Acta Microbiologica et Immunologica Hungarica 66 (4), pp. 509–527 (2019)
DOI: 10.1556/030.66.2019.034
First published online October 29, 2019

MOLECULAR DIVERSITY AND ANTIBIOTIC RESISTANCE GENE PROFILE OF *SALMONELLA ENTERICA* SEROVARS ISOLATED FROM HUMANS AND FOOD ANIMALS IN LAGOS, NIGERIA

ABRAHAM AJAYI[1]*, STELLA IFEANYI SMITH[2], JULIEN COULIBALY KALPY[3], IBIDUNNI OREOLUWA BODE-SOJOBI[4], YAO KOUAMÉ RENÉ[3] and ADEYEMI ISAAC ADELEYE[1]

[1]Department of Microbiology, University of Lagos, Akoka, Nigeria
[2]Molecular Biology and Biotechnology Department, Nigerian Institute of Medical Research, Lagos, Nigeria
[3]Laboratoire de Bactériologie et Virologie Institut Pasteur de Côte d'Ivoire, Centre Nationale de Référence de Salmonella, Paris, France
[4]Department of Medical Microbiology, Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria

(Received: 17 April 2019; accepted: 23 May 2019)

Outbreaks of *Salmonellosis* remain a major public health problem globally. This study determined the diversity and antibiotic resistance gene profile of *Salmonella enterica* serovars isolated from humans and food animals. Using standard methods, *Salmonella* spp. were isolated from fecal samples, profiled for antimicrobial susceptibility and resistance genes. Seventy-one *Salmonella* isolates were recovered from both humans and food animals comprising cattle, sheep, and chicken. Forty-four serovars were identified, with dominant *Salmonella* Budapest (31.8%). Rare serovars were present in chicken (*S*. Alfort, *S*. Wichita, *S*. Linton, *S*. Ealing, and *S*. Ebrie) and humans (*S*. Mowanjum, *S*. Huettwillen, *S*. Limete, and *S*. Chagoua). Sixty-eight percent of isolates were sensitive to all test antibiotics, while the highest rate of resistance was to nalidixic acid (16.9%; $n = 12$), followed by ciprofloxacin (11.3%; $n = 8$) and tetracycline (9.9%; $n = 8$). Five isolates (7%) were multidrug-resistant and antimicrobial resistance genes coding resistance to tetracycline (*tetA*), beta-lactam (bla_{TEM}), and quinolone/fluoroquinolone (*qnrB* and *qnrS*) were detected. Evolutionary analysis of *gyrA* gene sequences of human and food animal *Salmonella* isolates revealed variations but are evolutionarily interconnected. Isolates were grouped into four clades with *S*. Budapest isolate from cattle clustering with *S*. Budapest isolated from chicken, whereas *S*. Essen isolated from sheep and chicken was grouped into a clade. Diverse *S. enterica* serovars with high antibiotic resistance profile are

*Corresponding author; E-mail: ajayiabraham2013@gmail.com

ubiquitous in food animals; hence, there is a need for surveillance and prudent use of antibiotics in human and veterinary medicine.

Keywords: antibiotic resistance genes, serotype, quinolone, diversity

Introduction

Salmonellosis continue to pose substantial health challenges in developed and developing countries [1]. Over 2,500 *Salmonella enterica* serovars have been identified and some of which are the etiology of salmonellosis that is presented mainly as enteric fever in cases caused by *Salmonella* Typhi or Paratyphi and gastroenteritis caused by other *S. enterica* serovars. While the former is most of the time severe and invasive, the latter tends to be self-limiting; however, it could be severe and systemic in infants, older people, and immunocompromised individuals [2–4]. Epidemiological surveillance overtime has revealed that outbreaks of salmonellosis in human are linked to ingestion of foods mainly of animal origin that are contaminated with *Salmonella*; thus, most *Salmonella* infections could be considered as zoonotic. However, host-specific serovars like *S*. Typhi transmission is often through the fecal–oral route and not considered to be zoonotic. Establishing the route of transmission is more or less difficult, since the transmission chain is complex [2, 5, 6]. Food animals (chicken, pork, cattle, sheep, and even fish) are a major reservoir of diverse serovars of *S. enterica* as has been documented in several reports [7–11]. This persistence of *S. enterica* in the intestinal tract of food animals creates a chronic or non-symptomatic carrier state that provides for continuous shedding of bacteria in feces, thereby serving as a reservoir for subsequent spread by contaminated meat, milk, eggs, and agricultural products cultivated on *Salmonella*-containing manure lands [12]. Not only food animals are sources of *Salmonella* infections, they also serve as a channel for the dissemination of multidrug-resistant (MDR) serovars, which in recent times has emerged as a threat to the effective treatment of infections. In a 2014 World Health Organization (WHO) report on resistance of selected bacteria of international concern to antibacterial drugs, non-typhoidal *Salmonella* (NTS) resistance to fluoroquinolones made the list with a total number of reports with data sets based on ≥30 tested isolates to be as high as 75% [13]. Resistance of *Salmonella* spp. to most widely used classes of antibiotics spans from aminoglycosides, chloramphenicol, tetracycline, beta-lactams (including cephalosporin), quinolones, sulfonamide and trimethoprim to polymyxins [14–16]. In Nigeria, there has been reports of the isolation and antibiotic resistance profiling of *Salmonella* serovars isolated from humans, chicken, and pigs [17–19] but there is drought of information on studies that evaluates human and a broader range of food animals at once.

Hence, this study explores the diversity and antibiotic resistance genes profile of *Salmonella* serovars isolated from humans and three major food animals in Lagos, Nigeria, a cosmopolitan city in West Africa.

Materials and Methods

Study design

The study is composed of humans and food animals (cattle, sheep, and chicken).

Group 1: This group comprised human subjects who were apparently healthy food handlers recruited based on the following criteria: (1) adults with ≥18 years of age (both male and female) and (2) not on any form of antibiotic treatment. Sample size for human subject was defined as:

$$n = \frac{Z^2 pq}{d^2},$$

where:

$Z \rightarrow$ Standard normal deviate $= 1.96$

$p \rightarrow$ Prevalence $\cong 0.304$ according to Smith et al. [20]

$q \rightarrow 1 - \mathrm{p} = 1 - 0.304$

$d \rightarrow$ Tolerable margin of error $= 0.05$

$n = n = \frac{(1.96)^2 \times (0.304) \times (0.696)}{(0.5)^2 0.0025} = 0.8128211$

$n = 325.1.$

With a gazette 10% non-response rate

$n = 325 + 33 = 358.$

Group 2: This comprised apparently healthy food animals (cattle, sheep, and chicken) that were being processed for slaughter. One hundred and two fecal samples were collected from each animal type making the total sample size from animals to be 306. Therefore, the total number of samples collected for both group was 664.

Sample collection

Fecal samples were collected from humans, cattle, sheep, and chicken between June 2016 and March, 2017. Samples were collected from animals randomly selected each day of sample collection. Fecal samples were obtained after slaughter from viscera (intestine) in sterile specimen bottles appropriately

labeled and transported in a thermobox at 4 °C to the laboratory immediately. Fecal samples were also collected from humans in sterile sample bottles and transported immediately to the laboratory under the same conditions earlier stated.

Isolation and identification of isolates

Five grams of each fecal sample were enriched in 25 ml of selenite F broth (Oxoid, Basingstoke, UK) and incubated at 37 °C for 18–24 h. This was followed by plating onto *Salmonella–Shigella* agar (Oxoid) and incubated at 37 °C for 24 h. Presumptive colonies were further purified by subculturing on nutrient agar (Oxoid). Pure colonies were identified using biochemical tests including motility, indole, urease, mannitol, lysine decarboxylase, citrate, ortho-nitrophenyl-β-galactoside, Kliger Iron Agar to determine H_2S and gas production, lactose and glucose fermentation, oxidase and catalase.

Serotyping

Serotyping of all biochemically confirmed *Salmonella* isolates was carried out at Centre Nationale de Référence de Salmonella, Laboratoire de Bactériologie et Virologie Institut Pasteur de Côte d'Ivoire according to White Kauffmann–Le Minor scheme [21]. Serotyping was carried out by slide agglutination test to characterize O and H antigens using commercially available antisera (Bio-Rad, F-92430 Marnes-La-Coquette, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method according to the European committee on antimicrobial susceptibility testing [22] guidelines. *Salmonella* isolates were inoculated into brain–heart infusion broth (Oxoid) and incubated at 37 °C for 18 h after which they were subcultured on Mueller–Hinton agar (Hi-Media Laboratories Pvt. Ltd., India) at 37 °C for 24 h. Two to three distinct colonies were emulsified in 5 ml of sterile physiological saline and adjusted to 0.5 McFarland standard and then a sterile swab stick was in applying bacteria suspension to the surface of Mueller–Hinton agar. Antibiotic disks were then applied comprising ampicillin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), minocycline (30 μg), colistin (50 μg), tobramycin (10 μg), nalidixic acid (30 μg), norfloxacin (10 μg), imipenem (10 μg), cephalotin (30 μg), ceftazidime (10 μg), gentamycin (10 μg), aztreonam (30 μg), ceftriaxone (30 μg),

cefuroxime (30 μg), amikacin (30 μg), chloramphenicol (30 μg), cefotaxime (5 μg), trimethoprim–sulfamethoxazole (25 μg), amoxicillin +clavulanic acid (30 μg), and cefepime (30 μg) (Bio-Rad). The diameter of inhibition zone was measured using ADAGIO (Bio-Rad) and was interpreted as resistance (R), intermidiate (I), and sensitive (S). *Escherichia coli* ATCC 25922 was used as quality control organism.

DNA extraction

Phenol–chloroform–isoamyl alcohol method of DNA extraction according to Adi et al. [23] was adapted with modifications. Three to five colonies of bacterial isolates grown on Colombia Blood Agar overnight was emulsified in nuclease-free water to form a suspension. Cell suspension was refrigerated at −20 °C for 15 min and then transferred to thermomixer held at 95 °C and incubated for 15 min at 500 rpm and then centrifuged at 14,000 rpm for 10 min, after which the supernatant containing the DNA was separated into a new sterile Eppendorf tube. To the supernatant, 500 μl of phenol + chloroform + isoamyl (25:24:1) was added, vortexed, and then centrifuged at 13,000 rpm for 10 min at 4 °C. Eight hundred microliter of supernatant was obtained to which 80 μl of sodium acetate + 500 μl of absolute (100%) ethanol was added, vortexed gently, and stored at −20 °C overnight. After centrifugation at 13,000 rpm for 20 min at 4 °C, supernatant was discarded while 1 ml of ethanol (70%) was added to the residue, vortexed gently, and then centrifuged at 13,000 rpm for 10 min at 4 °C; then, supernatant was discarded; later, the residue was maintained at 70 °C to allow the leftover ethanol to evaporate for 25–30 min. Then, it was reconstituted with 60 μl of Elu Buf (NucliSENS, BioMerieux, France). Purity of DNA was determined using a spectrophotometer and DNA was stored for further use at −20 °C.

Detection of antibiotic resistance genes by polymerase chain reaction (PCR)

Nine antibiotic resistance genes (*tetA*, *tetB*, bla_{TEM}, bla_{SHV}, $bla_{\mathrm{CTX\text{-}M}}$, *qnrA*, *qnrB*, *qnrS*, and *gyrA*) were assayed by PCR using primers listed in Table I. A 50-μl PCR reaction was used that contained 28.8 μl of nuclease-free water, 3.5 μl $MgCl_2$ (25 mM), 10 μl of 5× PCR buffer, 1 μl of dNTPs (10 mM), 0.75 μl of each forward and reverse primers (10 μM), 0.2 μl of one Taq DNA polymerase (5,000 U/ml) (New England Biolabs, Hertfordshire, UK), and 5 μl DNA template. PCR was performed in a GeneAmp PCR system 9700 thermal cycler (AB Applied Biosystems, Singapore) with programming conditions determined empirically.

Table I. Primers used in the detection of antibiotic resistance genes

Primer	Sequence	Amplicon size	Reference
qnrA	F: 5′-GGATGCCAGTTTCGAGGA-3′	502	Cavaco et al. [24]
qnrA	R: 5′-TGCCAGGCACAGATCTTG-3′		
qnrB	F: 5′-GGMATHGAAATTCGCCACTG-3′	280	Cattoir et al. [25]
qnrB	R: 5′-TTTGCYGYYCGCCAGTCGAA-3′		
qnrS	F: 5′-TCGACGTGCTAACTTGCG-3′	480	Cavaco et al. [24]
qnrS	R: 5′-GATCTAAACCGTCGAGTTCGG-3′		
gyrA	F: 5′-TACCGTCATAGTTATCCACGA-3′	300	Wiuff et al. [26]
gyrA	R: 5′-GTACTTTACGCCATGAACGT		
bla_{TEM}	F: 5′-GCGGAACCCCTATTTG-3′	970	Olesen et al. [27]
bla_{TEM}	R: 5′-ACCAATGCTTAATCAGTGAG-3′		
$bla_{\text{CTX-M}}$	F: 5′-ATGTGCAGYACCAGTAARGTKATGGC-3′	605	Hendriksen et al. [28]
$bla_{\text{CTX-M}}$	R: 5′-TGGGTRAARTARGTSACCAGAAYSAGCGG-3′		
bla_{SHV}	F: 5′-TTATCTCCCTGTTAGCCACC-3′	796	Arlet et al. [29]
bla_{SHV}	R: 5′-GATTTGCTGATTTCGCTCGG-3′		
tetA	F: 5′-GTAATTCTGAGCACTGTCGC-3′	988	Waters et al. [30]
tetA	R: 5′-CTGCCTGGACAACATTGCTT-3′		
tetB	F: 5′-CTC AGT ATT CCA AGC CTT TG-3′	414	Sengeløv et al. [31]
tetB	R: 5′-ACT CCC CTG AGC TTG AGG GG-3′		

For *qnrA*, *qnrB*, *qnrS*, *gyrA*, bla_{SHV}, $bla_{\text{CTX-M}}$, and *tetB*, 35 cycles of initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 1 min, and final elongation at 72 °C for 10 min were used; whereas for bla_{TEM} and *tetA*, PCR conditions were similar only with a different annealing temperature of 56 °C. The negative control was water, whereas positive controls include *S.* Typhimurium P5002212DT104 for *gyrA*, *S.* Virchou 58.67 Holland for bla_{CTX}, *S.* Bredeney TEM-104 (Gisele) for bla_{TEM}, *S.* Keurmassar DAK2 for bla_{SHV}, *Enterobacter cloacae* 03-577 for *qnrA*, *Klebsiella pneumoniae* KP15 for *qnrB*, *Escherichia coli* pHC19 for *qnrS*, *E. coli* NCTC50078 for *tetA*, and *E. coli* CSH50:TN10 for *tetB*. PCR products were separated on a 1.5% agarose gel at 120 V and a 100-bp DNA ladder (New England Biolabs) was used as molecular weight maker.

DNA sequencing

PCR amplification products were sent to a commercial facility (Eurofins Genomics, France) for sequencing. Sequence results were analyzed and compared with sequences in the GenBank using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and CARD (https://card.mcmaster.ca/analyze/blast). Phylogenetic analysis was performed using the MEGA 7.0 software [32].

Statistical analysis

Statistical analysis and graphics were performed using GraphPad Prism software 5.01 (La Jolla, CA, USA) and Microsoft Excel (Microsoft Cooperation, 2013 USA). Statistical significance of proportions was assessed using the χ^2 test considering $p < 0.05$ as significant.

Results

Isolation and identification

A total of 71 *Salmonella* isolates were recovered from 664 stool samples collected from humans, cattle, sheep, and chicken with a recovery rate of 10.7%. Forty-eight percent of the isolates were obtained from chicken, whereas 22.5% was from sheep (Table II). Forty-four different serovars were identified with *S.* Budapest having the highest occurrence 31.8% (14/44) followed by *S.* Essen 15.9% (7/44) with *S.* Paratyphi C having the least with 4.5% (2/44) (Table III).

Antimicrobial susceptibility testing

Altogether 68% of all *Salmonella* isolates ($n = 48$) were susceptible to all 21 test antibiotics. The highest rates of resistance was found to nalidixic acid, (12/71; 16.9%) followed by ciprofloxacin 11.3%, while resistance to tetracycline and minocycline was 9.9%. Thirty-one percent ($n = 22$) of the entire isolates exhibited resistance to one or more of the test antibiotics, which was significant ($p < 0.001$) compared to the susceptible isolates. Seven percent ($n = 5$) of the antibiotic resistant isolates were MDR. Antibiotic resistance within population revealed

Table II. Distribution of the isolates from various sources and location

	Number of stool samples collected					Number of *Salmonella* spp. isolated from samples (%)				
Site source	H	S	C	CH	Total	H	S	C	CH	Total
Lagos Island	71	17	26	37	151	3	5	1	12	21
Mushin	76	0	0	18	94	2	0	0	5	7
Alabarago	55	22	68	7	152	2	2	9	6	19
Agege	74	47	8	22	151	2	8	1	8	19
Ikeja	49	0	0	0	49	1	0	0	0	1
Ikorodu	33	16	0	18	67	0	1	0	3	4
Total	358	102	102	102	664	10(14)	16(23)	11(15)	34(48)	71

Note: Sample source – H: human; S: sheep; C: cattle; CH: chicken.

Table III. The occurrence of *Salmonella* serovars identified

CODE	Antigenic formula	Serotype	Frequency of isolation
Isolates from chicken			
$A11_5$/190	(1,3,19: z: I,w)	*S.* Carno	1
CF10/191	(1,4,12,[27]: g,t: _)	*S.* Budapest	13
F2/194			
$A15_2$/198			
E14/200			
CF13/201			
A6/202			
CF3/206			
F4/207			
Fe/213			
CF6/215			
CF9/216			
F5/219			
AF13/220			
B11y/196	(35: gst: _)	*S.* Anecho	3
AF5/199			
D10/204			
E7/203	(3{10}{15}15,34:eh:1,5)	*S.* Muenster	1
D8/209	(4,12:eh:1,7)	*S.* Kaapstad	1
D6/205	(35: gt: _)	*S.* Agodi	2
E15/211			
A2/212	(8,20:Z_4Z_{23}:l,w)	*S.* Dabou	1
A11/214	(4,[5],12:g,z_{51}:e,n,z_{15})	*S.* Tennyson	1
$F1_2$/217	(6,8:r:l,w)	*S.* Goldcoast	1
AF1/218	(35:g,m,t:_)	*S.* Ebrie	1
AF6/221	(4,12:g,m:_)	*S.* Essen	3
E8/224			
CF14/226			
E6/222	(4,[5],12: l,v:e,n,z_{15})	*S.* Brandenburg	1
E4/225	(3,10:f,g:e,n,x)	*S.* Alfort	1
F1/227	(1,6,14,25:c:l,w)	*S.* Minna	1
D3/193	(13,23:r:1,6)	*S.* Linton	1
D25/210	(1,13,23:d:1,6)	*S.* Wichita	1
D14/223	(35:g,m,s:_)	*S.* Ealing	1
Total			34
Isolates from sheep			
S59/230	(6,7,14:d:l,w)	*S.* Livingstone	1
S63B/231	(6,7:d:1,6)	*S.* Kivu	1
S43/232	(17:c:1,5)	*S.* Berlin	1
S57/233	(4,12:g,m:_)	*S.* Essen	4
S38B/235			
S56/236			
S81/247			
S36C/237	(17:b:1,5)	*S.* Dahra	2
S36/241			
S37/240	(4,12:I,w:1,5)	*S.* Mono	1

Table III. The occurrence of Salmonella serovars identified *(Continued)*

CODE	Antigenic formula	Serotype	Frequency of isolation
S61/239	(1,4,12:z10:I,w)	*S.* Mura	1
S28/242	(8,20:z10:e,n,z15)	*S.* Chomedey	1
S38/243	(8,20:d:1,5)	*S.* Yovokome	1
S43/244	(16:d:1,5)	*S.* Sculcoates	1
S51/246	(1,4,12[27]:b:l,w)	*S.* Wien	1
S76/234	(3{10}{15}{15,34}:y:1,5)	*S.* Orion	1
Total			16
Isolates from cattle			
CO18/249	(3,10:d:1,7)	*S.* Onireke	1
CO67/248	(6,7:z_4z_{24}:_)	*S.* Somone	1
CO20/251	(13,23:z:1,6)	*S.* Farmsen	4
CO15/252			
CO26/257			
CO38/255			
CO101/250	(28: z_4z_{24}:_)	*S.* Ketheabarny	1
CO19/253	(1, 4,12[27]:gt:_)	*S.* Budapest	1
CO37/254	(43,:g,z_{62}:enx)	II	1
CO50/256	(1,4,12,27:l$z_{13}z_{28}$:e,n,z_{15})	*S.* Vom	1
CO12/258	(1,4,[5],12:eh:enz_{15})	*S.* Sandiego	1
Total			11
Isolates from human			
H132/266	(1,4,12,[27]:b:1,5)	*S.* Limete	1
H44/267	(9,12:z_{10}:1,5)	*S.* Portland	1
H45/268	(1,4,12:a:I,w)	*S.* Huettwillen	1
H63/269	(6,8:z:1,5)	*S.* Mowanjum	1
HL8/263	(6,7[vi]:c:1,5)	*S.* Paratyphi C	2
H418/274			
H363/271	(1,4,12,27:i:1,2)	*S.* Tyhpimurium	1
H209/272	(6,8:i:1,5)	*S.* Takoradi	1
H183/273	(1,4[5],12:b:1,2)	*S.* Paratyphi B	1
H117/275	(1,13,23:a:1,5)	*S.* Chagoua	1
Total			10

that 43.8% ($n = 7/16$) of isolates from sheep showed resistance to one or more antibiotics closely followed by isolates from humans with 40% (4/10) resistance. Percentage of antibiotic resistance of *Salmonella* isolates from chicken was 29.4% (10/34; Figure 1).

Detection of resistance genes by PCR

From PCR analysis, *Salmonella* isolates that displayed phenotypic resistance to antibiotics were positive for some antibiotic resistance genes assayed

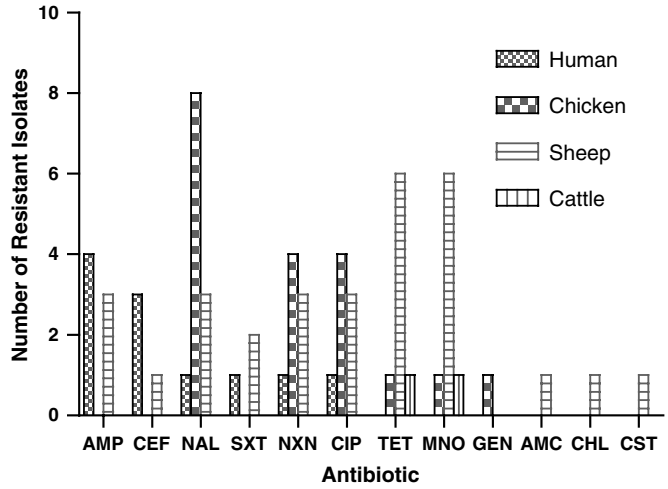

Figure 1. Distribution of resistance to antibiotics among *Salmonella* isolates from human, chicken, sheep and cattle. AMP: ampicillin; CEF: cephalotin; NAL: nalidixic acid; SXT: trimethoprim/sufamethoxazole; NXN: norfloxacine; CIP: ciprofloxacin; TET: tetracycline; MNO: minocycline; GEN: gentamycin

(Table IV). *qnrB* gene was detected in 10 (45.4%) of the isolates and *qnrS* was detected in 1 isolate (Figures 2 and 3). bla_{CTX}, bla_{SHV}, and *qnrA* were not detected in any of the isolates; however, 22.7% (5/22) was positive for bla_{TEM}*tetA* was also detected in 18.1% (4/22) of the isolates. The *gyrA* sequences of 12 isolates (GenBank accession numbers: MG593259–MG593270) were grouped into four clades with *S.* Typhimurium largely diverged. *S.* Budapest isolated from cattle and *S*. Budapest isolated from chicken were close, whereas *S*. Essen from sheep and chicken was grouped together in a clade, indicating evolutionary relatedness. Some isolates were grouped alongside *S.* Typhimurium (accession numbers: X78977 and EU512997) from GenBank (Figure 4).

Discussion

Diversity and gene variation among antibiotic-resistant *S. enterica* serovars of food animal sources continues to expand. Of the 71 *Salmonella* isolates from human, cattle, sheep, and chicken, 44 serovars were obtained with isolates from chicken accounting for 17 of these serovars. This is not surprising as poultry has been known to be a major reservoir and vehicle of transmission of *S.* serovars to humans [33]. *S.* Budapest had the highest rate of occurrence in poultry from different locations sampled. Although this serovar has not been reported in any

Table IV. Antimicrobial resistance phenotype, and genes of *Salmonella* serovars isolated from humans, chicken, sheep, and cattle

Code	Origin	Phenotype	Genes	Serotype
F5/219	Chicken	NAL, NXN, and CIP	*gyrA* and *qnrB*	*S.* Budapest
CF3/206	Chicken	NAL	*gyrA* and *qnrB*	*S.* Budapest
Fe/213	Chicken	NAL, NXN, and CIP	*gyrA* and *qnrB*	*S.* Budapest
$A15_2$/198	Chicken	NAL	*qnrB*	*S.* Budapest
A115/190	Chicken	NAL	*gyrA*	*S.* Carno
D6/205	Chicken	NAL, NXN, and CIP	*gyrA* and *qnrB*	*S.* Agodi
A11/214	Chicken	NAL,	*gyrA* and *qnrB*	*S.* Tennyson
AF6/221	Chicken	NAL, NXN, and CIP	*gyrA* and *qnrB*	*S.* Essen
A2/212	Chicken	TET and MNO	*qnrB, qnrS,* and *TetA*	*S.* Dabou
E14/200	Chicken	GMI	–	*S.* Budapest
CO19/253	Cattle	TET and MNO	*TetA*	*S.* Budapest
H117/275	Human	AM, NAL, SXT, NXN, and CIP	*gyrA* and *TetA*	*S.* Chagoua
H63/269	Human	AM and CEF	–	*S.* Mowanjum
H44/267	Human	AM and CEF	–	*S.* Portland
H363/271	Human	AM and CEF	–	*S.* Typhimurium
S57/233	Sheep	AM and TET	bla_{TEM} and *TetA*	*S.* Essen
S38B/235	Sheep	AM, TET, and SXT	bla_{TEM}	*S.* Essen
S56/236	Sheep	NAL, NXN, and CIP	*gyrA* and *qnrB*	*S.* Essen
S81/247	Sheep	MNO and TET	bla_{TEM}	*S.* Essen
S61/239	Sheep	NAL, NXN, and CIP	*gyrA*	*S.* Mura
S28/242	Sheep	NAL, TET, SXT, NXN, and CIP	*qnrB* and bla_{TEM}	*S.* Chomedey
S51/246	Sheep	MNO and TET	bla_{TEM}	*S.* Wien

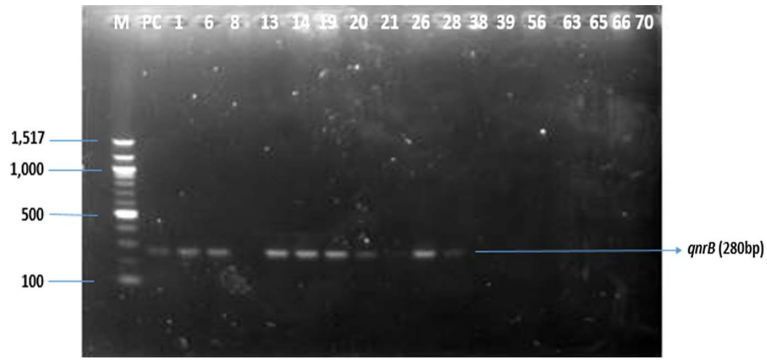

Figure 2. Agarose gel image of PCR products showing positive bands for *qnrB*. Lane 1 – M: 100-bp DNA marker, lane 2 – PC: positive control *Klebsiella pneumonia* KP15, lane 19 – NC: negative control, 1- *S.* Carno, 6, 8, 14, 20, 26- *S.* Budapest, 13- *S.* Agodi, 19- *S.* Dabou, 21- *S.* Tennyson, and 28- *S.* Essen

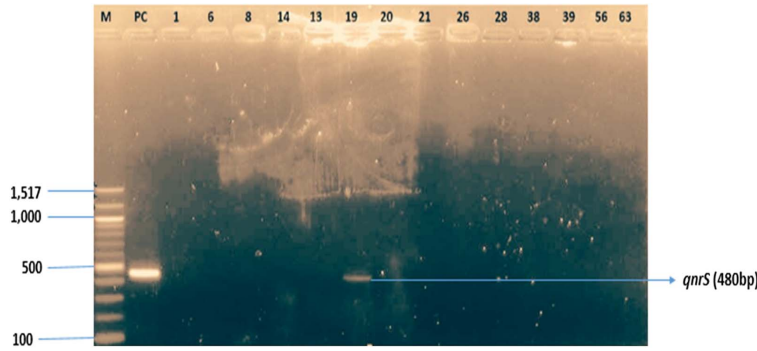

Figure 3. Agarose gel image of PCR products showing positive bands for *qnrS*. Lane 1 – M: 100-bp DNA marker, lane 2 – PC: positive control *Escherichia coli* pHC19, lane 16 – NC: negative control, 19- *S.* Dabou

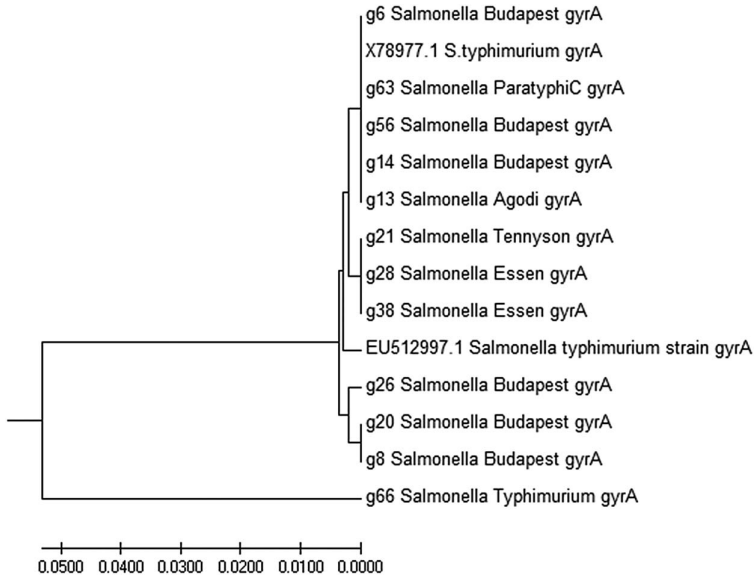

Figure 4. *gyrA* phylogenetic tree constructed based on an alignment of *gyrA* sequences of 12 isolates from human, chicken, cattle, and sheep with *gyrA* gene of *Salmonella* Typhimurium (EU512997 and X78977) from GenBank

human outbreak in Nigeria, a 2016 *Salmonella* annual report of the Centers for Disease Control and Prevention USA reported *S.* Budapest isolated from at least one patient with enteric fever in the USA between 2003 and 2013 [34]. NTS serovars accounted for majority of *S.* serovars from all three food animals

(cattle, sheep, and chicken). NTS serovars have a vast host range including ruminants and birds with their products (eggs, meat, and milk) acting as vehicle for the transmission of *Salmonella* infections to humans and have been implicated in bacterial bloodstream infections in children and adults in sub-Saharan Africa [35–37]. Rare serovars were present in chicken, which included *S*. Alfort, *S*. Wichita, *S*. Linton, *S*. Ealing, and *S*. Ebrie. In a similar study, Smith et al. [6] reported the occurrence of rare serovars including *S*. Amoutive, *S*. Ealing, *S*. Urbana, *S*. Bargny, *S*. Drac, and *S*. Nyborg in beef, chicken, goat, and pork sold in different markets in Lagos Nigeria. Food animals remain a major source of diverse serovars of *Salmonella*. This study reports the first isolation of rare serovars *S*. Mowanjum, *S*. Huettwillen, *S*. Limete, and *S*. Chagoua from apparently healthy humans in Nigeria. Although these serovars have been isolated from animals, animal products, and animal feeds in Denmark and Thailand, respectively [38, 39]. This finding is of public health relevance because these individuals who are food handlers are likely to disseminate the bacteria, since *Salmonella* has the tendency of persisting in macrophages and other immune cells coupled with chronic carrier status, which entails excreting the bacteria for some time that may be the case in this study [40]. No same serovar was recovered from human and animal sources, which indicates the difficulty in establishing the route of transmission of *Salmonella* serovars within the food chain. However, one isolate of *S*. Budapest was isolated from cattle and isolates of *S*. Essen were recovered from both chicken and sheep. *Salmonella* serovars displayed varied antibiotic susceptibility pattern with 16.9% resistance to nalidixic acid and 11.3% resistance to ciprofloxacin and norfloxacin. Most of these resistances to tested quinolone and fluoroquinolones were observed in isolates from chicken and sheep, which corroborates the findings of Raufu et al. [41] who reported a high-level resistance of *Salmonella* isolates of poultry origin to ciprofloxacin and nalidixic acid in the northern–eastern region of Nigeria. Zhu et al. [42] also reported a very high rate of resistance to nalidixic acid (99.5%) and ciprofloxacin (43%) in *Salmonella* isolates of poultry origin in China. This indicates a high use of quinolones in both human and veterinary medicine. Some isolates phenotypically exhibited resistance to some of the test antibiotics with no concomitant detection of resistance genes responsible for such resistance. This was observed in both human and animal isolates, for example *S*. Mowanjum, *S*. Portland, and *S*. Typhimurium were resistant to ampicillin and cephalotin yet no beta-lactamase (*bla*) genes were detected. In addition, *S*. Wien isolated from sheep was resistant to tetracycline but no *tetA* nor *tetB* genes were detected. Conversely, *S*. Chagoua, *S*. Essen, and *S*. Wien possessed the resistance genes but resistance phenotype was not observed. Similar observation was made by Afzal et al. [43] who reported *S*. Typhi isolates that were resistant to ampicillin and tetracycline with no detected corresponding

resistance genes. In the opinion of previous study of Adesiji et al. [44], such phenomenon may be due to non-expression of the genes responsible referred to as silent gene. *qnr* genes that indicates plasmid-mediated resistance of quinolones and fluoroquinolones were detected. Among antibiotic-resistant serovars, 43% were positive for *qnrB* gene with only one *S*. Dabou having *qnrS* in addition to *qnrB*; however, this isolate did not exhibit any phenotypic resistance to nalidixic acid nor ciprofloxacin. In a previous study, Fashae and Hendriksen [45] reported the detection of *qnrB19* in *S*. Corvallis and *qnrS1* in *S*. Derby isolated from pig farms in Ibadan, Nigeria. In this study, presumed mutations in *gyrA* gene would have resulted in ciprofloxacin resistance exhibited by serovars in which qnr genes were not detected, since mutation in *gyrA* gene resulting in ciprofloxacin resistance is widespread in Africa [46]. According to Baker et al. [47], selective and sustained pressure is the driving force for the evolution of antimicrobial resistance. Evolutionary analysis of *gyrA* genes in this study showed that *gyrA* gene in *S. enterica* from both human and food animals exhibits variations but are evolutionarily interconnected. Three major clades and a single largely diverged lineage were observed from the phylogenetic relationship analysis of isolates from both human and food animals using sequences of *gyrA* genes. The major clade had five *Salmonella* serovars from human (*S*. Paratyphi C) and chicken (*S*. Budapest and *S*. Agodi) clustering with X789771.1 *S*. Typhimurium serovar from the GenBank (NCBI), which was isolated from infected human subjects in the United Kingdom with a presumed zoonotic source: including chicken, turkey, eggs litter and imported exotic birds [48]. The second clade comprised *S*. Essen (g28) from chicken, *S*. Essen (g38) from sheep, and *S*. Tennyson (g21) from chicken, which was close to reference strain EU512997.1 *S*. Typhimurium from the GenBank, which was isolated from patients in Korea. Hence, a clonal expansion has been indicated by Chattaway et al. [49] who reported the difficulty in discerning clonal origin and distinguishing clonal expansion from evolutionary convergence of clonal lineages of fluoroquinolone-resistant *S*. Typhimurium DT104 and *S*. Kentucky. In this study, all bla_{TEM} genes detected were from isolates of sheep origin and belong to the TEM-144, TEM-135, and TEM-4 variants. This points to the fact that contaminated mutton could also be a potential source of beta-lactamase genes. Little or no attention has been paid to this food animal in terms of being a reservoir of antibiotic-resistant *Salmonella* serovars even when largely consumed in Nigeria. In this study, there was no resistance to third and fourth generation cephalosporins; however, utmost caution should be observed since dependency on this group of antibiotics in treatment most especially of children is high [37]. Thus, the need for prudent use of these drugs in both veterinary and human medicine is advocated to prevent the development of resistance.

Food-borne *Salmonella* serovars remains a public health risk; hence, there is a need for continuous antimicrobial resistance surveillance and monitoring in food animals and humans. Antibiotic surveillance systems is generally lacking in Africa. Thus, the results from this study can serve as a template for broader and further studies to provide a comprehensive data that can be used for formulating policies for antibiotics regulation in human and animal medicine to prevent and control outbreaks of multidrug resistance in food animals and humans.

Acknowledgements

To the authors would like to express their profound gratitude to the International Center for Genetic Engineering and Biotechnology (ICGEB) for supporting this study through ICGEB-SMART Fellowship (Fellowship number: S/NGA 16-02), National Food Institute (DTU Food) Denmark for providing all the reference strains used as positive controls, Prof. M. Dosso of Institute Pasteur Cote d'Ivoire for providing a research space for this work, and Mr. Perri Ehue and Mr. A. Sylla for their contributions to the success of this work.

Ethical approval

Ethical approval for this study was obtained from the Human Research and Ethical Committee (HREC) of the Lagos University Teaching Hospital with code number ADM/DCST/HREC/APP/1118 and Nigerian Institute of Medical Research Institutional Review Board, with project number IRB/12/180.

Conflict of Interest

The authors declare no conflict interest.

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