

# *bla*<sub>CTX-M-I</sub> group extended spectrum beta lactamase-producing *Salmonella typhi* from hospitalized patients in Lagos, Nigeria

Kabiru O Akinyemi<sup>1</sup>  
Bamidele A Iwalokun<sup>2</sup>  
Olajide O Alafe<sup>1</sup>  
Sulaiman A Mudashiru<sup>1</sup>  
Christopher Fakorede<sup>1</sup>

<sup>1</sup>Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria; <sup>2</sup>Biochemistry and Nutrition Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria



Correspondence: Kabiru O Akinyemi  
Department of Microbiology, Lagos State University, Badagry Expressway, Ojo, PMB 0001, Lasu Post Office, Ojo, Lagos, Nigeria  
Tel +234 80 2908 8676  
Email [akinyemiko@yahoo.com](mailto:akinyemiko@yahoo.com)

**Purpose:** The global spread of *bla*<sub>CTX-M-I</sub> extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* spp. remains a major threat to treatment and control. Evidence of emergence and spread of this marker are lacking in Nigeria. This study investigated *bla*<sub>CTX-M-I</sub> ESBL production among *Salmonella* isolates from hospitalized patients.

**Methods:** Patients (158 total) made up of two groups were evaluated. Group A was composed of 135 patients with persistent pyrexia and group B was composed of 23 gastroenteritis patients and their stool samples. Samples were cultured, and isolates were identified and were subjected to antibiotic susceptibility testing by standard methods. Isolates were further screened for ESBL production, *bla*<sub>CTX-M-I</sub> genes and transferability by double disk synergy test, plasmid extraction, polymerase chain reaction, and conjugation experiment.

**Results:** Thirty-five (25.9%) *Salmonella* isolates were identified from group A, of which 74.3% were *S. typhi*, 22.9% were *S. paratyphi* and two (5.7%) were invasive non-typhoidal *S. enteritidis*. Nine *Plasmodium falciparum* infections were recorded, four of which were identified as co-infections with typhoidal *Salmonella*. Only two (8.7%) *S. enteritidis* samples were obtained from group B ( $P > 0.05$ ). A total of 24 isolates were ESBL-positive, eliciting resistance to five to seven antibiotics, and were multiple-drug resistant. ESBL production due to the *bla*<sub>CTX-M-I</sub> gene cluster was detected in eleven (45.8%) *Salmonella* isolates. Nine (81.8%) of the eleven *bla*<sub>CTX-M-I</sub> ESBL producers were *S. typhi* and two (18.2%) isolates were *S. enteritidis*. Four of nine *S. typhi* *bla*<sub>CTX-M-I</sub> ESBL-producing strains harbored 23 kb self-transmissible plasmid that was co-transferred with cefotaxime and augmentin resistance to *Escherichia coli* j53-2 transconjugants.

**Conclusion:** This study revealed the emergence of *bla*<sub>CTX-M-I</sub> *S. typhi* as an agent of persistent pyrexia with potential to spread to other Enterobacteriaceae in Lagos, Nigeria. Cautionary prescription and judicious use of third-generation cephalosporins, particularly cefotaxime, for the treatment of typhoid fever and routine screening for *P. falciparum* co-infection with ESBL-producing *Salmonella* in the laboratories during diagnosis of persistent pyrexia conditions in patients are recommended.

**Keywords:** ESBL, emergence, plasmid, *Salmonella*, cephalosporin

## Introduction

In developing countries, diseases such as enteric fever and diarrhea are on the increase due to poor sanitation and inadequate potable water supply.<sup>1</sup> An estimated annual incidence of 540 per 100,000 for typhoid fever in developing countries and about 21 million cases worldwide had been reported.<sup>2</sup> The number of strains of *Salmonella enterica* serovars that have developed resistance to one or more antibacterial agent has steadily increased, probably due to continuous antibiotic pressure.<sup>3</sup> Resistance to third-generation cephalosporins (3GCs) due to acquisition and expression of

*bla*-CTX-M-mediated, extended-spectrum  $\beta$ -lactamase (ESBL) enzymes among Gram-negative bacteria in the family Enterobacteriaceae is also on the increase. These enzymes are produced exclusively by Gram-negative bacteria and are active against 3GCs, especially cefotaxime, compared to *bla*-TEM and *bla*-SHV ESBLs.<sup>3</sup> These ESBL enzymes are usually plasmid-mediated, but are susceptible to cephamycins such as ceftiofur, beta-lactamase inhibitors such as clavulanate, sulbactam, tazobactam, and carbapenems.<sup>4</sup>

ESBL-producing organisms are reported to account for a significant proportion of intensive care infections and mortality in children and immunocompromised patients.<sup>5</sup> Problems of ESBLs have led to limited as well as expensive treatment options, and have impacted negatively on clinical outcomes.<sup>6</sup> Growing incidences of ESBLs in *Salmonella* species have been identified in numerous countries of Latin America, Africa, Europe, and Asia.<sup>3,7,8</sup> In Nigeria, *bla*<sub>CTX-M-I</sub> ESBL-producing *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp. have been documented.<sup>9–11</sup> However, data on *bla*<sub>CTX-M-I</sub>-mediated ESBL-producing *S. enterica* serovars are currently not available in Nigeria. In a recent work carried out by our research team, we provided evidence of ESBL-*bla*-CTX-M-producing *S. enterica* serovars in Lagos.<sup>12</sup> Unfortunately, these strains were not characterized for carriage of the *bla*<sub>CTX-M-I</sub> gene cluster, which is associated with community-associated infections in many countries including Nigeria.<sup>11,13</sup> This family of *bla*<sub>CTX-M-I</sub>, reported to have replaced the *bla*-CTX-M-2 gene cluster in the late 1990s, have now spread worldwide among many members of the Enterobacteriaceae, including *Salmonella*.<sup>14</sup>

In recent times, increasing episodes of persistent fever among patients affected by *Salmonella typhi* and *Plasmodium falciparum* infections has been a major concern in hospitals and clinics in Lagos and other parts of Nigeria.<sup>15,16</sup> This is because of the perceived clinical failure associated with the use of 3GCs, particularly cefotaxime, ceftriaxone, and cefuroxime, and increasing drug pressure with artemisinin combination therapies.<sup>15,16</sup> The current World Health Organization (WHO) recommendation for parasite-based diagnosis of malaria in pyrexia cases before treatment in the era of artemisinin-based combination therapy (ACT) has further made it imperative to screen for malaria parasites by microscopic or rapid diagnostic method in countries like Nigeria, where ACT has replaced monotherapies as first- and second-line treatments for uncomplicated malaria.<sup>17,18</sup> In the current study, we investigated the carriage of *bla*<sub>CTX-M-I</sub> ESBL among *Salmonella* strains isolated from patients, and the possibility of *Plasmodium* spp. co-infection.

## Materials and methods

### Patient population and study design

A total of 158 patients who sought treatment at referral centers including Ikeja General Hospital, Lagos; Infectious Diseases Hospital Mainland, Lagos; Central Bank of Nigeria Clinics Satellite, Lagos; and Central Medical Laboratory Health Centre, Lagos from October 2010 to July 2011 were recruited for the study. Important biodata, history of vaccination, antimicrobial therapy, time of onset of illness, etc, of these patients were recorded. Ethics approval from the ethics committee of each institution was obtained prior to patients' enrollment.

### Case definition, sample processing, *Plasmodium* and *Salmonella* detection

Two categories of patients were demarcated. A total of 135 patients were assigned to group A, and had been diagnosed by a physician for persistent fever ( $\geq 37.5^\circ\text{C}$ ) in the previous 72 hours with or without one or more of the following symptoms: diarrhea, headache, abdominal pains, loss of appetite, vomiting, and/or nausea for 5 consecutive days. In detail, 4 mL of blood was collected from each of the patients at the early onset of symptoms. Three of the 4 mL blood samples in each case were inoculated into 27 mL of brain-heart infusion (BHI) broth (Oxoid, Basingstoke, UK) for bacteriological culture, while the remaining blood sample was used for the preparation of thick and thin blood films on grease-free slides (two per sample) for the detection and speciation of *Plasmodium* parasites by light microscopy. Group B was made up of 23 patients who had presented with frequent stools for 2 or more days (diarrhea). A fresh stool sample from each patient was inoculated into Cary-Blair transport medium (10 mL/tube) and was brought to the laboratory for bacteriological culture.

### Bacterial agent isolation

Blood samples in the inoculated BHI broth culture bottles were incubated overnight aerobically at  $37^\circ\text{C}$ . Similarly, stool samples from Cary-Blair medium were inoculated into enrichment Selenite F broth (Oxoid) and were incubated at  $37^\circ\text{C}$  for 18–24 hours aerobically. Thereafter, sub-cultures were made onto deoxycholate citrate agar, *Salmonella-Shigella* agar, and MacConkey agar plates. All the culture agar plates were incubated at  $37^\circ\text{C}$  aerobically for 18–24 hours. In negative blood samples, sub-cultures were repeated daily from the BHI broth cultures for 7 consecutive days, after which the samples were disposed of. After overnight incubation, culture plates were examined for colonial morphology and Gram

stained. Colonies were first identified by standard methods, as described in Cowan and Steel's Manual.<sup>19</sup> The Analytical Profile Index 20E identification system (Institut Mérieux, Marcy l'Etoile, France) was used for the confirmation of the *Salmonella* isolates. Further identification of *Salmonella* species based on their somatic (O) and flagella (H) antigen characteristics was done using polyvalent antisera (Wellcome Diagnostic, London, UK).

## Antimicrobial susceptibility testing

All *Salmonella* isolates were investigated for their in vitro susceptibilities to 13 antibiotics by disk diffusion, as described by Clinical and Laboratory Standard Institute (CLSI) guidelines.<sup>20</sup> Disks with the following preparations were used for susceptibility testing: ampicillin (25 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), tetracycline (25 µg), nalidixic acid (30 µg), ciprofloxacin (20 µg), ofloxacin (20 µg), gentamicin (10 µg), cefotaxime (30 µg), augmentin (30 µg; amoxicillin 20 µg/clavulanic acid 10 µg combination), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (30 µg), levofloxacin (10 µg), and azithromycin (15 µg) (Oxoid). The plates were incubated aerobically at 37°C for 18–24 hours. The diameter of the zones of inhibition were measured with a ruler and compared with a zone interpretation chart.<sup>14</sup> *E. coli* American Type Culture Collection (ATCC) 25922 was used as a control. Multidrug resistance phenotype was defined as resistance to three or more classes of antibiotics.

## ESBL assay

All the isolates that exhibited reduced susceptibility and/or resistance to 3GCs were screened for ESBL production, using the double disk synergy test method. This was done by placing the 3GC antibiotics, ie, ceftazidime (30 µg) and ceftriaxone (30 µg) at a distance of 15 mm (center to center) from 30 µg augmentin (20 µg amoxicillin combined with 10 µg clavulanic acid), using CLSI interpretative guidelines as the standard.<sup>21</sup> An aliquot of a 0.5 µl *Klebsiella pneumoniae* ATCC 700603 was used as the positive control and *E. coli* ATCC 25922 was used as the negative control in each test batch.

## Plasmid DNA extraction

Plasmid extraction was performed by a simplified alkaline lysis method described by Cheng et al.<sup>22</sup> Briefly, overnight culture (1.5 mL) was centrifuged at 5,000 rpm for 1 minute to pellet the cells. The supernatant was gently decanted. After washing, the cell pellet was re-suspended in 300 µL Tris (tris(hydroxymethyl)aminomethane)-EDTA

(ethylenediaminetetraacetic acid)-NaOH-SDS (sodium dodecyl sulfate) (TENS) buffer solution and mixed by gentle inversion (five times) within 3–5 minutes of incubation on ice to obtain a straw-like, sticky lysate. This was followed by neutralization by adding 200 µL of 5 M potassium acetate buffer (pH 5.2), followed by incubation on ice for 10 minutes. Plasmid DNA solution was recovered as supernatant after centrifugation at 10,000 rpm for 10 minutes. Plasmid pellets isolated with ice-cold absolute ethanol were washed in 70% ethanol before re-suspension in 20–40 µL Tris (10 mM)-EDTA (1 mM) buffer (pH 8.0). The plasmid DNAs were separated by electrophoresis on 0.8% agarose pre-stained with ethidium bromide (0.5 µg/mL).

## Detection of *bla*<sub>CTX-M-I</sub> gene cluster

Genomic DNA was extracted from the *Salmonella* isolates by boiling method and was used as a template for the detection of ESBL *bla*<sub>CTX-M-I</sub> gene cluster by polymerase chain reaction (PCR). The 25 µL PCR reaction mixture contained 10 pmol of each forward and reverse primer pair for the *bla*<sub>CTX-M-I</sub> gene cluster, 200 µM of deoxynucleotide triphosphate, 1 µL of Taq DNA polymerase, and 1 µL of DNA solution. The PCR program consisted of an initial denaturation step of 5 minutes at 94°C, a 30 cycle period (each cycle consisting of 30 seconds at 94°C, 40 seconds at 52°C, and 50 seconds at 72°C), and then a final extension step of 5 minutes at 72°C. The primer nucleotide sequences used for the 415 bp *bla*<sub>CTX-M-I</sub> group gene were 5'-AAA AAT CAC TGC GCC AGT TC-3' and 5'-AGC TTA TTC ATC GCC ACG TT-3' for Enterobacteriaceae species, as previously reported by Woodford et al.<sup>23</sup> The 415 bp *bla*<sub>CTX-M-I</sub> gene was resolved on 1.5% agarose gel pre-stained with ethidium bromide (0.5 µg/mL) by electrophoresis.

## Conjugation experiment

*Salmonella* isolates harboring the *bla*<sub>CTX-M-I</sub> group of ESBLs were selected for the conjugation experiment using the broth mating technique described by Chen et al.<sup>24</sup> with rifampicin-resistant *E. coli* j53-2 as the recipient. Transconjugants were selected on a BHI agar plate containing cefotaxime (4 µg/mL) and rifampicin (200 µg/mL). Transfer of *bla*<sub>CTX-M-I</sub> gene, plasmids, and co-dissemination of antibiotic resistance was confirmed by PCR, plasmid isolation, and antibiotic susceptibility testing for the transconjugants and recipient, as previously done for the donor.

## Statistical analyses

Data were expressed as frequencies or percentages. Fischer's exact test was performed on 2×2 and 2×3 contingency tables

of data for assessing disparity in *Salmonella* isolation rates between blood and stool samples, and ESBL patterns among the three *S. enterica* serovars isolated. Outcomes of  $P < 0.05$  were taken to be significant.

## Results

Out of the 135 samples screened in group A, 35 *Salmonella* isolates (25.9%) were made up of three serotypes; *S. typhi* (71.4%), *S. paratyphi* (22.9%), and two (5.7%) of *Salmonella enteritidis*. Nine *Plasmodium* spp. were identified. Three strains of *S. typhi* and a strain of *S. paratyphi* were isolated from patients with *Plasmodium* sp. and complications. Thirteen of the 23 stool samples from group B were confirmed positive for bacterial pathogens, made up of eleven strains of *E. coli* and two strains of *S. enteritidis* (Table 1). Further analysis revealed non-significant difference ( $P > 0.05$ ) in the observed disparity in the isolation rate of *S. enterica* isolates or *S. enteritidis* isolates between culture-positive stool and blood samples from the two groups of patients studied.

Antibiotic resistance patterns of the isolated 37 *S. enterica* serovars are presented in Table 2. A total of 24 isolates were ESBL producers, elicited resistance to 5–7 antibiotics, and accounted for eleven (78.6%) of the 14 antibiotic resistance patterns observed. On the whole, 75.7% of the recovered *S. enterica* serovars isolated were multidrug resistant. PCR analysis revealed ESBL production due to *bla*<sub>CTX-M-I</sub> gene cluster in eleven (45.8%) *S. enterica* serovars. These strains also accounted for 76.9% (ten of 13 cases) of augmentin resistance observed. Carriage of *bla*<sub>CTX-M-I</sub> was also significantly associated with cefotaxime resistance (Table 2). However, all the *Salmonella* isolates were sensitive to imipenem, levofloxacin, and azithromycin. Of the eleven *bla*<sub>CTX-M-I</sub> ESBL producers detected, nine (81.8%) were *S. typhi* and two (18.2%) were *S. enteritidis*. Non-production of ESBL by 24%, 75%, and 25% of *S. typhi*, *S. paratyphi*, and *S. enteritidis* ( $P = 0.03$ ), respectively, was also observed (Figure 1). Conjugation experiments revealed a 23 kb self-transmissible plasmid and co-transfer of cefotaxime

and augmentin resistance to *E. coli* j53-2 transconjugants by four of the eleven *bla*<sub>CTX-M-I</sub>-producing donor serovars, all of which were *S. typhi* strains (Figure 2 and Table 3).

## Discussion

3GCs remain the most commonly prescribed class of antibiotics for case management of typhoidal and non-typhoidal salmonellosis in many countries of the world, including Nigeria<sup>12,25</sup>. In this study, 37 *Salmonella* isolates, which included 35 (25.9%) from blood of patients with persistent pyrexia and two (8.7%) *S. enteritidis* from stool samples of patients with gastroenteritis were isolated to yield a prevalence of 20.8% salmonellosis. *S. typhi* was further found to account for 71.4% of persistent pyrexia cases due to *Salmonella* isolates producing *bla*<sub>CTX-M-I</sub> ESBLs that were mediated by a 23 kb plasmid. These phenotypic and molecular characteristics were observed in 75.6% of *Salmonella* isolates that were multidrug resistant.

The findings from the current study indicated that the epidemiology of *Salmonella* as a public health burden in Lagos has not changed from the previous reports from Lagos<sup>12,15,26</sup> and from other parts of Nigeria.<sup>16</sup> Interestingly, in this study, some of the bacteremic strains of *S. typhi* that produced *bla*<sub>CTX-M-I</sub> ESBL can be used to explain why persistent pyrexia occurs in affected patients despite them receiving treatment with 3GCs. The implication of this is the potential for spread of emerging *bla*<sub>CTX-M-I</sub>-producing *S. typhi* in Lagos, which will add to the prevailing public health burdens in the state. In our previous study in the same environment, we found 53.1% of *Salmonella* isolates, composed of *Salmonella typhimurium*, *S. enteritidis*, *S. paratyphi*, and *Salmonella choleraesuis* from gastroenteritis cases to be *bla*<sub>CTX-M-I</sub> positive.<sup>12</sup> The present result found eleven (nine *S. typhi* and two *S. enteritidis*) of 37 *Salmonella* isolates recovered to be *bla*<sub>CTX-M-I</sub>-positive, with four *S. typhi* isolates transferring this genetic marker to *E. coli* j53-2. This finding indicates that other groups of *bla*-CTX-M exist among clinical *Salmonella* isolates in Lagos and that *bla*<sub>CTX-M-I</sub> carriage may be an emerging trend with pathogenic

**Table 1** Percentage distribution of pathogens isolated according to clinical samples

Source (sample size)	No of positive samples (%)	<i>Salmonella enterica</i> serotypes isolated			Other pathogens obtained (% from total positive sample)		
		No of isolates (%)	<i>S. typhi</i>	<i>S. paratyphi</i>	<i>S. enteritidis</i>	<i>E. coli</i>	<i>Plasmodium</i> sp.
Blood (135)	46 (78.0)	35 (94.6) <sup>a</sup>	25 (71.4)	8 (22.9)	2 (5.7)	0 (0)	9 (19.6)
Stool (23)	13 (22.0)	2 (5.4)	0 (0.0)	0 (0.0)	2 (100) <sup>a</sup>	11 (84.6)	Not applicable
Total	59 (100.0)	37 (100)	26 (70.3)	8 (21.6)	3 (8.1)	11 (100)	9 (100)

**Notes:** <sup>a</sup> $P > 0.05$  (blood vs stool), Fischer's exact test.

**Abbreviations:** No, number; vs, versus.

**Table 2** Antibiotic resistance patterns of *Salmonella enterica* serovars producing bla-CTX-M-I extended-spectrum beta lactamase

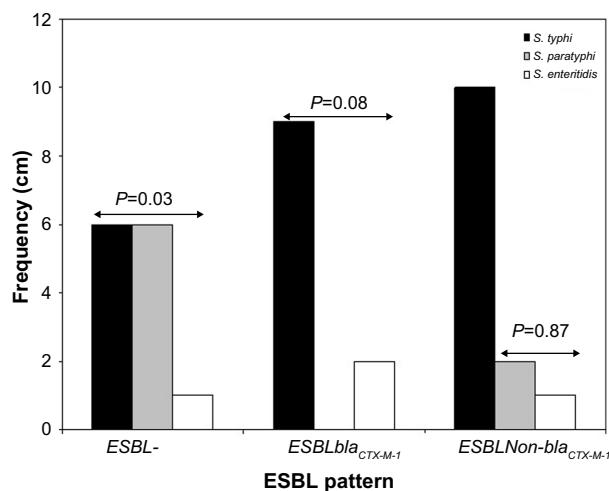
S/N	Resistance pattern	ESBL-	ESBL+	blaCTX-M-I	Non-blaCTX-M-I	P-value
1	AMPTETNALAUGCROCAZ	0	2	0	2	0.19
2	AMPTETCOTCOLAUGCROCTX	0	2	2	0	0.08
3	AMPTETNALCROCAZ	0	1	0	1	0.95
4	AMPTETCOTAUGCAZCROCTX	0	6	5	1	0.004
5	AMPTETCOTNALCAZ	0	2	0	2	0.2
6	AMPTETCOTGENCAZ	0	2	0	2	0.2
7	AMPCOTTETNALCRO	0	2	0	2	0.2
8	AMPTETNALCROCAZ	0	3	1	2	0.39
9	AMPTETNALCROCAZ	0	1	0	1	0.53
10	AMPNALAUGCROCTX	0	1	1	0	0.3
11	AMPCOTAUGCAZCTX	0	2	2	0	0.08
12	AMPCOTTETNAL	4	0	0	0	0.02
13	AMPTET	5	0	0	0	0.004
14	AMP	4	0	0	0	0.02
	TOTAL	13	24	11	13	

**Abbreviations:** AMP, ampicillin; TET, tetracycline; COT, cotrimoxazole; NAL, nalidixic acid; GEN, gentamicin; COL, colistin; STR, streptomycin; AUG, augmentin; CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; S/N, sample number; ESBL, extended-spectrum beta lactamase.

advantage to cause persistent pyrexia in patients with typhoid fever. The  $bla_{CTX-M-I}$  rate of 45.6% observed in this study is higher than the 7.7% detected among other clinical enteric pathogens from Abeokuta, Nigeria by Akinduti et al.<sup>27</sup> It is also higher than the 6% rate recently reported among clinical *Salmonella* isolates in France.<sup>28</sup> In previous studies on  $bla_{CTX-M-I}$  gene variants in Lagos,  $bla_{CTX-M-15}$  was detected in 17 urinary pathogens of *K. pneumoniae* by Soge et al.<sup>29</sup> and in *Enterobacter cloacae* and *Pantoea agglomerans* isolates by Aibinu et al.<sup>30</sup> Also in south-eastern Nigeria, Iroha et al.<sup>11</sup> reported the carriage of  $bla_{CTX-M-I}$  ESBL among 44 clinical isolates of *E. coli* from two hospitals in 2012. These findings

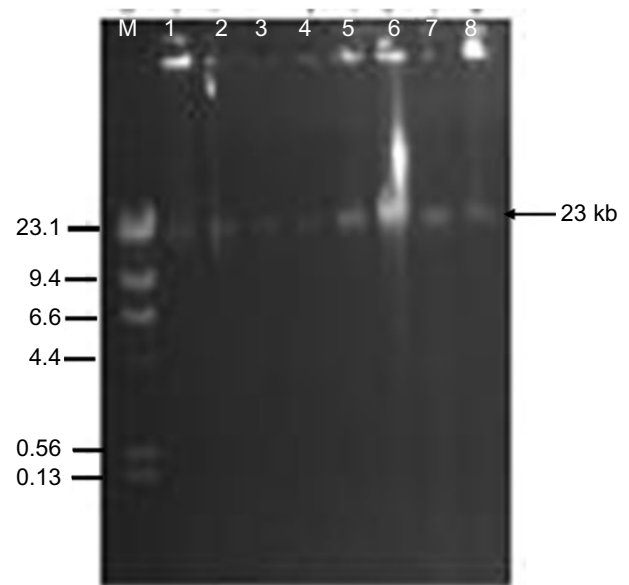
indicate increasing spread of  $bla_{CTX-M-I}$  among Enterobacteriaceae members in Nigeria.

Continuous surveillance of  $bla_{CTX-M-I}$ -producing pathogens is needed to guide preventive interventions in Nigeria.  $Bla_{CTX-M-I}$  gene cluster carriage is associated with increased resistance to cefotaxime. Therefore, the clinical practice of switching *Salmonella* bacteremic and febrile patients who failed treatment with ceftriaxone, cefuroxime, or ceftazidime empirically to cefotaxime also has the risk of treatment failure in this environment. For such patients, this

**Figure 1** Distribution of *Salmonella enterica* serovars producing extended spectrum beta lactamases.

**Notes:**  $P < 0.05$  was significant. *S. enterica* serovars as per ESBL patterns 2x3 contingency table, Fischer's exact test.

**Abbreviation:** ESBL, extended spectrum beta lactamase.

**Figure 2** Plasmid transfer by conjugation from donor *Salmonella typhi* strains to recipient *Escherichia coli* j53-2. Lanes 1, 3, 5, and 7 are *S. typhi* donors Lag-003, -004, -007, and -010. Lanes 2, 4, 6, and 8 are *E. coli* j53-2 transconjugants. Lane M represents Lambda DNA Hind III markers.

**Abbreviation:** DNA, deoxyribonucleic acid.

**Table 3** Antibiotic resistance and plasmid transfer by conjugation from the donor *Salmonella typhi* isolates to the *Escherichia coli* j53-2 recipient

Donor <i>S. typhi</i> isolate			<i>E. coli</i> j53-2 transconjugant	
Strain ID	Plasmid profile, kb	Antibiotic resistance profile	Plasmid transferred, kb	Transferred antibiotic resistant profile
Lag-001	30.5, 23, 9.0, 2.1	AMPTETCOTCOLAUGCROCTX	23	AMPCOTAUGCTX
Lag-004	23, 9.0	AMPTETCOTAUGCAZCROCTX	23	AMPAUGCAZCTX
Lag-007	23, 17, 2.1	AMPNALAUGCROCTX	23	AMPAUGCTX
Lag-010	23, 9.0, 5.1	AMPCOTAUGCAZCTX	23	AMPCTX

**Abbreviations:** ID, identification; AMP, ampicillin; TET, tetracycline; COT, cotrimoxazole; NAL, nalidixic acid; COL, colistin; AUG, augmentin; CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime.

study recommends the use of imipenem or levofloxacin, or azithromycin for case management. The clinical effectiveness of these antibiotics for treating multidrug-resistant infections has been reported in African countries such as Tanzania,<sup>31</sup> in North and South America,<sup>32</sup> Nepal,<sup>33</sup> and India.<sup>34</sup> These antibiotics are also included in the drug formulary of many hospitals in Lagos and other states in Nigeria, where they are used for case management of inpatients and outpatients affected by other bacterial infections.<sup>9–12</sup> The results of the conjugation experiment not only showed the *bla*<sub>CTX-M-I</sub> gene carried by *S. typhi* was plasmid-mediated, but also revealed the potential for rapid spread of this genetic marker to other members of Enterobacteriaceae such as *E. coli*, which are often encountered in polymicrobial infections in Lagos and other regions of Nigeria.<sup>35</sup>

Similar plasmid-mediated transfer of *bla*<sub>CTX-M-I</sub> and resistance to antibiotics such as cefotaxime, aminoglycosides, and beta-lactamase inhibitor-containing antibiotics such as augmentin, as demonstrated in the present study, have been documented by previous investigators from other countries of the world.<sup>31,36–38</sup> However, disparity can be seen in the molecular size of the plasmid transferred. Jin and Ling<sup>36</sup> reported the presence of *bla*-CTX-M genes on 62 kb, 70 kb, and 92 kb harbored by *S. enteritidis* and *S. typhimurium* from Hong Kong, while Mshana et al<sup>31</sup> found a 231 kb plasmid to be responsible for the carriage of *bla*-CTX-M-15 gene among *S. enterica* isolates from Tanzania. In a study carried out by Bado et al<sup>37</sup> in Uruguay, where the first description of *bla*<sub>CTX-M-I</sub> gene cluster in *S. enteritidis* was reported, a 195 kb plasmid was incriminated as a vector. In Germany, Fischer et al<sup>38</sup> reported the transferability of *bla*<sub>CTX-M-15</sub> among *Salmonella* isolates from horse and swine through 95 kb IncF and IncII plasmids. In Lagos, an earlier study by Soge et al<sup>29</sup> revealed the presence of the *bla*<sub>CTX-M-I</sub> gene on plasmids of sizes 58–320 kb in *K. pneumoniae*.

Nevertheless, it is sufficient to suggest that multiple self-transmissible plasmids are involved in the spread of

*bla*<sub>CTX-M-I</sub> across the different regions and countries of the world. Their active roles for dissemination of cefotaxime resistance appear to vary according to geographical region, reservoir of *Salmonella* isolates (ie, food animals or pets of humans), time of study, and their genetic environment. The latter can be used to explain why *bla*<sub>CTX-M-I</sub> carriage accounted for 76.9% resistance to augmentin, as seen in the current study. This was further confirmed by co-transfer of resistance to antibiotics to *E. coli* j53-2 by conjugation. Studies conducted in other countries have reported carriage of augmentin resistance markers such as *ampC*, aminoglycoside modification genes, and metallo beta lactamase genes on *bla*<sub>CTX-M-I</sub> plasmids.<sup>29–31,36–39</sup>

Furthermore, additional antibiotic resistance conferred by this genetic marker may also set the stage for the emergence of pan-resistant *S. typhi* in Nigeria, as recently documented in some Asian countries.<sup>40</sup> The emergence of multidrug-resistant typhoidal and non-typhoidal *Salmonella* have caused life-threatening invasive disease outbreaks in children and adults in many African countries, including Zaire,<sup>41</sup> Malawi,<sup>42</sup> and Kenya.<sup>43</sup> Similarly, multidrug-resistant *Salmonella* serotypes have been widely prevalent in Kuwait and India.<sup>44,45</sup>

It is worthy to note in this study that four *Salmonella* isolates with *P. falciparum*-associated co-infection were detected, and that *S. typhi* mono-infection was characterized by ceftriaxone and cefotaxime resistance; the current study has further revealed the relevance of *bla*<sub>CTX-M-I</sub> ESBL testing for rational use of both ACT and antibiotics for good outcomes in patients with persistent pyrexia-associated *Salmonella* or *Plasmodium*, or both. In this context, the reported risk of poor treatment outcomes in patients admitted in hospitals with laboratories that do not perform tests for detection of ESBLs and do not report ESBL producers as resistant to cephalosporins is now apparent in Lagos.<sup>46</sup> The potential spread of *bla*<sub>CTX-M-I</sub>-producing *S. typhi* to other states of the country and other neighboring countries is also a possibility.

The inability to confirm the variants of *bla*<sub>CTX-M-I</sub> gene cluster carried by the positive *S. typhi* strains, which is essential to infer clonal relatedness of these strains, is one of the limitations of this study. Molecular typing techniques such as multilocus sequence typing and pulse-field gel electrophoresis to reveal information about the upstream and downstream genetic environment of the recovered *bla*<sub>CTX-M-I</sub> plasmid of the positive *S. typhi* isolates is essential to assess their levels of homogeneity or heterogeneity. Such information will also be needed to further understand mechanisms of dissemination and extra-antibiotic resistance mechanisms of *bla*<sub>CTX-M-I</sub> plasmids among *S. typhi* isolates in our environment.

Despite these limitations, this study has revealed the emergence of resistance to 3GC antibiotics due to acquisition and expression of plasmid-borne *bla*<sub>CTX-M-I</sub> gene cluster among *S. typhi* strains, and this emergence calls for cautionary parenteral use of cefotaxime and other 3GCs for the treatment of typhoid fever in Lagos, Nigeria. A need for routine screening for *P. falciparum* co-infection with ESBL-producing *Salmonella* in the laboratories during diagnosis of persistent pyrexia conditions in patients is recommended.

## Acknowledgments

We are grateful to all the staff of the public hospitals used for this work. We are equally grateful to the Staff of the Department of Microbiology of Lagos State University and the Nigerian Institute of Medical Research, Yaba for their technical support.

## Disclosure

The authors report no conflicts of interest in this work.

## References

- Akinyemi KO, Iwalokun BA, Foli F, Oshodi K, Coker AO. Prevalence of multiple drug resistance and screening of enterotoxin gene (stn) in *Salmonella enterica* serovars from water sources in Lagos, Nigeria. *Public Health*. 2011;125:65–71.
- Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *B World Health Organ*. 2004;82:346–353.
- Goque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill*. 2008;13(47):19051.
- Jacoby AG, Munoz-Price SL. The new  $\beta$ -lactamases. *N Engl J Med*. 2005;352:380–391.
- Menashe G, Borer A, Yagupsky P, et al. Clinical significance and impact on mortality of extended-spectrum beta lactamase-producing Enterobacteriaceae isolates in nosocomial bacteremia. *Scand J Infect Dis*. 2001;33:188–193.
- Ndugulile F, Jureen R, Harthug S, Urassa W, Langeland N. Extended spectrum  $\beta$ -lactamases among gram-negative bacteria of nosocomial origin from an intensive care unit of a tertiary health facility in Tanzania. *BMC Infect Dis*. 2005;5:1–6.
- Tassios PT, Gazouli M, Tzelepi E, et al. Spread of a *Salmonella typhimurium* clone resistant to expanded-spectrum cephalosporins in three European countries. *J Clin Microbiol*. 1999;37(11):3774–3777.
- Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. *Clin Infect Dis*. 2001;32:S94–S103.
- Aibinu IE, Ohaegbulam VC, Adenipekun EA, Ogunsoola FT, Odugbemi TO, Mee BJ. Extended-spectrum beta-lactamase enzyme in clinical isolates of *Enterobacter* species from Lagos, Nigeria. *J Clin Microbiol*. 2003;41:2197–2200.
- Iroha IR, Amadi ES, Agabus AC, Oji AE. Susceptibility pattern of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* from clinical isolates. *Int J Microbiol*. 2008;5:34–37.
- Iroha IR, Esimone CO, Neumann S, et al. First description of *Escherichia coli* producing CTX-M-15-extended spectrum beta lactamase (ESBL) in out-patients from south eastern Nigeria. *Ann Clin Microbiol Antimicrob*. 2012;11:19.
- Akinyemi KO, Iwalokun BA, Agboyinu JA, Ogunyemi O, Fasure AK. Emergence of third generation cephalosporins and typing by rapid amplified polymorphic DNA among clinical *Salmonella* isolates from Lagos, Nigeria. *Brit Microbiol Res J*. 2014;6:668–677.
- Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum  $\beta$ -lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev*. 2013;26:744–758.
- Radice M, Power P, Di Conza J, Gutkind G. Early dissemination of CTX-M-derived enzymes in South America. *Antimicrob Agents Chemother*. 2012;46:602–604.
- Akinyemi KO, Oshundare YO, Oyeyinka OG, Coker AO. A retrospective study of community-acquired *Salmonella* infections in patients attending public hospitals in Lagos, Nigeria. *J Infect Dev Ctries*. 2012;6(5):387–395.
- Fashae K, Ogunshola F, Aerestrup FM, Hendriksen RS. Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. *J Infect Dev Ctries*. 2010;4:484–494.
- World Health Organization. *Guidelines for the Treatment of Malaria*. 2nd ed. Geneva: World Health Organization; 2010.
- World Health Organization. *The Implementation of the Global Malaria Control Strategy: WHO Technical Report Series No 839*. Geneva: World Health Organization; 1993.
- Cowan and Steel's Manual for the identification of Medical Bacteria. 3rd edn. Ed. Barrow GI, Feltham RKA, Cambridge University Press, 1993: pp331.
- National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing: Ninth Informational Supplement, Version 19*. Wayne, PA: National Committee for Clinical Laboratory Standards; 1999.
- Clinical Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: 19th Informational Supplement M100S19*. Wayne, PA: Clinical Laboratory Standards Institute; 2009.
- Cheng S, Zhao S, White DG, Meng J. Characterization of multiple antimicrobial resistant *Salmonella* serovars isolated from retail meats. *Appl Environ Microbiol*. 2004;70:1–7.
- Woodford N, Fagan EJ, Ellington LJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum  $\beta$ -lactamases. *J Antimicrob Chemother*. 2006;57:154–155.
- Chen L, Chen ZL, Liu JH, Zeng ZL, Ma JY, Jiang HX. Emergence of RmtB methylase-producing *Escherichia coli* and *Enterobacter cloacae* isolates from pigs in China. *J Antimicrob Chemother*. 2007;59: 880–885.
- Arlet G, Barrett TJ, Butaye P, Cloeckaert A, Mulvey MR, White DG. *Salmonella* resistant to extended-spectrum cephalosporins: prevalence and epidemiology. *Microbes Infect*. 2006;8:1945–1954.
- Akinyemi KO, Smith SI, Oyefolu AO, Coker AO. Multidrug resistance in *Salmonella enterica* serovar typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. *Public Health*. 2005;119: 321–327.
- Akinduti PA, Oluwadun A, Iwalokun BA, Onagbesan OM, Ejilude O. Community-acquired CTX-M beta lactamase isolates from Abeokuta, Nigeria. *Brit Microbiol Res J*. 2014;5:351–358.

28. Haenni M, Saras E, Métayer V, Médaille C, Madec JY. High prevalence of blaCTX-M-1/IncI1/ST3 and blaCMY-2/IncI1/ST2 plasmids in healthy urban dogs in France. *Antimicrob Agents Chemother.* 2014;58:5358–5362.
29. Soge OO, Adeniyi BA, Roberts MC. New antibiotic resistance genes associated with CTX-M plasmids from uropathogenic Nigerian *Klebsiella pneumoniae*. *J Antimicrob Chemother.* 2006;58(5):1048–1053.
30. Aibinu I, Pfeifer Y, Peters F, et al. Emergence of bla(CTX-M-15), qnrB1 and aac(6′)-Ib-cr resistance genes in *Pantoea agglomerans* and *Enterobacter cloacae* from Nigeria (sub-Saharan Africa). *J Med Microbiol.* 2012;61:165–167.
31. Mshana SE, Gerwing L, Minde M, et al. Outbreak of a novel Enterobacter sp. carrying blaCTX-M-15 in a neonatal unit of a tertiary care hospital in Tanzania. *Int J Antimicrob Agents.* 2011;38(3):265–269.
32. Biedenbach DJ, Toleman M, Walsh TR, Jones RN. Analysis of *Salmonella* spp. with resistance to extended-spectrum cephalosporins and fluoroquinolones isolated in North America and Latin America: report from the SENTRY Antimicrobial Surveillance Program (1997–2004). *Diagn Microbiol Infect Dis.* 2006;54(1):13–21.
33. Pathak A1, Marothi Y, Kekre V, Mahadik K, Macaden R, Lundborg C. High prevalence of extended-spectrum  $\beta$ -lactamase-producing pathogens: results of a surveillance study in two hospitals in Ujjain, India. *Infect Drug Resist.* 2012;5:65–73.
34. Pokharel BM, Koirala J, Dahal RK, Mishra SK, Khadga PK, Tuladhar NR. Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella enterica* (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. *Int J Infect Dis.* 2006;10:434–468.
35. Iwalokun BA, Gbenle GO, Smith SI, Ogunledun A, Akinsinde KA, Omonigbehin EA. Epidemiology of shigellosis in Lagos, Nigeria: trends in antimicrobial resistance. *J Health Popul Nutr.* 2001;19:183–190.
36. Jin Y, Ling JM. CTX-M-producing *Salmonella* spp. in Hong Kong: an emerging problem. *J Med Microbiol.* 2006;55(Pt 9):1245–1250.
37. Bado I, García-Fulgueiras V, Cordeiro NF, et al. First human isolate of *Salmonella enterica* serotype Enteritidis harboring bla-CTX-M-14 in South America. *Antimicrob Agents Chemother.* 2012;56:2132–2134.
38. Fischer J, Rodríguez I, Baumann B, et al. bla-CTX-M<sub>15</sub>-carrying *Escherichia coli* and *Salmonella* isolates from livestock and food in Germany. *J Antimicrob Chemother.* 2014;69:2951–2958.
39. Baba Ahmed-Kazi Tani Z, Arlet G. [News of antibiotic resistance among Gram-negative bacilli in Algeria]. *Pathol Biol (Paris).* 2014;62:169–178. French.
40. Tadesse G. A meta-analysis of the proportion of antimicrobial resistant human *Salmonella* isolates in Ethiopia. *BMC Pharmacol Toxicol.* 2014;15:51.
41. Milledge J, Calis, JC, Graham SM. The effect of HIV infection or neonate bacterial meningitis in Blantyre, Malawi. *Arch Dis Child.* 2003;88:1112–1118.
42. Karuiki S, Revathi G, Kariuki N, et al. Multidrug-resistant non-typhoidal *Salmonella* infection in Africa: zoonotic or anthroponotic transmission. *J Med Microbiol.* 2006;55:585–591.
43. Amatya NM, Shraatha B, Lekhak B. Etiological agents of bacteraemia and antibiotic susceptibility pattern in Kathmandu Model hospital. *JNMA J Nepal Med Assoc.* 2007;46:112–118.
44. Dimitrov T, Udo EE, Albaksami O, et al. Clinical and microbiological investigations of typhoid fever in an infectious disease hospital in Kuwait. *J Med Microbiol.* 2007;56:538–544.
45. Shukla I, Tiwan R, Agarwal M. Prevalence of Extended spectrum beta-lactamase producing *Klebsiella pneumoniae* in a tertiary care hospital. *Indian J Med Microbiol.* 2004;22:87–91.
46. Kruger T, Szabo D, Keddy KH, et al. Infections with Non-typhoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. *Antimicrob Agents Chemother.* 2004;48:4263–4270.

## Infection and Drug Resistance

### Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic

Submit your manuscript here: <http://www.dovepress.com/infection-and-drug-resistance-journal>

resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Dovepress