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Zoonotic and spoilage bacteria in a meat production and a processing line in Ethiopia

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To my mother *Anaajii Bakkali* and my families

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LIST OF ABBREVIATIONS

%	Percentage
µg	microgram
µl	microlitter
AAAE	Addis Ababa Abattoir Enterprise
AAU	Addis Ababa University
ALIPB	Aklilu Lemma Institute of Pathobiology
APC	Aerobic Plate Count
BHI	Brain Heart Infusion
BPLS	Brilliantgreen-Phenolred-bile-Lactoseagar
BPW	Buffer Peptone Water
CCPs	Critical Control Points
cfu	colony forming units
CI	confidence interval
CLSI	Clinical Laboratory Standards Institute
cm	centimeter
cm^2	centimeter square
$_{dd}H_2O$	double distilled water
DIAS-II	Digital Imaging and Analysis System II
EBC	*Enterobacteriaceae* count
EDTA	Ethylene diamine tetraacetic acid
FAO	Food and Agricultural Organization
g	gram
h	hours
H_2S	hydrogen sulfide
HACCP	Hazard analysis critical control points
HCl	Hydrochloric acid
kb	kilo base pair
kg	Kilogramm
km	Kilometer
l	liter
log_{10}	common logarithmic
MDR	Multidrug resistance
mg	milligram
min	minute
MKTTn	Muller Kaufmann Tetrathionat novobiocin
ml	milliliter
mM	milliMole
mm	millimeter
O/F	oxidation fermentation
^oC	degree Celsius
PFGE	Pulsed-field gel electrophoresis
RV	Rappaport-Vassiliadis Medium
TE	Tris-EDTA
VRBD	Violet Red Bile Dextrose Agar
XLT4	Xylose Lactose Tergitol™ 4

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1. INTRODUCTION

Food ecosystems are influenced by environment, technological impact and micro-organisms in the area (Montville *et al.*, 2012). Spoilage and zoonotic agents enter the chain at any point of the production line (Montville *et al.,* 2012; UADA, 2012; Jay *et al.*, 2005; Lewis *et al*., 2005) resulting in loss of shelf-life, economic loss and risk to public health (Jay *et al.*, 2005). Researchers like AMR (2009), Mead *et al. (*1999), CDC (2007) and CDC (2011) estimated a huge proportion of infections caused by contamination with *Salmonella*. *E. coli* is recognized as an indicator organism used to measure the hygienic conditions of surfaces in food production facilities before, during and after operations (Kornacki, 2011, Jay *et al.,* 2005). Its presence in water indicates the presence of fecal contamination and the likelihood of other pathogenic microbes (Zamxaka *et al.,* 2004). *E. coli* may also harbor human pathogenic gene sequences that cause diarrhea worldwide. This can be lethal particularly in children (Turner *et al.*, 2006; Hirsh and Zee, 1999). According to some researchers (Wagenlehner *et al.*, 2008; Kashef *et al.*, 2010; Khan *et al.*, 2002; and Biedenbach *et al.*, 2004, for example), complications are hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTCP).

Characterization of foods also includes testing for zoonotic agents with conventional microbiological, serological and molecular biological techniques. Testing for micro-organism spoilage includes the Aerobic Plate Count (APC) and the *Enterobacteriaceae* Count (EBC) as a verification of sanitation (Freier, 2004). Microbial load at a given point is useful to determine critical control points (CCPs) as an element of food safety (Smith *et al.,* 1999; Montville *et al.*, 2012, Jay *et al.*, 2005).

In Ethiopia, little information is available on the status of food safety. Animals such as cattle, sheep, goats, chicken and pigs are food animals in the country (Brighter Green, 2011) and there is a tradition of consuming raw meat from some of these animals. This means there are risks of infection with zoonotic agents (Hiko *et al.,* 2008; Nyeleti *et al.,* 2000; Molla *et al.,* 1999). Hence, pre-slaughter and slaughter, post-harvest, processing and preservation techniques are important for the assessment of hygienic quality of meat and meat products (Kerry *et al.,* 2002). Although slaughtering and processing has a long history in Ethiopia, meat processing to products such as mortadella is a relatively new technology.

1.1 Objectives of the study

The rationale of this study:

Globally, local food chains are differently organized, placed in different environments and using different technical means. For food animal chains, prerequisites for product safety are the health of animals and the hygiene along the complete chain from the place of origin of the animals up to consumption.

Considering local circumstances as important factors with regard to food safety, local insight is another important prerequisite for keeping an acceptable level of food safety.

In this study, two Ethiopian cattle chains (from an abattoir to local butchers and from a beef processing plant to supermarkets) were analysed in order to provide the information needed for an assessment of beef from selected Ethiopian cattle chains.

Hence, the objectives of this study are to:

- detect points of risk in meat production and processing lines particularly with regard to APC, *Enterobacteriaceae, Salmonella,* and *E. coli*
- assess the prevalence of *Salmonella* serotypes and *E. coli* in meat production and handling chains
- assess the source and transmission routes of *Salmonella* serotypes usi*ng* PFGE
- identify critical control points *(*CCPs) in beef production and processing chains using APC, EBC and *E. coli* as indicators
- evaluate the microbiological quality of the final product using APC and EBC
- perform antimicrobial susceptibility/resistance tests on the isolates

2. LITERATURE REVIEW

2.1 Spoilage bacteria

The examination of meats for the presence, types and numbers of micro-organisms in beef slaughtering at specific points in the process and/or in their products is basic to food microbiology (Freier, 2004; Schaffner and Smith, 2004). Assessment of the hygienic situation using enumeration of organisms is indicative of fecal contamination pointing to potential public health significance (Biss and Hathaway, 1995; Freier, 2004). Differences can be observed in total bacteria associated with dirty hides, rooms, workers' hands, clothes and equipment acting as intermediate sources of contamination of meat (Gilmour *et al.*, 2004; Abdalla *et al.,* 2009). They result in cross-contamination of carcasses along operations. Contaminations could extend to the end product recipient (Freier, 2004; Schaffner and Smith, 2004).

2.1.1 Aerobic Plate Count (APC)

According to Jay *et al.* (2005), there are four basic methods employed for identifying "total" numbers: standard plate counts (SPC) or aerobic plate counts (APC) for viable cells or colony forming units (cfu), the most probable numbers (MPN) method as a statistical determination of viable cells, dye reduction techniques to estimate numbers of viable cells that possess reducing capacities, and direct microscopic counts (DMC) for both viable and nonviable cells. However, standard plate counts (SPC) or aerobic plate counts (APC) for viable cells or colony forming units (cfu) are the most widely used methods (Freier, 2004; Schaffner and Smith, 2004; Jay *et al.,* 2005) that indicate verification of sanitation in a food product (Freier, 2004).

2.1.1.1 Examination procedure

Laboratory methods which allow rapid and accurate quantification of microbiological hazards enhance monitoring and investigation of contamination throughout the food chain (HPA, 2009). The Aerobic plate count (Freier, 2004) can easily be done by manual surface plating using 9 ml dilution blanks where only 0.1 ml of dilution is plated onto the agar surface,

followed by even distribution using a bent glass rod, incubation and enumeration (Montville *et al.*, 2012; Jay, 2004). Depending on availability, nutrient agar or blood agar can be used.

2.1.1.2 APC in food safety and quality

Examination of food for APC or Standard Plate Count (SPC) is an indicator of quality for extended shelf-life of foods, but not directly for a safety assessment of ready-to-eat food due to presence of product- specific bacteria such as lactic acid bacteria (mainly lactobacilli and streptococci)in certain food commpodities (HPA, 2009). Testing environmental samples from food makes a positive and additional contribution to food safety for hygiene investigation and follow-up assessment of the effectiveness of cleaning of premises (HPA, 2009; Freier, 2004).

2.1.1.3 APC reports along meat production chains

Literature on processing food of animal origin, their products, sources, spices, sample type, country of study, and reported load (Table 2 - 01) shows an increase in the total/aerobic bacterial load from abattoirs to processing plants, with an increase in the load along the steps. Abdalla *et al.* (2009) from Sudan reported absence of differences in total viable count (TVC) in three parts of a carcass (shoulder, neck and brisket) after skinning, evisceration and washing steps, and similarly on workers' hands after processing steps. Nouichi and Hamdi (2009) from Algeria reported slightly higher TVC in bovine carcasses than in ovine carcasses. A study conducted by Gurmu and Gebretinsae (2013) in Ethiopia showed an increase in standard plate counts at butchers', from pre- to post-processing on tables, workers' hands and knives.

To date, scarce data are available on microbial load examinations of meat processing technology in Ethiopia. Therefore, a study similar to the present one and undertaken by Barros *et al.* (2007) in Brazil was reviewed here. Barros *et al.* (2007) found an increase in mesophilic aerobic counts (MAC) in the processing steps at plant facility (equipment) points and in beef along the processing steps. Adzitey *et al.* (2014) reported higher APC from tables than from beef and aprons of butchers in Ghana. Of all, Omoruyi *et al.* (2011) reported higher Total Colony Countin meat contact surfaces in Nigeria than in other countries.

Table 2 - 01: Aerobic Plate Count (APC) samples from meat processing lines

Source	Species	Types of sample/site	Count	Unit	Mean ± SD	References	Country
Abattoir	Bovine	Meat sample	APC	log $_{10}$ cfu/g	5.21±0.46	Gebeyehu *et al.* (2013)	Ethiopia
		Carcass shoulder after:	TVC	log $_{10}$ cfu/cm^2		Abdalla *et al.* (2009)	Sudan
		skinning			3.03 ± 0.15		
		evisceration			2.73 ± 0.02		
		washing			2.79 ± 0.10		
		Carcass neck after:	TVC	log $_{10}$ cfu/cm^2			
		skinning			3.65 ± 0.02		
		evisceration			3.42 ± 0.02		
		washing			3.72 ± 0.02		
	Bovine	Carcass brisket after:	TVC	log $_{10}$ cfu/cm^2			
		skinning			3.1 ± 0.14		
		evisceration			3.71 ± 0.04		
		washing			3.65 ± 0.02		
		Workers hands after:	TVC	log $_{10}$ cfu/cm^2			
		skinning			3.74±0.02		
		evisceration			3.42±0.02		
		washing			3.71±0.02		
	Bovine	Bovine carcasses	TVC	log $_{10}$ cfu/g	4.48 ± 0.63	Nouichi and Hamdi (2009)	Algeria
	Ovine	Ovine carcasses	TVC	log $_{10}$ cfu/g	3.11 ± 0.68		
	Bovine	Beef contact surface	TCC	x 10^6cfu	26.50-592.50	Omoruyi *et al.* (2011)	Nigeria
Beef processing plant	Bovine	Equipment:	MAC	log $_{10}$ cfu/cm^2		Barros *et al.* (2007)	Brazil
		Knifes			4.06±1.07		
		Tables			4.42±1.06		
		Grinder			5.15±1.73		
		Meat saw/cutter			3.4±0.90		
		Mixer			5.25±2.83		
		Sausage stuffer			5.43±2.43		
		Installation	MAC	log $_{10}$ cfu/cm^2			
		Refrigeration systems			2.29±0.29		
		Floors			4.76±1.15		
		Products	MAC	log $_{10}$ cfu/g			
		Bovine carcasses			3.60±1.27		
		Ground beef			6.49±1.73		
		Fresh sausages			5.89±1.19		
		Cooked sausages			5.78±0.16		
Butchers'	Bovine	Pre-processing:	SPC	log $_{10}$ cfu/cm^2		Gurmu and Gebretinsae (2013)	Ethiopia
		Tables			6.28		
		Hands			5.67		
		Knifes			5.30		
		Post-processing:	SPC	log $_{10}$ cfu/cm^2			
		Tables			6.56		
		Hands			6.15		
		Knifes			6.89		
Retail shop	Bovine	Beef	APC	x cfu/cm^2	5.0×10^6	Adzitey *et al.* (2014)	Ghana
		Tables			3.7×10^7		
		Aprons			3.1×10^5		

TCC = Total colony count TVC = Total viable count
SPC = Standard plate count MAC = Mesophilic aerobe counts

2.1.2 *Enterobacteriaceae* Count (EBC)

Enterobacteriaceae counts are markers of fecal contamination and agents are, among others, responsible for meat spoilage. However, they may have zoonotic importance (Jay *et al.,* 2005; Quinn and Markey, 2003). The majority of *Enterobacteriaceae* on meat, meat products and environmental samples of meat production (Schaffner and Smith, 2004; Jay *et al.*, 2005) are indicators of food safety (Gree and Nattress, 2004).

2.1.2.1 Examination procedure

Dilution and inoculation procedures for EBC are principally the same as those of APC. However, for EBC, specific culture media, e-g-, Violet Red Bile Dextrose Agar (Oxoid, England) are used (Montville *et al.,* 2012; USDA, 2012). Then plates are incubated at 30^{o}C and colonies are counted after 48 hrs. of incubation.

2.1.2.2 EBC in food safety and quality

Enterobacteriaceae originate from the intestinal tract of animals and humans and also from plants and the environment (HPA, 2009; Roberts and Greenwood, 2003). They are used to assess the general hygiene status of a food product (HPA, 2009; Gree and Nattress, 2004). Indicator bacteria may be associated with an increased likelihood of the presence of pathogens which are useful for an assessment of food safety. They are relatively quick and easy to identify (HPA, 2009; Schaffner and Smith, 2004).

Table 2 - 02 shows EBC from abattoirs, meat processing plants and products from various countries and the respective microbial loads. Adetunji and Isola (2011) studied meat in Nigeria and reported a significantly higher microbial count on working tables after meat sales than before meat sales, demonstrating an increase in contamination on working tables. Nouichi and Hamdi (2009) from Algeria found similar FCC loads in bovine and ovine carcasses. Barros *et al.* (2007) reported low (1.95±0.99 log cfu/cm^2) total coliform counts (TCC) from meat saws/cutters, but an increasing count in the processing steps at processing plant facility (equipment) points and in beef products along the processing steps.

Table 2 - 02: *Enterobacteriaceae C*ount samples from meat processing lines

Source	Spec	Types of sample	Bact. count	Unit	Mean±SD	References	Country
Abattoir	Bov	Meat sample	TCC	log $_{10}$ cfu/g	1.72±0.63	Gebeyehu *et al.* (2013)	Ethiopia
	NM	Working tables:		log $_{10}$ cfu/g		Adetunji and Isola (2011)	Nigeria
		before sales	EBC		8.81±0.05		
		after meat sales	EBC		11.47±0.03		
		before sales	Coliform		8.35±0.07		
		after meat sales	Coliform		10.86±0.05		
	Bov	Beef contact surface	TCC	x 10^3cfu	14.25-33.75	Omoruyi *et al.* (2011)	Nigeria
		Bovine carcasses	TCC	log $_{10}$ cfu/g	2.92 ± 0.43	Nouichi and Hamdi (2009)	Algeria
		Bovine carcasses	FCC	log $_{10}$ cfu/g	2.60 ± 0.32		
	Ov	Ovine carcasses	FCC	log $_{10}$ cfu/g	2.55 ± 0.53		
Beef processing plant	Bov	Equipment:	TCC	log $_{10}$ cfu/cm^2		Barros *et al.* (2007)	Brazil
		Knifes			2.36±1.25		
		Tables			2.50±0.90		
		Grinder			3.11±1.29		
		Meat saw/cutter			1.95±0.99		
		Mixer			3.03±1.77		
		Sausage stuffer			3.04±2.86		
		Installation		log $_{10}$ cfu/cm^2			
		Refrigeration			1.75±0.57		
		systems Floors			2.26±1.23		
		Products		log $_{10}$ cfu/g			
		Bovine carcasses			1.49±1.15		
		Ground beef			3.32±0.98		
		Fresh sausages			3.27±1.13		
		Cooked sausages			not tested		
Abattoir	Ov	Carcass swab	EBC	cfu/cm^2	2.54±.44×10^3	Saad *et al.* (2011)	Egypt
	Bov				1.33±0.26×10^3		
	Cam				5.91±1.02×10^2		
	Ov	Carcass swab	TCF	cfu/cm^2	2.97±0.51×10^3		
	Bov				8.54±1.67×10^2		
	Cam				2.28± 0.75×10^2		
Abattoir	Bov	Beef processing stage	TCF	log cfu/g		Niyonzima *et al.* (2013)	Rwanda
		Skinning			3.1±0.5		
		Dressing			3.5±1.7		
		Transportation			3.9±0.5		
		Marketing			7±0.8		

TCC = total coliform counts,
EBC = *Enterobacteriaceae count*,
FCC = Fecal coliform counts,
- = not reported
Bact. = Bacterial

NM = Not mentioned,
Bov = Bovine
Ov = Ovine
Cam = Camele

2.2 *Salmonella*

2.2.1 Microbiology of *Salmonella*

Salmonellae are small, gram-negative, non-sporing rods (Jay *et al.,* 2005) distributed in nature, with humans and animals being their primary reservoirs (Nielsen, 2004). These organisms are able to grow on a large number of culture media and produce visible colonies within 24 hours at about 37^{o}C. They are generally unable to ferment sucrose and lactose but can do glucose and some other monosaccharides with production of gas. Although they normally utilize amino acids as nitrogen sources, in the case of *S.* Typhimurium, nitrate, nitrite, and NH_3 serve as sole sources of nitrogen. *Salmonella* usually produce hydrogen sulfide (H_2S) (Grimont and Weill, 2007). With the exception of *S.* Pullorum and *S.* Gallinarium, which are naturally non-motile, *Salmonella* serotypes are motile with atrichous or peritrichous flagella. But the motile ones can become non-motile if the flagella happen to dysfunction (D'Aoust, 1997). *Salmonella* are facultative intracellular bacteria found in a variety of phagocytic and non-phagocytic cells *in vivo* (Ibarra and Steele-Mortimer, 2009).

Some significant changes have been adopted for the taxonomy of *Salmonella*. These changes are based on DNA-DNA hybridization and multilocus enzyme electrophoretic characterizations of the *Salmonella.* Using somatic (O) and flagella (H) antigens, to date 2,579 serotypes have been identified (Grimont and Weill, 2007). All have been categorized in two species, *S. enterica* and *S. bongori* (Grimont and Weill, 2007; Quinn *et al.,* 2002). Most of them are classified under *S. enterica* as group I (*S. enterica* subsp. *enterica*), group II (*S. enterica* subsp. *salamae*), group IIIa (*S. enterica* subsp. *arizonae*), group IIIb (S. *enterica* subsp. *diarizonae*), group IV (*S. enterica* subsp. *houtenae*), and group V (*S. enterica* subsp. *indica*) (Grimont and Weill, 2007).

2.2.2 Sources and transmission of *Salmonella*

The primary habitat of *Salmonella* species is the intestinal tract of animals such as birds, reptiles, farm animals, and occasionally insects and humans (Nielsen, 2004). They may also be found in other parts of the body and environments including water. Once infected with these organisms, an individual can act as a common shedder of the organism, usually through

feces, but unnoticed (Nielsen, 2004; Jay *et al.,* 2005). Such distribution of *Salmonella* in the environment, their increase in prevalence in the global food chain, and their virulence and adaptability properties cause easy transmission, resulting in enormous medical, public health and economic impact worldwide (Molbak *et al.,* 2006).

2.2.3 Salmonellosis

According to Jay *et al.* (2005) and Krauss *et al.* (2003), for clinical and epidemiological purposes, *Salmonella* can be divided into three groups. The first group consists of *Salmonella* that infect humans only (*S.* Typhi, *S*. Paratyphi A and *S.* Paratyphi C), and *S*. Paratyphi B (humans and animals). These are the agents that specifically cause typhoid and paratyphoid fevers of humans. The second group encompasses host-adaptive serovars of which some are human pathogens and may be contracted from food. They are *S*. Gallinarum (in poultry), *S*. Dublin (in cattle), *S*. Abortus ovis (in sheep), *S*. Choleraesuis (in swine) and *S*. Abortus equi (in horses). The third group comprises of unadapted serovars (non-host preferences), pathogenic to humans and animals, so they are considered food borne agents (Jay *et al.,* 2005; Quinn *et al.,* 2004) that cause gastroenteritis and develop into a poisoning syndrome in 12-14 hrs. (Jay *et al.,* 2005). Symptoms usually include nausea, vomiting, abdominal pain, headache, chills, and diarrhea accompanied by prostration, muscular weakness, faintness, moderate fever, restlessness and drowsiness usually persisting for 2-3 days, bacteremia, and extra-intestinal localized infections involving many organs (Jay *et al.,* 2005).

The average mortality rate is 4.1%, varying from 5.8% during the 1st year of life to 2% between the 1st and 50th year, and is 15% in persons over 50 years. The non-typhoid *Salmonella* case-fatality rates for immuno-compromised infants and children are 43% and 10%, respectively, and 5% and 0% for non-immuno-compromised ones (Sirinavin *et al.,* 1999).

2.2.4 *Salmonella* characterization

Identification and characterization of *Salmonella* involve utilization of combined phenotypic and/or genotypic techniques for the differentiation of specific strains into species and sub-species (Adams and Moss, 2008). Serology based on surface antigens (Grimont and Weill,

2007), phage typing based on the bacteriophage host profile (Jay *et al.,* 2005), antimicrobial susceptibility and biotypes which use biochemical tests to reflect metabolic activities of *Salmonella* strains (WHO, 2010) are used for phenotypic characterization. According to Foley *et al.* (2009), genotyping techniques used here are grouped into three categories as: (1) restriction analysis of bacterial DNA; (2) amplification of particular genomic targets by Polymerase Chain Reaction (PCR); and (3) identification of DNA sequence polymorphisms. Genotypic analysis involves molecular genetic approaches using Restriction Fragment Length Polymorphism (RFLP), RFLP Ribotyping, Amplified Fragment Length Polymorphism (AFLP), Random Amplification of Polymorphic DNA (RAPD), Variable Number of Tandem Repeats (VNTR) and Multiple Locus VNTR Analysis (MLVA). Pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (Miller, 2013; PulseNet, 2013) are used for these purposes.

2.2.4.1 Salmonella serotyping

Serological identification of *Salmonella* was first established by White and Kauffmann in 1920. They identified 20 O-groups. Later on the procedure was used for a *Salmonella* classification system based on serological methods (Molbak *et al.,* 2006). Polymorphism of somatic lipopolyssacharide (O-antigens) and flagellar (H-antigens) were used for *Salmonella* serotyping (Appendix 10.2.2). Based on these antigens, 2,579 serotypes were identified from *S. enterica* (n=2,557) and *S. bongori* (n=22) (Grimont and Weill, 2007).

2.2.4.2 Pulsed-Field Gel Electrophoresis (PFGE)

PFGE is widely used for epidemiological investigations to determine primary sources to track transmission routes and for the distribution of food borne gram-negative pathogens (Foley *et al.*, 2009).

2.2.4.2.1 PFGE principle

Traditional phenotyping procedures such as serotyping has a limited significance for epidemiologic assessments of *Salmonella* transmission due to its poor ability to discriminate closely related isolates (Johnson *et al.,* 2001; Olsen *et al.,* 1994). Genotyping methods such as

Pulsed-field Gel Electrophoresis (PFGE) have been developed and standardized for genetic discrimination of *Salmonella* isolates from outbreaks and epidemiological investigations (Liebana *et al.*, 2001; Zou *et al.*, 2010; Fey *et al.*, 2012). The principle was based on whole chromosomal DNA digestion with the use of one or more specific restriction enzymatic endonucleases at selected genomic restriction sites. Between 8 and 25 high molecular weight DNA fragments of 10-800 kilobase (kb) size were results of this process (Wiedmann, 2002; Jay *et al.*, 2005). Some of the restriction enzymes used for *Salmonella* PFGE are *XbaI, BlnI; SpeI*, *SfiI*, *PacI* and *NotI*. Zheng *et al.* (2007) showed differences in the discriminary power of each of these enzymes independently and/or in different combinations. DNA fragments are separated by PFGE in agarose containing gels. This sub-typing method is characterized by variations in the polarity of the electric field at determined intervals of pulse time and the size of DNA molecules in kb (PulseNet, 2013). The fingerprints derived from the process aree analyzed by programmes to determine clonal diversity and relations between the isolates (Olive and Bean, 1999).

2.2.4.2.2 Computer assisted data analysis

Ethidium Bromide is a carcinogen that binds to DNA and allowss to visualize the bands under UV-light. When the gel is viewed, it is photographed and/or stored electronically. The digital image that is captured can then be examined with the aid of computer programs designed to carry out specific tasks related to PFGE.

According to the BioNumeric® (2011) http://www.applied-maths.com/bionumerics version 6.6, the markers that run on each gel are references that allow the bands to be compared on the basis of position, which corresponds to DNA fragment size in several lanes from one bacterium (resulting from a single colony).

The gels from PFGE are transferred into BioNumeric® software and are then processed in 4 steps (1. Strip; 2. Curves; 3. Normalization; and 4. Bands).

Step 1. Strip: At this step, strip patterns are defined following the program which asks the:

1. number of tracks
2. thickness in number of points (pts) of the image strips that the blue lines enclose in the complete patterns
3. number of nodes (possible 3-4) which allow to bend the strips locally
4. background subtraction and spot removal which allow gel scans with regular background and spots or artifacts to be cleaned up to a certain extent

Step 2. Curve: After defining strips, a densitometric curve is applied/preformed

Step 3. Normalization: This step is performed in reference to the position of the marker. The program extracts the densitometric curve from the image file using the information entered at the strip step. Reference positions are defined at the normalization step where the program automatically determines the molecular weight registered from the size entered at the image

Step 4. Bands: This is the last step in processing gels useful for defining and quantifying bands. At this step, the program defines bands according to band search filters which involve:

- percentage of minimum profiling (elevation of band with respect to the surrounding)
- percentage of gray zones which specify the band as uncertain bandminimum area as percentage of the total area of pattern, and shoulder sensitivity allows shoulders without a local maximum as well as doublets of bands with one maximum to be found

Using BioNumerics software for similarity by *Dice* coefficients, calculation with a) optimization (%) that allows between any two patterns to look for the best possible matching; and b) band position tolerance (%) with a maximum percentage of shift allowed between two bands for matching.

For analysis, the Unweight Pair Group Method with Arithmetic mean (UPGMA) is the result when applying cluster analysis. Following data selection from data setting entry using the comparison window in the BioNumerics software, a dendrogram is filed, experimental data and information filed are displayed for any analysis and interpretation. Tenover *et al.* (1995) defined epidemiologically related isolates and described criteria for interpreting PFGE patterns (Table 2 - 03). In addition, Barrett *et al.* (2006) argued that factors such as reproducibility of the method with a particular organism, the quality of the PFGE gel, the

variability of the organism being sub-typed, and the prevalence of the pattern in question must always be considered.

Table 2 - 03: Criteria for interpretation of strain relatedness using PFGE patterns

Category	No. of genetic differences compared with outbreak strain	Typical no. of fragment differences compared with outbreak pattern	Epidemiologic interpretation
Indistinguishable	0	0	Isolate is part of the outbreak
Closely related	1	2-3	Isolate is probably part of the outbreak
Possibly related	2	4-6	Isolate is possibly part of the outbreak
Different	>3	>7	Isolate is not part of the outbreak

Source: Tenover *et al.* (1995)

Tenover *et al.* (1995:2233) defined epidemiologically related isolates as "isolates cultured from specimens collected from patients, fomites, or the environment during a discrete time frame or from a well defined area as part of an epidemiologic investigation that suggests the isolates may be derived from a common source".

2.2.5 *Salmonella* along processing lines of food of animal origin

Salmonella remain an important concern in food processing (Mead *et al.*, 1999). Most often, food items that have a risk of contamination with *Salmonella* are foods of animal origin and they differ widely from one country to another in regards to infection risks. The presence of *Salmonella* in slaughter cattle and in slaughterhouse environments with potential cross-contamination of carcasses and edible organs can pose food safety hazards in many areas, including developed countries like UK (McEvoy *et al.*, 2003).

In a study from Denmark, it was found out that infected animals and individuals can act as common shedders of organisms usually through feces, noticed or unnoticed (Nielsen, 2004). Rhoades *et al.* (2009) summarized the prevalence of *Salmonella* as 2.9% (0.0-5.5%) in feces, 60% (15-71%) in hides, 1.3% (0.2-6.0%) in chilled carcasses and 3.8 % (0.0-7.5%) in raw beef products from different developed countries. Similarly, Kagambega *et al.* (2013) reported the prevalence of 52% in cattle from Burkina Faso.

Reports from examinations of fecal and gastrointestinal contents showed *Salmonella* prevalences of 6% to 19% (Sibhat *et al.*, 2011), 10% (Nyeleti *et al.*, 2000), 3.1% (Alemayehu *et al.,* 2003), all in cattle, and 15.1% in camels (Molla *et al.,* 2004). The lymphatic system,

particularly the lymphocytes are involved in the immune function by acting as a filtering mechanism of the body system, mainly of the blood (Arthur *et al.*, 2008). Research showed that the lymphatic system, specifically the lymph nodes are potential sources of pathogenic bacteria in beef. Mesenteric Lymph Nodes (MLN) are normally discarded during evisceration. However, examination of MLN would reflect the situation of the lymph nodes found in the fatty tissue of a beef carcass. Different studies showed different results for *Salmonella* from MLN of slaughtered cattle in Ethiopia, for example, 8% (Sibhat *et al.*, 2011), 19% (Nyeleti *et al.*, 2000), and 4.5% (Alemayehu *et al.,* 2003). Moreover, Molla *et al.,* (2004) reported 15.9% from a study on MNL of camels while Teklu and Nigussie (2011) reported 5.6% and 5.0%, respectively, from sheep and goats.

Reports for *Salmonella* such as 7.7% and 2.2% from slaughter personnel hands at different positions, 12% from pens in the slaughter house (Sibhat *et al.*, 2011), 7.4% from working knifes, 7.1% from water, and 8.9% from hands at a sheep/goat abattoir (Teklu and Nigussie, 2011) show the significance of environments as sources of *Salmonella* from carcass contamination. Reports of 10% from bovine carcasses (Nouichi and Hamid, 2009), 14.4% from minced beef (Ejeta *et al.*, 2004), 28.0% from livers (Tibaijuka *et al.*, 2003), 55% from chicken, 16% from swine (Kagambega *et al.*, 2013), 2.3% from fish (Zewdu and Cornelius, 2009), and 47.8% from dairy farms (Addis *et al.*, 2011) all show the contamination of meat, edible organs, fish and dairy products posing a risk of public infection from foods. Four percent of *Salmonella* were also reported from reheated processed sausages (Abd El-Atty and Meshref, 2007) from Egypt.

With regard to human cases, Reda *et al. (*2011) reported a 100% of *Salmonella* prevalence in stools sampled at the Harar hospital in Ethiopia. Beyene *et al. (*2011) for Ethiopia also reported prevalence rates of 6.7% and 2.5% in samples of blood and stools respectively taken from hospitals in Addis Ababa and from Jimma. Moreover, Tadesse (2014) published a systematic review of a meta-analysis on the prevalence of human salmonellosis in Ethiopia. Adabara *et al.* (2012) noted a prevalence of 75% in blood samples from hospital cases in Nigeria.

Table 2 - 04 shows reports of *Salmonella* prevalences from food, food products and food handling environments like abattoirs, supermarkets and dairy sectors and also from medical cases in hospitals.

Table 2 - 04: Reports on *Salmonella* prevalence from animals, foods of animal origin, production environments and hospitals

Source	Sample type, source (species) and *Salmonella* prevalence	References	Country
Abattoir	hand swabs (at flaying) (7.7%), evisceration hand swabs (2.2%), holding pens (12%); hide swabs (31%), rumen content (19%), caecal content (6%), MLN (8%) , and carcasses (2%)	Sibhat *et al*. (2011)	
	cattle feces (10.6%) ,MLN (19.6%), abdominal (9.8%), diaphragmatic muscles (11.9%)	Nyeleti *et al.* (2000)	Ethiopia
	pooled feces (3.1%), MLN (4.5%), abdominal muscle (2.8%) and diaphragmatic muscle (3.1%)	Alemayehu *et al.* (2003)	
	feces (19%), hides (12%), raw beef products (10%)	Rhoades *et al.* (2009)	Different countries
	ovine carcasses (1.11%), bovine carcasses (10%)	Nouichi and Hamdi (2009)	Algeria
	sheep carcasses and goat carcasses (12.4%)	Teklu and Nigussie (2011)	Ethiopia
	feces of cattle (52%), chicken (55%), swine (16%), hedgehogs (96%)	Kagambega *et al.* (2013)	Burkina Faso
	Sheep: skin swab s(4.9%), MLN (5.6%), caecal contents (2.1%), carcass swabs (14.1%), evisceration hand swabs (10.6%), knife swabs (8.5%), water samples (5.0%)	Teklu and Nigussie (2011)	Ethiopia
	Goats: skin swabs (5.0%), MLN (5.0%), caecal contents (6.7%), carcass swabs (8.3%), evisceration hand swabs (15.0%), knife swabs (5.0%), water samples (12.5%)		
	Camel feces (15.1%), MLN (15.9%), livers(11.8%), spleens (14.3%), abdominal muscles (21.0%), diaphragmatic muscles (19.3%)	Molla *et al.* (2004)	Ethiopia
Supermarkets	minced beef (14.4%), mutton (14.1%), pork (16.4%) samples	Ejeta *et al.* (2004)	
	sausage (4%)	Abd El-Atty and Meshref (2007)	Egypt
	chicken meat and giblets (21.1%)	Molla and Mesfin (2003)	Ethiopia
	chicken meat (12.3%), gizzard (53.1%), livers (28.0%)	Tibaijuka *et al.* (2003)	
	minced beef (7.9%)	Nyeleti *et al.* (2000)	
	luncheon meat (0%), fresh sausages (10 %) frozen minced (6%)	Edris *et al.* (2011)	
	chicken carcasses (13.9%), pork (11.3%), minced beef (8.5%), mutton (10.8%), fish (2.3%), cottage cheese (2.1%)	Zewdu and Cornelius (2009)	
Dairy sector	cows (10.7%), humans at dairy farm (13.6%)	Addis *et al.* (2011)	
	cheese (3.1%), butter (1.04%), milk (2.1%), and yogurt (0%)	Tesfaw *et al.* (2013)	
Hospital cases	blood and stools at Addis Ababa (6.7%) and Jimma (2.5%)	Beyene *et al.* (2011)	
	stoold at Harar (100%)	Reda *et al.* (2011)	
	blood samples (75%)	Adabara *et al.* (2012)	

MLN = mesenteric lymph nodes

2.2.6 *Salmonella* serovars in Ethiopia

Salmonella findings have been reported from meats and their production environments and also from human cases. To date, published or unpublished research reports from different health institutions in Ethiopia show that salmonellosis is a problem and that *Salmonella* exist in a number of sero-groups/serotypes in humans, animals, animal food products and other foods in the country. In a study conducted on a beef abattoir, Alemayehu *et al.* (2003) reported the presence of 48% of each of *S.* Dublin and *S.* Mishmarhaemek, and 20% of *S.* Typhimurium. Sibhat *et al.* (2011) reported 54% *S.* Anatum and 19% *S.* Newport. Molla *et al.* (2004) studied camels and reported 38.8% *S*. Saintpaul, 22.4% *S*. Braenderup and 8.6% *S*. Muenchen as major serovars.

Zewdu and Cornelius (2009) reported 2 isolates of *S.* Dublin from each of minced beef, pork and mutton. Further, they found 3 isolates of *S.* Newport from minced beef, 12 from pork, 12 from mutton and 2 from fish showing the occurrence of same serovars in different foods of animal origin at supermarkets. *S*. Dublin, *S*. Typhimurium, *S*. Anatum and *S.* Newport were frequently isolated from animals and foods of animal origin in abattoirs and supermarkets. *S*. Typhimurium, *S*. Infantis, *S*. Haifa and *S*. Enteritidis were also isolated from animals and human entities in Ethiopia showing diverse ranges of hosts (Alemayehu *et al.,* 2003; Ejeta *et al.*, 2004; Beyene *et al.,* 2011) for different *Salmonella* serovars. *S*. Muenchen has also been reported (Molla *et al.,* 2004; Aragaw *et al.*, 2007). Unidentified *Salmonella* serotypes were reported by Tibaijuka *et al.* (2003) from foods at supermarkets, Aragaw *et al.* (2007) from swine, and Beyene *et al.* (2011) from humans. Table 2 - 05, shows sources and quantities (in number or percentage) of some up-to-date *Salmonella* serotypes reported from Ethiopia.

Table 2 - 05: *Salmonella* serotypes isolated and reported from Ethiopia

Sources		*Salmonella* serotype (number or %)	Ref.
Abattoir	Cattle	*S*. Dublin (48%) *S*. Enteritidis (12%) *S*. Guildford (12%) *S*. Mishmarhaemek (48%) *S*. Typhimurium (20%) *S*. Anatum (54) *S*. Bredeney (1) *S*. Eastbourne (8)*S*. II 40:b (2) *S*. Newport (19) *S*. Typhimurium (1) *S*. Uganda (1) *S*. Saintpaul (38.8%) *S*. Braenderup (22.4%) *S*. Muenchen (8.6%), *S*. Kottbus (6.0%) *S*. Havana (5.2%)	Alemayehu *et al.* (2003) Sibhat *et al.* (2011) Molla *et al.* (2004)
	Camel		
Supermarket	Chicken carcass	S. Braenderup (12) S. Hadar (6) S. Newport (4) S. Typhimurium (3) S. Kentucky (2) S. Bovismorbificans (1) S. Anatum (1)	Zewdu and Cornelius (2009)
	Minced beef	S. Newport (3) S. Dublin (2) S. Anatum (2) S. Typhimurium (1) S. Infantis (1) S. Kentucky (1) S. Saintpaul (1) S. 1:9,12:-(1)	
	Pork	S. Newport (12) S. Haifa (5) S. Dublin (2) S. Infantis (2) S. Kottbus (1)	
	Mutton	S. Newport (12) S. Typhimurium (3) S. Hadar (2) S. Dublin (2) S. Bovismorbificans (2) S. Infantis (1) S. Zanzibar (1)	
	Fish	S. Newport (2) S. Zanzibar (1)	
Supermarket	Minced beef, mutton, pork	*S*. Uganda (11.1%) *S*. Braenderup (31.5%) *S*. Haifa (3.7%) *S*. Group B (3.7%) *S*. Typhimurium (3.7%) *S*. Virchow (1.8%) *S*. Saintpaul (14.8%) *S*. Anatum (25.9%)	Tibaijuka *et al.* (2003)
		S. Infantis (36.4%) *S.*Braenderup (29.5%) *S.* Anatum (9.1%) *S.* Bovismorbificans (9.1%) *S.* Vejle (4.5%) *S.* Dublin (2.3%) *S.* Saintpaul (2.3%) *Salmonella I: 8:20:*- (4.5%) *Salmonella I: 47:z4:z23* (2.3%)	Ejeta et la. (2004)
Abattoir and supermarkets		*S.* Dublin (54.1%) *S.* Muenchen (1.0%) *S.* rough form (3.1%) *S.* Meleagridis (5.1%) *S.* Saintpaul (9.2%) *S.* Anatum (27.6%)	Nyeleti *et al.* (2000)
Hospital cases		*S*. Infantis (1) *S*. Oskarshamn (1) *S*. Pomona (1) *Salmonella* group M (28:y:-) (1) untypeable sero-groups B and D (6) *S*. Butantan (2) *S*. Colindale (1) *S*. Colorado (1) *S*. Concord (82) *S*. Enteritidis (4) *S*. Garoli (1) *S*. Gatow (3) *S*. Haifa (1) *S*. Laronchelle (1) *S*. Paratyphi B (2) *S*. Typhi (2) *S*. Typhimurium (7)	Beyene *et al.* (2011) Beyene (2008)

Ref. = References

2.2.7 PFGE as a tool for tracking possible source and transmission routes of *Salmonella*

In a study that employed PFGE, Kagambega *et al.* (2013) from Burkina Faso reported that some *Salmonella* serotypes from production (cattle, poultry and swine), wild animals (hedgehog), and humans share similarity and have the potential risk of transmission to humans. They mentioned, that humans and animals often live in close vicinity in Africa and that the hygiene control of the meat retail chain is a major public health risk in Burkina Faso.

Using the PFGE phylogenetic tool, Vanhoof *et al.* (2012) tracked the transmission routes of *S.* Concord of different pulsotypes in children adopted from Ethiopia and reported their strong relationship with isolates from that country. Kagambega *et al.* (2013), in a PFGE analysis, found out a 90 to 95% genetic similarity of S. Muenster isolates obtained from the feces of all species of animals studied and of humans.

2.3 *Escherichia coli*

2.3.1 Microbiology of *E. coli*

Pathogenic *E. coli* are responsible for foodborne gastroenteritis in humans (Jay *et al.,* 2005). The bacteria are gram negative, rod shaped, non-spore forming, motile (use peritrichous flagella) or non-motile. They grow on MacConkey agar (colonies are 2-3 mm in diameter and red or colorless) (Farmer *et al.,* 2007). These bacteria are indole production and methyl red positive but Voges-Proskauer and Simmons citrate negative. The majority (> 90% of strains) ferment a range of monosaccharides such as lactose, sorbitol, mannitol and glucose acid/gas but not cellobiose (Jay *et al.,* 2005; Quinn *et al.,* 2002). Using O-antigens and H-antigens, *E. coli* are grouped into either *Enterotoxogenic E. coli* (ETEC), *Enteroaggregative E. coli (EAggEC), Enterohamorrhagic E. coli (EHEC), Enteroinvasive E. coli (EIEC),* or *Enteropathogenic E. coli (EPEC/AEEC)* (Jay *et al.,* 2005; Quinn and Markey, 2003). To date, using O-antigen, over 200 serotypes of *E. coli* have been identified whereas around 30 have been recognized using H-antigens (Quinn *et al.,* 2002). These bacteria can also release their genome during multiplication or upon their death (Jay *et al.,* 2005; Ercolini, 2004).

E. coli isolation and characterization involves microbiological-biochemical analysis, somatic (O) and flagella (H) antigen detection (Jay *et al.,* 2005; Quinn *et al.,* 2002) and molecular-biological procedures (Kudva *et al.*, 1997). Detection of toxin and genome is useful for

identifying respective pathogenic *E. coli* strains. PFGE is used for identifying chromosomal DNA, restriction fragment length polymorphism (RFLP) is utilized for identifying genomes coding toxins (Kudva *et al.*, 1997). Further, PCR is used for strain screening (Ali *et al*., 2012). The virulence mechanisms are genetically coded for chromosomal, plasmid and bacteriophage DNAs. They include heat-labile (LT-I, LT-IIa and LT-IIb) and heat-stable (ST-I and ST-II) toxins, Vero toxin types 1, 2 and 2e, cytotoxin necrotizing factor (CNF1 and CNF2), attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanisms (Eagg) and enteroinvasive mechanisms (En'v) (Pass *et al*., 2000). Kudva *et al.* (1997) detected virulence associated genes for Shiga toxin(s) and the attaching-and-effacing lesion (stx1, stx2 and eae) in *E. coli* (STEC) strains isolated from sheep, suggesting their potential for human pathogenicity.

2.3.2 Sources and transmission of *E. coli*

Cattle and their environment are important sources of pathogenic *E. coli.* Contamination of slaughter house facilities and meat and meat products occurs during operations, ending up in transmission to humans, as was shown for USA (Elder *et al.*, 2000). Rangel *et al.* (2005) identified six main transmission routes of *E. coli.* These, in a decreasing order of importance, are 52% foodborne, 21% unknown, 14% person-to-person, 6% recreation water and 3% drinking waterborne, 3% animal contact, and 0.3% laboratory related. Jay *et al.* (2005) and Doyle *et al.* (2006) also reported food and non-food sources of *E. coli* O157:H7.

2.3.3 *E. coli* infection

Pathogenic *E. coli* strains cause enteric/diarrheagenic and/or extra-intestinal pathogenic syndromes (Bien *et al.,* 2012). Symptoms begin with non-bloody diarrhea in one to five days after consumption of contaminated food and progress to bloody diarrhea, severe abdominal pain and moderate dehydration. In young children, hemolytic uremic syndrome (HUS) is a serious complication that can lead to renal failure and death. In adults, complications sometimes lead to thrombocytopenic purpura (TPP) characterized by cerebral nervous system deterioration, seizures and strokes. Three distinct clinical entities that result from infection with inherently pathogenic strains of *E. coli* are urinary tract infection, diarrheal disease, and neonatal sepsis/meningitis (Notaro and Levine, 1994; Johnson and Nolan, 2009). Based on the disease syndromes and their characteristics in humans, their effect on certain cell cultures, and

serological grouping, *E. coli* have been categorized into five virulent groups (Jay *et al.,* 2005; Quinn and Markey, 2003).

Virulence factors for *ETEC* are fimbria, adhesions and production of *heat labile* and *heat stable* enterotoxins. The factors for *EAggEC* are small fimbrial adhesions, toxin and transcripter activator genes (Mainil, 2012); for *EHEC* they are *Shiga-like toxins* and *Vero toxins (*Mainil, 2012; Johnson and Nolan, 2009); and for *EIEC* they are direct epithelial invaders (Johnson and Nolan, 2009). *EPEC/AEEC,* as virulence factors, have the ability to induce attaching and effacing (AE) lesions (Johnson and Nolan, 2009).

Pathogenic E. coli causes travelers' diarrhea, profuse neonatal diarrhea in babies, calves, piglets and post weaning diarrhea in piglets (Mainil, 2012), non-bloody diarrhea, hemolytic uremic syndrome (HUS), watery diarrhea, inflammatory colitis, or dysentery (Mainil, 2012; Johnson and Nolan, 2009) and persistent diarrhea (Johnson and Nolan, 2009).

From 1982 to 2002, 49 states of the USA reported 350 outbreaks of *E coli*, representing 8,598 cases which ended up in 17% in hospitalization, 4% in hemolytic uremic syndrome, and 0.5% in death (Rangel *et al.*, 2005). Of the outbreaks, 183 were foodborne, sources were ground beef (41%), unknown (23%), produce-associated (21%), other beef (6%), other foods (5%) and dairy products (4%) (Rangel *et al.*, 2005). Although the sources of infection being unknown, ETEC and EAggEC were more frequently isolated from fecal specimens from patients with acute watery diarrhea and acute bloody and persistent diarrhea in Ethiopia (Geyid *et al.,* 1998). Further, *E. coli* was isolated from medical cases of urine (45.5%), ear discharge (32.7%), wound swabs (18.7%), and eye discharge (14.2%) (Kibret and Abera, 2011) in the same country.

2.3.4 *E. coli* along meat production lines

Studies worldwide have shown that *E. coli* are often present in fresh meat and poultry (Kagambega *et al.*, 2012; Abdalla *et al.*, 2009; Zhao *et al*., 2001). However, there are differences in contamination rates along different supermarket chains with retail raw meat. Zhao *et al.* (2001) reported marked differences in the prevalence of such pathogens like *E. coli* in different meats (chicken, turkey, pork and beef). In a study conducted in Burkina Faso, Kagambega *et al.* (2012) reported a virulence gene of at least one diarrheagenic *E. coli* group

isolated from 49% of samples of feces taken from cattle, chicken, and pigs. A number of 2.8% of *E. coli* in meat and meat products examined in South Africa was positive for *E. coli* O157:H7 that carried fliC(H7), rfbE(O157) and eaeA genes (Abong'o and Momba, 2009). In Ethiopia, reports were made of 27.3% *E. coli* in fresh meat from the abattoir,meats from butchers (22.2%) and in meat sold in the streets (56.5%) of Mekele (Haileselassie *et al.*, 2013). Noted also from the same country was a *E. coli* O157:H7 prevalence of 4.2% in retail raw meat products from cattle, sheep and goats (Hiko *et al.,* 2008), 4.7% in feces, 8.7% in skin, and 8.1% in carcasses before and 8.7% after washing from slaughtered sheep and goats (Mersha *et al.,* 2010).

Regardless of strains, *E. coli* prevalences of 37% in feces of cattle (Kagambega *et al.,* 2012) and 44% in hides (Rhoades *et al.*, 2009) at abattoirs were reported. Further, a higher rate of 50% from tables of butchers was noted (Gurmu and Gebretinsae, 2013). However, when it came to processed products from supermarkets, the trend lowered to 1.17% in grilled meat (*suya*) (Enabulele and Uraih, 2009) in Nigeria, and to 2% in sausages (Abd El-Atty and Meshref, 2007) in Egypt.

Prevalence of *E. coli* in water used at an abattoir (4.2%) (Mersha *et al.*, 2010) in Ethiopia, surface waters (stream, river and dam water) (2.2%) and diarrhoeal patients (5.4%) (Chigor *et al.*, 2010) in Nigeria show risks of occurrences in ranges of environment and in human cases. Table 2 - 06 summarizes prevalence data for *E. coli* in general and of different *E. coli* strains in particular from various types of samples, sources, and countries.

Table 2 - 06: Reports on *E. coli* prevalence in animals, food of animals origin, and production environments

Source	Types of sample, source (species) and prevalence (%)	Strain	Reference	Country
Abattoir	Feces of cattle (37%), chicken (6%), pigs (30%) Feces of cattle (8%), chicken (37%), pigs (32%) Feces of cattle (4%), chicken (5%), pigs (18%) Feces of cattle (7%), chicken 6%, pigs (32%)	STEC EPEC ETEC EAEC	Kagambega *et al.* (2012)	Burkina Faso
	Feces (4.7%), skin swabs (8.7%), carcasses before washing (8.1%), after washing (8.7%), water samples (4.2%)	O157:H7	Mersha *et al.* (2010)	Ethiopia
	Feces (6.2%), hides (44%) , chilled carcasses (0.3%), raw beef products (1.2%)	VTEC	Rhoades *et al.* (2009)	Developed countries
	Carcasses (8.86%)	*E. coli*	Abdalla *et al.* (2009)	Sudan
	Beef (16.0%), lamb mutton (77.4%), goat meat (84.0%) at export	O157:H7	Hiko *et al.* (2008)	Ethiopia
	Beef (49.6%), lamb mutton (14.4%), goat meat (7.0%) at municipal	O157:H7		
Butchers	Tables (50%), knifes (25%), hand swabs (25%)	*E. coli*	Gurmu and Gebretinsae (2013)	
	Beef (34.4%), lamb mutton (8.2%), goat meat (9.0%)	O157:H7	Hiko *et al.* (2008)	
Butchers and processing plant	Before GMPs in minced meat (6.6%), 3.3% in each mincer, knifes and workers' hands; After GMPs in minced meat (3.3%), mincer (1.6%), not in knifes and workers' hands	*E. coli*	Attala and Kassem (2011)	Egypt
Butchers, supermarkets and open air markets	Meat and meat products (2.8%)	O157:H7	Abong'o and Momba (2009)	South Africa
Abattoir and supermarket	"Ready to eat" grilled meats (*suya*) (56.9%) Fresh meat at: abattoir (100%), open traditional market (100%)	*E. coli*	Enabulele and Uraih (2009)	Nigeria
Supermarkets	Sausage (2%)	O157	Abd El-Atty and Meshref (2007)	Egypt
Surface water	Water (2.2%)	*E. coli* O157	Chigor *et al.* (2010)	Nigeria
Health sector	Diarrhoeal stools (5.4%)	*E. coli* O157		

VTEC=verocytotoxigenic *E. coli*
STEC =Shiga toxin-producing *E. coli*
GMP= Good Manufacturing Practices

2.4 Antimicrobial susceptibility/resistance profiles

Antimicrobial resistance and bacterial infections indicate emerging antibacterial resistance threatening the management of bacterial infections. However, the prevention and containment has received far too little attention in Ethiopia (DACA, 2009).

2.4.1 *Salmonella*

An increase in resistance of *Salmonella* to commonly used antimicrobials has also been noticed in both public health and veterinary sectors in Ethiopia (Zewdu and Cornelius, 2009; Asrat, 2008; Molla *et al.,* 2003; Mache *et al.,* 1997). The most recent data from the EU on cephalosporin resistance in *Salmonella* are from 2006 and they showed resistance rates of *S.* Typhimurium and *S.* Enteritidis to cefotaxime to be 0.9% and 0.1%, respectively. *Salmonella* isolates from feces of different meat animals tested in Burkina Faso showed resistance to one or more antimicrobials (Kagambega *et al.*, 2013).

Data on outcomes of human infections with cephalosporin-resistant *Salmonella* are limited, although multi-drug resistant (MDR) *Salmonella* infections have shown more serious outcomes than infections with susceptible *Salmonella* (AMR, 2009). Multi-drug resistance was reported in 40% of *S.* Typhimurium and 0.7% of *S.* Enteritidis. According to a study by Ashenafi and Gedebou (1985), 22% of *Salmonella* isolates were MDR. Addis *et al.* (2011) reported 25% of isolates to be two-drug resistant. MDR rates of 16.7% to each of three, four and five drugs and 8.3% to six drugs were reported by Addis *et al.* (2011) in *Salmonella* obtained from cow milk, cow feces and human stools in Addis Ababa. Zewdu and Cornelius (2009) also reported MDR *Salmonella* from three to a maximum of ten drugs.

Table 2 - 07 below shows some *Salmonella* isolated from different sources having high susceptibility to most drugs but none (0%) to drugs of the tetracycline group (Tesfaw *et al.*, 2013), as well as those having a 14.2% susceptibility to the same group of drugs (Reda *et al.*, 2011).

Literature shows that (see Table 2 - 08) *Salmonella* isolated from different sources in Ethiopa reflect variable resistance profiles to different antimicrobial agents used in medical and veterinary medicine. In studies conducted in Ethiopia, Addis *et al.* (2011) reported low

resistance (0 - 16.7%) to chloramphenicol in *Salmonella* isolates from human cases, but Reda *et al.* (2011), Beyene *et al.* (2011) and Asrat (2008), respectively, reported 62.3%, 81.4% and 83.7% resistance to the same drug. In non-human cases, however, Wandili *et al* (2013), and Li *et al.* (2012) reported lower resistance rates to most of the drugs they used for investigation. Frequent resistance to tetracycline by *Salmonella* from animal and human cases was reported from Ethiopia (Beyene *et al.*, 2011; Addis *et al.*, 2011; Asrat, 2008).

Table 2 - 07: Reports on antimicrobial susceptibility of *Salmonella* to different drugs (in percentage)

Drug	Ishaku *et al.* (2013)	Wandili *et al.* (2013)	Abatcha *et al.* (2013)	Addis *et al.* (2011)			Reda *et al.* (2011)	Tesfaw *et al.* (2013)	Mengistu *et al.* (2014)
				Cow milk	Cow feces	Human stool			
PB	98.3	-	-	-	-	-	-	-	-
CN	98.3	100	100	66.7	73.3	100	92.8	100	72.5
C	64.5	88	100	66.7	93.3	100	28.6	83.3	52.5
W	-	-	-	-	-	-	-	-	-
SXT	59.3	100	100	83.3	93.3	100	-	100	57.5
N	-	-	25	-	-	-	-	-	-
OT*	40.7	94	100	33.3	53.3	33.3	14.2	0	7.5

B = Polymyxin B; SXT=Trimethoprim-sulphamethaxole/Cotrimaxazole; C=Chloramphenicol; OT=Oxytetracycline; CN=Gentamycin; -=not reported; *reports are for tetracycline

Table 2 - 08: Reports on antimicrobial resistance of *Salmonella* to different drugs (in percentage)

Drug	Wandili *et al.* (2013)	Li *et al.* (2012)	Reda *et al.* (2011)	Beyene *et al.* (2011)	Addis *et al.* (2011)			Zewdu and Cornelius (2009)	Asrat (2008)	Molla *et al.* (2003)
					Cow milk	Cow feces	Human stool			
PB	-	-	-	-	-	-	-	-	-	-
CN	0	1.9	3.6	74.3	33.3	13.3	0	3.1	75.6	-
C	6	1.9	62.3	81.4	16.7	6.7	0	0	83.7	30
W	-	1.0	-	-	-	-	-	31.3	-	21.2
SXT	0	2.8	-	80.5	0	0	0	31.3	75.7	21.2
N	-	-	-	-	-	-	-	0	-	-
OT*	6	24.1	71.4	39.8	50.0	26.7	33.3	46.9¶	94.5	41.2

PB = Polymyxin B; SXT=Trimethoprim-sulphamethaxole/Cotrimaxazole; C=Chloramphenicol; OT=OxyTetracycline; CN=Gentamycin; -=not reported; *reports are for tetracycline

2.4.2 *E. coli*

Studies investigated the susceptibility/resistance profiles of pathogenic *Escherichia coli* to antimicrobials used in human and veterinary medicine. The strains were isolated from food production processes, the environment of production, the food items (Ayl *et al.*, 2012) and from surface water and clinical sources (Chigor *et al.*, 2010). Arslan ans Eyi (2011)showed that 94.4% of the isolates were resistant to one or more antimicrobial agents while 56.9% were resistant to three or more antimicrobial drugs. However, a study from Ethiopia revealed 100% of the isolated strains of *E. coli* O157:H7 showing susceptibility to amikacin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B and trimethoprim-sulfamethoxazol. Multi-drug resistance (to three or more drugs) was detected in seven out of thirty one (22.6%) strains (Hiko *et al.,* 2008).

Reviews on susceptibility profiles of *E. coli* in Table 2 - 09 show a decrease in susceptibility to polymyxin B, from the latest drugs to drugs of ampicillin and tetracyclines (Ayl *et al.*, 2012; Kibret and Abera, 2011; Chigor *et al.,* 2010; Mohammad *et al.*, 2010).

Reviews in Table 2 - 10 reveale an increase in resistance of *E. coli* isolated from Ethiopia and other African countries to polymixin B and other commonly used drug classes such as amoxicillin and tetracycline (Ramos *et al.,* 2013; Ayl *et al.*, 2012; Kibret and Abera, 2011; Chigor *et al.*, 2010; Olaniran *et al.*, 2009; Hiko *et al.*, 2008).

Table 2 - 09: Reports on antimicrobial susceptibility of *E. coli* to different drugs (in percentage)

Drug	Gizachew *et al.* (2013)	Ayl *et al.* (2012)		Kibret and Abera (2011)	Mohammad *et al.* (2010)	Chigor *et al.* (2010)		Hiko *et al.* (2008)
		Clinical isolates	Food isolates			Clinical case	Water	
PB	-	98.5	97.5	-	-	-	-	100
CN	75.0	-	-	81.0	56	100	90.6	100
C	46.9	-	-	63.2	12.5	93.2	94.8	100
SXT	15.6	67	66	34.8	20.0	87.5	83.3	100
AML	0.0¶	15*	5	14.0	4¶	3.4¶	8.3¶	-
OT*	-	63	62.5	23.6	25	33.0	20.8	77.4

PB = Polymyxin B; AMX=Amoxicillin; SXT=Trimethoprim-sulphamethaxole/Cotrimaxazole; C=Chloramphenicol; OT=OxyTetracycline; CN=Gentamycin; -=not reported; *reports are for tetracycline; ¶Ampicillin and Amoxicillin are interchangeable (NCCL, 2001)

Table 2 - 10: Reports on antimicrobial resistance of *E. coli* to different drugs (in percentage)

Drug	Ramos *et al.* (2013)	Ayl *et al.* (2012)		Kibret and Abera (2011)	Chigor *et al.* (2010)	Olaniran *et al.* (2009)		Hiko *et al.* (2008)
		Clinical isolates	Food isolates			Palmiet River	Umgeni River	
PB	-	5	2.5	-	-	-	-	0
CN	3.1	-	-	13.0	1.1	0	3.85	0
C	15.6	-	-	35.3	5.4	14.7	7.69	0
SXT	33.9	33	34	62.9*	12.5	-	-	0
AML	35.4	85	95	86.0	83.7¶	55¶	40.38¶	-
OT*	63.0	37	37.5	72.2*	66.8	47	38.46	22.6*

PB = Polymyxin B; AMX=Amoxicillin; SXT=Trimethoprim-sulphamethaxole/Cotrimaxazole; C=Chloramphenicol; OT=OxyTetracycline; CN=Gentamycin; -=not reported; *reports are for tetracycline; ¶Ampicillin and Amoxicillin are interchangeable (NCCL, 2001)

3. THE GIVEN TECHNOLOGICAL PROCEDURES

An abattoir, meat processing facilities, butchers and supermarkets were visited and samples were taken.

3.1 Abattoir, butchers, processing plant and supermarkets

3.1.1 The abattoir line

The abattoir

Cattle are slaughtered weekly with daily variation of 30 up to 1500 heads at Addis Ababa Abattoir Enterprise (AAAE). The AAAE
abbatoir and butchers in Addis Ababa city served as sources of samples in this study.

Butchers in the city

From the abattoir, meat is transported to the city butchers, which run open meat shops in Addis Ababa city. They receive beef carcasses from the abattoir in trucks equipped with refrigerators so as to keep the meat cold. The city butchers handle the meat and supply it to the public under local environmental temperature of Addis Ababa city (20-27^{o}C).

3.1.2 The processing plant line

The meat processing plant

This plant is located in Bishoftu town, 47 km east of Addis Ababa. It receives raw beef from three abattoirs: AAAE (Addis Ababa), Adama Municipal Abattoir (Adama, 43 km east of Bishoftu) and Bishoftu Municipal Abattoir (Bishoftu). The plant processes beef, pork and poultry. Meat is transported from the abattoirs to the processing plant under cold conditions and is stored there in a refrigerator until it is processed. The product (beef mortadella) is distributed to supermarkets in Addis Ababa city in a refrigerated status.

Supermarkets

Supermarkets are private establishments supplying consumers with agro-industrial products and other items. Most of them are located in Addis Ababa.

3.2 The incoming animals

In Ethiopia, cattle, sheep, goats and poultry are reared in privately owned small scale farms. There, different animals are usually kept together and crop production also takes place in the same site. Among the animals pigs are used for local consumption, export of live animals, and export of processed meat.

A number of animals come to AAAE every day. Mentioning the fact that lots of animals come from different sources, Gudeta (2012) claims that their exact origin cannot be traced. However, once an animal arrives at abattoir, it gets an identification number.

3.3 The processing procedures

3.3.1 The abattoir line (Chain 1)

The abattoir

Procedures in AAAE start with reception and registration of animals for slaughter at lairage. The animals are kept in the lairage for 16-24 hours with water supply only. There, ante mortem examinations are made and the animals are moved to a different corner for slaughter steps. The majority of the steps take place in a single slaughter area and include:

- **Stunning** - This is done using captive bolt jut on the medulla oblongata.
- **Bleeding** - Immediately after stunning, bleeding is performed by opening the blood vessel with a knife. This knife is used at all steps of slaughtering operations.
- **Removal of hind feet and hanging -** Hind feet are cut and removed at the hook joint. Then the animal is hanged upside down on a hook at the hook joint. The hook goes with the carcass until it is deloaded.

- **Skinning and head removal -** At this step, skinning is done and the forefeet are cut at the carpal joint and removed. The head is also removed.
- **Evisceration -** This is done by opening the abdomen and removing the whole gastrointestinal tract.
- **Carcass coding** - Using ink, the same identification number that the animal had before slaughter is given to the carcass.
- **Carcass washing –** This is carried out using tap water directly from the pipeline.
- **Carcasses splitting and visceral organ removal** – This is performed after washing**.**
- **Quality inspection** – This is a routine procedure. Here, the carcass and visceral organs are inspected in parallel.

The whole carcass with its organs is then loaded on a truck for distribution to butchers in Addis Ababa city. Beef intended for processing plants is kept under refrigeration at the abattoir. A staff member doing the slaughter operation is involved in the various steps of handling the carcass.

City butchers

After reception, beef is kept in the shops at local temperature in the area, that is, meat is not kept under cold conditions. Beef and organs are sold to consumers on a kilo-basis and one can buy as much as one wants.

3.3.2 The beef processing plant line (Chain 2)

The beef processing plant

Beef intended for processing plants is kept under refrigeration at the abattoirs. Prior to operation procedures, personnel involved clean themselves and all other facilities they utilize using water and detergents. Then, extra fat of the meat is manually trimmed off using knives. After that the beef is cut into smaller sizes so as to fit into the grinding machine. Proportions of fat and lean meat are mixed according to the recipy. After it is grounded, the meat is thoroughly mixed in a mixer. Mixed beef is then cut into fine pieces where spices, salt and ice at appropriate rations are added. Spice mixtures are prepared in the processing plant from different spices (ginger, cardamom, black pepper, cinnamon, shallot, garlic, *Anethum* spp.,

Ociumum spp., mints and thyme) (Jansen, 1981) and stored in a refrigerator. When needed for use, they are weighed and utilized in the processing operation. The mixed items are fed into a stuffer and stuffed into polyvinyl thermos stable casings with different volume and then cooked using a steamer at 82^{o}C for 1 hr. Finally, when the temperature decreases, the product is taken out and stored in the refrigerator until it is dispatched to supermarkets.

Supermarkets

Products are kept in the refrigerator with other products of animal origin. Slicing is done using one slicing machine for all products.

4. MATERIALS AND METHODS

4.1 Sampling

4.1.1 Sample size determination

The daily slaughter ranges from 30 to 1,500 cattle, making an annual slaughter of about 16,000 animals, but no study was available at AAAE to show the daily slaughter population and to make estimates of the population size. So, using Raosoft© 2004 (Raosoft, 2011). the sample size was estimated supposing a infinitive population with 7.1% expected *Salmonella* prevalence in apparently healthy slaughtered cattle in Ethiopia (Alemeyehu *et al.,* 2003), at a 95% confidence interval and 9% precision.

- Prevalence of 14.7% *Salmonella* with 14.4% in minced beef, 14.1% in mutton and 16.4% in pork samples was reported in supermarkets at Addis Ababa, Ethiopia (Ejeta *et al.*, 2004).
- *Salmonella* prevalence of 5.0% and 5.6% in slaughtered goats and sheep, respectively, was also reported at Modjo (Teklu and Nigussie, 2011).

At the processing plant, sample size was calculated using a 50% expected prevalence at a 95% confidence interval and 9% precision.

The Raosoft© allows the use of as much as a maximum of 20,000 population without significant change of sample size. Hence, terms of the above mentioned values i. e the 20000 maximum population of cattle, 95% confidence interval, 9% precision and 7.1% expected prevalence (for abattoir line) but 50% expected prevalence for processing plant line, the sample size n and margin error E are given by:

$$n = N*x/((N-1)*E^2+x)$$

$$x = Z(^c/_{100})^2 * r * (100-r)$$

$$E = \text{Sqrt}\ [(N-n)*x/\ n * (N-1)]$$

Where: n = sample size; N = population size; r = the fraction of responses (samples) interested in; Z(c/100) = the critical value for confidence level c.

The resulting minimum sample size was 32 cattle at the abattoir and 118 raw beef and products at the processing plant. The number of environmental samples was determined using data from USAD (2012) with at least 2-10 samples from sampling locations. A total of 668 samples was collected: 237 samples including 101 environmental and 136 animal related samples from the abattoir, and 431 samples comprising 194 environmental, 118 raw beef and 119 product samples from the processing plant were taken. Products were sampled from 8 randomly selected supermarkets in Addis Ababa city. Fourteen samples were collected from one of the supermarkets while 15 were taken from each of the other seven.

4.1.2 Sample sources

Two lines were defined as “abattoir line” and “processing line”. Within each line, samples were collected from three sources: the environment, animal related samples and the end products in retail. End products were sampled from butchers (abattoir line) and supermarkets (processing line) (Fig. 4 - 01). Environmental samples were samples collected from the working environment of the abattoir and the beef processing plant. Animal-related materials were samples associated with the animals at the abattoir (feces, mesenteric lymph node and raw meat) and at the processing plant (raw meat). End products in retail were samples of raw beef from butchers and processed beef mortadella from supermarkets. The environmental samples at these stations were not sampled due to inaccessibility.

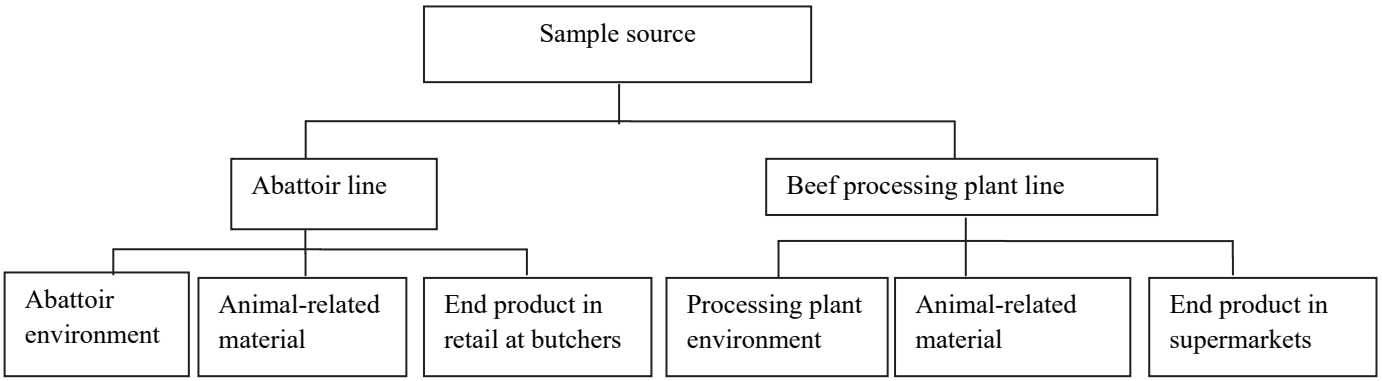


Fig. 4 - 01: Sample sources and categories

4.1.3 Sampling occasions

Sampling took place from December 2011 to April 2012 at 18 sampling occasions.

The abattoir line was sampled 5 times.

In the processing plant line, due to difference in beef production location i.e. at Bishoftu and the supermarkets, and at Addis Ababa city, sampling occasions of processing plant and supermarkets were different. Thus, the processing plant was sampled 8 times and supermarkets were visited 5 times. An overview of sample sources, distribution of samples by sampling date and occasion is seen in Table 4 - 01.

Table 4 - 01: List of sampling dates by sampling source and distributions of samples at abattoir[¶] and processing plant line

Abattoir line							Processing plant line							
			Abattoir		Butchers**				Processing plant			Supermarkets			
Sampling batch	Sampling occasion	Sampling Date	No. of environmental samples	No. of animal related samples*	No. of raw meat samples	Total No. of samples	Sampling occasion	Sampling date	No. of environmental samples	No. of animal related samples	Subtotal of samples	Sampling occasions	Date	No. of products sampled	Total No. of samples
1							P1	13.12.11	23	14	37				37
2							P2	21.12.11	20	14	34				34
3	A1	05.01.12	25	24	8	57									
4	A2	15.01.12	27	36	12	75									
5							P3	25.01.12	25	16	41				41
6							P4	01.02.12	20	16	36				36
7							P5	08.02.12	27	16	43				43
8												S1	16.02.12	21	21
9												S2	20.02.12	21	21
10												S3	24.02.12	26	26
11	A3	29.02.12	22	21	7	50									
12							P6	07.03.12	35	24	59				59
13												S4	13.03.12	26	26
14							P7	19.03.12	26	10	36				36
15							P8	26.03.12	18	8	26				26
16												S5	30.03.12	25	25
17	A4	07.04.12	11	9	3	23									
18	A5	13.04.12	16	12	4	32									
Total	**5**		**101**	**102**	**34**	**237**	**8**		**194**	**118**	**312**	**5**		**119**	**431**

[¶] 34 animals was sampled in total

*3 samples per animal were taken from the abattoir

** one sample per animal was taken from butchers

4.1.4 Sample types

Following Fig. 4 - 01 above, sampling stage with locations, types and number of samples from the studied beef line are shown in Table 4 - 02.

In the abattoir line

Abattoir:
Swab samples were taken from personnel's hands, knives, aprons, from rooms, refrigerators, hooks and meat transporting trucks. Water was sampled directly from the pipeline, and animal-related materials such as feces, mesenteric lymphnodes and raw beef were sampled at the abattoir and the processing plant.

Butchers:
End products in retail (raw beef) were sampled from city butchers.

In the processing plant line

Processing plant:
Swab samples were taken from personnel's hands, knives, aprons, from cutting plates, spice weighing equipment, rooms, refrigerators and working tables. Swabs from electrical processing machinery such as grinder, cutter, mixer and stuffer were also collected. Water was sampled directly from the pipeline. A sample from raw beef, as animal related sample, was taken at the processing plant.

At supermarkets:
After identification of the brand of the processing plant on the product, beef mortadella was purchased and sampled.

Table 4 - 02: Sampling stages with locations, types and number of samples from the studied beef production and processing lines

Line	Origin of sample		Processing stages/position	Sampling location	Sample type	No. of samples
Abattoir line	Abattoir	Environment	Before stunning and beginning of operation	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
			At carcass splitting	Rooms	Swabs from rooms	17
			Refrigeration	Refrigerators	Swabs from refrigerators	10
			Meat transport	Meat transport trucks	Swabs from trucks	11
			Sub total			101
		ARM	Before stunning	Stunning	Animal feces	34
			During evisceration	Evisceration	MLN* samples	34
			After washing when ready for distribution	Quality inspection	Raw meat samples	34
			Sub total			102
	Butchers'		Butchers, 6-8 hrs post delivery	Beef for public consumption	Retailed meat sample	34
	Total					237
Processing plant line	Processing plant	Environment	Manual production	Personnel's hands	Swabs from hands	19
				Aprons	Swabs from aprons	16
				Knives	Swabs from knives	15
				Cutting plates	Swabs from plates	13
			Cleaning water	Tap water	Samples from water	17
			Device-related materials	Working tables	Swabs from tables	17
				Room floors	Swabs from rooms	16
				Refrigerators	Swabs from refrigerators	15
			Spicing	Spices	Samples from spices	15
				SWE**	Swabs from SWE	15
			Beef processing electrical machinery	Grinder	Swabs from grinders	9
				Cutter	Swabs from cutters	9
				Mixer	Swabs from mixers	9
				Filler/Stuffer	Swabs from fillers	9
			Sub total			194
		ARM	Receiving raw beef from 3 abattoirs	Before processing	Samples from raw meat	118
	Supermarkets		End of production	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
			Sub total			119
	Total					431
Grand total						668

ARM = Animal-related materials
MLN = Mesenteric lymph node
SWE = Spice-weighing equipment

4.1.5 Sampling locations

4.1.5.1 The abattoir line

Area swabs and water samples were collected at respective stages. All were sampled before having contact with the meat material.

Abattoir

Swabs and water samples:

Samples from hands and aprons and knives used by the workers were collected before contact with the animals, just before the start of the slaughtering operations. 17 room samples were collected randomly from the slaughter hall in the post evisceration area used forcarcass splitting, 11 samples were taken from hooks in the hanging area. Again 11 samples were collected from the inside wall of meat transporting trucks. This was done in the beef loading area before the carcasses were loaded for transportation. 10 samples from refrigerator swabs were taken from the inside part. This was at beef storage after the refrigerators were made ready for loading. 12 water samples from the carcass cleaning area were taken directly from the pipeline into sterile calibrated glass bottle. This was before the water was used at the carcass washing point. Environmental sampling location, types and number of samples are shown in Table 4 - 03 below.

Table 4 - 03: Environmental sample distributions by location and occasions from the abattoir line

Sampling location	Types of sample	No. of samples	Sampling occasion¶ A1	A2	A3	A4	A5
A. Personnel related samples							
Personnel's hands	Hand swabs	13	3	4	3	1	2
Aprons	Apron swabs	14	4	4	3	1	2
Knives	Knife swabs	13	3	4	3	1	2
B. Tap water	Water samples	12	2	3	3	2	2
C. Device related samples							
Hooks (hanging part)	Hook swabs	11	4	3	3	1	*
Room floors	Room swabs	17	6	4	2	2	3
Refrigerators	Refrigerator swabs	10	1	3	1	2	3
D. Inside walls of transport trucks	Truck swabs	11	2	2	4	1	2
Total		101	25	27	22	11	16

¶ Refer to abattoir line of Table 4 -01

* Sample was not taken

Animal-related material samples:

Using a sterile glove, about 50-60 grams of 34 fecal samples were taken directly from the rectum of the animal in the stunning area just before stunning. With a sterile glove, forceps and scalpel blades, 34 samples of mesenteric lymph nodes were taken in the evisceration area after evisceration, and 34 samples from raw beef were taken in the inspection area of the abattoir just after inspection and approval for consumption.

City butchers

By combining the information from the abattoir with that of the butchers and following the identification number of the animals, 34 raw beef samples from different animals were collected from 34 butchers in Addis Ababa city within 6-8 hrs post-delivery.

Animal-related material sampling locations, types and number of samples at abattoir and at butchers are described in Table 4 - 04 below.

Table 4 - 04: Animal-related sample distribution by location and occasions from the abattoir line

			Sampling occasion¶				
Sampling location	**Type of sample**	**No. of samples**	**A1**	**A2**	**A3**	**A4**	**A5**
A. Abattoir							
	Feces	34	8	12	7	3	4
	MLN	34	8	12	7	3	4
	Raw meat	34	8	12	7	3	4
Sub total			24	36	21	9	12
B. Butchers**	Raw meat	34	8	12	7	3	4
Total		136	32	48	28	12	16

¶Refer to abattoir line of Table-4-01

*MLN = Mesenteric lymph node

** Samples from 34 different butchers on oneanimal from each

4.1.5.2 The processing plant line

Processing plant

At the beef processing plant, area swabs and samples from water and spices were collected at respective stages. All were sampled before contact with meat material.

Swabs, samples from water and spices:

At the processing plant, meat was sorted into fat and lean quality and cut on working tables and cutting plates. There, prior to the beginning of any processing operation, swab samples were collected from hands, aprons, knives, cutting plates and working tables.

At the beef processing positions, a total of 36 swab samples from the machinerfy were collected. These comprised 9 swab samples each from a beef grinder, a cutter, a mixer, and a stuffer (a grinder at the grinding area/ position, a cutter at the cutting position, a mixer at the mixing position and the stuffer at the stuffing position). Again, 15 swab samples were taken from spice-weighing equipment before contact with spices and 15 samples of spice were taken directly from its holding container. At cooling location, 15 swab samples were taken from refrigerators before storing the received beef in the processing room. While, 17 samples from tap water used for cleaning in the processing plant were taken directly from the pipeline into sterile glass bottles.

Animal-related material samples:

A total of 118 raw meat samples were taken as raw beef meat deliveries from abattoirs to the processing plant (before processing started).

Supermarkets

Using the brand of the processing plant for identification, 119 samples were taken from mortadella sausages purchased at different times from eight (8) randomly selected different supermarkets in Addis Ababa city. However, due to differences in the location, the sampling occasion from supermarkets was different from that of the processing plant.

Sampling location, types and number of samples from the processing plant line by sampling occasions is shown in Table 4 - 05.

Table 4 - 05: Sample distribution by sampling locations and occasions from the processing plant line

Source	Sampling location	No. of samples	Numbers of samples and sampling occasion[¶] P1	P2	P3	P4	P5	S1	S2	S3	P6	S4	P7	P8	S5
	A. Personnel-related swab samples														
	Personnel's hands	19	2	2	2	1	4				5		2	1	
	Aprons	16	2	2	2	1	3				3		2	1	
	Knives	15	2	2	2	1	3				3		2	*	
	Cutting plates	13	2	2	2	1	3				3		*	*	
	B. Tap water samples	17	3	2	3	2	1				2		2	2	
	C. Device-related swab samples														
Environment	Working tables	17	1	1	2	2	2				5		2	2	
	Room floors	16	2	1	2	2	2				3		2	2	
	Refrigerators	15	1	1	2	2	2				4		2	1	
	D. Spice-related samples														
	Spice samples	15	2	1	2	2	2				1		2	3	
	Spice-weighing equipment (swabs)	15	2	2	2	2	1				2		2	2	
	E. Electric machinery-related swab samples														
	Grinder	9	1	1	1	1	1				1		2	1	
	Cutter	9	1	1	1	1	1				1		2	1	
	Mixer	9	1	1	1	1	1				1		2	1	
	Filler	9	1	1	1	1	1				1		2	1	
	Sub total	194	23	20	25	20	27				35		26	18	
Animal-related	Raw beef	118	14	14	16	16	16				24		10	8	
Supermarket	Mortadella product samples														
	Supermarket A	15						4	2	2		4			3
	Supermarket B	15						4	2	2		4			3
	Supermarket C	15						3	4	4		2			2
	Supermarket D	14						2	2	4		3			3
	Supermarket E	15						2	4	4		2			3
	Supermarket F	15						2	4	4		2			3
	Supermarket G	15						2	3	2		4			4
	Supermarket H	15						2	*	4		5			4
	Sub total	119						21	21	26		26			25
Total		431	37	34	41	36	43	21	21	26	59	26	36	26	25

[¶] Refer to processing plant line of Table-4 - 01

*Sample was not taken

4.2 Sampling techniques

Environmental samples

Sterilized gauze with normal saline was used for all swabbing. Surfaces were aseptically measured as 50 cm^2 and swabbed. About 20 milliliters tap water was collected directly from the pipeline in sterile calibrated sampling glass bottles. About 50-60 grams of spices were directly put in sterile stomacher bags.

Samples from animal-related materials/products

A sterile glove was used to sample feces directly from the rectum of animals. Sterile glove, forceps and scalpels were used for sampling mesenteric lymph nodes and raw beef at the abattoir. At butchers, samples of raw beef were taken into sterile stomacher bags at sales. Similarly, about 50-60 gms of raw beef were taken at the processing plant using a sterile glove, forceps and scalpels. Following the brand of the processing plant for identification, 100 gms of the product (beef mortadella) were purchased from the 8 supermarkets.

4.3 Sample-handling and transport

All samples were placed in sterile stomacher plastic bags, immediately labeled with identification numbers and transported in an ice box at +4^{o}C to the Microbiology Laboratory, Aklilu Lemma Institute of Pathobiology, Addis Ababa University (ALIPB-AAU).

4.4. Laboratory techniques

This work was done in two laboratories, ALIPB-AAU, Ethiopia and Institute of Meat Hygiene and Technology, Panel Veterinary Public Health, FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Germany.

4.4.1 Sample preparation for microbiological examination

At ALIPB-AAU: For spoilage bacteria examination and specific microbiological examination for *Salmonella* and *E. coli*, all samples other than area swabs were aseptically measured at a 1:10 proportion of buffer peptone water (BPW) according to UADA (2012) and Montville *et al.* (2012). Ten grams of each of feces, MLN, raw beef, spices, mortadella product and 10 ml of water were aseptically measured. Swabs were directly used as 50 cm^2 sampling units in 50ml BPW.

Spoilage bacteria examination (APC and EBC) on samples from the beef processing plant line was also done immediately after treating samples with BPW. *Salmonella* examination was done on all samples which were also tested for *E. coli* isolation with a mobilirty test and biochemical for indole production and hydrogen sulfide (H_2S) production. All *Salmonella*, all *Staphylococcus* and some *E. coli* strains were tested for antimicrobial susceptibility/resistance using disc diffusion techniques. *Salmonella* and *E. coli* isolates were stored in cryogenic test tubes with Standard-II nutrient agar (Merck, Germany) and transported to the Institute of Meat Hygiene and Technology, Panel Veterinary Public Health, FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Germany, in July 2012.

At the Institute of Meat Hygiene and Technology, FU-Berlin, serological and PFGE analysis was done for *Salmonella*. *E. coli* was tested for gas production, oxidase, oxidation fermentation (O/F), sorbitol and mannitol utilization. The remaining *E. coli* strains were also tested for anti-microbial susceptibility/resistance using disc diffusion techniques.

4.4.2 Spoilage bacteria examination

Spoilage bacteria examination was done at ALIPB-AAU on samples from the beef processing plant line with slight modification made to Montville *et al.* (2012) and USDA (2012) test procedures, whereby one plate per dilution (modified) of examination was used for both APC and EBC (Appendix 10.1). Each sample was aseptically measured according to UAD (2012) and Montville *et al.* (2012). Ten grams of each of raw beef, spices and mortadellae, and 10 ml of water were aseptically measured. Swabs were directly used as 50 cm^2 sampling units.

4.4.2.1 Aerobic Plate Count (APC)

Ten-fold serial dilution steps were prepared and followed by the surface plating method. A one plate per dilution procedure was used on Standard I nutrient agar instead of the two plate per dilution procedure of Montville *et al.* (2012) and USDA (2012). Dilution steps for area swabs, solid sample materials (spices and meat) and liquid material (water) were considered independently. Dilution steps 10^{-2} and 10^{-3} were used for plating.

Area swabs: For each area, 50 cm^2 was sampled and samples were placed into 50ml BPW and homogenized with a stomacher (England 400). Of the original (10^0 dilution), 1 ml was transferred to 9 ml BPW (10^{-1} first dilution step). After mixing with a vortex, 1 ml was again transferred to 9 ml BPW (10^{-2} second dilution step). From this second dilution step, 1 ml was transferred to 9 ml BPW (10^{-3} third dilution). 0.1 ml from the second dilution containing 0.001 ml original sample was plated onto Standard-I nutrient agar (Merck). Similarly, 0.1 ml of dilution from the third dilution containing 0.0001 ml original sample was plated onto Standard-I nutrient agar (Merck). Plates were incubated at 30^oC over 48 hrs and colony forming units were counted. The outcome was expressed as cfu per 1 ml of 50 ml in 50cm^2 (Appendix 10.1.1).

S**pices and meat**: For 1:10 proportions, except for two product- samples in which 45 grams were used, 10 grams of each of spice and meat samples were diluted with 90 ml BPW and homogenized with a stomacher (England 400), as in the first dilution step10^{-1}. In the second dilution step, 1 ml of dilution was transferred to 9 ml BPW (10^{-2} dilution). From this second dilution step, 1 ml was transferred to 9 ml BPW (10^{-3} dilution). Then, 0.1 ml from the second dilution containing 0.001 g original sample was plated onto Standard-I nutrient agar (Merck). Similarly 0.1 ml of dilution from the third dilution containing 0.0001 gm of original sample was plated on Standard-I nutrient agar (Merck). Plates were incubated at 30^oC for 48 hrs (Appendix 10.1.2). The outcome was expressed as cfu per gram.

Water: After shaking, 10 ml of water was diluted with 90 ml of BPW (10^{-1} first dilution step). After mixing, 1 ml of the first dilution containing 0.1 ml of original sample was transferred to 9 ml BPW (10^{-2} second dilution step). One ml from the second dilution step was transferred to 9 ml BPW (10^{-3} dilution). 0.1 ml of dilution from the second dilution containing 0.001 ml original sample was plated onto Standard-I nutrient agar (Merck). Similarly 0.1 ml of dilution from the third dilution containing 0.0001 ml original sample was plated onto Standard-I

nutrient agar (Merck). The plates were incubated at 30^{o}C for 48 hrs (Appendix 10.1.3). The outcome was expressed as cfu/ml.

4.4.2.2 Enterobacteriaceae Count (EBC)

For EBC, the same procedure of dilution as described by Montville *et al.* (2012) and USDA (2012) was applied. Plating was done from 10^{-1} and 10^{-2} dilution steps. Thus, 0.1 ml of the respective dilution was plated on Violet Red Bile Agar (Oxoid, England) supplied with 1% Glucose (FIZMERK, India) (VRBG) agar by surface spreading, using one sterile spreader per plate. Plates were incubated at 30^{o}C for 48 hrs and colonies of dark violet with a precipitate zone were counted.

Area swabs: 0.1 ml of each first dilution containing 0.01 ml original sample was plated onto VRBG agar. Similarly, 0.1 ml of dilution from the second dilution containing 0.001 ml original sample was plated onto VRBG agar (Appendix 10.1.1). The outcome was expressed as cfu per 1 ml of 50 ml in 50cm^2.

Spices and meat: 0.1 ml of dilution from the second dilution containing 0.01g original sample was plated on to VRBG agar. Similarly, 0.1 ml of dilution from the third dilution containing 0.001g original sample was plated onto VRBG agar (Appendix 10.1.2). The outcome was expressed as cfu/g.

Water: 0.1 ml of dilution from the second dilution containing 0.01 ml original sample was plated onto VRBG agar. Similarly 0.1 ml of dilution from the third dilution containing 0.001 ml original sample was plated on to VRBG agar (Appendix 10.1.3). The outcome was expressed as cfu/ml.

4.4.2.3 Recalculation

Numbers of cfu per square of centimeter (cfu cm^{-2}) of areas, per milliliter (cfu ml^{-1}) of water and per gram (cfu g^{-1}) of meat and spices were determined. For all counted results, recalculation to the reference unit cm^2, gram and ml was done as follows (Meeyam, 2010):

For APC:

$$N = \frac{\Sigma C}{[(1 \times n1)+(0.1 \times n2)]} \times (d) \times 10 \times 100/10$$

Where N = number of colonies per ml of water or per cm^2 of area or per gm of weight

ΣC= sum of all colonies on all plates counted

n_1= number of plate in the first dilution

n_2= number of plate in the second dilution

d= dilution from which the first counts were obtained

For EBC:

$$N = \frac{\Sigma C}{[(1 \times n1)+(0.1 \times n2)]} \times (d) \times 10 \times 10/10$$

Where N = number of colonies per ml of water or per cm^2 of area or per gm of weight

ΣC= sum of all colonies on all plates counted

n_1= number of plate in first dilution

n_2= number of plate in the second dilution

d= dilution from which the first counts were obtained

Finally, counts were described using common logarithmic as $\log_{10}$ of cfu ml^{-1}, cfu g^{-1} or cfu cm^{-2} of microbiological load.

4.4.2.4 Determining microbiological quality of mortadella from supermarkets

According to NSW (2009), the beef product (mortadella) under investigation in "Category A" applies to ready-to-eat food, with all components fully cooked for immediate sale or consumption. From mean counts of APC and EBC, the mortadella product was classified as "*Good*", "*Acceptable*" or "*Unsatisfactory*" using parameters set by NSW (2009) and described in Table 4 - 06.

Table 4 - 06: Guideline levels for determining the microbiological quality of Mortadella product from supermarkets

	Microbiological result (cfu/g unless otherwise stated)			
Microbiological Test	**Good**	**Acceptable**	**Unsatisfactory**	**Potentially hazardous**
Standard plate count (APC)	$<10^4$	$<10^5$	$\geq 10^5$	N/A (not applicable)
Enterobacteriaceae (EBC)	$<10^2$	10^2 to $<10^4$	$\geq 10^4$	N/A (not applicable)

Source: (NSW, 2009)

4.4.3 *Salmonella* examination

4.4.3.1 Isolation

At ALIPB-AAU:

From a 1:10 BPW a pre-enriched sample, 0.1 ml and 1 ml respectively were transferred to 10 ml of Rappaport-Vassiliadis (RV) broth (Oxoid) and 10 ml of Muller Kaufmann tetrathionat novobiocin (MKTTn) (Merck) broth (selective enrichment). The inocula of RV and MKTTn were incubated for 18-24 hrs at 43ºC and 37ºC, respectively. A loopful was then plated on Brilliant-Phenol Lactose-Sucrose-agar (BPLS) (Merck) and Xylose Lactose Tergitol™ 4 (XLT4) in parallel and incubated at 37^{o}C for 24 hrs and 48 hrs, respectively (Appendix 10.2.1). Suspected colonies were exposed to Polyvalent-I and Polyvalent-II sera (Sifin, Berlin, Germany) agglutination as first serological screening tests at ALIPB-AAU.

4.4.3.2 Serotyping

At Institute of Meat Hygiene and Technology, FU Berlin:

Serotyping was done with the 86 *Salmonella* isolates obtained at ALIB-AAU using *Salmonella* antisera (Sifin, Berlin, Germany) against O-antigens and H-antigens for agglutination tests (Grimont and Weill, 2007).

4.4.3.3 Characterization by PFGE

With a slight modification of the Pulse Net protocol (Appendix 10.2.2), all *Salmonella* strains were tested by PFGE. The field strains and a reference *(S*. Braenderup STSAL 82) strain were cultured on Standard I nutrient agar (Merck, Germany) and incubated at 37^{o}C for 14-18 hrs. The serotype was proof-checked prior to the beginning of PFGE. The *Salmonella* cell suspension was prepared in cell suspension buffer (100mM Tris, 100mM EDTA, pH 8.0) at the level of MacFarland 5.0 concentration. The optical density of cell suspension was measured at 630nm of 0.55-0.60 (modified). An equal volume (200µl) of cell suspension and 2% Certified^{TM} Megabase Agarose in Tris-EDTA (TE) -Buffer (10mM Tris, 1mM EDTA, pH 8.0) was mixed with 10µl of proteinase K [20 mg/ml], and immediately poured in plug molds to solidify. Plugs were then placed into cell lysis buffer (50mM Tris: 50mM EDTA, pH 8.0;

1% Sarcosyl) with 25µl proteinase K solution [20 mg/ml] in 5 ml solution and incubated for 20 hrs in a shaking water bath at 54^{o}C.

Following lysis, the plugs were washed twice with 10-15 ml of pre-heated (50^{o}C) sterile double distilled water (ddH_2O) for 10-15 min at 50°C in a shaking water bath, then washed four times with pre-heated TE-buffer (10-15 min, 50°C). A about 0.5-1 mm slice of plug (modified) was incubated at 37^{o}C for 10-15 min in 200µl of 10xH-buffer® solution (in ddH_2O). Enzymatic restriction digestion was done with 60U *XbaI* (Roche Diagnostics GmbH, Germany) per sample for 2 hrs at 37^{o}C in a thermo shaker. This step ended by adding ES-solution (0.5mM EDTA, 1% Sarcosyl, pH 9.0). The sample was treated with 50µl of loading buffer (10mM EDTA, 40% Saccharose and 0.03% Bromophenol blue). A 50-1000kb Pulse marker^{TM} (Sigma-Aldrich co, USA), test strains and the reference strain were loaded into 1.2% Pulsed Field Certified Agrose® gel. The gel was run in a 0.5xTBE buffer (45mM Tris (Hydroxymethyl)-aminomethan, 45mM Boric acid and 1.25mM EDTE; 0.1mM Thiourea) in CHEF DR-II. The running condition was set with an initial pulse switch time of 2.2 sec and a final pulse switch time of 63.8 sec under a voltage of 200V (6V/cm) for 20 hrs at 14^{o}C cooling temperature. The gel was stained with 1mg/ethidium bromide solution for 20-30 min on a horizontal shaker (Certomat®U) and twice de-stained with distilled water for 20 min.

Using the Digital Imaging and Analysis System II (DIAS-II), the gel image was taken as ".jpg" and processed into a ".tif" file (Appendix 10.2.4.2). The file was processed using BioNumerics® software (Applied Maths BVBA, Kortrijk, Belgium). Isolates were compared using the genomic cluster analysis byBioNumerics®. The cluster analysis was based on a variety of algorithms that have the common feature of hierarchical relatedness between the origin, the domain, the sources and the types of samples by grouping them in a dendrogram or tree.

4.4.4 *Escherichia coli* examination

At ALIPB-AAU:

From a 1:10 BPW pre-enrichment, 1 ml was transferred to 10 ml Brilliant Green Broth (Oxoid, England) and incubated at 42^{o}C for 24 hrs. A loop of the inoculum was spread on

MacConkey Agar (Merck, Germany) and incubated at 37°C for 24 hrs. Two presumptive *E. coli* colonies then were collected and transferred to nutrient agar (Appendix 10.3).

Motility, indole and hydrogen sulfide (H_2S) production were tested using SIM media (Merck, Germany). Using a sterile needle, a well-isolated colony was picked and stabbed into the medium to about 1 cm of the bottom of the tube. The inoculum was incubated overnight at 37°C. Following registration of motility and H_2S production, 2-3 drops of Kovac's reagent were directly added for the indole test.

- Positive result shows swarming growth around the line of inoculation due to motility, no black color production (H_2S production), and a pink ring formation for indole production. Negative results show growth of bacteria only along the line of the inoculation (non motile), blackening of culture (H_2S production), and no pink ring formation (indole negative).

At the Institute of Meat Hygiene and Technology, FU Berlin:

For gas production and the oxidation fermentation (O/F), sorbitol and mannitol utilization tests, a single colony was inoculated into BHI and incubated for 16-18 hrs at 37°C as stock culture (Appendix 10.3).

From this stock culture, 0.1 ml was inoculated into a test tube containing 5% glucose-based broth with an inverted Durham tube for fermentation. The inoculum was incubated for 18-24 hrs at 37°C for the gas production test.

- Positive results showed a meniscus of gas in the inverted Durham tube while negative results showed no gas in the tube.

Again from this stock culture, 0.1 ml was inoculated into paired O/F basal medium with 1% glucose. One of the tubes was overlaid with paraffin while the other was paraffin-free. The tubes were incubated at 37°C for 18-24 hrs for oxidation fermentation (O/F) test.

- Positive results showed a change of the greenish color of media into yellow for both oxidation and fermentation whereas negative results showed no yellow color in one and/or both of the test media.

Further, from the same stock culture, 0.1 ml was inoculated into a test tube containing sorbitol broth with phenol red as indicator. The inoculum was incubated at 37°C for 18-24 hrs for the sorbitol utilization test.

- Positive results showed a change of the pink color of the broth into yellow for sorbitol fermentation, while negative results showed no change in color.

Following the same procedure, 0.1 ml of the same stock culture was inoculated into a test tube containing mannitol broth with phenol red as indicator. The inoculum was incubated at 37°C for 18-24 hrs for the mannitol utilization test.

- *E. coli* positive results showed no change in color as no mannitol was utilized, while negative results showed yellow color production.

On the other hand, for the oxidase test, an isolate was inoculated into Standard I Nutrient Agar (Merck, Germany) and incubated for 18-24 hrs at 37°C. A single and pure colony was applied directly onto a commercial test strip (Bactident®; Merck, Germany) using a sterile wire loop and the result was observed within 60 sec.

- Positive results revealed blue or violet blue color on the strip while negative result showed no change of color.

The total number of *E. coli* isolates was 307. Of these, 107 were from the abattoir line while 200 were from the processing plant line (Table 4 - 07).

Table 4 - 07: Total number of isolates by bacteria type and line of origin

		Number of bacterial isolates	
Origin	**Domain**	***Salmonella***	***E. coli***
Abattoir line	Environment	37	41
	Animal-related samples	15	53
	Butchers	11	13
	Sub total	63	107
Processing plant line	Environment	10	98
	Animal-related samples	12	67
	Supermarkets	1	35
	Sub total	23	200
Grand total		86	307

4.5 Resistance testing

All *Salmonella* and 124 *E. coli* isolates were tested at ALIPB-AAU for antimicrobial susceptibility using agar diffusion. The remaining 183 *E. coli* isolates were tested (CLSI, 2007) at the Institute of Meat Hygiene and Technology, FU-Berlin.

Tests were done on Mueller-Hinton agar (Oxoid, Hampshire, England) using slightly modified Kirby Bauer disc diffusion criteria of the National Committee for Clinical

Laboratory Standards Institute (CLSI, 2007; Bauer *et al.*, 1996). The isolates were sub-cultured on Standard-I Nutrient agar (Merck, Germany) and incubated at 37°C for 24 hrs. They were then inoculated into 3 ml of Brain Heart Infusion broth (BHI) (Merck, Germany) and again incubated for 1 hr at 37°C. The inoculum density was standardized using the 0.5 McFarland standard. 0.1 ml of standardized culture (modified) was spread on Mueller-Hinton agar (Oxoid, Hampshire, England) (Appendix 10.4). Antimicrobial substances (Oxoid) were used as follows: amoxicillin (AML 25 µg), chloramphenicol (C 50 µg), gentamycin (CN 10 µg), kanamycin (K 30 µg), neomycin (N 10 µg), oxytetracycline (OT 30 µg), polymyxin B (PB 300U), tetracycline (TE 10 µg), trimethoprim-sulfamethoxazol (STX 1.25/23.75 µg) and trimethoprim (W 5 µg). Based on the diameter of the inhibition zone for *Enterobacteriaceae* (Table 4 - 08), results were recorded as susceptible, intermediate and resistant (CLSI, 2007; National Committee for Clinical and Laboratory Standard (NCCL) (NCCL, 2001).

Table 4 - 08. Zone of inhibition diameter in mm and minimum inhibitory concentration of drugs for *Enterobacteriaceae* (CLSI, 2007; OXOID, 2011)

No.	Abbreviation	Drug used		*Enterobacteriaceae* (*E. coli* and *Salmonella*) Zone of inhibition (mm)			Tested strains*
		Drug name	Concentration	Resistant	Intermediate	Susceptible	
1	AML	amoxicillin	25 µg	≤13	14-16	≥17	*E. coli*
2	C	chloramphenicol	50 µg	-	-	21-27	*E. coli+Salm.*
3	CN	gentamycin	10 µg	≤12	13-14	≥15	*E. coli+Salm.*
4	K	kanamycin	30 µg	≤13	14-17	≥18	*E. coli*
5	OT	oxytetracycline	30 µg	≤14	15-18	≥19	*E. coli+Salm.*
6	PB	polymyxin B	300U	≤11	-	≥12	*E. coli+Salm.*
7	TE	tetracycline	10 µg	≤11	12-14	≥15	*E. coli+Salm.*
8	SXT	trimethoprim-sulfamethoxazol	1.25/23.75 µg	≤10	11-15	≥16	*E. coli+Salm.*
9	W	trimethoprim	5 µg	≤10	11-15	≥16	*E. coli+Salm.*
10	N	neomycin	30 µg	≤13	14-15	≥16	*Salmonella*

**E. coli+Salm. = E. coli* and *Salmonella*

4.6 Data processing and analysis

Data from APC, EBC, *Salmonella* and *E. coli* as well as the antimicrobial susceptibility profiles were entered into Excel Microsoft 2007©. Then they were analyzed using Excel, State 11 and SPSS 20 (BIM statistics). APC and EBC were expressed using mean and standard deviations in logarithmic function, based on the type, source and origin of samples. Linear regression and single t-test were used to determine mean logarithms of counts within a sampling location, among sampling occasion, and sample type. Paired t-tests were used to compare mean logarithms of APC and EBC counts within a sampling location. Percentage

and Midd Pexect (Midd. Pex.) 95% confidence intervals (CI) were calculated for *Salmonella* and *E. coli* prevalences. Significance of both spoilage and zoonotic bacteria along the line steps and the sampling points were determined at *P*-values <0.05.

Test similarity of PFGE results of *Salmonella* serovars' molecular biology was analyzed using BioNumerics software (Applied Maths BVBA, Kortrijk, Belgium). Thus, optimization of 1.0 and position tolerance of 1.5 among serovars were applied.

5. RESULTS

Both lines were investigated for the presence of Salmonella and E.coli, which were tested for resistance, too. The processing line was also tested for spoilage microorganisms.

5.1 Abattoir line

This part includes the microbiological results of the examined samples from the abattoir (environmental and animal-related materials) and the butchers at Addis Ababa City.

5.1.1 Spoilage bacteria

In contrast to the abattoir line, where a high contamination level was to be expected, the processing line contains a preservation technique (heat). For this, different to the abbatoir line, this line was additionally investigated for its content of spoilage microorganisms as indicated by the APC and the Enterobacteriaceae Count.

5.1.2 *Salmonella*

Prevalence data for *Salmonella* in general and at individual sampling location and occasions with identified serotypes and molecular biology (PFGE) profiles from the abattoir line in particular are included in this section. This part also includes tracing back to possible sources and transmission routes of *Salmonella* along the line.

5.1.2.1 Salmonella prevalence

In the abattoir line, higher prevalence in its environment (36.6%; 95%CI: 27.6-46.4) than in animal-related materials (14.7%; 95%CI: 8.7-22.9) was observed. Prevalence at butchers was 32.4% (95%CI: 18.3-49.3), similarto both the abattoir environment and the animal-related materials. Except for mesenteric lymph nodes taken at the evisceration positionc (8.8%) and beef at the quality inspection (11.8%) that showed considerable lower prevalences than rooms (52.9%) and refrigerator (60.0%), all prevalences at all other locations in the abattoir line were the same ($p>0.05$) (Table 5 - 01).

Table 5 - 01: *Salmonella* isolations by sampling location and type of samples from the abattoir line

Line	Sample origin		Processing stages/position	Sampling location	Sample type	No. of samples	No. (%) *Salmonella* positive	Mid-Pex. 95% CI
Abattoir line	Abattoir[1]	Environment	Before stunning and before operation starts	Personnel's hands	Hands swab	13	5 (38.5)	15.7-65.9
				Aprons	Aprons swab	14	5 (35.7)	14.4-62.4
				Knives	Knives swab	13	4 (30.7)	10.6-58.7
				Tap water	Water sample	12	1 (8.3)	0.4-34.7
				Hooks	Hooks swab	11	2 (18.2)	3.2-48.3
			At carcass splitting	Rooms	Rooms swab	17	9 (52.9)	29.7-75.2
			Refrigeration	Refrigerator	Refrigerator swab	10	6 (60.0)	29.1-85.8
			Meat transport	Meat transport trucks	Truck swab	11	5 (45.5)	18.9-74.1
			Sub total			101	37 (36.6)	27.1-46.4
		ARM[¶]	Before stunning	Stunning	Animal feces	34	8 (23.5)	11.6-37.8
			During evisceration	Evisceration	MLN* sample	34	3 (8.8)	2.3-22.2
			After washing, ready for distribution	Quality inspection	Raw meat sample	34	4 (11.8)	3.8-25.9
			Sub total			102	15 (14.7)	8.8-22.6
	Butchers		Butchers, 6-8 hrs post delivery	Beef for public consumption	Retailed meat sample	34	11 (32.4)	18.3-49.3
	Total					237	63 (26.6)	21.3-32.5

ARM[¶] = Animal-related materials
MLN* = Mesenteric lymph node
[1]Abbatoir chain after Gudeta Gudeta (2012)

Based on sampling occasions at the abattoir line (Table A 01), no differences in *Salmonella* prevalences ($p>0.05$) were observed in total between and among all sampling occasions. In particular, on each sampling occasion, no difference was observed between and among environment, animal-related materials and butchers samples ($p>0.05$) except for the second occasion when a statistically higher prevalence was observed in the abattoir environment (33.3%; 95%CI: 17.6-52.4) than in animal-related materials (5.6%; 95% CI: 0.9-17.2) ($p<0.05$).

As shown in Table A 02, at least one or more sampling location was found positive for *Salmonella* during the sampling occasions. On the third sampling occasion, except for the sample types aprons, tap water and hooks, all other sampling locations were positive for *Salmonella.*

5.1.2.2 Salmonella serovars

The high prevalence of 11.4% and the proportion of 42.8% of *S*. Saintpaul was followed by 5.9% prevalence and 22.2% proportion of *S*. Muenchen . Further prevalences were 4.6% for *S*. Larochelle, 1.7% for *S*. Dublin, 1.3% for *S*. Kastrup, and 0.24% for *S*. London. Unidentified cases accounted for about 1.3% (Table 5 - 02).

Table 5 - 02: Isolated *Salmonella* serotypes. their prevalences and proportions at the abattoir line

***Salmonella* Serotypes**	**Positive No. of isolates**	**Prevalence (%) in samples (n = 237)**	**Proportion (%) of isolates (n = 63)**
S. Saintpaul	27	11.4	42.8
S. Muenchen	14	5.9	22.2
S. Larochelle	11	4.6	17.5
S. Dublin	4	1.7	6.4
S. Kastrup	3	1.3	4.8
S. London	1	0.24	1.6
Unidentified	3	1.3	4.8
Total	63	26.3	100

As shown in Table A 03, *S.* Saintpaul was the predominant serotype and was observed at all sampling locations positive for *Salmonella*, with the exception of hooks. *S*. Larochelle and *S*. Muenchen were also major serotypes, they were observed in the majority of *Salmonella* positive sampling locations in the line. *S*. Dublin was observed only in room and animal feces samples at the abattoir and in retail meat at butchers. Unidentified isolates were observed in animal feces and beef at butchers.

Table 5 - 03 shows the diversity, frequencies and types of serovars in relation to sampling locations and occasions. With the exception of the 5th sampling occasion, where only two serotypes (*S*. Muenchen and *S*. Larochelle) were observed, on all other occasions 3 to 5 serovars with variable frequencies were obtained. *S*. Saintpaul, *S*. Muenchen and *S*. Larochelle were the serovars most frequently observed. *S*. Dublin and *S*. London were observed only during the 1st sampling occasion. All unidentified *Salmonella* isolates were observed on the 2nd sampling occasion. With regard to sampling location, personnel's hands, aprons and knives were frequently positive for *S*. Saintpaul, *S*. Muenchen and *S*. Larochelle. On the 4th sampling occasion, *S*. Muenchen and *S*. Larochelle showed a higher frequency in environmental samples than in animal-related materials and at butchers.

Table 5 - 03: Distribution of *Salmonella* serovars in positive sampling locations and occasions at the abattoir line

Origin/ source	Sampling locations*	Total No. of isolates	Sampling occasions				
			Occasion 1	Occasion 2	Occasion 3	Occasion 4	Occasion 5
			Serovar type and number in bracket				
Environment	Personnel's hands	5	*S*. Saintpaul (1)	*S*. Saintpaul (1) *S*. Kastrup (1)	*S*. Saintpaul (2)		
	Aprons	5		S. Saintpaul (1) *S*. Larochelle (1) *S*. Muenchen (1)			*S*. Muenchen (2)
	Knives	4		*S*. Saintpaul (1) *S*. Muenchen (1)	*S*. Larochelle (1)	*S*. Muenchen (1)	
	Water	1				*S*. Saintpaul (1)	
	Hooks	2	*S*. Muenchen (1)			*S*. Larochelle (1)	
	Rooms	9	*S*. Saintpaul (2) *S*. Dublin (1)	*S*. Saintpaul (1)	*S*. Saintpaul (1)	*S*. Larochelle (1) *S*. Muenchen (1)	*S*. Larochelle (1) S. Muenchen (1)
	Refrigerators	6			*S*. Saintpaul (1)	*S*. Muenchen (2)	*S*. Larochelle (1) S. Muenchen (2)
	Trucks	5	*S*. Saintpaul (1)	*S*. Saintpaul (1)	S. Saintpaul (2)		*S*. Muenchen (1)
	Subtotal n (%)	37	6 (16.2)	9 (24.3)	7 (18.9)	7 (18.9)	8 (21.6)
	Mid Pex. 95%CI		(6.8-30.7)	(12.6-39.9)	(8.6-33.8)	(8.6-33.8)	(10.6-36.9)
ARM	Feces	8	*S*. Dublin (1)	Unidentified (2)	*S*. Saintpaul (2) *S*. Kastrup (1)	*S*. Larochelle (1)	*S*. Larochelle (1)
	MLN*	3			*S*. Saintpaul (1) *S*. Muenchen (1)	*S*. Kastrup (1)	
	Raw meat	4	*S*. Dublin (1)		*S*. Saintpaul (2)	*S*. Larochelle (1)	
	Subtotal n (%)	15	2 (13.3)	2 (13.3)	7 (46.7)	3 (20.0)	1 (6.7)
	Mid Pex. 95%CI		(2.3-37.5)	(2.3-37.5)	(23.2-71.3)	(5.3-43.4)	(0.3-26.7)
Butchers	Beef at butchers	11	*S*. Saintpaul (2) *S*. London (1) *S*. Dublin (1)	*S*. Saintpaul (1) Unidentified (1)	*S*. Saintpaul (3)		*S*. Larochelle (2)
	Subtotal n (%)	11	4 (36.4)	2 (18.9)	3 (27.3)	0	2 (18.9)
	Mid Exe. 95%CI		(12.7-66.4)	(3.1-48.3)	(7.4-57.8)	(0.0-23.8)	(3.2-48.3)
	Grand total n (%)	63	12 (19.1)	13 (20.6)	17 (27.0)	10 (15.9)	11 (17.5)
	Mid Pex. 95%CI		(10.7-30.1)	(11.9-31.9)	(17.1-38.9)	(8.3-26.5)	(9.5-28.3)

ARM = Animal-related materials MLN* = Mesenteric lymph node n = Number

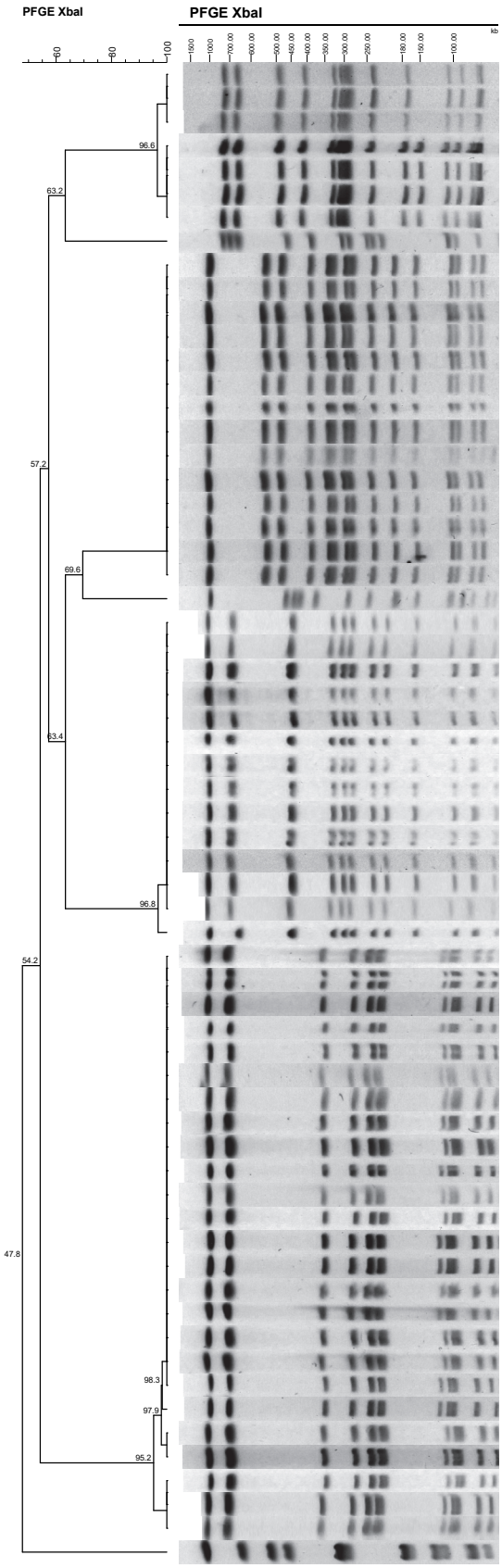
5.1.2.3 Pulsed-field gel electrophoresis (PFGE)

5.1.2.3.1 PFGE profiles of Salmonella

The PFGE patterns among serotypes and the dendrogram profiles of *Salmonella* serotypes obtained from the abattoir line are shown in Table 5 - 04 and Fig. 5 - 01, respectively. All 63 strains isolated from the abattoir line, analyzed and processed using BioNumerics®6.6, show 1, 2 and 6 pulsotypes with 1 to 14 ratios of isolates to pulsotypes in all isolates (*S*. Dublin, *S*. Kastrup, *S*. Larochelle, *S*. Saintpaul, *S*. London, *S*. Muenchen, as well as unidentified ones) (Table A - 04).

Table 5 - 04: Number of *Salmonella* serotypes isolated and PFGE patterns obtained for each serotype at the abattoir line

***Salmonella* Serotypes**	**Total number of isolates/serotypes**	**Number of pulsotypes**	**Ratio (isolates/pulsotypes)**
S. Saintpaul	27	6	4.5
S. Muenchen	14	1	14
S. Larochelle	11	2	5.5
S. Dublin	4	1	4
S. Kastrup	3	1	3
S. London	1	1	1
Unidentified	3	1	3
Total	63		



Key	Serovars	Sample	Source	Batch	Code	pulsoty
5340.	Unidentified (Rauhform)	Fecal	Abattoir	4th	11	UnX1
5341.	Unidentified (Rauhform)	Fecal	Abattoir	4th	13	UnX1
5342.	Unidentified (Rauhform)	Meat	Butchery	4th	13	UnX1
5325.	S. Dublin	Fecal	Abattoir	3rd	6	SDuX1
5326.	S. Dublin	Meat	Abattoir	3rd	6	SDuX1
5322.	S. Dublin	Room	Abattoir	3rd	-	SDuX1
5328.	S. Dublin	Meat	Butchery	3rd	6	SDuX1
5320.	S. Saintpaul	Room	Abattoir	3rd	-	SSaX5
5371.	S. Muenchen	Apron	Abattoir	18th	12	SMuX1
5372.	S. Muenchen	Apron	Abattoir	18th	13	SMuX1
5333.	S. Muenchen	Apron	Abattoir	4th	4	SMuX1
5323.	S. Muenchen	Hook	Abattoir	3rd	-	SMuX1
5362.	S. Muenchen	Knife	Abattoir	17th	11	SMuX1
5332.	S. Muenchen	Knife	Abattoir	4th	4	SMuX1
5357.	S. Muenchen	MLN	Abattoir	11th	26	SMuX1
5364.	S. Muenchen	Refrigerator	Abattoir	17th	-	SMuX1
5365.	S. Muenchen	Refrigerator	Abattoir	17th	-	SMuX1
5374.	S. Muenchen	Refrigerator	Abattoir	18th	-	SMuX1
5376.	S. Muenchen	Refrigerator	Abattoir	18th	-	SMuX1
5367.	S. Muenchen	Room	Abattoir	17th	-	SMuX1
5377.	S. Muenchen	Room	Abattoir	18th	-	SMuX1
5373.	S. Muenchen	Truck	Abattoir	18th	-	SMuX1
5330.	S. Saintpaul	Meat	Butchery	3rd	8	SSaX6
5334.	S. Larochelle	Apron	Abattoir	4th	5	SKLX1
5369.	S. Larochelle	Fecal	Abattoir	17th	29	SKLX1
5379.	S. Larochelle	Fecal	Abattoir	18th	31	SKLX1
5354.	S. Kastrup	Fecal	Abattoir	11th	24	SKLX1
5331.	S. Kastrup	Hand Swab	Abattoir	4th	4	SKLX1
5361.	S. Larochelle	Hook	Abattoir	17th	-	SKLX1
5350.	S. Larochelle	Knife	Abattoir	11th	10	SKLX1
5368.	S. Larochelle	Meat	Abattoir	17th	29	SKLX1
5370.	S. Kastrup	MLN	Abattoir	17th	29	SKLX1
5375.	S. Larochelle	Refrigerator	Abattoir	18th	-	SKLX1
5366.	S. Larochelle	Room	Abattoir	17th	-	SLKX1
5378.	S. Larochelle	Room	Abattoir	18th	-	SKLX1
5380.	S. Larochelle	Meat	Butchery	18th	32	SKLX1
5381.	S. Larochelle	Meat	Butchery	18th	33	SKLX2
5337.	S. Saintpaul	Apron	Abattoir	4th	7	SSaX2
5353.	S. Saintpaul	Fecal	Abattoir	11th	23	SSaX2
5355.	S. Saintpaul	Fecal	Abattoir	11th	26	SSaX2
5348.	S. Saintpaul	Hand Swab	Abattoir	11th	8	SSaX2
5349.	S. Saintpaul	Hand Swab	Abattoir	11th	9	SSaX2
5319.	S. Saintpaul	Hand Swab	Abattoir	3rd	2	SSaX2
5335.	S. Saintpaul	Hand Swab	Abattoir	4th	7	SSaX2
5351.	S. Saintpaul	Meat	Abattoir	11th	22	SSaX2
5356.	S. Saintpaul	MLN	Abattoir	11th	22	SSaX2
5346.	S. Saintpaul	Refrigerator	Abattoir	11th	-	SSaX2
5321.	S. Saintpaul	Room	Abattoir	3rd	-	SSaX2
5338.	S. Saintpaul	Room	Abattoir	4th	-	SSaX2
5344.	S. Saintpaul	Truck	Abattoir	11th	-	SSaX2
5345.	S. Saintpaul	Truck	Abattoir	11th	-	SSaX2
5324.	S. Saintpaul	Truck	Abattoir	3rd	-	SSaX2
5339.	S. Saintpaul	Truck	Abattoir	4th	-	SSaX2
5359.	S. Saintpaul	Meat	Butchery	11th	23	SSaX2
5360.	S. Saintpaul	Meat	Butchery	11th	25	SSaX2
5343.	S. Saintpaul	Meat	Butchery	4th	19	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
5352.	S. Saintpaul	Meat	Abattoir	11th	23	SSaX4
5347.	S. Saintpaul	Room	Abattoir	11th	-	SSaX4
5329.	S. London	Meat	Butchery	3rd	7	SLoX1

Fig. 5 - 01: Dendrogram of PFGE patterns (pulsotypes) of 63 *Salmonella* isolates obtained from the abattoir line

With the exception of tap water, where only one isolate was observed, 2 up to 7 pulsotypes were found in isolates from other positive locations. Ratios of isolates to serotype as well as isolates to pulsotype were 1 to 2.5 in isolates obtained from different sampling locations at the abattoir line (Table A - 04).

Pulsotype distribution of the 63 *Salmonella* serovars in positive sampling locations and occasions/batches at the abattoir line are described in Table 5 - 05. The number of pulsotypes of *S.* Saintpaul (SSa*X*)[1] observed during in the 1st to the 4th sampling occasions were 4, 2, 3 and 1, respectively, while only 2 pulsotypes of SKL[2] and 1 of SMuX[3] were observed on the 5th sampling occasion.

With regard to individual animals, pulsotypes of *Salmonella* isolated from animal-related materials and products (raw beef at the butchers) are described in Table 5 -06 and Fig 5 - 01. Isolates from animal number/code 6 and associated samples were 3 SDu*X*1[4]. They showed similar pulsotypes and isolates during the 1st sampling occasion. Two pulsotypes of SSa*X* were observed in each of samples from animals coded 22 and 23. Samples taken from an animal coded 29 were positive for *S.* Kastrup and *S.* Larochelle of a similar pulsotype (SKL*X*1).

[1] SSa*X*: S = *Salmonella*, Sa = Saintpaul, *X* = *Endonuclease enzyme XbaI*
[2] S<u>K</u>L*X*: *S.* Kastrup; SK<u>L</u>*X* = *S.* Larochelle
[3] SMu*X* = *S.* Muenchen
[4] SDu*X* = *S.* Dublin

Table 5 - 05: Pulsotype distribution of 63 *Salmonella* serovars in positive sampling locations and occasions at the abattoir line[¶]

Origin/ source	Sampling sources/ locations	Total No. of isolates	Sampling occasions					Total number of serotype
			Occasion 1/Batch 3 Pulsotype	Occasion 2/ Batch 4 Pulsotype	Occasion 3/ Batch 11 Pulsotype	Occasion 4/ Batch 17 Pulsotype	Occasion 5/ Batch 18 Pulsotype	
Environment	Personnel's hands	5	SSa*X*2	SSa*X*2 SKL*X*1	SSa*X*2 SSa*X*2			2
	Aprons	5		SSa*X*2 SKL*X*1 SMu*X*1			SMu*X*1 SMu*X*1	3
	Knives	4		SSa*X*4 SMu*X*1	SKL*X*1	SMu*X*1		3
	Water	1				SSa*X*3		1
	Hooks	2	SMu*X*1			SKL*X*1		2
	Rooms	9	SSa*X*2 SSa*X*5 SDu*X*1	SSa*X*2	SSa*X*4	SKL*X*1 SMu*X*1	SKL*X*1 SMu*X*1	4
	Refrigerators	6			SSa*X*2	SMu*X*1 SMu*X*1	SKL*X*1 SMu*X*1 SMu*X*1	3
	Trucks	5	SSa*X*2	SSa*X*2	SSa*X*2 SSa*X*2		SMu*X*1	2
	Subtotal	37	6	9	7	7	8	
ARM	Feces	8	SDu*X*1	Un*X*1 Un*X*1	SSa*X*2 SSa*X*2 SKL*X*1	SKL*X*1	SKL*X*1	5
	MLN*	3			SSa*X*2 SMu*X*1	SKL*X*1		3
	Raw meat	4	SDu*X*1		SSa*X*2 SSa*X*4	SKL*X*1		3
	Subtotal	15	2	2	7	3	1	
Butchers	Beef at butchers	11	SSa*X*1 SSa*X*6 SDu*X*1 SLo*X*1	SSa*X*2 Un*X*1	SSa*X*2 SSa*X*2 SSa*X*3		SKL*X*1 SKL*X*2	5
	Subtotal	11	4	2	3	0	2	
Grand total No.		63	12	13	17	10	11	

[¶]SSa*X* = *S.* Saintpaul; SDu*X* = *S.* Dublin; SLo*X* = *S.* London; SKL*X*1= *S.* Kastrup; SKL*X*1 = *S.* Larochelle; SMu*X* = *S.* Muenchen; Un*X* = Unidentified

Table 5 - 06: Pulsotype distribution of 26 *Salmonella serovars* from positive sampling locations and occasions with corresponding animal identification number at the abattoir line

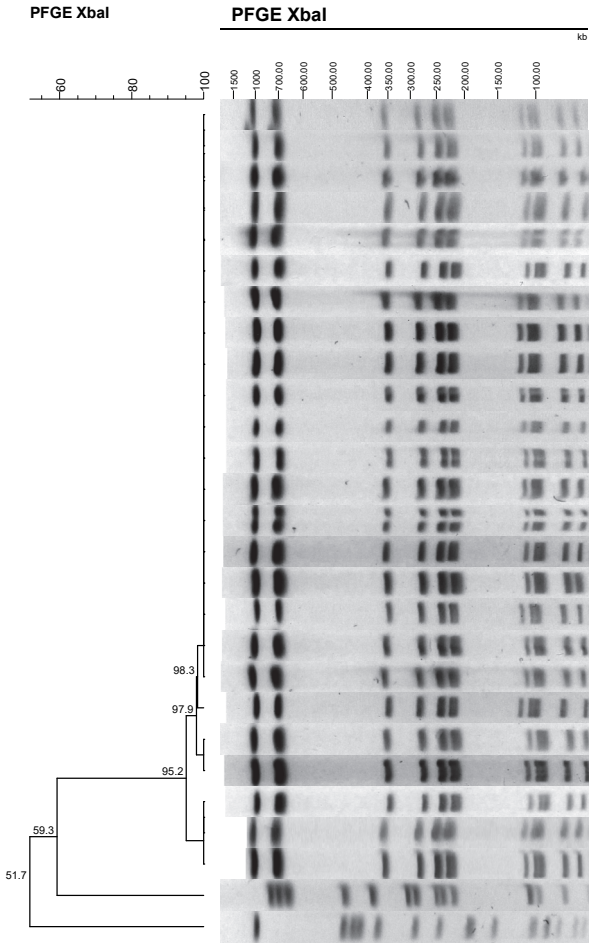
Origin/ source	**Sampling locations**	**Identification number of *Salmonella* positive animals**															
		#5	**#6**	**#7**	**#8**	**#11**	**#13**	**#19**	**#22**	**#23**	**#24**	**#25**	**#26**	**#29**	**#31**	**#32**	**#33**
		Occasion 1/Batch 3				**Occasion 2/ Batch 4**				**Occasion 3/ Batch 11**				**Occasion 4/ Batch 17**	**Occasion 5/ Batch 18**		
Abattoir (ARM)	Feces		SDu*X*1			Un*X*1	Un*X*1			SSa*X*2	S<u>K</u>L*X*1		SSa*X*2	SK<u>L</u>*X*1	S<u>K</u>L*X*1		
	MLN*								SSa*X*2				SMu*X*1	SK<u>L</u>*X*1			
	Raw meat		SDu*X*1						SSa*X*2	SSa*X*4				SK<u>L</u>*X*1			
Butchers	Beef at butchers	SSa*X*1	SDu*X*1	SLo*X*1	SSa*X*6		Un*X*1	SSa*X*2	SSa*X*3	SSa*X*2		SSa*X*2				S<u>K</u>L *X*1	SK<u>L</u>*X*2
Total No. of isolates			6				4				10			3		3	

\# = animal ID/ Code
ARM = Animal-related materials
MLN* = Mesenteric lymph node
SSa*X* = *S*. Saintpaul; SDu*X* = *S.* Dublin; SLo*X* = *S*. London; S<u>K</u>L*X*1: *S*. Kastrup; SK<u>L</u>*X*1 = *S*. Larochelle; SMu*X* = *S*. Muenchen; Un*X* = Unidentified

5.1.2.3.2 Tracking possible sources and transmission routes

S. Saintpaul

As shown in Fig. 5 - 02 and Table 5 - 07, different pulsotypes of *S*. Saintpaul were observed over the sampling occasions/batches in different sampling locations. Most of them are the SSaX2 pulsotype and are spread over different locations like personnel´s hand swabs, rooms, feces, trucks and meat at butchers. This was observed on different sampling occasions. However, other pulsotypes of *S*. Saintpaul like SSa*X*5 from room, SSa*X*3 from water and SSa*X*1 and SSa*X*6 from butchers were also observed.



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

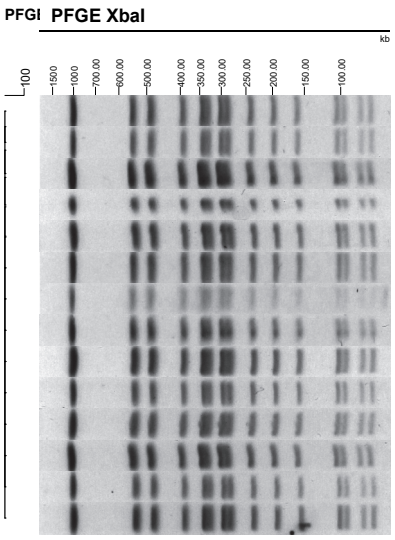
Fig. 5 - 02. Dendrogram profiles of *S*. Saintpaul isolated from the abattoir line

Table 5 - 07: Possible source and transmission routes of *S.* Saintpaul isolated along the abattoir line

Sampling		Samples and pulsotypes											
Batch	Occasion	Personnel's hands swab	Knifes	Aprons	Room	Water	Refriger. swab	Feces	MLN	Meat Abattoir	Truck	Meat at Butchers	Total No. of isolates
3	1	SSa*X*2			SSa*X*5 SSa*X*2						SSa*X*2	SSa*X*1 SSa*X*6	6
4	2	SSa*X*2	SSa*X*4	SSa*X*2	SSa*X*2						SSa*X*2	SSa*X*2	6
11	3	SSa*X*2 SSa*X*2			SSa*X*4		SSa*X*2	SSa*X*2 SSa*X*2	SSa*X*2	SSa*X*2 SSa*X*4	SSa*X*2 SSa*X*2	SSa*X*3 SSa*X*2 SSa*X*2	14
17	4					SSa*X*3							1
Total No.		4	1	1	4	1	1	2	1	2	4	6	27

S. Muenchen

All *S.* Muenchen obtained showed a similar pulsotype at different sampling locations on different sampling occasions. The pulsotype obtained from MLN of animals was also similar to all others obtained from environmental samples (Fig.5 - 03 and Table 5 - 08).



Key	Serovars	Sample	Source	Batch	Code	pulsotype
5323.	S. Muenchen	Hook	Abattoir	3rd	-	SMuX1
5332.	S. Muenchen	Knife	Abattoir	4th	4	SMuX1
5333.	S. Muenchen	Apron	Abattoir	4th	4	SMuX1
5357.	S. Muenchen	MLN	Abattoir	11th	26	SMuX1
5362.	S. Muenchen	Knife	Abattoir	17th	11	SMuX1
5364.	S. Muenchen	Refrigera.	Abattoir	17th	-	SMuX1
5365.	S. Muenchen	Refrigera.	Abattoir	17th	-	SMuX1
5367.	S. Muenchen	Room	Abattoir	17th	-	SMuX1
5371.	S. Muenchen	Apron	Abattoir	18th	12	SMuX1
5372.	S. Muenchen	Apron	Abattoir	18th	13	SMuX1
5373.	S. Muenchen	Truck	Abattoir	18th	-	SMuX1
5374.	S. Muenchen	Refrigera.	Abattoir	18th	-	SMuX1
5376.	S. Muenchen	Refrigera.	Abattoir	18th	-	SMuX1
5377.	S. Muenchen	Room	Abattoir	18th	-	SMuX1

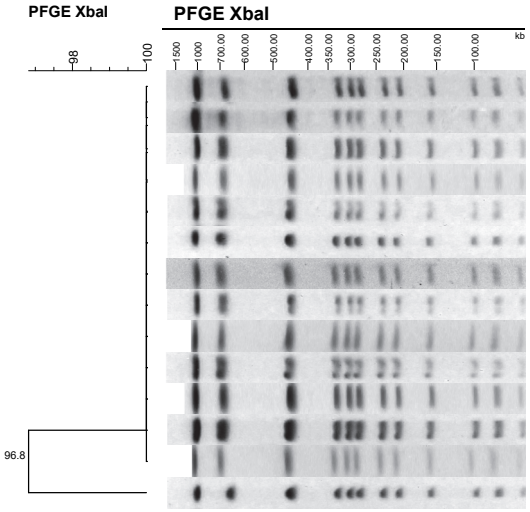
Fig. 5 - 03. Dendrogram profiles of *S.* Muenchen isolated from the abattoir line

Table 5 - 08: Possible source and transmission routes of *S.* Muenchen isolated along the abattoir line

Sampling		Samples and pulsotypes							Total No. of isolates
Batch	Occasion	Knifes	Aprons	Room	Hooks	Refrig.	MLN	Truck	
3	1				SMu*X*1				1
4	2	SMu*X*1	SMu*X*1						2
11	3						SMu*X*1		1
17	4	SMu*X*1		SMu*X*1		SMu*X*1 SMu*X*1			4
18	5		SMu*X*1 SMu*X*1	SMu*X*1		SMu*X*1 SMu*X*1		SMu*X*1	6
Total No.		2	3	2	1	4	1	1	14

S. Kastrup and *S.* Larochelle

As shown in Fig.5 - 04 and Table 5 - 09**,** the 3 *S.* Kastrup (S<u>K</u>L*X*1) and the 10 *S.* Larochelle (SK<u>L</u>*X*1) observed were similar in pulsotype with themselves and also with each other at different sampling locations and occasions. In fact, only one pulsotype of *S.* Larochelle (SK<u>L</u>*X*2) observed from a final product at a butcher was different from others.



Key	Serovars	Sample	Source	Batch	Code	pulsotype
5331.0	S. Kastrup	Hand S.	Abattoir	4th	4	SKLX1
5354.0	S. Kastrup	Fecal	Abattoir	11th	24	SKLX1
5370.0	S. Kastrup	MLN	Abattoir	17th	29	SKLX1
5334.0	S. Larochelle	Apron	Abattoir	4th	5	SKLX1
5350.0	S. Larochelle	Knife	Abattoir	11th	10	SKLX1
5361.0	S. Larochelle	Hook	Abattoir	17th	-	SKLX1
5366.0	S. Larochelle	Room	Abattoir	17th	-	SLKX1
5368.0	S. Larochelle	Meat	Abattoir	17th	29	SKLX1
5369.0	S. Larochelle	Fecal	Abattoir	17th	29	SKLX1
5375.0	S. Larochelle	Refriger.	Abattoir	18th	-	SKLX1
5378.0	S. Larochelle	Room	Abattoir	18th	-	SKLX1
5379.0	S. Larochelle	Fecal	Abattoir	18th	31	SKLX1
5380.0	S. Larochelle	Meat	Butchery	18th	32	SKLX1
5381.0	S. Larochelle	Meat	Butchery	18th	33	SKLX2

Fig. 5 - 04. Dendrogram profiles of *S.* Kastrup and *S.* Larochelle isolated from the abattoir line

Table 5 - 09: Possible source and transmission routes of *S*. Kastrup and *S*. Larochelle isolated along the abattoir line

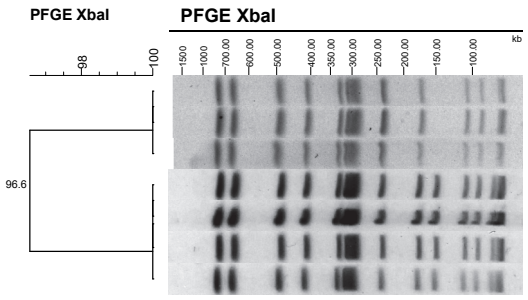
Sampling		Swab Samples and pulsotypes										
Batch	Occasion	Personnel's hand	Knifes	Aprons	Room	Hooks	Refrigerator	Feces	MLN	Meat at Abattoir	Meat at butchers	Total No. of isolates
4	2	SKL*X*1		SKL*X*1								2
11	3		SKL*X*1					SKL*X*1				2
17	4				SKL*X*1	SKL*X*1		SKL*X*1	SKL1	SKL*X*1		5
18	5				SKL*X*1		SKL*X*1	SKL*X*1			SKL*X*1	4
											SKL*X*2	1
Total No.		1	1	1	2	1	1	3	1	1	2	14

SKL*X*1 = S. Kastrup; SKL*X*1 = S. Larochelle

S. Dublin and unidentified strains

All *S*. Dublin isolates (SDu*X*1) obtained during the 1st sampling occasion showed a similar pulsotype. Three of them were obtained from samples taken from an animal coded with number "6" (Fig. 5 - 05 and Table 5 - 10).

Some unidentified *Salmonella* strains that were obtained during the 2nd sampling occasion also showed similar pulsotypes. They were all from animal related samples (Fig. 5 - 05 and Table 5 - 10).



Key	Serovars	ETHID	Sample	Source	Batch	Code	pulsotype
5340.	Unidentified (R.	A087	Fecal	Abattoir	4th	11	UnX1
5341.	Unidentified (R.	A089	Fecal	Abattoir	4th	13	UnX1
5342.	Unidentified (R.	A125	Meat	Butchery	4th	13	UnX1
5322.	S. Dublin	A019	Room	Abattoir	3rd	-	SDuX1
5325.	S. Dublin	A031	Fecal	Abattoir	3rd	6	SDuX1
5326.	S. Dublin	A047	Meat	Abattoir	3rd	6	SDuX1
5328.	S. Dublin	A055	Meat	Butchery	3rd	6	SDuX1

Fig. 5 - 05. Dendrogram profiles of *S*. Dublin and unidentified isolates from the abattoir line

Table 5 - 10: Possible source and transmission routes of *S*. Dublin and unidentified isolates along the abattoir line

Serotype	Sampling		Sample and pulsotype				Total No. of isolates
	Batch	Occasion	Room swab	Fecal	Meeat at abattoir	Meat at butchers'	
S. Dublin	3	1	SDu*X*1	SDu*X*1	SDu*X*1	SDu*X*1	4
Total No.			1	1	1	1	4
Unidentified	4	2		Un*X*1		Un*X*1	3
				Un*X*1			
Total No.				2		1	3

5.1.3 *E. coli* along the abattoir line

In this section, prevalence of *E. coli* overall and at every sampling location and occasion from the abattoir and butchers are reported.

5.1.3.1 Overall E. coli prevalence

Overall prevalence of *E. coli* at the abattoir line (45.1%; 95%CI: 38.9-51.5) showed the same result as prevalence in the environment (40.6%; 95%CI: 31.4-50.4), animal-related materials at the abattoir (52.0%; 95%CI: 42.3-61.5) or in retailed meat at butchers (38.2%; 95%CI: 23.2-55.2). Similarly, prevalence rates at the majority of individual sampling locations were not different from each other, ranging from 33.3% (95%CI: 11.6-62.3) in tap water to 55.9% (95%CI: 39.1-71.7) in raw beef at the abattoir (Table A 05). By comparing the 95% confidence intervals, no significant difference in *E. coli* prevalences isseen between locations.

Observations from the abattoir line (Table A - 06) generally indicate similar but lower *E. coli* prevalence on the 1st (35.1%; 95% CI: 23.6-48.1) and 3rd (16.0%; 95%CI: 7.7-28.1) sampling occasion as compared to the 2nd, 4th and 5th occasions ($p<0.05$). Within each sampling occasion no principal difference ($p>0.05$) in prevalence between and among environment, animal-related materials and butchers ($p>0.05$) was observed.

5.1.3.2 Prevalence in the abattoir

Seen from the point of view of sources, prevalence in the environmental samples collected during the 1st, 2nd and 3rd sampling occasion was similar ($p>0.05$) but it was lower compared to the 5th occasion (93.8%; 95%CI: 72.8-99.0) ($p<0.05$). Prevalence of the 3rd sampling occasion was lower than that of the 4th.

When it comes to animal-related materials, prevalences of samples taken during the 2nd, 4th and 5th occasion were similar but higher compared to the prevalences at the 1st and the 3rd occasion. Prevalences at these two occasions weresimilar ($p>0.05$) (Table A - 06).

5.1.3.3 Prevalence at the butchers

Regardless of the number of samples collected, respective prevalences at the butchers were 37.5%, 41.7%, 14.3%, 66.7% and 50.0% on the 1st, 2nd, 3rd, 4th and 5th sampling occasions. No differences in prevalence were observed between and among these sampling occasions at the butchers ($p>0.05$) (Table A - 06).

As shown in Table A - 07, only samples from personnel's hand swabs, all animal related sample materials at the abattoir, and retailed meat at butchers were positive throughout all sampling occasions. In contrast, However, all samples without exception were positive on the 5th sampling occasion.

5.2 Processing plant line

As described in the section "materials and methods", this part contains the microbiological results from samples taken from the processing plant (environmental and animal-related materials) at Bishoftu town and from the recipient supermarkets at Addis Ababa City.

5.2.1 Spoilage bacteria

5.2.1.1 Overall spoilage bacteria

Samples for spoilage bacterial load examination were taken from the processing plant line (the processing plant itself and supermarkets). As seen from Table 5 - 11**,** very high (5.09±0.42 and 5.28±0.24) log APCs were observed in samples from personnel's hands and room floor swabs, respectively, while very low (3.99±0.75) log APC were detected in samples from spices.

Relatively high counts (3.19±0.55 and 3.03±0.59) of log EBC were observed in swab samples from room floors and the grinder while very low counts (2.08±0.19) of log EBC were noted in products at the Supermarket(G). Differences as high as 2.75±0.65 ($\log_{10}$ APC – $\log_{10}$ EBC) between Supermarket-A and 1.71±0.70 ($\log_{10}$ APC – $\log_{10}$ EBC) for spices (Processing plant/ Environment) were observed (Table 5 – 11).

Spoilage bacteria in all the 23 locations showed significant differences within a sampling location for APC ($p<0.05$) and for EBC ($p<0.05$). Significantly higher APC than EBC were observed within each sampling location ($p<0.05$), too. Higher bacterial load on aprons

(5.28±0.27 log APC; 95%CI: 5.14-5.42) than in most of the locations was observed. A higher APC count was obtained in raw beef (4.82±0.52 log APC; 95%CI: 4.73-4.91) than in products (4.52±0.72 log APC; 95%CI: 4.39-4.69) as well as a higher EBC count in raw beef (2.77±0.59 log EBC; 95%CI: 2.66-2.88) than in products (2.21±0.39 log EBC; 95%CI: 2.24-2.28). The differences were significant in raw beef (2.05±0.64 log APC-EBC; 95%CI: 1.93-2.17) and product (2.31±0.74 log APC-EBC; 95%CI: 2.18-2.45).

Using APC and EBC criteria of NSW (2009), in total and for each supermarket, the product was found "*acceptable*" at all supermarkets.

Table 5 - 11: Status of spoilage bacteria loads by sampling location at the processing plant line

Sample origin		Sampling location	No. of samples	Unit	Log $_{10}$ APC			Log $_{10}$ EBC			Log $_{10}$ APC – Log $_{10}$ EBC		
					Mean ±SD	t-value	Mean 95%CI	Mean ±SD	t-value	Mean 95%CI	Mean ±SD	t-value	Mean 95%CI
Processing plant	Environment	Personnel's hands	19	cm^2	5.09±0.42	53.0	4.89-5.29[a]	2.94±0.73	17.5	2.59-3.29	2.16±0.82	11.5	1.76-2.55
		Aprons	16	cm^2	5.28±0.27	79.2	5.14-5.42[c]	3.07±0.63	19.4	2.73-3.40	2.21±0.66	13.5	1.86-2.56
		Knives	15	cm^2	4.94±0.38	50.0	4.73-5.15	2.84±0.66	16.5	2.47-3.21	2.09±0.68	12.0	1.72-2.47
		Cutting plates	13	cm^2	4.79±0.49	35.1	4.50-5.09[d]	2.99±0.61	17.7	2.62-3.36	1.81±0.52	12.6	1.45-2.12
		Tap water	17	ml	4.54±0.45	42.0	4.31-4.77	2.49±0.59	17.3	2.19-2.80	2.04±0.52	15.6	1.76-2.32
		Working tables	17	cm^2	5.05±0.47	44.0	4.80-5.29	2.86±0.76	15.5	2.47-3.26	2.18±0.78	11.5	1.78-2.59
		Room floors	16	cm^2	5.01±0.48	41.3	4.75-5.27	3.19±0.55	23.0	2.89-3.48	1.83±0.27	26.6	1.68-1.97
		Refrigerators	15	cm^2	4.75±0.56	32.8	4.44-5.06[d]	2.94±0.79	14.4	2.50-3.38	1.81±0.64	10.9	1.46-2.16
		Spices	15	grams	3.99±0.75	20.5	3.57-4.40	2.28±0.52	17.1	1.99-2.56	1.71±0.70	9.4	1.32-2.09
		SWE**	15	cm^2	4.71±0.58	31.6	4.39-5.04[d]	2.81±0.80	13.6	2.36-3.25	1.91±0.67	10.9	1.54-2.28
		Grinders	9	cm^2	4.99±0.59	25.5	4.53-5.44	3.03±0.59	15.5	2.58-3.48	1.96±0.62	9.5	1.49-2.43
		Cutters	9	cm^2	4.32±0.70	18.5	3.79-4.86[b]	2.37±0.45	15.9	2.03-2.72	1.94±0.68	8.5	1.42-2.47
		Mixers	9	cm^2	4.92±0.64	23.2	4.43-5.41	2.98±0.93	9.6	2.26-3.69	1.94±0.77	7.6	1.35-2.54
		Fillers/Stuffers	9	cm^2	4.57±0.68	20.3	4.05-5.09[d]	2.47±0.57	13.1	2.04-2.91	2.10±0.59	10.6	1.64-2.56
	ARM[¶]	Raw beef	118	grams	4.82±0.51	102.5	4.73-4.91	2.77±0.59	51.4	2.66-2.88	2.05±0.64	35.6	1.93-2.17
Supermarkets		Supermarket-A	15	grams	4.97±0.50[A]	38.6	4.69-5.25[e]	2.22±0.46 [A]	18.7	1.97-2.47	2.75±0.65	16.5	2.39-3.11
		Supermarket-B	15	grams	4.47±0.69 [A]	24.8	4.09-4.86	2.18±0.32 [A]	26.5	2.00-2.36	2.29±0.76	11.7	1.87-2.71
		Supermarket-C	15	grams	4.26±0.71 [A]	23.4	3.87-4.65[f]	2.16±0.37 [A]	22.7	1.95-2.36	2.11±0.71	11.6	1.71-2.49
		Supermarket-D	14	grams	4.15±0.83 [A]	18.8	3.68-4.63[f]	2.22±0.38 [A]	21.9	2.00-2.44	1.93±0.77	9.4	1.49-2.38
		Supermarket-E	15	grams	4.30±0.75 [A]	22.3	3.89-4.71	2.29±0.52 [A]	17.1	2.00-2.58	2.00±0.63	12.4	1.66-2.36
		Supermarket-F	15	grams	4.51±0.81 [A]	21.5	4.06-4.96	2.24±0.44 [A]	19.6	1.99-2.48	2.27±0.86	10.2	1.79-2.75
		Supermarket-G	15	grams	4.72±0.54 [A]	33.8	4.42-5.02	2.26±0.38 [A]	23.3	2.06-2.47	2.46±0.74	12.9	2.05-2.87
		Supermarket-H	15	grams	4.74±0.49 [A]	37.8	4.47-5.01	2.08±0.19 [A]	42.5	1.97-2.19	2.66±0.52	19.9	2.38-2.95
		Total	119	grams	4.52±0.71 [A]	69.9	4.39-4.65	2.21±0.39 [A]	62.4	2.14-2.28	2.31±0.74	33.9	2.18-2.45

ARM[¶] = Animal-related materials
SD = Standard deviation
A = Acceptable according to NSW (2009)
$p<0.05$: for [a] and [b]; [c] and [d]; [e] and [f]
SEW** = Spice-weighing equipment

5.2.1.2 Spoilage bacteria in the processing plant

Aerobic bacteria were observed on all sampling occasions in all types of samples and locations in the processing plant. They ranged from 3.99±0.75 log APC in samples collected from spices on the 3rd occasion to as high as 5.14±0.31 log APC in samples taken from aprons on the 5th occasion. Findings from raw beef also showed a range spanning from as low as 3.99±0.36 log APC on the 7th sampling occasion to as high as 5.21±0.42 log APC on the 5th occasion (Table A - 08).

At the processing plant, the lowest log EBC (1.96) was noted on most sampling occasions in the majority of the environmental sampling locations while the highest (4.69) was observed for a mixer on the 3rd sampling occasion. In raw beef, the lowest (2.54±0.69) log EBC was detected on the 5th sampling occasion whereas the highest (3.26±0.37) was found on the 1st occasion (Table A - 09).

5.2.1.3 Spoilage bacteria at supermarkets

The total amount of aerobic bacteria at supermarkets ranged between 4.09±0.96 log APC and 4.90±0.50 log APC on various sampling occasions. The lowest (3.04±0.15 log APC) was observed for a sample collected from Supermarket “D”, while the highest (5.54±0.01 log APC) was detected from one taken from Supermarket “G”, both on the 3rd occasion (Table 5 - 12).

Using APC criteria of NSW (2009) for each sampling occasion, the product was generally found to be “*acceptable*” at all supermarkets during all sampling occasions.

Table 5 - 12: APC in mortadella by sampling occasions at supermarkets

Supermarket Code	Numbers of samples and APC counts by sampling occasion											
	Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Total	
	No. of samples	Mean ± SD Log 10	No. of samples	Mean ± SD Log 10	No. of samples	Mean ± SD Log 10	No. of samples	Mean ± SD Log 10	No. of samples	Mean ± SD Log 10	No. of samples	Mean ± SD Log 10
A	4	5.43±0.08 U	2	5.07±0.12 U	2	5.24±0.12 U	4	4.88±0.42 A	3	4.26±0.42 A	15	4.97±0.50 A
B	4	4.79±0.25 A	2	4.27±0.57 A	2	4.21±1.77 A	4	4.66±0.79 A	3	4.12±0.30 A	15	4.47±0.69 A
C	3	4.41±0.77 A	4	5.03±0.34 U	4	3.52±0.40 G	2	4.03±0.67 A	2	4.21±0.30 A	15	4.26±0.71 A
D	2	4.77±0.20 A	2	3.93±0.10 G	4	3.04±0.15 G	3	4.92±0.33 A	3	4.62±0.38 A	14	4.15±0.83 A
E	2	4.61±0.43 A	4	4.75±0.87 A	4	3.54±0.19 G	2	4.85±0.11 A	3	4.15±0.78 A	15	4.30±0.75 A
F	2	4.09±0.60 A	4	3.93±0.71 G	4	4.56±1.07 A	2	5.46±0.14 U	3	4.87±0.15 A	15	4.51±0.81 A
G	2	4.56±0.63 A	3	4.29±0.38 A	2	5.54±0.01 U	4	5.06±0.29 U	4	4.39±0.33 A	15	4.72±0.54 A
H	2	5.00±0.01 U	-	-	4	4.45±0.45 A	5	5.10±0.23 U	4	4.47±0.60 A	15	4.74±0.49 A
Total	21	4.77±0.52 A	21	4.49±0.67 A	26	4.09±0.96 A	26	4.90±0.50 A	25	4.40±0.46 A	119	4.52±0.71 A

G = Good
A = Acceptable
U = Unsatisfactory
SD = Standard deviation
- = sample was not taken

As indicated in Table A - 10, low (1.96) counts of log EBC were observed in most supermarkets on most sampling occasions. However, one count was high (3.07±0.30) in Supermarket-A on the 2nd sampling occasion. In the majority of the supermarkets, a relatively high EBC was observed on the 1st, 2nd and 5th sampling occasion.

Using EBC criteria of NSW (2009), in total the product was found "*acceptable*" on all sampling occasions, and "*good*" on the 3rd sampling occasion. Products from all supermarkets on the 3rd sampling occasion were "*good*", except for Supermarket-H which showed an "*acceptable*" level.

5.2.1.4 Microbiological quality of the product (Mortadella)

With regard to the microbiological quality of the products studied, they in totalwere more frequently at *acceptable* level of APC (44.5%) than *good* (24.4%). The reverse was true when EBC was considered, in that *good* quality products accounted for 64.7% of EB positive samples, while those *acceptable* accounted for only 35.5% ($p<0.05$). Using APC, the products with unsatisfactory quality accounted for 31.1%. For individual supermarkets, using APC, no significant differences were observed between quantities of good, acceptable, and unsatisfactory product qualities ($p>0.05$). Similarly, the use of EBC showed no differences between frequencies of products with good and acceptable quality ($p>0.05$) (Table 5 - 13).

Table 5 - 13: Microbiological quality profiles of products from individual supermarkets

Supermarket code	No. of samples examined	Microbiological criteria				
		Using APC			Using EBC*	
		Good No. (%)	Acceptable No. (%)	Unsatisfactory No. (%)	Good No. (%)	Acceptable No. (%)
A	15	1 (6.7)	5 (33.3)	9 (60.0)	11 (73.3)	4 (26.7)
B	15	4 (26.7)	9 (60.0)	2 (13.03)	9 (60.0)	6 (40.0)
C	15	6 (40.0)	6 (40.0)	3 (20.0)	11 (73.3)	4 (26.7)
D	14	5 (35.7)	6 (42.9)	3 (21.4)	9 (64.3)	5 (35.7)
E	15	6 (40.0)	7 (46.7)	2 (13.3)	9 (60.0)	6 (40.0)
F	15	4 (26.7)	5 (33.3)	6 (40.0)	10 (66.7)	5 (33.3)
G	15	1 (6.7)	8 (53.3)	6 (40.0)	8 (53.3)	7 (46.7)
H	15	2 (13.3)	7 (46.7)	6 (40.0)	10 (66.7)	5 (33.3)
Total	119	29 (24.4)	53 (44.5)	37 (31.1)	77 (64.7)	42 (35.5)

*unsatisfactory product was not observed using EBC

Using APC and EBC, six different combinations (Table 5 - 14) of microbiological quality did arise; good and/or acceptable product combinations were more frequent (68.9%; 95%CI: 60.2-76.7) than unsatisfactory combinations (31.1%; 95%CI: 23.3-39.8) ($p<0.05$). For individual supermarkets, the frequencies of combinations remained the same ($p>0.05$).

Table 5 - 14: Product quality at individual supermarkets using APC and EBC

Supermarket code	No. of Samples examined	Microbiological criteria					
		APC Good		APC Acceptable		APC Unsatisfactory	
		EBC Good No. (%)	EBC Acceptable No. (%)	EBC Good No. (%)	EBC Acceptable No. (%)	EBC Good No. (%)	EBC Acceptable No. (%)
A	15	1 (6.7)	0	3 (20.0)	2 (13.3)	7 (46.7)	2 (13.3)
B	15	3 (20.0)	1 (6.7)	4 (26.7)	5 (33.3)	2 (13.3)	0
C	15	6 (40.0)	0	3 (20.0)	3 (20.0)	2 (13.3)	1 (6.7)
D	14	4 (28.6)	1 (7.1)	3 (21.4)	3 (21.4)	2 (14.3)	1 (7.1)
E	15	5 (33.3)	1 (6.7)	4 (26.7)	3 (20.0)	0	2 (13.3)
F	15	4 (26.7)	0	1 (6.7)	4 (26.7)	5 (33.3)	1 (6.7)
G	15	1 (6.7)	0	2 (13.3)	6 (40.0)	5 (33.3)	1 (6.7)
H	15	2 (13.3)	0	4 (26.7)	3 (20.0)	4 (26.7)	12 (3.3)
Total	119	26 (21.9)	3 (2.5)	24 (20.2)	29 (24.4)	27 (22.7)	10 (8.4)
Grand total %		68.9%; 95%CI: 60.2-76.7)				31.1%; 95%CI: 23.3-39.8)	

As shown in Table 5 - 15**,** the use of combinations of both APC and EBC parameters revealed fluctuations and differences in the quality of products on different sampling occasions.

Table 5 - 15: Quality of products using combinations of both APC and EBC on different sampling occasions

Sampling occasion	No. of samples examined	Microbiological criteria				
		Using APC			Using EBC	
		Good No. (%)	Acceptable No. (%)	Unsatisfactory No. (%)	Good No. (%)	Acceptable No. (%)
1st occasion	21	2 (9.5)	12 (57.1)	7 (33.3)	12 (57.1)	9 (42.9)
2nd occasion	21	6 (28.6)	10 (47.6)	5 (23.8)	8 (38.1)	13 (61.9)
3rd occasion	26	15 (57.7)	3 (11.5)	8 (30.8)	25 (96.1)	1 (3.8)
4th occasion	26	2 (7.7)	10 (38.5)	14 (53.8)	22 (84.6)	4 (15.4)
5th occasion	25	4 (16.0)	18 (72.0)	3 (12.0)	10 (40.0)	15 (60.0)
Total	119	29 (24.4)	53 (44.5)	37 (31.1)	77 (64.7)	42 (35.5)

A higher level of good quality products using APC and EBC combinations (57.7%; 95%CI: 38.4-75.4) was observed on the 3rd sampling occasion rather than on the other occasions (Table 5 - 16).

Table 5 - 16: Product quality on sampling occasions using APC and EBC

Sampling occasion	Microbiological criteria						
	No. of samples examined	APC & EBC Good	APC Good & EBC Acceptable	APC Acceptable & EBC Good	APC & EBC Acceptable	APC Unsatisfactory & EBC Good	APC Unsatisfactory & EBC Acceptable
1st occasion	21	2 (9.5)	0	4 (19.1)	8 (38.1)	6 (28.6)	1 (4.8)
2nd occasion	21	3 (14.3)	3 (14.3)	4 (19.1)	6 (28.6)	1 (4.8)	4 (19.1)
3rd occasion	26	15 (57.7)	0	2 (7.7)	1 (3.8)	8 (30.8)	0
4th occasion	26	2 (7.7)	0	9 (34.6)	1 (3.8)	11 (42.3)	3 (11.5)
5th occasion	25	14 (6.0)	0	5 (20.0)	13 (52.0)	1 (4.0)	2 (8.0)
Total	119	26 (21.9)	3 (2.5)	24 (20.2)	29 (24.4)	27 (22.7)	10 (8.4)

5.2.2 *Salmonella*

This section covers prevalences of *Salmonella* in overall and individual sampling locations and occasions with identified serotypes, and the molecular biology of PFGE profiles from the processing plant and supermarkets. Tracking and tracing of possible sources of *Salmonella* and their transmission routes along these lines are also presented.

5.2.2.1 Salmonella prevalence

At the processing plant line, an overall *Salmonella* prevalence of 5.3% was recorded. The respective prevalence rates at environment, animal-related materials, and supermarkets were 5.2% (95%CI: 2.6-8.9), 10.2% (95%CI: 5.6-16.6) and 0.8% (95%CI: 0.04-4.1) ($p>0.05$). Prevalence was higher in animal related materials at the processing plant than in the final product at supermarkets ($p<0.05$). Only one isolate could be identified in one sample at single supermarket, none in the rest (Table 5 - 17).

Table 5 - 17: *Salmonella* isolates by sampling locations and type of samples from the processing plant line

Origin of sample		Processing stages/position	Sampling location	Sample type	No. of samples	No. (%) *Salmonella* positive	Mid-Pex 95% CI
Processing plant	Environment	Manual production	Personnel's hands	Hands swab	19	1 (5.2)	0.3-23.3
			Aprons	Aprons swab	16	0	0-17.1
			Knives	Knives swab	15	0	0-18.1
			Cutting plates	Plates swab	13	1 (7.7)	0.3-25.7
		Cleaning water	Tap water	Water sample	17	0	0-16.2
		Device related materials	Working tables	Tables swab	17	3 (17.7)	4.7-40.9
			Room floor	Room swab	16	3 (18.7)	5.0-43.0
			Refrigerators	Refrigerator swab	15	0	0-18.1
		Spice adding	Spices	Spices sample	15	0	0-18.1
			SWE**	SWE swab	15	0	0-18.1
		Beef processing electrical machinery	Grinders	Grinder swab	9	0	0-28.3
			Cutters	Cutter swab	9	1 (11.1)	0.5-43.9
			Mixers	Mixer swab	9	0	0-28.3
			Fillers/Stuffers	Filler swab	9	1 (11.1)	0.5-43.9
		Sub total			194	10 (5.2)	2.6-8.9
	ARM¶	Receiving raw beef from 3 abattoirs	Before processing	Raw meat sample	118	12 (10.2)	5.6-16.6
		Sub total			312	22 (7.1)	5.6-10.3
Supermarkets		End of production	Supermarket-A	Product sample	15	0	0-18.1
			Supermarket-B	Product sample	15	1 (6.7)	0.3-28.7
			Supermarket-C	Product sample	15	0	0-18.1
			Supermarket-D	Product sample	14	0	0-19.3
			Supermarket-E	Product sample	15	0	0-18.1
			Supermarket-F	Product sample	15	0	0-18.1
			Supermarket-G	Product sample	15	0	0-18.1
			Supermarket-H	Product sample	15	0	0-18.1
		Sub total			119	1 (0.8)	0.04-4.1
Total					431	23 (5.3)	3.5-7.8

ARM¶ = Animal-related materials
SEW** = Spice-weighing equipment

As described in Table –A - 11, in the processing plant line, no differences in prevalence were observed between each of the 8 sampling occasions. Similarly, no differences were detected between environment and animal-related materials within one sampling occasion ($p>0.05$). With regard to supermarkets, one isolate was identified on the 1st sampling occasion. On the remaining 4 sampling occasions, results were negative for *Salmonella*.

As shown in Table A - 12; on different sampling occasions, at least one or more sampling locations were found positive for *Salmonella*. The lowest prevalence (2.3%) was observed on the 5th sampling occasion while the highest (14.7%) was noticed on the 2nd occasion. With the exception of the 4th and 5th sampling occasions, which showed negative results, raw beef samples were found positive for *Salmonella* on all occasions.

5.2.2.2 Salmonella serovars

Examination of *Salmonella* serovars revealed a high (1.4%) prevalence and preponderance (26.1%) of *S*. London. The prevalence was 0.23% for each *S*. Typhimurium and *S*. Saintpaul (Table 5 - 18).

Table 5 - 18: Isolated *Salmonella* serotypes and their prevalences at the rocessing plant line

***Salmonella* Serotypes**	**Positive no. of isolates**	**Prevalence (%) in samples (n=431)**	**Proportion (%) in isolates (n=23)**
S. London	6	1.4	26.1
S. Muenchen	3	0.7	13.0
S. Eastbourne	3	0.7	13.0
S. Anatum	2	0.46	8.7
S. Concord	2	0.46	8.7
S. Typhimurium	1	0.23	4.3
S. Saintpaul	1	0.23	4.3
Unidentified	5	1.2	21.7
Total	23	5.3	100

An unidentified *Salmonella* strain was observed in each of swabs from personnel's hands, room, stuffer and raw meat. One to five *Salmonella* serotypes were observed in different positive sampling locations. Furthermore, *S*. Eastbourne was detected in environment and animal-related materials while *S*. Muenchen was found in raw beef and end products at the supermarket. Most of *S*. London was observed in raw beef (Table 5 - 19).

Table 5 - 19: Distribution of *Salmonella* serovars in positive sampling location at the processing plant line

Orgin/source	**Sampling locations***	**Total No. of isolates**	**Serovar type and number in bracket**
Environment	Personnel's hands	1	Unidentified (1)
	Cutting plates	1	*S*. Estbourne (1)
	Working tables	3	*S*. London (1), *S*. Concord (2)
	Rooms	3	*S*. Typhimurium (1), *S*. Eastbourne (1), Unidentified (1)
	Cutters	1	Unidentified (1)
	Fillers/Stuffers	1	Unidentified (1)
	Sub-total	10	
ARM	Raw meat	12	*S.* Saintpaul (1), *S*. Anatum (2), *S*. London (5), *S*. Muenchen (2), *S*. Eastbourne (1), Unidentified (1)
Supermarkets*	Supermarket-B	1	*S*. Muenchen (1)
Total		23	

ARM = Animal-related materials *other supermarkets were negative for *Salmonella*

At the processing plant line, as shown in Table 5 - 20, *S*. London was common and was observed on the 1st and 2nd sampling occasions while *S*. Eastbourne was observed on the 5th, 7th and 8th sampling occasions. All other serovars were observed only once on a single sampling occasion.

Table 5 - 20: Distribution of *Salmonella* serovars in positive sampling locations and occasions at the processing plant line

Origin/ source	Sampling locations*	Positive	Sampling occasions							
			Occasion 1 Serotype (n)	**Occasion 2 Serotype (n)**	**Occasion 3 Serotype (n)**	**Occasion 4 Serotype (n)**	**Occasion 5 Serotype (n)**	**Occasion 6 Serotype (n)**	**Occasion 7 Serotype (n)**	**Occasion 8 Serotype (n)**
Environment	Personnel's hands	1							Unidentified (1)	
	Cutting plates	1					S. Eastbourne (1)			
	Working tables	3		S. London (1)				S. Concord (2)		
	Rooms	3				*S.* Typhimurium (1)			S. Eastbourne (1)	Unidentified (1)
	Cutters	1								Unidentified (1)
	Fillers/Stuffers	1	Unidentified (1)							
ARM	Raw meat	12	S. Anatum (2) *S.* London (1)	S. London (4)	*S.* Saintpaul (1)			*S.* Muenchen (2)	Unidentified (1)	*S.* Eastbourne (1),
Grand total (%)		22	4 (18.2)	5 (22.7)	1 (4.5)	1 (4.5)	1 (4.5)	4 (18.2)	3 (13.6)	3 (13.6)
Mid Pex. 95%CI of total			6.0-38.2	8.8-43.4	0.22-20.4	0.22-20.4	0.22-20.4	6.0-38.2	3.5-32.8	3.5-32.8

ARM = Animal-related materials
MLN* = Mesenteric lymph node

5.2.2.3 Pulsed-field gel electrophoresis (PFGE)

5.2.2.3.1 PFGE profiles of Salmonella

PFGE patterns among serotypes and dendrogram profiles of *Salmonella* serotypes obtained from the processing plant line are shown in Table 5 - 21 and Fig. 5 - 6, respectively. Use of BioNumerics®6.6 revealed that, except for the unidentified *Salmonella* strains which showed three different pulsotypes, each of the other *Salmonella* serotypes obtained from the line showed one pulsotype. However, the ratio of isolates to pulsotypes ranged from 1 to 6.

Excluding a strain with a single isolate, serotype diversities (isolates/serotypes) of 2-6 as well as variability among pulsotypes (isolates/pulsotype) of 1.6 to 6 were recorded for *Salmonella* in the processing plant line (Table 5 - 21).

Table 5 - 21: Number of *Salmonella* serotypes isolated and PFGE patterns obtained for each serotype from the processing plant line

***Salmonella* Serotypes**	**Total number of isolates/serotypes**	**Pulsotypes**	**Ratio (isolates/pulsotype)**
S. London	6	1	6
S. Muenchen¶	3	1	3
S. Eastbourne	3	1	3
S. Anatum	2	1	2
S. Concord	2	1	2
S. Typhimurium	1	1	1
S. Saintpaul	1	1	1
Unidentified	5	3	1.6
Total	23		

¶ One *S.* Muenchen was identified from a supermarket.

PFGE patterns among serotypes and dendrogram profiles of *Salmonella* serotypes obtained from sampling locations in the processing plant line are shown in Table 5 - 22. From there, pulsotypes of 2, 3 and 6 were observed in working tables, room floors and raw beef, respectively. A 1:2 ratio of isolates to serotype as well as isolates to pulsotypes was also observed at each positive sampling location in the processing plant line (Table 5 - 22).

Table 5 - 22: Distribution of 23 *Salmonella* isolates among serotypes and *XbaI* pulsotypes by sampling location and type of samples from a beef processing plant line

Sample origin		Sampling location	Sample type	No. of isolates	No. of *Salmonella* serotypes	Pulsotypes	Ratio isolates/ serotype	Ratio isolates/ pulsotype
Processing plant	Environment	Personnel's hands	Hands swab	1	1	1	1	1
		Cutting plates	Plates swab	1	1	1	1	1
		Working tables	Tables swab	3	2	2	1.5	1.5
		Room floor	Room swab	3	3	3	1	1
		Cutters	Cutter swab	1	1	1	1	1
		Fillers/Stuffers	Filler swab	1	1	1	1	1
	ARM¶	Before processing	Raw meat sample	12	6	6	2	2
Supermarkets		Supermarket B	Product sample	1	1	1	1	1
Total				23				

ARM¶ = Animal-related materials
SEW** = Spice-weighing equipment

Pulsotype distribution of 22 *Salmonella* serovars identified at positive sampling locations and on different occasions from the processing plant line are presented in Table 5 - 23. *S.* London (SLo*X*1) obtained on the 1st and 2nd sampling occasions shows similar pulsotypes. A *S.* Muenchen obtained from supermarket B (Table 5 - 22) also shows similar pulsotypes with two SMu*X*1 obtained on the 6th sampling occasion from raw beef at the processing plant.

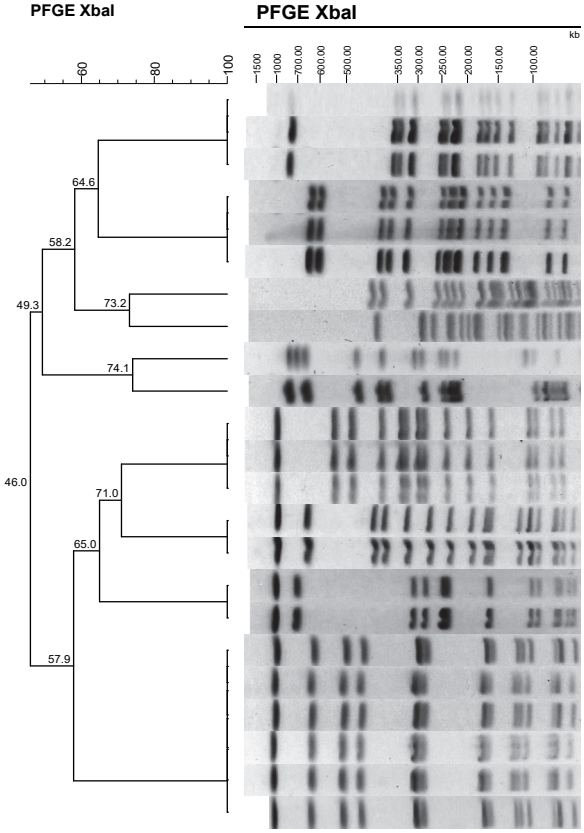
Table 5 - 23: Pulsotype distribution of 22 *Salmonella* serovars in positive sampling locations and occasions at the processing plant line

Origin/ source	Sampling locations*	Positive	Sampling occasions								Total number of serotype
			Occasion 1/ Batch 1	Occasion 2/ Batch 2	Occasion 3/ Batch 5	Occasion 4/ Batch 6	Occasion 5/ Batch 7	Occasion 6/ Batch 12	Occasion 7/ Batch 14	Occasion 8/ Batch 15	
			Pulsotype	Pulsotype	Pulsotype	Pulsotype	Pulsotype	Pulsotype	Pulsotype	Pulsotype	
Environment	Personnel's hands	1							Un*X*2		1
	Cutting plates	1					SEa*X*1				1
	Working	3		SLo*X*1				SCo*X*1			2
	Tables							SCo*X*1			
	Rooms	3				STy*X*1			SEa*X*1	Un*X*1	3
	Cutters	1								Un*X*3	1
	Fillers/Stuffers	1	Un*X*1								1
ARM	Raw meat	12	SAn*X*1 SAn*X*1 SLo*X*1	SLo*X*1 SLo*X*1 SLo*X*1 SLo*X*1	SSa*X*5			SMu*X*1 SMu*X*1	Un*X*1	SEa*X*1	6
Grand total No. of isolates		22	4	5	1	1	1	4	3	3	

SAn*X*1 = *S.* Anatum; SLo*X*1 = *S.* London; SSa*X*5 = *S.* Saintpaul; SMu*X*1 = *S.* Muenchen; STy*X*1 = S. Typhimurium; SCo*X*1 = S. Concord; SEa*X*1 = *S.* Eastbourne; Un*X*1 = Unidentified

5.2.2.3.2 Tracking possible sources and transmission routes

Similar pulsotypes of *S.* London (SLo*X*1) were observed in raw beef on the 1st sampling occasion and samples from working tables on the 2nd sampling occasion. Further, pulsotypes of *S.* Anatum (SAn*X*1), *S.* London (SLo*X*1) and *S.* Muenchen (SMu*X*1) were detected in raw beef on the 1st, 2nd and 6th sampling occasions, respectively. The *S.* Muenchen (SMu*X*1) obtained from a processed beef product during the 8th sampling occasion was similar to the one found in raw beef sampled from the processing plant line on the 12th sampling occasion. The SCo*X*1 pulsotype of *S.* Concord was observed only on a working table during the 6th sampling occasion (Fig. 5 - 6 and Table 5 - 24).



Key	Serovars	Sample	Source	Batch	Code	pulsoty
5293.	Unidentified (Pol.	Filler	Meat processi.	1st	-	UnX1
5313.	Unidentified (Pol.	Meat	Meat processi.	14th	-	UnX1
5318.	Unidentified (Pol.	Room	Meat processi.	15th	-	UnX1
5304.	S. Eastbourne	Plate	Meat processi.	7th	9	SEaX1
5314.	S. Eastbourne	Room	Meat processi.	14th	-	SEaX1
5316.	S. Eastbourne	Meat	Meat processi.	15th	-	SEaX1
5315.	Unidentified (Pol.	Hand.	Meat processi.	14th	16	UnX2
5317.	Unidentified (Ra.	Cutter	Meat processi.	15th	-	UnX3
5302.	S. Saintpaul	Meat	Meat processi.	5th	-	SSaX5
5303.	S. Typhimurium	Room	Meat processi.	6th	-	STyX1
5311.	S. Muenchen	Meat	Meat processi.	12th	-	SMuX1
5312.	S. Muenchen	Meat	Meat processi.	12th	-	SMuX1
5307.	S. Muenchen	Produ.	Supermaket	8th	-	SMuX1
5294.	S. Anatum	Meat	Meat processi.	1st	-	SAnX1
5295.	S. Anatum	Meat	Meat processi.	1st	-	SAnX1
5308.	S. Concord	Table	Meat processi.	12th	-	SCoX1
5309.	S. Concord	Table	Meat processi.	12th	-	SCoX1
5296.	S. London	Meat	Meat processi.	1st	-	SLoX1
5297.	S. London	Table	Meat processi.	2nd	-	SLoX1
5298.	S. London	Meat	Meat processi.	2nd	-	SLoX1
5299.	S. London	Meat	Meat processi.	2nd	-	SLoX1
5300.	S. London	Meat	Meat processi.	2nd	-	SLoX1
5301.	S. London	Meat	Meat processi.	2nd	-	SLoX1

Fig. 5 - 06: Dendrogram profiles of *Salmonella* serovars isolated from the processing plant line

Table 5 - 24: Possible sources and transmission routes of *Salmonella* serovars along the processing plant line

Sampling		Sample and pulsotype								
Batch	Occasion	Personnel's hands	Working table	Plate	Room	Cutter	Filler/stuffer	Meat	Supermarket (Product)*	Total of isolates
1	1						Un*X*1	SAn*X*1		4
								SAn*X*1		
								SLo*X*1		
2	2		SLo*X*1					SLo*X*1		5
								SLo*X*1		
								SLo*X*1		
								SLo*X*1		
5	3							SSa*X*5		1
6	4				STy*X*1					1
7	5			SEa*X*1						1
8	1								SMu*X*1	1
12	6		SCo*X*1					SMu*X*1		4
			SCo*X*1					SMu*X*1		
14	7	Un*X*2			SEa*X*1			Un*X*1		3
15	8				Un*X*1	Un*X*3		SEa*X*1		3
Total		1	3	1	3	1	1	12	1	23

SAn*X*1 = *S.* Anatum; SLo*X*1 = *S.* London; SSa*X*5 = *S.* Saintpaul; SMu*X*1 = *S.* Muenchen; STy*X*1 = S. Typhimurium; SCo*X*1 = S. Concord; SEa*X*1 = *S.* Eastbourne; Un*X*1 = Unidentified
* only one isolate was obtained

5.2.3 *E. coli*

This section deals with the prevalence of *E. coli* overall and at individual sampling locations and occasions in samples collected from the processing plant and from supermarkets.

5.2.3.1 Overall E. coli prevalence

The overall prevalence at the processing plant line (PPL) (46.4%; 95%CI: 41.7-51.1) was the same than the prevalence of the environment (50.5%; 95%CI: 43.5-57.5) and of animal-related materials (56.8%; 95%CI: 47.7-65.5) ($p > 0.05$). These prevalences were at these locations higher than that of the end product at supermarkets (29.4%; 95%CI: 21.7-38.1) ($p < 0.05$). With regard to sampling locations in this study, no significant differences were observed between them with the exception of the processing plant rooms where a higher prevalence was recorded (75%; 95%CI: 50.1-91.5) compared to tap water (23.5%; 95%CI: 8.0-47.5), spices (13.3%; 95%CI: 2.3-37.5), Supermarkets A and H each (20%; 95%CI: 5.4-45.3) and SupermarketF (16.7%; 95%CI: 2.3-37.5). No difference in prevalence did exist between and within products from all supermarkets ($p > 0.05$) (Table 5 - 25).

Table 5 - 25: *E. coli* isolations by sampling location and type of samples from the processing plant line

Sample origin	Processing stage/position	Sampling location	Source of swab/ Sample type	No. of samples	No. (%) *E. coli* positive	Mid-Pex 95% CI
Processing plant Environment	Manual production	Personnel's hands	Hands	19	10 (52.6)	30.6-73.9
		Aprons	Aprons	16	10 (62.5)	37.6-83.2
		Knives	Knives	15	8 (53.5)	28.7-76.8
		Cutting plates	Plates	13	8 (61.5)	34.1-84.3
	Cleaning water	Tap water	Water sample	17	4 (23.5)	7.9-47.5
	Devices related materials	Working tables	Tables	17	8 (47.1)	24.8-70.3
		Room floor	Rooms	16	12 (75.0)	50.1-91.5
		Refrigerators	Refrigerators	15	10 (66.7)	40.8-86.6
	Spices adding	Spices	Spices sample	15	2 (13.3)	2.3-37.5
		SWE**	SWE	15	7 (46.7)	23.2-71.3
	Beef processing electrical machinery	Grinders	Grinders	9	6 (66.7)	33.2-90.7
		Cutters	Cutters	9	3 (33.4)	9.3-66.8
		Mixers	Mixers	9	5 (55.6)	24.0-83.9
		Filler/Stuffer	Fillers	9	5 (55.6)	24.0-83.9
	Sub total			194	98 (50.5)	43.5-57.5
ARM¶	Receiving raw beef from 3 abattoirs	Before processing	Raw meat sample	118	67 (56.8)	47.7-65.5
Sub total				312	165 (52.9)	47.3-58.4
Supermarkets	End of production	Supermarket-A	Product sample	15	3 (20.0)	5.4-45.4
		Supermarket-B	Product sample	15	4 (26.7)	9.1-52.5
		Supermarket-C	Product sample	15	4 (26.7)	9.1-52.5
		Supermarket-D	Product sample	14	6 (42.9)	19.6-68.8
		Supermarket-E	Product sample	15	5 (33.3)	13.4-59.2
		Supermarket-F	Product sample	15	2 (13.3)	2.3-37.5
		Supermarket-G	Product sample	15	8 (53.3)	28.7-76.8
		Supermarket-H	Product sample	15	3 (20.0)	5.4-45.4
	Subtotal			119	35 (29.4)	21.7-38.1
Total				431	200 (46.4)	41.7-51.1

ARM¶ = Animal-related materials
MLN* = Mesenteric lymph nodes
SEW** = Spice-weighing equipment

5.2.3.2 Prevalence at the processing plant

In total at the processing plant line: A similar prevalence rate was observed on the 1st, 2nd and 3rd sampling occasions. In contrast, the rate was higher on the 5th to the 8th sampling occasions than on the 3rd one.

Environment: A similar prevalence was observed on the 2nd and 3rd sampling occasions, which was lower than those of the 5th to 8th occasions.

Raw beef: No differences in prevalence were observed on all sampling occasions ($p > 0.05$).

Between environment and raw beef: Except on the 2nd sampling occasion, where higher *E. coli* in raw beef (78.6%) than in environmental samples (25%) were observed, no other

difference in prevalence was observed between environmental samples and raw beef on any sampling occasion (p >005) (Table –A - 13).

With regard to the different sampling locations of the processing plant (Table –A - 14), all were found positive for *E. coli* on at least one or more sampling occasions.

5.2.3.3 Prevalence at supermarkets

Prevalences at supermarkets were only different on the 2nd sampling occasion (76.2%; 95% CI 54.8-90.0) compared to the 3rd, 4th and 5th occasions. However, no difference was observed among the latter three and the 1st sampling occasion (Table –A - 15). At the majority of the supermarkets, high *E. coli* were observed on the 1st and 2nd sampling occasions.

5.3 Antimicrobial susceptibility/resistance profiles of isolates

This part includes the antimicrobial susceptibility/resistance profiles of *Salmonella* and *E. coli* isolates to identify important antimicrobial agents of medicinal importance from different classes (WHO, 2011) in relation to the zoonotic relevance of theses microbial agents.

5.3.1 *Salmonella* isolates

5.3.1.1 Overall susceptibility/resistance

Fig. 5 - 07 shows the overall susceptibility/resistance profiles of 86 *Salmonella* isolates from the investigated abattoir and processing plant line. Susceptibility to PB, CN, C, W and SXT was similar (p >0.05) but higher than susceptibility to N (45.4%; 95%CI: 35.0-55.9) and OT (44.2%; 95%CI: 33.9-54.8) (p <0.05). Resistance was higher to N (15.1%) and OT (53.5%) than to the former five drugs (p <0.05) was obtained. Intermediate reaction to N (39.5%) was higher than reaction to all other evaluated drugs (p <0.05).

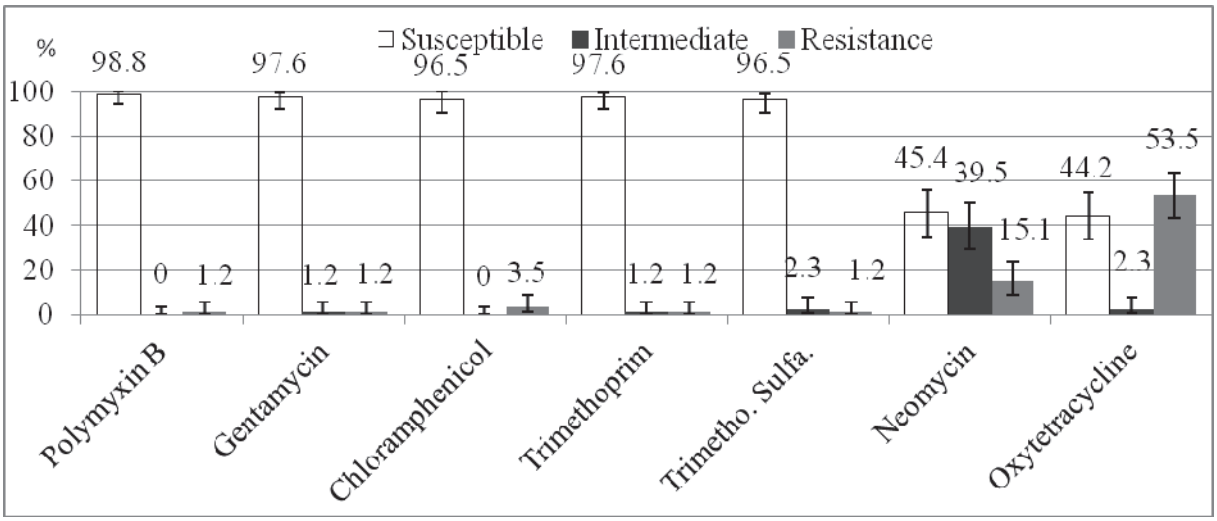


Fig. 5 - 07: Overall antimicrobial susceptibility/resistance profiles (%) of *Salmonella* isolates (n=86)

5.3.1.2 Abattoir line

As shown in Fig. 5 - 08, susceptibility to PB, CN, C, W and SXT was similar with each other ($p>0.05$) but higher than susceptibility to N (50.1%; 95%CI: 38.5-62.9) and OT (31.7%; 95%CI: 21.2-43.9) ($p <0.05$). A significantly higher resistance was observed for N and OT than for the former five drugs ($p <0.05$). Intermediate reaction to N (33.3%) was higher than reaction to all other evaluated drugs ($p <0.05$).

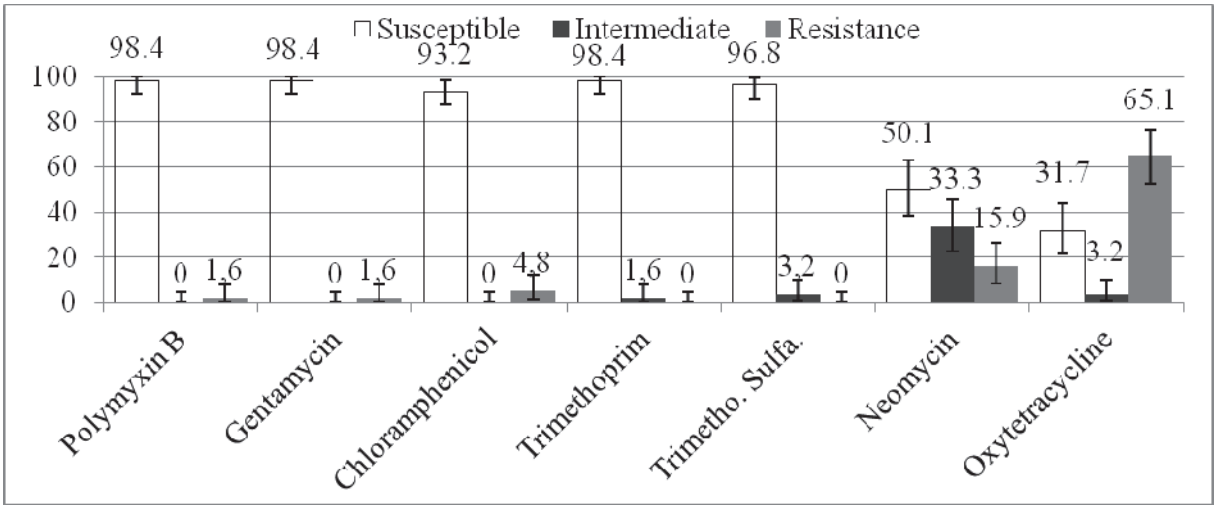


Fig. 5 - 08: Susceptibility/resistance profiles of *Salmonella* strains (%) from the abattoir line (n=63)

With regard to *Salmonella* isolates, a similar susceptibility and resistance to the study drugs was observed on the various sampling occasions. A 100% susceptibility to PB and CN was

observed on all sampling occasions. On the other hand, high resistance to OT was observed also on all sampling occasions. With the exception of the 1st sampling occasion, resistance to N was observed on all the other occasions (Table –A - 16).

As shown in Table -A - 17, save for isolates from water samples which were 100% susceptible to all drugs, one or more isolate(s) were at least resistant to one of the study drugs at all other locations in the abattoir line. Intermediate reaction to N (20-100%) was equally observed in isolates from all locations other than tap water.

5.3.1.3 Processing plant line

For the 23 *Salmonella* isolates obtained from the processing plant line, susceptibility to N (30.4%; 95%CI: 14.4-51.1) was lower than to all other drugs ($p < 0.05$). These did not differ in their susceptibilities ($p > 0.05$). Resistances equally were the same to all tested drugs ($p > 0.05$) (Fig. 5 - 09).

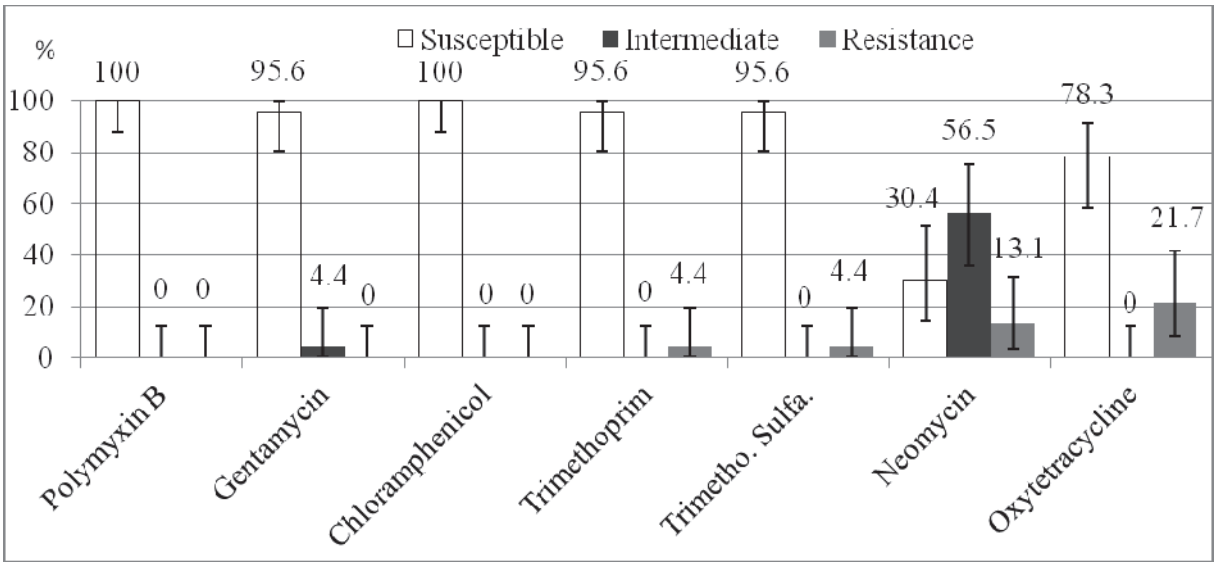


Fig. 5 - 09: Susceptibility/resistance profiles of *Salmonella* strains (%) from the processing plant line (n=23)

No isolate resistant to all drugs was detected from among those sampled on the 3rd, 4th, and 5th occasions. Resistant isolates to N were observed only on the 1st (50%) and 2nd (20%) sampling occasions and to both W and SXT on the 7th (33.3%) occasion. Furthermore, the isolates showed resistance to OT on the 6th (50%), 7th (33.3%) and 8th (33.3%) occasions, and to CN on the 8th (33.3%) occasion (Table –A - 18).

With regard to origin of isolates, those from plates, stuffers and supermarket-B were 100% susceptible to all drugs tested. Isolates from human hand swabs were 100% resistant to W, SXT and OT while those from tables were 66.7% resistant to N. Moreover, isolates from rooms were 33.3% resistant to OT, whereas those from raw meat were 8.3% resistant to N and 16.7% to OT (Table –A - 19).

Susceptibility/resistance of *Salmonella* serotypes to the tested drugs is shown in Table 5 - 26. *S.* Typhimurium was susceptible to all drugs. All *S.* Anatum were of intermediate reaction to N, but susceptible to all the other drugs. *S.* Saintpaul was 100% susceptible to CN, SXT and W but 3.6% resistant to each of PB, C, and N. Resistance to OT was 82.2% (the highest). *S.* London was 100% susceptible to all drugs but 28.6% were resistant to N and 14.3% to OT. *S.* Muenchen was resistant to OT with a percentage of 94.1%.

Table 5 - 26: Antimicrobial susceptibility/resistance (no. (%)) of 86 *Salmonella* serotypes to individual drugs

***Salmonella* serotypes**	**No. Tested**	**Antimicrobials**																		
		PB*		**CN**			**C****		**W**			**SXT**			**N**				**OT**	
		S. No. (%)	**R. No. (%)**	**S. No. (%)**	**I. No. (%)**	**R. No. (%)**	**S. No. (%)**	**R. No. (%)**	**S. No. (%)**	**I. No. (%)**	**R. No. (%)**	**S. No. (%)**	**I. No. (%)**	**R. No. (%)**	**S. No. (%)**	**I. No. (%)**	**R. No. (%)**	**S. No. (%)**	**I. No. (%)**	**R. No. (%)**
S. Typhimurium	1	1 (100)	0 (0)	1 (100)	0 (0)	0(0)	1 (100)	0 (0)	1 (100)	0 (0)	0(0)	1 (100)	0 (0)	0(0)	0(0)	1 (100)	0(0)	1 (100)	0 (0)	0(0)
S. Anatum	2	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)
S. Saintpaul	28	27 (96.4)	1 (3.6)	28 (100)	0 (0)	0 (0)	27 (96.4)	1 (3.6)	28 (100)	0 (0)	0 (0)	28 (100)	0 (0)	0 (0)	16 (57.1)	11 (39.3)	1 (3.6)	3 (10.7)	2 (7.1)	23 (82.2)
S. London	7	7 (100)	0 (0)	7 (100)	0 (0)	0 (0)	7 (100)	0 (0)	7 (100)	0 (0)	0 (0)	7 (100)	0 (0)	0 (0)	2 (28.6)	3 (42.8)	2 (28.6)	6 (85.7)	0 (0)	1 (14.3)
S. Larochelle	11	11 (100)	0 (0)	10 (90.9)	0 (0)	1 (9.1)	10 (90.9)	0 (0)	11 (100)	0 (0)	0 (0)	11 (100)	0 (0)	0 (0)	3 (27.3)	5 (45.4)	3 (27.3)	8 (72.7)	0 (0)	3 (27.3)
S. Concord	2	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	1 (50.0)	1 (50.0)	2 (100)	0 (0)	0 (0)
S. Dublin	4	4 (100)	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	2 (50.0)	2 (50.0)	0 (0)	3 (75.0)	0 (0)	1 (25.0)
S. Kastrup	3	3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	1 (33.3)	0 (0)	2 (66.7)	3 (100)	0 (0)	0 (0)
S. Estbourne	3	3 (100)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	2 (66.7)	0 (0)	1 (33.3)
S. Muenchen	17	17 (100)	0 (0)	17 (100)	0 (0)	0 (0)	15 (88.2)	2 (11.8)	16 (96.1)	1 (5.9)	0 (0)	15 (88.2)	2 (11.8)	0 (0)	6 (35.3)	7 (41.2)	4 (23.5)	2 (11.8)	0 (0)	15 (88.2)
Unidentified**	8	8 (100)	0 (0)	8 (100)	0 (0)	0 (0)	8 (100)	0 (0)	7 (87.5)	0 (0)	1(12.5)	7 (87.5)	0 (0)	1 (12.5)	6 (75.0)	2 (25.0)	0 (0)	6 (75.0)	0 (0)	2 (25.0)
Total	86	85 (98.8)	1 (1.2)	84 (97.6)	1 (1.2)	1 (1.2)	83 (96.5)	3 (3.5)	84 (97.6)	1 (1.2)	1 (1.2)	83 (96.5)	2 (2.3)	1 (1.2)	39 (45.4)	34 (39.5)	13 (15.1)	38 (44.2)	2 (2.3)	46 (53.5)

*no intermediate was observed for PB;
C* has only susceptibility break point

Here, only *Salmonella* strains resistant to drugs were assessed in relation to serovars and sources/origin. Antimicrobial resistance of *Salmonella* to one or more drugs of medicinal importance is an emerging global challenge (Plumb, 2008; Kasper *et al.*, 2005).

A total of 55 (63.9%) isolates were found to be resistant to at least one of the antimicrobial agents used in this study. As shown in Fig. 5 - 10, the distribution of strains resistant to individual drugs at the abattoir and at the PPL line was similar. The distribution of resistance overall shows no difference compared to the distribution for individual drugs ($p > 0.05$). In addition, an overall resistance of 1.8% to each of PB, CN, W and SXT, 5.4% to C, 26.6% to N and 83.6% to OT was observed. In isolates from the abattoir line, a significantly higher difference was observed in resistances to N and OT ($p < 0.05$) as compared to the resistance of respective isolates to all other drugs ($p < 0.05$).

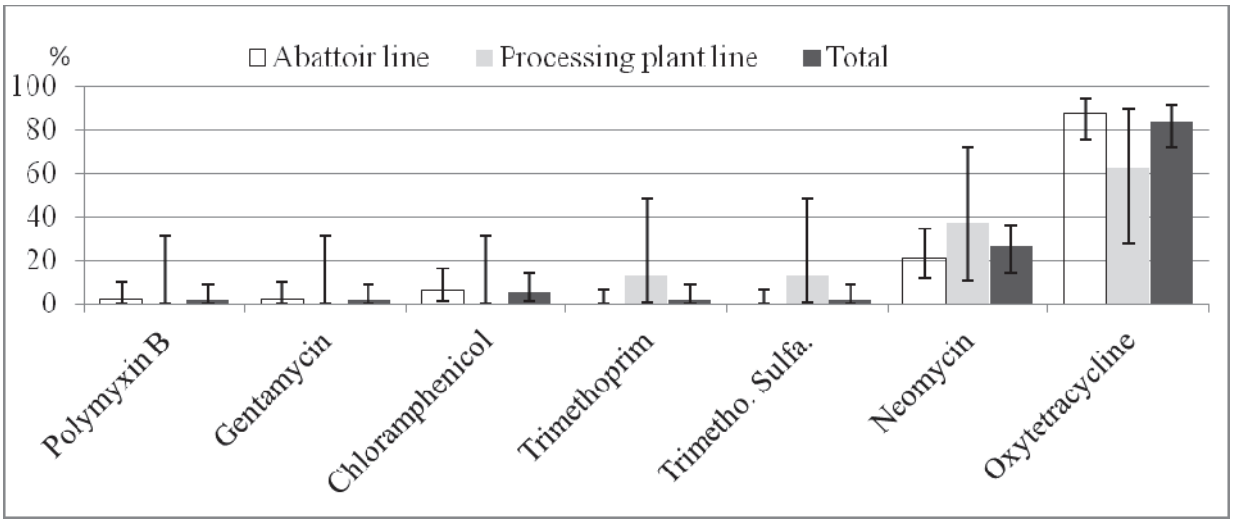


Fig. 5 - 10: Distribution of resistant strains (%) in the abattoir and processing plant lines

In terms of resistance, no difference was found between *S.* Saintpaul (41.8%) and *S.* Muenchen (30.9%) ($p > 0.05$) but a higher degree of resistance was observed in *S.* Muenchen isolates (30.9%) than in *S.* Larochelle ones (10.9%; 95%CI: 4.5-21.3) ($p < 0.05$). Again, no difference in resistance was detected among *S.* Larochelle, *S.* London, *S.* Kastrup, *S.* Concord, *S.* Dublin strains and the unidentified ones ($p > 0.05$) (Fig. 5 - 11).

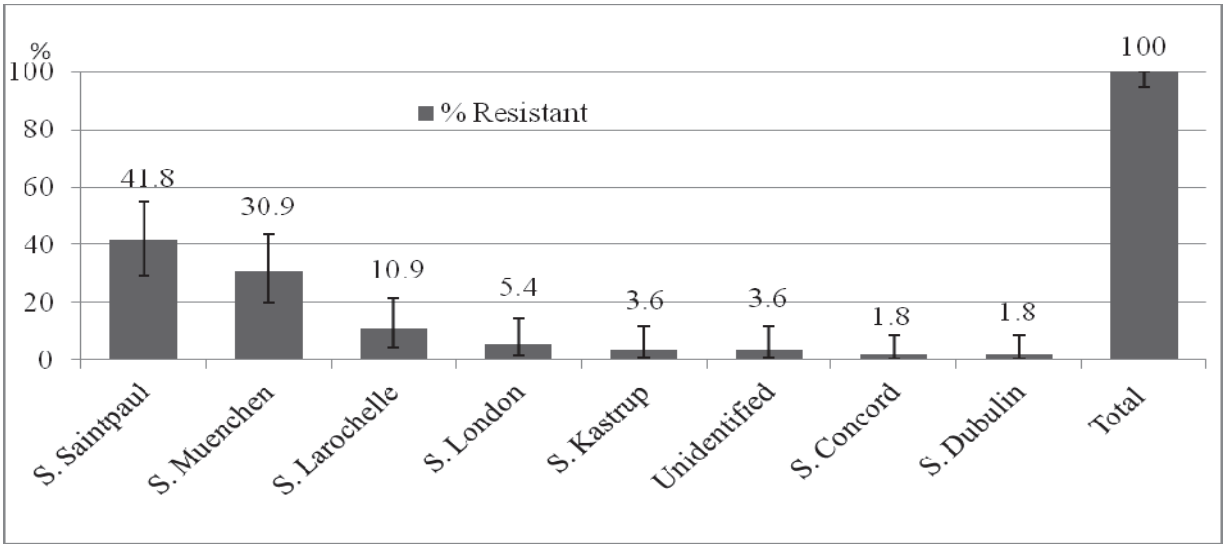


Fig. 5 - 11: Resistance of isolates (%) by serotypes

The proportion of *Salmonella* isolates tested in both, the abattoir and processing plant lines showed no difference (p>0.05) (Table –A - 17). This is also shown in Fig. 5 - 12**.** In the abattoir line, a higher resistance in the environment (54.6%; 95%CI: 41.3-67.3) than in animal-related materials (21.8%; 95%CI: 12.3-34.1) and samples from butchers (9.1%; 95%CI: 3.4-19.0) was observed (p <0.05). However, no difference was found between the latter two sample types (p >0.05). With regard to the processing plant line, no difference in resistant isolates was observed between and among the environment, animal related material and products (supermarkets) (p >0.05).

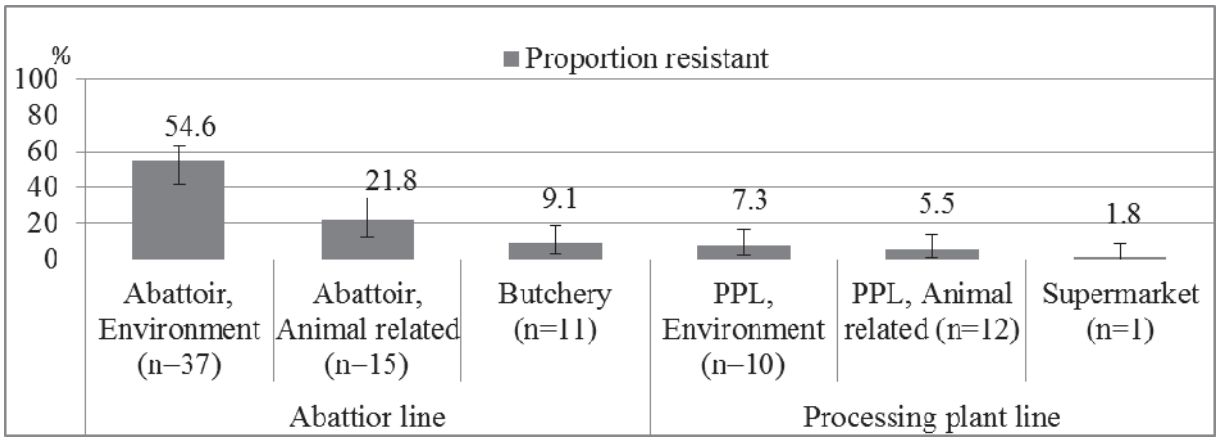


Fig. 5 - 12: Proportion of resistant isolates to at least one drug along the lines studied (n = number of isolates tested)

In total, 63.9% (55/86) of the isolates showed resistance to at least one or more drugs . Resistance of isolates to individual drugs or combination in both lines is presented below in XXTable 5 - 27. Strains resistant to single drugs accounted for 83.6% and were more frequent than those resistant to two drugs (12.7%) and to MDR (3.6%) (p <0.05). Resistance to OT

was observed when used as a single drug, together with another drug, or as MDR in one or more strains from all sources/origin (Table 5 - 27).

Table 5 - 27: Resistance of *Salmonella* isolates to single and combined antimicrobials by origin/source line

Line	Sources/origin	No. of resistant isolate	Resistance to individual drugs/drug combinations		
			Single drug	Two drugs	MDR* resistant
Abattoir line	Abattoir, Environment	30	1: C 22: OT	1 :C, OT 5: N, OT	1: C, OT, PB
	Abattoir, ARM	12	1: C 4: N 6: OT	1: N, OT	0
	Butchers	5	5: OT	0	0
	Subtotal No. (%)	47	39 (82.9)	7 (15.0)	1 (2.1)
Processing plant line	PPL, Environment	4	2: N 1: OT	0	1: OT, SXT, W
	PPL, ARM	3	1: N 2: OT	0	0
	Supermarkets	1	1: OT	0	0
	Subtotal No. (%)	8	7 (87.5)	0	1 (12.5)
	Total No. (%)	55	46 (83.6)	7 (12.7)	2 (3.6)

The proportions of *Salmonella* isolates resistant to single or combined drugs is presented in Table 5 – 28. Results show, that there was no difference in the proportion of isolates and the corresponding resistant isolates ($p > 0.05$) in both the abattoir and the PPL lines. Nevertheless, a significantly more frequently resistant strain was observed at the abattoir line (85.5%; 95%CI: 74.2-93.0) than at the PPL line (14.5%; 95%CI: 6.9-25.7) ($p<0.05$). Resistant strains to a single and to two drugs were more frequent the abattoir than at the PPL line, while frequencies of multiple-drug resistant strains were similar at both lines.

Table 5 - 28: Proportion of *Salmonella* isolates and resistance to individual drugs or drug combinations at the study lines

Line	No. (%) proportion tested	No. (%) proportion resistant	Resistance to individual drugs/drug combinations, No. (%)		
			Single drug	Two drugs	MDR*
Abattoir	63 (73.3)	47 (85.5)	39 (84.8)	7 (100)	1 (50)
Processing plant	23 (26.7)	8 (14.5)	7 (15.2)	0	1 (50)
Total	86 (100)	55 (100)	46 (100)	7 (100)	2 (100)

*Resistance to ≥3 drugs

As shown in Table 5 - 29 below, 12, 5 and 4 isolates of *S.* Saintpaul were resistant to OT at abattoir environment, animal-related materials and butchers, respectively. From the abattoir environment, all 8 strains of *S.* Muenchen showed resistance to OT while only 3 were resistant to a combination of N and OT. One isolate of *S.* Saintpaul from butchers and one

unidentified isolate from the processing plant environment showed MDR to C-OT-PB and to OT-W-SXT, respectively.

Table 5 - 29: *Salmonella* isolates resistant to single or multiple antimicrobials by serotype and source/origin of the beef lines

Line	Sources/origin	No. (%) proportion resistant	Single drug			Two drugs			MDR*		
			Drugs	No.	Serotypes	Drugs (n)	No.	Serotypes	Drugs (n)	No.	Serotypes
Abattoir	Abattoir, Environment	30 (54.6)	12: OT	12	*S.* Saintpaul	5: N, OT	1	*S.* Saintpaul			
			1: CN	1	*S.* Larochelle		1	*S.* Larochelle			
			2: OT	2	*S.* Larochelle		3	*S.* Muenchen			
			1: C	1	*S.* Muenchen						
			8: OT	8	*S.* Muenchen						
	Abattoir Animal-related materials	12 (21.8)	5: OT	5	*S.* Saintpaul	1: C, OT	1	*S.* Muenchen			
			2: CN	2	*S.* Larochelle						
			1: OT	1	*S.* Dublin	1: N. OT	1	*S.* Muenchen			
			2: N	2	*S.* Kastrup						
	Butchers	5 (9.1)	5: OT	4	*S.* Saintpaul				1: C, OT, PB	1	*S.* Saintpaul
				1	*S.* London						
PPL	PPL, Environment	4 (7.3)	2: N	1	*S.* London				1: OT, W, SXT	1	unidentified
				1	*S.* Concord						
			1: OT	1	Unidentified						
	PPL Animal-related materials	3 (5.5)	1: N	1	*S.* London						
			2: OT	2	*S.* Muenchen						
	Supermarkets	1 (1.8)	1: OT	1	*S.* Muenchen						
Total No. (%)		55 (100)	46 (83.6)			7 (12.7)			2 (3.6)		

*resistant to ≥ 3 drugs
PPL = Processing plant

5.3.2 *E. coli* isolates

This section describes the overall susceptibility/resistance profile of *E. coli* isolates to selected drugs of veterinary and public health importance.

5.3.2.1 Overall susceptibility/resistance

All the 307 *E. coli* isolates showed sizeable susceptibility/resistance to antimicrobials. The susceptibility to PB, C and CN was similar. In contrast, a difference, though low, was observed in their susceptibility to SXT (92.2%; 95%CI 88.8-94.8), AML (76.2%; 95%CI 72.2-80.7) and OT (56.4%; 95%CI 50.8-61.8) ($p < 0.05$). Resistance to SXT (7.5%), AML (21.2%) and OT (39.7%) was significantly highr ($p < 0.05$) (Fig. 5 - 13).

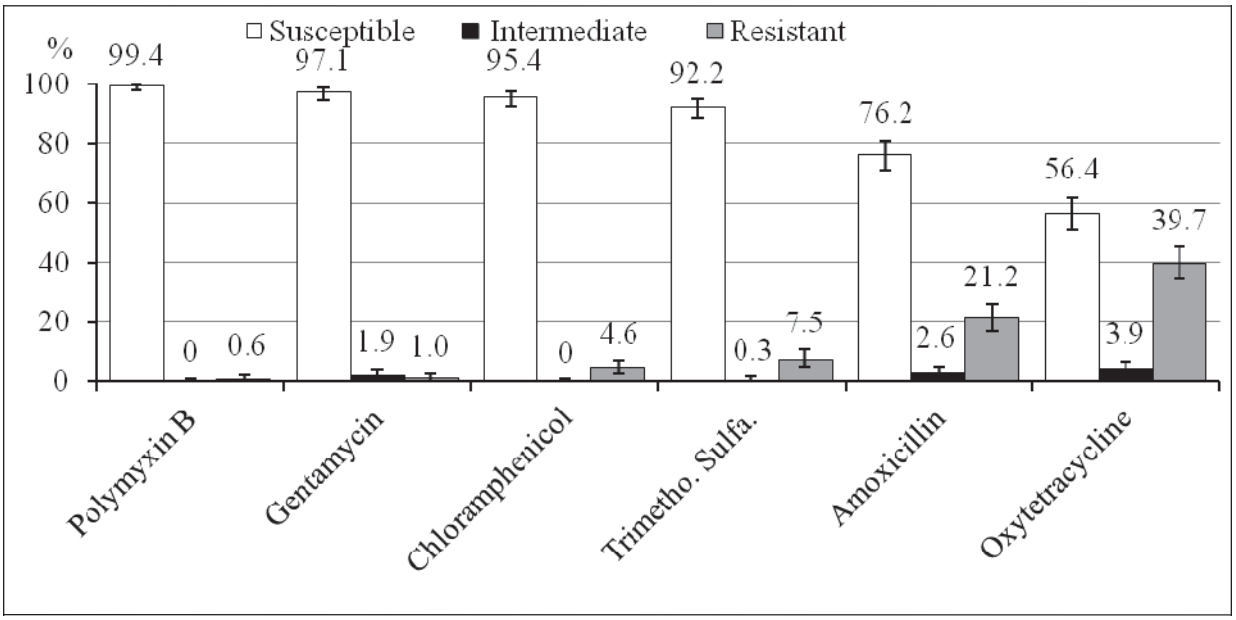


Fig. 5 - 13: Overall antimicrobial susceptibility/resistance (%) of total *E. coli* isolated from both beef lines

5.3.2.2. Abattoir line

As shown in Fig. 5 - 14, all 107 *E. coli* isolates from the abattoir line showed 100% susceptibility to PB. Further, their susceptibility to C, CN and SXT was similar in all cases ($p > 0.05$) but higher to that of AML and OT ($p < 0.05$). A difference in susceptibility was also observed between AML and OT ($p < 0.05$), although both were similar in terms of resistance ($p > 0.05$).

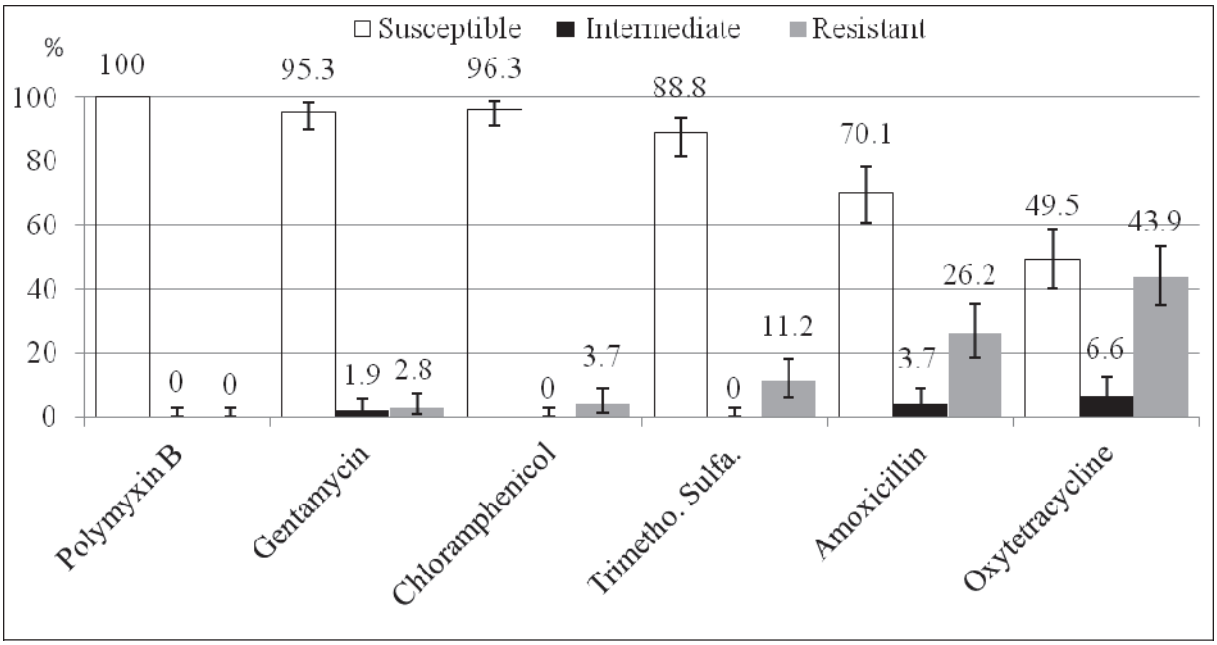


Fig. 5 - 14: Susceptibilities/resistances of isolates (%) from the abattoir line (n=107)

As shown in Table –A - 20, variability in susceptibility/resistance of strains to the drugs used for investigation was observed on different sampling occasions. On the 5th sampling occasion, strains were found resistant to all drugs other than PB. Resistance to AML, OT and SXT fluctuated at the abattoir line on all sampling occasions.

With regard to the sample type from this line, 14.3% -100% of *E. coli* were found resistant to OT, regardless of sample (Table –A - 21). A resistant strain to AML was also found in in the majority of samples, except in swab samples from personnel's hands, knives and refrigerators.

5.3.2.3 Processing plant line

As shown in Fig. 5 - 15, susceptibility to PB, C, CN and SXT of 200 *E. coli* isolates from the the processing plant line was similar (94.0%-99.0%) in each case ($p > 0.05$) but higher than susceptibility to AML and OT ($p < 0.05$). A difference was also detected in both susceptibility and resistance of the isolates from this line to AML and OT ($p < 0.05$).

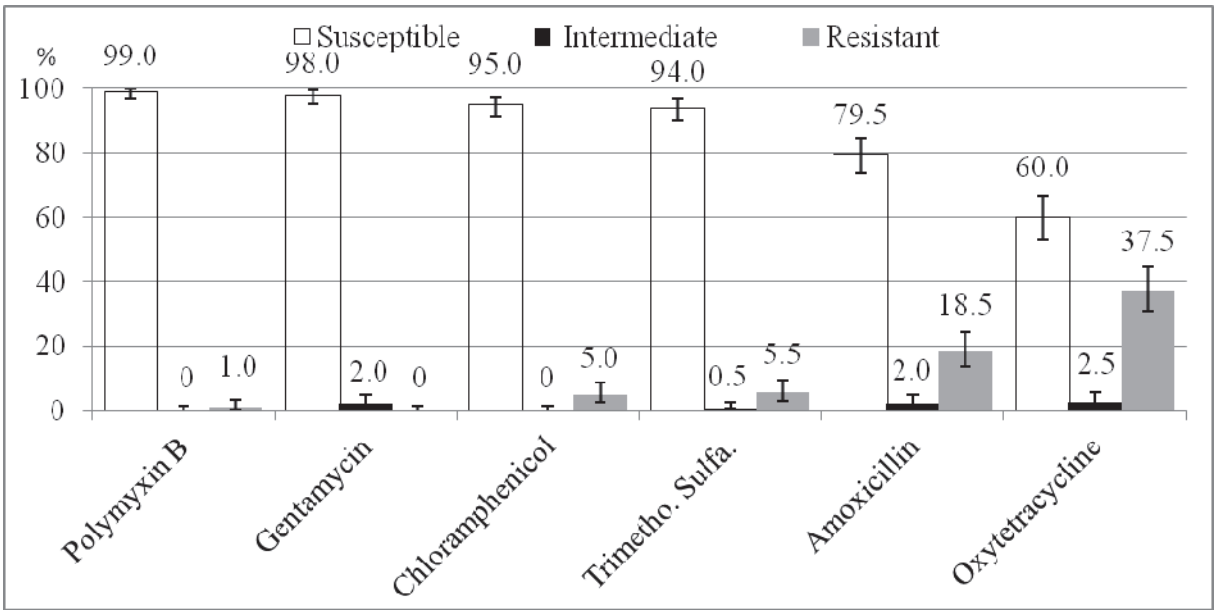


Fig. 5 - 15: Susceptibility/resistance (%) of *E. coli* isolates from the processing plant line (n=200)

As given in Table –A - 22, variability in susceptibility/resistance to drugs evaluated in this study was observed on various sampling occasions in isolates from the processing plant. Except for the 8th sampling occasion, resistance to AML was observed in the isolates on all sampling occasions. Resistance to OT was observed in the isolates throughout all sampling occasions. PB resistance was only detected on the 6th sampling occasion, in an isolate from the processing plant.

With regard to supermarkets (Table –A - 23), an isolate obtained on the 5th occasion was found to be resistant to PB. Furthermore, a strain resistant to AML was recorded on all sampling occasions except the 4th one. A OT strain was also found resistant on all sampling occasions.

As shown in Table 5 - 30, PB resistant isolates were obtained from a swab from personnel's hands at the processing plant line and from the final product at a supermarket. Except for isolates from cutting plates, water, spices and products from Supermarket-A, strains resistant to OT were observed in all other processing plant environment and animal related samples. Strains resistant to AML were also widely present in considerable types of samples in this line.

Table 5 - 30: Antimicrobial susceptibility/resistance of *E. coli* isolates from the processing plant line by origin and type of samples

Sample origin	Source of sample	No. of tests	PB*		CN**			C***		SXT		AML			OT		
			S.* No. (%)	R*. No. (%)	S.* No. (%)	I.* No. (%)	S*. No. (%)	R.* No. (%)	S*. No. (%)	I.* No. (%)	R.* No. (%)	S*. No. (%)	I.* No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)
Processing plant Environment	Personnel's hands	10	9 (90)	1 (10)	10 (100)	0	9 (90)	1 (10)	10 (100)	0	0	8 (80)	1 (10)	1 (10)	6 (60)	0	4 (40)
	Aprons	10	10 (100)	0	10 (100)	0	10 (100)	-	10 (100)	0	0	8 (80)	0	2 (20)	7 (70)	0	3 (30)
	Knives	8	8 (100)	0	8 (100)	0	8 (100)	-	7 (87.5)	0	1 (12.5)	7 (87.5)	0	1(12.5)	7 (87.5)	0	1 (12.5)
	Plates	8	8 (100)	0	8 (100)	0	7 (87.5)	1 (12.5)	8 (100)	0	0	6 (75.0)	1(12.5)	1(12.5)	8 (100)	0	0
	Water	4	4 (100)	0	4 (100)	0	4 (100)	-	4 (100)	0	0	4 (100)	0	0	4 (100)	0	0
	Tables	8	8 (100)	0	8 (100)	0	7 (87.5)	1 (12.5)	6 (75)	0	2 (25)	5 (62.5)	0	3(37.5)	3 (37.5)	0	5 (62.5)
	Rooms	12	12 (100)	0	12 (100)	0	12 (100)	-	11 (91.7)	0	1 (8.3)	12(100)	1 (8.3)	1 (8.3)	7 (58.3)	0	5 (41.7)
	Refrigerators	10	10 (100)	0	10 (100)	0	9 (90)	1 (10)	10 (100)	0	0	9 (90)	0	1 (10)	6 (60)	0	4 (40)
	Spices	2	2 (100)	0	2 (100)	0	2 (100)	-	2 (100)	0	0	2 (100)	0	0	2 (100)	0	0
	SWE **	7	7 (100)	0	7 (100)	0	7 (100)	-	7 (100)	0	0	7 (100)	0	0	4 (57.1)	0	3 (42.9)
	Grinders	6	6 (100)	0	6 (100)	0	6 (100)	-	6 (100)	0	0	5 (83.3)	0	1(16.7)	4 (66.7)	0	2 (33.3)
	Cutters	3	3 (100)	0	3 (100)	0	3 (100)	-	3 (100)	0	0	3 (100)	0	0	2 (66.7)	0	1 (33.3)
	Mixers	5	5 (100)	0	5 (100)	0	5 (100)	-	5 (100)	0	0	5 (100)	0	0	3 (60)	0	2 (40)
	Fillers	5	5 (100)	0	5 (100)	0	5 (100)	-	5 (100)	0	0	5 (100)	0	0	3 (60)	0	2 (40)
ARM¶	Raw meat	67	67 (100)	0	65 (97)	2 (3)	62 (92.5)	5 (7.5)	62 (92.5)	0	5 (7.5)	49 (73.1)	0	18(26.9)	36 (53.7)	4 (6)	27 (40.3)
Supermarkets	Supermarket-A	3	3 (100)	0	3 (100)	0	3 (100)	-	2 (66.7)	0	1 (33.3)	3 (100)	0	0	2 (66.7)	1(33.3	0
	Supermarket-B	4	3 (100)	0	3 (75)	1(25)	3 (100)	-	3 (75)	0	1 (25)	5 (50)	0	2 (50)	2 (50)	0	2 (50)
	Supermarket-C	4	4 (100)	0	4 (100)	0	4 (100)	-	4 (100)	0	0	4 (100)	0	0	3 (75)	0	1 (25)
	Supermarket-D	6	6 (100)	0	6 (100)	0	5(83.3)	1 (16.7)	5 (83.3)	1 (16.7)	0	4 (66.7)	0	2 (33.3)	3 (50)	0	3 (50)
	Supermarket-E	5	5 (100)	0	5 (100)	0	5 (100)	-	5 (100)	0	0	5 (100)	0	0	4 (80)	0	1 (20)
	Supermarket-F	2	2 (100)	0	2 (100)	0	2 (100)	-	2 (100)	0	0	1 (50)	0	1 (50)	1 (50)	0	1 (50)
	Supermarket-G	8	7 (87.5)	1(12.5)	8 (100)	0	7 (87.5)	-	8 (100)	0	0	5 (62.5)	1(12.5)	2 (25)	3 (37.5)	0	5 (62.5)
	Supermarket-H	3	3 (100)	0	2 (66.7)	1(33.3	3 (100)	-	3 (100)	0	0	2 (66.7)	0	1 (33.3)	0	0	3 (100)

PB* = no PB intermediate was observed SWE** = Spice-weighing equipment ARM¶ = Animal-related materials CN** no CN resistance was observed
C*** = S and R are the only available breakpoint is for susceptibility S* = Susceptible I* = Intermediate R* = Resistant

In Figure 5 – 16 below, *E. coli* strains are assessed in relation to their resistance to drugs and their sources/origin. Antimicrobial resistant *E. coli* are becoming a global challenge as they harbour resistant genes that directly come from contact with animals, food of animal origin, or from the environment (EFSA, 2013; Plumb, 2008; Anupurba and Sen, 2005).

A total of 143 (46.6%) isolates was found resistant to at least one of the six classes of antimicrobial agents tested against in this study. As shown in the figure below, the distribution of strains resistant to individual drugs at the abattoir and at the processing plant line was similar to each other and equal to the total distribution of resistant strains ($p > 0.05$). The extent of resistance to each of the drugs in an increasing order was PB (1.4%), CN (2.1%), C (8.4%), SXT (16.1%), AML (45.5%) and OT (85.3%). Differences were observed among isolates resistant to SXT, AML and OT ($p < 0.05$).

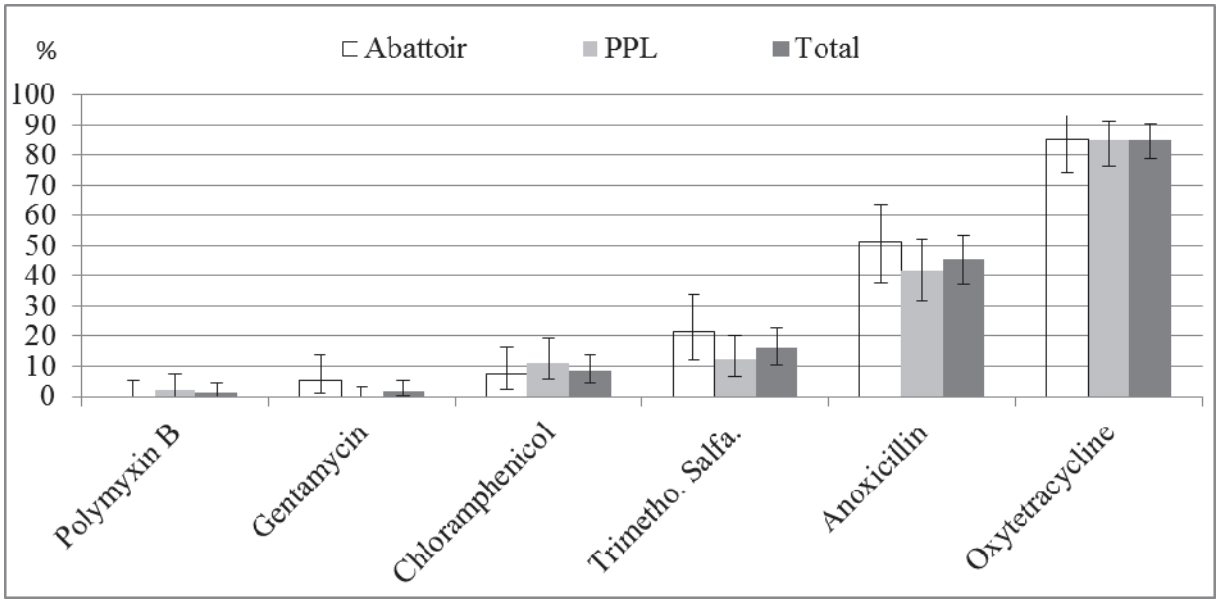


Fig. 5 - 16: Distribution (%) of resistant *E. coli* strains in both study lines

Between all resistant strains, a difference ($p < 0.05$) was observed between those from the abattoir line (38.5%; 95%CI 30.7-46.6) and the processing plant line (61.5%; 95%CI 53.4-69.3). As shown in Fig. 5 - 17 and Table 5 - 30, 55.9% of isolates were resistant to a single and 28.7% resistant to two drugs. Further, 15.4% of the strains were found to be MDR. For these three categories of drugs, no difference in resistance was observed between strains from the abattoir line and the processing plant line ($p > 0.05$).

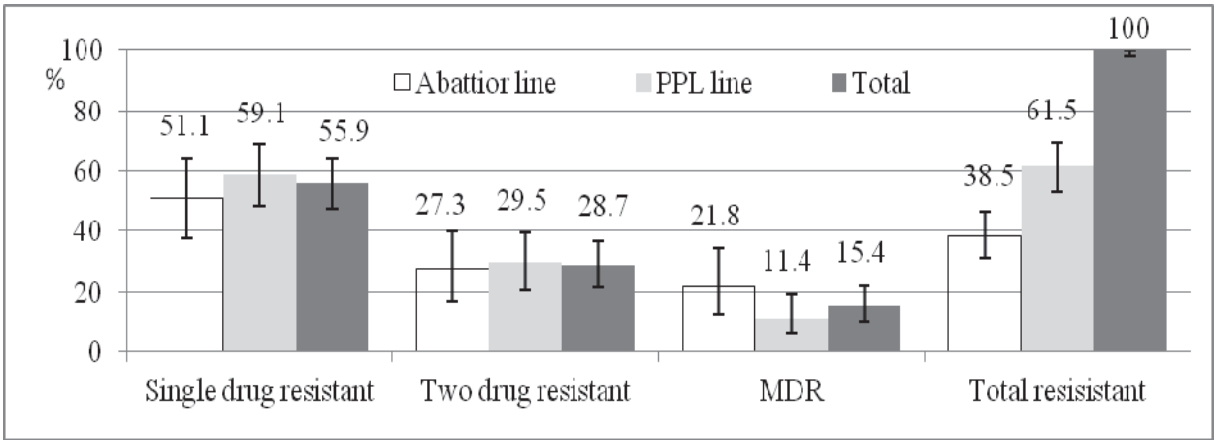


Fig. 5 - 17: Resistance *(%) of *E. coli* strains from the abattoir and the processing plant lines to different combinations of drugs

In total 55.9%, 28.7% and 15.4% of the isolates showed resistance to a single drug, two drugs and multiple drugs (MDR), respectively (Table 5 -31). No strains were resistant to multiple drugs at butchers, but some were to AML and OT along both study lines. Resistance to AML and OT was frequently observed for single drugs, their pairs and in multiple drug form.

Table 5 - 31: Isolates resistant to single to multiple antimicrobials by origin/source line

Line	Sources/origin	No. (%) of resistant isolates	Resistance by individual drugs/drug combinations		
			Single drug	Two drugs	MDR* resistant
Abattoir line	Abattoir, Environment	20 (36.4)	1: AML 1: C 8: OT	2: AML, OT 2: C, OT 1: CN, OT	5: AML, OT, SXT
	Abattoir, ARM	30 (54.5)	2: AML 1: C 1: CN 11: OT	8: AML, OT	7: AML, OT, SXT
	Butchers	5 (9.1)	2: AML 1: OT	1: AML,OT 1:CN, OT	0
	Subtotal (%)	55 (100)	28 (51.1)	15 (27.3)	12 (21.8)
Processing plant line	PPL, Environment	37 (42.0)	2: AML 2: C 22: OT	1: AML, SXT 5: AML, OT 1: C, OT 1: OT, SXT	1: AML, C, OT, SXT, PB 2: AML, OT; SXT
	PPL, ARM	32 (36.4)	2: AML 1: C 11: OT	2: AML, SXT 9: AML, OT 2: C, OT	2: AML; C; OT 3: AML; OT; SXT
	Supermarkets	19 (21.6)	1: AML 10: OT 1: SXT	1: AML, PB 4: AML; OT	1: AML, C, OT 1: AML, OT, SXT
	Subtotal (%)	88 (100)	52 (59.1)	26 (29.5)	10 (11.4)
	Total No. (%)	143	80 (55.9)	41 (28.7)	22 (15.4)

*Resistance to ≥3 drugs

The proportion of *E. coli* isolates tested and the corresponding resistant isolates showed no difference ($p > 0.05$) in regards to origin/source (Fig. 5 - 18). In the abattoir line, a significantly lower proportion (3.5%) of resistant isolates was registered in samples from the butchers compared to isolates from the environment (Env't) (14.0%) and animal-related materials (21.0%) ($p < 0.05$). However, no difference did exist between the latter two (p

>0.05). As regards the processing plant line, no difference in resistant isolates was observed in terms of origin/source (p >0.05).

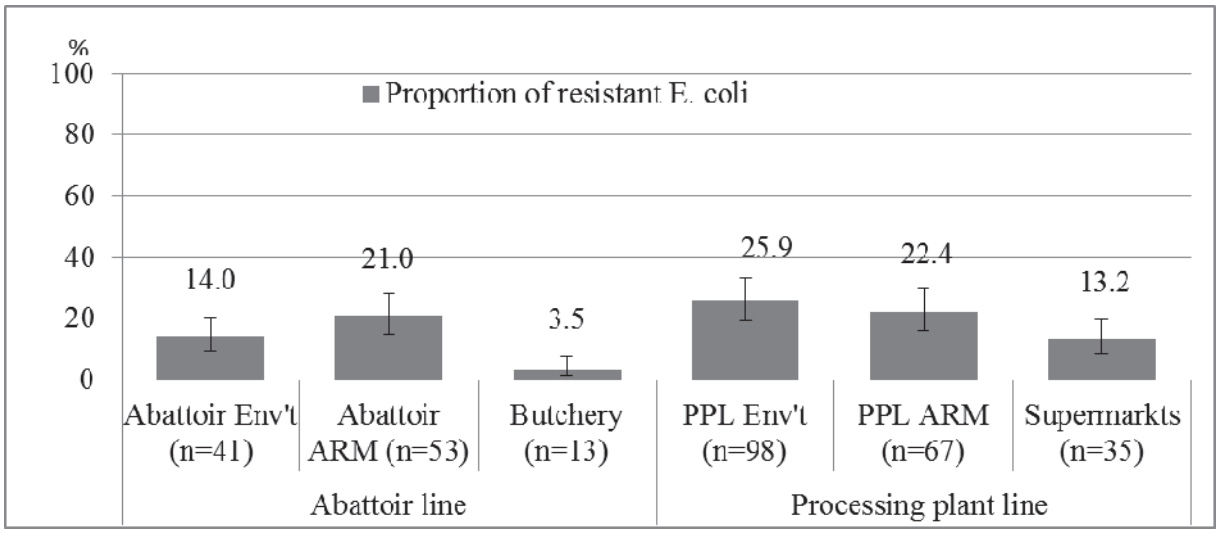


Fig. 5 - 18: Proportion of *E. coli* tested and corresponding resistance (%) by source/origin in both lines (n = number of isolates tested)

The proportions of *E. coli* isolates tested and their resistance to individual or combined drugs by study line are presented in Table 5 - 32 below. Results show that there was no difference in the proportion of isolates and of the resistant isolates (p >0.05) in both, the abattoir and the processing plant lines. On the other hand, while the proportion of isolates was higher at the processing plant line (65.2%) than at the abattoir line (34.8%), the corresponding numbers of resistant strains were higher at the processing plant line (61.5%) than at the abattoir line (38.5%). Strains resistant to a single and to two drugs were more frequent at the processing plant than at the abattoir line while the number of those resistant to multiple-drugs was higher at the abattoir line (54.5%) than at the processing plant line (45.5%) (Table 5 - 32).

Table 5 - 32: Proportion of *E. coli* isolates and resistance to individual drugs or drug combinations in both lines

Line	No. (%) proportion tested	No. (%) proportion resistant	Resistance to individual drugs/drug combinations		
			Single drug	Two drugs	MDR*
Abattoir	107 (34.8)	55 (38.5)	28 (35.0)	15 (36.6)	12 (54.5)
Processing plant	200 (65.2)	88 (61.5)	52 (65.0)	26 (63.4)	10 (45.5)
Total No. (%)	307 (100)	143 (100)	80 (100)	41 (100)	22 (100)

*Resistance to ≥3 drugs

6. DISCUSSION

6.1 Materials and methods

In this section, the characteristics of the studied abattoir and processing plant lines, the corresponding characteristics of samples and study/samples shortcomings are discussed. The parameters and aims of the methods used for the spoilage and zoonotic microbes' (APC, EBC, *Salmonella* and *E. coli*) examinations as well as the objectives of the antimicrobial susceptibility/resistance tests are also included in this discussion.

6.1.1 Materials

The Addis Ababa Abattoir Enterprise (AAAE) is mandated to slaughter cattle and supply consumers with meat from local slaughters through the city butchers. The abbatoir was constructed in the center of the city in 1957 and transferred 1992 as a public enterprise, 100% owned by the Addis Ababa City Government. Contrary to other Government infrastructure services, the AAAE however was given an autonomous status to operate totally as an independent business enterprise. As the aim is full financial self sufficiency, investments into the infrastructure were low. An average of 1.200 cattle were slaughtered per day. At the time of study, the abbatoir was very old, effectively having reached the end of its useful life. In 1993, a tender was launched for the turnkey construction of a new slaughterhouse on a new location, with a capacity of 5.000 cattle per day, also for export slaughter.

Samples for this study were taken from locations along the beef slaughtering and the processing line of this abbatoir up to butchers in the city. These operate private, mostly small-scale businesses in usually open-air stalls. About 1400 butcher shops in the city receive somewhat cooled raw beef from the abattoir. On average, 300kg meat is sold by a shop per month. The meat is stored during the day at room temperature and displayed open-air on open surfaces or trays. The minority of shops has refrigeration for nightly storage, the meat consequently is stored in a shop at room temperature for an average of 2 to 2 1/2 days, with a frequent maximum of 5 days. While environmental samples at the butchers could not be drawn, raw beef samples could. About 120 supermarkets are located in Addis Ababa. There are six supermarket chains, the rest are small to medium-sized individual stores, catering for

higher income ethiopian consumers and for faranjiis. Only 0.5% of groceries are currently bought through modern outlets, the number is slowly but steadily rising. Supermarkets usually have refrigeration for overnight storage of meats and meat products.

The beef processing plant used in the study is located in Bishoftu town, 47 km east of Addis Ababa. It is one of the first few (10) small scale manufacturers processing meat. It receives raw beef from different abattoirs. Because of some irregularity in the processing operation and due to differences in the locations of source abattoirs, raw beef samples could not be identified and drawn at their source abbatoir in each case. Consequently, steps examined here started in the processing plant. Its end product in the supermarkets of Addis Ababa city were traced using the the processing company's brand name. In fact, due to differences in locations between the processing plant and the supermarkets and also due to irregularity in production at the processing plant, tracking of products had to depend on the brand name, not on the date of manufacturing of the product.

The abattoir and processing plant lines studied have certain similarities and differences in beef production procedures, available facilities, production steps and processing technologies, end products and product stations. Details are given in section 6.2 of this discussion part where samples from both lines are examined from different perspectives.

Due to logistic reasons such as time limitation, restricted number of environmental samples to be taken (2-10 samples) from a sampling location, and limited laboratory capacity to handle primary samples in time, the study was limited to small numbers of samples in most of the sampling locations in both lines. However, following Gudata (2012) and taking meat hygiene into consideration, samples were collected from almost all important points/locations at AAAE. Moreover, it is felt that using a unique identification of slaughtered animals, end products (raw beef) from butchersin the city were suited to principally conclude on the abattoir line hygiene and to draw a basic safety picture of the chain. Similarly, following FAO (2007) which suggested control points for hazards directly related to meat processing, samples were collected from such points of fresh raw meat, cold storages, meat cutting and preparing facilities, non-meat additives, handling facilities, meat commuting units, and filling equipment at the processing plant. The end product (processed mortadella) at the recipient supermarkets were sampled to assess the hygiene of the processing plant line as well as of a supermarket and, thus, to draw basic chain safety conclusions under present production and retail conditions.

6.1.2 Methods

Samples assumed representative were aseptically taken from selected locations. Then samples were defined and grouped into environmental, animal-related materials, and product at final stations (butchers and supermarkets). Environmental samples are samples collected from spots that could have contact with a product during production and processing. Animal-related materials are samples associated with the source animals at the production and processing stages. Products at final stations are products ready for sale at the butchers and for retail at supermarkets.

Surface area swabs were taken from personnel's hands, working facilities, meat transporting trucks, and samples of water and spices. These were considered environmental samples. Animal-related materials such as feces, mesenteric lymph nodes and raw beef were samples from the abattoir. Raw beef was also sampled at reception in the processing plant.

Samples from the abattoir line were examined for *Salmonella* and *E. coli* while those from the processing plant line were examined additionally also for spoilage bacteria (APC and EBC). *Salmonella* strains isolated from both beef lines were serotyped and exposed to PFGE genomic analysis. Using genomic relatedness and differences of *Salmonella* serotypes, possible sources and transmission routes were determined. *E. coli* isolates were biochemically identified. *Salmonella* and *E. coli* strains were tested for antimicrobial susceptibility/resistance to selected drugs of critical importance (WHO, 2011).

The aim of collecting samples from the environment was to assess the magnitude of occurrence of zoonotic agents and their possible contribution as source of contamination of products . Animal-related materials were sampled and analysed to identify the number of carrier animals at slaughter, to assess possible contamination of raw products throughout and at the final stage of slaughter as well as to examine transmission agents (*Salmonella*) that can act as source of infection for consumers.

As shown in Table 6 - 01, a difference in resistance ($p<0.05$) was observed only for AML between the two laboratories performing the resistance tests (Section 4.5), i.e., those strains tested at the Institute of Meat Hygiene and Technology, FU-Berlin, showed higher resistance to AML (27.9%, 95%CI 21.7-34.7) than those tested at ALIPB-AAU (11.3%, 95%CI 6.6-17.8).

Table 6 - 01: Susceptibility/resistance profiles of *E. coli* by the laboratories employed in the study

Drug	Aklilu Lemma Institute of Pathobiology AAU (No. of *E. coli* tested = 124)			Institute of Meat Hygiene and Technology, FU-Berlin (No. of *E. coli* tested = 183)		
	S* No. (%)	I* No. (%)	R* No. %	S* No. (%)	I* No. (%)	R* No. %
PB	124 (100)	0	0	181 (98.9)	0	2 (1.1)
CN	121 (97.6)	0	3 (2.4)	177 (96.7)	6 (3.3)	0
C*	121 (97.6)	-	3 (2.4)	171 (94.0)	-	11 (6.0)
SXT	115 (92.7)	0	9 (7.3)	168 (91.8)	1 (0.6)	14 (7.6)
AML	103 (83.1)	7 (5.7)	14 (11.3)	131 (83.1)	1 (0.6)	51 (27.9)
OT	70 (56.5)	6 (4.8)	48 (38.7)	103 (56.3)	6 (3.3)	74 (40.4)

S*= susceptible; I*= intermediate; R*= resistant C*= the only available breakpoint is for susceptibility

6.2 Results

The presence of *Salmonella* serovars along the abattoir line and that of spoilage bacteria (APC and EBC) and *E. coli* along the processing plant line in at least one or more sampling location on one or more sampling occasion shows the weak points in hygiene throughout, resulting in considerable spoilage and zoonotic agents along the examined beef lines. The presence of *E. coli* serves as an indicator of hygienic deficits and the acquisition of *Enterobacteriaceae* organisms (Ramos *et al.*, 2013). Isolates of *Salmonella* and *E. coli* tested for drug susceptibility/resistance showed variable results.

6.2.1 Spoilage bacteria in general

Examination of spoilage bacterial loads from the processing plant line showed the presence of aerobic bacteria and *Enterobacteriaceae* at all sampling locations, implying extensive contamination of the studied line. Freier (2004) and Schaffner and Smith (2004) emphasized the importance of examinations of meat production, processing and/or products for basic food microbiology and the understanding of their hygiene. In general, high log APC from personnel's hands and room floor swabs, respectively, to lower log APC from spices were observed. Further, a high count of log EBC in samples from room floors to a low one in samples from mortadella at Supermarket-G was detected. The presence of spoilage bacteria in all locations along the processing plant and in the end products is obvious. A significantly higher APC than EBC within examined locations ($p < 0.05$) was observed. Further, a significant difference between and among locations ($p > 0.05$) was also detected. Within a sampling location all APC were similar ($p < 0.05$) and EBC ($p < 0.05$) imply consistent and

similar occurrences of spoilage bacteria along the processing plant line. Abdalla *et al. (*2009) also reported TVC of 3.03±0.15 log_{10} from carcass shoulders, 3.74±0.02 log_{10} from personnel's hands post skinning, 2.25±0.03 log_{10} from knives, 3.71±0.04 log_{10} from briskets post evisceration, 2.79±0.10 log_{10} from shoulders, and 3.72±0.02 log_{10} from necks post carcass washing. Barros *et al.* (2007) reported mesophilic aerobic bacterial counts ranging from 2.29±0.29 log_{10} in a refrigeration system of a beef processing plant to 6.49±1.73 log_{10} in ground beef.

Adetunji and Isola (2011) on working tables at an abattoir found an increase in EBC from 8.35±0.07 log_{10} to 10.85±0.06 log_{10} before and after meat sale respectively; and an alsio an increase in coliforms from 8.81±0.05 log_{10} to 11.47±0.04 log_{10} before and after meat sale. Freier (2004), Schaffner and Smith (2004) and Gilmour *et al.* (2004) also reported occurrences of and obvious differences in spoilage bacterial loads along meat production lines.

6.2.1.1 Aerobic Plate Count

Examination of APC at identified locations (points) in the beef processing plant line shows its occurrences with different degrees of contamination. The locations are discussed as follows:

Personnel hands: The present log cfu/cm^2 APC reported in samples from personnel hands swab is higher than the 2.49±0.73 log cfu/cm^2 and 3.06±0.1 log cfu/cm^2 reported by Attala and Kassem (2011) in Egypt.

Aprons: The highest APC observed in this study is similar to the $3.1x10^5$ cfu/cm^2 APC reported by Adzitey *et al.* (2014) on aprons of staff handling retailed meat in Ghana, but higher than the 5-7.9 $x10^1$ cfu/cm^2 total viable count (TVC) reported by Lues and Tonder (2007) from South Africa. Such a result in the processing plant could be due to overuse of aprons and improper cleaning.

Knives and cutting plates: The APC of knives and cutting plates in the present study was similar to each other and to the 4.42±0.14 log cfu/cm^2 reported by Attala and Kassem (2011) from Egypt, but lower than the 6.7±5.3 log cfu/cm^2 found in cutting instruments by Bello and Son (2009) from Russia and also the 10.2 log cfu/cm^2 TVC on knives by Ali *et al.* (2010) from Pakistan.

Water: The present APC in water was higher than the 1.71±0.1 log. cfu/g total plate counts in ice water reported by Güngör and Gökoglu (2010) from Turkey and lower than the 13.68 log cfu/ml in washing water used in a goat abattoir as reported by Adetunji and Odetokun (2011) from Nigeria. These differences could be due to differences in water type and the studied meat production locations.

Working tables: APC on working tables at the studied beef processing plant was slightly higher than the 4.42±1.06 log cfu/cm^2 mesophilic aerobes reported by Barros *et al.* (2007), and lower than $3.7x10^7$ cfu/cm^2 in the reports of Adzitey *et al.* (2014) from Ghana.

Processing room: The present APC observed in the processing room was similar to the 4.76±1.15 log cfu/cm^2 mesophilic aerobes reported by Barros *et al.* (2007), lower than the 6.8 log cfu/cm^2 total viable count identified by Ali *et al.* (2010) and higher than in investigations by Bradeeba and Sivakumaar (2013), where 1.10±0.03 log cfu/cm^2 on floors and 1.89±0.05 log cfu/cm^2 on walls were detected.

Processing machinery/devices: Microbial load at meat commuting units (grinding, cutting, mixing and filling) and cooling points (FAO, 2007) in this study is similar to each other, showing similar levels of spoilage bacteria on those working surfaces. The APC on the machinery was similar to the 4.79±0.17 log cfu/cm^2 and 4.2±0.21 log cfu/cm^2 of meat mincers reported by Attala and Kassem (2011) from Egypt and the 5.24±2.83 log. cfu/cm^2 of mixers and the 5.15±1.73 log cfu/cm^2 on grinders reported by Barros *et al.* (2007). Loads were, however, lower than the 7.5 log cfu/cm^2 TVC of a meat mixer reported by Ali *et al.* (2010).

Spices and Spice-weighing apparatus: The present APC observed in spices and the spice-weighing apparatus shows the importance of this point as CCPs. The APC observed in spices was similar and in the range of 2-6 log cfu/g spore-forming bacteria and thermophiles counts reported by Witkowska *et al.* (2011), but much lower than the $2.96x10^8$cfu/g in spices reported by Shamsuddeen (2009) from Nadu. The difference could be due to differences in the quality of spices and and in the safety of their handling. Cohen *et al*. (2008) also reported higher bacteria in beef processed with spices (7.4±0.4 log cfu/cm^2) than without spices (6.8±0.4 log cfu/cm^2) in Casablanca, Marocco, implying spices as possible source of bacterial load in final products.

Raw beef: The total mean of 4.82±0.51 log APC cfu/g in the present study is similar to that of each of the sampling occasions and to the mean APC of 1.62×10^5 cfu/g reported by

Gebeyehu *et al.* (2013) in beef of Arsi cattle in Adama Town, Oromia, Ethiopia, and the 5.8±0.16 log cfu/g before and 5.25±0.5 log cfu/g after GMP's application in minced beef reported by Attala and Kassem (2011) from Egypt, but lower than the $5.0x10^6$ cfu/cm^2 reported by Adzitey *et al.* (2014) from Ghana, and the 10.2 log cfu/g TVC in retail beef meat reported by Ali *et al.* (2010). Obviously large differences in meat handling conditions in the study areas are responsible.

Product: Examination of final mortadella products showed APCs ranging from 4.15±0.83 log cfu/g to 4.97±0.50 log cfu/g in the supermarkets, similar to the overall count. It is also similar to the 3.93±0.1 log cfu/g, 3.93±0.09 log cfu/g and 3.66±0.18 log cfu/g in cooked, peeled and pasteurized sausages, respectively, reported by Güngör and Gökoglu (2010) for Turkey. HPA (2009) listed factors such as poor quality of raw materials or food components, undercooking, cross-contamination, poor cleaning, poor temperature and time control as responsible factors for the presence of indicator bacteria in ready-to-eat food.

6.2.1.2 Enterobacteriaceae Counts

Examination of locations for EBC shows different degrees of contamination for the beef processing plant line. The situation for each location is discussed below.

Personnel hands: The present 3.9±0.8 log cfu/cm^2 EBC in swabs from personnel hands is higher than the range from 5 to $1.8x10^1$ cfu/cm^2 EBC reported by Lues and Tonder (2007) from the South Africa. This shows the essential role of hygiene of personnel hands; hands with no or poor hygiene harbour microbial agents, which, during processing and handling act as source of contamination of beef.

Aprons: The highest 3.07±0.65log cfu/cm^2 EBC found on aprons in this study without doubt is due to non-changing of aprons and non-cleaning. The distribution was considerably higher than the 2-9 cfu/cm^2 coliforms reported by Lues and Tonder (2007) in their South Africa stuydy.

Knives and cutting plates: The EBCs detected on knives and cutting plates are much lower than the 13.58 log cfu/cm^2 and 13.53 log cfu/cm^2 counts found on knives before and after use, respectively, in goat abattoir (Adetunji and Odetokun, 2011) in Nigeria. Contamination in both facilities whereever meat is cut though is considerable. Lower EBCs at the processing plant compared to the abbatoir in all likelihood are due to the involvement of gastro-intestinal contents in the latter one.

Water: The EBC observed in water in this study was lower than the $8.6x10^3 \pm 5.2x10^2$ coliforms in tap water reported by Garba *et al.* (2009) during the wet season in north-western Nigeria. Local waters obviously differ a lot in their contamination rate.

Working tables: The EBC observed on working table in this study is considerably lower than the of 8.81 log cfu/cm^2 and 11.47 log cfu/cm^2 on meat tables before and after sales of meat, respectively, at an abattoir in Nigeria (Adetunji and Isola, (2011)). A abattoir can be a source of *Enterobacteriaceae,* from which the working environment of a processing plant is further contaminated.

Processing room: The present finding of 3.19±0.55 log cfu/cm^2 EBC from the processing room is the highest of all examined locations in this study and underlines the importance of spoilage bacteria contaminating meat and affiliated facilities, as also determined by a 2.26±1.23 log TCC from a processing room reported by Barros *et al.* (2007) for Brazil. show the .

Machinery and devices: The presence of EBC on such locations shows consistency in occurrence, loads are similar. Meat might already be contaminated and kept in the refrigerator before further processed by machines. The present finding of EBC on machineries is similar to the coliform count of 2.29±0.11 log cfu/cm^2 observed before the application of GMP on mincing equipment, which helped to reduce the load to 1.2±0.21 log cfu/cm^2(Attala and Kassem, 2011). The effect of GMP as one example of a hazard analysis critical control point tool for risk minimization is clearly demonstrated. Algino *et al.* (2007) from Wisconsin also reported on the effectiveness of intervention treatments at small beef slaughter facilities to reduce indicator organisms. Contamination levels up to 10^5 CFU/cm^2 indicate good hygienic conditions during slaughtering; meat contamination of 10^6 CFU/cm^2 indicates a deterioration process (Barros *et al.*, 2007).

Spices and spice-weighing apparatus: The present 2.28±0.52 log cfu/g EBC in spices shows their safety and quality, based on their *Enterobacteriaceae* indicator microorganisms counts. ASTA (2011) suggests a possibility of destruction of micro-organisms during drying of spices but still many of the bacteria such as *Salmonella, E. coli, L. monocytogenes, S. aureus* and *Aspergillus flavus/parasiticus* which have the potential for spoiling food and threatening public health, could survive. Meat processed with spices showed higher bacterial counts than one processed otherwise (Cohen *et al.,* 2008).

Raw beef: The mean total EBC detected in raw beef in this study is similar to that of each individual sample taken on different occasions. It is higher though than the mean total coliform 5.29×10^{1} cfu/g reported by Gebeyehu *et al.* (2013) for another abbatoir in Ethiopia, 100 km east of Addis Ababa, with equally poor hygienic conditions than in Addis Ababa. Further, it is considerably lower than the 6.54-6.98 log cfu/g EBC in fresh, minced and unpacked beef reported by Crowley *et al.* (2005) from the Republic of Ireland and the 6.6×10^{6} cfu/g count in red meat reported by Nel *et al.* (2004) from South Africa.

Product: The EBC in the mortadella product in this study is by far lower than the 10.14×10^{5}, 5×10^{4} and 37.8×10^{4} cfu/g EBCs in Egyptian meat products kofta, sausage, and shawerma, respectively (Al-Mutairi, (2011). Observation of *Enterobacteriaceae* final products without doubts point to principal as lack of quality and sanitation in meat handling as argued by HPA (2009).

6.2.2 *Salmonella*

Results show the epidemiological and serotypic prevalence and the genomic diversity of *Salmonella* at the locations along the beef lines. An assessment of possible sources and transmission routes of the strains is attemted. The isolates show varying susceptibility/resistance to antimicrobial substances.

6.2.2.1 Salmonella prevalence

6.2.2.1.1 Overall Salmonella situation

Abattoir line: The overall 26.6% *Salmonella* prevalence at the abattoir line, which is roughly similar to that at the butchers (32.4%), in the abattoir environment (36.6%) and in animal-related materials (14.7%), indicates the possible contamination threat for consumers . With the exception of mesenteric lymph nodes at evisceration (8.8%) and raw beef at quality inspection (11.8%), with lower prevalences than in rooms (52.9%) and the refrigerator (60.0%), findings at all other locations were similar, indicating the risk of *Salmonella* presence.

Processing plant line: Overall, the 5.3% *Salmonella* prevalence at the processing plant line was similar to that of the environment (5.2%) and of raw beef (10.2%) The prevalence in the

final product at supermarkets (0.8%) was lower than that in raw material, showing *Salmonella* reduction effects duringprocessing.

Comparing the two lines, the overall 26.6% *Salmonella* positives at the abattoir line were to higher than the 5.3% at the processing plant line. The prevalence at individual locations like theabattoir environment (36.6%), animal-related materials (14.7%) and meats for sale to butchers (32.4%) were equally higher than the respective prevalences of 5.2%, 10.2% and 0.8% at their counterpart locations in the processing plant line. This finding indicates a principally more extensive contamination with *Salmonella* at the abattoir than at the processing plant line. The higher *Salmonella* prevalence at the abattoir can be attributed to the involvement of the slaughter animals and their contaminationof areas, equipment, floors and personnel. From the abattoir, these pathogens principally proliferate in meat of butchers and to raw beef at the processing plant. The surface for bacterial growth is principally enlarged when beef is cut into smaller pieces. Comparing the present findings with reports of others, the overall high *Salmonella* prevalence of 26.6% at the abattoir line was similar to the 23.6% in the previous report by Molla *et al.* (2003) from the same abbatoir in Ethiopia. It is, however, higher than the 11.5% reported by Reda *et al.* (2011), 10.8% by Sibhat *et al.* (2011) and 7.2% by Teklu and Nigussie (2011) from other localities in Ethiopia. The 5.3% prevalence at the processing plant lines in this study shows how pathogens from meat of abattoirs, being the main sources of *Salmonella* and possibly other zoonotic agents, eventually reach the consumption line. The low prevalence of 0.8% in the final product at supermarkets though shows how heat as processing technology can have a pathogen hurdling effect. Hygiene though has to be stringently maintained throughout; the positive *Salmonella* sample at a supermarket indicates that heat treatment is not carried out correctly throughout, or that cross or re-contamination from contaminated raw meat occurs at supermarkets, from slicing machines or from working personnel.

6.2.2.1.2 Salmonella situation at sample locations

All sampling locations at the abattoir line were positive for *Salmonella,* at least on one sampling occasion. In contrast, at the processing plant line, no *Salmonella* ever were identified in samples from aprons, knives, tap water, refrigerators, spices and their weighing equipment, meat grinder and mixer, as well as in 7 of the 8 supermarkets. This shows a principal lower *Salmonella* risk in the processing plant line due to the absence of slaughtering activities.

Personnel's hands*:* Regardless of the small sample size, *Salmonella* were identified from swabs from personnel's hands, both at the abattoir line (38.5%) and at the processing plant line (5.2%). These prevalences, based on the widths of the 95% confidence intervals were similar and agree with the 42.86% from personnel hands at butcheries from another recent investigation in Ethiopia (Gurmu and Gebretinsae, 2013).

Because of the small sample sizes in this study, results have to be interpreted and comparisons made with other results from Ethiopia like the 9%, 7.4% and 6.0% in reports by Sibhat *et al.* (2011), Teklu and Nigussie (2011) and Molla *et al.* (2003) with care. However, all investigations identified non-trivial *Salmonella* contamination of personnel directly handling meat.

Aprons: A prevalence of 35.7% on aprons at abattoir was much higher than the zero (0) result obtained at the processing plant. Contamination during slaughter and evisceration at the abattoir obviously is high. Similarly, Stevens *et al.* (2006) showed specific work clothing used at meat sales, modern butchers, permanent markets and itinerant retailers in Dakar districts, Senegal, as risk points for *Salmonella,* all leading to or fostering meat contamination.

Knives: The present 30.7% *Salmonella* observed on knives used at the abattoir also was higher than the zero (0) result obtained for knives used at the processing plant. It is quite likely that contamination of knifes at the abattoir occurs particularly from evisceration. Staff at the abattoir use a single knife throughout the slaughtering steps. Knifes are not cleaned or disinfected throughout a day.

On the other hand, this finding for knives at the abattoir was higher than the 7.4% of the report by Teklu and Nigussie (2011) for knives used for sheep and goat eviscerations and the 14.29% reported by Gurmu and Gebretinsae (2013) for knives used by butchers.

Working tables: The 17.7% prevalence obtained from working tables at the processing plant was lower than the 96.4% at permanent markets and the 70% on wood and cardboards at district sales shops in Dakar, Senegal (Stevens *et al.*, 2006), and the 42.86% from tables of butchers in Mekelle, Ethiopia (Gurmu and Gebretinsae, 2013). In the present study, cutting plates that could have had contact with working tables both were positive for Salmonella.

Water: The present 8.3% prevalence in water at the abattoir was higher than the 0% result from the processing plant line, showing the differences in the microbiological quality of water

used in both studied beef lines. As the investigated water was used for cleaning of facilities and the final products, it principally can act as sources of contamination of those points.

The 8.3% *Salmonella* abbatoir water prevalence in this study was similar to the 7.1% reported by Teklu and Nigussie (2011) in water used for slaughtering sheep and goats. ***Rooms:*** Occurrence of *Salmonella* in rooms at the abattoir (52.9%) and at the processing plant (18.7%) was similar and suggests that rooms are possible sources of contamination duringmeat production as well as manufacturing products from meat. ***Refrigerator:*** A prevalence of 60% *Salmonella* was observed for refrigerators at the abattoir but no *Salmonella* was detected in refrigerators at the processing plant. The presence of *Salmonella* in beef stored in refrigerators shows the persistence of the agent; refrigerator temperatures do not impact *Salmonella*. Stevens *et al.* (2006) for Senegal also reported *Salmonella* from meat storage areas at temperatures of 0-10^{o}C and <0^{o}C.

Feces: The identified 23.5% *Salmonella* from animal feces was similar to the 19% reported in rumen contents by Sibhat *et al.* (2011). It is higher though than the 2.2% in cattle feces (Nyeleti *et al.*, 2000), 1.9% in cattle feces (Molla *et al.,* 2003), 3.1% in pooled feces (Alemayehu *et al.,* 2003) and 15.1% in camel feces (Molla *et al*., 2003), all reports done in Ethiopia. Ddifferences in the study area, samples and species of animals obviously can explain these differences.

Mesenteric lymph nodes: The occurrence of *Salmonella* in mesenteric lymph nodes of cattle in this study was similar to the 8% reported by Sibhat *et al.* (2011) in mesenteric lymph nodes of beef cattle, but higher than the 4.2% (Nyeleti *et al.*, 2000) and the 4.5% (Alemayehu *et al.,* 2003) of slaughtered cattle and the 5.0% and 5.6% of slaughtered goats and sheep, respectively, reported by Teklu and Nigussie (2011) at Modjo. Gragg *et al.* (2013) also reported prevalences as high as 91.2% in mesenteric, 76.5% in sub-iliac, 55.9% in mandibular, and 7.4% in mediastinal lymph nodes s from slaughtered cattle.

Raw meat product: The prevalence in raw beef at the abattoir (11.8%) in this study was lower than the 32.4% in raw beef at the butchers and similar to 10.2% at the reception point of meat in the processing plant. The prevalence in raw beef at the butchers in all likelihood is due to the open environmental conditions of their shops and the handling of non-cooled temperatures facilitating microbial multiplication. Present findings in raw beef at the abattoir and the processing plant wwere similar to the 9.8% (Nyeleti *et al.,* 2000), but higher than the 2.8% (Alemayehu *et al.,* 2003) and 2% (Sibhat *et al*., 2011) in carcass swabs at abattoirs and

lower than the 42.8% (Stevens *et al.*, 2006) in meat from slaughterhouses, modern butchers, supermarkets, district retailers and itinerant retailers in Dakar, Senegal. Differences can be attributed to differences in sample types and studied abattoirs. Prevalence in raw beef at the processing plant was similar to the 11.9% in diaphragms and the 9.8% in abdominal muscles (Nyeleti *et al.*, 2000) at the abattoir. The prevalence of 32.4% in raw beef at butchers was lower than the 87.4% reported by Stevens *et al.* (2006) in retailed beef in Senegal and the 60% in slaughter house samples (Nel *et al.,* 2004). This could be either an extension from abattoir or a cross-and recontamination of carcasses during handling and transportation. In this study, meat transporting trucks were found with a prevalence of 45.5% which is as high as that of the butchers.

Processed meat products (mortadella)*:* The 0.8% *Salmonella* found in mortadella from a single supermarket was in the range of 0-35% reported by Ejeta *et al.* (2004) but lower than the 7.9% in minced beef (Nyeleti *et al.,* 2000), 14.4% in minced beef, 14.1% in mutton and 16.4% in pork from supermarkets (Ejeta *et al.,* 2004). Samples in the latter studies were from ordinary raw meat, while the heat treated products in the present study may imply the microbial hurdling effect of heat technology.

6.2.2.2 Salmonella serotypes

A total of 10 different serovars, most frequently (28 isolates) being *S.* Saintpaul, and 8 unidentified serovars were identified. The occurrence of compositions of serovars in meat and its production environments in Ethiopia is clearly demonstrated. *S.* Saintpaul, *S.* Muenchen and *S.* Larochelle were the prevailing serotypes observed in most sampling locations in the abattoir line of this investigation. Numbers and serovar composition were different from findings by e.g. Stevens *et al.* (2006) from a slaughterhouse/meat retailer scenario Senegal, where *S.* Bredeney, *S.* Corvalli, *S.* Kentucky, *S.* Muenste and *S.* Waycross were found, from Gebreyehu et al. (2013) who found no *Salmonella* at all on beef carcasses of the Adama abbatoir in Ethiopia and from Gebremedhin (2012) who found a completely different fauna of 14 serotypes in chicken carcasses and pork in Addis Ababa supermarkets.

It is worth noting that the occurrence of *Salmonella* serovars (*S.* Saintpaul, *S.* Muenchen, *S.* London and the unidentified ones) was mostly similar, both the abattoir and the processing plant line. On the other hand, some serovars were only observed at the abattoir line (*S.* Dublin, *S.* Kastrup and *S.* Larochelle) while others were detected only at the processing plant

line (*S*. Typhimirium, *S*. Anatum, *S*. Concord and *S*. Eastbourne). Similar serovars from both lines point to the possibility that the beef at the processing plant already was contaminated with thwe abbatoir serovars and no further serovars were added during subsequent work at the plant. If serovars different from the abbatoir would have been detected at the plant, this would indicate that the *Salmonella* fauna would have been different in the other two abbatoirs from which the plant, additional to the Addis abbatoir, did buy meat.

***S*. Saintpaul:** *S*. Saintpaul was the most predominant serotype isolated in this investigation. Its 42.8% prevalence at the abattoir line was higher than the 4.3% at the processing plant line. Present findings at the abattoir were similar to the 38.8% reported by Molla *et al.* (2004) in camels but higher than the 2.3% reported by Ejeta *et al.* (2004) from minced beef. They were similar, however, to findings of Ejeta *et al.* (2004).

***S*. Muenchen:** The 22.2% proportion of *S*. Muenchen at the abattoir line was higher than the 13.0% at the processing plant line, the 8.6% reported by Molla *et al.* (2004) in camels, and the two isolates in pigs by Aragaw *et al.* (2007) in Ethiopia. On the other hand, the proportion of isolates from the processing plant line was similar to those reported ts by Molla *et al.* (2004) and Aragaw *et al.* (2007).

***S*. London:** To the best of our knowledge, *S*. London has not yet been reported from Ethiopia. Thus, the present observation of the isolate with prevalence/proportion of 0.24%/1.6% at the abattoir line and the 1.4%/26.1% at the processing plant line constitutes that *S*. London is also present in Ethiopia.

***S*. Kastrup:** In this study, this isolate exhibited a prevalence/proportion of 1.3%/4.8% in samples collected from animal feces, swabs from personnel hands and MLN, all at the abattoir line. Available research does do not show any report of *S*. Kastrup from Ethiopia yet. Menghistu *et al.* (2011) reported an overall prevalence of 2.7% *Salmonella* ,including *S*. Kastrup, from poultry in India.

***S*. Larochelle:** The 11 (4.6%) *S*. Larochelle seen in the abattoir line was similar to the single isolate identified from a hospital case by Beyene (2008). The presence of this serotype in a food chain, including the environment (6), animal feces (2) and raw beef (3) at the abattoir line points to the risk of the public of becoming infected with this serotype.

***S*. Dublin:** The 6.4% proportion of *S*. Dublin in this study was similar to the 2.4% reported by Ejeta *et al.* (2004) but lower than the 48% reported by Alemayehu *et al.* (2003) from Ethiopia.

***S.* Typhimurium:** In this study, the proportion of *S.* Typhimurium was 4.3%, which is higher than the 0.9% detected by Molla *et al.* (2004) but lower than the 20% reported by Alemayehu *et al.* (2003). Beyene *et al.* (2011) also identified 0.7% of 1225 samples of hospital cases to be infected with this serotype in Ethiopia.

***S.* Anatum:** The 8.7% proportion of *S.* Anatum in this study was similar to the 9.1% reported by Ejeta *et al.* (2004), but slightly higher than the 2.6% reported by Molla *et al.* (2004) from Ethiopia.

***S.* Concord:** The 8.7% proportion of *S.* Concord in this study underlines its occurrence in both the beef production and the processing line. Its 0.46% prevalence though was lower than the overall prevalence of 4.2% (5.2% in Addis Ababa and 2.3% in Jimma Hospitals) reported by Beyene *et al.* (2011). Their study showed that *S.* Concord was a major pathogen in diarrheic children in Ethiopia. Vanhoof *et al.* (2012) reported 83.4% (30 out of 36) *S.* Concord isolates in children adopted from Ethiopia by people of different developed countries.. In addition to reports by Beyene *et al.* (2011) and Vanhoof *et al.* (2012) for human cases, the two isolates of *S.* Concord in this study were isolated from working tables which are frequently visited by different personnel. It is well possible that the serotype is associated with humans and their activities contaminating their working environment.

***S.* Eastbourne:** The 0.7% *S.* Eastbourne of cutting plates and rooms was similar to the 3/277 in carcass swabs but lower than the 15/278 in caecal contents and the 21/278 in mesenteric lymph nodes reported by Aragaw *et al.* (2007) from a pig abattoir in Ethiopia. The spread of this serotype between slaughter places and meats of different animal species may be quite easily facillitated.

Unidentified strains: The eight unidentified *Salmonella* isolates (three from the abattoir and five from the processing plant line) are consistent with the six non-typed ones reported by Aragaw *et al.* (2007) in animals and Beyene *et al.* (2011) in humans in Ethiopia. Investigations so far are rudimentary, the spectrum of *Salmonella* by far is not investigated yet (Alemayehu *et al.,* 2003; Ejeta *et al.*, 2004; Beyene *et al.*, 2011).

General: The prevalence of serotypes in the studied beef lines only partly can be compared with previous findings from similar or related food animals, meats and environments as well as from medical centers in Ethiopia because of the often sketchy nature of such investigations (Table 6 - 02).

Table 6 - 02: Comparison of *Salmonella* serovars obtained from abattoir and processing plant lines in the present study with those from previous studies in Ethiopia

Present findings of *Salmonella* prevalence and serotypes in studied beef lines ¶				Previous reports in Ethiopia with numbers and/or % reported in the bracket			
Serovars	Source line¶	No.	%	Source	Sample type (No. or %)	References	Remarks
S. Typhimurium	PPL line	1	0.23	Pig	Caecal content (2), Mesenteric lymph node (13), Carcass swab (0)	Aragaw *et al.* (2007)	
				Children	Addis Ababa (0.8%) and Jimma (0.3%) with overall 0.7% in hospitals	Beyene *et al.* (2011)	
S. Anatum	PPL line	2	0.46	Pig	Caecal content (2), Mesenteric lymph node (0), Carcass swab (1)	Aragaw *et al.* (2007)	
				SMKT	Minced beef (3 (13%), Mutton (1 (8.3%)	Ejeta *et al.* (2004)	
				Cattle	Cattle hides (23), hand swabs (6), rumen content (12), caecal content (2), Mesenteric lymph nodes (2) and holding pens (9)	Sibhat *et al.* (2011)	
S. Saintpaul	AAB line	27	11.4	Pig	Caecal contents (10), Mesenteric lymph nodes (27), Carcass swabs (0)	Aragaw *et al.* (2007)	
				SMKT	Minced beef (1 (4.3%))	Ejeta *et al.* (2004)	
	PPL line	1	0.23	Camel	Feces (6), abdominal muscle (10), diaphragmatic muscle (10), liver (9), Mesenteric lymph nodes (6) and spleens (4)	Molla *et al.* (2004)	
S. London	AAB line	1	0.24		Raw beef at butchers (1)	This study	First report in Ethiopia
	PPL line	6	1.4		Working tables (1), and raw beef meat (4)		
S. Larochelle	AAB line	11	4.6	Hospitals	From clinically presented children at two hospitals, Ethiopia 1(0.9%)	Beyene, 2008	
S. Concord	PPL line	2	0.46	Hospitals	Children at Addis Ababa (5.4%) and Jimma (2.3%) with overall 4.2%	Beyene *et al.* (2011)	
S. Dublin	AAB line	4	1.7	SMKT	Minced beef (1 (4.3%))	Ejeta *et al.* (2004)	
				Abattoir	Cattle, personnel and minced beef 54.1%	Nyeleti *et al.* (2000)	
S. Kastrup	AAB line	3	1.3		Personell hands (1), animal feces (1) and mesenteric lymph nodes	This study	First report in Ethiopia
S. Eastbourne	PPL line	3	0.7	Pig	Caecal contents (15), Mesenteric lymph nodes (21), Carcass swabs (3)	Aragaw *et al.* (2007)	
				Cattle	Mesenteric lymph nodes (5) and carcass swabs (1)	Sibhat *et al.* (2011)	
S. Muenchen	AAB line	14	5.9	Pig	Caecal contents (1), Mesenteric lymph nodes (1), Carcass swabs (0)	Aragaw *et al.* (2007)	
	PPL line	3	0.7	Camel	Feces (4), abdominal muscle (3), diaphragmatic muscle (1) and Mesenteric lymph nodes (1)	Molla *et al.* (2004)	
Unidentified	AAB line	3	1.3	Pig	Non-typed report (6)	Aragaw *et al.* (2007)	
	PPL line	5	1.2				
Total.		86	12.9				

PPL = Processing plant
AAB = Abattoir
¶number of sample (n = 431 at PPL, n = 237 at AAB)
SMKT = Supermarkets

6.2.2.3 Transmission routes of Salmonella

The need for PFGE dendrogram-based *Salmonella* analysis was emphasized by Sibhat *et al.* (2011), being one deficit in their study conducted in Ethiopia. In this study, PFGE was used to examine *Salmonella* strains obtained from the studied beef lines. Results are related to the epidemiological and genotypic diversity of the isolates. Possible sources, contamination and transmission routes are discussed here.

6.2.2.3.1 Molecular diversity of Salmonella obtained from both lines

PFGE profiles of 51.7% to 100% similarities within the same *Salmonella* serotypes were observed in this study. Similarly, Kagambega *et al.* (2013), in their study undertaken in Burkina Faso, found an approximate 70% to 100% genetic relatedness in *Salmonella* of the same serotype.

***S.* Saintpaul**: The present study showed a genotypic diversity ranging from 51.7% to 100% similarity in the 28 isolates (27 from the abattoir and 1 from the processing plant line) of *S.* Saintpaul grouped into 6 different pulsotypes[5] consisting of 1, 19, 2, 3, 2 and 1 isolates. The genetic diversity of this serotype is concomitant with reports of Kerouanton *et al.* (2007), Wasyl *et al.* (2012) and Fey *et al.* (2012). On the other hand, regardless of sample source and geographic distribution, a high degree of genetic diversity of *S.* Saintpaul has been reported by Kerouanton *et al.* (2007) who identified 20 pulsotypes from 30 isolates; 82 *XbaI* PFGE profiles were identified from 159 isolates (Wasyl *et al.,* 2012). Fey *et al.* (2012) also showed genotypic and global genetic diversity of *S.* Saintpaul within a same geographic location and area.

S. Muenchen: The investigation of 17 *S.* Muenchen (14 from the abattoir and 3 from the processing plant line) in which *XbaI* restriction enzyme was used showed a 100% genotypic similarity with SMu*X*1, implying a strong genotypic relatedness of this serovar. Using PFGE *XbaI* restriction enzyme, Thong *et al.* (2007) showed a better resolution of 16 *S.* Muenchen isolates into 12 pulsotype than in using *AvrII* and *SpeI* restriction enzymes which respectively distinguished those 16 *S.* Muenchen into 11 and 9 distinct pulsotypes. Furthermore, Thong *et al.* (2007) found 13 different sub-types of *S.* Muenchen strains using a combination of the three aforementioned enzymes, implying a further need for the analysis of this serovar.

[5] Unless otherwise, terms for: pulsotype(s) = PFGE pattern(s) = cluster(s) = clone(s) = PFGE types are interchangeably used in this text

***S.* Kastrup** and ***S.* Larochelle:** To some extent, *S.* Kastrup (6,7:e,n,z_{15}:1,6) and *S.* Larochelle (6,7:e,h:1,2) (Grimont and Weill, 2007) are antigenically differentiated by serotyping, but using PFGE *XbaI*, the present three isolates of *S.* Kastrup and the eleven isolates of *S.* Larochelle in this study showed genetically indistinguishable profiles. Using *XbaI* PFGE, it was possible to differentiate only one isolate of *S.* Larochelle (SKL*X*2) from the rest (Table 5 - 9).

***S.* Dublin**: Findings from the present PFGE *XbaI* profiles of S. Dublin show indistinguishable serovars. However, Liebana *et al.* (2002) differentiated 100 *S.* Dublin isolates (50 from humans and 50 from animals) into 21 PFGE types using a combination of plasmid profiling, *XbaI*-PFGE and *PstI-SphI* ribotyping, further distinguishing them into 43 clones or strains. Zou *et al.* (2010) also differentiated 25 *S.* Dublin isolates from food-producing animals, production facilities and clinical diagnostic samples into 5 clusters. Using *XbaI* PFGE, Kerouanton *et al.* (2007) differentiated 27 isolates into 10 pulsotypes. However, the indistinguishable properties of *S.* Dublin in the present finding could be due to the same animal-related material sources used in this study. Geographical differences of serovars could also explain some of the difference of the present result from other findings.

***S.* London:** Pulsotypes from this study (one isolate from the abattoir and five from the processing plant lines) showed 100% similarity. However, Kerouanton *et al.* (2007) observed 13 PFGE patterns from 21 *S.* London obtained from animal health and production sources, feed products and ecosystems. Differences thus are quite likely due to differences in the source, sample type and geographical locations of studies. The identical result here indicates the same origin in the lines, which should be rechecked.

Although the number of ***S.* Anatum** isolated and tested was small, results showed 100% similarity in pulsotypes. Kerouanton *et al.* (2007), however, distinguished 31 isolates of *S.* Anatum into 15 PFGE patterns.

Using *XbaI,* no distinguishable property was found between the present two isolates of ***S.* Concord.** This is similar to findings by Vanhoof *et al.* (2012) who also reported genetic similarity of *S.* Concord isolated from children adopted from Ethiopia.
Although the strains were isolated from different locations during different sampling occasions, the three ***S.* Eastbourne** also show 100% similarity.

Genetic similarities among the six unidentified *Salmonella* isolates (three Un*X*1 from each of the abattoir (Table 5 - 10) and the processing plant line (Table 5 - 23) in the results section

above) showed a possible relatedness of these strains in the investigated beef lines. On the other hand, diversity of the other two isolates (one Un*X*2 and one Un*X*3 from the processing plant) with different pulsotypes suggest diverse sources. Although genotypic examinations were not done, Tibaijuka *et al.* (2003) and Aragaw *et al.* (2007) previously reported rough forms and untypable *Salmonella,* respectively, from Ethiopia, too.

6.2.2.3.2 Tracking possible sources and transmission routes

Using PFGE molecular biological profiles of *Salmonella* serotypes, the possible sources of contamination and transmission routes of individual isolated *Salmonella* serotypes along the abattoir and the processing plant lines, to the level of consumer supply, were assessed. Using the same techniques, Kagambega *et al.* (2013) from Burkina Faso also tried to investigate transmission routes of some of the same *Salmonella* serotypes from the production process and wild animals to humans.

I. Abattoir line

***S*. Saintpaul**: As described in Table 5 - 7, the 100% genotypic similarity among pulsotypes SSa*X*2 of S. Saintpaul in isolated sampling locations, at the same or different sampling occasions and across locations in the abattoir line shows direct and/or indirect transmission from one stage to another, up to the level of consumer supply at the butchers. On the other hand, results from *S.* Saintpaul serotypes with different pulsotypes indicate possible transmission from different sources (knifes and room for SSa*X*4; room for SSa*X*5 and water for SSa*X*3). Contamination was also possible at various stages (SSa*X*4 in raw beef at the abattoir, SSa*X*1, SSa*X*3 and SSa*X*3 in meat at butchers) along the production and handling steps and stages (Table 5 - 7). Similarly, Fey *et al.* (2012), using PFGE, indicated the transmission of closely related S. Saintpaul isolates from a single source and outbreak during a particular period of time (1 February – 15 March 2009). However, they also differentiated non-outbreak S. Saintpaul strains from the outbreak ones. Further, in the analysis of isolates from different geographic regions, *Wasyl et al.* (2012) used PFGE showing similar profiles of S: Saintpaul which had been obtained from several food animals. They also found indistinguishable profiles of S: Saintpaul - to use their term – isolated from different sources of food animals, foods and different animal meat production types like duck breeders, laying hens or fattening turkeys.

***S.* Muenchen:** 100% similarity of 14 *S.* Muenchen (SMu*X*1) in the abattoir (Table 5 - 8), almost all from environmental samples, shows extensive cross-contamination of the abattoir. In contrast, Thong *et al.* (2007) indicated distinguishable properties of *S.* Muenchen from different sources. The present finding also shows that the abbatoir environment could act as a possible source for carcass contamination anytime during slaughtering and processing procedures.

***S.* Kastrup**: Observation of genetically similar *S.* Kastrup (SKL*X*1) from personnel's hands, MLN and feces of animals (Table 5 - 9) shows, that these locations are possible sources of carcasses contamination.

***S.* Larochelle**: The 100% similarity of *S.* Larochelle SKL*X*1 isolated from the 17th and 18th sampling batches/occasions at the abattoir line shows the potential of cross-contamination and cross-transmission to the level of meat supply (butchers). On the other hand, pulsotype SKL*X*2, observed at the butchers, points to a contamination of carcasses during handling and to the supply from other sources than Addis Ababa abbatoir (Table 5 - 9).

***S.* Dublin**: Regardless of the small number (four) of *S.* Dublin strains identified and the use of only one restriction enzyme (*XbaI*) in this study, the indistinguishable properties (SDu*X*1) obtained from a room at the abattoir show contamination of the abattoir from animals slaughtered the days before sampling, Detection of the SDu*X*1 pulsotype from the feces of animals, on carcasses at the abattoir and at the butchers shows a clear fecal contamination of carcasses with genetically similar *S.* Dublin and transmission to the butchers. *S.* Dublin is a group D serotype, regarded as a strongly host-adapted type for cattle; it occasionally infects other animal species including man. It can be transmitted to man via meat and dairy products (Liebana *et al.*, 2002). Additionally, observing an unidentified strain (Un*X*1) and S. Saintpaul (SSa*X*2) from feces of animals coded 13 and 23, respectively, and from their corresponding carcasses at butchers, shows fecal contamination and transmission to carcasses, then to the beef supply station on.

II. Processing plant line

Present findings show 100% genotypic similarity of ***S.* Concord** in samples from working tables, showing the contamination with this serotype from the same meat. Vanhoof *et al.* (2012), using PFGE, also traced back the transmission routes of four isolates of *S.* Concord

(different pulsotypes) in children adopted from Ethiopia to the US. Isolates identified, according to the authors, have a strong relationship with Ethiopian isolates.

A ***S.* London** with strain similar pulsotype (SLo*X*1 Figure 5-6) obtained on the 1st sampling occasion from meat and on the 2nd occasion from a table at the processing plant line indicates the possibility of contamination of tables from contaminated beef. The remaining four isolates obtained from raw beef also showed 100% similarity, implying contamination of the beef with *S.* London. Unlike the present finding, Kerouanton *et al.* (2007) distinguished *S.* London isolates from different samples into diverse pulsotypes.

Similarly, ***S.* Anatum** and ***S.* Muenchen,** with respective pulsotypes SAn*X*1 and SMu*X*1, obtained from raw beef on the 1st and 6th sampling occasions, respectively, showed similar sources. One isolate each of ***S.* Typhimurium, *S.* Saintpaul** and ***S.* Muenchen** obtained from room, raw beef and the final product at supermarkets on different sampling occasions showed contamination of each of the locations with respective serovars. Ejeta *et al.* (2004) also reported *S.* Muenchen in meat from Addis Ababa supermarkets.

6.2.2.4 Antimicrobial susceptibility/resistance of Salmonella

6.2.2.4.1 Overall susceptibility/resistance

For comparison, preferably data from the same country should be used. In fact, there are several studies from Ethiopia available (Tables 2 – 7 and 2 – 8). Susceptibility to Polymyxin B was 98.8% with only one (1.6%) strain from the abbatoir line showing resistance. Cardoso *et al.* (2007) reported 100% susceptibility of *Salmonella* isolates. Susceptibility to gentamycin was comparable with the 92.8% reported by Reda *et al.* (2011); and the 1.2% resistance was similar to the 8.6% reported by Yan *et al.* (2010) and the 3.6% by Reda *et al.* (2011). The degrees of resistances above were lower than the 75.6% reported by Asrat (2008). Regardless of differences in drug concentration, the 96.5% susceptibility to chloramphenicol in this study was higher than the 28.6% reported by Reda *et al.* (2011), while the resistance was lower than the 30% reported by Molla *et al.* (2003), the 83.7% by Asrat (2008), the 62.3% by Reda *et al.* (2011), and the 12.3% by Yan *et al.* (2010). This could be attributed to differences in geography and concentration of the drug used. In addition, for 50µg were used for testing, while other authors used a 30µg concentration. The present 1.2% resistance to each of sulfamethoxazol-trimethoprim and trimethoprim was lower than the 21.2% resistance to

trimethoprim in chicken carcasses and giblets (Molla *et al.*, 2003), the 48.1% in retailed food (Yan *et al.,* 2010) and the 75.7% in human cases (Asrat (2008). Although the substances belong to different chemical classes, susceptibility to neomycin (45.4%) was similar to that of oxytetracycline (44.2%) in this study. A high (39.5%) intermediate reaction was observed to neomycin. The 15.1% resistant isolates to neomycin in this study was higher than the 3.75% reports by Cardoso *et al.* (2007). Tetracycline and oxytetracycline belong to the same chemical class (WHO, 2011). The 44.2% susceptibility to oxytetracycline detected in the present study was higher than the 14.2% susceptibility to tetracycline reported by Reda *et al.* (2011), while resistance was frequent (53.5%) in this study and similar to the 41.2% resistance to tetracycline reported by of Molla *et al.* (2003). However, it is lower than the 94.5% report in *Salmonella* from stool samples by Asrat (2008), the 71.4% in a report by Reda *et al.* (2011) in isolates from stool samples, but higher than the 31.8% by Aragaw *et al.* (2007) in pigs from Ethiopia.

6.2.2.4.2 Susceptibility/resistance of Salmonella strains from both lines

Polymyxin B: The susceptibility to Polymyxin B of almost all strains isolated from the abattoir and the processing plant lines shows the effectiveness of this substance for the treatment of infection cases. Low level/ absence of resistance to this substance could be due to uncommon use in veterinary medicine in Ethiopia.

Chloramphenicol, Gentamycin, Trimetoprim and Trimethoprim sulfametozazole: Susceptibility of *Salmonella* strains isolated from both beef lines to the above drugs ranged from 93.2% to 100% while resistance ranged from 0.5% to 4.8%, indicating their therapeutic efficiency for *Salmonella* infection. The high susceptibility to these drugs could again be due to their limited use in veterinary medicine in Ethiopia.

Neomycin: Susceptibility of isolates from the abattoir line (50.1%) was similar to those (30.4%) from the processing plant line. The corresponding resistance of strains to this drug was similar (15.9% for the abattoir line and 13.1% for the processing plant line). Again, high and similar intermediate reactions to this drug were observed in strains from the abattoir (33.3%) and processing plant line (56.5%), respectively.

Oxytetracycline: Susceptibility of strains to this substance from the abattoir line was lower (31.7%) than that of strains from the processing plant line (78.3%), the reverse obviously is true in regards to resistance. Isolates from the abattoir line showed resistance of 65.1% while those from the processing plant line exhibited a resistance of 21.7%. In all likelihood animals

being shipped to the abattoir carry considerable resistance against oxytetracycline and are sources of oxytetracycline-resistant *Salmonella* strains.

6.2.2.4.3 Resistant Salmonella strains

The total of 55 (63.9%) strains resistant to at least one or more of the drugs tested in this Addis Ababa study is higher than the 20.7% detected by Sibhat *et al.* (2011) in their investigation of beef cattle and around a slaughterhouse in Debre Zeit, similar to 58% resistance to (only) *S.* aureus from the meat production and sale scenario in the smaller Jimma town (Tassew *et al.* 2000) and, for comparison, the 63.7% resistance of *Salmonella* reported by Molla *et al.* (2003) for another animal food line (chicken carcasses and giblets from a processing plant in Debre Zeit and from Addis supermarkets) . It is, Wandili *et al.* (2013) reported a 100% reasisrtance in Kenya, a country neighbouring Ethiopia and frequently exchanging livestock. Regardless of the number of strains isolated, a higher number resistant strains was observed in the abattoir line (85.5%) than in the processing plant line (14.5%). This seems to indicate that animals destined for slaughter at the study abattoir carry a heavy load of resistant *Salmonella* serovars. Considering the use of antibiotic substances in Ethiopia, the resistance of strains is the result of treatments and treatment attempts of cattle (DACA, 2009). Anibiotics are freely sold and are frequently misused, particularly under-dosed (Essack, 2006).

Slaughter of animals harboring resistant strains results in contamination of the abattoir, of meats and meat products. The present investigation does throw a spot-light on such a situation: similar proportions of *Salmonella* isolates and corresponding resistant isolates are present in the environment, in cattle-related materials and in respective end products in both lines. Due to insufficient sampling, elaborations on differences of loads at different locations do not stand statistical analysis. Important is that contamination lines run throughout the slaughtering and processing processes.

Single-drug resistant *Salmonella* are widely present in this scenario, particularly *S.* Saintpaul (41.8%), *S.* Muenchen (30.9%) and *S.* Larochelle (10.9%). Resistance to two drug substances was also observed Except for *S.* Kastrup and *S.* London, the proportion of resistant serovars against oxytetracycline is a particular concern.

6.2.3 *E. coli*

6.2.3.1 E. coli prevalence

6.2.3.1.1 Overall E. coli situation

In this study, the overall prevalence of *E. coli* along the abattoir line was 45.1% with averages of 40.6% at the environment, 52.0% in animal-related materials, 38.2% at butchers and 33.3% - 55.9% prevalences at individual locations. The total prevalence rates of 46.4% at the processing plant line, with 50.5% prevalence in the environment and 56.8% in animal related samples (raw beef), were similar to each other. At supermarkets, 29.4% positive samples could be identified . Prevalence of *E. coli* between the sampled locations of the processing plant line were the same , except for, maybe lower 23.5% from water and 13.3% from spices which and higher 57.0% from rooms and 66.7% from refrigerators. The entire line and its end products are extensively contaminated.

The presence of *E. coli* in all examined locations in this study agree with reports from other investigations in Ethiopia like the 26.6% prevalence in the beef scenario of the provincial town Jimma (Tassew *et al.,* 2010) or the 10.2% E. coli 0157:H7 positivity of raw meat in Addis Ababa. Gurmu and Gebretinsae (2013) found a 32% E. coli prevalence at butchers in Ethiopia. Compared to animals and animal foods the 14.2% from human cases in Ethiopia was lower (Kuibret and Abera, 2011). For orientation: The European Union (EFSA, 2013) reported that mandatory hygienic systems with microbiological testing of carcasses in 2011 achieved an average EU VTEC contamination level of 1.4% in fresh beef, with individual member countries reporting 0%. Still, close to 40% of meat retailed at individual small butchers and nearly 30% of sausages sold at supermarkets did not satisfy the hygienic standards set by the EU (EFSA, 2013) for *E. coli.*

6.2.3.1.2 E. coli situation at different locations

All sampling locations at the abattoir and processing plant lines were found positive for *E. coli* at at least on one sampling occasion. ***Personnel hands:*** Prevalences of were 46.2% and 52.6% on personnel hands at the abattoir and at the processing plant lines, respectively. urmu and GeAttala and Kassem (2011) demonstrate the virtues good manufacturing practices (GMPs) for a scenario in Egypt, the contamination rates of personnel hands in butcheries could be reduced

from an already low 3.3% to 0%. ***Aprons:*** Prevalences of 42.9% and 62.5% on staff aprons at the abattoir and at the were equally (high). ***Knives:*** Prevalences of 38.5% and 53.5% on knives in the abattoir and in the processing plant were similar and agree with the 25% prevalence on knives of butchers reported by Gurmu and Gebretinsae (2013). ***Working tables:*** 50% occurrence of *E. coli* on working tables at the processing plant also agrees with the 50% on working tables of butcheries found by Gurmu and Gebretinsae (2013). ***Water:*** Tap water was found to be highly contaminated throughout, 33.3% at the abattoir and 23.5% at the processing plant line. Contamination levels of surface water even are higher. Bahiru *et al.* (2013) determined 84.3% *E. coli* contamination. Mersha *et al.* (2009) specified the *E. coli* O157:H7 prevalence in tap water of sheep and goat abattoirs in Ethiopia; it was on average 4.2%.

Rooms: Regardless of sample size, the prevalence of *E. coli* observed in rooms at the abattoir (41.2%) and the processing plant (75%) was similarily high. Hooks used for hanging carcasses at the abbatoir were equally highly contaminated (36.2%).

Refrigerators: Findings of contaminations of the chilling rooms to the level of 50.0% and 66.7% at the abattoir and at the processing plant, respectively, were similar to each other. ***Feces:*** In this study, a fecal *E. coli* prevalence of 52.9% was detected in slaughtered cattle at the abattoir.

Mesenteric lymph nodes: The prevalence of 47.1% detected in mesenteric lymph nodes of animals in this investigation indicates the sub-clinical disease level of the slaughter cattle.

Raw meat product: Contamination rates of raw beef with *E. coli* at the abattoir (55.9%), at the butchers (38.2%) and at the receiving point of the processing plant (56.8%) in this investigation were similarly high The extensive contamination of the slaughter chain is clarly demonstrated, occasional washing with cold water is unable to decrease the *E. coli* in the beef. Water itself may have contributed to contamination Similar to this study, Mersha *et al.* (2009) also in Ethiopia, found no difference in *E. coli* O157:H7 in sheep and goat carcasses before (8.1%) and after (8.7%) washing.

Processed meat products (mortadella)*:* The similar occurrence of *E. coli* (20.0% to 53.3%) in mortadella samples from 8 supermarkets ($p>0.05$ among them all) shows that contamination of products continues at any point during handling at retail and final sale to customers.

It is well established that contamination levels of *E. coli* are location specific, highly dependent on a range of factors including geographical factors, farming and/or meat production

practices, the technology in use and the level of hygiene at the abattoir. Even in the best abattoirs, complete absence of *E. coli* throughout meat production is unachievable under commercial conditions (Ramos *et al.*, 2013; Nørrung and Buncic, 2008). However, levels of contamination of slaughter cattle, absence of elementary hygiene and low levels of slaughter and processing technology, among other factors, lead to high *E. coli* level in meat and meat products as determined not only in the present study but also in other studies conducted under African conditions. Studies from Ethiopia (Gurmu and Gebretinsae, 2013; Bahiru *et al.*, 2013; Kibret and Abera, 2011) and from Sudan (Abdalla *et al.*, 2009) in each case confirm the high contamination level of feces of communal cattle. Meat from these cattle essentially is carried through all slaughter without any measure to at least reduce contamination. Hygiene management systems like HACCP plans to keep *E. coli* levels at least at acceptable levels are in place but are not used. Also, sanitary procedures such as steam pasteurization, hot water washes, organic acid washes, or combinations of these treatments (Elder *et al.*, 2000) are more than well known.

6.2.3.2 Antimicrobial susceptibility/resistance of E. coli isolates

6.2.3.2.1 Overall susceptibility/resistance to individual drugs

Reports about antimicrobial resistance of *E. coli* in animals and animal products in Ethiopia are scarce. The World Health Organization in 2011 published its 3rd revision report on critically and highly important antimicrobial agents for human medicine (WHO, 2011). For *E coli*, 15 antibiotic groups are listed as critically important and 13 groups as highly important. Three of the six antibiotics tested in this study belong to the critically important and three to the highly important groups. At least one or more resistant strains to one or more of these drugs were observed in this study.

The overall 99.4% susceptibility to polymyxin B showed its efficacy still for treatment of infections. The still high susceptibility could be due to the fact that the drug is not used in veterinary medicine. . Susceptibility to chloramphenicol and to gentamycin was similar to reported 92.3% (Lagacé-Wiens *et al.*, 2008) and 93.2% (Hiko *et al.,* 2008), respectively. This is higher than the 79.6% susceptibility to gentamycin of *E. coli* isolated from human samples reported by Kibret and Abera (2011) from Ethiopia. This could be due to infrequent use of these drugs in food animals in Ethiopia. Susceptibility of the strains to amoxicillin was similar to the 14.0% finding reported by Kibret and Abera (2011). The strains were also 100%

susceptible to trimethoprim-sulfamethoxazol, similar to an earlier report by Hiko *et al.* (2008). The 7.3% resistance to trimethoprim-sulfamethoxazol recorded in this study was lower than the 37% (Meyer *et al.*, 2008) and the 29% to 43% (Jouini *et al.,* 2009) resistance rates of *E. coli* elsewhere. The 21.2% resistance to amoxicillin in the present investigation is lower than 96% (Bahiru *et al.*, 2013) and 86.0% (Kibret and Abera, 2011) reported from Ethiopia. Resistance to tetracycline (39.7%), frequently observed in both study lines, was similar to the 41.35% reported earlier for Ethiopia by Apun *et al.* (2008), but lower than the 50% by Bahiru *et al.* (2013), the 77.4% by Hiko *et al.* (2008), the 72.6% by Kibret and Abera (2011) and the 57% by Meyer *et al.* (2008). The frequent resistance to oxytetracycline revealed in this study could be attributed to its wide use in the veterinary sector. Frequent exposure results in increased resistance development in the strains (Hirsh and Zee, 1999; Plumb, 2008; Sayah *et al.*, 2005).

6.2.3.2.2 Susceptibility/resistance of E. coli strains by source line

Polymyxin B: The susceptibility of almost all strains isolated from the abattoir and the processing plant lines was similar to the susceptibility of isolates from clinical cases (98.5%), food items (97.5%) (Aly *et al*., 2012) and retailed meat (100%) (Hiko *et al*., 2008), Low/absence of resistance to this drug could be due to its uncommon use in veterinary medicine in Ethiopia.

Chloramphenicol and gentamycin: Susceptibility of *E. coli* isolated from both beef lines ranged from 95.0% to 98.0% and was similar with the 75.0% to 100% susceptibility to gentamycin (Gizachew *et al.,* 2013; Kibret and Abera, 2011; Chigor *et al.*, 2010; Hiko *et al.*, 2008), and the 93.2% to 100% susceptibility to chloramphenicol (Chigor *et al.*, 2010; Hiko *et al.*, 2008). Both drugs apparently still have a high therapeutic effectiveness for the treatment of infections.

Trimethoprim-sulfamethoxazol: 88.8 % and 94.0% of *E. coli* isolated from the abattoir and the processing plant lines, respectively, were susceptible at similar level. This was comparable with the 87.5% from clinical cases, 83.3% from water (Chigor *et al.*, 2010) and 100% from retailed beef (Hiko *et al.*, 2008) but higher than the15.5% from human cases (Gizachew *et al*., 2013) and the 34.8% (Kibret and Abera, 2011) and 20.0% (Mohammad *et al.*, 2010) susceptibility reported.

Amoxicillin: Similar susceptibility rates of isolates from the abattoir line (70.1%) and the processing plant line (79.5%) were observed. These rates are higher than the 0-15%

susceptibility rates reported by several authors for Ethiopia (Gizachew *et al.,* 2013; Ayl *et al.*, 2012; Kibret and Abera, 2011; Chigor *et al.*, 2010). The corresponding resistance to this drug was 26.2% for strains from the abattoir line and 18.5% for those from the processing plant line. With susceptibility rates huigher (above), corresponding resistance rates in above studies were lower than the 83.7% to 95% resistances (Ayl *et al.*, 2012; Kibret and Abera, 201; Chigor *et al.*, 2010).

Oxytetracycline: Susceptibility to this drug of *E. coli* from the abattoir line (49.5%) and from the processing plant line (60.0%) was similar with each other and with the susceptibility to tetracycline of 63% from clinical cases and 62.5% from food (Aly *et al.*, 2012) and the 77.4% from retailed meat (Hiko *et al.*, 2008). Again, the corresponding resistance of the strain from the respective lines was 43.9% and 37.5%. not too different . The result basically agrees with reported 37% (Aly *et al.*, 2012) and 38.5% resistance (Olaniran *et al.*, 2009) but was lower than the 63.0% (Ramos *et al.,* 2013), 72.2% (Kibret and Abera, 2011) and 66.8% (Chigor *et al.*, 2010) having been established for other Ethiopian scenarios.

Variable but frequent resistance to oxytetracycline, amoxicillin and trimethoprim-sulfamethoxazol was registered in strains obtained from different samples and occasions at both beef lines. Primary entry of bacteria is from bacterial loads of the cattle used for slaughter, secondary entries from slaughter and handling environments and cross-contaminations from handlings at the different slaughter and meat cutting stations. Sayah *et al.* (2005) rightly point to the risk of contamination from water, with resistant strains from the environment.Animal products such as meats generally have to be regarded as risk-commodities in respect to pathogen contents, being an unavoidable consequence of failures/deficits of meat processing (Jones *et al.,* 2008).

6.2.3.2.3 Resistant E. coli strains

The 46.6% resistance to at least one or more of the drugs tested in this study was lower than the 90% in the report by Aly *et al.* (2012). With regard to *E. coli* isolates resistant to critically important antimicrobial agents for human medicine (WHO, 2011), resistance rates of 1.4% to polymyxins B, 2.1% to gentamycin and 45.5% to amoxicillin were determined. For the highly important drug groups, the rates were 8.4% for chloramphenicol, 16.1% for trimethoprim-sulfamethoxazol and 85.3% for oxytetracycline. The occurrence of *E. coli* resistant to drugs of

public health importance in meat production environments, raw and final products is clearly demonstrated.

Resistant *E. coli* detected in the environment (14%) and in animal-related materials (21%) obtained from abattoir were more frequent than in samples collected from butchers (3.5%). Fig. 5 - 18 identifies in particular the abattoir as source of resistant strains, from where strains are further distributed to product receiving stations like butchers and processing plants. The similarity in resistant *E. coli* in the processing plant environment (25.9%), animal-related materials (22.4%) and in products at supermarkets (13.2%) demonstrate this line. Hirsh and Zee (1999) suggest the abattoir to act as main source of resistant strains, receiving meat from different animals (Woteki and Kineman, 2003), whereby a single piece of meat may act as source of contamination at a processing plant already (Woteki and Kineman, 2003; USDA, 2011). Processed meat products are usually treated at temperatures of 80^{o}C for some minutes (part of processing plant procedure), which is lethal for most bacteria, with the exception of spores (FAO, 2010). As resistant *E. coli* strains were identified in the mortadella product, the used time and temperature at the processing plant obviously was not efficient to kill off *E. coli*. Recontamination with resistant strains after heating may also have occurred.

Of 22 MDR, one isolate was resistant to five drugs. The remaining ones were resistant to three drugs of which three were resistant to amoxicillin, chloramphenicol and oxytetracycline, and 18 to amoxicillin, oxytetracycline and trimethoprim-sulfamethoxazol. By far, the majority (55.9%) were resistant to single antibiotics, 15.4% were MDR cases. This is lower than the 35% reported by Aly *et al*. (2012). Using targeted antibiotics for animal therapy is not widely practiced in Ethiopia. Of concern may be the range of structurally unrelated antibiotic classes involved in resistance to more than one antibiotic. More than 85% of resistance in this study involved tetracycline in combination with another antibiotic, be it either penicillin, the amino glycoside, the amphenicole or the folate pathway inhibitor antibiotic classes.

For serious human diseases, for which the use of a particular class of antibiotics is the sole or one of limited available therapy, first resistances, although at lower levels, were determined for antibiotics of the critically important groups used for therapy. Of more concern eventually may be antimicrobial resistant bacteria that are transmitted to man from non-human sources including meat, meat products and the environment. This situation may arise when in Ethiopia annual per capita consumption of meat increases. With annual just 2.4 kg beef per person in

rural areas and 6.8 kg in urban areas, meat consumption is extremely low in Ethiopia, even by African standards (Tafere and Worku, 2012). Development of meat consumption should be monitored and principal awareness raised for bacteria resistance in animal products in Ethiopia. Risk-management strategies related to non-human antimicrobial use seem unachievable under present conditions.

Resistance of *E. coli* isolates to individual antibiotics was highest for oxytetracycline (85.3%) which could be due to the fact that the drug is marketed as "broad-spectrum antibiotic", popular and widely used in the veterinary sector in the world in general (Ateba and Bezuidenhout, 2008) and in Ethiopia in particular also by animal owners (DACA, 2009). Antibiotics are freely available, often of doubtful origin and widely used by the unskilled animal owners themselves. Most of the cattle slaughtered at the AAAE are older age cull animals, which have reached the end of their productive life. It can be argued that they were repeatedly, but unprofessionally, treated over their lifespan with widely available and low price antibiotics which results in MDR. Gebrekidan *et al.* (2009) showed that female cattle slaughtered at AAAE were of older age and slaughtered due to various chronic health problems.

6.2.4 Conclusions

6.2.4.1 Data obtained in this study

Materials and methods: This study had to be content with small sample sizes due to logistics and time factors. These investigations do not claim to provide authoritative data from all aspects of two entire study lines or of individual sampling locations. Data rather provide snap-shots on meat safety and hygiene of the two study meat production and the processing lines. The study approach may permit to execute throughout-designed investigations of 'food safety chains'.

Hygiene and quality: Spoilage bacteria were equally present at similar magnitudes, species and compositions at all sampling locations and occasions. The slightly higher APC than EBC in all production areas and raw materials underline the importance of processes in the 'dirty sectors' of slaughters. Also, high amounts of spoilage bacteria in tap water, only used as cold water for cleaning all facilities and meat, make them another source of cross- and re-contamination.

***E. coli*:** Observation of similar *E. coli* prevalences in almost all sampling points to a lack of any hygiene measures throughout the studied lines.

***Salmonella* prevalence, serotypes and epidemiology:** Most of the serotypes recorded in this study were previously reported from Ethiopia; *S*. Kastrup and *S*. London were reported for the first time for the country. Using *XbaI*, indistinguishable properties of *S*. Kastrup and *S*. Larochelle were established.

PFGE of *Salmonella* reflects similarity of serotypes on the same and/or different sampling occasions or locations. The transmission of the same *Salmonella* serotypes within a location and/or from another location over different periods of time, under direct and/or indirect conditions, is an important result of this study. Different PFGE profiles of a *Salmonella* serotype may indicate possible contamination of the beef lines from other sources.

Antimicrobial susceptibility/resistance: The 63.9% *Salmonella* and 46.6% *E. coli* resistant isolates from both beef lines to at least one drug is exclusively the result of inadequate treatment of cattle (DACA, 2009; Essack, 2006). The risk exists, that resistant zoonotic agents along beef lines already exist or can enter them.

The present high resistance profiles of both *Salmonella* and *E. coli* isolates to oxytetracycline is of concern. Oxytetracycline is widely used for animal treatment and is freely available. The 83.6% and 85.3% resistance of *Salmonella* and *E. coli*, respectively, to oxytetracycline, underline this risk, too.

6.2.4.2 Conclusions from practical points of view

The results obtained here show high levels of spoilage and zoonotic agents in both chains due to the absence of essentially any effective hygiene. The abattoir was not divided into a dirty and a clean side, personal and equipment hygiene of the slaughter personnel were in a dismal state, meat on its long way to consumers was essentially non-refrigerated and even ‘modern’ supermarkets in single cases were unable to guarantee the hygienic safety of their products. Beef lines including raw materials, facilities, water used along the lines, personnel hygiene and associated facilities were not controlled regularly or efficiently enough. Implementation of control measures such as regular cleaning and disinfection, chlorination of water, and training of meat production, handling and supply/sale personnel would be important. Unless principles of food hygiene are employed, foods of animal origin tend to be critical.

Antibiotics used in animal production as growth promoters are uncommon in Ethiopia and can be excluded to have led to antimicrobial resistance. Hence, resistant bacteria are the result of misuse of drugs for therapeutic purposes. Farmers often treat their animals themselves, veterinary services often are non-accessible and inefficient.

6.2.4.3 Future emphasis

Findings from this study offer advanced knowledge on the hygienic status of beef lines in Ethiopia.

- Further steps may involve the use of molecular techniques for epidemiological and transmission route investigations. Multiple endo-nucleus restriction enzymes could be used to examine indistinguishable properties observed in this study of *Salmonella* serotypes such as *S*. Kastrup and *S*. Larochelle. *E. coli* isolates equally could be screened into specific serotypes *like Enterotoxogenic E. coli*, *Enteroaggregative E. coli, Enterohamorrhagic E. coli, Enteroinvasive E. coli* or *Enteropathogenic E. coli*.
- With regard to resistance, detection of resistant genes for corresponding drug(s) in both *Salmonella* and *E. coli* could be further research areas.
- Additionally, the occurrence of *Salmonella* and *E. coli* in one or more locations points to the risk other zoonotic agents entering beef lines.

Similar studies with stepwise analysisfor other zoonotic agents should be applied to other species of meat animals (sheep, goats, pig and chicken), their production, processing, handling and supply chains and,local and export abattoirs of the country.

7. SUMMARY

Zoonotic and spoilage bacteria in a meat production and a processing line in Ethiopia

Background:
In Ethiopia, little information is available on the status of food safety. It was the aim of this study to detect points of risk in meat and product contamination; to assess the prevalence of *Salmonella* serotypes and *E. coli* in meat production and handling chains and to track possible sources and transmission routes of *Salmonella*; to evaluate the microbiological quality of the final product using the Aerobic Plate Count (APC) and the *Enterobacteriaceae* count (EBC); and to perform antimicrobial susceptibility/resistance tests on the isolates.

Materials and methods:
The study was conducted from December 2011 to April 2012 in two beef production and supply lines. The first was the Addis Ababa Abattoir Enterprise (AAAE) abbatoir with its recipient city butchers' in Addis Ababa city - "the abattoir line" - while the second was a beef processing plant located some 47 km out of Addis Ababa at Bishoftu/Debre Zeit town with its product recipient supermarkets in Addis Ababa city - "the processing plant line". For this purpose, from the abattoir line, different samples were taken along cattle slaughter steps from the abattoir environment (n=101), animal-related materials (n=102) and from raw beef from city butchers' (n=34). Similarly, samples were collected in the processing plant line along the processing steps from the processing environment (n=194), animal-related materials (n=118) and products from 8 selected supermarkets (n=119) . All samples were examined for *Salmonella* and *E. coli*. In addition, samples from the processing plant line were examined for APC and EBC. *Salmonella* isolates were serotyped and exposed to Pulsed Field Gel Electrophoresis (PFGE) using *XbaI*® enzymatic genomic digestion. Possible sources and transmission routes of *Salmonella* serotypes were tracked based upon genetic similarity and differences of the obtained same serotype relation. All *Salmonella* and *E. coli* isolates were tested for susceptibility/resistance to antimicrobial agents of six different chemical classes of drugs and each with their respective concentrations (Oxoid) used for sensitivity testing. The drugs were β-lactams - amoxicillin (AML 25 µg), amphenicols - chloramphenicol (C 50 µg), amino glycosides - gentamycin (CN 10 µg) and neomycin (30 µg), tetracycline - oxytetracycline (OT 30 µg), polymyxins - polymyxin B (PB 300IU), and folate pathway

inhibitors - trimethoprim (W 5 µg) and trimethoprim-sulfamethoxazol (SXT 1.25/23.75 µg). Within both lines, sampling occasions, locations and directions in the production, processing and supply of the final product were considered and taken into account for data management and analysis. Numerical data from spoilage bacteria counts were calculated in logarithmic function and compared using single and paired t-tests. Categorical data for prevalence and antimicrobial susceptibility/resistance were calculated using percentages and the95% mid-prevalence exact confidence interval was used to assess associations. PFGE data were analyzed using the BioNumerics® 6.6 version.

Results:

Abattoir line

Spoilage bacteria: Due to non-expected heavy contamination of the abattoir spoilage bacteria were only assessed at the processing plant line.

***Salmonella*:** 63 isolates resulting in a 26.6% prevalence were identified in the abattoir line. *Salmonella* prevalence was higher for abattoir environment (36.6%) than for animal-related materials (14.7%). Prevalence at butchers' was 32.4%, similarly to abattoir environment but not animal-related materials. Six different serotypes with higher prevalences/proportions of 11.4%/42.8% for *S.* Saintpaul, followed by 5.9%/22.2% for *S.* Muenchen were observed. In this line, a prevalence of *S.* Larochelle (4.6%), *S.* Dublin (1.7%), *S.* Kastrup (1.3%), *S.* London (0.24%) and unidentified ones (1.3%) was found. The PFGE pattern showed 1, 2 and 6 pulsotypes with 1 to 14 ratios of isolates to pulsotypes in serotypes and 1 to 7 pulsotypes with 1 to 2.5 ratios of isolates to pulsotypes in positive locations. Based on genomic similarity, transmission routes were identified for *S.* Saintpaul (SSa*X*2), S. Muenchen (SMu*X*1), S. Larochelle (SKL*X*1) and *S.* Dublin (SDu*X*1). Findings revealed similar pulsotypes over sampling occasions/batches in different sampling locations for the corresponding serotypes. Other isolates of the same serotype but of a different pulsotype were determined as possible sources and/or contaminants of the production line from other sources.

E. coli: With regard to *E. coli* from this line, an overall prevalence of 45.1% was observed. This prevalence essentially agreed with prevalences of the environment (40.6%), animal-related materials at the abattoir (52.0%) and with retailed meat at butchers (38.2%). Prevalence at individual sampling locations ranged from 33.3% in tap water to 55.9% in raw beef at the abattoir with no principal differences among and between sampled locations.

Processing plant line

Parameters tested here were spoilage bacteria, *Salmonella* and *E. coli.*

Spoilage bacteria: Highest APC of 5.28±0.24 $\log_{10}$ cfu/cm^2 in room floor swabs to lowest 3.99±0.75 $\log_{10}$ cfu/g in spices wwere observed. EBC ranged from high 3.19±0.55 $\log_{10}$ cfu/cm^2 for room floors to low 2.08±0.19 $\log_{10}$ cfu/g in products at Supermarket-G. The paired t-test on the count difference ($\log_{10}$ APC – $\log_{10}$ EBC) within locations showed higher APC than EBC. This difference ranged from 2.75±0.65 cfu/g in products to 1.71±0.70 cfu/g in spices. Using APC, microbiological quality of products in total was found to be good in 24.4% of cases, 44.5% acceptable and 31.1% unsatisfactory. In contrast, by EBC 64.7% of products were good and 35.5% acceptable.

Salmonella: A total of 23 *Salmonella* isolates (5.3% prevalence) was observed in this line. Prevalence in theenvironment (5.2%) was similar to that of animal-related materials (10.2%) and final products from supermarkets (0.8%), but in comparison it was higher in animal-related materials than in final products. Only one isolate was observed in a single supermarket, the others being negative. A total of seven serotypes with a high ratio of prevalence to proportion (1.4%/26.1%) of *S*. London was observed. Unidentified *Salmonella* strains were also obtained from this line. Possible transmission of *S*. London (SLo*X*1) was observed on the 1st sampling occasion from raw beef samples to working tables which were also positive on the 2nd sampling occasion. A similar pulsotype within each of *S*. Anatum (SAn*X*1), *S*. London (SLo*X*1) and *S.* Muenchen (SMu*X*1) from raw beef on the 1st, 2nd and 6th sampling occasions was observed, respectively showing that the contaminated meat was coming from the same sources.

E. coli: The overall prevalence along the processing plant line (46.4%) was found to be the same as the prevalence in the environment (50.5%) and in animal-related materials at the processing plant (56.8%) but was higher than in the end products at supermarkets (29.4%). Differences were not observed between and among locations, with the only exception beingprocessing plant rooms for whom a higher prevalence was recorded (75%) than in tap water (23.5%), spices (13.3%), Supermarkets-A and H (each with 20%) and Supermarket-F (16.7%). There was no difference in prevalence among and between products from all supermarkets.

Antimicrobial susceptibility/resistance:

Salmonella: Susceptibility to PB, CN, C, W and SXT ranged from 93.2% to 100% and from 30.4% to 50.4% to N for all isolates in general and for those from the abattoir line and

processing plant line in particular. Susceptibility to OT was 44.2% overall but it was 31.7% and 78.3% in isolates from abattoir and processing plant lines, respectively. Resistance was high (53.5%) overall. High (65.1%) resistance to oxytetracycline was found in isolates from the abattoir line was low (21.7%) in isolates from the processing plant line. A total of 55 (63.9%) isolates was found to be resistant to at least one of the antimicrobial agents used in this study. High resistance was detected in *S.* Saintpaul (41.8%) and in *S.* Muenchen (30.9%). The proportion of *Salmonella* isolates tested and the corresponding resistant isolates showed no difference ($p>0.05$) within origin/source. Strains of higher resistance were found at the abattoir line (85.5%) than at the processing plant line (14.5%). Single-drug resistant strains were more frequent (83.6%) than two-drug resistant ones (12.7%). Multiple drug resistance (MDR) in the strains was only 3.6%. Resistance to OT was frequently observed. Distribution of strains resistant to individual drugs in both abattoir and processing plant lines was 1.8% to each of PB, CN, W and SXT, 5.4% to C, 26.6% to N and 83.6% to OT, in increasing order.

E. coli: Susceptibility of *E. coli* isolates to PB, C, CN and SXT ranged from 92.2% to 100%. Furthermore, their susceptibility to AML was between 70.1% and 79.5% while to OT it spanned between 49.5% and 60.0%. This was true overall for isolates from the abattoir line and the processing plant line. High frequencies of resistant strains to OT (37.5% to 43.9%) and to AML (18.5% to 26.2%) were observed in total as well as in individual isolates from abattoir and processing plant lines. A total of 143 (46.6%) isolates were found resistant to at least one of the antimicrobial agents used in this study. Higher frequencies of resistant strains were observed at the processing plant line (61.5%) than at the abattoir plant line (38.5%). Single-drug resistant isolates (55.9%) were more frequent than two-drug resistant ones (28.7%) and MDR accounted for 15%. Isolates resistant to OT were frequently observed. The total distribution of strains resistant to individual drugs both at the abattoir and at the processing plant line showed differences. The extent of resistance to each drug in increasing order was PB (1.4%), CN (2.1%), C (8.4%), SXT (16.1%), AML (45.5%) and OT (85.3%).

Discussion:

Observation of *Salmonella* and *E. coli* in all sampling locations on one or more sampling occasions in this investigation demonstrated the presence of zoonotic and spoilage bacteria, particularly with higher loads along the abattoir line. Higher *Salmonella* prevalence (26.6%) at the abattoir line than at the processing plant line (5.3%) identified the abattoir to be the main source of *Salmonella* transmission along beef production, processing and handling lines. In this study, occurrence of *S.* Kastrup and *S.* London was reported for Ethiopia for the first

time. With *XbaI*® PFGE profiles, *S.* Kastrup and *S.* Larochelle showed indistinguishable profiles. Antimicrobial susceptibility/resistance of *Salmonella* and *E. coli* from both studied lines showed high susceptibility to PB, CN, C, SXT and W. Resistance was high to oxytetracycline.

Conclusions:

This investigation involved two beef lines to study spoilage and zoonotic bacteria and their antimicrobial susceptibility/resistance. Findings revealed prevalence of the agents and the need for improvement, regular monitoring and application of HACCP during production, processing, handling and supply of products. Furthermore, resistance of *Salmonella* and *E. coli* to oxytetracycline was high and accounted for 83.6% and 85.3%, respectively. This drug is marketed, it is popular and widely used in the veterinary sector in Ethiopia. The further use of this drug thus should be questionned in light of the high prevalence of resistant strains in the country. Future emphasis should be put tohygiene toimprove beef production line, to antimicrobial susceptibility testing, to further screening of the present isolates of *E. coli* strains and to the extension of this type of study to meat production lines of other food animals of the country.

8. ZUSAMMENFASSUNG

Zoonose- und Verderbnisserreger in zwei Fleisch produzierenden und verarbeitenden Linien in Äthiopien (Rind)

Hintergrund:

Für Äthiopien existieren nur wenige Informationen zur Lebensmittelsicherheit. Ziele der vorliegenden Untersuchung waren:

- Abschätzung der Salmonella- und E. coli-Prävalenz in Fleischproduktions- und –bearbeitungsketten mit Zurückverfolgung der Transferrouten von Salmonella,
- Beurteilung der mikrobiologischen Qualität des Endprodukts mit Hilfe von Gesamtkeimzahl (GKZ) und Enterobacteriaceae-Zahl (EBZ) sowie
- Resistenztests an den gewonnenen Isolaten

Material und Methoden:

Die Untersuchungen wurden von Dezember 2011 bis April 2012 in zwei Fleischproduktionsketten in Äthiopien durchgeführt. Die „Abattoir Line“ wurde aus dem städtischen Schlachthof in Addis Ababa (Addis Ababa Abattoir Entreprise (AAAE)) sowie Verkaufsstätten gebildet, die Fleisch direkt von diesem Schlachtbetrieb für den Weiterverkauf erhielten. Die „Processing Plant Line“ bestand aus dem Zerlege- und Verarbeitungsbetrieb in Bishoftu / Debre Zeit mit 3 unterschiedlichen Zulieferschlachtbetrieben sowie Supermärkten in Addis Ababa, die fertige Produkte aus dem Bearbeitungsbetrieb erhielten.

In der „Abattoir Line“ wurden entlang der Schlachtlinie an unterschiedlichen Positionen Proben genommen (n = 101) sowie Proben von Tieren bzw. den Schlachttierkörpern (n = 102). In den Verkaufsstätten wurden Fleischproben genommen (n = 34). In der „Processing Plant Line“ wurden von den einkommenden Tierkörpern (n = 118) und Umgebungsproben an unterschiedlichen Positionen im Verarbeitungsbetrieb (n = 194) genommen. In insgesamt 8 Supermärkten wurden Produktproben (Mortadella) (n = 119) entnommen.

Alle Proben wurden auf Salmonella und E. coli untersucht, die Proben der „Processing Plant Line“ wurden zusätzlich auf GKZ und EBZ geprüft. Die Salmonella-Isolate wurden serotypisiert und mit Hilfe einer Pulsfeldgelelektrophorese (XbaI) mögliche Übertragungsrouten mit Hilfe bestimmter Serotypen verfolgt. Die gewonnenen Salmonella und E. coli-Isolate wurden auf antimikrobielle Resistenzen / Empfindlichkeiten gegenüber sechs Chemotherapeutika überprüft: β-Laktame: Amoxicillin (AML 25 µg); Amphenicole: Chloramphenicol (C 50 µg); Aminoglykoside: Gentamycin (CN 10 µg), Neomycin (30 µg);

Tetracycline: Oxytetracyclin (OT 30 µg); Polymyxine: Polymycin B (PB 300 IU) und Folsäureinhibitoren: Trimethoprim (W 5 µg), Trimetoprim-Sulfamethoxazol (SXT 1,25/23,75 µg).

Ergebnisse:

Abattoir Line

Salmonella: Insgesamt wurden 63 Salmonella-Isolate in der „Abattoir Line" gewonnen, dies entsprach einer Prävalenz von 26,6 % (Umgebungsproben: 36,6 %, Tierproben: 14,7 %; Proben aus den Verkaufsstätten: 32,4 %). Sechs unterschiedliche Serotypen wurden gefunden: S. Saintpaul, S. Muenchen, S. Larochelle, S. Dublin, S. Kastrup und S. London sowie unidentifizierbare Serotypen. Mit Hilfe der PFGE konnten bei S. Saintpaul, S. Muenchen, S. Larochelle und S. Dublin jeweils ähnliche Pulstypen, die auf eine genetische Verwandtschaft der jeweiligen Serotypen hinweisen, identifiziert werden. Für diese Serotypen wurden mögliche Transferrouten gezeigt.

E. coli: Für E. coli lag die Prävalenz bei 45,1 % (Umgebungsproben: 40,6 %, Tierproben: 52 %; Metzgereien: 38,2 %).

Processing Plant Line

Hygieneparameter GKZ: Die GKZ lag zwischen 3,99 ± 0,75 log10 KbE/g (Gewürze) und 5,28 ± 0,24 log10 KbE/cm2 (Bodentupferproben). Die EBZ lag zwischen 2,08 ± 0,19 log10 KbE/g (Produktproben aus Supermarkt G) und 3,19 ± 0,55 log10 KbE/cm2 (Bodentupferproben).

Salmonella: Insgesamt wurden 23 Salmonella-Isolate in der „Processing Plant Line" gefunden (Prävalenz: 5,3 %). Für S. London, S. Anatum und S. Muenchen konnten mögliche Transferrouten identifiziert werden.

E. coli: Für E. coli lag die Prävalenz bei 46,4 % (Umgebungsproben: 50,5 %; Tierproben: 56,8 %; Produktproben: 29,4 %).

Empfindlichkeitsprüfungen gegenüber Chemotherapeutika:

Salmonella: Die Empfindlichkeit von den aus beiden Linien gewonnenen Salmonella-Isolaten gegenüber PB, CN C, W und SXT schwankte zwischen 93,2 und 100 %; gegenüber N zwischen 30,4 und 50,4 %.

Insgesamt 55 Isolate waren gegen mindestens ein Chemotherapeutikum resistent (63,9 %).

E. coli: Von den gefundenen E. coli-Isolaten waren 92,2 bis 100 % empfindlich gegenüber PB, C, CN und SXT. Insgesamt waren 143 Isolate gegen mindestens ein Chemotherapeutikum resistent (46,6 %).

83,6 % der *Salmonella*-Isolate bzw. 85,3 % der *E. coli*-Isolate waren gegenüber Oxyterazyklin resistent.

Diskussion:

Salmonella und *E. coli* wurden an unterschiedlichen Probenahmetagen innerhalb beider Ketten gefunden, die Nachweisrate in der „Abattoir Line“ war höher (26,6 %) als in der Processing Plant line (5,3 %). Erstmals in Äthiopien wurden S. Kastrup und S. London nachgewiesen. Die XbaI® PFGE Profile beider Serotypen waren nicht unterscheidbar.

Bezüglich der Antimikrobiellen Empfindlichkeit / Resistenz war bei Salmonella und E.coli eine hohe Empfindlichkeit gegenüber PB, CN, C, SXT und W erkennbar sowie eine hohe Resistenz gegenüber Oxytetracyclin.

Schlussfolgerungen:

Diese Untersuchung bezog sich auf zwei Prozeßlinien (Schlacht- und Verarbeitungslinie) und auf Zoonoseerreger, Verderbsmikroflora und das Resistenzgeschehen. Die Ergebnisse lassen die Notwendigkeit regelmäßiger Kontrollen, so etwa eines HACCP- Systems erkennen. Es ergab sich eine hohe Resistenz gegemnüber Oxytetracyclin in beiden Linien (83,6% und 85,3%). Oxytetracyclin ist in Äthiopien häufig in Benutzung, was angesichts der hohen Resistenzen hinterfragt werden sollte. In Bezug auf die Hygiene sollten die Abläufe verbessert werden, es sollten Resistenztests und Untersuchungen auf die nähere Natur der auftretenden E.coli durchgeführt werden. Untersuchungen wie die hier vorgelegte Studien sollten auch in anderen Nutztier- Linien durchgeführt werden.

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10. ANNEX

Annex 10 - 01. Methods of enumerating spoilage bacteria

10.01.1. Samples from surface area and

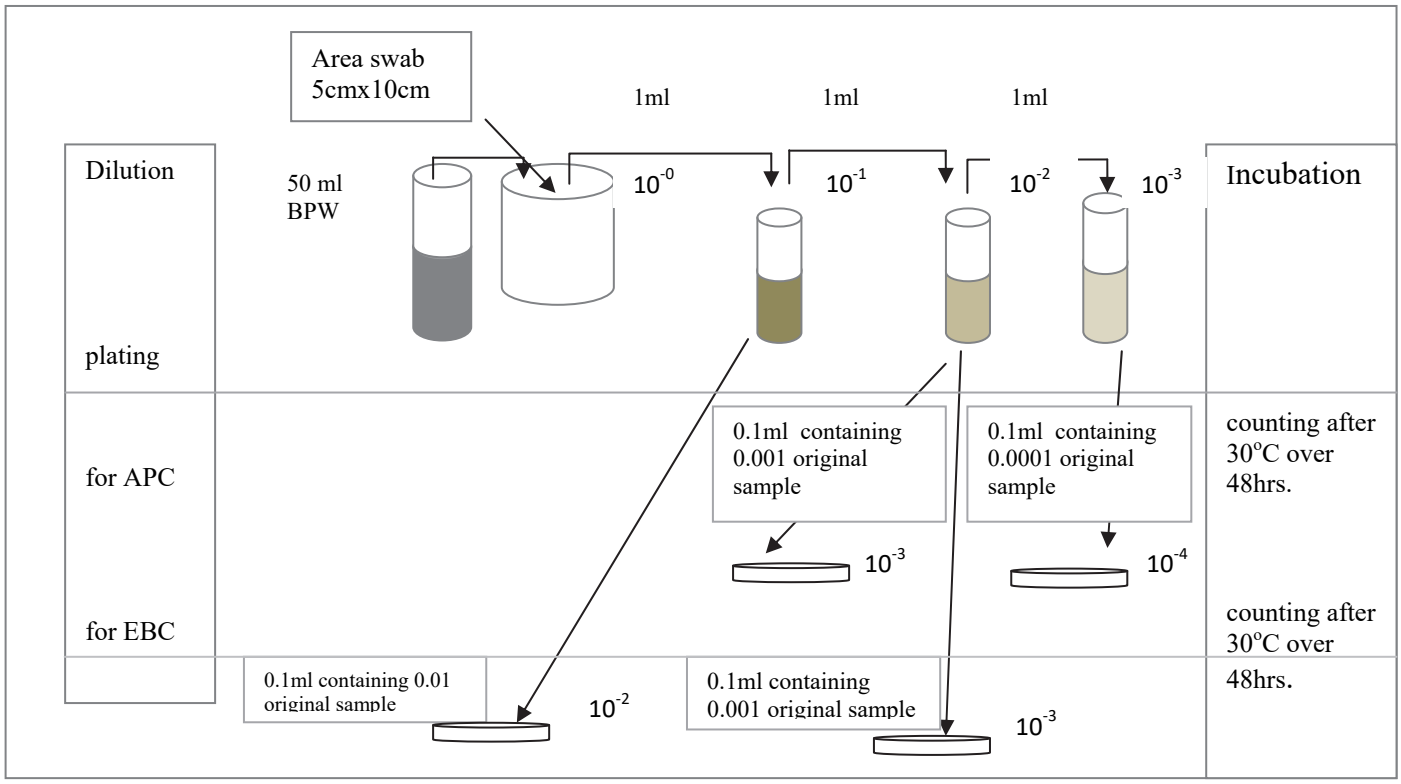


Fig. 10 - 01: Methods for enumerating aerobic bacteria and *Enterobacteriaceae* from area swab samples

10. 01.2. Solid (meat and spices) samples

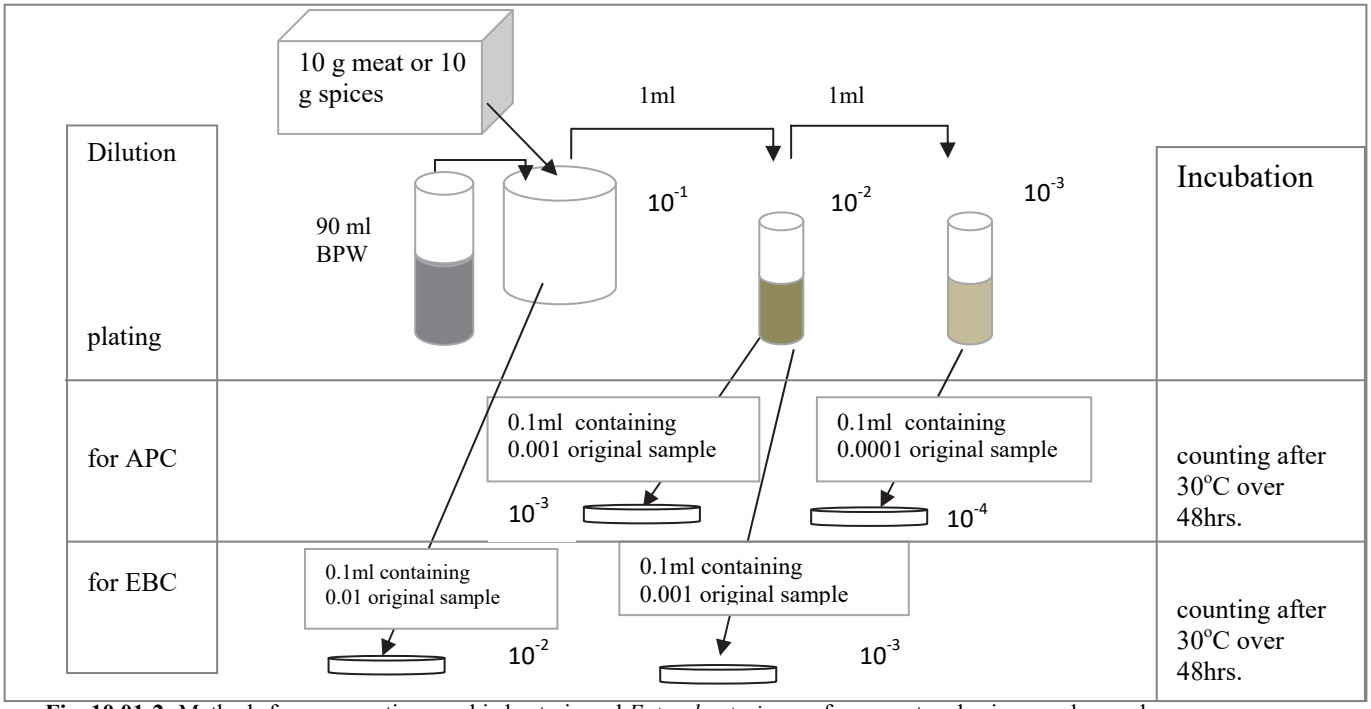


Fig. 10 01-2: Methods for enumerating aerobic bacteria and *Enterobacteriaceae* from meat and spices swab samples

10.01.3. Liquid (water) samples

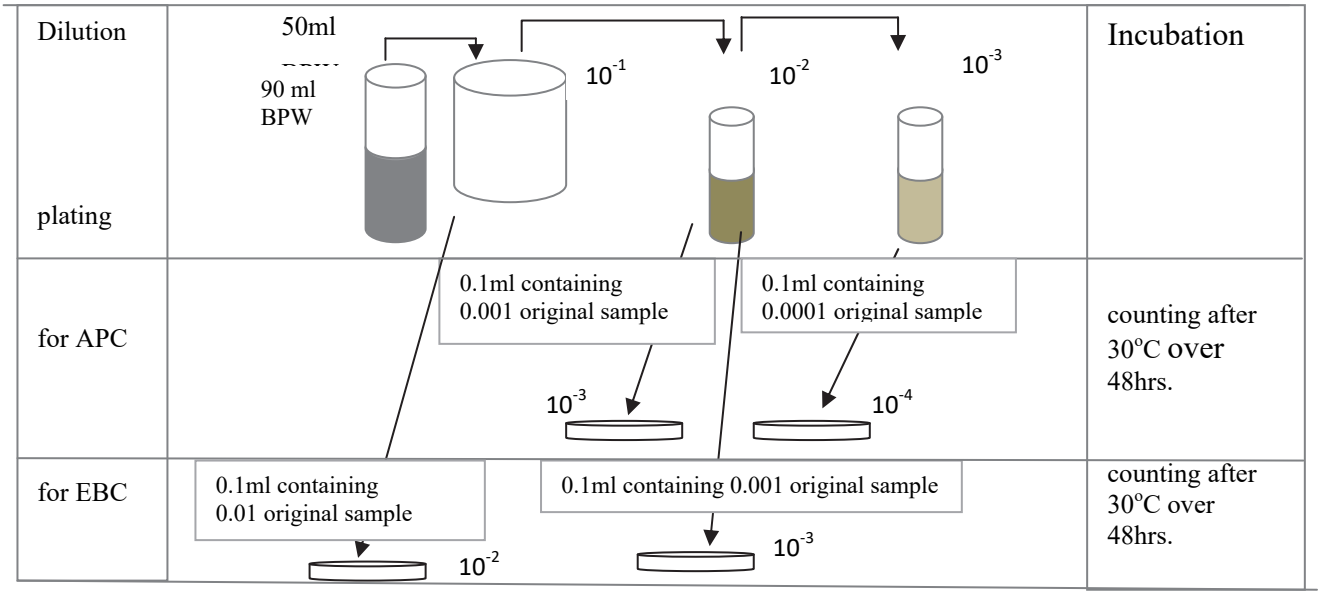
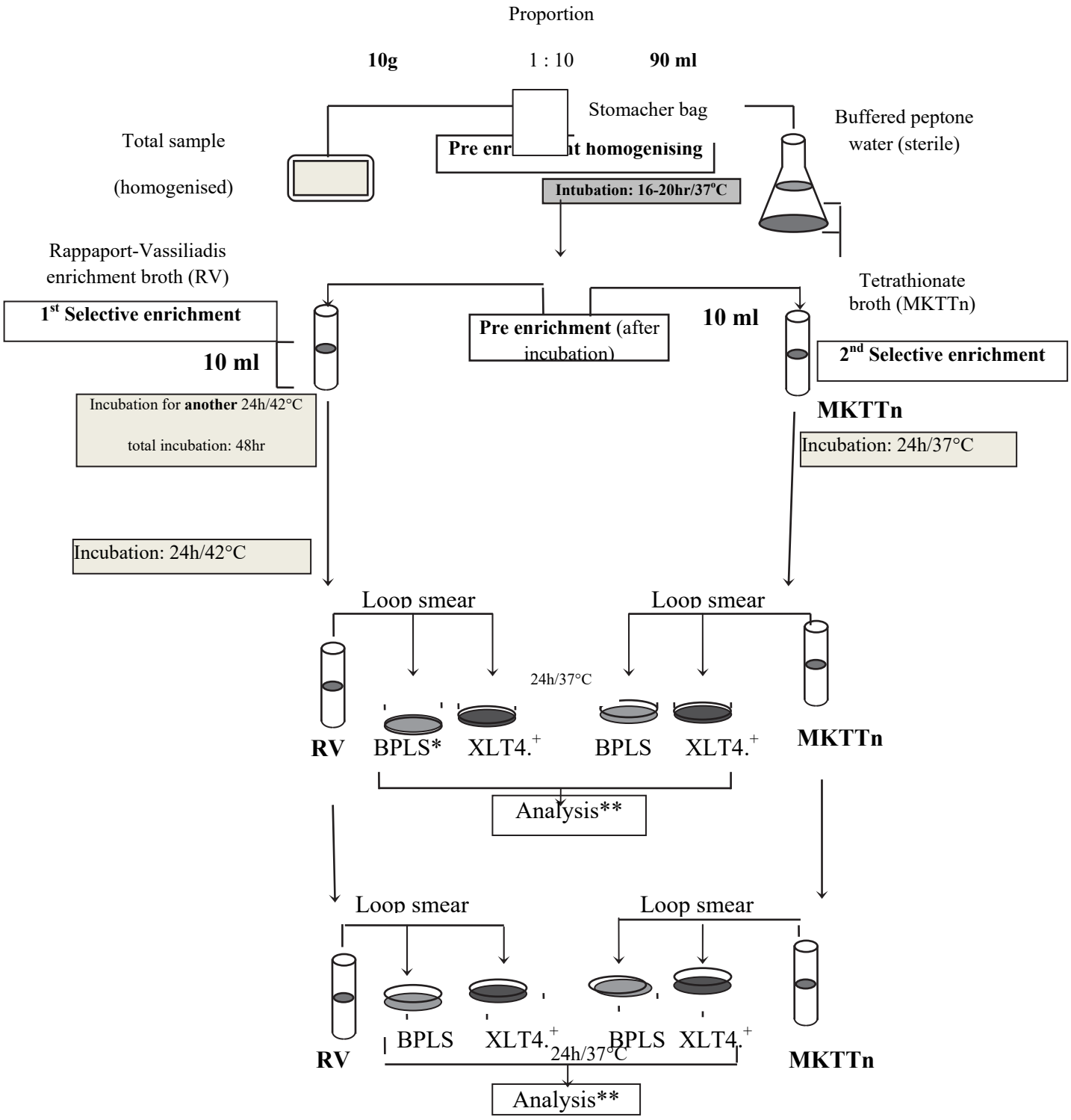


Fig. 10-01-3: Methods for enumerating aerobic bacteria and *Enterobacteriaceae* from water samples

Annex 10 - 02. Identification and differentiation of *Salmonella*

10. 02.1. *Salmonella* isolation



**: In case of finding of suspicious colonies on the selective media a biochemical/serological confirmation was made

10.02.2 PFGE techniques for *Salmonella* (*Sanguankiat 2013; PulsNet 2013*)

Day1:

1. Fresh culture preparation: Glycerol on Standard I, incubate at 37 oC for 18 hrs

PFGE Day 0: One colony Subculture-→Serology→Steps

2. Streak single colony from first culture (**Day 1 above**) on Standard I, incubate at 37 oC for 18 hrs

PFGE Day 1

3. Weigh 90mg Certified ™ Megabase Agrose (CMBA) (-weigh in 15ml centrifuge test tube), (For preparing 2% Certified ™ Megabase Agrose)
4. Prepare 10xTE buffer (10mM Tris: 1mM EDTA, pH 8.0) as follows:
   a. 10ml of 1M Tris, pH 8.0 *(see 10.2.4)*
   b. 5ml of 0.2M EDTA, pH 8.0 *(see 10.2.4)*
   c. Diluted to 1000ml with double-distilled water (ddH_2O)
   d. Sterilization at 100^oC for 6hr
5. 0.5%MacFarland Proteinase K-(20mg/ml)** (-20^oC) (10 µl/sample) 10% Sarcosyl (10.0g Sodium Lauroyl Sarcosinate in 100.0 ddH_2O)** for Cell Lysis and ES solutions preparation
6. Cell suspension Buffer (CSB) (100mM Tris: 100mM EDTA), pH 8.0: For Cell Lysis solution. As:
   a. 10ml of 1M Tris, pH8.0 *(see 10.2.4)*
   b. 50ml of 0.2M EDTA, pH 8.0 *(see 10.2.4)*
   c. 40ml of sterile ddH_2O
   d. Keep it in refrigerator before use
7. Turn on water bath (54^oC); hot plate (100^oC) and spectrophotometer with 630nm wave length
8. Prepare **2%CMBA** to boil 100^oC (for 10-15') loose cap
   a. 90 mg (0.09g) of CBMA (*from 2*)
   b. Add 4.5ml sterile TE-Buffer; Swirl gently to disperse agarose
   c. Loose cap and heat at 100 ^oC in beaker of water on the hot plate until the agarose is completely dissolved (about 15-20 minutes).
   d. Check water bath working at (54^oC) and cooling agrose in the water bath at (54^oC) before use of agarose.
9. Label 10ml test tube
10. Dispense 2 ml of Cell suspension Buffer (CSB) into it. Using sterile loop, transfer chaenk of colony into this solution to the level of 0.5 NacFarland.
11. Adjust concentration of cell suspension
    a. dispense **100µl** sterile distilled water in 1st row of 12 well of Micro titer Plate
    b. Pipette **100 µl** of prepared Cell suspension into 2nd and 3rd row of Micro titer Plate
    c. Measure Optical Density /OD/ **(0.55-0.60)** in 360nm UV Wave Length

OD	CSB µL
0.601-0.620	50µL
0.621-0.630	100 µL
0.631-0.640	200 µL
0.641-0.650	300 µL
0.651-0.680	400 µL
0.681-0.700	500 µL
0.701-0.720	600 µL
0.721-0.740	700 µL
0.741-0.760	800 µL
0.761-0.800	900 µL

Reference table for section 11.d

d. If high adjust OD, add CSB (*see table*), or if low OD, add some colony in both cell suspension test tubes.
e. Re-check with (Pipette 100 µl) using 5th and 6th row of MTP.

12. Label 1.5 ml micro-centrifuge tubes with PFGE culture numbers
13. Transfer ***200 µl*** of ADJUSTED/ Corrected Cell suspension into 1.5ml pre-labeled Micro centrifuge tubes.
14. *Add 10µl of Proteinase K* (20mg/ml stock) (*120 µl for 12 cells*) and gently mix by tips. Speed of application is required here. Proteinase K is cold and kept in deep freezer, and warm to room temperature before working with it.
15. Transfer a ***200 µl*** of Boiled Megabase Arose into the same 1.5ml sample Soln.;
16. Mix thoroughly by the same micro tip and **Immediately** dispense into Plug Mold (2 per-sample is required)
17. Keep to cool down for 10-15minut /in +4 ^{o}C refrigerator for 5 min.
18. Label 20 ml glass tube with PFGE number and place in the rack.
19. Prepare Cell Lysis Buffer (100mM Tris: 100mM EDTE, pH 8.0 + 1%Sarcosyl): **5ml per sample**; as bellow:

	Cell Lysis Buffer	
Reagents	**5ml for 1 sample**	**60ml/12 sample**
CSB	2.5 ml	30 ml
10%Sarcosyl +	0.5 ml	6 ml
Sterile ddH2O +	2 ml	24 ml
Proteinase K*	> 25µl	> 300 µl
	Proteinase K must be Stored in Ice	

20. Gently remove the white tape from bottom of Plug Mold, Disposal into Waste beaker, transfer the Pluh Mold into respective glass tube (*from 17*).
21. Using 10ml pipette, flush with **"5 ml Lysis buffer"!** Be sure that the blocks are under!
22. Properly cover the test tube with Paraffin then with Aluminum foil in Plastic rack, -Place in **(54^{o}C)** water bath for **20hrs** under shaking condition 150-175 rpm. *Make sure that the level of water in the water bath is above the level of lysis volume!*

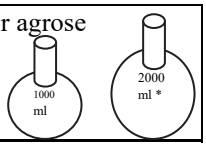
PFGE Day 2

23. Remove the glass from water bath.
24. Turn on water bath (50^{o}C, 4-5 cm depth of distilled water).
25. **Washing steps:** Pre-heated **50^{o}C** 240-360ml of sterile water in water bath as pre-heat water for 2X washing (10-15ml/sample X2 times) **and** 480-720ml Heated **10xTE buffer** in water bath as pre-heat TE for 4Xwashing (10-15ml/sample X4 times).
26. Carefully pour off lysis buffer into west beaker, use Absolute Ethanol for disinfection in between activities. Add 10-15 ml of pre-heated water. Wash under shaking water bath (**50^{o}C) for 10-15minutes (X2)** by pouring off water and repeat.
27. Pour off water and add 10-15ml pre-heated **10xTE buffer.** Wash under shaking water bath (**50^{o}C) for 10-15minutes (X4)** by pouring off water and repeat.
28. Descant the last wash and continue step 29, or store the plugs in 1.5 ml micro tube with 500 µl **10xTE buffer.**
29. Turn on water bath (55-60^{o}C to cool down and keep the running Gel).

30. Prepare 3000ml 0.5XTries-Borate Buffer (**TBE**) **(in 1000ml** and **2000ml**).

No.	***Reagent***	***Volume***		
1	*10XTBE*	*50ml*	*100ml*	***150ml***
2	*0.5M Thiourea*	*200µl*	*400 µl*	***600 µl***
3	*Dilute with ddH_2O*	*to 1000*	*to 2000**	***3000ml***

100ml of (*) used for agrose



31. Pulsed Field Certifid Agrose preparation:

a. Weigh 1.2g of Pulsed Field Certifid Agrose in 250 ml screw capped bottle.

b. Add **100ml of 0.5x TBE** (*from 30*) and use sterile magnetic streare.

c. Loose cap and heat at 300°C hot plate with 20-25min, must be completely boiled and dissolved.

d. Tighten screw and carfully place boiled gel in 55-60°C water bath, 10-15min (*from 24*).

e. Carfully clean gel apparatus with absolute ethanol and fix (set **including the Combe).** The smaller side is alwys at front adge of gel.

f. Carfully pour down gel of 55-60°C, avoid bubble; For closing comb whole, **leave small** amount of gel in the bottle and replace in warm water bath. Keep solidified.

32. **Pre-restriction incubation steps:** Prepare 10X H buffer (1:10 dilution)in 15 centerfuge test tube.

Reagent	***µl/ sample***	***µl/ 12 sample***
Sterile ddH_2O	*180µl*	*2160 µl*
H buffer (Roche Applied Sci.) It is kept Deep frezer, Warm before use	*20 µl*	*240 µl*
Total Volume	***200 µl***	*2400 µl*

33. Label a pair of 1.5Micro center tube (1 for slices of 1.0-2.0mmI and 1 for sample storage).
34. Carefully cut 1.0-2.0mmI of plug of sample and place in 1.5 ml Micro center tube (*from 32).*
35. Carefully cut 1.0-2.0mmI of plug of S. Braenderup STSAL 82 standard and transfer into 1.5 ml Micro center tube (*from 32).*
36. Carfully and Completely ***remove TE*** from slices *(if it had been prepered and stored!)*¶
37. Add **200 *µl*** *of H-buffer to sample (from 32)*
38. Incubate at 37°C for 10-15min, or room temperature, for 15min.
39. Prepare Resteriction incubation: according to the following table**;** *XbaI (50U* /sample*)*

Reagent	**µl/ plug sample**	**µl/12 plug sample**
Sterile ddH_2O	43.5 µl	522 µl
10XH buffer (Roche Applied Science)	5 µl	60 µl
*Concentrated Enzyme XbaI (40U /*µl*) Kept in Deep freezer*	1.5 µl	18 µl
Total	**50 µl**	600 µl

40. After 15min incubation (*from 38*), gently remove H-buffer (*careful not to damage plug and not to lose, use 95µl tip*) and continue **Step 41.**
41. Add 200µl of restriction enzyme (*from 39*), close the cap, be sure the plug slices under the solution.
42. Incubate for 2hrs under Thermomixer of 37°C
43. Put the black gel frame in the electrophoresis chamber. Add 2.9 litter of prepared 0.5xTBE (*from 30 &31b*). Close cover of unit.
44. Turn on cooling module (14 °C), power supply, and pump aprox. 30 minutes before gel is to be run.
45. After 2 hrs of incubation with restrction (*from 42*), add "**STOP" solution** (ES- Solution/EDTA Sarcosyl soln.) **ES** *prepare as follows:*

Reagent	**Amount and unit**
Na^+ free EDTA	*1.46g*
Sterile ddH_2O	*9ml*
3 pieces of NaOH	*Chak pH to 9.0*
Check 9.0pH *with pH indicator (pH 7.5-14). Adjust to 9.0 pH by adding /removing NaOH*	

10% Sarcosyl	*1000µl i.e 1ml*

46. *Preparation of Loading Buffer sulution (100 µl/sample***)**

Reagent

a. *0.04g Na^+ free EDTA in 10ml Sterile ddH_2O*
b. *4 g Saccharose*
c. *0.003g Bromphnenle*

47. Remove restricted plug from 37°C.

48. Directly add **25 *µl* ES solution** into restricted plug.

49. Directly add **50 *µl* loading buffer**.

50. Remove the comb from gel after it has been solidified (*from 31f*).

51. Cut marker (Puls Marker™ 50-1000kb)as thin as possible and load the marker in wells (lanse) **1, 8** and **15**. After finshing, replace the marker in 2-8°C temperature.

52. Remove the restricted silices plug. Use tapped end of spatula, petri dish, scalpel blade. Gently load in wells (lanse) 2nd -7th and 9th -13th, use 14th for STSAL 28. Avoid bubble, flat side should be to the running direction of gel.

53. Fill the wells (lanse) with Pulsed Field Certifid Agrose of 55-60°C (from 31f) "that was left in small amount". Avoid bubble. Keep solidified.

54. After 53rd step, unscrew, remove end gel, gently remove the gel with black form and remove excess gel from the bottom. Keep the cassete inside the black frame in Electrophoreses chamber. Close cover unit.

55. Set Electrophoroses condtion on CHEF DR-II. Programming as folllows:

a. Initial A time →2.2s
b. Final A timer →63.8s
c. Voltage →200V (6V/cm)
d. Run time 19-20hr

56. Check correct setting of all programs again. Start electrophorosis.

PFGE Day 3

57. When electrophoresis run is over, turn off apparatus; remove gel into covered container for staining (on empty covered staining entirey)

58. During working with Ethidium bromide (carcinogenic), use a Blue glove! Using proper Box for gel staining, Stain gel with Ethidium bromide (dilute 90µl of Ethidium bromide stock Soln. (10mg/ml) with 1000ml of Distilled water). Stain for **20-30 min** in covered container on horizontal shaker (Certomat(R) with speed **40/min**)

59. Pour off Ethidium bromide into its own specific container!

60. To have clear picture, the gel shall be 2X de-stained in approximately 500 ml Distilled water for **20 min** each in horizontal shaker (Certomat(R) with speed 40/min)!

61. On UV light; Gently place the gel in imaging equipment to serve the gel image as an '.img', or '.lsc' file; convert this file into '.tif' file (***look 10.2.5.2***) for analysis with BioNumeric® software program.

62. The camera apparatus; Keep gel still with tissue paper; Close camera apparatus; Take picture and save following the duration!

63. Clean chamber of Electrophoresis by draining buffer and discard into sink. Clean apparatus circuit with **(2 Liters)** of distilled water by apparatus pumping. Then flush again the chamber with distilled water and drain off all apparatus to keep air dry!
64. Sterilization of solution was at 100^{o}C for 6 hrs; disinfection was with absolute (95%) Ethanol.

10.2.1. Preparation of Stock solutions for PFGE

1M Tris, pH 8.0**

Tris base (1Mof Tris: 121.14g)	121.1 gram
Double distilled water (ddH_2O) to	1000.0milliliter

Note: Dissolve in 800 ml of ddH_2O, adjust pH with Conc. HCl, and add ddH_2O to 1Lt. **Autoclave to sterile**. Store at room Temperature

0.2M EDTA, pH 8.0

Na_2EDTA (1M of EDTA: 372.3g)	74.46 gram
Double distilled water (ddH_2O) to	1000.0milliliter

Note: Dissolve in 800 ml of ddH_2O, adjust pH with NaCl, and add distilled water to 1Lt. **Autoclave to sterilize**. Store at room temperature.

10xTris-Borate EDTA buffer (10xTBE)

Composition for 1000ml

0.9 M Tris (Hydroxymethyl)-aminomethan	109.0 gram/Liter
0.9 M Boric acid	55.6 gram/Liter
0.025 EDTA with Na	9.3 gram/Liter

Note: Dissolve in 800 ml of ddH_2O, and add ddH_2O to 1Liter. **Autoclave to sterilize**. Store at room temperature.

10% Sarkosyl

Sodium Lauroyl Sarcosinate	10.0 gram
Double distilled water	100.0 milliliter

Proteinase K (20 gm/ml)

Proteinase K	25.0 mg
Double distilled water	12.5 milliliter

Note: Dissolve in 12.5 ml of ddH_2O into vial of Proteinase K powder, mix and transfer into small tubes and store in a freezer (-20^{o}C).

0.5 Thiourea

Thiourea (1M: 76.12)	38.06 gram
Double distilled water	100.0 milliliter

** Autoclaving is at 100^{o}C for 6hrs

10.02.3 The laboratory record for PFGE

10.02.3.1 Measuring bacterial cell density

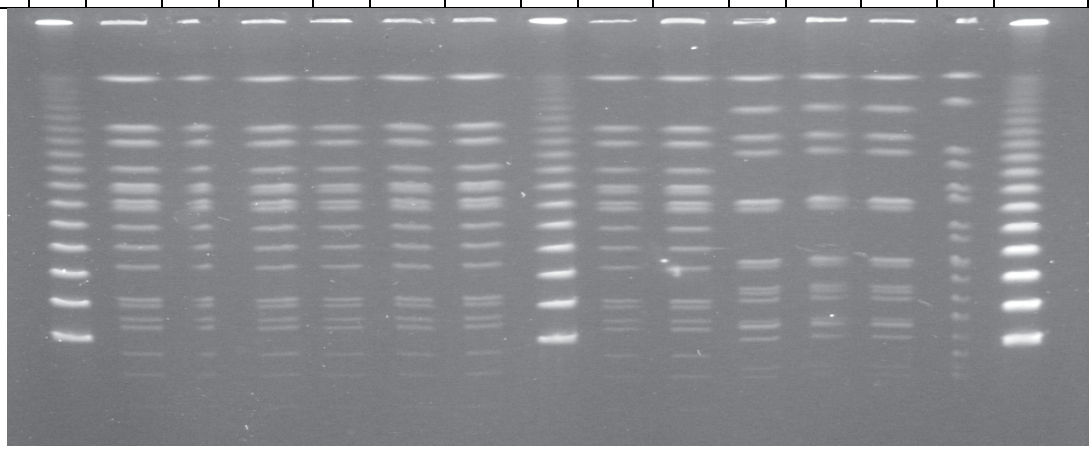
Date re-serotyping was made:_____ Optical density test result examination

	1	2	3	4	5	6	7	8	9	10	11	12
A	water	water	water	water	water	water	water	water	water	water	water	water
A	water	water	water	water	water	water	water	water	water	water	water	water
B	Isolate$_1$	…..$_2$	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..
C	Isolate$_1$	…..$_2$	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..
D												
F	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..
G	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..	…..
H												

10.02.3.2 PFGE result registration protocol

Date: From ___ to __ **Restriction Enzyme**: *XbaI* **Isolates from**: _____

Microbiology Lab.Nr	50-1000 kb							50-1000 kb						ST SAL 82	50-1000 kb
	M	**1**	**2**	**3**	**4**	**5**	**R**	**M**	**7**	**8**	**9**	**10**	**11**	**R**	**M**

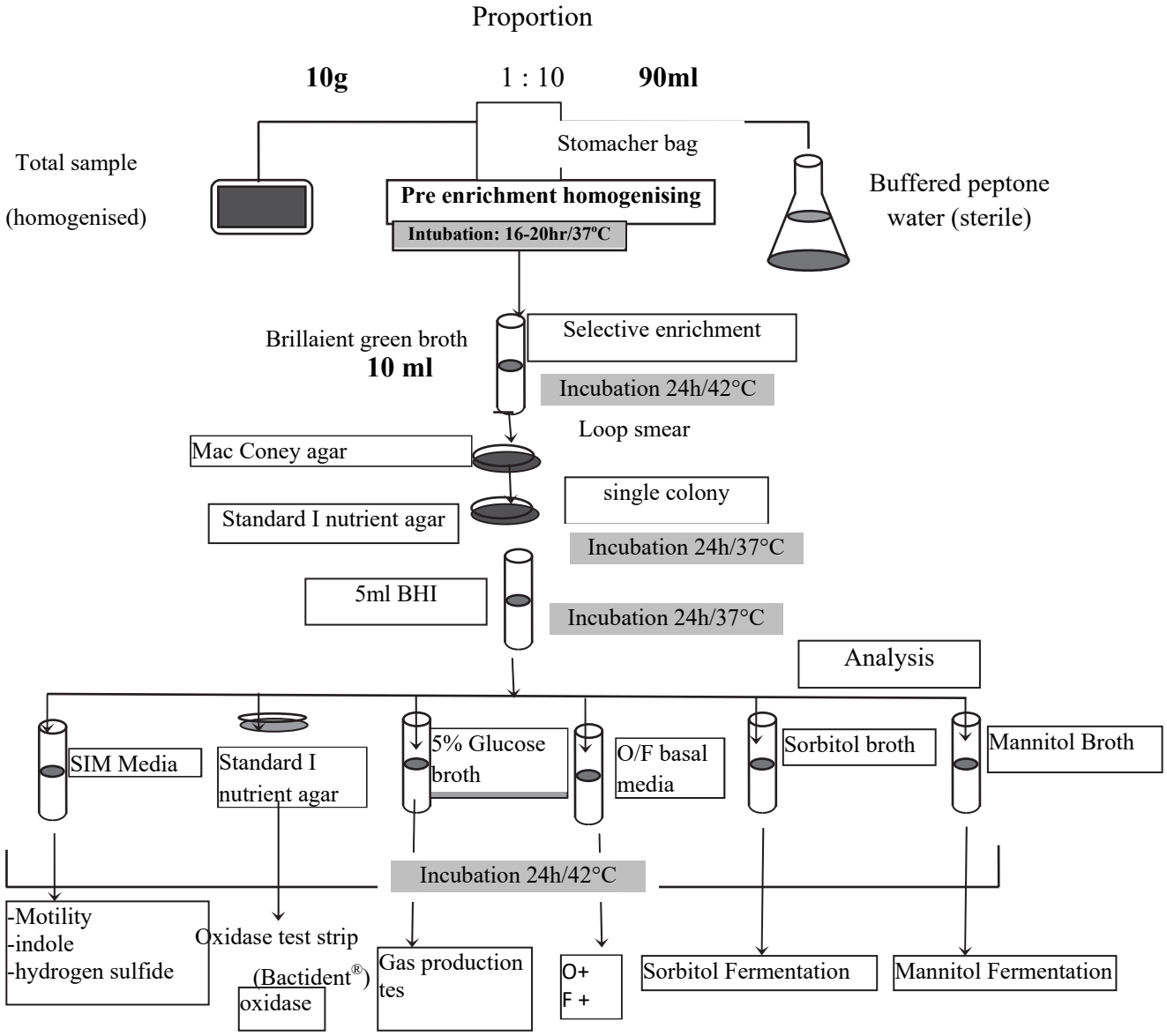


	M	1	2	3	4	5	R	M	7	8	9	10	11	R	M
Microbiology Lab.Nr															
Molecular biology Lab. Nr.															
Sample															

M = Pulse Marker™ 50-1,000kb

An example of PFGE result image and documentation parameters used in this study

Annex 10 - 03. *E. coli* isolation and identification

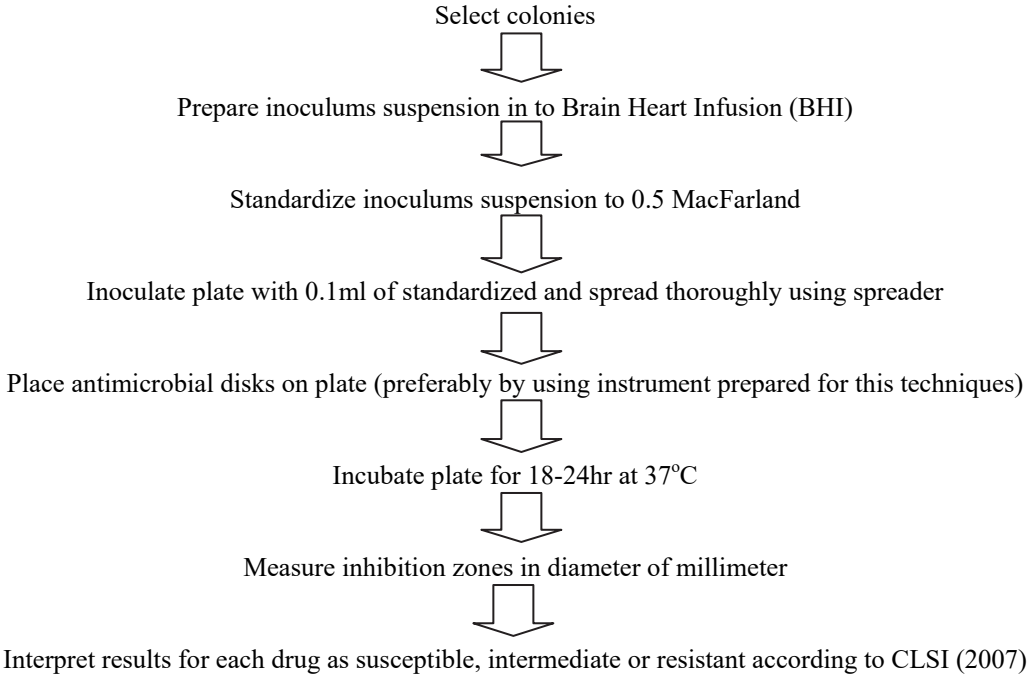


Result interpretation:

Test	*E. coli* Positive reaction	*E. coli* Negative reaction
Motility	swarming growth around inoculation line	growth only along line of inoculation
Indole	pink ring formation for indole test	no pink ring formation
H_2S	no black color production	blackening of culture
Oxidase	blue or violet blue on the strip	shows no change of color
Gas production	gas in the inverted Durham tube	no gas in the Durham tube
O/F O	Acid production	No color production
test F	Acid production	No color production
Sorbitol fermentation	Acid production	No color production
Mannitol fermentation	Acid production	No color production

Annex 10 - 04. Antimicrobial susceptibility/resistance test

Disc diffusion test



Source: CLSI (2007)

Annex 10 - 05. List of media, chemical reagents and equipments

10.05.1. Media and chemical reagents used for microbiological

Media/chemical	Article number	Company
Buffer peptone water (BPW)	1.07228.0500/5007	Merck
Standard-I nutrient agar	1.07881.0500	Merck
Standard-II nutrient agar	1.07883.0500	Merck
Muller Kaufmann tetrathionat novobiocin	1.07878.0500	Merck
Briliant green bile 2% broth	CM0031	Oxoid
Brilliant-green Phenol red Lactose-sucrose-agar	1.07232.0500	Merck
Xylose Lactose Tergitol™ 4		
- XLT4 Agar, Base	1.13919.0500	Merck
- XLT4 Agar Supplement (Sodium teteradecyl sulfate 26-28%)	1.08980.0100	Merck
Rappaport-Vassiliadis	1.07700.0500	Merck
Violet Red Bile Agar	CM107T	Oxoid
Glucose		FIZMERK
Brilliant Green Broth	CM0031	Oxoid
MacConkey Agar	1.05465 .0500	Merck
SIM media	1.05470.0500	Merck
Kovac's reagent	60983	Sigma
Oxidase test strip (Bactident®)	1.13300.0001	Merck
Sorbitol		
Mannitol		
Brain Heart Infusion	48200	Merck
Iodine	1.04761.0100	Merck
Potassium iodide	1.05043.0250	Merck
Media/chemical	**Article number**	**Company**
Anti-*Salmonella* sera		
Polyspecific Enteroclone Anti-*Salmonella* -I (A-E)	TR 1111	Sifin
Polyspecific Enteroclone Anti-*Salmonella* -I (F-67)	TR 1121	Sifin
Enteroclone Anti-*Salmonella* B	TR 1201	Sifin
Enteroclone Anti-*Salmonella* C	TR 1202	Sifin
Enteroclone Anti-*Salmonella* D	TR 1203	Sifin
Enteroclone Anti-*Salmonella* E	TR 1204	Sifin
Monospecific Enteroclones Anti-*Salmonella* and test sera Anti-*Salmonella* O, Vi		
Anti-*Salmonella* O 4	TR 1302	Sifin
Anti-*Salmonella* O 5	TR 1303	Sifin
Anti-*Salmonella* O 6	TR 1304	Sifin
Anti-*Salmonella* O 7	TR 1305	Sifin
Anti-*Salmonella* O 8	TR 1306	Sifin
Anti-*Salmonella* O 9	TR 1307	Sifin
Anti-*Salmonella* O 10	TR 1308	Sifin
Anti-*Salmonella* Vi	TR 1316	Sifin
Monospecific Enteroclones test sera Anti-*Salmonella* H		
Anti-*Salmonella* Hd	TR 1404	Sifin
Anti-*Salmonella* HE	TR 1405	Sifin
Anti-*Salmonella* Hg	TR 1406	Sifin
Anti-*Salmonella* Hg	TR 5406	Sifin
Anti-*Salmonella* Hh	TR 1409	Sifin
Anti-*Salmonella* Hi	TR 1410	Sifin
Anti-*Salmonella* HL	TR 1412	Sifin
Anti-*Salmonella* Hn	TR 1438	Sifin
Anti-*Salmonella* Hp	TS 1414	Sifin
Anti-*Salmonella* Hv	TS 1420	Sifin
Anti-*Salmonella* Hz	TR 1424	Sifin
Anti-*Salmonella* Hz_{15}	TS 1428	Sifin
Anti-*Salmonella* H1	TR 1437	Sifin

Anti-*Salmonella* H2	TR 1433	Sifin
Anti-*Salmonella* H5	TS 1434	Sifin
Anti-*Salmonella* H6	TS 1435	Sifin
Anti-*Salmonella* H7	TS 1436	Sifin

10.05.2. Media and chemical reagents used for Molecular biological analysis

Media and reagents	**Article number**	**Campany**
EPS solutions		
- 0.5% EDTA	E 2.628-2	Sigma Aldrich
- 1% N-Lauroyl-Sarcosine Sarkosly	L-9150	Sigma Aldrich
- 1 mg/ml proteinase k, pH 9	03 115 801 001	Roche
Ethanol	9065.4	Carl Roth
Ethidium bromide	E-8751	Sigma Aldrich
Megabase Agarose	161-3108	Biorad
10xTBE buffer (10X)		
- 0.9 M Tris (Hydroxymethyl)-aminomethan	5429.3	Carl Roth
- 0.9 M Boric acid	15165	Serva
- 0.025 EDTA with Na	39760	Serva
TE Buffer		
- 10 mM Tris	4855.2	Carl Roth
- 1 mM EDTA	39760	serva
Restriction endonuclease XbaI	REF 11047663001	Roche
Pulse marker ^{TM} 50-1000kb	D-2416	Sigma Aldrich
Pulsed Field Certified Agrose® gel		
N-Lauroyl-Sarcosine sodium salt	L9150-1000	Sigma Aldrich
Sodium hydroxide pellets	1.06498.1000	Merck
Succharose		
Bromphenolblau		
Na_2EDTA		
Thiourea (1M: 76.12)		

10.05.3. Media and chemical reagents used for antimicrobial susceptibility resistance test

Media/chemical	**Article number**	**Company**
MacFarland		
Brain heart infusion (BHI)	48200	Merck
Standard I nutrient agar	1.07881.0500	Merck
Mueller-Hinton agar	CM0337	Oxoid
Amoxicillin (AML 25 µg),).	CT0061B	Oxoid
Chloramphenicol (C 50 µg),	CT0014B	Oxoid
Gentamycin (CN 10 µg),	CT0024B	Oxoid
Kanamycin (K 30 µg),	CT0026B	Oxoid
Neomycin (N 10 µg),	CT0032B	Oxoid
Oxytetracycline (OT 30 µg),	CT0041B	Oxoid
Polymyxin B (PB 300U),	CT0044B	Oxoid
Trimethoprim-sulfamethoxazol (STX 1.25/23.75 µg)	CT0052B	Oxoid
Trimethoprim (W 5 µg)	CT0076B	Oxoid

10.05.4. Equipments used

10.05.4.1. Equipments used for Microbiology and Antimicrobial susceptibility test

Equipment	Article number	Company
Balance	L2200S-D	Sartorius
Densitometer	O50102-1302-006	DEN-1
Disc dispenser	X3199A	Oxoid
Freezer (-30 °C)	Premium	Liebherr
Incubator for 37 °C	Kelvitront®	Heraeus
Incubator for 42 °C		Melag
Laboratory blender	Stomacher 400	Seward
Refrigerator	Standard 430	Kirsch
Refrigerator	Export	Bosch
Thermometer/pH meter	Profi line FKS2600	Schott

10.05.4.2. Equipment used for molecular biology

Equipments	Article number	Company
Auto pipette, 0.5-10 µl	4910 000.018	Eppendorf
Autopipette, 10-100µl	4910 000.042	Eppendorf
Autopipette, 100-1000µl	4910 000.069	Eppendorf
Balance	LP2200P	Sartorius
Balance	A200S	Sartorius
CHEF-DR®II System	170-3612	Bio-Rad
CHEF-DR Disposable Plug Mold	170-3713	Bio-Rad
Digital Imaging and Analysis System		Serva
- Cabinet incl.Power cable	DISA-II	
- Canon Power Shot G9 12.1MP digital camera incl. Power adaptor		
- CD Glescan 6.0 Software incl. manual	GS-V60	
Magnetic stirrer	MR2002	Heidolph
Refrigerator 4/-20 °C	KGE2612	Bosh
Referigerator 4	Laber-461	Bosch
Spectrophotometer	Multiskan®Plus	Titertek

Annex 10 - 06. Supplementary results

Table A - 01: *Salmonella* prevalence by sampling occasion and sample source from the abattoir line

	No. of samples and *Salmonella* positives by sampling occasion																	
	Occasion 1			Occasion 2			Occasion 3			Occasion 4			Occasion 5			Total		
Source	No. of sample	Positive No. (%)	Mid-Pex. 95% CI	No. of	Positive No. (%)	Mid-Pex. 95% CI	No. of	Positive No. (%)	Mid-Pex. 95% CI	No. of sample	Positive No. (%)	Mid-Pex. 95% CI	No. of sample	Positive No. (%)	Mid-Pex. 95% CI	No. of sample	Positive No. (%)	Mid-Pex. 95% CI
Env't	25	6 (24.0)	10.4-43.4	27	9 (33.3)	17.6-52.4	22	7 (31.8)	15.1-53.1	11	7 (63.6)	33.6-87.2	16	8 (50.0)	26.6-73.4	101	37 (36.6)	27.1-46.4
ARM	24	2 (7.3)	1.4-24.9	36	2 (5.6)	0.9-17.2	21	7 (33.3)	15.9-55.1	9	3 (33.3)	9.3-66.8	12	1 (7.3)	0.4-34.7	102	15 (14.7)	8.8-22.6
BUT	8	4 (50.0)	18.4-81.6	12	2 (16.7)	2.9-45.1	7	3 (42.8)	12.3-78.4	3	0	0-63.2	4	2 (50)	9.4-90.6	34	11 (32.4)	18.3-49.3
Total	57	12 (21.1)	11.9-33.1	75	13 (17.3)	9.9-27.2	50	17 (34.0)	21.9-47.9	23	10 (43.5)	24.6-63.9	32	11 (34.2)	19.9-5.9	237	63 (26.6)	21.3-32.5

Env't = Environment
ARM[¶] = Animal-related materials;
BUT = Butchers

Table A - 02*:* *Salmonella* positive samples and prevalences by sampling occasion and locations from the abattoir line

Source/ origin	Sampling location	*Salmonella* positive samples by numbers and sampling occasion											
		Occasion 1 No. of samples		Occasion 2 No. of samples		Occasion 3 No. of samples		Occasion 4 No. of samples		Occasion 5 No. of samples		Total	
		Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)
Abattoir Environment	Personnel related swab samples												
	Personnel's hands	3	1 (33.3)	4	2 (50.0)	3	2 (66.7)	1	0	2	0	13	5 (38.5)
	Aprons	4	0	4	3 (75.0)	3	0	1	0	2	2 (100)	14	5 (35.7)
	Knives	3	0	4	2 (50.0)	3	1 (33.3)	1	1 (100)	2	0	13	4 (30.7)
	Tap water	2	0	3	0	3	0	2	1 (50.0)	2	0	12	1 (8.3)
	Device related swab samples												
	Hooks (hanging part) samples	4	1 (25.0)	3	0	3	0	1	1 (100)	*	-	11	2 (18.2)
	Room floor samples	6	3 (50.0)	4	1 (25.0)	2	1 (50.0)	2	2 (100)	3	2 (66.7)	17	9 (52.9)
	Refrigerators	1	0	3	0	1	1 (100)	2	2 (100)	3	3 (100)	10	6 (60.0)
	Transport trucks	2	1 (50.0)	2	1 (50.0)	4	2 (50.0)	1	0	2	1 (50.0)	11	5 (45.5)
	Total	25	6 (24.0)	27	9 (33.3)	22	7 (31.8)	11	7 (63.6)	16	8 (50.0)	101	37 (36.6)
Abattoir Animal related	Animal feces	8	1 (12.5)	12	2 (16.7)	7	3 (42.8)	3	1 (33.3)	4	1 (25.0)	34	8 (23.5)
	MLN* sample	8	0	12	0	7	2 (28.5)	3	1 (33.3)	4	0	34	3 (8.8)
	Raw beef	8	1 (12.5)	12	0	7	2 (28.5)	3	1 (33.3)	4	0	34	4 (11.8)
	Subtotal	24	2 (7.3)	36	2 (5.6)	21	7 (33.3)	9	3 (33.3)	12	1 (7.3)	102	15 (14.7)
Butchers	Retail meat sample	8	4 (50.0)	12	2 (16.7)	7	3 (42.8)	3	0	4	2 (50)	34	11 (32.4)
Grand total		57	12 (21.1)	75	13 (17.3)	50	17 (34.0)	23	10 (43.5)	32	11 (34.2)	237	63 (26.6)
	Mid-Pex. 95%CI of grand total		11.9-33.1		9.9-27.2		21.9-47.9		24.6-63.9		19.9-5.9		21.3-32.5

*Sample was not taken

Table A - 03: Distribution of *Salmonella* serovars in positive sampling locations of the abattoir line

Orgin/ Source	Sampling locations*	Total No. of isolates	*Salmonella* serovar type and number in bracket
Environment	Personnel's hands	5	*S.* Saintpaul (4), *S.* Kastrup (1)
	Aprons	5	*S.* Saintpaul (1), *S.* Larochelle (1), S. Muenchen (3)
	Knives	4	*S.* Saintpaul (1), *S.* Larochelle (1), *S.* Muenchen (2)
	Water	1	*S.* Saintpaul (1)
	Hooks	2	*S.* Larochelle (1), *S.* Muenchen (1)
	Rooms	9	*S.* Saintpaul (4), *S.* Muenchen (2), *S.* Larochelle (2), *S.* Dublin (1)
	Refrigerators	6	*S.* Saintpaul (1), *S.* Larochelle (1), *S.* Muenchen (4)
	Trucks	5	*S.* Saintpaul (4), *S.* Muenchen (1)
	Sub total	37	
ARM	Feces	8	*S.* Saintpaul (2), *S.* Larochelle (2), *S.* Dublin (1), *S.* Kastrup (1), unidentified (2)
	MLN*	3	*S.* Saintpaul (1), *S.* Muenchen (1), *S.* Kastrup (1)
	Raw meat	4	*S.* Saintpaul (2), *S.* Larochelle (1), *S.* Dublin (1)
	Sub total	15	
Butchers	Beef at butchers	11	*S.* Saintpaul (6), *S.* Larochelle (2), *S.* London (1), *S.* Dublin (1), Unidentified (1)
Total		63	

ARM = Animal-related materials
MLN* = Mesenteric lymph node

Table A - 04: Distribution of 63 *Salmonella* isolates among serotypes and *XbaI* pulsotypes by sampling location and type of samples from the abattoir line

Sample origin	Sampling location	Source of swab/ Sample type	No. of isolates	No. of *Salmonella* serotypes	Pulsotypes	Ratio isolates/ serotypes	Ratio isolates/ pulsotypes
Abattoir Environment	Personnel's hands	Hands	5	2	2	2.5	2.5
	Aprons	Aprons	5	3	3	1.67	1.67
	Knives	Knives	4	3	3	1.33	1.33
	Tap water	Water	1	1	1	1	1
	Hooks	Hooks	2	2	2	1	1
	Rooms	Rooms	9	4	6	2.25	1.5
	Refrigerator	Refrigerators	6	3	3	2	2
	Meat transport truck	Trucks	5	2	2	2.5	2.5
ARM¶	Stunning	Animal feces	8	5	4	1.6	2
	Evisceration	MLN* sample	3	3	3	1	1
	Quality inspection	Raw meat sample	4	3	3	1.33	1.33
Butchers	Beef for public consumption	Retailed meat sample	11	5	7	2.2	1.56
Total			63				

ARM¶ = Animal-related materials MLN* = Mesenteric lymph node

Table A - 05: *E. coli* isolations by sampling locations and type of samples from the abattoir line

Line	Sample origin	Processing stages /position	Sampling location	Source of swab/ Sample type	No. of samples	No. (%) *E. coli* positive	Mid-Pex. 95% CI
Abattoir line	Abattoir[1] Environment	Before stunning and before operation starts	Personnel's hands	Hands	13	6 (46.2)	21.3-72.6
			Aprons	Aprons	14	6 (42.9)	19.6-68.8
			Knives	Knives	13	5 (38.5)	15.7-65.9
			Tap water	Water sample	12	4 (33.3)	11.6-62.3
			Hooks	Hooks	11	4 (36.2)	12.8-66.4
		At carcass splitting	Rooms	Rooms	17	7 (41.2)	20.1-65.0
		Refrigeration	Refrigerators	Refrigerator	10	5 (50.0)	21.2-78.8
		Meat transport	Meat transport trucks	Truck	11	4 (36.4)	12.8-66.4
		Sub total			101	41 (40.6)	31.4-50.4
	ARM[¶]	Before stunning	Stunning	Animal feces	34	18 (52.9)	36.3-69.1
		During evisceration	Evisceration	MLN* sample	34	16 (47.1)	30.9-63.7
		After washing, ready for distribution	Quality inspection	Raw meat sample	34	19 (55.9)	39.1-71.7
		Sub total			102	53 (52.0)	42.3-61.5
	Butchers	Butchers, 6-8 hrs post delivery	Beef for public consumption	Retail meat sample	34	13 (38.2)	23.2-55.2
	Total				237	107 (45.1)	38.9-51.5

ARM[¶] = Animal-related materials, MLN* = Mesenteric lymph node, [1]Abbatoir chain after Gudeta (Gudeta, 2012)

Table A - 06: *E. coli* prevalences by sampling occasions and sample sourcesfrom the abattoir line

Source**	No. of samples	Positive No. (%)	Mid-Pex.95% CI	No. of	Positive No. (%)	Mid-Pex.95% CI	No. of	Positive No. (%)	Mid-Pex.95% CI	No. of samples	Positive No. (%)	Mid-Pex.95% CI	No. of samples	Positive No. (%)	Mid-Pex.95% CI	No. of samples	Positive No. (%)	Mid-Pex.95% CI
	No. of samples and *E. coli* positives by sampling occasion																	
	Occasion 1			**Occasion 2**			**Occasion 3**			**Occasion 4**			**Occasion 5**			**Total**		
Env't	25	7 (28.0)	13.2-47.7	27	8 (29.6)	14.8-48.6	22	3 (13.6)	3.6-32.8	11	8 (72.7)	42.2-92.5	16	15 (93.8)	72.8-99.7	101	41 (40.6)	31.4-50.4
ARM	24	10 (41.7)	23.5-61.8	36	23 (63.9)	47.4-78.2	21	4 (19.0)	6.4-39.8	9	7 (77.8)	43.8-96.1	12	9 (75.0)	45.9-93.2	102	53 (52.0)	42.3-61.5
BUT	8	3 (37.5)	10.6-72.2	12	5 (41.7)	17.2-69.8	7	1 (14.3)	0.7-53.0	3	2 (66.7)	13.2-98.3	4	2 (50.0)	9.4-90.6	34	13(38.2)	23.2-55.2
Total	57	20 (35.1)	23.6-48.1	75	36 (48.0)	36.9-59.3	50	8 (16.0)	7.7-28.1	23	17 (73.9)	53.4-88.7	32	26 (81.3)	65.0-92.0	237	107 (45.1)	38.9-51.5

** Env't and ARM are in the abattoir (Env't = Environment and ARM¶ = Animal-related materials)

BUT = Butchers

Table A - 07: *E. coli* prevalence by sampling occasions and locations from the abattoir line

Source	Sampling location	No. of samples and *E. coli* positives by sampling occasion											
		Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Total	
		No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)
Environment	Personnel's related swab samples												
	Personnel's hand swabs	3	1 (33.3)	4	1 (25.0)	3	1 (33.3)	1	1 (100)	2	2 (100)	13	6 (46.2)
	Aprons	4	2 (50.0)	4	1 (25.0)	3	0	1	1 (100)	2	2 (100)	14	6 (42.9)
	Knives	3	0	4	3 (75.0)	3	0	1	0	2	2 (100)	13	5 (38.5)
	Tap water	2	0	3	0	3	1 (11.1)	2	1 (50)	2	2 (100)	12	4 (33.3)
	Device swabs												
	Hooks	4	2 (50.0)	3	2 (66.7)	3	0	1	0	-	-	11	4 (36.2)
	Room floors	6	2 (33.3)	4	1 (25.0)	2	0	2	2 (100)	3	2 (66.7)	17	7 (41.2)
	Refrigerator	1	0	3	0	1	0	2	2 (100)	3	3 (100)	10	5 (50.0)
	Transporting truck	2	0	2	0	4	1 (25.0)	1	1 (100)	2	2 (100)	11	4 (36.4)
	Total	25	7 (28.0)	27	8 (29.6)	22	3 (13.6)	11	8 (72.7)	16	15 (93.8)	101	41 (40.6)
ARM¶	Animal feces	8	5 (62.5)	12	6 (50.0)	7	2 (28.6)	3	1 (33.3)	4	4 (100)	34	18 (52.9)
	MLN* sample	8	4 (50.0)	12	7 (58.3)	7	1 (14.3)	3	3 (100)	4	1 (25.0)	34	16 (47.1)
	Raw beef	8	1 (12.5)	12	10 (83.3)	7	1 (14.3)	3	3 (100)	4	4 (100)	34	19 (55.9)
	Subtotal	24	10 (41.7)	36	23 (63.9)	21	4 (19.0)	9	7 (77.8)	12	9 (75)	102	53 (52.0)
BUT	Retail meat samples	8	3 (37.5)	12	5 (41.7)	7	1 (14.3)	3	2 (66.7)	4	2 (50.0)	34	13 (38.2)
	Grand total	57	20 (35.1)	75	36 (48.0)	50	8 (16.0)	23	17 (73.9)	32	26 (81.3)	237	107 (45.1)
Grand total Mid-Pex.95% CI			23.6-48.1		36.9-59.3		7.7-28.1		53.4-88.7		65.0-92.0		38.9-51.5

ARM¶ = Animal-related materials
MLN* = Mesenteric lymph node
- = sample was not taken
BUT = Butchers

Table A - 08: APC by sampling occasions and locations at the processing plant line

Source	Sampling location	Numbers of samples and APC counts by sampling occasion															
		Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Occasion 6		Occasion 7		Occasion 8		Total	
		No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}	No. of samples	Mean ± SD Log_{10}
Environment	Personnel related swab samples																		
	Personnel's hands	2	5.19±0.21	2	5.43±0.04	2	4.92±0.35	1	5.32	4	4.63±0.57	5	5.43±0.14	2	4.98±0.08	1	4.76	19	5.09±0.42
	Aprons	2	5.22±0.08	2	5.38±0.15	2	5.05±0.14	1	5.52	3	5.14±0.31	3	5.32±0.41	2	5.33±0.36	1	5.59	16	5.28±0.27
	Knives	2	4.92±0.57	2	5.39±0.05	2	4.84±0.11	1	4.96	3	4.94±0.53	3	4.94±0.39	2	4.59±0.38	-	-	15	4.94±0.38
	Cutting plates	2	4.58±0.76	2	4.94±0.04	2	4.66±0.13	1	4.48	3	4.92±0.69	3	4.93±0.70	-	-	-	-	13	4.79±0.49
	Tap Water samples	3	4.88±0.06	2	4.51±0.11	3	4.83±0.58	2	4.49±0.08	1	3.86	2	4.55±0.09	2	3.86±0.42	2	4.68±0.51	17	4.54±0.45
	Device swab samples																		
	Working tables	1	4.96	1	5.04	2	5.35±0.12	2	4.93±0.00	2	4.77±1.14	5	4.93±0.59	2	5.38±0.29	2	5.14±0.26	17	5.05±0.47
	Rooms floor	2	5.31±0.07	1	5.45	2	5.26±0.02	2	4.22±0.37	2	4.91±0.04	3	4.58±0.03	2	5.64±0.18	2	5.17±0.45	16	5.01±0.48
	Refrigerator	1	4.96	1	5.18	2	4.90±0.03	2	4.66±0.01	2	5.09±0.50	4	4.49±0.71	2	4.76±1.27	1	4.32	15	4.75±0.56
	Spices related samples																		
	Spices samples	2	4.56±0.42	1	3.96	2	3.80±0.34	2	4.36±0.50	2	4.96±0.66	1	3.43	2	2.96±0.00	3	3.71±0.84	15	3.99±0.75
	SWE**	2	4.41±0.71	2	5.21±0.18	2	5.37±0.16	2	5.29±0.26	1	4.26	2	4.24±0.47	2	4.05±0.26	2	4.66±0.07	15	4.71±0.58
	Electrical machinery																		
	Grinder	1	4.67	1	5.38	1	5.82	1	4.86	1	4.86	1	4.43	2	5.41±0.47	1	4.04	9	4.99±0.59
	Cutter	1	4.57	1	4.36	1	3.96	1	5.18	1	4.63	1	4.46	2	4.02±1.50	1	3.66	9	4.32±0.70
	Mixer	1	4.58	1	4.42	1	5.86	1	5.43	1	4.97	1	4.87	2	5.21±0.40	1	3.74	9	4.92±0.64
	Filler/Stuffer	1	4.26	1	3.96	1	4.58	1	5.49	1	4.53	1	3.56	2	5.02±0.95	1	3.71	9	4.57±0.68
ARM¶	Raw beef	14	5.06±0.29	14	4.97±0.37	16	4.88±0.34	16	4.69±0.34	16	5.21±0.42	24	4.89±0.50	10	3.99±0.36	8	4.29±0.39	118	4.82±0.51

ARM¶ = Animal-related materials
SWE** = Spice-weighing equipment
SD = Standard deviation
- = sample was not taken

Table A - 09: EBC by sampling occasions and locations at the processing plant line

Source	Sampling location	Numbers of samples and EBC counts by sampling occasion																	
		Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Occasion 6		Occasion 7		Occasion 8		Total	
		No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$
Environment	Personnel related swab samples																		
	Personnel's hands	2	3.31±0.49	2	3.94±0.42	2	3.50±0.11	1	2.54	4	2.54±0.41	5	2.59±0.87	2	2.91±0.49	1	3.46	19	2.94±0.73
	Aprons	2	2.86±1.27	2	3.67±0.09	2	3.31±0.70	1	1.96	3	2.70±0.67	3	3.20±0.32	2	3.14±0.08	1	3.42	16	3.07±0.63
	Knives	2	3.35±1.29	2	2.61±0.07	2	3.23±0.52	1	1.96	3	2.84±0.83	3	2.70±0.69	2	2.86±0.42	-	-	15	2.84±0.66
	Cutting plates	2	3.00±1.05	2	3.73±0.74	2	3.07±0.30	1	1.96	3	2.87±0.19	3	2.91±0.46	-	-	-	-	13	2.99±0.61
	Tap Water samples	3	2.48±0.66	2	2.50±0.67	3	2.67±0.61	2	2.85±0.59	1	1.96	2	1.96±00	2	2.20±0.34	2	3.00±1.05	17	2.49±0.59
	Device swab samples																		
	Working tables	1	3.26	1	2.56	2	3.45±0.18	2	1.96±00	2	2.35±0.55	5	2.49±0.72	2	3.90±0.02	2	3.56±0.05	17	2.86±0.76
	Rooms floor	2	3.41±0.64	1	3.71	2	3.44±0.25	2	2.61±0.49	2	2.80±0.34	3	2.80±0.06	2	4.09±0.36	2	3.09±0.33	16	3.19±0.55
	Refrigerator	1	3.28	1	3.83	2	3.48±0.38	2	2.20±0.33	2	2.11±0.21	4	2.97±0.81	2	3.31±1.48	1	2.91	15	2.94±0.79
	Spices related samples																		
	Spices samples	2	3.27±0.74	1	1.96	2	2.31±0.49	2	2.46±0.28	2	1.96±0.00	1	1.96	2	1.96±0.00	3	2.12±0.27	15	2.28±0.52
	SWE**	2	2.62±0.93	2	3.83±0.30	2	3.95±0.28	2	1.96±0.00	1	2.56	2	2.20±0.34	2	2.26±042	2	2.96±0.06	15	2.81±0.80
	Electrical machinery																		
	Grinder	1	3.00	1	4.07	1	3.96	1	2.66	1	2.91	1	2.65	2	2.55±0.16	1	2.91	9	3.03±0.59
	Cutter	1	2.89	1	3.04	1	2.26	1	1.96	1	2.81	1	1.96	2	2.26±0.42	1	1.96	9	2.37±0.45
	Mixer	1	2.96	1	1.96	1	4.69	1	2.86	1	3.32	1	3.30	2	2.87±1.28	1	1.96	9	2.98±0.93
	Filler/Stuffer	1	2.81	1	2.43	1	2.56	1	3.74	1	1.96	1	1.96	2	2.41±0.21	1	1.96	9	2.47±0.57
ARM	Raw beef	14	3.26±0.37	14	2.71±0.61	16	2.82±0.50	16	2.87±0.44	16	2.54±0.69	24	2.72±0.68	10	2.28±0.33	8	2.91±0.33	118	2.77±0.59

ARM[¶] = Animal-related materials
SD = Standard deviation
SWE** = Spice-weighing equipment
- = sample was not taken

Table A - 10: EBC in mortadella by sampling occasions at supermarkets

Source Supermarket Code	Numbers of samples and *EBC* by sampling occasion											
	Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5			
	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$	No. of samples	Mean ± SD Log $_{10}$
A	4	2.16±0.39 ^{A}	2	3.07±0.30 ^{A}	2	1.96±0.00 ^{G}	4	1.96±0.00 ^{G}	3	2.26±0.52 ^{A}	15	2.22±0.46 ^{A}
B	4	2.37±0.49 ^{A}	2	2.50±0.09 ^{A}	2	1.96±0.00 ^{G}	4	1.96±0.00 ^{G}	3	2.16±0.17 ^{A}	15	2.18±0.32 ^{A}
C	3	2.06±0.17 ^{A}	4	2.17±0.43 ^{A}	4	1.96±0.00 ^{G}	2	1.96±0.00 ^{G}	2	2.85±0.29 ^{A}	15	2.16±0.37 ^{A}
D	2	2.86±0.07 ^{A}	2	2.50±0.09 ^{A}	4	1.96±0.00 ^{G}	3	2.22±0.45 ^{A}	3	1.96±0.00 ^{G}	14	2.22±0.38 ^{A}
E	2	2.39±0.60 ^{A}	4	2.87±0.61 ^{A}	4	1.96±0.00 ^{G}	2	1.96±0.00 ^{G}	3	2.12±0.27 ^{A}	15	2.29±0.52 ^{A}
F	2	2.26±0.42 ^{A}	4	2.17±0.43 ^{A}	4	1.96±0.00 ^{G}	2	1.96±0.00 ^{G}	3	2.87±0.40 ^{A}	15	2.24±0.44 ^{A}
G	2	1.96±0.00 ^{G}	3	2.12±0.27 ^{A}	2	1.96±0.00 ^{G}	4	2.19±0.29 ^{A}	4	2.76±0.23 ^{A}	15	2.26±0.38 ^{A}
H	2	2.11±0.21 ^{A}	-	-	4	2.04±0.15 ^{A}	5	2.02±0.13 ^{A}	4	2.19±0.29 ^{A}	15	2.08±0.19 ^{A}
Total	21	2.26±0.38 ^{A}	21	2.44±0.49 ^{A}	26	1.97±0.06 ^{G}	26	2.04±0.20 ^{A}	25	2.38±0.43 ^{A}	119	2.21±0.39 ^{A}

- = sample was not taken SD = Standard deviation ^{G} = Good ^{A} = Acceptable ^{U} = Unsatisfactory

Table A - 11: *Salmonella* prevalences by sampling occasions, type and source of samples from the processing plant line

Source	Sample type	Numbers of samples and *Salmonella* positives by sampling occasion																	
		Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Occasion 6		Occasion 7		Occasion 8		Total	
		No. of samples	Positive No (%)	No. of samples	Positive No (%)	No. of samples	Positive No (%)	No. of samples	Positive No (%)	No. of samples	Positive No (%)	No. of samples	Positive No (%)	No. of samples	Positive No (%)	No. of samples	Positive No (%)	No. of samples	Positive No (%)
Env't	Env't samples	23	1 (4.3)	20	1 (5.0)	25	0	20	1 (5.0)	27	1 (3.7)	35	2 (5.7)	26	2 (7.7)	18	2 (11.1)	194	10 (5.2)
	Mid-Pex 95% CI		0.2-19.6		0.2-22.3		0-11.3		0.2-22.3		0.2-16.9		0.9-17.6		1.3-23.2		1.9-32.1		2.6-8.9
ARM[¶]	Raw beef	14	3 (21.4)	14	4 (28.6)	16	1 (6.3)	16	0	16	0	24	2 (8.3)	10	1 (10.0)	8	1 (12.5)	118	12(10.2)
	Mid-Pex 95% CI		5.7-47.9		9.8-55.5		0.3-27.2		0-17.1		0-17.1		1.4-24.9		0.5-40.4		0.6-48.0		5.6-16.6
Total		37	4 (10.8)	34	5 (14.7)	41	1 (2.4)	36	1 (2.8)	43	1 (2.3)	59	4 (6.8)	36	3 (8.3)	26	3 (11.5)	312	22 (7.1)
Mid-Pex. 95% CI of total			3.5-24.1		5.5-29.6		0.1-11.4		0.1-12.9		0.1-10.9		2.1-15.5		2.1-21.0		3.0-28.3		5.6-10.3

Env't = Environmental ARM[¶] = Animal-related materials

Table A - 12*: Salmonella* positive samples and prevalences by sampling occasions and locations from the processing plant line

Source	Sampling location	*Salmonella* positive samples by numbers and sampling occasions																	
		Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Occasion 6		Occasion 7		Occasion 8		Total	
		No. of samples		No. of samples		No. of samples		No. of samples		No. of Samples		No. of samples		No. of samples		No. of samples		No. of samples	
		Tested	Positive No. (%)	Tested	Positive No. (%)	Tested	Positive No. (%)	Tested	Positive No. (%)	Tested	Positive No. (%)	Tested	Positive No. (%)	Tested	Positive No. (%)	Tested	Positive No. (%)	Tested	Positive No. (%)
Environment	Personnel related swab samples																		
	Personnel's hands	2	0	2	0	2	0	1	0	4	0	5	0	2	1 (50.0)	1	0	19	1 (5.2)
	Aprons	2	0	2	0	2	0	1	0	3	0	3	0	2	0	1	0	16	0
	Knives	2	0	2	0	2	0	1	0	3	0	3	0	2	0			15	0
	Cutting plates	2	0	2	0	2	0	1	0	3	1 (33.3)	3	0					13	1 (7.7)
	Tap water	3	0	2	0	3	0	2	0	1	0	2	0	2	0	2	0	17	0
	Device related swab samples																		
	Working tables	1	0	1	1 (100)	2	0	2	0	2	0	5	2 (40.0)	2	0	2	0	17	3 (17.7)
	Rooms floor	2	0	1	0	2	0	2	1 (50.0)	2	0	3	0	2	1 (50.0)	2	1 (50.0)	16	3 (18.7)
	Refrigerator	1	0	1	0	2	0	2	0	2	0	4	0	2	0	1	0	15	0
	Spices related samples																		
	Spices	2	0	1	0	2	0	2	0	2	0	1	0	2	0	3	0	15	0
	SWE	2	0	2	0	2	0	2	0	1	0	2	0	2	0	2	0	15	0
	Electrical machinery related swab samples																		
	Grinder	1	0	1	0	1	0	1	0	1	0	1	0	2	0	1	0	9	0
	Cutter	1	0	1	0	1	0	1	0	1	0	1	0	2	0	1	1 (100)	9	1 (11.1)
	Mixer	1	0	1	0	1	0	1	0	1	0	1	0	2	0	1	0	9	0
	Filler/Stuffer	1	1 (100)	1	0	1	0	1	0	1	0	1	0	2	0	1	0	9	1 (11.1)
	Sub total	23	1 (4.3)	20	1 (5.0)	25	0	20	1 (5.0)	27	1 (3.7)	35	2 (5.7)	26	2 (7.7)	18	2 (11.1)	194	10 (5.2)
ARM	Raw beef	14	3 (21.4)	14	4 (28.6)	16	1 (6.3)	16	0	16	0	24	2 (8.3)	10	1 (10.0)	8	1 (12.5)	118	12 (10.2)
Total		37	4 (10.8)	34	5 (14.7)	41	1 (2.4)	36	1 (2.8)	43	1 (2.3)	59	4 (6.8)	36	3 (8.3)	26	3 (11.5)	312	22 (7.1)
	Mid Pex. 95%CI of total		(3.5-24.1)		(5.5-29.6)		(0.1-11.4)		(0.1-12.9)		(0.1-10.9)		(2.1-15.5)		(2.1-21.0)		(3.0-28.3)		5.6-10.3

ARM = Animal related SWE = Spice-weighing equipment swabs

Table A - 13: *E. coli* prevalence by sampling occasions of environment and animal-related materials from the processing plant line

		Numbers of samples and *E. coli* positives by sampling occasion																	
		Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Occasion 6		Occasion 7		Occasion 8		Total	
Source	**Sample type**	**No. of samples**	**Positive No. (%)**	**No. of**	**Positive No. (%)**	**No. of samples**	**Positive No. (%)**	**No. of**	**Positive No. (%)**	**No. of**	**Positive No. (%)**	**No. of**	**Positive No. (%)**	**No. of**	**Positive No. (%)**	**No. of**	**Positive No. (%)**	**No. of samples**	**Positive No. (%)**
Env't	Environmental samples	23	9 (39.1)	20	5 (25.0)	25	5 (20.0)	20	8 (40.0)	27	18 (66.7)	35	22 (62.9)	26	17 (65.4)	18	14 (77.8)	194	98 (50.5)
	Mid-Pex. 95% CI		21.1-59.8		9.8-40.0		7.7-38.9		20.6-62.1		47.6-82.4		46.1-77.5		45.9-81.6		54.7-92.5		43.5-57.5
ARM ¶	Raw beef	14	5 (35.7)	14	11 (78.6)	16	5 (31.3)	16	11 (68.7)	16	8 (50.0)	24	15 (62.5)	10	6 (60.0)	8	6 (75.0)	118	67 (56.5)
	Mid-Pex. 95% CI		14.4-62.4		52.1-94.2		12.5-56.3		43.7-87.5		26.6-72.4		42.2-79.9		29.1-85.8		38.8-95.6		47.7-65.5
Total		37	14 (37.8)	34	16 (47.1)	41	10 (24.4)	36	19 (52.8)	43	26 (60.5)	59	37 (62.7)	36	23 (63.9)	26	20 (76.9)	312	165 (52.9)
Mid-Pex. 95% CI of total			23.4-54.1		30.8- 63.7		13.1-39.2		36.5-68.5		45.4-74.2		49.9-74.3		47.4-78.2		58.0-90.0		47.3-58.4

Env't = Environment
ARM¶ = Animal-related materials

Table A - 14: *E. coli* prevalence by sampling occasions and locations from the processing plant line

Source	Sampling location	Numbers of samples and *E. coli* positives by sampling occasion																Total	
		Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Occasion 6		Occasion 7		Occasion 8			
		No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)
Environment	Personnel related swab samples																		
	Personnel's hands	2	1 (50.0)	2	1 (50.0)	2	0	1	0	4	3 (75.0)	5	2 (40.0)	2	2 (100)	1	1 (100)	19	10 (52.6)
	Aprons	2	0	2	0	2	2 (100)	1	0	3	2 (66.7)	3	3 (100)	2	2 (100)	1	1 (100)	16	10 (62.59
	Knives	2	2 (100)	2	0	2	0	1	0	3	2 (66.7)	3	2 (66.7)	2	2 (100)	-	-	15	8 (53.5)
	Cutting plates	2	1 (50.0)	2	1 (50.0)	2	0	1	0	3	3 (100)	3	3 (100)	-	-	-	-	13	8 (61.5)
	Tap water samples	3	1 (33.3)	2	0	3	0	2	1 (50.0)	1	0	2	0	2	0	2	2 (100)	17	4 (23.5)
	Device swab samples																		
	Working tables	1	0	1	0	2	0	2	0	2	1 (50.0)	5	3 (60.0)	2	2 (100)	2	2 (100)	17	8 (47.1)
	Rooms floor	2	0	1	1 (100)	2	0	2	2 (100)	2	2 (100)	3	3 (100)	2	2 (100)	2	2 (100)	16	12 (75.0)
	Refrigerator	1	1 (100)	1	1 (100)	2	2 (100)	2	1 (50.0)	2	1 (100)	4	3 (75.0)	2	1 (50.0)	1	0	15	10 (66.7)
	Spices related samples																		
	Spices samples	2	0	1	0	2	0	2	1 (50.0)	2	0	1	0	2	0	3	1 (33.3)	15	2 (13.3)
	SWE**	2	2 (100)	2	1 (50.0)	2	0	2	0	1	1 (100)	2	1 (50.0)	2	0	2	2 (100)	15	7 (46.7)
	Electrical machinery																		
	Grinder	1	0	1	0	1	0	1	1 (100)	1	1 (100)	1	1 (100)	2	2 (100)	1	1 (100)	9	6 (66.7)
	Cutter	1	0	1	0	1	0	1	0	1	1 (100)	1	1 (100)	2	1 (50.0)	1	1 (100)	9	3 (33.4)
	Mixer	1	0	1	0	1	1 (100)	1	1 (100)	1	1 (100)	1	1 (100)	2	1 (50.0)	1	0	9	5 (55.6)
	Filler/Stuffer	1	1 (100)	1	0	1	0	1	1 (100)	1	0	1	0	2	2 (100)	1	1 (100)	9	5 (55.6)
	Sub total	23	9 (39.1)	20	5 (25.0)	25	5 (20.0)	20	8 (40.0)	27	18(66.7)	35	22(62.9)	26	17(65.4)	18	14(77.8)	194	98 (50.5)
ARM[¶]	Raw beef	14	5 (35.7)	14	11 (78.6)	16	5 (31.3)	16	11 (68.7)	16	8 (50.0)	24	15 (62.5)	10	6 (60.0)	8	6 (75.0)	118	67 (56.8)
Total		37	14 (37.8)	34	16 (47.1)	41	10 (24.4)	36	19 (52.8)	43	26 (60.5)	59	37 (62.7)	36	23 (63.9)	26	20 (76.9)	312	165 (52.9)
Mid-Pex. 95% CI of total		23.4-54.1		30.8- 63.7		13.1-39.2		36.5-68.5		45.4-74.2		49.9-74.3		47.4-78.2		58.0-90.0		47.3-58.4	

ARM[¶] = Animal-related materials;
- = sample was not taken;
SWE** = Spice-weighing equipment

Table A - 15: *E. coli* prevalence in beef mortadella by sampling occasions at supermarkets

Source Supermarket Code	Number of samples and *E. coli* positives by sampling occasion											
	Occasion 1		Occasion 2		Occasion 3		Occasion 4		Occasion 5		Total	
	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)	No. of samples	Positive No. (%)
A	4	1 (25.0)	2	2 (100)	2	0	4	0	3	0	15	3 (20.0)
B	4	1 (25.0)	2	2 (100)	2	0	4	0	3	1 (33.3)	15	4 (26.7)
C	3	1 (33.3)	4	2 (50.0)	4	1 (25.0)	2	0	2	0	15	4 (26.7)
D	2	2 (100)	2	2 (100)	4	0	3	1 (33.3)	3	1 (33.3)	14	6 (42.9)
E	2	1 (50.0)	4	4 (100)	4	0	2	0	3	0	15	5 (33.3)
F	2	1 (50.0)	4	1 (25.0)	4	0	2	0	3	0	15	2 (13.3)
G	2	0	3	3 (100)	2	1 (50.0	4	2 (50.0)	4	2 (50.0)	15	8 (53.3)
H	2	1 (50.0)	-	-	4	1 (25.0)	5	1 (20.0)	4	0	15	3 (20.0)
Total	21	8 (38.1)	21	16 (76.2)	26	3 (11.5)	26	4 (15.4)	25	4 (16.0)	119	35 (29.4)
Mid-Pex.95% CI of total		19.5-59.7		54.8-90.7		3.0-28.3		5.1-33.8		5.3-34.2		21.7-38.1

- = sample was not taken

Table A - 16: Antimicrobial susceptibility/resistance of S*almonella* isolates from the abattoir line by sampling occasion

Drug	Antimicrobial susceptibility/resistance of *Salmonella* by sampling occasion														
	Occasion 1 (n= 12)			Occasion 2 (n=13)			Occasion 3 (n=17)			Occasion 4 (n= 10)			Occasion 5 (n=11)		
	S* No. (%)	I* No. (%)	R* No. %	S* No. (%)	I* No. (%)	R* No. %	S* No. (%)	I* No. (%)	R* No. %	S* No. (%)	I* No. (%)	R* No. %	S* No. (%)	I* No. (%)	R* No. %
PB	11 (91.7)	0	1 (8.3)	13 (100)	0	0	17 (100)	0	0	9 (90.0)	0	1 (10.0)	11 (100)	0	0
CN	12 (100)	0	0	13 (100)	0	0	17 (100)	0	0	10 (100)	0	0	11 (100)	0	0
C*	11 (91.7)	0	1 (8.3)	13 (100)	0	0	17 (100)	0	0	10 (100)	0	0	9 (18.8)	0	2 (18.2)
W	12 (100)	0	0	13 (100)	0	0	17 (100)	0	0	10 (100)	0	0	10 (90.9)	0	1 (9.1)
SXT	12 (100)	0	0	13 (100)	0	0	17 (100)	0	0	9 (90.0)	1 (10.0)	0	11 (100)	0	0
N	6 (50.0)	6 (50.0)	0	8 (61.5)	3 (23.1)	2 (15.4)	9 (52.9)	5 (29.4)	3 (17.7)	3 (30.0)	4 (40.0)	3 (30.0)	6 (54.5)	3 (27.3)	2 (18.2)
OT	4 (33.3)	0	8 (66.7)	4 (30.8)	1 (7.7)	8 (61.5)	2 (11.8)	0	15 (88.2)	5 (50.0)	1 (10.0)	4 (40.0)	5 (45.5)	0	6 (54.5)

S* = susceptible; I* = intermediate; R* = resistant; C* = the only available breakpoint is for susceptibility

Table A - 17*:* Antimicrobial susceptibility/resistance of *Salmonella* isolates by sample type from the abattoir line

Sampling location	***No. of Salmonella***	**PBÞ**		**CNÞ**		**CÞ**		**Wdb**		**SXTdb**		**N**		**OT**			
		S.* No. (%)	**R*. No. (%)**	**S.* No. (%)**	**R*. No. (%)**	**S.* No. (%)**	**R*. No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**
Personnel related swab samples																	
Personnel's hands	5	5(100)	0	5(100)	0	5(100)	0	5(100)	0	5(100)	0	4(80)	1(20)	0	1(20)	0	4 (80)
Aprons	5	5(100)	0	5(100)	0	4(80)	1 (20)	4(80)	1(20)	4(80)	1(20)	3 (60)	1 (20)	1 (20)	0	0	5 (100)
Knives	4	4(100)	0	4(100)	0	4(100)	0	4(100)	0	3(75)	1 (25)	1 (25)	1 (25)	2 (50)	0	0	4(100)
Tap water	1	1(100)	0	1(100)	0	1(100)	0	1(100)	0	1(100)	0	1(100)	0	0	0	1(100)	0
Swabs from devices																	
Hooks	2	2(100)	0	2(100)	0	2(100)	0	2(100)	0	2(100)	0	0	2(100)	0	1(50)	0	1(50)
Room floor	9	8(88.9)	1(11.1)	8(88.9)	0	7 (77.8)	2 (22.2)	8(88.9)	0	8(88.9)	0	3 (33.3)	5(55.6)	1(11.1)	4(44.4)	0	5(55.6)
Refrigerators	6	6(100)	0	6(100)	0	6(100)	0	6(100)	0	6(100)	0	4(66.7)	2(33.3)	0	1(16.7)	0	5(83.3)
Transport truck	5	5(100)	0	5(100)	0	5(100)	0	5(100)	0	5(100)	0	3(60)	1(20)	1(20)	0	0	5(100)
Animal feces	8	8(100)	0	8(100)	0	8(100)	0	8(100)	0	8(100)	0	3(37.5)	2(25)	3(37.5)	5(62.5)	0	3(37.5)
MLN* sample	3	3(100)	0	3(100)	0	3(100)	0	3(100)	0	3(100)	0	0	1(33.3)	2(66.7)	1(33.3)	0	2(66.7)
Raw beef	4	4(100)	0	3(75)	1(25)	4(100)	0	4(100)	0	4(100)	0	3(75)	1(25)	0	2(50)	0	2 (50)
Retail meat sample	11	11(100)	0	11(100)	0	11(100)	0	11(100)	0	11(100)	0	7(63.6)	4(36.4)	0	5(45.5)	1(9.0)	5(45.4)

*sample was not taken

Þ = intermediate was not observed; ɗb = resistance was not observed

S* = susceptible; I* = intermediate; R* = resistant; C* = the only available breakpoint is for susceptibility

MLN* = Mesenteric lymph node

Table A - 18: Antimicrobial susceptibility/resistance of *Salmonella* isolates from the processing plant by sampling occasions

	Antimicrobial susceptibility/resistance of *Salmonella* by sampling occasion																					
	Occasion 1 (n=4)			Occasion 2 (n= 5)			Occasion 3 (n=1)ɗb	Occasion 4 (n=1)			Occasion 5 (n=1) ɗb	Occasion 6 (n=4)			Occasion 7 (n=3)			Occasion 8 (n=3)		
Drugs	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)
PB	4(100)	0	0	5(100)	0	0	1(100)	1(100)	0	0	1(100)	4(100)	0	0	3(100)	0	0	3(100)	0	0
CN	4(100)	0	0	5(100)	0	0	1(100)	1(100)	0	0	1(100)	4(100)	0	0	3(100)	0	0	2(66.7)	0	1(33.3)
C*	4(100)	0	0	5(100)	0	0	1(100)	1(100)	0	0	1(100)	4(100)	0	0	3(100)	0	0	3(100)	0	0
W	4(100)	0	0	5(100)	0	0	1(100)	1(100)	0	0	1(100)	4(100)	0	0	2(66.7)	0	1(33.3)	3(100)	0	0
SXT	4(100)	0	0	5(100)	0	0	1(100)	1(100)	0	0	1(100)	4(100)	0	0	2(66.7)	0	1(33.3)	3(100)	0	0
N	1(25)	2(50)	1(50)	1(20)	3(60)	1(20)	1(100)	1(100)	0	0	1(100)	3(75)	1(25)	0	2(66.7)	1(33.3)	0	2(66.7)	1(33.3)	0
OT	4(100)	0	0	5(100)	0	0	1(100)	1(100)	0	0	1(100)	2(50)	0	2(50)	2(66.7)	0	1(33.3)	2(66.7)	0	1(33.3)

S* = susceptible; I* = intermediate; R* = resistant; C* = the only available breakpoint is for susceptibility; ɗb = all isolates are susceptible

Table A - 19: Antimicrobial susceptibility/resistance of *Salmonella* isolates by origin and type of sample from the processing plant line[¶]

Sample origin		Sample type	No. of tests	CN*	C*		W**		SXT**		N			OT**		
				S.* No. (%)	S.* No. (%)	I.* No. (%)	S*. No. (%)	R.* No. (%)	S*. No. (%)	R.* No. (%)	S*. No. (%)	I.* No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)
Processing plant	Environment	Hand swab	1	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)	0	1(100)	0	0	0	1(100)
		Plate swab	1	1(100)	1(100)	0	1(100)	0	1(100)	0	1(100)	0	0	1(100)	0	0
		Table swab	3	3(100)	3(100)	0	3(100)	0	3(100)	0	0	1(33.3)	2(66.7)	3(100)	0	0
		Room swab	3	3(100)	3(100)	0	3(100)	0	3(100)	0	2(66.7)	1(33.3)	0	2(66.7)	0	1(33.3)
		Cutter swab	1	1(100)	1(100)	0	1(100)	0	1(100)	0	0	1(100)	0	1(100)	0	0
		Filler/Stuffer swab	1	1(100)	1(100)	0	1(100)	0	1(100)	0	1(100)	0	0	1(100)	0	0
	ARM[¶]	Raw meat	12	12(100)	11(91.7)	1(8.3)	12(100)	0	12(100)	0	3(25)	8(66.7)	1(8.3)	10(83.2)	0	2(16.7)
Supermarkets		Supermarket-B	1	1(100)	1(100)	0	1(100)	0	1(100)	0	1(100)	0	0	1(100)	0	0

[¶]All are susceptible to PB
No resistant strain for CN* and C*
No intermediate strain for C*; W**, SXT** and OT**
S* = susceptible; I* = intermediate; R* = resistant
ARM[¶] = Animal-related materials
SWE** = Spice-weighing equipment

Table A - 20: Antimicrobial susceptibility/resistance of *E. coli* isolates from the abattoir line by sampling occasion

Drug	Antimicrobial susceptibility/resistance of *E. coli* by sampling occasion														
	Occasion 1 (n= 20)			Occasion 2 (n=36)			Occasion 3 (n=8)			Occasion 4 (n= 17)			Occasion 5 (n=26)		
	S*	I*	R*	S* No.	I*	R*	S*	I*	R*	S*	I*	R*	S*	I*	R*
	No. (%)	No. (%)	No. (%)	(%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
PB	20 (100)	0	0	36 (100)	0	0	8 (100)	0	0	17 (100)	0	0	26 (100)	0	0
CN	18 (90.0)	0	2 (10.0)	34 (94.4)	2 (5.6)	0	8 (100)	0	0	17 (100)	0	0	25 (96.2)	0	1 (3.8)
C*	20 (100)	-	-	34 (94.4)	-	2 (5.6)	8 (100)	-	-	17 (100)	-	-	24 (92.3)	-	2 (7.7)
SXT	16 (80.0)	0	4 (20.0)	34 (94.4)	0	2 (5.6)	5 (62.5)	0	3 (37.5)	16 (94.1)	0	1 (5.9)	24 (92.3)	0	2 (7.7)
AML	12 (60.0)	1 (5.0)	7 (35.0)	23 (63.9)	3 (8.3)	10 (27.8)	4 (50.0)	0	4 (50.0)	15 (88.2)	0	2 (11.8)	21 (80.8)	0	5 (19.2)
OT	8 (40.0)	0	12 (60.0)	18 (50.0)	2 (5.6)	16 (44.4)	2 (25.0)	2 (25.0)	4 (50.0)	9 (52.9)	3 (17.6)	5 (29.4)	16 (61.5)	0	10 (38.5)

S* = susceptible I* = intermediate R* = resistant C* = the only available breakpoint is for susceptibility

Table A - 21: Antimicrobial susceptibility/resistance of *E. coli* isolates by sample sources from the abattoir line

Sample origin	Sample type	No. tests	CN			C*			SXT			AML			OT		
			S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)	S.* No. (%)	I*. No. (%)	R.* No. (%)
Environment	Hand swabs	6	5 (83.3)	0	1 (16.7)	6 (100)	-	-	6 (100)	0	0	6 (100)	0	0	3 (50.0)	0	3 (50.0)
	Apron swabs	6	6 (100)	0	0	6 (100)	-	-	5 (83.3)	0	1 (16.7)	4 (66.7)	0	2 (33.3)	3 (50.0)	0	3 (50.0)
	Knive swabs	5	5 (100)	0	0	5 (100)	-	-	5 (100)	0	0	5 (100)	0	0	3 (60.0)	0	2 (40.0)
	Water sample	4	4 (100)	0	0	2 (50.0)	-	2(50.0)	2 (50.0)	0	2 (50.0)	2 (50.0)	0	2 (50.0)	0	0	4 (100)
	Hook swabs	4	4 (100)	0	0	4 (100)	-	-	2 (50.0)	0	2 (50.0)	2 (50.0)	0	2 (50.0)	2 (50.09	0	2 (50.0)
	Room swabs	7	7 (100)	0	0	6 (85.7)	-	1(14.3)	7 (100)	0	0	6 (85.7)	0	1 (14.3)	5 (71.4)	1 (14.3)	1 (14.3)
	Refrigerator	5	5 (100)	0	0	5 (100)	-	-	5 (100)	0	0	5 (100)	0	0	3 (60.0)	1 (20.0)	1 (20.0)
	Truck swabs	4	4 (100)	0	0	4 (100)	-	-	4 (100)	0	0	3 (75.0)	0	1 (25.0)	2 (50.0)	0	2 (50.0)
ARM¶	Animal feces	18	16 (88.9)	2 (11.1)	0	18 (100)	-	-	14 (77.8)	0	4 (22.2)	11 (61.1)	0	7 (38.9)	7 (38.9)	0	11 (61.1)
	MLN*	16	16 (100)	0	0	16 (100)	-	-	15 (93.7)	0	1 (6.3)	12 (75.0)	0	4 (25.0)	9 (56.3)	0	7 (43.7)
	Raw meat	19	18 (94.7)	0	1 (5.3)	19(100)	-	-	17 (89.5)	0	2 (10.5)	12 (63.1)	1 (5.3)	6 (31.6)	8 (42.1)	3 (13.8)	8 (42.1)
BUT	Retailed meat sample	13	12 (93.3)	0	1 (7.7)	13(100)	-	-	13 (100)	0	0	7 (53.8)	3 (23.1)	3 (23.1)	8 (61.5)	2 (15.4)	3 (23.1)

¶BP: all are PB susceptible C* = the only available breakpoint is for susceptibility S* = susceptible I* = intermediate R* = resistant
ARM¶ = Animal-related materials BUT = Butchers MLN* = Mesenteric lymph node

Table A - 22: Antimicrobial susceptibility/resistance of *E. coli* isolates from the processing plant by sampling occasion

	Antimicrobial susceptibility/resistance of *E. coli* by sampling occasion																			
	Occasion 1 (n=14)			**Occasion 2 (n= 16) ¶**		**Occasion 3 (n=10) ¶**		**Occasion 4 (n=19) ¶**		**Occasion 5 (n=26)¶**		**Occasion 6 (n=37)**			**Occasion 7 (n=23)**			**Occasion 8 (n=20)**		
Drugs	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**
PB	14 (100)	0	0	16 (100)	0	10 (100)	0	19 (100)	0	26 (100)	0	36 (97.3)	0	1 (2.7)	23 (100)	0	0	20 (100)	0	0
CN	14 (100)	0	0	16 (100)	0	10 (100)	0	19 (100)	0	26 (100)	0	35 (94.6)	2 (5.4)	0	23 (100)	0	0	20 (100)	0	0
C*	13 (92.9)	-	1 (7.1)	16 (100)	-	9 (90.0)	1 (10.0)	16 (84.2)	3 (15.8)	26 (100)	-	33 (89.2)	-	4 (10.4)	23 (100)	-	-	20 (100)	-	-
AML	12 (85.8)	1 (7.1)	1 (7.1)	14 (87.5)	2 (12.5)	9 (90.0)	1 (10.0)	17 (89.5)	2 (10.5)	21 (80.8)	5 (19.2)	23 (62.2)	1 (2.7)	13 (35.1)	17 (73.9)	1 (4.4)	5 (21.7)	20 (100)	0	0
SXT	14 (100)	0	0	15 (93.8)	1 (6.2)	9 (90)	1 (10)	18 (94.7)	1 (5.3)	26 (100)	0	34 (91.9)	0	3 (8.1)	20 (87)	0	3 (13)	20 (100)	0	0
OT	12 (85.7)	0	2 (14.3)	13 (81.3)	3 (18.7)	6 (60.0)	4 (40.0)	10 (52.6)	9 (47.4)	13 (50.0)	13 (50.0)	20 (54.1)	2 (5.4)	15 (40.5)	13 (56.5)	0	10 (43.5)	15 (75)	2 (10)	3 (15)

S* = Susceptible I* = Intermediate R* = Resistant
C* = the only available breakpoint is for susceptibility
¶ No intermediate resistant strain was observed

Table A - 23: Antimicrobial susceptibility/resistance of *E. coli* isolates from the supermarkets by sampling occasion

	Antimicrobial susceptibility/resistance of *E. coli* by sampling occasion															
	Occasion 1 (n=8)			**Occasion 2 (n=16)**			**Occasion 3 (n=3)**			**Occasion 4 (n=4)**			**Occasion 5 (n=4)**		
Drugs	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**	**S.* No. (%)**	**I*. No. (%)**	**R.* No. (%)**
PB	8 (100)	0	0	16 (100)	0	0	3 (100)	0	0	4 (100)	0	0	3 (75.0)	0	1 (25.0)
CN	8 (100)	0	0	15 (93.8)	1 (6.2)	0	2 (66.7)	1 (33.3)	0	4 (100)	0	0	4 (100)	0	0
C*	7 (87.5)	-	1 (12.5)	16 (100)	-	-	3 (100)	-	-	4 (100)	-	-	4 (100)	-	-
SXT	8 (100)	0	0	14 (87.5)	1 (6.3)	1 (6.3)	3 (100)	0	0	4 (100)	0	0	3 (75.0)	0	1 (25.0)
AML	7 (87.5)	0	1 (12.5)	13 (81.3)	0	3 (18.7)	1 (33.3)	0	2 (66.7)	3 (75.0)	1 (25.0)	0	2 (50.0)	0	2 (50.0)
OT	3 (37.5)	0	5 (62.5)	11 (68.8)	1 (6.2)	4 (25.0)	1 (33.3)	0	2 (66.7)	2 (50.0)	0	2 (50.0)	1 (25.0)	0	3 (75.0)

S* = Susceptible; I* = Intermediate; R* = Resistant; C* = the only available breakpoint is for susceptibility

11. PUBLICATIONS, ORAL PRESENTATIONS AND CONFERENCE

➢ Paper publications

- **Hiko, A**., Irsigler, H., Ameni, G., Zessin, K., Fries, R.. **(2016).** *Salmonella* serovars along two beef chains in Ethiopia. *The Journal of Infection in Developing Countries*, 10 (11): 1168-1176. doi:10.3855/jidc.6354.
- **Hiko A**, Gobena Ameni, Nina Langkabel, Reinhard Fries: (**2015**). Microbiological Load and Zoonotic Agents in Beef Mortadella from Addis Ababa City Supermarkets. *Journal of Food Protection,* **78** (5): 1043–1045
- **Hiko, A**., Bräutigam L., Ameni, G., Zessin, K., Fries, R.. **(Submitted). *XbaI* PFGE pattern of Salmonella serovars isolated along two beef chains in Ethiopia.** *Journal of Food Protection,*

➢ Oral and poster presentations

- **Hiko A.** (2015): Microbiological quality of beef mortadella: In the view of public health and meat export alternatives. 3rd International Conference on Vet Education **June 17-19, 2014/2015, Haramaya University**, Ethiopia.
- **Hiko A.,** Bräutigam L., Irsigler H., Ameni G., Zessin K-H., Fries R. (2014): Antimicrobial resistance and genotypic diversity of *Salmonella Saintpaul* isolated from beef production lines in Ethiopia 54th Interscientific Conference on Antimicrobial Agents and Chemotherapy (ICAAC®) 2014, **Washington DC, USA 5-9, September 2014.**
- **Hiko, A.,** Bräutigam, L., Irsigler, I., Ameni, G., Zessin, K.-H., Fries, R. (2014): Identification of possible transfer routes of *Salmonella* along two beef lines in Ethiopia. 14. Fachtagung Fleisch- und Geflügelfleischhygiene, **Berlin, Germany 4-5 March 2014.**
- **Hiko A**., Fries R. (2014): *Salmonella*: Cases for Ethiopia. International Postgraduate Masters Course of Masters of Veterinary Public Health (6th Batch). Institute of Meat Hygiene and Technology, Panel Veterinary Public Health and FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, **23 April 2014**.
- **Hiko A.,** Ameni G., Zessin K.-H., Fries R. (2013): Antimicrobial susceptibility and resistance profiles of *E. coli* isolates from meat processing chains. 54. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene (54 Workshop of the study area food hygiene) der

DVG, Garmisch-Partenkirchen, **Garmisch Partenkirchen, Germany, 24-27 September 2013.**

- **Participation on conference and events (major)**

  - ZIBI Summer Symposium "**One health-Maintaining a Healthy Planet"**. **Humboldt University , Berlin Germany, 3-11, June 2013**
  - Genomic sequencing analysis with focus on the in *Mycobacterium Tuberculosis* complex, AHRI, **Addis Ababa, Ethiopia,15-18, May 2012.**
  - Participating on 1st International Congress on Pathogens at Human-Animal Interface (ICOPHI): Impact, Limitation, and Needs in Developing Countries, **United Nation Conference Addis Ababa, Ethiopia, 15-17, Sept. 2011.**

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13. DECLARATION

I, **Adem Hiko**, declared that this thesis entitled with "**Zoonotic and spoilage bacteria in a meat production and a processing line in Ethiopia**" and the work presented in it are my own and has been generated by me as the result of my orgional research.

Adem Hiko
Date: 27.09.2017