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Serogenotyping and antimicrobial susceptibility testing of *Salmonella* spp. isolated from retail meat samples in Lagos, Nigeria

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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.

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 *fljB*,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,

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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.* Hiduddify 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 2013into 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].