Received: 9 February 2017

DOI: 10.1111/zph.12427

ORIGINAL ARTICLE

WILEY

Phenotypic and genotypic comparison of salmonellae from diarrhoeic and healthy humans and cattle, Nigeria

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Summary

The sources and modes of transmission of non-typhoidal *Salmonella* particularly zoonotic transmission are poorly understood in Africa. This study compared phenotypic and genotypic characteristics of Salmonellae isolated from cattle and humans. Faecal samples of diarrhoeic patients (n = 234), and a healthy population (n = 160), beef cattle at slaughter (n = 250), farms (n = 72) and market (n = 100) were cultured for salmonellae and serotyping and antimicrobial susceptibility were determined. Whole-genome sequence typing (WGST) of selected isolates and bioinformatic analysis were used to identify the multilocus sequence type (MLST), plasmid replicons, antimicrobial resistance genes and genetic relatedness by single nucleotide polymorphism (SNP) analysis. The *Salmonella* isolates, diarrhoeic patients (n = 17), healthy population (n = 13), cattle (abattoir, n = 67; farms, n = 10; market n = 5), revealed 49 serovars; some serovars were common to humans and cattle. Rare serovars were prevalent: Colindale (cattle and humans); Rubislaw and Bredeney (humans); and Dublin, Give, Eastbourne, Hadar, Marseille, Sundsvall, Bergen, Ekotedo, Carno and Ealing (cattle). The sequence types (ST) include ST 584, ST 198, ST 562 and ST 512 for *S*. Colindale, *S*. Kentucky *S*. Rubislaw and *S*. Urbana, respectively. Clonal cluster shared by cattle and human WGST isolates was not found. Antimicrobial resistance rates were generally low and towards only chloramphenicol, ampicillin, gentamicin, ciprofloxacin, tetracycline and streptomycin, range 2.7% (chloramphenicol) to 8.9% (streptomycin). Multiply resistant isolates included serovars Kentucky, 4,5,12:i:- and Typhimurium. The study presents a baseline description of the prevalence, serotypes, antimicrobial resistance phenotypes and genetic relatedness of *Salmonella* isolated from healthy and diarrhoeic humans, and cattle at harvest, on farm and at market. Cattle are a reservoir of diverse salmonellae with shared serovars with humans, but WGST does not support zoonotic transmission. Further study with larger samples is recommended to determine whether epidemiological link exists between cattle and humans.

KEYWORDS

cattle, gastroenteritis, human, multilocus sequence type, Nigeria, *Salmonella*, single nucleotide polymorphism

1 | INTRODUCTION

Salmonella infections generally present as self-limiting gastroenteritis. Non-typhoidal *Salmonella* is a major cause of gastroenteritis estimated at 93.8 million cases and 155,000 deaths globally (Majowicz et al., 2010). Of the over 2,500 *Salmonella* serovars, the few causing infections are dominated by *S*. Typhimurium (ST) and *S*. Enteritidis (SE) globally. There are sparse data on prevalent S*almonella* serovars associated with gastroenteritis in humans in sub-Saharan Africa, but ST and SE and *S*. Collindale have been reported prevalent in Kenya and Gambia (Dione, Ikumapayi, Saha, Mohammed, & Geerts, 2011; Kariuki et al., 2006; O'Reilly, Jaron, Ochieng, Nyaguara, & Tate, 2012). Reports on *Salmonella* serovars associated with gastroenteritis in Nigeria are few (Ifeanyi, Bassey, Ikeneche, & Al-Gallas, 2014; Raufu et al., 2013) and limited by small sample size and geographical location.

The routes of transmission of *Salmonella* include animals, water, foods, person-to-person contact and the environment. While in the developed countries, the majority of human *Salmonella* infection are foodborne (Jackson, Griffin, Cole, Walsh, & Chai, 2013; Majowicz et al., 2010), the relative importance of the transmission routes in Africa is poorly understood (Dione et al., 2011; Kariuki et al., 2006). Source attribution studies based on genomic subtyping have the potential to provide valuable information (Onsari & MacLennan, 2014) but are scarcely performed in Nigeria (Leekitcharoenphon et al., 2016); serotyping, which might not translate to genetic similarity, has been used (Raufu et al., 2013).

Food animals (like cattle) play a significant role in the epidemiology of *Salmonella*, because they act as reservoirs and excrete salmonellae in their faeces (Rodriguez-Rivera et al., 2014); therefore, the meat and meat products can be contaminated during slaughter and processing, posing health risks; infections can also result from cattle contact (Cummings et al., 2012; Leekitcharoenphon et al., 2016). Beef cattle are an important potential source of salmonellae in Nigeria as they are widely consumed. The potential for zoonotic transmission is further increased given limited capacity for sanitation and comingling of livestock with human population. Subtyping *Salmonella* remains key to identify sources of human infections for implementation of interventions and control measures. Next-generation sequencing technologies have made whole-genome sequencing (WGS) of foodborne bacterial pathogens a realistic and superior alternative to traditional subtyping methods; routine, real-time and widespread application of WGS in food safety and public health is now possible (Deng, den Bakker, & Hendriksen, 2016). WGS evaluation of salmonellae isolated from humans and cattle is therefore warranted to lay the foundation for being able to use WGS for source attribution in this part of the world.

Antimicrobial resistance has increased and is widespread, hence a public health concern (Crump, Sjölund-Karlsson, Gordon, & Parry, 2015) with indiscriminate use of antimicrobials being a factor (Collignon, Powers, Chiller, Aidara-Kane, & Aarestrup, 2009) and calls for continuous surveillance and control.

This study elucidated the prevalence, serotypes and antimicrobial resistance phenotypes of salmonellae and genetic relatedness (using WGS) of shared serovars isolated from healthy and diarrhoeic humans and cattle at harvest, cattle at market and on farms in Nigeria, focusing at establishing baseline data to eventually make trace-back possible.

Impacts

- There was some overlap between the *Salmonella* serotypes observed between humans and cattle.
- But the WGS data do not support an epidemiological link between the strains from humans and cattle.
- Further study is needed to determine whether an epidemiological link exists.

2 | MATERIALS AND METHODS

2.1 | Collection of faecal samples

Faecal samples were randomly collected from human cases of diarrhoea (n = 234), apparently healthy pregnant women attending an antenatal clinic at Adeoyo Hospital (n = 160) and beef cattle at slaughter (n = 250), cattle market (n = 100) and two cattle farms (n = 30; n = 42, respectively) (Figure 1). Faecal samples were collected between September 2011 and August 2013: cattle (2011–2012), pregnant women (2011) and diarrhoeic cases (April 2012 to October 2013). The cattle faecal samples were obtained from Bodija abattoir where most cattle were slaughtered and butchered for consumption by the population of Ibadan; the Akinyele cattle market was one of the main markets while the cattle farms were chosen based on accessibility. The human diarrhoeic samples were obtained from three hospitals (Adeoyo and Oni and Son General and University College Hospitals) and a Mokola Primary Health Centre 111 of the patients were females and 143 were less than 15 years old. Figure 1 (map) shows the hospitals, primary health centre, Bodija abattoir, cattle market and cattle farms where faecal samples were collected. Informed consent (written or verbal) of all human subjects was obtained; parents or guardians of children gave informed consent on behalf of the children. At slaughter, cattle faecal samples were collected into sterile bottles from the cattle rectum using a pair of gloves per animal by the veterinarians on duty at the abattoir, while the cattle faecal samples collected from the farms and market were randomly obtained from the floor.

2.2 | Isolation of *Salmonella*

Each 5 g of faecal sample was inoculated into 25 ml of selenite F broth (Lab M, Lancashire, UK) and incubated aerobically at 37°C for 24 hr (Fashae, Ogunsola, Aarestrup, & Hendriksen, 2010). Thereafter, a loopful of the broth was plated onto *Salmonella-Shigella* agar (SSA; Lab M) by streaking and the SSA plate was then incubated aerobically at 37°C for 24 hr. Then, presumptive *Salmonella* colonies from the SSA plate were re-subcultured by streaking onto fresh SSA plates

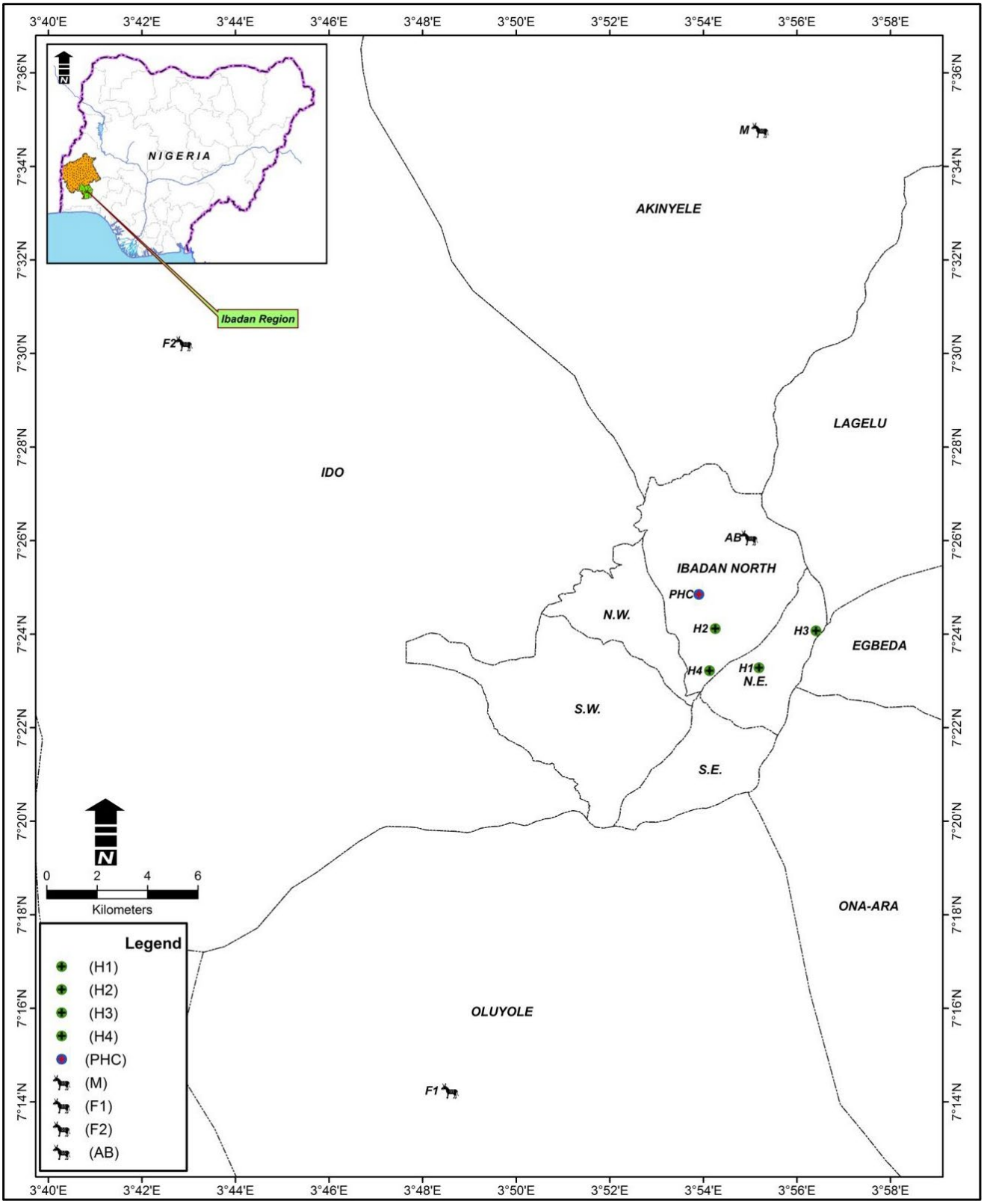

FIGURE 1 Hospitals (H1 to H4), public health centre (PHC), farms (F1 and F2), abattoir (AB), and cattle market (M) where faecal samples were collected. [Colour figure can be viewed at wileyonlinelibrary.com]

and incubated as above for purification. All presumptive *Salmonella* isolates were then identified by standard methods (Cowan & Steel, 1974); isolates that were Gram-negative rods, oxidative negative, glucose fermenters, non-lactose fermenters and hydrogen sulphide producers were subjected to serotyping for the identification of *Salmonella* serovars.

2.3 | Serotyping of *Salmonella* isolates

Only 85 of the 112 biochemically confirmed isolates were serotyped by slide agglutination using *Salmonella* polyvalent and monovalent O and H antisera (Diagnostic Pasteur, Paris, France) according to the Kauffmann–White classification scheme (Grimont & Weill, 2007). These included 40 of the cattle isolates and all others.

2.4 | Antimicrobial susceptibility testing of *Salmonella* isolates

The susceptibility of all the 112 *Salmonella* isolates to 12 antimicrobials was determined by disc diffusion method on Mueller-Hinton agar (Merck, Hamburg, Germany) plates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M02-A12, 2015a). The *Escherichia coli* ATCC 25922 was used as the quality control strain. The antimicrobial discs (Oxoid, Basingstoke, Hampshire, UK) tested were ampicillin (10 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), nalidixic acid (30 μg), streptomycin (10 μg), sulphonamides (300 μg), tetracycline (30 μg) and trimethoprim (5 μg). Diameters of zones inhibition were measured with a ruler and interpreted by the CLSI guidelines (CLSI M100-S25 2015b). *Salmonella* isolates with ciprofloxacin disc diffusion zones of inhibition in the intermediate range (<30 mm) were interpreted as showing reduced susceptibility to ciprofloxacin (CLSI M100-S25b).

2.5 | Whole-genome sequencing

Genomes of the following isolates of *S*. Colindale (*n* = 7), *S*. Rubislaw (*n* = 3), *S*. Urbana (*n* = 2), *S*. Agama (*n* = 2) and *S*. Kentucky (*n* = 4) (selected based on isolation from more than one source or multiplicity of isolates and cost consideration) were sequenced using a Illumina MiSeq platform (Illumina, Inc., San Diego, CA) as described earlier (Hendriksen et al., 2015). The quality of the reads was assessed using the FastQC quality control tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), reads with a quality score of below 20 were filtered out, and all the remaining reads were assembled using the ASSEMBLER PIPELINE (version 1.4) available from the Center for Genomic Epidemiology (CGE) (http://cge.cbs.dtu.dk/services/all.php). Raw sequence data were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under study accession no.: PRJEB21564 (http://www.ebi.ac.uk/ena/data/view/PRJEB21564). A complete list of genomic sequence data is available in the Table S1. The assembled sequences were analysed to identify the MLST sequence type (ST) for *Salmonella enterica*, plasmid replicons and acquired antimicrobial resistance genes using the pipelines MLST (version 1.7), PLASMIDFINDER (version 1.2, 80% threshold for %ID) and RESFINDER (version 2.1, 80% threshold for %ID/60% minimum length) available from CGE (Carattoli et al., 2014; Larsen et al., 2012; Zankari et al., 2012). Single nucleotide polymorphisms (SNPs) were determined using the pipeline CSI PHYLOGENY (version 1.4) available from the CGE (http://cge.cbs.dtu.dk/services/all.php). The reference genomes used for SNP analysis were *S*. Colindale (HD 3A), one of the contemporary isolates due to lack of appropriate reference genome; others were *S*. Kentucky Str CMV 29188 and *S*. Rubislaw Str 10717. The pipeline is a suite of different public programmes. The reads were aligned against the genome of *S*. Colindale (HD 3A), *S*. Kentucky Str CMV 29188 or *S*. Rubislaw Str 10717 depending on the *Salmonella* serovar using the Burrows-Wheeler Aligner (BWA) version 0.7.2 (Li & Durbin, 2009). SNP calling was by SAMTOOLS version 0.1.18 (Li et al., 2009) "mpileup" commands. Subsequently, SNPs were selected when meeting the following criteria: (i) a minimum distance of 15 bps between each SNP (pruning); (ii) a minimum of 10% of the average depth; (iii) the mapping quality was above 30; (iv) the SNP quality was more than 20; and (v) all indels were excluded. The qualified SNPs from each genome were concatenated to a single alignment corresponding to position of the reference genome. The concatenated sequences were subjected to tree construction using maximum likelihood from FastTree (Price, Dehal, & Arkin, 2010).

2.6 | Ethical approval

The study was approved by the Ethic Committee of the Ministry of Health, Ibadan, Oyo State and University of Ibadan.

3 | RESULTS

3.1 | *Salmonella* serovars distribution

Overall *Salmonella* prevalence was 13.7% (*n* = 112); the serotyped isolates revealed 49 serovars from both the human and cattle sources (Table 1). The prevalence varied with source: the highest was observed in beef cattle at slaughter (*n* = 67; 26.8%) followed by healthy women (*n* = 13; 8.1%) and diarrhoeic humans (*n* = 17; 7.3%) with cattle at the market having the least prevalence (*n* = 5; 0.5%). Cattle at slaughter also had the highest serovar diversity (*n* = 28) followed by diarrhoeic humans (*n* = 13) and healthy women (*n* = 9) (Table 1). The least serovar diversity was observed in cattle at the market (*n* = 3).

S. Colindale and *S*. Rubislaw co-predominated (each with a prevalence of 17.6%) in diarrhoeic humans. *S*. Bredeney (*n* = 3; 23%) predominated in healthy women; *S*. Dublin (*n* = 5; 13%) in cattle at slaughter; *S*. Ekotedo co-predominated with *S*. Carno in one of the cattle farms (*n* = 3; 33% each) while *S*. Ekotedo was also the only *Salmonella* isolated in the other farm. In the cattle market, both *S*. Kentucky and *S*. Ealing co-predominated (*n* = 2; 40% each). There was overlapping of *Salmonella* serovars between humans and cattle and also between the diarrhoeic patients and healthy women; such serovars included Colindale, Give, Corvallis, Oranienburg and Kentucky. Furthermore, one isolate of serovar 4,5,12:i:- (monophasic variant of *S*. Typhimurium) was recovered from a diarrhoeic woman and a *S*. Typhimurium isolate from a healthy woman.

3.2 | Antimicrobial susceptibility testing of *Salmonella* isolates

Only 9.8% (11/112) of the *Salmonella* isolates showed resistance to at least one of the tested antimicrobials (Table 1). The antimicrobial

TABLE 1 *Salmonella* serovars distribution from the different sources

Serovars	Sources of isolation						Total
	Abattoir	Market	Farm 1	Farm 2	Healthy women	Diarrhoeic humans	
4,5,12: i: -	0	0	0	0	0	1	1
43: d:-	0	0	0	0	0	1	1
6,7,d	1	0	0	0	0	0	1
Agama	0	0	0	0	1	1	2
Agoueve	1	0	0	0	0	0	1
Altendorf	1	0	0	0	0	0	1
Bere	1	0	0	0	0	0	1
Bergen	2	0	0	0	0	0	2
Bredney	0	0	0	0	3	0	3
Butantan	0	0	0	0	0	1	1
Carno	0	0	0	3	0	0	3
Chandans	0	0	0	0	0	1	1
Chester	1	0	0	0	0	0	1
Colindale	2	0	0	0	2	3	7
Corvallis	0	0	0	1	1	0	2
Dakar	0	0	0	0	0	1	1
Derby	1	0	0	0	0	0	1
Dublin	5	0	0	0	0	0	5
Ealing	0	2	0	0	0	0	2
Eastbourne	3	0	0	0	0	0	3
Ekotedo	0	0	1	3	0	0	4
Essen	0	1	0	0	0	0	1
Gatehead	0	0	0	0	0	1	1
Give	3	0	0	0	0	1	4
Glostrup	0	0	0	1	0	0	1
Hadar	2	0	0	0	0	0	2
Hato	1	0	0	0	0	0	1
Houtenae ser.44:z4z23:-	1	0	0	0	0	0	1
Johannesburg	1	0	0	0	0	0	1
Kentucky	0	2	0	1	0	1	4
Kibusi	0	0	0	0	1	0	1
Kingston	1	0	0	0	0	0	1
Liverpool	0	0	0	0	1	0	1
Livingstone	1	0	0	0	0	0	1
Marseille	2	0	0	0	0	0	2
Muenster	1	0	0	0	0	0	1
Nigeria	0	0	0	0	0	1	1
Oranienburg	1	0	0	0	1	0	2
Plymouth	1	0	0	0	0	0	1
Poona	0	0	0	0	0	1	1
Rough	1	0	0	0	0	0	1
Typhimurium	0	0	0	0	1	0	1
Rubislaw	0	0	0	0	0	3	3
Stanleyville	1	0	0	0	0	0	1

(Continues)

TABLE 1 (Continued)

Serovars	Sources of isolation						Total
	Abattoir	Market	Farm 1	Farm 2	Healthy women	Diarrhoeic humans	
Sundsvall	2	0	0	0	0	0	2
Takoradi	1	0	0	0	0	0	1
Tees	1	0	0	0	0	0	1
Urbana	0	0	0	0	2	0	2
Worthington	1	0	0	0	0	0	1
Total	40	5	1	9	13	17	85

resistance was observed only towards ampicillin, chloramphenicol, ciprofloxacin, gentamicin, streptomycin and tetracycline with resistance prevalences ranging from chloramphenicol (*n* = 3, 2.7%) to streptomycin (*n* = 10, 8.9%).

The majority (83.3%) of the resistant isolates was obtained from the healthy women and comprised six serovars while all the three resistant isolates from cattle were *S*. Kentucky. All the ciprofloxacin-resistant isolates were also belonging to *S*. Kentucky, while one *S*. Corvallis isolate, obtained from a healthy woman, showed reduced susceptibility to ciprofloxacin. Furthermore, seven (63.6%) of the resistant isolates showed multidrug resistance (MDR) (resistance to ≥3 antimicrobials); majority (57.1%) of the MDR isolates were *S*. Kentucky (Table 2).

3.3 | Whole-genome sequence analysis

All the human and cattle isolates of *S*. Colindale except one (cattle) showed the same MLST loci and alleles; aroC-6, dnaN-18, hemD-46, hisD-124, pure-2, sucA-175, thrA-12; ST 584. The only exceptional isolate (cattle, DE 34) was a single locus variant with an alteration in allele dnaN-116, unknown ST (Figure 2).

Two of the *S*. Kentucky isolates (cattle) showed the MLST loci and alleles; aroC-76, dnaN-14, hemD-2, hisD-77, purE-64, sucA-64, thrA-67; ST 198. The remaining two isolates (human and cattle; AK 584 and HD 68L) were single locus and double loci variants with alterations in alleles hisD-517 and aroC-174 and hisD-100, respectively, unknown ST (Figure 3). One of the *S*. Rubislaw isolates showed MLST and alleles aroC-37, dnaN-4, hemD-46, hisD-205, pure-8, sucA-175, thrA-164; ST 562. The remaining two isolates, HD 41G and HD 43H, were two and three loci variants, with alterations in alleles dnaN-180 and pure-134; and aroC-166, dnaN-180 and pure-134, respectively, unknown ST (Figure 4). The *S*. Urbana isolates showed ST 512 and those of *S*. Agama, unknown ST.

The phylogenetic SNP analysis (maximum-likelihood trees; branch length represents SNP distance and the distance is related to the scale below each tree) revealed no clonal cluster shared by human and cattle *Salmonella* isolates (Figures 2 and 3); the isolates generally formed individual lineages, with pairwise SNP differences of ≥48 (*S*. Colindale with the exception of two human diarrhoeic isolates HD 34E and HD 32D) and ≥60 (*S*. Kentucky) showing existence of different genetic subpopulations of strains (Figures 2 and 3). Nevertheless, the two human diarrhoeic isolates (HD 34E and HD 32D) were, however, genetically related, separated by only five SNPs (Figure 2). All the human isolates of *S*. Rubislaw were genetically different revealing individual lineages with pairwise-genome differences of 38 to 24,090 SNPs (Figure 4).

Antimicrobial resistance genes were only detected in the WGST MDR Kentucky isolates, and these correlated with the observed

TABLE 2 Antimicrobial susceptibility of the *Salmonella* isolates

Serovars	No. isolates	Sources	No. (%) of isolates resistant to antimicrobials					
			Ampicillin	Chloramphenicol	Gentamicin	Streptomycin	Tetracycline	Ciprofloxacin
4,5,12:i:-	1	Diarrhoea	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Kentucky	4	Diarrhoea/cattle	1 (25.0)	0 (0.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)
Corvallis	1	Healthy women	1 (00.0)	1 (100.0)	0 (0.0.)	1 (100.0)	0 (0.0)	0 (0.0)
Liverpool	1	Healthy women	0 (0.0)	0 (0.0)	0 (0.0)	1 (00.0)	0 (0.0)	0 (0.0)
Bredeney	1	Healthy women	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Typhimurium	1	Healthy women	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Colindale	1	Healthy women	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Kibusi	1	Healthy women	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
Others	101		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	112		4 (3.6)	3 (2.7)	4 (3.6)	10 (8.9)	5 (4.5)	4 (3.6)

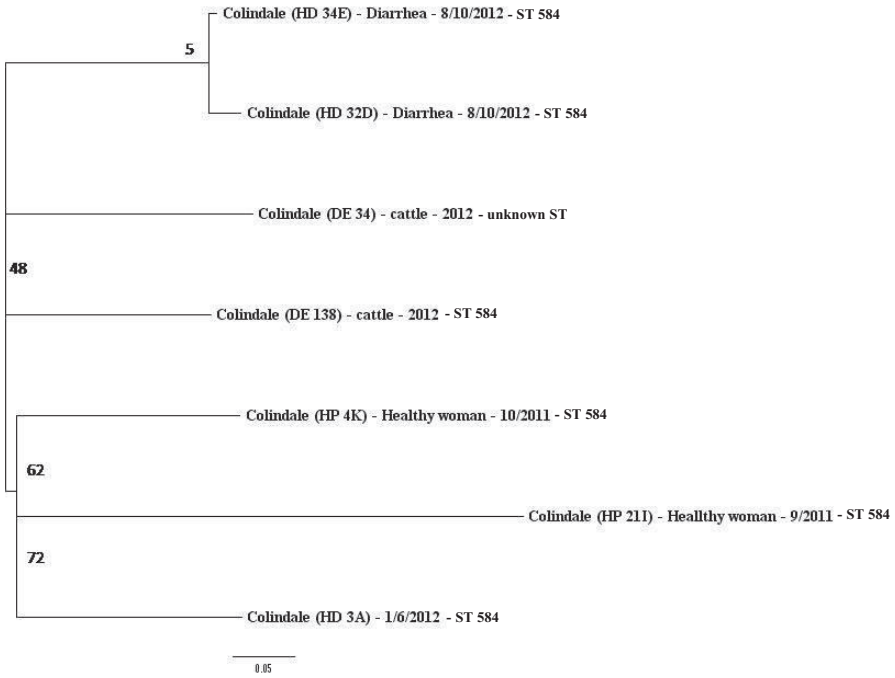

FIGURE 2 Phylogenetic tree of *S.* Colindale isolates

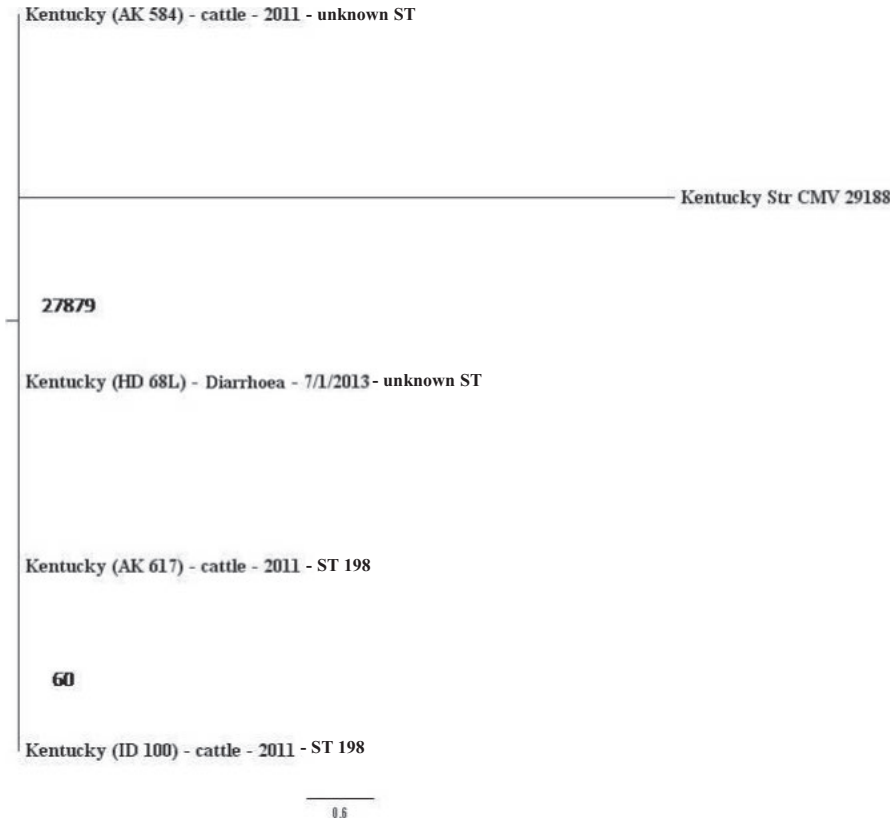

FIGURE 3 Phylogenetic tree of *S.* Kentucky isolates

antimicrobial resistance phenotypes: $bla_{\text{TEM-1B}}$ (ampicillin), *aac*(3)-*Id* (gentamicin), *aad*A7, *str*A, *str*B (streptomycin) and *tet*A (tetracycline). No resistance gene was detected using the WGST in serovars: Colindale, Urbana and Agama isolates (all were pansusceptible). The $bla_{\text{TEM-1B}}$ gene was detected in the only one Kentucky isolate from a diarrhoeic human. Plasmid replicons were not detected.

4 | DISCUSSION

This study showed cattle at harvest to be a reservoir of salmonellae, harbouring diverse serovars, many of which are important potential human pathogens. Although there was some overlap between the serotypes observed between cattle and humans, genetic relatedness does not

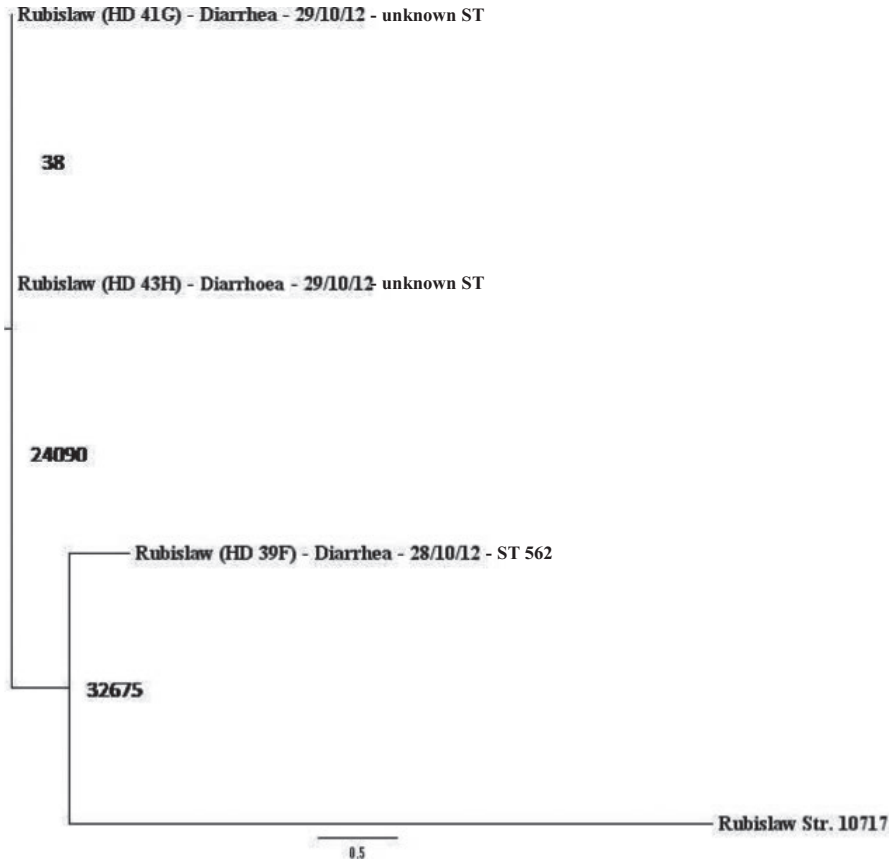

FIGURE 4 Phylogenetic tree of *S.* Rubislaw isolates

support zoonotic transmission or epidemiological link. More data are needed before concluding on the epidemiological link between the two populations. The observation of serovar overlap contrasted with reports from some African countries (Dione et al., 2011; Kariuki et al., 2006).

This study marks the first isolation of some serovars in cattle in Nigeria; *S*. Ekotedo, *S*. Bere, *S*. Begen and *S*. Takoradi that have been implicated in human infections but never cultured from non-human sources or cattle (Collard & Sen, 1960) suggesting cattle as the reservoir and probable source.

Other potential pathogens were found in cattle but not in humans in the present study: *S*. Dublin, an important causative agent of human invasive salmonellosis in Nigeria (Fashae et al., 2010; Obaro et al., 2015); *S*. Hadar, *S*. Muester, *S*. Stanleyville, *S*. Johanesburg, *S*. Livingstone and *S*. Chester (Harrois et al., 2014; Ktari et al., 2009); *S*. Ealing (Rowe et al., 1987); *S*. Kingston (Filip, Chihu-Amparan, Coman, Velázquez, & Silva, 2004); *S*. Eastbourne (Craven, Mackel, Baine, Barker, & Gangarosa, 1975); *S*. Worthington (Kapoor et al., 2006) and *S*. Essen and *S*. Hato (Osman, Marouf, & Alatfeehy, 2013). *S*. Dublin is the third commonest cause of *Salmonella* bacteremia after both *S*. Typhimurium and *S*. Enteritidis in Nigeria; accounting for 18% (in a south-western city) and 5% (in the Federal Capital Territory and a north-western city) of cases (Fashae et al., 2010; Obaro et al., 2015). *S*. Stanleyville and *S*. Johanesburg are probably persistent in cattle in Nigeria (Collard & Sen, 1960).

Overall, rare serovars were prevalent in the human infections with *S*. Colindale, *S*. Rubislaw, *S*. Bredeney and *S*. Urbana being the prevalent serovars. These serovars were less prevalent in human infections some decades ago (Collard & Sen, 1960), thus indicating persistence and increased public health importance. Interestingly, *S*. Colindale is also prevalent in cattle in this study; the similar prevalence in pigs in Nigeria (Fashae & Hendriksen, 2014) suggests dissemination in food animals; therefore, food animals are probable source of infection. Other probable sources of *S*. Colindale are poultry and well water (Dekker et al., 2015; Tabo et al., 2013). It is noteworthy that *S*. Colindale was also prevalent in human gastroenteritis in Gambia (Dione et al., 2011) and was associated with bacteremia (Kalonji et al., 2015; Obaro et al., 2015). Cattle are also reservoirs of S. Rubislaw coupled with pigs (Collard & Sen, 1960; Fashae & Hendriksen, 2014) though found only in humans in this study. The serovars *S*. Bredeney and *S*. Urbana in addition to others that were found presently only in humans were probably contracted from sources other than cattle (Fashae & Hendriksen, 2014; Fashae et al., 2010; Smith et al., 2016). The occurrence of *S*. Give in gastroenteritis and its high prevalence in cattle is also noteworthy; the prevalence is comparable to that in pigs in Nigeria (Fashae & Hendriksen, 2014); therefore, this serovar is probably an important animal pathogen in Nigeria (Higgins et al., 1997).

The healthy women carriers constitute public health risk as they excreted some clinical important serovars; *S*. Bredeney (Cormican et al., 2002; Moore et al., 2003), *S*. Urbana (Kocianová et al., 2010; Minami et al., 2004), *S*. Orianieburg (Werber et al., 2005), *S*. Corvalis (Langendorf et al., 2015) and *S*. Typhimurium (Im et al., 2016).This coupled with the high *Salmonella* carriage rate suggests the importance

of person-to-person *Salmonella* transmission (Im et al., 2016) particularly intrafamilial spread (Niizuma, Terada, Matsuda, Ogita, & Kataoka, 2002).

Furthermore, the *S*. Typhimurium monophasic variants 4,5,12:i:-, *S*. 43:d:-, *S*. Gatehead, *S*. Corvallis, *S*. Liverpool, *S*. Dakar and *S*. Kibusi serovars found only in humans are being reported for the first time in humans in Nigeria. Human infection by serovar 4,5,12:i:- (monophasic variant of *S*. Typhimurium) indicates the occurrence of this emerging epidemic pathogen in Nigeria thus warranting surveillance.

The prevalence of rare serovars corroborates reports from some African countries (Harrois et al., 2014; Osman et al., 2013), while it contrasts with others (Bonkoungou et al., 2013; Langendorf et al., 2015; Moyo et al., 2011), suggesting complex epidemiology of *Salmonella* in Africa which may complicate the control. The observed different serovar patterns are probably a reflection of local climatic conditions, culture and animal husbandry.

The *S*. Colindale strains were divided into more than one MLST as a result of a single locus variation with a maximum pairwise-genome difference of 77 SNPs suggesting a monomorphic serovar like *S*. Typhi (Hendriksen et al., 2015); this conclusion should be taken with caution as good reference genome and more temporal and spatial strains (presently unavailable) are required for confirmation. Both *S*. Kentucky and *S*. Rubislaw strains were also divided into more than one MLST based on variation in one to two (*S*. Kentucky) and two to three (*S*. Rubislaw) loci suggesting polymorphic serovars. With the collection of contemporary strains of the serovars Kentucky and Rubislaw diverging greatly from the corresponding reference genome by 27,879 SNPs and 32,675 SNPs, respectively, it indicates polymorphic serovars.

Phylogenetic SNP analysis of *Salmonella* isolates of the shared serovars (observed between cattle and humans) revealed circulation of different genetic populations of strains indicating lack of epidemiological link; however, this requires confirmation by large contemporaneous isolates. The genetic relatedness of the two human diarrhoeic isolates (HD 34E and HD 32D) separated by only five SNPs (Figure 1) indicates clonal dissemination.

The overall low prevalence of antimicrobial resistance is noteworthy; this coupled with the finding of rare *Salmonella* serovars suggests exposure from environment with less antimicrobial selective pressure such as reptiles.

These findings underscore the importance of cattle at harvest as an important reservoir of salmonellae; therefore, the sources of cattle infection need to be identified for targeted control measures. Cattle could be infected through feeds, water, grazing pasture and the environment. Indiscriminate grazing of cattle in open and unhygienic pasture and drinking of water from contaminated streams and rivers is common in this environment. Cattle are also allowed to stray on the streets scavenging domestic and market wastes. Biosecurity of cattle farms is also poor as they are bushy and not properly fenced to prevent infestation by small animals like rodents and reptiles coupled with poor hygienic condition of the farms. All these factors should be a focus of regulatory policies by appropriate Government Agencies to safeguard public health.

This study is limited by small sample size and evaluation of only some of the *Salmonella* isolates by WGST due to cost. Furthermore, one faecal sample was evaluated per individual instead of multiple samples which enhances isolation of *Salmonella*. The time span between collection of animal and human samples could be responsible for lack of clonal clusters formed by cattle and human *Salmonella* isolates.

5 | CONCLUSION

The study provides baseline data of *Salmonella* (human and cattle) prevalence, ST, antimicrobial resistance phenotypes and genetic relatedness, laying the framework for using WGS for source attribution in Nigeria, but more analysis is needed of *Salmonella* isolated from a broader array of sample types to further facilitate this application of WGS. There is need for continuous antimicrobial resistance surveillance.

ACKNOWLEDGEMENTS

We appreciate Fatma Aderibigbe, Tayo Akinsete, Asishia, Esther Enigah, Seun Ohu and Muyiwa Akerele for helping in sample collection and Dr. Olutoyin Fashae for assistance with the map. The following were appreciated for support during implementation of the project: late Prof. A.A. Oni, Mrs Kolade, Mrs Ogunleye, Mrs Nwachukwu and Mr Odekanmi of Medical Microbiology and Parasitology, University College Hospital; medical doctors and laboratory staff of Oni and Sons Hospital; and nurses at Adeoyo Hospital and Mokola Primary Health Centre.

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SUPPORTING INFORMATION

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How to cite this article: Fashae K, Leekitcharoenphon P, Hendriksen RS. Phenotypic and genotypic comparison of salmonellae from diarrhoeic and healthy humans and cattle, Nigeria. *Zoonoses Public Health*. 2017;00:1–11. https://doi.org/10.1111/zph.12427