Antimicrobial resistance profiles of *Salmonella* serovars isolated from dressed chicken meat at slaughter in Kaduna, Nigeria

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Summary

Invasive non-typhoidal salmonellosis characterized by gastroenteritis and bacteremia is endemic in sub-Sahara Africa. Most infections are foodborne with animals serving as asymptomatic carriers. We investigated *Salmonella* serovars and associated resistance genes in chicken meat using culture, minimum inhibitory concentrations and PCR amplification of resistance genes. Of 100 samples examined, 28 (28%) were *Salmonella* positive and spread across six serovars: Haifa (71.4%), Chomedey (7.1%), Saintpaul (7.1%), Kainji (7.1%), Derby (3.6%), and Blockley (3.6%). Antimicrobial resistance was observed to ciprofloxacin (85.7%), nalidixic acid (75.0%), sulfamethoxazole (67.8%), tetracycline (89.3%), trimethoprim (42.9%), gentamicin (35.7%), streptomycin (32.1%), ampicillin (10.7%), chloramphenicol (10.7%), kanamycin (7.1%) and florfenicol (3.6%). All isolates were susceptible to cefotaxime, ceftazidime and colistin, whereas 19 (67.9%) showed multidrug resistance to at least three antimicrobials. The predominant resistance type was Cip-Gen-Nal-Smx-Tet-Tmp detected in six (21%) isolates. Multidrug resistance of *Salmonella* serovars was high in the sampled chicken meat with resistance most observed against ciprofloxacin. This suggests possible horizontal transfer of plasmid-mediated quinolone resistance genes, which may compromise the clinical use of fluoroquinolones. Thus, improved hygiene and provision of adequate facilities at meat processing centers could help limit meat contamination and foodborne transmission of multi-resistant non-typhoidal *Salmonella* serovars from chickens to humans.

■ INTRODUCTION

Salmonella is an enteric bacterial pathogen of the family Enterobacteriaceae and has the potential to invade tissue and cause bacteremia (de Jong et al., 2012). Globally, *Salmonella* serovars are among the leading cause of foodborne gastroenteritis and constitute health and socioeconomic burdens in both developed and developing countries. Non-typhoidal *Salmonella enterica* causes an annual estimate of 93.8 million cases of gastroenteritis and 155,000 deaths worldwide, with over 85% of *Salmonella* gastroenteritis attributed to foodborne transmission (Majowicz et al., 2010). Food-producing animals have been implicated as major reservoirs and sources of zoonotic transmission of non-typhoidal salmonellosis (Kagambèga et al., 2013). In particular, poultry and poultry products play significant roles in the transmission of salmonellosis to humans. Apparently, healthy chickens may harbor non-typhoidal zoonotic *S. enterica* serovars in their gastrointestinal tracts, which are transmitted to humans through contact with carrier birds, feces-contaminated environment and consumption of contaminated food and water (Kagambèga et al., 2011; 2013).

In developing countries, cases of salmonellosis are often misdiagnosed because of inadequate laboratory investigations (Feasey et al., 2012). Moreover, unhygienic practices in food processing and in distribution chains, low-level awareness of non-typhoidal salmonellosis, malnutrition and concurrent infections may contribute to spread and severity of

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foodborne infections. Self-medication, delayed or lack of access to good health care, inappropriate prescriptions and prevailing cultural practices are additional factors that may contribute to complications, widespread dissemination, increased morbidity and case fatality of *Salmonella* gastroenteritis (Morpeth et al., 2009). Generally, the incidence of foodborne infection in developing countries is grossly underestimated because of poor record keeping, inadequate disease reporting and lack of coordinated monitoring and surveillance of important foodborne pathogens.

The increasing incidence of antimicrobial resistance by pathogenic and commensal bacteria is of public health concern. In sub-Saharan Africa, antimicrobial chemotherapy is an integral part of the management of invasive non-typhoidal *Salmonella* infections (Feasey et al., 2012). However, widespread acquisition and dissemination of antimicrobial resistance traits are continuously narrowing the antimicrobial therapeutic options available for the treatment of bacterial infections. Antimicrobial-resistant *Salmonella* spp. have been isolated from different foods of animal origin around the world (Garedew et al., 2015; Löfström et al., 2015; Saravanan et al., 2015). Extensive use of antimicrobial agents in animal production is common and selects for multidrug resistant (MDR) pathogens (Marshall and Levy, 2011; Oluwasile et al., 2014). Fluoroquinolones are broad-spectrum antimicrobials effective in the treatment of a wide variety of clinical and veterinary infections, particularly for life-threatening salmonellosis treatment.

Quinolone resistance in *Salmonella* is mainly attributed to point mutations in chromosomal genes encoding gyrase subunits A (*gyrA*) and B (*gyrB*), and topoisomerase subunits C (*parC*) and E (*parE*) (Hopkins et al., 2005). The fluoroquinolone resistance phenotype might be further modulated with the appearance of several plasmid-mediated quinolone resistance mechanisms (PMQR) (Luo et al., 2011; Wasyl et al., 2014) encoded by *qnrA*, *qnrB*, *qnrS*, *qepA* genes.

Factors responsible for the endemicity of non-typhoidal *Salmonella* infections in Africa are poorly understood (Morpeth et al., 2009). There is a dearth of information on the dynamics of *Salmonella* serovars and their antimicrobial resistance properties in livestock and food of animal origin. To prevent the continuous spread of antimicrobial resistance and zoonotic transmission of foodborne pathogens from animals to humans, there is a need for regular monitoring and reporting of resistant bacteria in food of animal origin. The present study investigated the occurrence of *Salmonella* serovars as well as their phenotypic and genotypic antimicrobial resistance properties in freshly dressed chicken meat in Kaduna in Northern Nigeria.

■ MATERIALS AND METHODS

Sample collection

One hundred neck skin samples were collected from freshly dressed chicken meat sold to consumers at two chicken processing facilities in Kaduna, capital of Kaduna State, Nigeria. Neck skin sampling was selected in this study since it provides higher *Salmonella* yields than whole carcass rinse and other skin parts (Diezhang et al., 2014). Fifty samples were collected from each facility, and sampling was carried out three times daily (morning, afternoon and evening) for two weeks in March 2012. In each center, one and sometimes two samples per visit were collected from different processors selling dressed chickens. Samples were collected in pre-labeled sample bags and preserved with ice pack in sterile containers before transport to the laboratory for microbiological analysis.

Isolation and presumptive identification of **Salmonella**

Salmonella isolation was performed according to ISO 6579 standard (2002) with minor modifications. In particular, each neck sample (10 g) was pre-enriched after thorough homogenization in 90 ml of sterile buffered peptone water (BPW, Oxoid CM0509, UK), then incubated at 37°C for 18–24 hours. From the BPW culture, 0.1 ml was transferred into 9.9 ml of modified semi-solid Rapport-Vassiliadis (MSRV) selective enrichment broth (Oxoid CM0910) supplemented with novobiocin (Oxoid SR0161), then incubated at 42°C for 24–48 hours. Afterwards, MSRV was observed for the typical migration pattern of *Salmonella enterica* (≥ 2.0 mm migration) every 24 hours until 72 hours. A loopful of observable bacterial spread was taken from the periphery of MSRV medium and streaked simultaneously onto plates of brilliant green agar (BGA, Oxoid CM0263) supplemented with sulphamandelate (Oxoid SR0087) and onto xylose lysine desoxycholate agar (XLD, Oxoid). The inoculated plates were incubated at 37°C for 18–24 hours, then examined for bacterial colonies. Typical *Salmonella* colonies were picked and subjected to biochemical and serological tests.

Serotyping of **Salmonella** *isolates*

Presumptive *Salmonella* isolates were shipped to the Office International des Epizooties (OIE) Reference Laboratory for *Salmonella*, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, Italy). All strains were serotyped by agglutination tests with specific O and H antisera and classified according to Kauffman-White's scheme as previously described (Grimont and Weill, 2007).

Phenotypic antimicrobial susceptibility testing

Susceptibility to 14 antimicrobials was carried out to determine the minimum inhibitory concentrations by means of broth microdilution following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The CMV2AGNF (sensititre, Trek Diagnostic Systems, USA) susceptibility plates of the National Antimicrobial Monitoring System containing 14 antimicrobials was used. The antimicrobials tested were ampicillin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, florfenicol, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim. The breakpoints used for sulfamethoxazole and kanamycin were CLSI (2009) breakpoints, whereas for other antimicrobials, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off was used (Aarestrup and McDermott, 2007; EUCAST, 2012). *Escherichia coli* (ATCC 25922) was used as the quality control strain.

Determination of antimicrobial resistance genes

DNA templates used for PCR were prepared by boiling as described by Barco et al. (2011). Molecular characterization of genetic antimicrobial resistance determinants in all isolates was undertaken by PCR amplification according to Beutlich et al. (2011). The isolates were analyzed for the presence of resistance genes corresponding to their resistance phenotypes. The resistance-associated genes investigated included those encoding resistance to beta-lactam antibiotics (bla_{PSE1}, $bla_{TEM1-like}$, $bla_{OXA1-like}$), chloramphenicol and florfenicol (*catA1*, *cmlA*, *floR*), fluoroquinolones (*gyrA*, *qnrA*, *qnrB*, *qnrS*, *qepA*), gentamicin [*aacC2*, *aacC4*, *aac*(3)-Ie (*aacC5*), *aadB*, *armA*], kanamycin [*aphA1*, *aphA2*, *aac*(6)-1b], streptomycin (*aadA1*-like, *strA*, *strB*), sulfamethoxazole (*sul1*, *sul2*, *sul3*), tetracycline [*tet*(A), *tet*(B), *tet*(G)], and trimethoprim (*dfrA1*-like, *dfrA5*-14, *dfrA7*-17, *dfrA12*). In particular, PCR amplification of *gyrA* gene and sequencing of purified amplicons were performed on both nucleotide strands to detect substitutions using ABI 100 DNA sequencer (Applied Biosystems, USA) as previously reported (Capoor et al., 2009). In each case, the *gyrA* sequence obtained was compared with wild-type sequences (GenBank accession number AE006468.1).

■ RESULTS

Prevalence of **Salmonella** *serovars in chicken meat*

Culture and serology confirmed almost one third (28%, 28/100) of the samples as *Salmonella* positive; they were spread across six serovars:

tYpage moléculaire de Salmonella dans la viande de poulet

S. Haifa, *S*. Chomedey, *S*. Saintpaul, *S*. Derby, *S*. Blockley and *S*. Kainji (Table I).

Phenotypic antimicrobial resistance of S. isolates

The highest antimicrobial resistance was to ciprofloxacin (85.7%, 24/28), followed by nalidixic acid (75%), sulfamethoxazole (67.8%) and tetracycline (89.3%) (Table I). About half of *Salmonella* isolates showed resistance to trimethoprim, gentamicin and streptomycin, whereas small numbers of isolates were resistant to ampicillin, chloramphenicol, kanamycin and florfenicol. All isolates were susceptible to cefotaxime, ceftazidime and colistin, whereas the two representatives of *S*. Kainji were susceptible to all tested antimicrobial agents (Table I). Of the 26 resistant isolates, nineteen (67.9%) showed multidrug resistance to at least three different classes of antimicrobials. The most prevalent resistance type (R-type) was Cip-Gen-Nal-Smx-Tet-Tmp observed in six (21%) isolates, followed by Cip-Nal-Tet in four (14%) isolates (Table II).

Antimicrobial resistance genes in Salmonella isolates

The *bla*$_{TEM}$ gene coding for beta-lactam resistance was detected in all three ampicillin-resistant isolates (Table I). The *floR* gene encoded resistance to chloramphenicol and florfenicol in one isolate. Two chloramphenicol-resistant isolates carried *cmlA* gene. Streptomycin was mainly encoded by *strB* (6 isolates) and/or *strA* (1 isolate) genes except for two isolates that showed only *aad1*-like gene. Gentamicin resistance was mediated by *aacC2* (7/10 resistant isolates) and *acc(3)-le* (3/10

resistant strains) genes. Sulfamethoxazole resistance was mostly associated with *sul1* (15/19), however, *sul2* (2/19) and *sul3* (2/19) were also detected. Tetracycline resistance was uniquely encoded by *tet*(A) in 25 isolates. Trimethoprim resistance was uniquely encoded by *dfrA5-14* (12 isolates). Substitutions in the codons of *gyrA* were responsible for fluoroquinolone resistance in 21 isolates. Sequence analysis revealed mutation of amino acid at position 83 (TCC→TAC, Ser83→Tyr) in 17 isolates and (TCC→TTC, Ser83→Phe) in four isolates. Three fluoroquinolone-resistant isolates with similar R-type (Cip-Gen-Nal-Smx-Str-Tet-Tmp) had double point mutations in their *gyrA* gene. The first mutation occurred at position 83 (TCC→TTC, Ser83→Phe), the second at position 87 (GAC→GGC, Asp87→Gly). Three (10.7%) of the *Salmonella* isolates carried a *qnr* gene. These isolates were characterized by a reduced level of fluoroquinolone resistance (0.5–1 mg/l) and no simultaneous chromosomal mutations occurred in gyrase or topoisomerase. Two isolates (*S*. Chomedey) carried *qnrB* gene and one isolate (*S*. Derby) *qnrS* gene.

■ DISCUSSION

The results showed high contamination of poultry meat during processing with potentially zoonotic non-typhoidal *Salmonella* serovars. The serovars detected have been associated throughout the world with foodborne infections in humans. For instance, Kaibu et al. (2005) report fatal cases of food intoxication by *Salmonella* Haifa in an older person and in a child. *S*. Derby was reported in an outbreak of foodborne

Table I

Distribution of resistant isolates and resistance genes among *Salmonella* serovars isolated from fresh chicken meat in Kaduna, Nigeria

Antimicrobial agent	Resistance gene	*Salmonella* serovars[*]					Total (n = 28)	% (95% CI)
		S. Haifa (n = 20)	S. Derby (n = 1)	S. Chomedey (n = 2)	S. Blockley (n = 1)	S. Saintpaul (n = 2)		
Ampicillin	*bla*$_{TEM}$	1	–	–	–	2	3	10.7 (2.9–28.0)
Cefotaxime	–	–	–	–	–	–	–	–
Ceftazidime	–	–	–	–	–	–	–	–
Chloramphenicol	*cmlA1*	–	–	–	–	2	2	7.1 (0.9–23.7)
Chloramphenicol/ florfenicol	*floR*	1	–	–	–		1	3.6 (0.0–19.2)
Colistin	–	–	–	–	–	–	–	–
Fluoroquinolone (ciprofloxacin)	*gyrA* mutation	20	–	–	1	–	21	75 (56.4–87.6)
Fluoroquinolone (ciprofloxacin)	*qnrB*	–	–	2	–	–	2	7.1 (0.9–23.7)
Fluoroquinolone (ciprofloxacin)	*qnrS*	–	1	–	–	–	1	3.6 (0.0–19.2)
Gentamicin	*aacC2*	7	–	–	–	–	7	25 (12.4–43.6)
Gentamicin	*aac(3)-le*	3	–	–	–	–	3	10.7 (2.9–28.0)
Kanamycin	*aphA1*	–	–	–	1	1	2	7.1 (0.9–23.7)
Nalidixic acid	–	–	20	–	–	1	21	75 (56.4-87.6)
Streptomycin	*strA*	1	–	–	–	–	1	3.6 (0.0–19.2)
Streptomycin	*strB*	3	–	2	1	–	6	21.4 (9.9–39.9)
Streptomycin	*aad-1like*	–	–	–	–	2	2	7.1 (0.9–23.7)
Sulfamethoxazole	*sul1*	15	–	–	–	–	15	53.6 (35.8–70.5)
Sulfamethoxazole	*sul2*	–	–	2	–	–	2	7.1 (0.9–23.7)
Sulfamethoxazole	*sul3*	–	–	–	–	2	2	7.1 (0.9–23.7)
Tetracycline	*tet*(A)	19	1	2	1	2	25	89.3 (72.0–97.1)
Trimethoprim	*dfrA5-14*	12	–	–	–	–	12	42.9 (26.5–61.0)

[*] No resistant isolates were found for *S*. Kainji (n = 2)

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Table II

Phenotypic and genotypic resistance patterns of *Salmonella* serovars isolated
from fresh chicken meat in Kaduna, Nigeria

Serial Num.	Serovar	Phenotypic resistance pattern	Resistance genes (mutations)
1	*S.* Haifa	CIP-NAL	*gyrA* (Ser83→Tyr)
2	*S.* Derby	CIP-TET	*qnrS, tet*(A)
3	*S.* Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
4	*S.* Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
5	*S.* Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
6	*S.* Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
7	*S.* Chomedey	CIP-SMX-STR-TET	*qnrB, sul2, strB, tet*(A)
8	*S.* Chomedey	CIP-SMX-STR-TET	*qnrB, sul2, strB, tet*(A)
9	*S.* Blockley	CIP-KAN-NAL-STR-TET	*gyrA* (Ser83→phe), *aphA1, strA, strB, tet*(A)
10	*S.* Haifa	CIP-NAL-SMX-TET	*gyrA* (Ser83→Tyr), *sul1, tet*(A)
11	*S.* Haifa	CIP-NAL-SMX-TET	*gyrA* (Ser83→Tyr), *sul1, tet*(A)
12	*S.* Haifa	CIP-NAL-SMX-TET	*gyrA* (Ser83→Tyr), *sul1, tet*(A)
13	*S.* Haifa	CIP-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *sul1, tet*(A), *dfrA5-14*
14	*S.* Saintpaul	AMP-CHL-FFN-SMX-STR-TET	*bla*$_{TEM,}$ *floR, sul3, aad1-like, tet*(A)
15	*S.* Saintpaul	AMP-CHL-KAN- SMX-STR-TET	*bla*$_{TEM,}$ *cmlA, aphA1, sul3, aad1-like, tet*(A)
16	*S.* Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
17	*S.* Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
18	*S.* Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
19	*S.* Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
20	*S.* Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
21	*S.* Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
22	*S.* Haifa	AMP-CIP-GEN-NAL-SMX-TET-TMP	*bla*$_{TEM,}$ *gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
23	*S.* Haifa	CIP-CHL-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→Tyr), *cmlA, aad1-like, sul1, tet*(A), *dfrA5-14*
24	*S.* Haifa	CIP-GEN-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→phe, Asp87→Gly), *aac(3)Ie, sul1, strB, tet*(A), *dfrA5-14*
25	*S.* Haifa	CIP-GEN-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→phe, Asp87→Gly), *aac(3)Ie, sul1, strB, tet*(A), *dfrA5-14*
26	*S.* Haifa	CIP-GEN-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→phe, Asp87→Gly), *aac(3)Ie, sul1, strA, strB, tet*(A), *dfrA5-14*

AMP: ampicillin; CHL: chloramphenicol; CIP: ciprofloxacin; FFN: florfenicol; GEN: gentamicin; KAN: kanamycin; NAL: nalidixic acid; STR: streptomycin; SMX: sulfa-methoxazole; TET: tetracycline; TMP: trimethoprim

Florfenicol: *floR*; fluoroquinolones: *gyrA, qnrB, qnrS*; gentamicin: *aacC2, aac*(3)-Ie; kanamycin: *aphA1*; streptomycin: *aadA1*-like, *strA, strB*; sulfamethoxazole: *sul1*, *sul2*, *sul3*; tetracycline: *tet*(A); trimethoprim: *dfrA5-14*

salmonellosis following the consumption of grilled meat in Fukuka City in Japan (Ebuchi et al., 2006). *S*. Saintpaul was responsible for a large foodborne outbreak of salmonellosis involving 1500 cases with 21% hospitalizations and two deaths in the United States (Barton et al., 2011). Gonose et al. (2012) reported *S*. Blockley as the etiology of food-borne illness characterized by diarrhea, stomach cramp and headaches in three adult males in South Africa.

The 28% overall prevalence rate of *Salmonella* observed in dressed chicken in our study was higher than those of 11% reported in the feces of on-farm chickens in Ibadan (Fashae et al., 2010), and of 2.3–5.2% in feces and intestinal samples of chickens in Maiduguri, Nigeria (Raufu et al., 2009). Unhygienic processing including lack of proper separa-tion of dirty and clean areas during evisceration in the slaughterhouse, unhygienic processors and equipment contributed to the contamination of carcasses during processing. In addition, the extent of colonization of the crop due to feed withdrawal during transportation and resting exposes carcasses to possible contamination (Ramirez et al., 1997). *Salmonella* contamination of the neck skin has been linked to coloni-zation of the crop since this organ is more likely to be ruptured during processing than other parts of the gastrointestinal tract such as the ceca (Hargis et al., 1995). Finally, successful contamination of carcasses and slaughter lines may also depend on the toughness of the contaminating *Salmonella* strain, considering that such strains survive environmental

stress usually associated with slaughterhouses (Olsen et al., 2003; Rasschaert et al., 2007).

S. Haifa was the predominant (71.4%) serovar detected in the present study. Five other serovars were also detected suggesting that chic-ken meat may act as a vehicle for the transmission of diverse ranges of *Salmonella* serovars. Fashae et al. (2010) detected eight *S*. serovars (Virchow, Bredeney, Derby, Haifa, Havana, Muenster, Mbandaka and Onireke) in the feces of on-farm chickens in Ibadan, and *S*. Virchow accounted for 71% of all isolates. In Maiduguri, *S*. Hiduddify was the main (95%) serovar isolated from chicken feces and tissues (Raufu et al., 2009). Differences in geographical location, sampling site and type may account for the observed disparities betwen these studies. Although the *S*. serovars detected in this study could originate from the intestinal content of chickens, they may have originated from extraneous sources including the persons involved in processing chicken meat.

Fluoroquinolones, especially ciprofloxacin, are the drugs of choice in the treatment of human salmonellosis (Akinyemi et al., 2007) and they are also used frequently in livestock medicine. Resistance to ciprofloxa-cin was found in over 85% of all isolates. Most fluoroquinolone resis-tance (87.5%) was mediated by chromosomal mutations on *gyrA* gene. Resistance mutations occurring on *gyrA* gene, corresponding to amino acid changes at Ser-83 (to Phe or Tyr) or at Asp-87 (to Gly), are the most frequently observed in fluoroquinolone resistant strains (Piddock,

2002; Hopkins et al., 2005). In addition to these site mutations, there were occurrence and dissemination of *qnr* genes in *Salmonella* isolates in this study, even though they were quite low (l0.7%). This suggests horizontal spread of resistance plasmid determinants in Kaduna State as reported by Fortini et al. (2011). However, it is notable that wide dissemination of PMQR genes mainly in commensal or pathogenic Gram-negative bacteria is mostly associated with defined successful plasmids which could be positively selected by other drugs because of the co-location of multiple resistance determinants (Fortini et al., 2011). In general, the acquisition of PMQR genes decreases susceptibility to fluoroquinolones and may accelerate selection of fluoroquinolone-resistant mutants (Martínez-Martínez et al., 2003; Rodríguez-Martínez et al., 2007). Given their transferability and the possibility that they may cause increases in resistance thereby affecting the clinical response to treatment, data on the occurrence of PMQR genes are important for the surveillance of quinolone resistance in humans and animals. In developing countries where poor facilities hamper accurate and timely diagnosis, antimicrobial agents are administered empirically for the treatment of suspected cases of salmonellosis without recourse to laboratory investigation. Resistance may complicate *Salmonella* infections as resistant strains are more likely to be associated with tissue invasion, severe illness and death (Helms et al., 2004).

In this study there was a high resistance to (fluoro)quinolones, sulfonamide and tetracycline, a moderate resistance to trimethoprim and aminoglycosides, and a low resistance to ampicillin and phenicol antimicrobials. This could be a reflection of the pattern of antimicrobial use in the poultry industry. Usage of any particular antimicrobial agent could exert selective pressure leading to the emergence, proliferation and spread of bacterium strains showing resistance to the antimicrobial agent. Previous studies have shown that fluoroquinolones, sulfonamides and tetracyclines are the most widely used antimicrobial agents in the Nigerian poultry industry (Ogunleye et al., 2008; Oluwasile et al., 2014). In our study resistance to tetracycline was found in five out of six serovars and was uniquely associated with *tet*(A) gene. Tetracycline is used as an additive in water and feed to improve performance, whereas ciprofloxacin is the ready choice for the treatment of bacterial infections and sometimes for prophylaxis (Oluwasile et al., 2014).

Although three serovar types (Haifa, Saintpaul and Chomedey) showed resistance to sulfamethoxazole, the resistance was mediated by different genes. The *sul1* gene was responsible for resistance in all isolates belonging to serovar Haifa. In *S.* Chomedey, sulfamethoxazole resistance was due to *sul2*, whereas in *S.* Saintpaul it was due to *sul3*. Two isolates of *S.* Saintpaul possessed *cmlA*, which accounted for resistance to chloramphenicol, whereas in *S.* Haifa resistance to chloramphenicol and its derivative (florfenicol) was mediated by *floR*. All gentamicin-resistant *Salmonella* isolates belonged to serovar Haifa and resistance was attributable mainly to *aacC2* (seven isolates) but also to *aac(3)-Ie* (three isolates). Similarly, trimethoprim resistance was observed only in *S.* Haifa and was encoded solely by *dfrA5-14* gene. Two *S.* Saintpaul isolates and

one *S.* Haifa showed resistance to ampicillin and possessed *bla*$_{TEM}$ gene. Resistance to streptomycin was detected in nine isolates belonging to four different serovars. Three streptomycin-associated genes were detected: *strA* and *strB* in *S.* Haifa, *strB* in *S.* Chomedey and Blockley, and *aadA-like* in *S.* Saintpaul. The *Salmonella* isolates were completely susceptible to cefotaxime, ceftazidime and colistin. These drugs are not known to be used in poultry production. Susceptibility to cefotaxime and ceftazidime indicated that cephalosporins including highly important third and fourth generation antimicrobials will be very effective in the treatment of life-threatening and complicated salmonelloses in humans.

These findings are subject to at least two limitations. Firstly, these data were mainly collected from chickens originating from Kaduna State and other parts of Northern Nigeria which produce live chickens, and secondly, the relatively small sample size, which depended on budgetary constraints since whole chickens had to be purchased to allow for sampling. Notwithstanding these limitations, this study offers some insight into the circulating non-typhoidal *Salmonella* serovars and their antibiogram.

■ CONCLUSION

The study showed that chicken meat may contribute to the transmission of non-typhoidal *Salmonella* serovars in the area. In addition, the *Salmonella* observed might have been attributed to unhygienic handling during processing. This included observed poor personal hygiene among processors during processing as well as poor slaughterhouse sanitation due to inadequate infrastructure and unclean running water. Improved hygiene, provision of slaughter facilities, as well as adequate inspection at meat processing centers may limit meat contamination and reduce the risk of zoonotic transmission of non-typhoidal salmonellosis to humans. Furthermore, the study revealed circulation of *S.* serovars that are predominantly multidrug resistant. Preventive measures against misuse of antibiotics, including strict monitoring of antibiotics use both for prophylaxis and growth stimulation in poultry farms will mitigate antibiotic resistance development.

Author contributions statement

MA, AAL, OEO, MAD designed and planned the study; MA, AL, EM, KA, PZ, BBO, EOO performed experiments and collected data; MA, AAL, OEO analyzed and interpreted data; MA, AAL, OEO, BBO, AL, EM, KA, PZ, EOO drafted the first version of the manuscript; MA, AL, OEO, MAD critically reviewed the manuscript.

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Résumé

Agbaje M., Lettini A.A., Ojo O.E., Longo A., Marafin E., Antonello K., Zavagnin P., Oluwasile B.B., Omoshaba E.O., Dipeolu M.A. Profils de résistance antimicrobienne de sérotypes de salmonelles isolés à partir de viande de poulet apprêtée à l'abattoir à Kaduna, Nigeria

La salmonellose invasive non typhique, caractérisée par une gastro-entérite et une bactériémie, est endémique en Afrique subsaharienne. La plupart des infections sont d'origine alimentaire, les animaux servant de porteurs asymptomatiques. Nous

Resumen

Agbaje M., Lettini A.A., Ojo O.E., Longo A., Marafin E., Antonello K., Zavagnin P., Oluwasile B.B., Omoshaba E.O., Dipeolu M.A. Perfiles de resistencia antimicrobiana a serovares de *Salmonella* aislados de carne de pollo en mataderos de Kaduna, Nigeria

La salmonelosis invasiva no tifoidea, caracterizada por gastroenteritis y bacteriemia es endémica en África subsahariana. La mayoría de las infecciones son de origen alimenticio, con animales actuando como portadores asintomáticos. Investigamos

avons étudié les sérotypes de *Salmonella* et les gènes de résistance associés dans la viande de poulet à l'aide de cultures, de concentrations minimales inhibitrices et de l'amplification par PCR des gènes de résistance. Sur 100 échantillons examinés, 28 (28 %) étaient positifs pour *Salmonella* et répartis dans six sérotypes : Haïfa (71,4 %), Chomedey (7,1 %), Saintpaul (7,1 %), Kainji (7,1 %), Derby (3,6 %) et Blockley (3,6 %). Une résistance antimicrobienne à la ciprofloxacine (85,7 %), à l'acide nalidixique (75,0 %), au sulfaméthoxazole (67,8 %), à la tétracycline (89,3 %), au triméthoprime (42,9 %), à la gentamicine (35,7 %), à la streptomycine (32,1 %), à l'ampicilline (10,7 %), au chloramphenicol (10,7 %), à la kanamycine (7,1 %) et au florfenicol (3,6 %) a été observée. Tous les isolats ont été sensibles à la céfotaxime, à la ceftazidime et à la colistine ; 19 (67,9 %) ont présenté une multirésistance à au moins trois antimicrobiens. Le type de résistance prédominant était Cip-Gen-Nal-Smx-Tet-Tmp, détecté dans six isolats (21%). La multirésistance des sérovars de *Salmonella* était élevée dans la viande de poulet échantillonnée, et la résistance la plus souvent observée était contre la ciprofloxacine. Cela suggère la possibilité d'un transfert horizontal des gènes de résistance à la quinolone à médiation plasmidique, ce qui pourrait compromettre l'utilisation clinique des fluoroquinolones. Ainsi, l'amélioration de l'hygiène et la mise en place d'installations adaptées dans les centres de transformation de la viande pourraient contribuer à limiter la contamination de la viande et la transmission aux humains des sérotypes multirésistants non typhiques de *Salmonella* par la viande de poulets.

Mots-clés : *Salmonella*, résistance aux antibiotiques, viande de poulet, abattage d'animaux, Nigeria

serovares de *Salmonella* y los genes de resistencia asociados en carne de pollo, mediante cultivo, concentraciones mínimas inhibitorias y amplificación de PCR de los genes de resistencia. De las 100 muestras examinadas, 28 (28%) fueron positivas a *Salmonella* y se distribuyeron en seis serovares: Haifa (71,4%), Chomedey (7,1%), Saintpaul (7,1%), Kainji (7,1%), Derby (3,6%), y Blockley (3,6%). La resistencia antimicrobiana se observó a ciprofloxacina (85,7%), ácido nalidíxico (75,0%), sulfametoxazol (67,8%), tetraciclina (89,3%), trimetoprima (42,9%), gentamicina (35,7%), estreptomicina (32,1%), ampicilina (10,7%), cloranfenicol (10,7%), kanamicina (7,1%) y florfenicol (3,6%). Todos los aislamientos fueron susceptibles a cefotaxima, ceftazidima y colistina, mientras que 19 (67,9%) mostraron resistencia múltiple a por lo menos tres antimicrobianos. El tipo de resistencia predominante fue Cip-Gen-Nal-Smx-Tet-Tmp, detectado en seis (21%) aislamientos. La resistencia múltiple a fármacos de serovares de *Salmonella* fue elevada en las muestras de carne de pollo, con una mayor resistencia observada a la ciprofloxacina. Esto sugiere una posible transmisión horizontal de genes resistentes a quinolonas mediados por plásmidos, lo que podría comprometer el uso clínico de fluoroquinolonas. Por lo tanto, la mejora de la higiene y la provisión de instalaciones adecuadas en los centros de procesamiento de carne podrían ayudar a limitar la contaminación de la carne y la transmisión a través de los alimentos de los serotipos multi resistentes de *Salmonella* no tifoidea de pollos a humanos.

Palabras clave: *Salmonella*, resistencia a los antibióticos, carne de pollo, sacrificio, Nigeria