Antimicrobial resistance and genotypic profiles of Salmonella Saintpaul isolated along beef processing and distribution continuum

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Abstract

Salmonella Saintpaul (SSa) is increasingly reported from food and foodborne outbreak cases. Pulsed field gel electrophoresis (PFGE) is used for screening and tracking of Salmonella infections. Widespread use of antimicrobial agents in humans and food animals could result in antimicrobial resistant Salmonella serotypes. The aim of this study was to characterize S. Saintpaul (n = 28) isolated from various sampling locations at abattoir and meat processing plant lines in Ethiopia for phenotypic antimicrobial resistance and genotypic diversity, and to track its transfer routes. Sampling location, steps and occasions were considered for each isolate description. Antimicrobial sensitivity testing was performed against seven different antimicrobial agents using disc diffusion method. PFGE with XbaI® enzymatic genomic digestion with BioNumerics® analysis was used for genotypic diversity. Of all the isolates tested, only 17.9% were pan susceptible, and 82.1% were resistant to at least one and at most to three antimicrobials. All isolates were susceptible to gentamycin, trimethoprim-
sulfamethoxazol and trimethoprim. Resistance to oxytetracycline (82.2%) was predominant followed by 3.6% resistance to each of chloramphenicol, neomycin and polymyxin B. PFGE analysis revealed three distinguishable clusters of pulsotypes but the majority of the isolates (25/28) belonged to cluster-I (SSaX1-4) pulsotype. Indistinguishable/similar cluster of (SSaX 1-4) isolates among and between sampling location, steps and occasions were observed. Majorities of S. Saintpaul (88%) in the cluster-I pulsotype were resistant to oxytetracycline. Our study indicated that oxytetracycline resistance is very common among the S. Saintpaul isolates studied; and the isolates were diverse with similar resistance profiles within the same genomic pulsotypes. Transfer of S. Saintpaul within, between and across sampling locations, during the same or different occasion were determined from SSaX 1-4 pulsotype while cluster-II (SSaX5) indicates transfer from abattoir to butchery. The unique isolate in cluster-III (SSaX6) shows the presence of other possible source of S. Saintpaul for the beef chain contamination.

Keywords: Food science, Food safety, Microbiology

1. Introduction

*Salmonella* species are inhabitant of the intestinal tract of animals and distributed in the environment which results in increasing prevalence in the global food chain and their virulence [1]. Their adaptability properties favors easy transmission result in an enormous medical, public health and economic impact worldwide [1, 2]. CDC [3] reported a total of 84 persons infected with the outbreak strain of *Salmonella* Saintpaul with 28% of ill hospitalized and no deaths were reported. Due to contamination with *Salmonella*, Mead et al. [4] estimated a huge proportion of infections, case-fatality rates of 43% and 10% for immunocompromised and 5% and 0% for non-immunocompromised due to non-typhoid *Salmonella* in respective of infants and children were also reported by Sirinavin et al. [5]. Characterization of *Salmonella* involves utilization of combined phenotype and/or genotypic techniques for the differentiation of strains specific spices and sub spices [6]. Serology based on surface antigen [7], phage typing based on bacteriophage host profile [8], antimicrobial susceptibility and biotypes of *Salmonella* strain [9] were used for phenotypic characterization. Pulsed field gel electrophoresis (PFGE) and whole-genome sequencing [10, 11] are used for genetic discrimination of *Salmonella* isolates including S. Saintpaul from outbreaks and epidemiological investigations [12, 13]. In Ethiopia, little information is available on the status of food safety where there is a tradition raw meat consumption which could carry risks of infection with zoonotic agents [14, 15], where different *Salmonella* serotypes were isolated from food animal, food and production environment [14, 15, 16, 17, 18, 19, 20] with antimicrobial resistance test on
isolates [21] in Ethiopia. However, survey on genotypic diversity and transfer route investigation were scares in the country. The aim of this study was to characterize S. Saintpaul isolated from various sources at abattoir and meat processing plant in Ethiopia for phenotypic antimicrobial resistance and genotypic diversity as well as tracking its transfer routes along meat production and processing lines.

2. Materials and methods

2.1. Sampling, sample source and locations

The studied strains were originated from different samples collected from abattoir at Addis Ababa City and from processing plant line at Bishoftu town, 47 Km from East of Addis Ababa City. Following the beef production and supply procedure chain described by FAO [22], different samples were collected from abattoir line and the beef processing plant line. A total of 237 and 431 samples were aseptically collected from the abattoir and the processing plant lines respectively (Table 1).

2.2. Laboratory procedure

2.2.1. Bacterial isolation

Bacterial isolation was conducted at Food Hygiene and Microbiology Laboratory, Akililu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia following standard protocols [7]. Pre-enrichment was performed using one portion of sample by volume or gram was homogenized with 10 portions of buffer peptone water (BPW) (Merck, Germany) at 1:10 proportion. From the pre-enriched samples, 0.1 ml and 1 ml was transferred to 10 ml of Rappaport-Vassiliadis (RV) medium (Oxoid Hampshire, England) and 10 ml of Muller Kaufmann tetrrathionate with novobiocin (MKTTn) (Merck) broths respectively for selective enrichment. RV and MKTTn broth cultures were then incubated at 43 °C and 37 °C respectively for 18-24 hrs. A loop full was plated on Brilliant phenol lactose sucrose agar (BPLS) (Merck) and Xylose lactose Tergitol™ 4 (XLT4) (Merck) in parallel and incubated at 37 °C for 24 hrs and 48 hrs, respectively. Presumptive colonies based on their characteristic morphological appearances on the selective agar plates were sub-cultured onto standard-I nutrient agar (Merck) and biochemically confirmed for serotyping.

2.2.2. Serotyping

The isolates were serotyped at Microbiology Laboratory, Institute of Meat Hygiene and Technology, Panel Veterinary Public Health, FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Germany. Serotyping was performed
using *Salmonella* antisera (Sfin, Berlin, Germany) with O-antigens and H-antigens agglutination test [7].

### 2.2.3. Antimicrobial resistance testing

All of the isolates were tested for their phenotypic antimicrobial resistance by agar disc diffusion method with antimicrobial impregnated discs (Oxoid, Hampshire, England) against polymyxin-B (PB; 300 U), trimethoprim-sulfamethoxazole (STX;
1.25/23.75 μg), chloramphenicol (C; 50 μg), gentamycin (G; 10 μg), trimethoprim (W; 5 μg), neomycin (N; 10 μg) and oxytetracycline (OT; 30 μg). Antimicrobial resistance tests were performed on Mueller-Hinton agar (Oxoid) according to Bauer Kirby agar disc diffusion [23] following Clinical Laboratory Standards Institute’s protocol [24]. The isolates were sub-cultured onto standard-I nutrient agar (Merck) and incubated at 37 °C for 24 hrs. They were then inoculated into 3 ml of brain heart infusion broth (BHI) (Merck) and again incubated for 1 hr at 37 °C. The inoculum density was standardized to 0.5 McFarland standard; from which 0.1 ml was spread onto Mueller-Hinton agar (Oxoid). After the plates were allowed to absorb the moisture; antimicrobial impregnated discs were applied; and the plates were incubated at 35 ± 2 °C for 16-18 hr. Based on the diameter of zone inhibition for Enterobacteriaceae, results were recorded as susceptible, intermediate or resistant [24].

2.2.4. Pulsed filed gel electrophoresis (PFGE) procedure

The PFGE examination of the isolates were performed following PulseNet protocol [11] at Molecular Biology Laboratory, Institute of Meat Hygiene and Technology, Panel Veterinary Public Health, FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Germany. Agarose-embedded whole genomic DNA of the isolates was digested with the restriction enzyme XbaI® (60 U) (Roche Diagnostics GmbH, Germany) enzymatic restriction. DNA fragments were separated by PFGE in agarose gels. S. Braenderup STSAL82 (Merck, Germany) was used as a reference strain. A 50–1000 kb Pulse marker™ (Sigma-Aldrich Co, USA), test strains and reference strain were loaded into 1.2% Pulsed Field Certified Agarose® gel. The gel running condition was set with initial pulse switch time of 2.2 seconds and the final pulse switch time of 63.8 seconds under 200 V (6 V/cm) voltage for 20 hrs at 14 °C according to Pulse Net [11]. Then, the gel was stained with 1 mg/l ethidium bromide solution for 20-30 min on a horizontal shaker (Certomat®U) and twice de-stained with distilled water for 20 min. The PFGE files were processed using BioNumerics® Ver. 6.6 software (Applied Maths BVBA, Kortrijk, Belgium).

2.3. Data analysis

Phenotypic antimicrobial resistance profiles and genotypic diversity were combined in data entry for analysis. Antimicrobial susceptibility and resistance profiles were presented as percentage. The PFGE results were analyzed by using BioNumerics® Version 6.6 software (Applied Maths BVBA, Kortrijk, Belgium) with optimization of 1.0 and position tolerance of 1.5. For beef line S. Saintpaul transfer route determination, considerations were made on sampling occasion/batch (date of sampling), sample source and locations in the studied beef production lines.
3. Results and discussion

3.1. Occurrence and drug resistance profile of S. Saintpaul

Except the hooks swab samples which was not positive for S. Saintpaul, all other sampling locations were found positive ranging from 2.9% from MLN to 36.4% from beef transport truck in the abattoir line. However, only one isolate (0.23%) of S. Saintpaul from total 431 samples at beef processing plant line which is 0.8% in raw beef was observed with other samples being negative for the serotype (Table 2). This finding indicates abattoir is highly contaminated and can act as sources of microbial pathogens including S. Saintpaul. The single isolate observed on meat at processing plant line also show transfer of *Salmonella* via raw beef. Regardless of number, observing the 28 isolates of S. Saintpaul from different meat production and processing environmental, animal and beef product locations indicates wide distribution of this serotype in Ethiopia. The present finding was consistent with the 45 (38.8%) [19] from camel and its meat, one isolate [17] from minced beef, 14.8% [20], 4.3% [18] in minced beef from supermarkets previously reported in Ethiopia showing its distribution and occurrence in meat and its production area in the country. S. Saintpaul was also reported as dominant serotype 20 (76.9%) of all isolates from poultry in Ethiopia [25].

The pan (100%) susceptible of strain to gentamicin, trimethoprim and trimethoprim-sulfamethoxazol (Table 3) show the effectiveness of these antimicrobials for treatment of cases of S. Saintpaul in Ethiopia. Kikuvi *et al.* [26] also showed effectiveness of trimethoprim-sulfamethoxazol against *Salmonella* isolate including S. Saintpaul

### Table 2. *Salmonella* Saintpaul positive samples along studied beef line in Ethiopia.

| Source/origin      | Sampling location         | No. of examined samples | No. (%) Positive |
|--------------------|----------------------------|-------------------------|------------------|
| Abattoir Environment | Personnel related swab samples |                          |                  |
|                    | Personnel hands            | 13                      | 4 (30.8)         |
|                    | Aprons                      | 14                      | 1 (7.1)          |
|                    | Knives                      | 13                      | 1 (7.8)          |
|                    | Tap water                   | 12                      | 1 (8.3)          |
|                    | Device related swab samples |                          |                  |
|                    | Hooks samples               | 11                      | 0                |
|                    | Rooms floor samples         | 17                      | 4 (23.5)         |
|                    | Refrigerator                | 10                      | 1 (10.0)         |
|                    | Beef transport truck        | 11                      | 4 (36.4)         |
|                    | Subtotal                    | 101                     | 16 (15.8)        |
| Abattoir Animal related | Animal feces               | 34                      | 2 (5.9)          |
|                    | MLN* sample                 | 34                      | 1 (2.9)          |
|                    | Raw beef                    | 34                      | 2 (5.9)          |
|                    | Subtotal                    | 102                     | 5 (4.9)          |
| Butchery           | Retail meat sample          | 34                      | 6 (17.6)         |
| Beef PPL**         | Raw meat sample             | 118                     | 1 (0.8)          |

*Mesentric lymph node; PPL = Processing plant line; ** *Salmonella* Saintpaul was not isolated from other samples.*
isolated from pig in Kenya. However, Beutlich et al. [27] reported 78% intermediate or full resistance of *S*. Saintpaul to gentamicin and 11% resistance to trimethoprim. The observation of one isolate (3.6%) resistant to chloramphenicol in this study was similar to reports of Kikuvi et al. [26] who reported one resistant isolate from Kenya and five (9%) Beutlich et al. [27] from Germany. On the other hand, the 82.2% resistant isolates to oxytetracycline in the present study was higher than the reported in other studies from Kenya [26] and 31% [27] from Germany. The high resistant isolates to oxytetracycline in this investigation could be due to frequent uses of this drug where it is marketed as ‘broad spectrum antibiotic. The popular and widely uses of oxytetracycline in the veterinary sector in global [28] and in Ethiopia with associated resistance [29], were reported, too. A total of 26/28 (82.1%) of the isolates were resistant to at least one and at most to three antimicrobials. The present finding was lower than the phenotypic and genotypic profiles isolates resistant for one or more antimicrobials among the 76 isolates (2.18%) reported [30] among Non-typhoidal *S*. Enterica. High frequencies of resistance to tetracycline 26.27% were also reported [30] in Non-typhoidal *S*. Enterica. Presence of single to multiple drug resistant *Salmonella* isolates including *S*. Saintpaul was also reported from poultry in Ethiopia [25].

### 3.2. Genomic diversity and phenotypic drug resistance profile of *S*. Saintpaul

Using genotypic PFGE and phenotypic drug susceptibility/resistance profiles, three different clusters of *S*. Saintpaul were observed along the beef production and processing lines. Sibhat et al. [16] investigated *Salmonella* prevalence in abattoir and

| Types of Drug used            | Concentration of drugs used       | *Salmonella Saintpaul (n = 28)* |
|------------------------------|-----------------------------------|--------------------------------|
|                              |                                   | **S** No. (%) | **I** No. (%) | **R** No. (%) |
| Polymyxin B                  | PB 300 IU                         | 27 (96.4)     | 0             | 1 (3.6)       |
| Gentamycin                   | CN 10 µg                          | 28 (100)      | 0             | 0             |
| Chloramphenicol              | C 50 µg                           | 27 (96.4)     | 0             | 1 (3.6)       |
| Trimethoprim                 | W 5 µg                            | 28 (100)      | 0             | 0             |
| Trimethoprim-sulfamethoxazol | STX 1.25/23.75 µg                 | 28 (100)      | 0             | 0             |
| Neomycin                     | N 10 µg                           | 16 (57.1)     | 11 (39.3)     | 1 (3.6)       |
| Oxytetracycline              | OT 30 µg                          | 3 (10.7)      | 2 (7.1)       | 23 (82.2)     |

Note: S* = susceptible; I* = intermediate; R* = resistance.
Fig. 1. PFGE analysis of S. Saintpaul isolates from a cattle abattoir line, Addis Ababa, Ethiopia, 2011–2012. Hand Sw = hand swabs; MLN = mesenteric lymphnode sample; Refrigera. = chilling room sample.

recommend the need for further analysis of isolates using PFGE clustering as a tool to assess the epidemiological and genotypic diversity in Ethiopia. Thus, the present study shows 51.3—100% genomic relatedness of the 28 (27 from abattoir and 1 from processing plant line) S. Saintpaul. They are differ into three (3) different clusters of pulsotypes\(^1\) consisted of 25 isolates in cluster-I, 2 isolates in cluster-II and 1 isolate in cluster-III (Fig. 1). These different clusters shows the genotypic diversity of S. Saintpaul in Ethiopia. Regardless of sample source and geographic distribution, high degree of genetic diversity of S. Saintpaul were also reported by Kerouanton et al. [31] showing 20 pulsotypes among the 30 isolates. Moreover, 82 of the 159 isolates from animals, food of animal origin and humans shows only 42.6% similarity [32].

About 88% of the isolates within cluster-I were resistant to oxytetracycline with multiple drug resistance profile of C-OT-PB in one of them but one isolate resistant to N and OT (Fig. 2). Besides their genotypic similarity, 22 (88%) of the 25 isolates in cluster-I shows resistant to oxytetracycline but all (100%) of isolates in cluster II shows susceptibility to oxytetracycline. This indicated the phenotypical drug response similarity of isolates within a cluster.

\(^1\) Unless otherwise indicated terms for: pulsotype(s) = PFGE pattern(s) = cluster(s) = clone(s) = PFGE type are interchangeably used in this article.
3.3. Tracking and tracing the possible sources and transfer routes

The 95.2% PFGE genomic similarity among isolates in cluster-I (Fig. 1) indicates the occurrence indistinguishable S. Saintpaul in different sampling occasion/batch and location including on personal hand, the abattoir room, in animal feces, meat transporting truck and on the meat. All of isolates within this cluster are from abattoir line. This indicated highly contamination of the abattoir with similar clonal of S. Saintpaul. Laconch et al. [33] confirming spread of a single clone of Salmonella serotype over a large geographical area. Using 2000 isolates, Sandvang et al. [34] the spread between farms, survival and transmission of specific clone of Salmonella enterica serotype Typhimurium using their identical PFGE patterns among the fecal and environmental isolates from pig production farms units. Using PFGE generated whole genome mapping data, Fey et al. [13] tracked the distinguishable S. Saintpaul serotype from outbreak in relation to the temporal periods.

3.4. PFGE as tool for determining the Salmonella source and tracing its transfer routes

The present possible sources, contamination and transfer routs of the studied S. Saintpaul was assessed. PulseNet [11] recommended PFGE as a tool for tracking and trace of sources of pathogens in food and outbreaks. The significantly high (95.2%)
genotypic PFGE similarities among S. Saintpaul within cluster-I indicates the possible transfer of the agent during the same or different sampling occasions as well as across locations in the abattoir line to the level of consumer supply at the butcheries. Fey et al. [13] used to track and trace the sources of Salmonella strains for the outback in time period using their PFGE indistinguishable properties. One of isolate obtained from abattoir room in cluster-II was found indistinguishable from an isolate from processing plant line (Fig. 1). This indicates abattoir could be the sources of beef contamination which extended to the processing plant. In fact, the studied abattoir is one of sources of raw beef for the processing plant. Using PFGE enzymatic digestion and clustering into close relationship among the phage types of Salmonella, sources of isolates were confirmed [33], spread between locations, the survival and the transmission of Salmonella were determined [34] from an outbreaks on temporal periods [13]. Kagambega et al. [35] also try to assess potentially transmit of some of the same Salmonella serotypes from wild animals to humans using the same techniques in the Burkina Faso. On the other hand the one PFGE distinguishable S. Saintpaul in cluster-III was observed in beef at butchery. This indicates the possibility of contamination of raw beef at public supply location from other sources along the handling steps or at supply stages. Such occurrence, distribution and transfer of S. Saintpaul within a particular studied meat production and processing lines in Ethiopia indicates the need for further investigation for other serotypes and pathogens based on geographical region, during particular period of time in the country [13, 32].

4. Conclusion

Our study indicated that phenotypic oxytetracycline resistance was very common among the S. Saintpaul isolated from Ethiopia. The serotype was also found diverse having similar genotypic and phenotypic (drug susceptibility/resistance profiles) within the same genomic pulsotypes. Moreover, the presence and transfer of indistinguishable S. Saintpaul serotype within same sampled location, during same and/or different sampling occasion along beef abattoir line were observed. Transfer of the serotype from abattoir to the butchery shop and the beef processing plant via raw beef were confirmed using PFGE. Contamination of beef line from other possible sources with S. Saintpaul serotype indicates the risk of public acquiring infection. Hygiene application along the beef production and processing line with regular drug susceptibility test may reduce risks posed for contamination with Salmonella and public infection.

Declarations

Author contribution statement

Adem Hiko: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Herlinde Irsigler, Lieselotte Bräutigam: Performed the experiments; Analyzed and interpreted the data.

Gobena Ameni: Conceived and designed the experiments.

Reinhard Fries: Contributed reagents, materials, analysis tools or data.

Baumann Maximilian: Analyzed and interpreted the data; Wrote the paper.

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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