Heliyon

Received:
13 April 2018
Revised:
22 September 2018
Accepted:
5 December 2018

Cite as: Adem Hiko, Herlinde Irsigler, Lieselotte Bräutigam, Gobena Ameni, Reinhard Fries, Baumann Maximilian. Antimicrobial resistance and genotypic profiles of *Salmonella* Saintpaul isolated along beef processing and distribution continuum. Heliyon 4 (2018) e01025. doi: 10.1016/j.heliyon.2018.e01025

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-

https://doi.org/10.1016/j.heliyon.2018.e01025

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 (SSa*X1*-4) pulsotype. Indistinguishable/similar cluster of (SSa*X* 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 SSa*X 1*-4 pulsotype while cluster-II (SSa*X*5) indicates transfer from abattoir to butchery. The unique isolate in cluster-III (SSa*X*6) 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 ttradition 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

https://doi.org/10.1016/j.heliyon.2018.e01025

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 tetrathionate 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

https://doi.org/10.1016/j.heliyon.2018.e01025

Table 1. Description of studied sample origin, location, type and numbers used for isolation of *Salmonella* Saintpaul along studied beef lines in Ethiopia.

Line	Origin of sample		Sampling location	Sample type	No. of samples
Abattoir line	Abattoir	Environment	Personnel's hands	Swabs from hands	13
			Aprons	Swabs from aprons	14
			Knives	Swabs from knives	13
			Tap water	Water samples	12
			Hooks	Swabs from hooks	11
			Rooms	Swabs from rooms	17
			Refrigerators	Swabs from refrigerators	10
			Meat transport trucks	Swabs from trucks	11
		ARM*	Stunning	Animal feces	34
			Evisceration	Mesenteric lymph node samples	34
			Quality inspection	Raw meat samples	3
	Butchers'		Beef for public consumption	Retailed meat sample	34
	Total				237
Processing plant line	Processing plant	Environment	Personnel's hands	Swabs from hands	19
			Aprons	Swabs from aprons	16
			Knives	Swabs from knives	15
			Cutting plates	Swabs from plates	13
			Tap water	Samples from water	17
			Working tables	Swabs from tables	17
			Room floors	Swabs from rooms	16
			Refrigerators	Swabs from refrigerators	15
			Spices	Samples from spices	15
			Spice-weighing equipment	Swabs from SWE	15
			Grinder	Swabs from grinders	9
			Cutter	Swabs from cutters	9
			Mixer	Swabs from mixers	9
			Filler/Stuffer	Swabs from fillers	9
			Subtotal		194
		ARM*	Before processing	Samples from raw meat	118
	Supermarkets		Supermarket A	Samples from product	15
			Supermarket B	Samples from product	15
			Supermarket C	Samples from product	15
			Supermarket D	Samples from product	14
			Supermarket E	Samples from product	15
			Supermarket F	Samples from product	15
			Supermarket G	Samples from product	15
			Supermarket H	Samples from product	15
			Subtotal		119
	Total				431
Grand total					668

*ARM = Animal-related materials.

using *Salmonella* antisera (Sifin, 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;

https://doi.org/10.1016/j.heliyon.2018.e01025

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 refernce 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.

https://doi.org/10.1016/j.heliyon.2018.e01025

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.

https://doi.org/10.1016/j.heliyon.2018.e01025

Table 3. Phenotypic drug susceptibility/resistant profile of the isolated *S.* Saintpaul isolated from beef line in Ethiopia.

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)

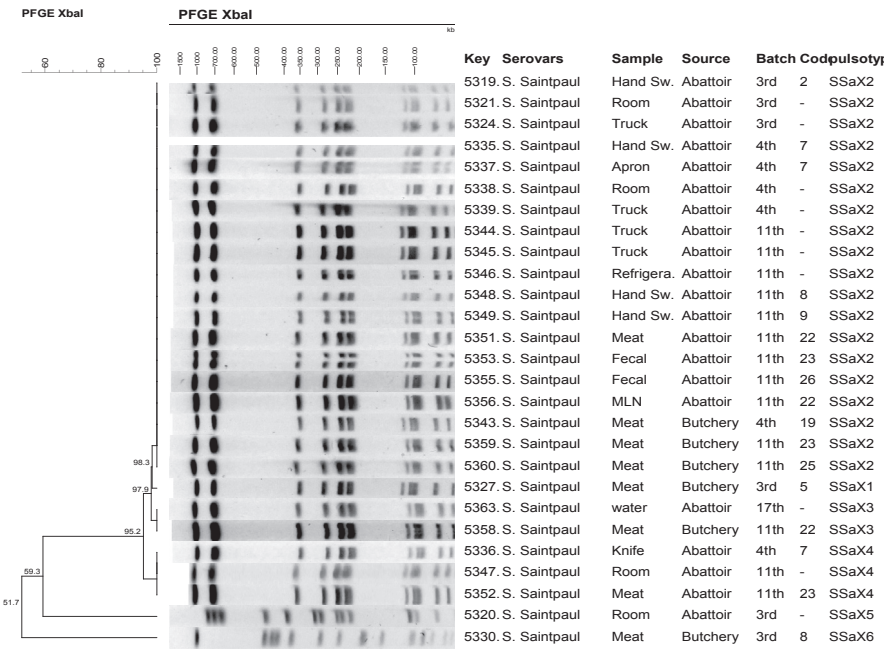
Note: S* = susceptible; I* = intermediate; R* = resistance.

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

https://doi.org/10.1016/j.heliyon.2018.e01025



Key	Serovars	Sample	Source	Batch	Cod	pulsotyp
5319.	S. Saintpaul	Hand Sw.	Abattoir	3rd	2	SSaX2
5321.	S. Saintpaul	Room	Abattoir	3rd	-	SSaX2
5324.	S. Saintpaul	Truck	Abattoir	3rd	-	SSaX2
5335.	S. Saintpaul	Hand Sw.	Abattoir	4th	7	SSaX2
5337.	S. Saintpaul	Apron	Abattoir	4th	7	SSaX2
5338.	S. Saintpaul	Room	Abattoir	4th	-	SSaX2
5339.	S. Saintpaul	Truck	Abattoir	4th	-	SSaX2
5344.	S. Saintpaul	Truck	Abattoir	11th	-	SSaX2
5345.	S. Saintpaul	Truck	Abattoir	11th	-	SSaX2
5346.	S. Saintpaul	Refrigera.	Abattoir	11th	-	SSaX2
5348.	S. Saintpaul	Hand Sw.	Abattoir	11th	8	SSaX2
5349.	S. Saintpaul	Hand Sw.	Abattoir	11th	9	SSaX2
5351.	S. Saintpaul	Meat	Abattoir	11th	22	SSaX2
5353.	S. Saintpaul	Fecal	Abattoir	11th	23	SSaX2
5355.	S. Saintpaul	Fecal	Abattoir	11th	26	SSaX2
5356.	S. Saintpaul	MLN	Abattoir	11th	22	SSaX2
5343.	S. Saintpaul	Meat	Butchery	4th	19	SSaX2
5359.	S. Saintpaul	Meat	Butchery	11th	23	SSaX2
5360.	S. Saintpaul	Meat	Butchery	11th	25	SSaX2
5327.	S. Saintpaul	Meat	Butchery	3rd	5	SSaX1
5363.	S. Saintpaul	water	Abattoir	17th	-	SSaX3
5358.	S. Saintpaul	Meat	Butchery	11th	22	SSaX3
5336.	S. Saintpaul	Knife	Abattoir	4th	7	SSaX4
5347.	S. Saintpaul	Room	Abattoir	11th	-	SSaX4
5352.	S. Saintpaul	Meat	Abattoir	11th	23	SSaX4
5320.	S. Saintpaul	Room	Abattoir	3rd	-	SSaX5
5330.	S. Saintpaul	Meat	Butchery	3rd	8	SSaX6

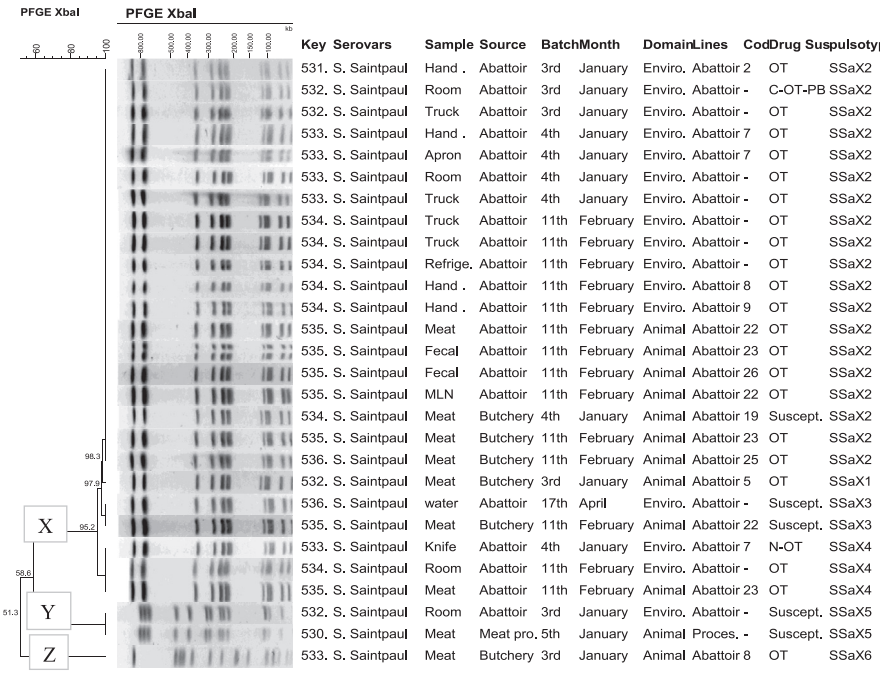
Fig. 1. PFGE analysis of *S.* Saintpaul isolates from a cattle abattoir line, Addis Ababa, Ethiopia, 2011−2012. Hand Sw = hand swabs; MLN = mesenteric lymphnode sample; Refrigera. = chilling room sample.

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 differed 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.

https://doi.org/10.1016/j.heliyon.2018.e01025



Key	Serovars	Sample	Source	Batch	Month	Domain	Lines	Cod	Drug Susp	ulsoty
531.	S. Saintpaul	Hand .	Abattoir	3rd	January	Enviro.	Abattoir 2	OT		SSaX2
532.	S. Saintpaul	Room	Abattoir	3rd	January	Enviro.	Abattoir -	C-OT-PB		SSaX2
532.	S. Saintpaul	Truck	Abattoir	3rd	January	Enviro.	Abattoir -	OT		SSaX2
533.	S. Saintpaul	Hand .	Abattoir	4th	January	Enviro.	Abattoir 7	OT		SSaX2
533.	S. Saintpaul	Apron	Abattoir	4th	January	Enviro.	Abattoir 7	OT		SSaX2
533.	S. Saintpaul	Room	Abattoir	4th	January	Enviro.	Abattoir -	OT		SSaX2
533.	S. Saintpaul	Truck	Abattoir	4th	January	Enviro.	Abattoir -	OT		SSaX2
534.	S. Saintpaul	Truck	Abattoir	11th	February	Enviro.	Abattoir -	OT		SSaX2
534.	S. Saintpaul	Truck	Abattoir	11th	February	Enviro.	Abattoir -	OT		SSaX2
534.	S. Saintpaul	Refrige.	Abattoir	11th	February	Enviro.	Abattoir -	OT		SSaX2
534.	S. Saintpaul	Hand .	Abattoir	11th	February	Enviro.	Abattoir 8	OT		SSaX2
534.	S. Saintpaul	Hand .	Abattoir	11th	February	Enviro.	Abattoir 9	OT		SSaX2
535.	S. Saintpaul	Meat	Abattoir	11th	February	Animal	Abattoir 22	OT		SSaX2
535.	S. Saintpaul	Fecal	Abattoir	11th	February	Animal	Abattoir 23	OT		SSaX2
535.	S. Saintpaul	Fecal	Abattoir	11th	February	Animal	Abattoir 26	OT		SSaX2
535.	S. Saintpaul	MLN	Abattoir	11th	February	Animal	Abattoir 22	OT		SSaX2
534.	S. Saintpaul	Meat	Butchery	4th	January	Animal	Abattoir 19	Suscept.		SSaX2
535.	S. Saintpaul	Meat	Butchery	11th	February	Animal	Abattoir 23	OT		SSaX2
536.	S. Saintpaul	Meat	Butchery	11th	February	Animal	Abattoir 25	OT		SSaX2
532.	S. Saintpaul	Meat	Butchery	3rd	January	Animal	Abattoir 5	OT		SSaX1
536.	S. Saintpaul	water	Abattoir	17th	April	Enviro.	Abattoir -	Suscept.		SSaX3
535.	S. Saintpaul	Meat	Butchery	11th	February	Animal	Abattoir 22	Suscept.		SSaX3
533.	S. Saintpaul	Knife	Abattoir	4th	January	Enviro.	Abattoir 7	N-OT		SSaX4
534.	S. Saintpaul	Room	Abattoir	11th	February	Enviro.	Abattoir -	OT		SSaX4
535.	S. Saintpaul	Meat	Abattoir	11th	February	Animal	Abattoir 23	OT		SSaX4
532.	S. Saintpaul	Room	Abattoir	3rd	January	Enviro.	Abattoir -	Suscept.		SSaX5
530.	S. Saintpaul	Meat	Meat pro.	5th	January	Animal	Proces. -	Suscept.		SSaX5
533.	S. Saintpaul	Meat	Butchery	3rd	January	Animal	Abattoir 8	OT		SSaX6

Fig. 2. Dendrogram and resistance profiles of *S*. Saintpaul isolated from beef abattoir and processing plant line. X = cluster-I; Y = cluster-II; Z = cluster-III. Drug code for which the isolate resistant: C = Chloramphenicol; OT = Oxytetracycline; PB = Polymyxin B; N = Neomycin.

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%)

https://doi.org/10.1016/j.heliyon.2018.e01025

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 asses 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 susceptibly 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.

https://doi.org/10.1016/j.heliyon.2018.e01025

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.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We appreciate the cooperation of Addis Ababa City Abattoir, the Beef Processing Plant and the Addis Ababa City Supermarkets during sample collection. Ethiopian Engineering Capacity Building was also appreciated for facilitation of the research.

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