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Co-existence of extended-spectrum β -lactamases $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-15}}$ genes in *Salmonella* species isolated from febrile and diarrhoeagenic patients in Lagos, Nigeria: a cross-sectional study

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Abstract

Background: Resistance to different antimicrobial classes by *Salmonella* species has generated a global public health concern. The spread of extended-spectrum β -lactamases (ESBLs) bla_{CTX} gene variants is also increasing. This study aimed to investigate the antibiotic resistance and the carriage of $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-15}}$ as well as the quinolone resistance gene (*qnrB19*) among *Salmonella* species from hospitalised patients in Lagos, Nigeria.

Methods: In this cross-sectional study from April 2021 to August 2021, a total of 508 samples were collected from hospitalised patients. The samples were subjected to standard microbiological investigation. All the isolates were identified using API 20E kits and real-time polymerase chain reaction (RT-PCR). The in vitro antibiotic susceptibility testing (AST) was investigated using the disk diffusion method. Detection of antibiotic resistance and virulence gene makers was conducted using RT-PCR.

Results: In total, 24 *Salmonella* species were identified. All the isolates were non-typhoidal *Salmonella* isolates. None of the isolates screened was *S. Typhi* and *S. Paratyphi*. Most of the isolates were susceptible to imipenem, ciprofloxacin, ofloxacin and gentamycin, while a high level of resistance to all cephalosporins, penicillin, and some carbapenems was observed. In total, 79.2% (19/24) of the *Salmonella* isolates harboured the $bla_{\text{CTX-M}}$ variant including 54.2% (13/24) $bla_{\text{CTX-M-9}}$ and 12.5% (3/24) $bla_{\text{CTX-M-15}}$, while co-habitation of $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-15}}$ was observed in 12.5% (3/24) of the isolates, respectively. None of the isolates harboured quinolone-resistant *qnrB19* gene and virulence gene *stn*. However, *invA* gene was present in 66.7% (16/24) of all isolates.

Conclusions: This study is considered the first report of $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-15}}$ variants in *Salmonella* species in Nigeria. The continued existence of cefotaximase (CTX-M)-producing *Salmonella* within our environment calls for the prudent use of cephalosporins.

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Keywords: Non-typhoidal *Salmonella*, Diarrhoeal, MDR, $Bla_{CTX-M-9}$, $Bla_{CTX-M-15}$

Background

Salmonella infections are recognised as a global public health challenge and are one of the most common causes of human gastroenteritis worldwide, sometimes with negative clinical outcomes especially among vulnerable individuals [1–3]. Invasive salmonellosis is presented in approximately 6% of patients with a clinical prognosis of diarrhoeal enterocolitis. Invasive non-typhoidal *Salmonella* (iNTS) is endemic especially in sub-Saharan Africa occurring in 79% of the estimated 535, 000 global cases of iNTS infection and 85% of the estimated 77, 500 death globally giving a 15.8% case fatality ratio [4, 5].

Salmonella-associated infections are treated with ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole in the 1980s. However, the emergence of resistance clones to these antimicrobials resulted in the use of other antibiotic classes. Currently, several classes of antibiotics are available such as 3rd and 4th generation cephalosporin (cefepodoxime, ceftriaxone, ceftazidime, cefotaxime), fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin) and carbapenems (ertapenem, doripenem, imipenem) which are essential in the treatment of salmonellosis with good therapeutic potentials. However, the misuse of these antibiotics classes in agricultural, animal and human medicine has been attributed to the emergence, persistence and eventual spread of resistant strains [6]. The continuous spread of multidrug-resistant bacterial pathogens has been attributed to prolonged hospitalisation causing increased morbidity and mortality [7].

Salmonella is increasingly becoming resistant from an estimated rate of 20–30% in the 1990s and increasing to over 30% a decade later [8]. There has been a global increase in the number of bacteria exhibiting resistance to third and fourth-generation cephalosporin, most times due to the production of extended-spectrum β -lactamases (ESBLs) and AmpC-type β -lactamases. Cefotaximase (CTX-M) type of ESBLs remains the widely distributed ESBL enzymes globally [9]. The prevalence rate of CTX-M-type β -lactamases differs among members of the family *Enterobacteriaceae* [10]. The earliest CTX-M-1 clone was detected in Germany in the late 1980s [11], consequently, an explosion of CTX-M-type β -lactamases was witnessed in the twenty-first century, with a report of CTX-M-2, 3, 4, 5, 6, 7, 9, 15 in South America, the Mediterranean and within the European countries [12].

The CTX-M-type β -lactamases belong to a novel Ambler molecular class A ESBL and are mostly

plasmid-mediated with a preference for hydrolysing cefotaxime [13] with $bla_{CTX-M-15}$ being most prevalent [14]. In Nigeria, the first $CTX-M-1$ clone was reported in 2015 [15]. Incidentally, only $bla_{CTX-M-1}$ and $bla_{CTX-M-3}$ clones have been documented by separate studies in *Salmonella enterica* [15, 16]. So far, the report on CTX-M-9 and 15 have only been documented in *Escherichia coli* isolated from clinical and animal sources in Nigeria [17, 18]. Eguale et al. [19] reported $bla_{CTX-M-15}$ in *Salmonella* from Ethiopia. Many other studies in Egypt have recorded both $bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ in *E. coli* [20, 21]. The distribution of CTX-M type of ESBLs among *Salmonella* species is becoming a serious challenge to global health. The continued dissemination of this resistant clone among members of the family *Enterobacteriales* in Nigeria is also becoming worrisome. There is a paucity of reports on the CTX-M type of ESBLs among *Salmonella* serovars in Nigeria, especially from clinical sources. This study aimed to investigate the prevalence of *Salmonella* in febrile and diarrhoeal patients, their antibiotic resistance, and the carriage of $bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ ESBLs genes using real-time polymerase chain reaction (RT-PCR) in Lagos, Nigeria.

Methods

Ethics clearance

Ethical approvals from the Human Research and Ethics Committee of Lagos State University Teaching Hospital with reference number LREC/06/10/1012 and the Lagos State Health Service Commission with reference number LSHSC/2222/VOL.VC/352 were obtained prior to patients' enrolment in accordance with 1964 Helsinki declaration and its later amendments or comparable ethical standards. The written informed consent was obtained from all subjects and/or their legal guardians.

Study population, case definition and sample size

In this cross-sectional study, a total of 508 in-patients and out-patients who sought treatment at some public health care facilities in Lagos State, Nigeria, were recruited from April 2021 to August 2021. The patient's history and demography were obtained. Two categories of patients were recruited for this study: category A includes patients with febrile illness. Patients with febrile illness were defined as a patient whose body temperature is greater than (≥ 37.5 °C) in the last 3 days with one or more of the following symptoms: abdominal pain, loose stool, headache, vomiting, and/or nausea, loss of appetite for up to 5 days. Category

B includes patients with the diarrhoeal disease and was defined as a patient who had been diagnosed by a clinician with persistent loose stool for up to 72 h or more. The sample size was estimated using the formula for estimating the minimum sample for descriptive studies: $N = Z^2 p (1-p) / d^2$, where, N = sample size, $Z = 1.96$ (standard error) at 95% confidence interval, p = prevalence of the disease as reported by Smith et al. [22] = 74%, and d = level of precision at 5% (0.05). Therefore, $N = 295649536$. Approximately 296 samples per 10,000 population. However, five hundred and eight (508) samples were collected to make the inference valid.

Collection and processing of samples

Blood samples (10 ml from adults and 5 ml from children 1–15 yrs) were aseptically obtained and inoculated into blood culture bottles containing brain heart infusion (BHI) broth (LAB M, Lancashire, UK) and were gently mixed. The bottles were incubated at 37 °C aerobically for 7 days. Turbid bottles were sub-cultured on xylose lysine agar (XLD) (HIMEDIA, Mumbai, India), MacConkey agar (HIMEDIA, Mumbai, India), and *Salmonella–Shigella* Agar (SSA) (HIMEDIA, Mumbai, India) and were further incubated for 18–24 h at 37 °C. Non-turbid blood culture bottles were also sub-cultured and repeated daily from the BHI broth for up to 7 days, after which the blood-broth suspension was finally discarded. A loop full of stool samples was inoculated into 10 ml of alkaline peptone water (HIMEDIA, Mumbai, India) for pre-enrichment. The suspension was incubated for 18 h at 37 °C. One ml of the bacterial suspension was dispensed into 9 ml of Selenite F broth (HIMEDIA, Mumbai, India), and the suspension was incubated for at 37 °C for 18 to 24 h. After incubation, a loop full of the bacteria suspension was inoculated into MacConkey agar (HIMEDIA, Mumbai, India), blood agar (LAB M, Lancashire, UK), bismuth sulphite agar (BSA) (LAB M, Lancashire, UK), *Salmonella–Shigella* agar (SSA) (HIMEDIA, Mumbai, India), and xylose lysine agar (XLD) (HIMEDIA, Mumbai, India). The media were incubated at 37 °C for 18 to 24 h.

Bacterial identification

Bacteria identification was carried out using the analytical profile index (API) 20E identification system (Institut Mérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The results of several biochemical parameters were used to generate a profile index code. The profile index code generated was used for the identification of all the isolates in the API-WEB database (<https://apiweb.biomerieux.com>).

Antimicrobial susceptibility testing (AST)

The in vitro antimicrobial susceptibilities of all identified *Salmonella* isolates to the commonly prescribed antibiotics in Nigeria were investigated by disk diffusion method, as described by the Clinical and Laboratory Standard Institute (CLSI) guidelines [23]. The following antibiotic discs were used: cefpodoxime (CPD) (30 µg), cefoxitin (FOX) (30 µg), cefotaxime (CTX) (30 µg), ceftazidime (CAZ) 30 µg, ampicillin (AMP) 10 µg, ciprofloxacin (CPR) 5 µg, augmentin (AUG) 30 µg, ofloxacin (OFL) 5 µg, imipenem (IMP) 10 µg, ertapenem (ETR) 10 µg, doripenem (DOR) 10 µg, amikacin 30 µg (AMK), and gentamycin (GEN) 10 µg (Oxoid, Hampshire, UK). Each of the antibiotic's discs was placed aseptically on Mueller Hinton agar (HIMEDIA, Mumbai, India) plates that were previously inoculated with the *Salmonella* suspension equal to 0.5 McFarland standard and the plates were incubated aerobically at 37 °C for 18–24 h. The diameter of the zones of inhibitions was measured in millimetres and compared with a zone interpretation chart of CLSI [23]. *E. coli* ATCC® 25922™ was used as a control.

Extended-spectrum β-lactamase (ESBL) assay

All *Salmonella* isolates with reduced susceptibility and/or resistance to 3rd generation cephalosporin (3GCs) were screened for ESBL production phenotypically using the double disk synergy test (DDST) method as previously described [16]. *Escherichia coli* ATCC® 25922™ was used as negative control while *Klebsiella pneumoniae* ATCC® 700603™ was used as a positive control.

DNA extraction from bacterial isolate

The DNA extraction was carried out using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions as described by Akinyemi et al. [16].

RT-PCR

The RT-PCR Rotor gene Q 2plex (Qiagen, Hilden, Germany) was used for the amplification and detection of STY0313/SPA2475/t2576 and STY0316/t2574 for *Salmonella* Typhi and *Salmonella* Paratyphi A. *Salmonella* virulence genes *invA*, *stn*, and antibiotics resistance genes *bla*_{CTX-M-9}, *bla*_{CTX-M-15}, and *qnrB19* were screened using the TaqMan technology. The following previously published oligonucleotides sequences specific for detection of *invA*, *stn*, *bla*_{CTX-M-9}, *bla*_{CTX-M-15}, and *qnrB19* were used [16, 24–27] (Table 1). The qPCR assays were performed in a Qiagen rotor gene Q 2plex thermocycler (Qiagen, Hilden, Germany) with 72 well reaction tubes, closed with 4-Cap Strips. The reaction was performed by dispensing 12.5 µl of 2 × SYBR®Green Taq PCR master

Table 1 Primers with nucleotide sequence use in the study

Primers	Oligonucleotide sequence	References
<i>InvA</i> -F	5'- ACCACGCTCTTTCGTCTGG-3'	[16]
<i>InvA</i> -R	5'- GAACTGACTACGTAGACGCTC-3'	
<i>CTX-M-9</i> -F	5'-TTACAAACCGTCGGTGACGA-3'	[24]
<i>CTX-M-9</i> -R	5-GT GAC AAA GAG AGT GCA ACG G-3	
<i>CTX-M-15</i> -F	5'-GGTTAAAAAATCACTGCGTC-3'	[25]
<i>CTX-M-15</i> -R	5'-TTACAAACCGTCGGTGACGA-3'	
<i>qnrB19</i> -F	5'-CRATGTGCAGYACCAGTAA-3'	[25]
<i>qnrB19</i> -R	5'-CGCRATATCRTTGGTGGTG-3'	
<i>Stn</i> -F	5'- ACCACGCTCTTTCGTCTGG-3'	[26]
<i>Stn</i> -R	5'- GAACTGACTACGTAGACGCTC-3'	
STY0313/SPA2475/t2576-F	5-CTTGACGTACCGGTAGAGAT ATACTGGCT-3	[27]
STY0316/t2574-R	5-CTTGACGTACCGGTAGAGAT ATACTGGCT-3	

mix (Qiagen, Hilden, Germany) into 0.1 ml PCR tubes (Eppendorf, Germany). Then, 1.5 µl of both forward and reverse primers (Inqaba Biotec West Africa, Nigeria) and 10.5 µl of nucleic acid-free water (Qiagen, Hilden, Germany) were added. Finally, 1.5 µl of bacterial DNA (approximately 10⁴ copies of gDNA) was added to the mixture and amplified using the following protocol: hold temperature of 95 °C for 3 min, 40 cycles of denaturation at 94 °C for 1 min, annealing 64 °C for 30 s and extension at 72 °C for 30 s [16]. The threshold limit setting was performed in automatic mode. A “no template” control (NTC) was included in each reaction. Only signals generated in SYBR[®]Green qPCR analysis that displayed amplification above the threshold level were considered positive. While those signals with no C_t value obtained were considered negative.

Statistical analysis

These data generated were entered into Microsoft Excel spreadsheet 2010 and were analysed using Statistical Package for Social Sciences (SPSS) Windows (Version 15.2; Chicago, IL) and Epi Info (Version 6.0, USD, Stone Mountain, GA). Prevalence of *Salmonella* and its association with demographic determinants among febrile and diarrhoeagenic patients in selected health care facilities were determined. The prevalence of salmonellosis was calculated as the number of positive cultures for *Salmonella* divided by the total number of samples collected. The data were interpreted using the Chi-square test of independence, while the strength of association was determined by estimating the odds ratio (OR). The statistical test was considered significant if the *P*-value was ≤ 0.05. A correlation analysis was also performed to determine the association of virulence genes and antimicrobial resistance genes belonging to the

same antimicrobial class or different classes among the investigated *Salmonella* isolates. Binary data (0) indicating absent and (1) indicating presence of virulence and resistance genes were imported into an R software (version 4.1.3 and RStudio IDE. <https://www.r-project.org>; <https://www.rstudio.com>. Accessed, 3 September 2022). The correlations were then calculated with package “ggcorrplot” using the function cor.mtest” at a significance of *P* < 0.05.

Results

Salmonella and other bacterial isolates

In total, 229 bacterial pathogens were isolated from 508 samples obtained from febrile and diarrhoeagenic patients. Twenty-four *Salmonella* species were identified from the total sample. Other non-*Salmonella* bacterial pathogen isolated were as follows: *Proteus vulgaris* (12.2%), *Proteus mirabilis* (9.6%), *E. coli* (9.3%), *Klebsiella oxytoca* (4.3%), *Klebsiella pneumoniae* (3.3%), *Edwardsiella* species (2.0%), *Enterobacter* species (1.4%), *Citrobacter* species (0.6%), *E. coli* 0157H7 (0.4%), and *Staphylococcus* species (0.4%).

Demographic distribution of *Salmonella* isolates

The proportion of *Salmonella* species was 6.3% (15/240) in males and 3.4% (9/268) in females. Out of the 24 *Salmonella* species detected, 15/24 representing 62.5%, were isolated from children between the ages of 1–10 years, with a mean age of 4.7 years. Also, 25.0% (6/24) of the total *Salmonella* isolates were detected from patients between the ages of 11–20 years, with a mean age of 14.3 years. Meanwhile, the remaining 12.5% (3/24) was isolated from patients within the age bracket 21–30 years with a mean age of 24.7 years (Tables 2 and 3). The prevalence of *Salmonella*-associated bacteraemia stood at 3.9%

Table 2 Distribution of *Salmonella* and other bacterial pathogens isolated from febrile and diarrhoeagenic patients in Lagos, Nigeria

Variables	Category	Number tested	Number positive culture	<i>Salmonella</i> species	<i>E. coli</i>	<i>E. coli</i> 0157H7	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>Citrobacter sp</i>	<i>P. vulgaris</i>	<i>Staphylococcus sp</i>	<i>Klebsiella oxytoca</i>	<i>Enterobacter sp</i>	<i>Edwardsiella sp</i>
Gender	Male	240	117	15 (6.3)	17 (7.1)	0 (0)	21 (8.8)	8 (3.33)	1 (4.6)	32 (13.33)	2 (0.83)	10 (4.2)	5 (2.08)	6 (2.5)
	Female	268	128	9 (3.4)	30 (11.2)	2 (0.7)	28 (10.5)	9 (3.4)	2 (0.8)	30 (11.2)	(0.90)	12 (4.5)	2 (0.8)	4 (1.5)
Age	1–10 years	129	77	15 (11.6)	22 (17.1)	2 (1.6)	12 (9.3)	2 (1.6)	1 (0.78)	20 (15.5)	1 (0.78)	6 (4.7)	2 (1.6)	4 (3.1)
	11–20 years	104	50	6 (5.8)	10 (9.6)	0 (0)	10 (9.6)	4 (3.8)	0 (0)	14 (13.5)	0 (0)	6 (5.8)	2 (1.9)	2 (1.9)
	21–30 years	78	32	3 (3.8)	5 (6.4)	0 (0)	8 (10.3)	2 (2.6)	1 (1.3)	12 (15.4)	0 (0)	1 (1.3)	1 (1.3)	1 (1.3)
	31–40 years	63	23	0 (0)	3 (4.8)	0 (0)	4 (6.3)	3 (4.8)	0 (0)	8 (12.7)	0 (0)	4 (6.3)	0 (0)	1 (1.6)
	41–50 years	42	14	0 (0)	2 (4.8)	0 (0)	3 (7.1)	3 (7.1)	0 (0)	2 (4.8)	0 (0)	2 (4.8)	1 (0.2)	1 (0.2)
Clinical prognosis/sample type	51 years and above	31	12	0 (0)	2 (6.5)	0 (0)	3 (9.7)	1 (3.2)	1 (3.2)	2 (6.5)	0 (0)	1 (3.2)	1 (3.2)	1 (3.2)
	No age (adults)	61	21	0 (0)	3 (4.9)	0 (0)	9 (14.8)	2 (3.3)	0 (0)	4 (6.6)	1 (1.6)	2 (3.3)	0 (0)	0 (0)
	Febrile illness (blood)	378	147	15 (3.96)	0 (0)	0 (0)	41 (10.8)	15 (3.96)	1 (0.3)	51 (13.5)	2 (0.5)	19 (5.02)	5 (1.3)	8 (2.1)
Study centres	Diarrhoeal disease (stool)	130	82	9 (6.9)	47 (36.2)	2 (1.5)	8 (6.2)	2 (1.5)	2 (1.5)	11 (8.5)	0 (0)	3 (2.3)	2 (1.5)	2 (1.5)
	Lagos State University teaching Hospital (blood abd stool)	170	83	3 (1.8)	27 (15.9)	0 (0)	17 (10)	8 (4.7)	1 (0.6)	17 (10)	1 (0.6)	3 (1.8)	4 (2.4)	4 (2.4)
Clinical prognosis/sample type	Randle General Hospital (blood)	140	62	12 (8.6)	0 (0)	0 (0)	13 (9.3)	1 (0.7)	1 (0.7)	27 (19.3)	0 (0)	12 (8.6)	0 (0)	4 (2.9)
	Life font Hospital (blood)	70	24	3 (4.3)	0 (0)	0 (0)	6 (8.6)	3 (4.3)	0 (0)	9 (12.9)	0 (0)	4 (5.7)	0 (0)	1 (1.4)
	Alimosho General Hospital (blood and stool)	128	60	6 (4.7)	20 (15.6)	2 (1.6)	13 (10.2)	5 (3.9)	1 (0.8)	9 (7.0)	1 (0.8)	3 (2.3)	3 (2.3)	1 (0.8)
Total		508	245	24 (4.7)	47 (9.3)	2 (0.4)	49 (9.6)	17 (3.3)	3 (0.6)	62 (12.2)	2 (0.4)	22 (4.3)	7 (1.4)	10 (1.96)

Table 3 Prevalence of *Salmonella* and its association with demographic determinant among febrile and diarrhoeagenic patients in selected health care facilities

Variable	Category	No. tested	No. of +ve culture	No. of <i>Salmonella</i> Detected	Occurrence (%) (n-24)	Prevalence (%)	Odds ratio	95% CI	z statistic	P-value
Gender	Male	240	107	15	62.5	6.25	0.0667	0.0384–0.1158	9.608	<0.0001
	Female	268	122	9	37.5	3.4	0.0347	0.0175–0.0690	9.601	<0.0001
Age distribution	1–10	129	77	15	62.5	11.6	0.1316	0.0729–0.2376	6.725	<0.0001
	11–20	104	50	6	25	5.8	0.0612	0.0257–0.1458	6.308	<0.0001
	21–30	78	32	3	12.5	3.8	0.0400	0.0121–0.1323	5.275	<0.0001
Diagnosis	Febrile illness	378	147	15	62.5	3.9	0.0413	0.0242–0.0706	11.657	<0.0001
	Diarrhoeal disease	130	82	9	37.5	6.9	0.0744	0.0362–0.1527	7.079	<0.0001
Study centre	LASUTH	170	83	3	12.5	1.8	0.0180	0.0056–0.0574	6.783	<0.0001
	RGH	140	62	12	50	8.6	0.0938	0.0496–0.1772	7.290	<0.0001
	LFMC	70	24	3	12.5	4.3	0.0448	0.0134–0.1491	5.060	<0.0001
	AGH	128	60	6	25	10.0	0.0492	0.0209–0.1157	6.902	<0.0001

LASUTH Lagos State University Teaching Hospital, RGH Randle General Hospital, LFMC Life-Font Medical Centre, AGH Alimosho General Hospital, ND not determined, NA not determined

(15/378), while *Salmonella*-associated gastroenteritis was 6.9% (9/130). The relationship between *Salmonella* detections, gender, age distribution, clinical prognosis, and study centres reveals a significant association with a P -value < 0.0001 , odd ratio (OR) ranging from (0.0180–0.1316), at 95% CL (Table 3).

Antibiotic susceptibility and pattern of resistance

The result of the in vitro antimicrobial susceptibility testing showed some level of susceptibility to imipenem (75.0%), ciprofloxacin (62.5%), gentamycin (62.5%) and ofloxacin (50.0%), while all the isolates developed resistance (100.0%) to ceftazidime, cefotaxime, ceftiofloxacin, augmentin, ampicillin, ertapenem, and doripenem. However, 75.0% of the isolates showed resistance to cefpodoxime. Resistance to ofloxacin and amikacin was 50.0% each. All *Salmonella* isolates were multiple drug resistant (MDR). MDR was defined as resistance to at least one agent in three or more antimicrobial categories (Table 4). All the isolates developed resistance to between 7 and 13 antibiotics, with 5 patterns of resistance observed. Six isolates each have pattern CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR, and CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR, five isolates with pattern CAZ-CTX-FOX-AUG-AMP-ETR-DOR, while three isolates each have CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-ETR-DOR and CAZ-CPD-CTX-FOX-AUG-AMP-AMK-OFL-IMP-ETR-DOR as their pattern of resistance and one isolate with resistant pattern CAZ-CPD-CTX-AUG-AMP-ETR-DOR (Table 5). High multiple antibiotic resistance (MAR) index (ranging from 0.5 to 1.0) was observed for all the *Salmonella* isolates. All the *Salmonella* isolates from this study were negative when screened for *Salmonella* Typhi and *Salmonella* Paratyphi A gene using STY0313/SPA2475/t2576-F and STY0316/t2574-R primer pairs (Table 6).

Virulence and CTX-M genes

Sixteen of the isolates (66.7%) harboured the invasive (*invA*) gene, while none of the isolates harboured the enterotoxin (*stn*) gene. In total, 79.2% (19/24) of the *Salmonella* isolates harboured the *bla*_{CTX-M} variant including 52.0% (13/24) *bla*_{CTX-M-9} and 12.5% (3/24) *bla*_{CTX-M-15}, respectively. Six *Salmonella* isolates from diarrhoeal patients and seven *Salmonella* isolates from patients with febrile illness harboured *bla*_{CTX-M-9} only. Two *Salmonella* isolates from febrile patients and one from diarrhoeal patients harboured *bla*_{CTX-M-15} only, respectively. Of note was the co-existence of *bla*_{CTX-M-9} and *bla*_{CTX-M-15} in 3 (12.5%) *Salmonella* isolates from febrile patients with similar resistance pattern CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR. All the isolates

were negative for the plasmid-mediated quinolone resistance gene *qnrB19* (Table 6).

Correlation analysis

The result of the correlation analysis revealed a significant association between the *invA* gene and *bla*_{CTX-M-9} and a strong association between *bla*_{CTX-M-9} and *bla*_{CTX-M-15}. Significance was calculated at $P < 0.05$, and boxes with non-significant correlations were left blank as such genes were not detected (Fig. 1).

Discussion

Salmonella-associated infection caused by the genus *Salmonella* remains a public health challenge in Africa. *Salmonella* has been associated with an increasing number of reported cases of community-acquired bloodstream infections, self-limiting gastroenteritis to more severe infections resulting in a high rate of morbidity and mortality [16]. Fluoroquinolone and extended-spectrum cephalosporin are used as first-line antibiotics for the treatment of salmonellosis. However, treatment failure due to resistance to these classes of antibiotics has resulted in prolonged hospitalisation with negative clinical outcomes [7]. In this study, 24 *Salmonella* species were detected from 508 samples with an overall prevalence of 4.7%. The prevalence of *Salmonella*-associated bacteraemia was 3.9%, while *Salmonella*-associated gastroenteritis was 6.9%. This result was similar to 3.9% prevalence of *Salmonella*-associated bacteraemia reported in Nigeria by Akinyemi et al. [16], but at variance with the 1.9% recorded by the same author in a separate study [28]. All the *Salmonella* species detected in this study were non-typhoidal *Salmonella* because none of the primer pairs used for the detection of *Salmonella* Typhi and *Salmonella* Paratyphi A yielded Ct value. The Prevalence of *Salmonella*-associated gastroenteritis (SAG) in this study was 6.9%. The result was lower when compared to the results of similar studies; 9.6%, 16.3% and 16.6% that were conducted in different periods in 2021, 2018 and 2007 in Lagos, Nigeria [16, 29, 30], respectively. However, this study was consistent with the 6.2% (59/957) reported in Ethiopia [31]. Conversely, lower prevalence of 1.3% and 2.7% were reported in separate studies in Ethiopia [32, 33], 3.0% in Kenya [34] and 3.5% in Egypt [35] compared to 6.9% SAG reported in this study. Also, the 6.9% SAG recorded in this study was lower when compared to the 13.80% in Tanzania [36] 17.86% in Thailand [37] and 20.39% in China [38]. The observed variations in the prevalence of *Salmonella* infection in this study when compared to other studies, could be attributed to various factors such as sample size, gender, and age distribution, location of sampling and seasonal variation [36].

Table 4 Antibiogram of non-typhoidal *Salmonella* isolates from clinical samples by disc diffusion method

Class of antibiotics	Antimicrobial agent	Resistant %	Susceptible %
Cephalosporin	Ceftazidime (CAZ) (30 µg)	24 (100.0)	0 (0.0)
	Cefpodoxime (CPD) (30 µg)	18 (75.0)	6 (25.0)
	Cefotaxime (CTX) (30 µg)	24 (100.0)	0 (0.0)
	Cefoxitin (FOX) (30 µg)	24 (100.0)	0 (0.0)
Penicillin	Augmentin (AUG) 30 µg	24 (100.0)	0 (0.0)
	Ampicillin (AMP) 10 µg	24 (100.0)	0 (0.0)
Fluoroquinolone	Ciprofloxacin (CPR) 5 µg	9 (37.5)	15 (62.5)
	Ofloxacin (OFL) 5 µg	12 (50.0)	12 (50.0)
Carbapenem	Doripenem (DOR) 10 µg	24 (100.0)	0 (0.0)
	Ertapenem (ETR) 10 µg	24 (100.0)	0 (0.0)
	Imipenem (IMP) 10 µg	6 (25.0)	18 (75.0)
Aminoglycoside	Gentamycin (GEN) 10 µg	9 (37.5)	15 (62.5)*
	Amikacin (AMK) 30 µg	12 (50.0)	12 (50.0)*

*In vitro susceptibility does not mean the antibiotics are effective in vivo and should be considered as resistant

Table 5 Antibiotics resistant pattern of MDR-*Salmonella* species isolated from febrile and diarrhoeal patients

No. of profile	Antibiotics resistant pattern	No. of antibiotics resisted	<i>Salmonella</i> species
1	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	13	6
2	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-ETR-DOR	12	3
3	CAZ-CPD-CTX-FOX-AUG-AMP-AMK-OFL-IMP-ETR-DOR	11	3
4	CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR	8	6
5	CAZ-CTX-FOX-AUG-AMP-ETR-DOR	7	5
6	CAZ-CPD-CTX-AUG-AMP-ETR-DOR	7	1

* Ceftazidime (CAZ), cefpodoxime (CPD), cefotaxime (CTX), cefoxitin (FOX), augmentin (AUG), ampicillin (AMP), ciprofloxacin (CPR), ofloxacin (OFL), doripenem (DOR), ertapenem (ETR), imipenem (IMP), gentamycin (GEN)

Fifteen out of the 20 (15/24) *Salmonella* species detected representing 62.5% were isolated from children between the ages of 1–10 years, with a mean age of 4.7 years, standard deviation (SD) ± 2.859 , margin of error (ME) ± 0.2517 (4.7364 ± 0.494 ($\pm 10.42\%$) at 95% confidence level. The prevalence of *Salmonella* infection in this study was higher among children < 10 years when compared to other age groups. Children have been identified as a vulnerable population group at risk of *Salmonella* infection due to underdeveloped immune system and exhibition of poor hygienic habits [38]. Report on high prevalence of *Salmonella* infection in children is not uncommon. Several studies have documented a high prevalence of *Salmonella* in children in different countries such as Nigeria [16, 39], China [38, 40], and Italy [41]. There were variations in the observed clinical presentations of the patients in this study and intravenous saline (0.8%) was administered in those patients with severe dehydration. The result of the in vitro antimicrobial susceptibility testing showed that all the 24 *Salmonella*

enterica detected were MDR as they were resistant to three or more classes of antibiotics. Notably, 100% resistance to all the following β -lactam antibiotics (ceftazidime, cefotaxime, cefoxitin, augmentin and ampicillin) and carbapenems (ertapenem and doripenem) were observed in all the *Salmonella* species, while 75% (18/24) *Salmonella* isolates showed resistance to cefpodoxime. Regrettably, in Nigeria, similar level of resistance was reported by other studies [16, 42, 43]. Studies conducted elsewhere recorded similar resistance in *Salmonella* species. In Ethiopia, 88.9% of all the isolates were resistant to ampicillin [28], 88% in Bangladesh [40] and 74% resistance to cefotaxime was reported by Harakeh et al. [44] in Lebanon. High-level resistance to β -lactam antibiotics was also reported in Burkina Faso [45], Canada [46], and the USA [47]. The high level of resistance among *Salmonella* to β -lactam antibiotics and carbapenems as revealed in this study is worrisome and calls for urgent attention. The differences in the percentage of resistance from different countries may be due to different risk factors such as

Table 6 Characteristics of *invA*, *bla_{CTX-M-9}* and *bla_{CTX-M-15}* gene-harbouring *Salmonella* isolates

Isolate ID	Sample type	Age	Sex	Clinical prognosis	Resistance pattern	No of antibiotics resisted (%)	MAR Index	S. Typhi/S. Paratyphi A	Virulence gene		Antibiotics resistant gene		
									<i>invA</i> (C _t value)	<i>Stm</i>	<i>qnrB19</i>	<i>bla_{CTX-M-9}</i> (C _t value)	<i>bla_{CTX-M-15}</i> (C _t value)
S101	Stool	6	M	Diarrheal disease	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	13 (100.0)	1.0	-	+ (20.4)	-	-	+ (37.15)	-
S102	Blood	8	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	13 (100.0)	1.0	-	+ (23.30)	-	-	-	+ (29.29)
S103	Blood	4	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	12 (92.3)	0.9	-	+ (23.59)	-	-	+ (33.14)	-
S104	Blood	13	F	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-AMK-OFL-IMP-ETR-DOR	11 (84.6)	0.8	-	+ (39.53)	-	-	-	-
S105	Blood	20	F	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR	8 (61.5)	0.6	-	-	-	-	+ (25.94)	+ (38.33)
S106	Stool	2	F	Diarrheal disease	CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR	8 (61.5)	0.6	-	-	-	-	-	-
S107	Stool	5	M	Diarrheal disease	CAZ-CTX-FOX-AUG-AMP-ETR-DOR	7 (53.6)	0.5	-	+ (27.15)	-	-	+ (30.62)	-
S108	Blood	28	M	Febrile illness	CAZ-CTX-FOX-AUG-AMP-ETR-DOR	7 (53.6)	0.5	-	+ (27.60)	-	-	+ (29.60)	-
S109	Blood	8	F	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR	8 (61.5)	0.6	-	+ (39.53)	-	-	+ (25.40)	+ (30.29)
S110	Blood	2	F	Febrile illness	CAZ-CTX-FOX-AUG-AMP-ETR-DOR	7 (53.6)	0.5	-	+ (31.60)	-	-	+ (21.70)	-
S111	Blood	1	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-ETR-DOR	12 (92.3)	0.9	-	-	-	-	+ (29.72)	-

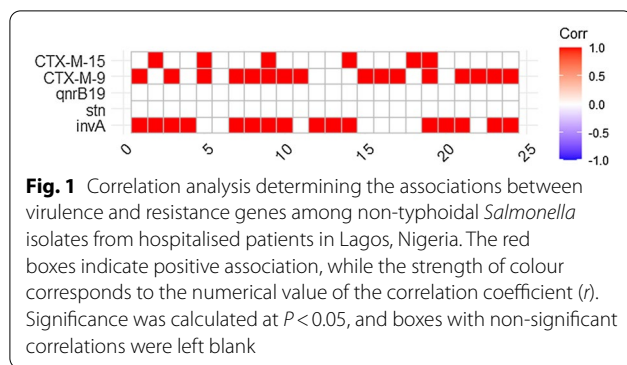
Table 6 (continued)

Isolate ID	Sample type	Age	Sex	Clinical prognosis	Resistance pattern	No of antibiotics resisted (%)	MAR Index	S. Typhi/S. Paratyphi A	Virulence gene		Antibiotics resistant gene				
									invA (C _t value)	Stn	qnrB19	bla _{CTX-M-9} (C _t value)	bla _{CTX-M-15} (C _t value)		
S112	Blood	15	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-AMK-OFL-IMP-ETR-DOR	11 (84.6)	0.8	-	+	(23.30)	-	-			
S113	Blood	20	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR	8 (61.5)	0.6	-	+	(22.21)	-	-			
S114	Stool	7	F	Diarrheal disease	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	13 (100.0)	1.0	-	+	(28.09)	-	+	(20.31)		
S115	Stool	4	M	Diarrheal disease	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	13 (100.0)	1.0	-	-	-	+	(30.20)	-		
S116	Stool	24	F	Diarrheal disease	CAZ-CTX-FOX-AUG-AMP-ETR-DOR	7 (53.6)	0.5	-	-	-	-	+	(33.64)	-	
S117	Stool	5	F	Diarrheal disease	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	13 (100.0)	1.0	-	-	-	-	+	(33.24)	-	
S118	Stool	9	M	Diarrheal disease	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-IMP-ETR-DOR	13 (100.0)	1.0	-	-	-	-	-	+	(29.29)	
S119	Blood	2	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR	8 (61.5)	0.6	-	+	(23.30)	-	+	(20.23)	+	(31.47)
S120	Blood	16	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR	8 (61.5)	0.6	-	+	(27.15)	-	-	-	-	
S121	Blood	19	F	Febrile illness	CAZ-CTX-FOX-AUG-AMP-ETR-DOR	7 (53.6)	0.5	-	+	(28.06)	-	+	(37.15)	-	
S122	Blood	5	M	Febrile illness	CAZ-CPD-CTX-AUG-AMP-ETR-DOR	7 (53.6)	0.5	-	-	-	-	+	(25.94)	-	

Table 6 (continued)

Isolate ID	Sample type	Age	Sex	Clinical prognosis	Resistance pattern	No of antibiotics resisted (%)	MAR Index	S. Typhi/S. Paratyphi A	Virulence gene		Antibiotics resistant gene		
									<i>invA</i> (C _t value)	<i>Stn</i>	<i>qnrB19</i>	<i>bla</i> _{CTX-M-9} (C _t value)	<i>bla</i> _{CTX-M-15} (C _t value)
S123	Stool	7	M	Diarrheal disease	CAZ-CPD-CTX-FOX-AUG-AMP-GEN-AMK-CPR-OFL-ETR-DOR	12 (92.3)	0.9	-	+ (28.06)	-	-	+ (33.40)	-
S124	Blood	30	M	Febrile illness	CAZ-CPD-CTX-FOX-AUG-AMP-AMK-OFL-IMP-ETR-DOR	11 (84.6)	0.8	-	+ (31.60)	-	-	+ (29.20)	-

* Cefazidime (CAZ), cefpodoxime (CPD), cefotaxime (CTX), cefoxitin (FOX), augmentin (AUG), ampicillin (AMP), ciprofloxacin (CPR), ofloxacin (OFL), doripenem (DOR), eripenem (ETR), imipenem (IMP), gentamycin (GEN)



sickle cell disease, HIV, malnutrition, tuberculosis and other respiratory infections, all of which are conditions known to be associated with frequent antibiotic use [39]. Reduced susceptibility to imipenem (25.0%), ciprofloxacin (37.5%), ofloxacin (50.0%), gentamycin (37.5%), and amikacin (50.0%) was recorded in this study. Reduced susceptibility to fluoroquinolones is a serious public health concern, as this class of antibiotics remains among the antimicrobials of choice for the treatment of invasive and systemic salmonellosis in human medicine and animals' husbandry [48, 49]. This study revealed high multiple antibiotic resistance (MAR) index (ranging from 0.5 to 1.0) in all the *Salmonella* isolates with five heterogeneous patterns of resistance. *Salmonella* isolates have been reported in Nigeria to display similar resistant phenotype [16, 42]. Studies elsewhere have also shown to express similar resistance phenotypes consistent with this study. For instance, Asfaw Ali et al. [50] reported that 11 out of the 43 *Salmonella* isolates exhibited the same resistance phenotype. Also, Eguale et al. [19] reported high MAR index with similar resistance pattern in *S. Virchow*, *S. Typhimurium*, *S. Saint-Paul*, *S. Kentucky*, *S. Heidelberg* and *S. Concord* in their study. The expression of similar resistance phenotype by different *Salmonella* serovars makes the resistance profile an unreliable typing tool. Despite the phenotypic expression of high-level resistance to β -lactam antibiotics, phenotypic expression of ESBL was not recorded in this study with the double disc synergy test used. The negative impact of the two enzyme groups (AmpC and ESBL) which have overlapping hydrolysis spectra and the presence of ESBL genes have been reported to affect the phenotypic expression of ESBL using the convectional screening methods [51]. It is noteworthy that, 79.2% (19/24) *Salmonella* isolates harboured bla_{CTX} genes. Thirteen of the *Salmonella* isolates carry $bla_{CTX-M-9}$ genes only, while three of the *Salmonella* isolates harboured $bla_{CTX-M-15}$ genes only. Both

$bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ were detected in *Salmonella* isolates from diarrhoeal patients and febrile patients with persistent pyrexia. Interestingly, co-existence of $bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ was observed in three *Salmonella* spp isolated from febrile patients with resistance pattern CAZ-CPD-CTX-FOX-AUG-AMP-ETR-DOR. Detection of $bla_{CTX-M-15}$ and $bla_{CTX-M-9}$ from the clinical samples have been reported in Iran [52]. Ethiopia [19], India [9] Zambia [53], Kuwait [54], Japan [53], France and Senegal [25], and Germany [55]. Therefore, this study has demonstrated the potential dissemination of bla_{CTX-M} variants in *Salmonellae* from our environment with a prolonged hospital stay of patients and consequently resulting in treatment failure. Resistance to this class of antibiotic by *Salmonella* has generated global public health concern [56], and this is now a major concern in Nigeria as 50% of the patients harbouring *Salmonella* with $CTX-M$ genes in this study are children < 10 years of age. There have been successful global dissemination of $CTX-M$ genes, two of these most common variant $bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ were detected in this study. Although, in Nigeria, $bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ have been reported in *E. coli* isolated from stool samples of pregnant women [17] and from animal sources [18]. To the best of our knowledge, this is the first report of $bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ in *Salmonella* isolated from clinical sources in Nigeria. In this study, out of the $bla_{CTX-M-9}$ and $bla_{CTX-M-15}$ -producing *Salmonella* strains detected, 50% (12/24) were simultaneously resistant to ciprofloxacin and ofloxacin in vitro. However, none of these isolates harboured plasmid-mediated quinolone-resistant gene $qnrB19$ genes. This gene if present would have enhance the development of quinolone resistance strains [57]. None of the *Salmonella* isolates from this study harboured the enterotoxin (stn) gene. The expression of this gene is responsible for *Salmonella*-associated gastroenteritis by inducing previously been reported in Nigeria [59] and Egypt [60]. However, the detection of invasive ($invA$) gene which is a common molecular target for *Salmonella*-specific detection was recorded in 66.7% (16/24) of *Salmonella* species investigated, an indication that qPCR investigation of $invA$ gene is a suitable target for the confirmation of putative *Salmonella* isolates. The $invA$ gene of *Salmonella* species is associated with invasion of host epithelial cell and it is located on the pathogenicity island 1 (SPI-1). The detection of this virulence gene in *Salmonella* as a diagnostic application had been reported by several authors with varying degree of sensitivity and specificity ranging from 55 to 100% [16, 61, 62].

Conclusion

This study revealed prevalence of 4.7% *Salmonella*-associated infections, with 62.5% of the *Salmonella* isolates detected found in children ≤ 10 years. All *Salmonella* isolated exhibited high level of resistance to β -lactams antibiotics and carbapenems with reduced susceptibility to fluoroquinolone. The study revealed for the first time the carriage of *bla*_{CTX-M-9} and *bla*_{CTX-M-15} variants among the *Salmonella* species from Nigeria. Complete therapeutic failure of first-line antibiotics is eminent, if the policy-makers do not only ensure a complete ban on the over-the-counter-sale of antibiotics without doctor's prescription, but also a routine follow-up programme for enforcement, to curb the spread of antibiotics resistance organisms.

Abbreviations

AST: Antibiotic susceptibility testing; CTX-M: Cefotaximase; ESBLs: Extended-spectrum β -lactamases; iNTS: Invasive non-typhoidal *Salmonella*; qPCR: Quantitative polymerase chain reaction.

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Author contributions

KOA* conceived the study, KOA* and COF designed this study. COF collected the data, KOA*, COF and KOA performed the experimental methods, COF and MS performed statistical analysis. COF drafted and wrote the manuscript, KOA*, KOA and MS edited and revised the manuscript. KOA* supervised the work, KOA co-supervised. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included here and are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Human Research and Ethics Committee of Lagos State University Teaching Hospital with reference number LREC/06/10/1012 and the Lagos State Health Service Commission with reference number LSHSC/2222/VOL.VC/352 in accordance with 1964 Helsinki declaration and its later amendments or comparable ethical standards. The written informed consent was obtained from all subjects and/or their legal guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Raymond M, Gibani MM, Day NPJ, Cheah PY. Typhoidal *Salmonella* human challenge studies: ethical and practical challenges and considerations for low-resource settings. *Trials*. 2019;20(2):704.
2. Borges KA, Furian TQ, Souza SN, Salle CTP, Moraes HLS, Nasimento VP. Antimicrobial resistance and molecular characterization of *Salmonella enterica* serotypes from poultry sources in Brazil. *Braz J Poult Sci*. 2019;21(1):1–8.
3. Keestra-Gounder AM, Tsois RM, Baumler AJ. Now you see me, now you don't: the interaction of *Salmonella* with innate immune receptors. *Nat Rev Microbiol*. 2019;13:206–16.
4. Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* (London, England). 2018;392:1789–858.
5. Tack B, Vanaenrode J, Verbakel JY, Toelen J, Jacobs J. Invasive non-typhoidal *Salmonella* infections in sub-Saharan Africa: a systematic review on antimicrobial resistance and treatment. *BMC Med*. 2020;18:212.
6. World Health Organization. Food Safety Fact Sheet. 2020. <https://www.who.int/news-room/fact-sheets/detail/food-safety>. Accessed 4 Jun 2021.
7. WHO. Antimicrobial resistance. Why is antimicrobial resistance a global concern? Fact sheet. Nov 2021. <https://www.who.int/news-room/fact-sheets/detail/Antimicrobial-resistance>. Accessed 17 June 2021.
8. Katiyo S, Muller-Pebody B, Minaji M, Powell D, Johnson AP, De Pinna E, Day M, Harris R, Godbole G. Epidemiology and outcomes of nontyphoidal *Salmonella* bacteremias from England, 2004 to 2015. *J Clin Microbiol*. 2019;57(1):e01189–e1218.
9. Ramchandran A, Shanthi M, Sekar U. Detection of *bla*_{CTX-M} extended spectrum beta-lactamase producing *Salmonella enterica* serotype Typhi in a tertiary care centre. *J Clin Diagn Res*. 2017;11(9):DC21–24.
10. Bialvaei AZ, Kafili HS, Asgharzadeh M, Aghazadeh M, Yousefi M. CTX-M extended-spectrum β -lactamase-producing *Klebsiella* spp, *Salmonella* spp, *Shigella* spp and *Escherichia coli* isolates in Iranian hospitals. *Braz J Microbiol*. 2016;47(3):706–11.
11. Simarro E, Navarro F, Ruiz J, Miró E, Gómez J, Mirelis B. *Salmonella enterica* Serovar Virchow with CTX-M-Like β -Lactamase in Spain. *J Clin Micro*. 2000;38(12):4676–8.
12. Jin Y. CTX-M-producing *Salmonella* spp in Hong Kong: an emerging problem. *J Med Microbiol*. 2006;55(9):1245–50.
13. Nagshetty K, Shilpa B, Patil S, Shivannavar C, Manjula N. An overview of extended spectrum beta lactamases and metallo beta lactamases. *Adv Microbiol*. 2021;11:37–62.
14. Saravanan M, Ramchandran B, Barabadi H. The prevalence and drug resistance pattern of extended spectrum β -lactamases (ESBLs) producing *Enterobacteriaceae* in Africa. *Microb Pathog*. 2018;114:180–92.
15. Akinyemi KO, Iwalokun BA, Alafe OO, Mudashiru SA, Fakorede CO. *Bla*_{CTX-M-15} group extended spectrum beta lactamase-producing *Salmonella Typhi* from hospitalized patients in Lagos, Nigeria. *Infect Drug Resist*. 2015;8:99–106.
16. Akinyemi KO, Fakorede CO, Abegunrin RO, Ajose SO, Anjorin AA, Amisu KO, et al. Detection of *invA* and *bla*_{CTX-M}-genes in *Salmonella* spp. isolated from febrile patients in Lagos hospitals in Nigeria. *Ger J Microbiol*. 2021;1(3):1–10.
17. Fortini D, Fashae K, Villa L, Feudi C, Garcia-Fernandez A, Carattoli A. A novel plasmid carrying *bla*_{CTX-M-15} identified in commensal *Escherichia*

- coli* from healthy pregnant women in Ibadan, Nigeria. *J Glob Antimicrob Resist*. 2015;3(1):9–12.
18. Fashae K, Engelmann I, Monecke S, Braun SD, Ehrlich R. Molecular characterisation of extended spectrum β -lactamase producing *Escherichia coli* in wild birds and cattle, Ibadan, Nigeria. *BMC Vet Res*. 2021;17:33.
 19. Eguale T, Birungi J, Asrat D, et al. Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal *Salmonella* isolates from humans and animals in central Ethiopia. *Antimicrob Resist Infect Control*. 2017;6:13.
 20. Badr H, Reda RM, Hagag NM, Kamel E, Elnomrosy SM, Mansour AI, et al. Multidrug-resistant and genetic characterization of extended-spectrum beta-lactamase-producing *E. coli* recovered from chickens and humans in Egypt. *Animals (Basel)*. 2022;12(3):346.
 21. Moawad AA, Hotzel H, Hafez HM, Ramadan H, Tomaso H, Braun SD, et al. Occurrence, phenotypic and molecular characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* in healthy turkeys in Northern Egypt. *Antibiotics*. 2022;11(8):1075.
 22. Smith S, Opere B, Fowora M, Aderohunmu A, Ibrahim R, Omonigbehin E, et al. Molecular characterization of *Salmonella* spp directly from snack and food commonly sold in Lagos, Nigeria. *Southeast Asian J Trop Med*. 2012;43:718–23.
 23. Clinical Laboratory Standard Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 31th edn. Clinical and Laboratory Standards Institute, M100: PA, USA, 2021.
 24. Novais A, Canton R, Valverde A, Machado E, Galán JC, Peixe L, et al. Dissemination and persistence of *bla*_{CTX-M-9} are linked to class 1 integrons containing CR1 associated with defective transposon derivatives from Tn402 located in early antibiotic resistance plasmids of IncHI2, IncP1-alpha, and IncFI groups. *Antimicrob Agents Chemother*. 2006;50(8):2741–50.
 25. Weill F, Perrier-Gros-Claude J-D, Demartin M, Coignard S, Grimont PA. Characterization of extended-spectrum- β -lactamase (CTX-M-15)-producing strains of *Salmonella enterica* isolated in France and Senegal. *FEMS Microbiol*. 2004;238(2):353–8.
 26. Choudhury M, Borah P, Sarma HK, Barkalita LM, Deka NM, Hussain I, et al. Multiplex-PCR assay for detection of some major virulence genes of *Salmonella enterica* serovars from diverse sources. *Curr Sci*. 2016;111(7):1252–8.
 27. Nagarajan AG, Karnam G, Lahiri A, Allam US, Chakravorty D. Reliable means of diagnosis and serovar determination of blood-borne *Salmonella* strains: quick PCR amplification of unique genomic loci by novel primer sets. *J Clin Microbiol*. 2009;47(8):2435–41.
 28. Akinyemi KO, Ajoseh SO, Fakorede CO. A systemic review of literature on human *Salmonella enterica* serovars in Nigeria (1999–2018). *J Infect Dev Ctries*. 2021;15(9):1222–35.
 29. Akinyemi KO, Ajoseh SO, Iwalokun BA, Oyefolu AOB, Fakorede CO, Abegunrin RO, et al. Antimicrobial resistance and plasmid profiles of *Salmonella enterica* serovars from different sources in Lagos, Nigeria. *Nigeria Health*. 2018;10:758–72.
 30. Akinyemi KO, Bamiro BS, Coker AO. Salmonellosis in Lagos, Nigeria: incidence of *Plasmodium falciparum*-associated co-infection, patterns of antimicrobial resistance, and the emergence of reduced susceptibility to fluoroquinolones. *J Health Popul Nutr*. 2007;25(3):351.
 31. Eguale T, Gebreyes WA, Asrat D, Alemayehu H, Gunn JS, Engidawork E. Non-typhoidal *Salmonella* serotypes, antimicrobial resistance and co-infection with parasites among patients with diarrhoea and other gastrointestinal complaints in Addis Ababa, Ethiopia. *BMC Infect Dis*. 2015;15:497.
 32. Tosisa W, Mihret A, Ararsa A, Eguale T, Abebe T. Prevalence and antimicrobial susceptibility of *Salmonella* and *Shigella* species isolated from diarrheic children in Ambo town. *BMC Pediatr*. 2020;20:91.
 33. Deksissa T, Gebremedhin Z. A cross sectional study of enteric fever among patients at Ambo hospital: prevalence, risk factors comparison of Widal test and stool culture antimicrobials susceptibility pattern of isolates. *BMC Infect Dis*. 2019;19:288.
 34. Paton S, Nicolle L, Mwangera M, Kabiru P, Mirza N, Plummer F, Wamola I. *Salmonella* and *Shigella* Gastroenteritis at a Public Teaching Hospital in Nairobi, Kenya. Published online by Cambridge University Press; 2006.
 35. Kadyr M, Nader SM, Dorgham SM, Kandil MM. Molecular diversity of the *invA* gene obtained from human and egg samples. *Vet World*. 2019;12(7):1033–8.
 36. Ngogo FA, Joachim A, Abade AM, Rumisha SF, Mizinduko MM, Majigo MV. Factors associated with *Salmonella* infection in patients with gastrointestinal complaints seeking health care at a regional hospital in Southern Highland of Tanzania. *BMC Infect Dis*. 2020;20:135.
 37. Wu X, Angkititrakul S, Richards LA, Pulsrikarn C, Khaengair S, Keosengthong A, et al. Risk of antimicrobial resistant non-typhoidal *Salmonella* during asymptomatic infection passage between pet dogs and their human caregivers in Khon Kaen, Thailand. *Antibiotics (Basel)*. 2020;9:477.
 38. Gong B, Li H, Feng Y, Zeng S, Zhuo Z, Luo J, Chen X, Li X. Prevalence, serotype distribution and antimicrobial resistance of non-typhoidal *Salmonella* in hospitalized patients in Conghua District of Guangzhou, China. *Front Cell Infect Microbiol*. 2022;12: 805384.
 39. Obaro SK, Hassan-Hanga F, Olateju EK, Umoru D, Lawson L, Olanipekun G, et al. *Salmonella* bacteraemia among children in central and northwest Nigeria, 2008–2015. *Clin Infect Dis*. 2015;61(4):S325–31.
 40. Liu YH, Zhang Y, Hu JQ. Clinical epidemiological characteristics of non-typhoid *Salmonella* infection in children in Guangzhou from 2016 to 2018. *Guangdong Med J*. 2021;42:936–9.
 41. Mascaro V, Pileggi C, Crinò M, Proroga YTR, Carullo MR, Graziani C, et al. Non-typhoidal *Salmonella* in Calabria, Italy: a laboratory and patient-based survey. *BMJ Open*. 2017;7: e017037.
 42. Adamu U, Yusha'u M, Usman AD, Abdulhadi SK. Antibiotic resistance pattern of *Salmonella* species isolated from Typhoid patients in Jigawa state, Nigeria. *Nov Res Microbiol J*. 2020;4:696–703.
 43. Igbinosa EO, Beshiru A, Igbinosa IH, Okoh AI. Antimicrobial resistance and genetic characterisation of *Salmonella enterica* from retail poultry meats in Benin City, Nigeria. *LWT*. 2022;169: 114049.
 44. Harakeh S, Yassine H, Gharios M, Barbour E, Hajjar S, El-Fadel M, et al. Isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* and *Escherichia coli* isolates from meat-based fast food in Lebanon. *Sci Total Environ*. 2005;341(1–3):33–44.
 45. Bonkougou IJ, Haukka K, Österblad M, Hakanen AJ, Traoré AS, Barro N, et al. Bacterial and viral aetiology of childhood diarrhoea in Ouagadougou. *Burkina Faso BMC Pediatr*. 2013;13:36.
 46. McEwen SA, Prescott JF, Boerlin P. Antibiotics and poultry—a comment. *Can Vet J*. 2010;51:561–2.
 47. M'ikanatha NM, Sandt CH, Localio AR, Tewari D, Rankin SC, Whichard JM, et al. Multidrug-resistant *Salmonella* isolates from retail chicken meat compared with human clinical isolates. *Foodborne Pathog Dis*. 2010;7:929–34.
 48. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin Microbiol Rev*. 2015;28:901–37.
 49. Palma E, Tilocca B, Roncada P. Antimicrobial resistance in veterinary medicine: an overview. *Int J Mol Sci*. 2020;21:1914.
 50. Asfaw Ali D, Tadesse B, Ebabu A. Prevalence and antibiotic resistance pattern of *Salmonella* isolated from caecal contents of exotic chicken in Debre Zeit and Modjo, Ethiopia. *Int J Microbiol*. 2020;2020:1910630.
 51. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Böttger EC, Hombach M. Evaluation of a diagnostic flow chart for detection and confirmation of extended spectrum β -lactamases (ESBL) in *Enterobacteriaceae*. *Clin Microbiol Infect*. 2012;18(12):1194–204.
 52. Tajbakhsh M, Avini MY, Alikhajeh J, Tadjeddin E, Rahbar M, Eslami P, et al. Emergence of *bla*_{CTX-M-15}, *bla*_{TEM-169} and *bla*_{PER-1} extended-spectrum β -lactamase genes among different *Salmonella enterica* serovars from human faecal samples. *Infect Dis (Lond)*. 2016;48(7):550–6.
 53. Ramatla T, Mileng K, Ndou R, Mphuti N, Syakalima M, Lekota KE, et al. Molecular detection of integrons, colistin and β -lactamase resistant genes in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from chickens and rats inhabiting poultry farms. *Microorganisms*. 2022;10:313.
 54. Rotimi V, Jamal W, Pal T, Sovenned A, Albert M. Emergence of CTX-M-15 type extended-spectrum beta-lactamase producing *Salmonella* spp. in Kuwait and the United Arab Emirates. *J Med Microbiol*. 2008;57(7):881–6.
 55. Fischer J, Rodriguez I, Baumann B, Guiral E, Beutin L, Schroeter A, et al. *bla*_{CTX-M-15} carrying *Escherichia coli* and *Salmonella* isolates from livestock and food in Germany. *J Antimicrob Chemother*. 2014;69(11):2951–8.
 56. Arlet G, Barrett TJ, Butaye P, Cloeckaert A, Mulvey MR, White DG. *Salmonella* resistant to extended-spectrum cephalosporin: prevalence and epidemiology. *Microb Infect*. 2006;8:1945–54.

57. Acheampong G, Owusu M, Owusu-Ofori A, Osei I, Sarpong N, Sylverken A, et al. Chromosomal and plasmid-mediated fluoroquinolone resistance in human *Salmonella enterica* infection in Ghana. *BMC Infect Dis*. 2019;19:898.
58. De Jong B, Ekdahl K. The comparative burden of salmonellosis in the European Union member states, associated and candidate countries. *BMC Public Health*. 2006;6:4.
59. Akinyemi KO, Iwalokun BA, Foli F, Oshodi K, Coker AO. Prevalence of multiple drug resistance and screening of enterotoxin (stn) gene in *Salmonella enterica* serovars from water sources in Lagos, Nigeria. *Public Health*. 2011;125:65–71.
60. Abou Elez R, Elsohaby I, El-Gazzar N, Tolba H, Abdelfatah EN, Abdellatif SS, et al. Antimicrobial resistance of *Salmonella* Enteritidis and *Salmonella* Typhimurium isolated from laying hens, table eggs, and humans with respect to antimicrobial activity of biosynthesized silver nanoparticles. *Animals*. 2021;11(12):3554.
61. Anejo-Okopi JA, Isa SE, Audu O, Fagbamila IO, Iornenge JC, Smith IS. Isolation and polymerase chain reaction detection of virulence *invA* gene in *Salmonella* spp. from poultry farms in Jos Nigeria. *J Med Trop*. 2016;18:98–102.
62. Sharma I, Das K. Detection of *invA* Gene in isolated *Salmonella* from marketed poultry meat by PCR assay. *J Food Process Technol*. 2016;7:564.

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