fox* gene, with the highest occurrence found in *S. typhi* strains (43.8%) followed by *S. typhimurium* (25%). There was no AmpC *fox* gene detected in *S. paratyphi* strains. Interestingly, coproduction of enzymes occurred in some of the isolates, raising fears of resistance to a multitude of antibiotics in the treatment of bacterial infections.

**Conclusion:** Emergence of AmpC  $\beta$ -lactamase-producing *Salmonella* isolates in our environment was recorded for the first time, raising concern on increased antibiotic resistance among strains of *Salmonella* serovars in Lagos. Further genotypic study of the isolates could answer the questions on strain sources, clonal relatedness, and mechanism of spread.

**Keywords:** *fox* gene, plasmid, minimum inhibitory concentration, resistance, *Salmonella*

## Introduction

*Salmonella* spp. are important pathogens that cause foodborne and waterborne diseases. The diseases associated with this pathogen in patients are generally treated using  $\beta$ -lactam antibiotics, particularly third- and fourth-generation cephalosporins.  $\beta$ -Lactamase production is an important mechanism of resistance to  $\beta$ -lactam antibiotics.<sup>1</sup> The  $\beta$ -lactamase enzyme deactivates  $\beta$ -lactam antibiotics, thereby breaking and opening the common element in their molecular structure, that is,  $\beta$ -lactam. Some of these enzymes include extended-spectrum  $\beta$ -lactamase (ESBL), AmpC  $\beta$ -lactamase, and carbapenemase.<sup>2</sup> In the last one decade, efforts to detect other  $\beta$ -lactamases, such as AmpC enzymes, in *Klebsiella* spp. and *Escherichia coli* isolates have been intensified, while those of *Salmonella* spp. seem to be scanty, particularly in developing countries. Fortunately, unlike the past two decades when the lack of standard guidelines

for detecting AmpC-producing isolates was a problem, the situation is no longer the same as the expression of AmpC  $\beta$ -lactamases can be generated either by chromosomal or plasmid genes.<sup>3</sup>

The origin of plasmid-mediated AmpC  $\beta$ -lactamases was initially traceable to the chromosomes of several Enterobacteriaceae species and is rarely inducible.<sup>3</sup> The transfer has resulted in plasmid-mediated AmpC  $\beta$ -lactamases in the isolates of *E. coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Citrobacter freundii*, *Enterobacter aerogenes*, *Serratia* spp., and *Proteus mirabilis*.<sup>4-6</sup> There have been increasing trends in the number of infections caused by ESBL and AmpC-producing organisms. Several studies have reported that  $\beta$ -lactamases producing gram-negative bacteria are recovered from hospitalized patients after they receive treatment with  $\beta$ -lactam antibiotics such as cefoxitin and undergo a prolonged stay in hospital.<sup>4-7</sup>

Although plasmid-mediated AmpC  $\beta$ -lactamases had been discovered two decades ago, most clinical laboratories and physicians are still unaware of their clinical significance. The aftermath effect of this is that organisms producing these types of  $\beta$ -lactamases often go undetected, thus resulting in hospital outbreaks.<sup>3</sup>

In Nigeria, there is a paucity of information on the documentation of AmpC  $\beta$ -lactamases producing *Salmonella* spp. Resistance to broad-spectrum  $\beta$ -lactams, mediated by ESBL, is fast emerging and has had negative impacts on the clinical outcomes.<sup>5,8</sup> The detection of AmpC- and ESBL-producing *Salmonella* strains is paramount, as it has both epidemiologic and therapeutic significance. Therefore, in view of the potential impact of  $\beta$ -lactamase-producing organisms on the clinical outcomes among infected patients in our environment, this study was undertaken to investigate the occurrence of ESBLs and AmpC enzymes among multidrug-resistant *Salmonella* isolates.

## Materials and methods

### Bacterial isolates

A total of 40 nonreplicate stock cultures of clinical *Salmonella* isolates from the Microbiology Division of Lagos State University and Nigerian Institute of Medical Research, Lagos, were used. The institutional review boards of Lagos State University and Nigerian Institute of Medical Research approved the study. All patients who provided samples gave written informed consent for their specimens to be used in this research. The organisms were isolated from the patients attending public hospitals; Nigerian Institute of Medical Research clinics; Lagos State University Teaching Hospitals; Central Public Health Laboratory Services, Lagos; and infec-

tious disease hospitals, Yaba, Lagos, between February 2014 and July 2015, who were diagnosed with pyrexia of unknown origin and/or gastroenteritis. The organisms were isolated from blood and stool specimens by standard procedures and the isolates were identified by standard methods as described by Cowan and Steel.<sup>9</sup> Confirmation of *Salmonella* spp. was done with API 20E identification system (Bio-Mérieux, Saint Vulbas, France). Further identification of *Salmonella* spp.-based somatic (O) and flagella (H) antigen characteristics was done using polyvalent antisera (Wellcome Diagnostic, Temple Hill, Dartford, UK).

### Antimicrobial susceptibility tests

All *Salmonella* isolates were investigated for their in vitro susceptibilities to 13 antibiotics by disk diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI) guidelines.<sup>10</sup> The disks (Oxoid, Basingstoke, Hampshire, UK) with the following were used for susceptibility testing: ampicillin (25  $\mu$ g), chloramphenicol (30  $\mu$ g), cotrimoxazole (25  $\mu$ g), tetracycline (25  $\mu$ g), nalidixic acid (30  $\mu$ g), ciprofloxacin (20  $\mu$ g), ofloxacin (20  $\mu$ g), gentamicin (10  $\mu$ g), cefotaxime (30  $\mu$ g), augmentin (30  $\mu$ g) (amoxicillin [20  $\mu$ g]/clavulanic acid [10  $\mu$ g] combination), ceftriaxone (30  $\mu$ g), ceftazidime (30  $\mu$ g), imipenem (30  $\mu$ g), levofloxacin (10  $\mu$ g), and azithromycin (15  $\mu$ g). Agar dilution test was also performed to determine the minimum inhibitory concentration (MIC) of some of the antibiotics tested against plasmid-mediated AmpC-carrying isolates. Each of the disk diffusion or agar dilution tests carried out was performed in triplicate, and the mean values expressed as mm or  $\mu$ g/mL were computed. Interpretation of results was done in accordance with the CLSI guidelines.<sup>11,12</sup> *E. coli* ATCC 25922 was used for quality control of the disk diffusion and agar dilution tests. Multidrug-resistant phenotype was defined as resistance to three or more classes of antibiotics.<sup>11</sup>

### Detection of ESBL production

All *Salmonella* isolates that exhibited resistance to third-generation cephalosporin antibiotics were screened for ESBL production by Double Disk Synergy Test Method using CLSI interpretative standard guidelines.<sup>12</sup> An isolate was considered ESBL positive if the diameter of zone of inhibition around the clavulanate or tazobactam containing disk was at least 4 mm greater than that of ceftazidime or cefotaxime alone.<sup>12</sup>

### Detection of inducible AmpC production Disk antagonism test

*Salmonella* isolates that fell within the susceptibility range to cefotaxime, ceftazidime, or ceftriaxone (third-generation

cephalosporins) and cefoxitin in-line with CLSI were subjected to disk antagonism test for inducible AmpC detection method described by Sanders et al.<sup>13</sup>

## Detection of plasmid-mediated AmpC production

### Amp disk approximation test

Isolates that yielded a cefoxitin zone diameter less than 18 mm and that were resistant to third-generation cephalosporins were further screened for AmpC enzyme production using AmpC disk test method described by Black et al.<sup>14</sup> and Yang et al.<sup>15</sup> Control strain *E. coli* ATCC 25922 was included.

## Molecular detection of plasmid-mediated AmpC $\beta$ -lactamases

### Detection of AmpC fox genes

This was done by polymerase chain reaction (PCR) using the primers designed by Pérez-Pérez and Hanson,<sup>16</sup> with a modification in the amplification condition after PCR optimization in our laboratory. The primers were synthesized and supplied by Fermentas (Carlsbad, Canada). Each PCR is of 20  $\mu$ L volume, comprising 1 $\times$  PCR buffer (pH 8.3), 1.5 mM of MgCl<sub>2</sub>, 200 nM each of the deoxynucleotide triphosphates, and 40 pmol each of the forward primer 5'-AAC ATG GGG TAT CAG GGA GAT G-3' and the reverse primer 5'-CAA AGC GCG TAA CCG GAT TGG-3'. The reaction was carried

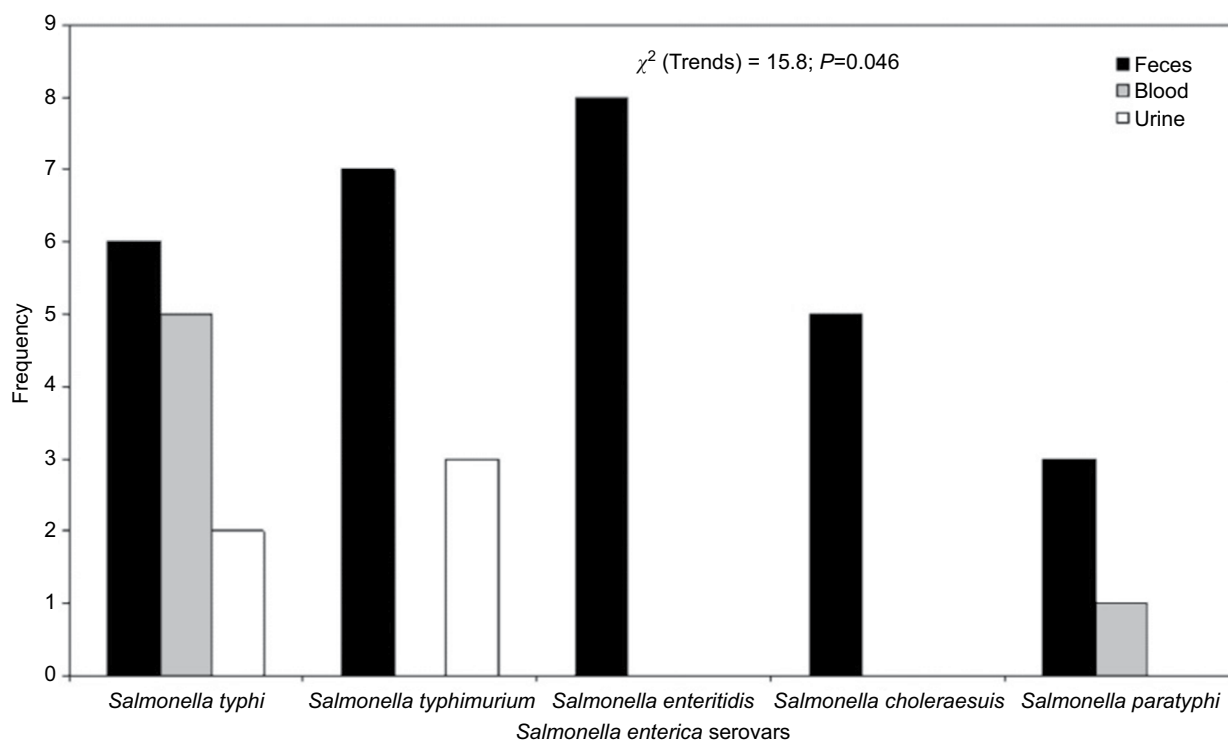
out in a Techne™ thermal cyclor (TC-312; Thermo Fisher Scientific, Waltham, MA, USA) with a program that consisted of an initial denaturation at 94°C for 5 min, followed by 25 cycles of DNA denaturation at 94°C for 30 s, primer annealing at 58°C for 30 s, and primer extension at 72°C for 60 s. This was completed by a final extension step at 72°C for 7 min. Aliquots of PCR products (10  $\mu$ L each) were separately loaded into wells created on 2% agarose gel prestained with ethidium bromide at 0.5  $\mu$ g/mL and electrophoresed at 70 V for 1 h. This was followed by visualization on a UV transilluminator. A 100 bp DNA ladder from Promega (Mannheim, Germany) was used as a marker. A fox AmpC carrying *E. coli* ATCC 8739 was used for quality control.

## Statistical methods

Data were expressed as numbers and percentages. Comparison was done using chi-square or Fischer's exact test for percentages and Student's *t*-test for mean values. *P* values <0.05 were considered to be significant. Data were analyzed using the statistical package for social sciences SPSS version 15.

## Results

In this study, five distinct species of *Salmonella* were isolated from fecal samples, while only *Salmonella typhi* (n=7) was recovered from blood (Figure 1). All ESBL producers showed enhanced inhibition of one or more extended-spectrum



**Figure 1** Distribution of *Salmonella* isolates in the clinical samples studied.

**Table 1** ESBL, AmpC production and AmpC *fox* gene detection and coexistence of two enzymes among the *Salmonella* isolates

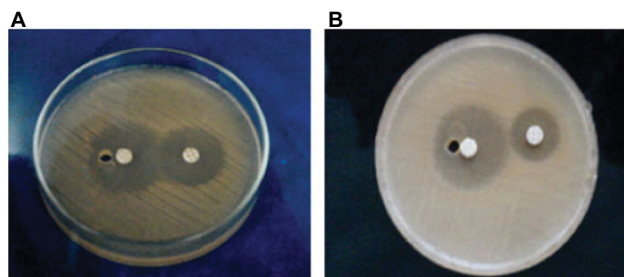
<i>Salmonella</i> spp. (number screened)	Cefoxitin resistance test, +ve (%)	ESBL, +ve (%)	Plasmid-mediated AmpC $\beta$ -lactamase production (%)	AmpC <i>fox</i> gene (%)	ESBL + AmpC (%)	ESBL + AmpC <i>fox</i> gene (%)
<i>Salmonella typhi</i> (13)	12 (92.3)	6 (46.2)	4 (30.8)	7 (53.8)	2 (15.4)	2 (15.4)
<i>Salmonella typhimurium</i> (10)	8 (80.0)	3 (30.0)	2 (20.0)	4 (40.0)	0 (0.0)	0 (0.0)
<i>Salmonella enteritidis</i> (8)	8 (100)	4 (50.0)	3 (37.5)	3 (37.5)	1 (12.5)	1 (12.5)
<i>Salmonella paratyphi</i> (4)	2 (50.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Salmonella choleraesuis</i> (5)	2 (40.0)	2 (40.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)
Total (40)	32 (80.0)	16 (40.0)	10 (25.0)	16 (40.0)	3 (7.5)	3 (7.5)

**Abbreviation:** ESBL, extended-spectrum  $\beta$ -lactamase.

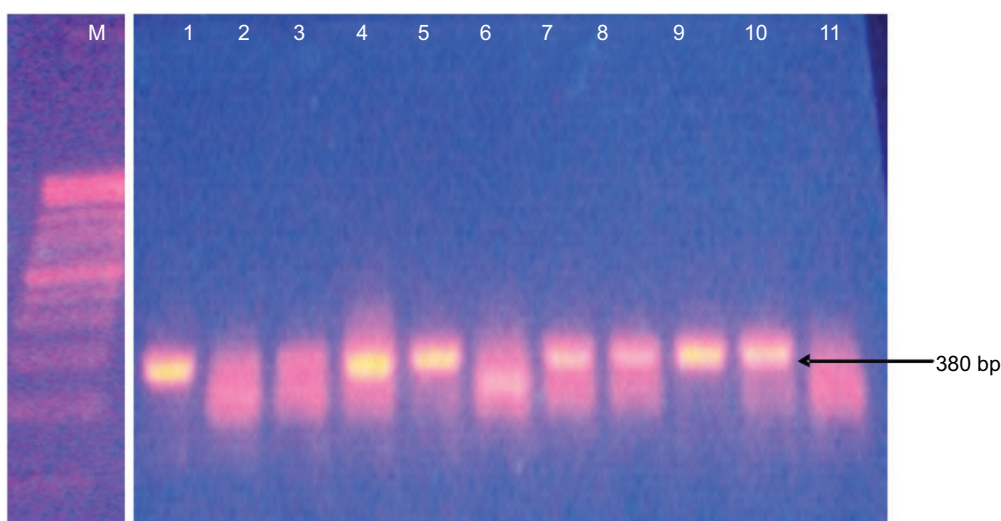
$\beta$ -lactams by the disk potentiation method and exhibited a 5 mm or greater increase in the zone diameter of ceftazidime plus inhibitor with respect to ceftazidime alone. ESBL enzymes were detected in 16/40 (40%) of the *Salmonella* isolates. *S. typhi* recorded the highest number of ESBL-positive serovars (6), followed by *Salmonella enteritidis* (4) in this study (Table 1; Figure 2).

The AmpC-producing isolates demonstrated cefoxitin zone diameters <18 mm. The results showed that 32/40 (80%)

of the *Salmonella* isolates were cefoxitin resistant. None of the isolates were positive for inducible AmpC  $\beta$ -lactamases. Phenotypically, plasmid-mediated AmpC  $\beta$ -lactamase was recorded in 10/40 (25%) *Salmonella* isolates. AmpC maker *fox* gene was used to detect plasmid-mediated AmpC  $\beta$ -lactamase in *Salmonella* isolates by molecular method, and 16/40 (40%) of the *Salmonella* isolates were positive (Table 1). Consequently, 10/40 (25%) of the AmpC  $\beta$ -lactamase producers screened phenotypically were positive, compared to 16/40 (40%) AmpC *fox* genes detected by PCR molecular method. All the plasmid-mediated AmpC  $\beta$ -lactamase *Salmonella* isolates possessed 380 bp AmpC *fox* genes (Figure 3), with the highest occurrence of *fox* gene found in *S. typhi* strains (43.8%) followed by *Salmonella typhimurium* (25%). There was no AmpC *fox* gene detected in *S. paratyphi* strains (Table 1). In this study, coexistence of ESBL and AmpC  $\beta$ -lactamases (*fox* gene) occurred in 3/40 (7.5%) of the isolates, mainly in two strains of *S. typhi* and one strain of *S. enteritidis* (Table 1).



**Figure 2** Extended-spectrum  $\beta$ -lactamase production by *Salmonella enteritidis* and *Salmonella typhi* in plates (A) and (B), respectively.



**Figure 3** Detection of 380 bp AmpC *fox* gene by PCR among the *Salmonella* isolates.

**Notes:** Lane M = 100 bp ladder; lanes 1–3 = *Salmonella typhimurium*; lanes 4–7 = *Salmonella typhi*; lanes 8 and 9 = *Salmonella enteritidis*; lane 10 = *Salmonella choleraesuis*; lane 11 = *Salmonella paratyphi*.

**Abbreviation:** PCR, polymerase chain reaction.

Sixteen of 40 (50%) of the cefoxitin-resistant isolates were AmpC  $\beta$ -lactamase producers and 50% of non-AmpC *Salmonella* isolates were also recorded. In all, 60% (24/40) of the *Salmonella* isolates were non-AmpC  $\beta$ -lactamase producers. On comparison of the resistance ability of *fox* gene-positive and -negative isolates to other antibiotics tested, data presented in Table 2 show significant association ( $P < 0.05$ ) between *fox* AmpC carriage and resistance to nitrofurantoin, nalidixic acid, and augmentin (amoxicillin/clavulanic acid). The mean MIC values of antibiotics used against *fox* AmpC and ESBL *Salmonella* isolates varied among the serotypes. For example, an average MIC value of 0.19  $\mu\text{g/mL}$  was obtained for levofloxacin against *fox* AmpC *Salmonella choleraesuis* producer similar to *S. enteritidis* (Table 3). There was a general increase in the average MIC values recorded for most of the antibiotics evaluated against *Salmonella* serotypes with dual carriage of *fox* AmpC and ESBL, compared with

*fox* AmpC carriage alone. For example, there was an increase in the mean MIC values for ceftazidime and cotrimoxazole from 7.1 and 9.7  $\mu\text{g/mL}$  against *fox* AmpC *S. typhi* single carriage to 19.4 and 11.4  $\mu\text{g/mL}$  against dual carriers of *fox* AmpC and ESBL *S. typhi*, respectively (Table 3).

## Discussion

The prevalence of ESBL among *Salmonella* serotypes in this study was 16/40 (40%). This result is on the high side when compared with earlier studies conducted in Nigeria and elsewhere, particularly among the bacterial genera of the family Enterobacteriaceae. For example, in two separate studies conducted in Kano State, Nigeria by Yusha'u et al<sup>17</sup> and Yusuf and Haruna,<sup>5</sup> ESBL prevalence of 9.3% and 15.8%, respectively, was recorded in gram-negative bacteria. This is an indication of the increasing trend of ESBL in the clinical settings in our environment. In our previous report, ESBL production due to *bla*CTX-M-I gene cluster was detected in 45.8% of *Salmonella* isolates.<sup>18</sup> This finding further affirmed the circulation of ESBL-producing *Salmonella* spp. in our locality.

In this study, none of the isolates was positive for inducible AmpC  $\beta$ -lactamases, thus ruling out the presence of chromosomal-mediated AmpC  $\beta$ -lactamase resistance in the *Salmonella* isolates evaluated. Consequently, 10/40 (25%) of the AmpC  $\beta$ -lactamase producers screened phenotypically were positive, compared to 16/40 (40%) AmpC *fox* genes detected by PCR molecular method. The observed difference in the number of AmpC  $\beta$ -lactamase producers by the molecular and phenotypic methods used may be attributed to the false-negative and false-positive results associated with the phenotypic method. It has been documented that possible misleading results arising from the phenotypic tests for AmpC are one of the biggest challenges associated with its specificity and sensitivity, which may impact negatively on the accuracy of surveillance and hospital infection control measures.<sup>19</sup>

**Table 2** Comparison of AmpC-producing and nonproducing *Salmonella enterica* serovars in relation to antibiotic resistance

Antibiotics	AmpC-producing and nonproducing <i>Salmonella enterica</i> serovars for antibiotic resistance			
	<i>fox</i> AmpC (+ve, n=16, %)	<i>fox</i> AmpC (-ve, n=24, %)	$\chi^2$	P-value
AMP	16 (100)	22 (91.7)	0.19	>0.05
COT	16 (100)	21 (87.5)	0.74	>0.05
NIT	9 (56.3)	2 (8.3)	8.7	<0.01
GEN	13 (81.3)	20 (83.3)	0.06	>0.05
NAL	16 (100)	13 (54.2)	7.9	<0.05
OFL	0 (0)	0 (0)	ND	ND
AUG	16 (100)	16 (66.7)	4.8	<0.05
TET	16 (0)	24 (100)	ND	ND
IMP	2 (12.5)	0 (0)	1.07	>0.05

**Notes:** Data are number (%) with disparity in % antibiotic resistance between *fox* AmpC (+ve) and *fox* AmpC (-ve) analyzed by chi-square ( $\chi^2$ ) or Fischer's exact test.  $P < 0.05$  was considered to be significant.

**Abbreviations:** AMP, ampicillin; AUG, augmentin; COT, cotrimoxazole; GEN, gentamicin; IMP, imipenem; NAL, nalidixic acid; ND, not determined; NIT, nitrofurantoin; OFL, ofloxacin; TET, tetracycline.

**Table 3** MIC of antibiotics against *fox* AmpC and ESBL-producing isolates

Antibiotics	AmpC		AmpC + ESBL		P-value	AmpC		AmpC + ESBL		P-value
	<i>Salmonella choleraesuis</i>	<i>Salmonella enteritidis</i>	<i>Salmonella typhimurium</i>	<i>Salmonella typhi</i>						
	LAEV	0.19 (0.12–0.25)	ND	0.19 (0.12–0.5)		0.2 (0.12–0.25)	>0.05	0.47 (0.12–1.0)	ND	
IMP	0.18 (0.25–0.5)	ND	0.42 (0.25–0.5)	0.42 (0.25–0.5)	>0.05	0.56 (0.25–1.0)	ND	0.71 (0.25–2.0)	1.07 (0.5–2.0)	>0.05
COT	8.5 (4–16)	ND	8 (4–16)	13.3 (8–16)	>0.05	10 (4–16)	ND	9.7 (4–16)	11.4 (4–16)	>0.05
GEN	1.5 (1–2)	ND	2.3 (1–4)	3.3 (2–4)	>0.05	2.75 (1–4)	ND	3 (1–4)	3.4 (1–4)	>0.05
CAZ	6 (4–8)	ND	5.3 (4–8)	13.3 (8–16)	<0.05	4.5 (2–8)	ND	7.1 (2–16)	19.4 (8–32)	<0.05

**Notes:** MIC, mean (range),  $\mu\text{g/mL}$ . Difference in mean MIC values between AmpC + ESBL versus AmpC alone was analyzed by Student's t-test.  $P < 0.05$  was considered to be significant.

**Abbreviations:** CAZ, ceftazidime; COT, cotrimoxazole; ESBL, extended-spectrum  $\beta$ -lactamase; GEN, gentamicin; IMP, imipenem; LAEV, levofloxacin; MIC, minimum inhibitory concentration; ND, not determined.

Plasmid-mediated AmpC  $\beta$ -lactamases were first reported in the late 1980s, but till date, many infectious disease personnel are still unaware of their clinical significance due to lack of surveillance or inaccurate laboratory testing. The number of infections caused by AmpC-producing pathogens in the family Enterobacteriaceae is increasing in several countries like Italy,<sup>20</sup> India,<sup>4</sup> Egypt,<sup>6</sup> and Japan.<sup>21</sup> All the plasmid-encoded AmpC  $\beta$ -lactamase *Salmonella* isolates detected in this study possessed 380 bp AmpC *fox* genes.

The overall results of 40% AmpC  $\beta$ -lactamase producers detected by *fox* group genes are on the high side. However, scanty data on the prevalence of plasmid-mediated AmpC  $\beta$ -lactamase-producing *Salmonella* spp. in this part of the world and elsewhere had made objective comparison of these results very difficult. Nevertheless, we recorded the highest occurrence (43.8%) of AmpC *fox* genes in *S. typhi* strains followed by *S. typhimurium* (25%). Our finding is similar to that of the study carried out by El-Hady and Adel<sup>6</sup> who reported 30.4% AmpC  $\beta$ -lactamase producers detected by *fox* group genes, 56.5% by *cit* group genes (including CMY-2), and 73.9% by *mox* group genes (including CYM-1) in the clinical isolates of Enterobacteriaceae. There was no AmpC *fox* gene detected in *S. paratyphi* strains (Table 2). Interestingly, we observed varying capability of *Salmonella* serotypes to produce AmpC and express *fox* genes. For example, AmpC *fox* gene in *S. typhi* was found among three ESBL positives, but not expressed. In *S. typhimurium*, two of the ESBL-positive isolates also carried AmpC *fox* gene, but did not express detectable level by the AmpC disk test. These observations may be due to other possible AmpC types and/or mechanisms mediating cefoxitin resistance in *Salmonella* isolates. In *S. choleraesuis*, *fox* AmpC gene carriage was not detected by PCR. Cefoxitin resistance found in ESBL-positive isolates (n=2) could have been mediated by other mechanisms, including other AmpC  $\beta$ -lactamases not analyzed in this study.

To the best of our knowledge, this is the first report to describe the detection of plasmid-mediated AmpC *Salmonella* spp. in Lagos and, thus, calls for serious concerns.

Coexistence of ESBL and AmpC  $\beta$ -lactamases (*fox* gene) was detected in three (9.1%) isolates, particularly in *S. typhi* and *S. enteritidis* strains (Table 1). The dual carriage of the *fox* AmpC gene and the phenotypic expression of ESBL observed in *S. enteritidis* and *S. typhi* isolates resulted in enhanced ceftazidime resistance (>2-fold increase), which was statistically significant ( $P < 0.05$ ) when compared with *fox* AmpC gene carriage alone. Reduced, but not significant

( $P > 0.05$ ) susceptibility to levofloxacin, imipenem, cotrimoxazole, and gentamicin was also observed among these isolates ( $P > 0.05$ ; Table 3). The coexistence of bacteria in the family Enterobacteriaceae has been documented. For instance, Yusuf and Haruna<sup>5</sup> reported a prevalence of 5.8% in *E. coli*. Similar observations were observed in *Klebsiella* spp. (11.1%) and *Proteus* spp. Sinha et al<sup>22</sup> recorded 8% coexistence among *E. coli*. Also, coexistence of ESBL *bla*SHV28 and the AmpC *bla*CMY-2 in *K. pneumoniae* isolated from animals has been documented in Italy.<sup>20</sup> Therefore, the observed coproduction of ESBL and AmpC  $\beta$ -lactamases in our isolates suggests that ESBL coexistence with AmpC works synergistically and increases the MIC (Table 3). It also suggests the possible spread of plasmid-mediated multiple drug resistance (MDR) isolates to aminoglycosides, trimethoprim-sulfamethoxazole, and fluoroquinolones.

The inability to use other AmpC makers such as *mox* group genes, *cit* group genes, and *ebc* group genes to accommodate other most common types of plasmid-mediated AmpC  $\beta$ -lactamases is one of the limitations of this study.

## Conclusion

This study revealed the occurrence of plasmid-mediated AmpC  $\beta$ -lactamases producing MDR *Salmonella* isolates in our environment for the first time. It also showed the coexistence of ESBL and AmpC  $\beta$ -lactamases (*fox* gene) in some strains of *Salmonella* isolates. A need to screen the ESBL potential of MDR *Salmonella* isolates for plasmid-mediated AmpC  $\beta$ -lactamases, along with performing antibiogram is advocated before making therapeutic decisions in patient management in our hospitals. This is essential to prevent treatment failure and stem mortality. Further investigations are required on other families of AmpC  $\beta$ -lactamase-encoded genes, to study the mechanisms of cefoxitin resistance among *Salmonella* isolates, and to sequence *fox* AmpC genes detected for source attribution. Therefore, application of molecular typing techniques such as multilocus sequencing techniques and pulse field gel electrophoresis on plasmid-mediated AmpC  $\beta$ -lactamases and ESBL-producing strains of *Salmonella enterica* serovars is envisaged. This is essential in order to assess the levels of homogeneity and heterogeneity and for better understanding of the mechanisms of antibiotic resistance and spread.

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## Disclosure

The authors report no conflicts of interest in this work.

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