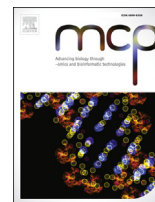




Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

Original research article

Serogenotyping and antimicrobial susceptibility testing of *Salmonella* spp. isolated from retail meat samples in Lagos, NigeriaStella Smith^{a,*}, Sascha Braun^{b,e,1}, Faith Akintimehin^c, Toun Fesobi^d, Moses Bamidele^a, Akito Coker^c, Stefan Monecke^{b,e,f}, Ralf Ehricht^{b,e}^a Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria^b Alere Technologies GmbH, Jena, Germany^c College of Medicine, University of Lagos, Lagos, Nigeria^d Public Health Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria^e InfectoGnostics Research Campus, Jena, Germany^f TU Dresden, Institute for Medical Microbiology and Hygiene, Dresden, Germany

ARTICLE INFO

Article history:

Received 23 February 2016

Received in revised form

22 April 2016

Accepted 24 April 2016

Available online xxx

Keywords:

Salmonella

Africa

DNA-based microarray

Retail meat

Genotyping

Serotyping

Serogenotyping

ABSTRACT

Microarray-based serogenotyping, antimicrobial susceptibility tests and the detection of relevant resistance genes were performed on isolates of *Salmonella* spp. from retail meat samples obtained in Lagos, Nigeria.

Out of 151 meat samples, 33 *Salmonella* isolates were obtained. Nine different *Salmonella* serovars (*S. Amoutive*, *S. Bargny*, *S. Drac*, *S. Ealing*, *S. Urbana*, *S. Hadar*, *S. Nyborg*, *S. Anatum* and *S. Havana*) were identified by microarray-based serogenotyping and confirmed afterwards using classical serotyping. Antibiotic susceptibility tests with 17 antibiotics showed that almost all isolates were fully susceptible to this panel.

The results of this study indicated a high prevalence of *Salmonella* in retail meat, the presence of some previously rather rarely described Serovars in retail meat samples from Lagos, and a need to monitor for *Salmonella* and their antibiotic resistance determinants. The microarray-based system used herein proved to be perfectly suited as epidemiological tool to replace classical serotyping.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Food-borne salmonellosis is one of the most prevalent zoonotic diseases worldwide. It often follows consumption of contaminated animal products, which usually originate from infected animals used in food production or from post mortem contamination of carcasses and/or meat [1]. *Salmonella* infections of live animals, including cattle, swine and sheep, arise from intensive rearing practices and from the use of contaminated feeds [2]. Stress associated with transport of the animals to an abattoir augments shedding of *Salmonella* by carrier animals, and this may contribute

to the spread of the organism to other animals in the slaughter plants [3].

Serotyping is an epidemiological tool for *Salmonella* characterization helping to determine identity/non-identity of isolates to enable detection of case clusters and outbreaks and to link human cases to e.g. brands or batches of foodstuff [4]. Therefore, it is pertinent to carry out serotyping for all culture confirmed cases of *Salmonella* infection. For typing, a scheme was developed that bases on antigenic variations affecting O (somatic) as well as H (flagellar) antigens. The number of *Salmonella* spp. serotypes covered by this so-called White-Kaufmann-LeMinor scheme expanded from 44 serovars known in 1934 to 2587 serovars currently known [5,6].

Currently, 46 *Salmonella* O-serogroups have been described. The genes of the O-antigen, flippase (wzx) and polymerase (wzy), are highly variable and specific for their respective serogroup [7–9]. For the H-antigen, there are two known flagella structural genes, *fliC* and *fliB*, which are highly conserved at their 5' and 3' ends and variable in their central regions [10].

Aside from the fact that classical serotyping is labor intensive,

* Corresponding author.

E-mail addresses: stellaismith@yahoo.com (S. Smith), sascha.braun@clondia.com (S. Braun), akintimehinopeoluwas@yahoo.com (F. Akintimehin), wuratoun@yahoo.co.uk (T. Fesobi), mosesbamidele@yahoo.com (M. Bamidele), aocoker@hotmail.com (A. Coker), stefan.monecke@clondia.com (S. Monecke), ralf@clondia.com (R. Ehricht).

¹ Contributed equally.

time consuming and expensive (requiring 250 sera to characterize 2500 serovars), this method is certainly out of reach of most laboratories, especially in the developing countries. As an alternative method, serogenotyping using unique DNA sequence information for identification, is highly reproducible, accessible and can easily be standardized worldwide. This has led to the development of different molecular typing systems such as DNA sequencing approaches [11], microarrays [7–9] and ligation-based microarrays [12]. Some are however fraught with the challenges of typing only a small sub-set of serotypes and are also expensive and/or labor intensive to be implemented in diagnostic or public health laboratories.

In Nigeria, there are limited data on the prevalence of *Salmonella* serovars causing disease in humans as well as of those that can be encountered in livestock or food products. Only the study by Fashae et al. (2010) gave information on the serovars prevalent in humans as well as animals, and the authors found different serovars present in both the human and animal isolates, with some rare otherwise serovars predominating amongst the animal and human isolates [13]. Another study from the city of Maiduguri, Nigeria, described observations of the rare serovar *S. Hiduiddif* that was isolated from chicken and poultry meat [14]. According to the report by Akinyemi et al. (2015) [15], the *Salmonella* spp. isolated from patients in Lagos, Nigeria with pyrexia and gastroenteritis were *S. Enteritidis*, *S. Paratyphi* and *S. Typhi*, while Ifeanyi et al. (2014) [16], isolated *S. Enteritidis*, *S. Zanzibar* and *S. Brancanstar* from diarrhoeic children in Abuja, Nigeria. Another report from North west Nigeria, showed cases of *S. Typhi*, *S. Paratyphi A*, *S. Arizonae*, *S. Typhimurium* and *S. Enteritidis* amongst children and adults presenting with pyrexia and gastroenteritis Abdullahi et al. (2013) [17].

To our best knowledge, this is the first study for Lagos, Nigeria that investigates the direct isolation of *Salmonella* from retail meat purchased in different market places. All isolates confirmed as *Salmonella* were subsequently serogenotyped using the technique developed previously [8], as well as screened phenotypically and genotypically for antimicrobial susceptibility patterns. The main aim of this study was to evaluate the microarray-based technique to supplement or to replace the classical serotyping as epidemiological tool in countries where standardized sera are too expensive and/or unavailable.

2. Materials and methods

2.1. Sample collection, bacterial isolation and preliminary identification

One hundred and fifty one samples of meat, consisting of beef ($n = 81$), chicken ($n = 30$), pork ($n = 16$), and goat ($n = 24$) from different parts of these animals, were purchased from various abattoirs and markets in six locations of different local government areas (LGA) in different districts of Lagos city. This included facilities in the towns of Mushin (in the Mushin LGA; $n = 64$), Yaba (Mainland LGA; $n = 40$), Ketu (Kosofe LGA; $n = 16$), Berger (Ikeja LGA; $n = 9$), Ojota (Kosofe LGA; $n = 5$) and Surulere (Surulere LGA; $n = 17$). All samples were collected between May and August 2013 into sterile plastic containers at room temperature and transferred to the laboratory within 2 h of collection. Each sample was stored at 4 °C and put up for culture within 2 h. Briefly, 10 g of each meat sample were homogenized in 90 ml of sterile Rappaport-Vasiliadis enrichment broth (Oxoid, Basingstoke, UK) using a sterile homogenizer in a sterile bottle. The homogenized samples were incubated at 37 °C for 18–24 h. An inoculation loop of the homogenate was inoculated onto Deoxycholate Citrate Agar (DCA, Oxoid Limited, Basingstoke, UK), at 37 °C for 18–24 h. Colonies were subsequently subcultured and incubated at 37 °C for 24 h on

Salmonella-Shigella-Agar (SSA, Oxoid Limited, Basingstoke, UK) in order to obtain discrete pure colonies. Colony material of all isolates was Gram stained to verify that they were Gram-negative bacilli. Isolates were further identified on XLD agar by identifying colony morphology that was oxidase negative, non-lactose fermenters and hydrogen producers (colonies with black dots). Thirty-three isolates identified presumptively as *Salmonella* spp. were confirmed using the Salm-SeroGenoTyping AS-1 Kit (Alere Technologies GmbH, Jena, Germany) and the VITEK 2 system (BioMerieux, Nuertingen, Germany).

2.2. Serogenotyping of *Salmonella* serovars

Isolates were cultivated on tryptone-yeast agar (TY-agar), and genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. If necessary, DNA was concentrated to at least 100 ng/μl using a Speed Vac centrifuge (Eppendorf, Hamburg, Germany) at 1400 rpm at 30 °C for 30 min. All isolates were genotyped by the array-based serogenotyping assay Salm-SeroGenoTyping AS-1 Kit according to manufacturer's instructions. The DNA-based microarray used includes 255 different targets to analyze O- and H-phases and assign the genotype to the antigenic formula according to the White-Kauffmann-LeMinor scheme. Additionally, the genus-specific genes *invA* (M90846.1), *galF* (X56793.1) and *manC* (X59886.1) were used as marker to identify genus *Salmonella*. With this microarray-based method, 132 different *Salmonella* serovars could be identified. Also, 77 targets were included that were related to antimicrobial resistance. Details of used targets and software analysis are described in Ref. [8].

After DNA isolation, a multiplex linear DNA amplification was used for site-specific internal labelling of the corresponding target region using dUTP linked biotin as a marker. Briefly, the linear amplification steps included 5 min of initial denaturation at 96 °C, followed by 50 cycles with 20 s of annealing at 50 °C, 40 s of elongation at 72 °C, and 60 s of denaturation at 96 °C. This reaction results in a multitude of specifically amplified, single-stranded, biotin-labeled DNA molecules for subsequent hybridization to the corresponding DNA microarray built in the ArrayStrips delivered with Salm-SeroGenoTyping AS-1 Kit. The ArrayStrips were placed in a BioShaker iQ thermomixer (Q. Instruments, Jena, Germany) and subsequently washed with 200 μl of de-ionized water for 5 min at 55 °C/550 rpm and with 100 μl hybridization buffer C1 for 5 min at 55 °C/550 rpm. All liquids were always completely removed using a soft plastic pipette to avoid scratching of the chip surface. In a separate tube, 10 μl of the labeled, single-stranded DNA were diluted in 90 μl hybridization buffer C1. The hybridization was carried out at 55 °C, shaking at 550 rpm for 1 h. After hybridization, the ArrayStrips were washed twice for 5 min with 200 μl washing buffer C2 at 45 °C, shaking at 550 rpm. Peroxidase-streptavidin conjugate C3 was diluted 1:100 in buffer C4. A total of 100 μl of this mixture was added to each cavity of the ArrayStrip, and subsequently incubated for 10 min at 30 °C and 550 rpm. Afterwards, washing was carried out twice at 550 rpm with 200 μl C5 washing buffer at 30 °C, with each step performed for 5 min. Visualization was achieved by adding 100 μl of a locally precipitating dye, D1, to the ArrayStrips. Microarrays were analyzed using the ArrayMate device with IconoClust version 3.2r1. Results, including serovar assignment and identification of AMR genes, are then described in detail in the result-HTML-files (supplementary information 1). Microarray raw data of all isolates are provided in the supplementary information 2. Classical serotyping using slide agglutination was carried out by the National Reference Laboratory for Salmonellosis in Cattle, at the Friedrich-Loeffler-Institute (FLI, Jena, Germany), according to ISO 6579:2002 [12].

2.3. Antimicrobial susceptibility testing

All *Salmonella* isolates were tested using an automated susceptibility testing technique (VITEK 2, bioMérieux, Nuertingen, Germany). Briefly, all isolates were cultured overnight on TY-agar for 12 h at 37 °C. Single colonies were used to prepare a 0.5 McFarland standard solution in 0.45% saline. This solution was used to load VITEK 2 cards AST-N289 (bioMérieux REF NO. 415 057). Nalidixic acid was additionally tested using the disk diffusion method in accordance with National Committee for Clinical Laboratory Standards [18].

2.4. Statistical tests

Statistical tests were performed to discriminate the prevalence of *Salmonella* isolates between study sites and type of meat. The significance (p) were conducted using the Fisher's exact test on Refs. [19,20].

3. Results

Out of the 151 food samples collected, 33 samples (21.8%) were tested positive for *Salmonella*. All obtained isolates (n = 33) were confirmed as *Salmonella* using the Salm-SeroGenoTyping AS-1 Kit and the VITEK 2 System, and were tested positive for *invA*, *galF* and *manC* as species marker for *Salmonella* using the microarray technique (Table 1).

(18/81) of identified *Salmonella* isolates originated from beef. Smaller numbers originated from chicken (10/30), goat (4/24) and pork (1/16). Table 1 show all details, including host species and sampling site, for each single isolate of *Salmonella* spp.

The isolates were identified to belong to nine different serovars. S. Amoutive was the most common serovar accounting for 24.2% (n = 8), and it was present in all meat samples (Table 1). The isolates from beef samples included: S. Anatum (n = 3), S. Ealing (n = 4), S. Havana (n = 1), S. Nyborg (n = 2), S. Urbana (n = 1), S. Bargny (n = 3), and S. Amoutive (n = 4). The isolates from chicken samples included: S. Anatum (n = 1), S. Hadar (n = 1), S. Drac (n = 2), S. Havana (n = 1), S. Amoutive (n = 2), S. Bargny (n = 2) and S. Nyborg (n = 1). The only isolate obtained from pork was S. Amoutive. Isolates from goat included: S. Anatum (n = 1), S. Ealing (n = 1), S. Amoutive (n = 1) and S. Drac (n = 1). In the present study S. Hadar was only found in samples from chicken while S. Urbana was unique in samples collected from beef. S. Drac was found in both, goat and chicken samples. The serogenotyping results were confirmed by classical serotyping using agglutination (Table 1) indicating that all *Salmonella* isolates were correctly typed using the Salm-SeroGenoTyping AS-1 Kit.

Due to missing O-antigen signals of three isolates, analyses via the PatternMatch algorithm (as described in Ref. [8]) could be not performed. Nevertheless, all three isolates showed the same H-antigens signals; H1:l,w and H2:e,n,x. Classical analysis for all three isolates by serotyping yielded the O-antigen O:47. The serological results for H-antigens H1 and H2 were in concordance with the results of the array-based serogenotyping. These isolates were assigned as S. Drac (47:l,w:e,n,x).

Antibiotic susceptibility testing revealed that only S. Amoutive isolate Nig-120 and S. Nyborg isolate Nig-135 were resistant to ampicillin, while S. Amoutive isolate Nig-120 was also resistant to ampicillin/sulbactam, cefuroxime, cefuroxime-axetil, cefotaxime and ceftazidime (supplementary information 3). No beta-lactamase gene to which this phenotype possibly could be attributed was found in these two isolates. All other isolates were sensitive to the 17 antimicrobials included into the AST-N289 panel and did not yield signals for the resistance markers screened. However, the

resistance genes *sul1* and *sul2* were found in two isolates from beef assigned as S. Amoutive (Nig-082) and S. Ealing (Nig-149), respectively. These genes are known to mediate resistance to sulfamethoxazole. Phenotypic tests for sulfamethoxazole were not performed since the VITEK AST-N289 panel considered only co-trimoxazole, i.e., a combination of trimethoprim and sulfamethoxazole, and isolates were found to be susceptible to co-trimoxazole.

Other resistance genes covered by the described test were not found using the Salm-SeroGenoTyping Kit. The isolates S. Amoutive (Nig-082), S. Bargny (Nig-129-2), S. Ealing (Nig-077), S. Drac (Nig-107) and S. Havana (Nig-009) were resistant to nalidixic acid.

4. Discussion

To our best knowledge this is the first report about *Salmonella* in retail meat from Lagos, Nigeria. The results of our study showed that *Salmonella* spp. were more isolated from beef than any other of the food source sampled during the time period in this study. Nevertheless, statistical analyses yielded no significant differences in the prevalence of *Salmonella* between chicken, pork, beef and goat (p = 0.19, Fisher's Exact test). However, differed the prevalence of *Salmonella* significant between different study sites (p = 0.005, Fisher's Exact test).

Food samples from Ojota (Kosofe LGA) market were found to have the highest number of *Salmonella* spp. (3 of 5 samples). This high prevalence could be explained by the low number of samples collected and also due to the fact that all the samples were collected from the same market with a common abattoir. Food samples from Surulere (Surulere) (9/17 samples) and Ketu/Ojota (Kosofe) (7/21 samples) were found to have very high prevalence of *Salmonella*. Although Surulere is not close to Ketu/Ojota, the latter two are close to each other and could likely to have been supplied by the same abattoirs. However, Surulere is one of the dirty regions in Lagos city, hence the high prevalence of *Salmonella* spp.

S. Nyborg and S. Urbana were peculiar to Mushin (Mushin LGA) area, while S. Hadar was found only in Berger (Ikeja LGA). Both LGAs are far from each other and markets where the samples were taken might have been supplied from different abattoirs. In a study from South West and North East of Nigeria, S. Kentucky was isolated from their chicken isolates in 4.9% of samples [21]. In our study S. Kentucky was not found in the meat samples. Fashae et al., [13] reported S. Virchow to be more prevalent (71.0%) among their chicken isolates, in contrast to our study where we did not isolate any S. Virchow in chicken or other food samples. Interestingly, none of the *Salmonella* serovars isolated from the chicken farms during the Fashae study [13] belonged to the same serovars as those from chicken in our study. The absence of serovars S. Kentucky and S. Virchow might be related to the samples and sampling method. In both studies [13,21] only fecal samples taken from living animals in poultry farms were analyzed. In contrast, in the present study samples were taken directly from different abattoirs and markets where the chicken was already handled. The source of sampling makes a big difference as contaminations with other *Salmonella* serovars may occur during processing. Nevertheless, other parameters like the geographic distance, temporal changes (i.e., a change of the population structure during several years), seasonal variability, local outbreak situations, and/or different races or breeds of chicken could play a major role for the serovar composition.

In another study from Africa, Burkina Faso, S. Drac was isolated from feces of cattle [22]. In our study S. Drac was isolated from chicken and goat meat samples, collected in three closely located LGAs (Surulere, Mushin and Mainland). We assume that all three markets were supplied from the same major abattoir and contamination with S. Drac occurs during the slaughtering process. In a study from Tunisia, S. Hadar was isolated from poultry meat

Table 1
Sample source and *Salmonella enterica* spp. *enterica* serovars according to sites.

Sample source	District Lagos city (LGA ^a)	Number of isolates	Results of microarray-based serotyping					Results of classical serotyping ^b		
			Species	Serovar	Serogroup	Antigenic formula	invA/galF/manC	O-antigen	H1-antigen	H2-antigen
Beef	Surulere (Surulere)	3	<i>S.e. enterica</i>	Amoutive	M (O:28)	28:d:1,5	+/+/+	28	d	1,5
Beef	Surulere (Surulere)	3	<i>S.e. enterica</i>	Bargny	C2–C3 (O:8)	8,20:i:1,5	+/+/+	8,20	i	1,5
Beef	Mushin (Surulere)	2	<i>S.e. enterica</i>	Ealing	O (O:35)	35:g,m,s:-	+/+/+			
Beef	Mushin (Mushin)	2	<i>S.e. enterica</i>	Nyborg	E1 (O:3,10)	3,{10}{15}:e,h:1,7	+/+/+	3,10	e,h	1,7
Beef	Yaba (Mainland)	1	<i>S.e. enterica</i>	Amoutive	M (O:28)	28:d:1,5	+/+/+			
Beef	Ketu (Kosofe)	1	<i>S.e. enterica</i>	Anatum	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:1,6	+/+/+			
Beef	Mushin (Mushin)	1	<i>S.e. enterica</i>	Anatum	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:1,6	+/+/+			
Beef	Ojota (Kosofe)	1	<i>S.e. enterica</i>	Anatum	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:1,6	+/+/+			
Beef	Surulere (Surulere)	1	<i>S.e. enterica</i>	Ealing	O (O:35)	35:g,m,s:-	+/+/+			
Beef	Yaba (Mainland)	1	<i>S.e. enterica</i>	Ealing	O (O:35)	35:g,m,s:-	+/+/+	35	g,m,s	—
Beef	Mushin (Mushin)	1	<i>S.e. enterica</i>	Havana	G (O:13)	1,13,23:f,g,[s]:-	+/+/+	13,23	f,g	—
Beef	Mushin (Mushin)	1	<i>S.e. enterica</i>	Urbana	N (O:30)	30:b:e,n,x	+/+/+	30	b	e,n,x
Chicken	Ojota (Kosofe)	1	<i>S.e. enterica</i>	Amoutive	M (O:28)	28:d:1,5	+/+/+			
Chicken	Yaba (Mainland)	1	<i>S.e. enterica</i>	Amoutive	M (O:28)	28:d:1,5	+/+/+			
Chicken	Ketu (Kosofe)	1	<i>S.e. enterica</i>	Anatum	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:1,6	+/+/+	3,10	e,h	1,6
Chicken	Surulere (Surulere)	1	<i>S.e. enterica</i>	Bargny	C2–C3 (O:8)	8,20:i:1,5	+/+/+			
Chicken	Yaba (Mainland)	1	<i>S.e. enterica</i>	Bargny	C2–C3 (O:8)	8,20:i:1,5	+/+/+			
Chicken	Mushin (Mushin)	1	<i>S.e. enterica</i>	Drac	n.d.	n.d.:l,v:e,n,x	+/+/+	47	l,v	e,n,x
Chicken	Yaba (Mainland)	1	<i>S.e. enterica</i>	Drac	n.d.	n.d.:l,v:e,n,x	+/+/+	47	l,v	e,n,x
Chicken	Berger (Ikeja)	1	<i>S.e. enterica</i>	Hadar	C2–C3 (O:8)	6,8:z10:e,n,x	+/+/+	8	z10	e,n,x
Chicken	Ketu (Kosofe)	1	<i>S.e. enterica</i>	Havana	G (O:13)	1,13,23:f,g,[s]:-	+/+/+			
Chicken	Mushin (Mushin)	1	<i>S.e. enterica</i>	Nyborg	E1 (O:3,10)	3,{10}{15}:e,h:1,7	+/+/+			
Goat	Mushin (Mushin)	1	<i>S.e. enterica</i>	Amoutive	M (O:28)	28:d:1,5	+/+/+			
Goat	Ketu (Kosofe)	1	<i>S.e. enterica</i>	Anatum	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:1,6	+/+/+			
Goat	Surulere (Surulere)	1	<i>S.e. enterica</i>	Drac	n.d.	n.d.:l,v:e,n,x	+/+/+	47	l,v	e,n,x
Goat	Mushin (Mushin)	1	<i>S.e. enterica</i>	Ealing	O (O:35)	35:g,m,s:-	+/+/+			
Pork	Ojota (Kosofe)	1	<i>S.e. enterica</i>	Amoutive	M (O:28)	28:d:1,5	+/+/+			
Total number of isolates		33								

^a LGA: Local government area.^b Classical serotyping was done for at least one serovar which was detected by the Salm-SeroGenoTyping AS-1 Kit. All Isolates belonging to serovar S. Drac were tested by classical serotyping.

[23]. Also in our study one *S. Hadar* serovar was isolated from a chicken sample collected from a market in Berger (Ikeja LGA).

Antimicrobial susceptibility testing showed that the isolates *S. Amoutive* Nig-120 and *S. Nyborg* Nig-135 were resistant to ampicillin, with *S. Amoutive* Nig-120 being resistant also to ampicillin/sulbactam, cefuroxime, cefuroxime-axetil, cefotaxime and ceftazidime. In this study all isolates were sensitive to ofloxacin and ciprofloxacin. A report by Akinyemi et al. [24], in 2003 from Lagos, Nigeria (although from humans and typhoid fever patients), stated the fact that ofloxacin and ciprofloxacin are the drugs of choice for *S. Typhi*, current findings also from humans show that our local *Salmonella* spp. are still sensitive to ofloxacin and ciprofloxacin [16,17]. Our data also reported all *Salmonella* isolates to be sensitive against both antibiotics. All isolates were sensitive to tetracycline, which is contrary to that reported by both Fashae et al. (2010) and Adeyanju & Ishola [25] (which was also from South West of Nigeria) in which the majority of chicken isolates were resistant to tetracycline (93%) [13]. Data are not available to compare the possibilities for this discrepancy. Other Nigerian studies by Refs. [13,21,23] with isolates from poultry, showed high prevalence rates of

resistance to nalidixic acid and high level of reduced susceptibility to ciprofloxacin, in contrast to our study, where the isolates were all sensitive to ciprofloxacin with only five isolates (15.2%) resistant to nalidixic acid. These results could be due to unregulated use of antibiotics in our environment, coupled with different sample types and different years of isolation. Although *S. Amoutive* isolate Nig-120 was resistant to 2nd and 3rd generation cephalosporins as identified by phenotypically VITEK 2 system, analysis of the isolate revealed no antibiotic resistance genes (i.e., *blaCTX-M-1/15* or *blaOXA*) which normally mediated such a phenotype. Possible explanations are that i) there was a beta-lactamase gene present in the isolate that was not covered by the microarray at all, or ii) that a primer or probe binding site of a beta-lactamase gene was significantly altered hindering detection by the array or that iii) another mechanism like a multidrug-resistance efflux pump [26] mediated the resistance. The resistance genes *sul1* and *sul2* found in *S. Amoutive* (Nig-082) and *S. Ealing* (Nig-149) are known to mediate resistance to sulfamethoxazole. Phenotypic tests (VITEK 2) considered only co-trimoxazole and isolates were found to be susceptible to co-trimoxazole. This could be explained by the fact

that resistance to co-trimoxazole requires the presence of both, *sul* and *dhfrA* genes. The gene *dhfrA* was not detected in both isolates. These results show once again the limitations of molecular tests [27]. Genotypes as defined by molecular assays do not always reflect the phenotype and vice versa, not for all phenotypes the responsible genes are detected. Therefore, a direct comparison of phenotype and genotype is necessary to validate a molecular assay [28–30]. The different results between this study and other studies regarding population structure and antibiotic resistance could be explained by the samples described in this study. *Salmonella* isolated from fecal samples of industrial livestock farming may be more resistant to antibiotics due to the high tasks of antibiotics in such areas. In this study *Salmonella* were isolated from handled meat of different animals and the sources of *Salmonella* contamination are unknown. We assume that the *Salmonella* population structure described in this study differs in their resistance in comparison to isolates from livestock farming.

5. Conclusion

To our knowledge this study is the first report of serogenotyping of food samples in Lagos Nigeria. Previously studies about *Salmonella* were mainly conducted in industrial animal farms using samples from living animals [13,21,31]. The prevalence of confirmed *Salmonella* spp. amongst the samples tested was about 21.8%. To our opinion, such a percentage of handled and retailed meat is alarmingly high. Therefore, any handled meat should be regularly monitored. Serovars, that are rare elsewhere, were isolated from these food samples, and occurred in all the food types. In addition, resistance was detected amongst our chicken and beef isolates, with isolates from Ketu/Ojota and Surulere having the highest prevalence. Continuous monitoring of antibiotic resistance should be carried out in these areas to prevent further spread of antibiotic resistant *Salmonella*. Taken together, microarray-based serogenotyping is a method which is affordable, easy to perform around different laboratories all over the world and easily to extend and adapt. The increasing availability of sequencing data will improve the technology rapidly.

Competing interests

SDB, RE and SM are employees of Alere Technologies, the company that manufactures the microarrays also used in this study. This has no influence on study design, data collection and analysis, and this does not alter the authors' adherence to all the Molecular and Cellular Probes policies on sharing data and materials. The other authors declare that no competing interests exist.

Authors contribution

SIS and SDB conceived of the study, and participated in its design and coordination. SDB and RE carried out the geno- and serotyping. SDB carried out the antimicrobial resistance pattern by VITEK 2. FOA, TWF, MB and AOC participated in preliminary design as well as bacteriological analysis of part of the study. SIS, SDB, SM and RE drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank Ullrich Methner (FLI, Jena, Germany) and Silke Keiling (FLI, Jena, Germany) for support with the classical serotyping of the *Salmonella* isolates, Keri Clack (Alere, Jena, Germany) for proof reading the manuscript, Annett Reißig (Alere, Jena, Germany) for excellent technical support, Christina Braun for continuous support

as well as for proof reading of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.mcp.2016.04.001>.

References

- [1] D. Alemayehu, B. Molla, A. Muckle, Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia, *Trop. Anim. Health Prod.* 35 (2003) 309–319.
- [2] J.Y. D'Aoust, Psychrotrophy and foodborne *Salmonella*, *Int. J. Food Microbiol.* 13 (1991) 207–215.
- [3] A.C. Baird-Parker, Foodborne salmonellosis, *Lancet* 336 (1990) 1231–1235.
- [4] C. Dorneles, J.M. Correa, S. Mendes, S. Haas, M.L. Tiba, R. Campanher, Microbiological analysis of the foods involved in foodborne disease outbreaks occurring in the Rio Grande do Sul State, *Rev. Bras. Biocienc.* 8 (2010) 44–48.
- [5] Anonymous, The genus *Salmonella* lignières, 1900, *J. Hyg. (Lond)* 34 (1934) 333–350.
- [6] P.A. Grimont, F.X. Weill, Antigenic Formulae of the *Salmonella* Serovars, ninth ed., WHO Collaborating Centre for Reference and Research on *Salmonella*, Paris, 2007.
- [7] K. Ballmer, B.M. Korczak, P. Kuhnert, P. Slickers, R. Ehricht, H. Hächler, Fast DNA serotyping of *Escherichia coli* by use of an oligonucleotide microarray, *J. Clin. Microbiol.* 45 (2007) 370–379.
- [8] S.D. Braun, A. Ziegler, U. Methner, P. Slickers, S. Keiling, S. Monecke, et al., Fast DNA serotyping and antimicrobial resistance gene determination of *Salmonella enterica* with an oligonucleotide microarray-based assay, *PLoS One* 7 (2012) e46489.
- [9] K. Franklin, E.J. Lingohr, C. Yoshida, M. Anjum, L. Bodrossy, C.G. Clark, et al., Rapid genoserotyping tool for classification of *Salmonella* serovars, *J. Clin. Microbiol.* 49 (2011) 2954–2965.
- [10] B.J. Masten, T.M. Joys, Molecular analyses of the *Salmonella* g... flagellar antigen complex, *J. Bacteriol.* 175 (1993) 5359–5365.
- [11] S. Sukhnanand, S. Alcaine, L.D. Warnick, W.L. Su, J. Hof, M.P. Craver, et al., DNA sequence-based subtyping and evolutionary analysis of selected *Salmonella enterica* serotypes, *J. Clin. Microbiol.* 43 (2005) 3688–3698.
- [12] P. Wattiau, T. Weijers, P. Andreoli, C. Schliker, H.V. Veken, H.M. Maas, et al., Evaluation of the Premi Test *Salmonella*, a commercial low-density DNA microarray system intended for routine identification and typing of *Salmonella enterica*, *Int. J. Food Microbiol.* 123 (2008) 293–298.
- [13] K. Fashae, F. Ogunsola, F.M. Aarestrup, R.S. Hendriksen, Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria, *J. Infect. Dev. Ctries.* 4 (2010) 484–494.
- [14] I.A. Raufu, R.S. Hendriksen, J.A. Ameh, F.M. Aarestrup, Occurrence and characterization of *Salmonella* Hiduiddify from chickens and poultry meat in Nigeria, *Foodborne Pathog. Dis.* 6 (2009) 425–430.
- [15] K.O. Akinyemi, B.A. Iwalokun, O.O. Alafe, S.A. Mudashiru, C. Fakorede, blaCTX-M-I group extended spectrum beta lactamase-producing *Salmonella typhi* from hospitalized patients in Lagos, Nigeria, *Infect. Drug Resist.* 8 (2015) 99–106.
- [16] C.I. Ifeanyi, B.E. Bassey, N.F. Ikeneche, N. Al-Gallas, Molecular characterization and antibiotic resistance of *Salmonella* in children with acute gastroenteritis in Abuja, Nigeria, *J. Infect. Dev. Ctries.* 8 (2014) 712–719.
- [17] B. Abdullahi, K. Abdulfatai, J.R. Wartu, I. Mzungu, H.I.D. Muhammad, A.O. Abdulsalam, Antibiotic susceptibility patterns and characterization of clinical *Salmonella* serotypes in Katsina State, Nigeria, *Afr. J. Microbiol.* 8 (2014) 915–921.
- [18] NCCLS, Performance Standards for Antimicrobial Susceptibility Testing: 11th Informational Supplement, Approved Standard M2-A7 and M7-A5, seventh ed., 2001. Wayne, Pennsylvania.
- [19] R-Development-Core-Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2008, ISBN 3-900051-07-0. <http://www.R-project.org>.
- [20] R.A. Fisher, On the interpretation of χ^2 from contingency tables, and the calculation of P, *J. R. Stat. Soc.* 85 (1922) 87–94.
- [21] I.A. Raufu, K. Fashae, J.A. Ameh, A. Ambali, F.T. Ogunsola, A.O. Coker, et al., Persistence of fluoroquinolone-resistant *Salmonella enterica* serovar Kentucky from poultry and poultry sources in Nigeria, *J. Infect. Dev. Ctries.* 8 (2014) 384–388.
- [22] A. Kagambega, T. Lienemann, L. Aulu, A.S. Traore, N. Barro, A. Siitonen, et al., Prevalence and characterization of *Salmonella enterica* from the feces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human *Salmonella* isolates, *BMC Microbiol.* 13 (2013) 253.
- [23] L. Soufi, Y. Saenz, M. de Toro, M.S. Abbassi, B. Rojo-Bezares, L. Vinue, et al., Phenotypic and genotypic characterization of *Salmonella enterica* recovered from poultry meat in Tunisia and identification of new genetic traits, *Vector Borne Zoonotic Dis.* 12 (2012) 10–16.
- [24] K.O. Akinyemi, S.I. Smith, A.O. Oyefolu, A.O. Coker, Multidrug resistance in *Salmonella enterica* serovar *typhi* isolated from patients with typhoid fever complications in Lagos, Nigeria, *Public Health* 119 (2005) 321–327.

- [25] G.T. Adeyanju, O. Ishola, Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria, Springerplus 3 (2014) 1–9.
- [26] L.J. Piddock, Multidrug-resistance efflux pumps - not just for resistance, Nat. Rev. Microbiol. 4 (2006) 629–636.
- [27] A. Lauri, P.O. Mariani, Potentials and limitations of molecular diagnostic methods in food safety, Genes Nutr. 4 (2009) 1–12.
- [28] S.D. Braun, S. Monecke, A. Thurmer, A. Ruppelt, O. Makarewicz, M. Pletz, et al., Rapid identification of carbapenemase genes in gram-negative bacteria with an oligonucleotide microarray-based assay, PLoS One 9 (2014) e102232.
- [29] L. Geue, S. Monecke, I. Engelmann, S. Braun, P. Slickers, R. Ehricht, Rapid microarray-based DNA genoserotyping of *Escherichia coli*, Microbiol. Immunol. 58 (2014) 77–86.
- [30] L. Geue, B. Stieber, S. Monecke, I. Engelmann, F. Gunzer, P. Slickers, et al., Development of a rapid microarray-based DNA subtyping assay for the alleles of Shiga toxins 1 and 2 of *Escherichia coli*, J. Clin. Microbiol. 52 (2014) 2898–2904.
- [31] H. Barua, P.K. Biswas, K.E. Olsen, J.P. Christensen, Prevalence and characterization of motile *Salmonella* in commercial layer poultry farms in Bangladesh, PLoS One 7 (2012) e35914.