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Isolation, DNase-cross-Coagulase test and antimicrobial resistance test on *Staphylococcus* along beef abattoir line in Addis Ababa Ethiopia

*Adem Hiko**

College of Veterinary Medicine, Haramaya University, Po. Box. 138, Dire Dawa, Ethiopia.

Correspondence: E. mail: adex.2010ph@gmail.com

Abstract

Staphylococci are responsible for foodborne infection and intoxication with the spread of antibiotic resistance. The aims of the study were to investigate beef abattoir line contamination with *Staphylococcal*, to evaluate DNase test for alternative of the tube coagulase test, and to assess isolates drug resistance in Ethiopia. A total of 169 samples from slaughter environment, raw beef at inspection and at public supply along Addis Ababa Abattoir Enterprise line were examined for *Staphylococci.* The isolates were tested against DNase, tube plasma coagulase, and eight medicinal drugs. A total proportion of 35.5% (60/169) isolates with 13.6% *S. aureus* and 21.9% coagulase negative *Staphylococcus* (CNS) were observed. All sampling locations were found positive for *Staphylococcus* environmental samples ranged from 18.2% - 46.2% with no difference ($p > 0.05$) among locations. In raw beef, it was 23.5% at abattoir and 52.9% at butchers. Three (1.9%), 13 (7.7%) and 23 (13.6%) of locations were positive for *S. aureus* only, CNS only and both as a mixed, respectively. Of all 60 *Staphylococci* isolates, the DNase test and coagulase tests were in agreement for 56 isolates (21 for positive, 35 for negative) showing DNase test was strong agreement with the gold standard test (coagulase tests), kappa=0.86)). *S. aureus* was 38.3% but CNS was 61.7%. Resistant isolates were observed for trimethoprim (35.0%), polymyxin-B (33.7%), oxytetracycline (31.7%), trimethoprim-sulfamethoxazole (20.0%), chloramphenicol (8.3%), oxacillin (6.7%), and gentamycin (5.0%) but not for tetracycline. Thirty-one (51.7%) isolates were resistant for at least one drug with multiple drugs resistance (MDR) of three to six in 17 isolates. Contamination of all sampling locations with *Staphylococcus* including with resistant isolate to medically used drugs warrants the application of good hygienic practices along the abattoir line. Due to availability and cost effectiveness, DNase can be used as alternative to the gold standard, coagulase test, for diagnosis of *Staphylococcus*.

Key words: Abattoir line; beef; drug resistance; foodborne intoxication; *Staphylococci*

Introduction

Staphylococcus species are classified as classical pathogen, causing infections (Waldvogel, 1995; Lowry, 1998). But, heterogeneous group of *coagulase-negative Staphylococci* (CNS) those historically as being less or nonpathogenic (Becker *et al.,* 2014). They are differentiated based on their diagnostic reaction. Carriers are at higher risk of infection and they are presumed to be an important source of spread of *S. aureus* strains among individuals, to food and food production and processing chain (Muto *et al*., 2003; Kazakova *et al*., 2005; Miller and Diep, 2008). A fundamental biological property of *S. aureus* is the ability to asymptomatically colonize normal people. Approximately 30% of humans are asymptomatic nasal carriers of *S. aureus* (Kluytmans *et al*., 1997; Gorwitz *et al.,* 2008). Due to patient and procedure related changes, CNS also represent one of typical opportunistic major nosocomial pathogens having a substantial impact on human life and health, and also food associated saprophytes resulted in clinically manifested infections (Becker *et al.,* 2014).

The ideal identification of clinical *S. aureus* isolates requires a battery of tests and this is costly in resource limited settings. *S. aureus* identification was routinely done using either human or sheep plasma. Study was also conducted using mannitol salt agar and the deoxyribonuclease (DNase) test for improving the efficiency of the tube coagulase test in resource limited settings (Kateete *et al*., 2010). Indicating the absence of single phenotypic test (including tube coagulase) that can guarantee as reliable method for identification of *S. aureus,* Kateete *et al.* (2010) suggested the need for sequel testing of the isolates with Mannitol salt agar, DNase and Tube coagulase. On the other hand, the coagulase test is a golden standard method to differentiate *S. aureus* from coagulase negative *Staphylococcus (*Koneman *et al.,* 1997*).* Coagulase production can be detected using either slide coagulase test or the tube coagulase test. The tube coagulase test is better than the slide coagulase test in that it detects the secreted extracellular free coagulase which reacts with a substance in plasma called "Coagulase-Reacting Factor" (CRF) to form a complex clot (Koneman *et al.,* 1997; Winn *et al.,* 2006).

The extended use and misuse of antibiotics in agriculture, livestock farming and human medicine have rapidly facilitated the emerging of resistant bacteria strains including *Staphylococci* (Valsangiacomo *et al*., 2000). Antibiotic resistance leads to prolonged hospital stay and increased costs in terms of treatment (Kitara *et al*., 2011). Multidrug resistance is norm among *Staphylococci* pathogens (Lowy, 2003).

Study along milk production, Gizaw (2014) reported *Staphylococci* species at 47.1% from udder milk, 58% from tank milk of the selected dairy farms, 34.4% from cow bucket swab, 38% from farm tank swab, 38% from milkers hand and 70% from nasal swab of milkers. Similarly, prevalence of 38.5% from carcass swab, 37.8% from knife swab, 48.6% from slaughter line swab, 37.8% from abattoir worker hand and 46.7% nasal swab of butchers were reported in Ethiopia (Gizaw, 2014). Indicating the significance of drug resistance, pooled prevalence of methicillin resistant *S. aureus* was reported at rate of 32.5% from several report meta-analysis data in Ethiopia (Eshetie *et al*., 2016). On the other hand, structured abattoir line-based *Staphylococci* survey with DNase test were not yet conducted in Ethiopia. Therefore, the aims of the study were to investigate beef abattoir line contamination with *Staphylococcal* strain, to evaluate DNase test as alternative of the tube coagulase test and to assess drug resistance status of the isolates against medicinal drugs frequently used in Ethiopia.

Materials and Methodology

Abattoir lines description

The study was conducted at Addis Ababa Abattoir Enterprise (AAAE) located in Addis Ababa city. Multi-purpose cattle stocks purchased from extensive or semi-intensive management systems in different parts of the Ethiopia, transported to AAAE and slaughtered for human consumption were used. The AAAE has a capacity to slaughter up to 1,200 cattle in 8 hours with a staff of about 700 persons. After slaughter, carcasses were examined through routine meat inspection procedures. The raw beef, immediately or after a short cooling interval were loaded into meat transport trucks and delivered to city butcheries. Butcheries are mostly small open-stall shops, handling the meat at 20°C–27°C, which is the ambient temperature in Addis Ababa City.

Sampling and sample types

Samples were taken from December 2011 to April 2012 from the abattoir and beef retailer butchery. In the abattoir, different samples were taken from the operation environment. Sample of raw beef were also taken directly after quality inspection at abattoir. Following the beef identification number, same raw beef product were sampled at butchery houses located at Addis Ababa. A total of 169 samples from 10 sampling locations (Table 1) were taken. For swabs, a 50 cm^2 area was swabbed with sterilized gauze moistened with normal saline solution. Water (20 mL) sample was filled directly from the tap into sterile calibrated glass bottles. Meat samples (34 samples from AAAE and 34 samples from butcheries) were taken aseptically and placed in sterile stomacher bags. The number and types of samples were described within Table 1 with the results. Samples were immediately transported to Microbiology Laboratory, Akililu Lemma Institute of Pathobiology, Addis Ababa University (ALIPB-AAU), Ethiopia on the day of sampling using an ice box at +4°C.

Sample preparation

Each sample was aseptically taken. For pre-enrichment, buffered peptone water (Merck, Darmstadt, Germany) was used and the inoculate were incubated at 37°C for 18–20 hours. The first 1:10 dilution was homogenized with a Stomacher 400 (Seward Laboratory, London, UK) and incubated at 37°C for 18–20 hours to be used as pre-enrichment (Monttville *et al.,* 2012; USDA, 2012).

Staphylococcus isolation and characterization

A loopfull of pre-enrichment was inoculated on whole-hen-egg based Kranep Agar (Merck, Germany). One to five presumptive *Staphylococci* colonies were taken per-each sample. Colonies were screened using 3% hydrogen peroxide. The presumptive positive *S. aureus* colonies isolate was transferred to Standard-II Nutrient Agar (Merck, Germany) incubated at 37°C for 24 hr, stored at +4°C. The strain was exposed to deoxyribonuclease (DNase) and tube plasma coagulase test in parallel. An overnight cultured colony was inoculated in forms of line (Fig. 1) on DNase Agar (Merck, Germany) and incubated for 18-24 hr at 37°C. The culture was over flushed with 1 ml 1 mole/ml Hydrochloric acid. Based up on DNA digestion zone of clear transparence surrounding the culture, the strains were termed as DNase positive. Parallel to DNase test, tube plasma coagulase test (Bactident Merck, Germany) was done. The colony was inoculated in Brain Heart Infusion (BHI) (Merck, Germany) incubated at 37°C

for 18-24hr. A 0.5ml of rabbit plasma (Bactident Merck, Germany) was added in to sterile test tube followed by addition of 0.1ml of culture suspension. The mixture was incubated in 37°C water bath with 1hr interval up to 4 hr with registration of degrees of coagulation. Finally, the tubes were kept overnight at room temperature and re-examined.

Considered and used test protocol

In *Staphylococcus* identification, coagulase production can be detected using either the slide coagulase test or the tube coagulase test (Winn *et al*., 2006). The tube coagulase is better than the slide coagulase test in that it detects the secreted extracellular free coagulase which reacts with a substance in plasma called "Coagulase-Reacting Factor" to form a complex clot (Koneman *et al*., 1997; Winn *et al*., 2006). Thus, coagulase testing was used as gold standard for differentia between *S. aureus* and coagulase negative *Staphylococcus (*CNS*).* Control reactions included templates of *Staphylococcus aureus* ATCC 25923 (positive control), *Staphylococcus epidermidis* ATCC 12228 and nuclease free water (negative controls).

Ethical Considerations

Samples were also collected from adult personnel's hands who are working in the abattoir. Individuals involved on the sample were informed regarding the details of the research objectives. Hence, informed consent was collected.

Antimicrobial resistance test

The test was done on Mueller-Hinton agar (Oxoid, Hampshire, England) using Kirby Bauer disc diffusion criteria of the National Committee for Clinical Laboratory Standards (Bauer *et al*., 1996; CLSI, 2012). The following antimicrobial substances (Oxoid UK) were used: chloramphenicol (C 50 µg), gentamycin (CN 10 µg), oxytetracycline (OT 30 µg), oxacillin (OX 1 µg), polymyxin B (PB 300U), tetracycline (TE 10 µg), trimethoprim-sulfamethoxazole (STX 1.25/23.75 µg) and trimethoprim (W 5 µg (Oxoid, Hampshire, England). They were selected using the fact that they are used in health sectors of Ethiopia and their availability on the markets. The isolates were sub-cultured on Nutrient agar (Merck, Germany) and incubated at 37°C for 24 hrs. They were then inoculated into 5 ml of Brain Heart Infusion broth (BHI) (Merck, Germany) and again incubated for 1 hr at 37°C. The inoculum density was standardized using a 0.5 McFarland standard. Volume of 0.1 ml of standardized culture was

spread on Mueller-Hinton agar (Oxoid, Hampshire, England). The antibiotics disc was mounted and incubated at 37°C for 18–20 hours. Based on the diameter of inhibition zone, the results were recorded as susceptible, intermediate and resistant according to the rules of National Committee for Clinical Institute laboratory standards (CLSI, 2012).

Data analysis

Data were entered in to Microsoft Excel 2007® (Microsoft Corp., Redmond, USA) and analyzed using Excel, State 11, and SPSS version 20 (IBM Corp., Armonk, USA). Percentage and 95% confidence intervals (CI) of the percentage were used to demonstrate prevalence differences between and among the sampling occasions and types of samples. Using the coagulase test as gold standard (Winn et al., 2006; Koneman et al., 1997), the diagnostic test agreement (Kappa), sensitivity and specificity were also calculated. The cut-off Kappa value and interpretation was according to Viera, and Garrett (2005).

Results

Out of the total 169 samples, 35.5%; 95% CI 28.6%-42.9% were found positive for Staphylococcus. The prevalence ranges of 18.2% in hooks to 46.2% from personal hand swab with no difference ($p > 0.05$) were observed in environmental samples. In raw beef, it was 23.5% from when ready for distribution at abattoir and 52.9% in post distribution at butchers (Table 1).

Table 1: *Staphylococcus* by sampling locations and sample types along the Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample		Processing stages/position	Sampling location	Total № of sample	Total Staphylococcus	
					№ (%)	95% CI percentage.
Abattoir	Environment	Before beginning of operation	Personnel's hands	13	6 (46.2)	23.0-71.1
			Aprons	14	6 (42.9)	21.3-67.7
			Knives	13	4 (30.8)	12.8-58.1
			Hooks	11	2 (18.2)	5.5.-48.4
		Cleaning water	Tap water	12	3 (25.0)	9.1-53.8
		At carcass splitting	Rooms	17	5 (29.4)	13.3-53.5
		Refrigeration	Refrigerators	10	3 (30.0)	10.9-60.9
		Meat transport	Meat transport trucks	11	5 (45.5)	21.1-72.3
	Raw beef*	After washing for distribution	Beef inspection	34	8 (23.5)	12.5-40.1
		Sub total		135	42 (31.1)	23.4-39.6
Butchers		Butchers, 6-8 hours post delivery	Beef for consumption	34	18 (52.9)	35.1-70.2
Total				169	60 (35.5)	28.6-42.9

Note: CNS = Coagulase negative Staphylococci; CI = Confidence interval; Mid Pexct. = Mid prevalence exact; * Raw beef at abattoir

From a total sample, 13.6% S. aureus and 21.9% CNS were observed (Table 2). Among environmental samples, S. aureus ranged from 0% on knives to 36.4% in meat transport trucks while CNS ranged from 0% on hooks to 46.2% on personnel's hands swabs. In raw beef, S. aureus was 8.8% at ready for distribution but raised to 20.6% post-delivery at public supply. Similarly, CNS was 14.7% at ready for distribution but raised to 32.4% post-delivery at public supply.

Table 2: Distribution of *S. aureus* and CNS by sampling locations and sample types along Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample		Sampling location	TN№ of sample	*S. aureus* № (%)	CNS № (%)
Abattoir	Environment	Personnel's hands	13	3 (23.1)	3 (23.1)
		Aprons	14	2 (14.3)	4 (28.6)
		Knives	13	0	4 (30.8)
		Hooks	11	2 (18.2)	0
		Tap water	12	1 (8.3)	2 (16.7)
		Rooms	17	2 (11.8)	3 (17.6)
		Refrigerators	10	2 (20.0)	1 (10.0)
		Meat transport trucks	11	1 (9.1)	4 (36.4)
	Raw beef*	Beef inspection	34	3 (8.8)	5 (14.7)
	Subtotal		135	16 (11.9)	26 (19.3)
Butchers		Beef for consumption	34	7 (20.6)	11 (32.4)
Total			169	23 (13.6)	37 (21.9)

* Raw beef at abattoir

Mixed occurrence of *S. aureus* and CNS by sampling locations was shown in Table 3. *S. aureus* only, CNS only and co-occurrence were 1.9%, 7.7% and 13.6% respectively. Except on knives and hooks, mixed occurrences of *S. aureus* and CNS were observed in all other sampling locations.

Table 3: Mixed occurrence of *S. aureus* and CNS by sampling locations and sample types along Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample		Sampling location	Total № of sample	S. aureus only № (%)	CNS only № (%)	Mixed isolation № (%)
Abattoir	Environment	Personnel's hands	13	0	0	3 (23.1)
		Aprons	14	0	2 (14.3)	2 (14.3)
		Knives	13	0	4 (30.8)	0
		Hooks	11	2 (18.2)	0	0
		Tap water	12	0	1 (8.3)	1 (8.3)
		Rooms	17	0	1 (5.9)	2 (11.8)
		Refrigerators	10	1 (10.0)	0	1(10.0)
		Meat transport trucks	11	0	1 (9.1)	4 (36.4)
	Raw beef*	Beef inspection	34	0	2 (5.9)	3 (8.8)
	Subtotal		135	3 (2.2)	9 (6.6)	16 (11.9)
Butchers		Beef for consumption	34	0	4 (11.8)	7 (20.6)
Total			169	3 (1.9)	13 (7.7)	23 (13.6)

* = Raw beef at abattoir

The coagulase test was used as gold standard. Of all 60 isolates exposed to DNase (Figure 1) and coagulase tests, 21 were positive for both tests whereas 35 were negative for both test reactions showing (Kappa = 0.86; 95% CI = 0.73-0.99**)** Sensitivity = 91.3% and Specificity = 94.6%) (Table 4). Out of the total 60 isolates, 23 (38.3%) and 37 (61.7%) isolates were coagulase positive **Staphylococcus** (CPS) and coagulase negative **Staphylococcus** (CNS), respectively.

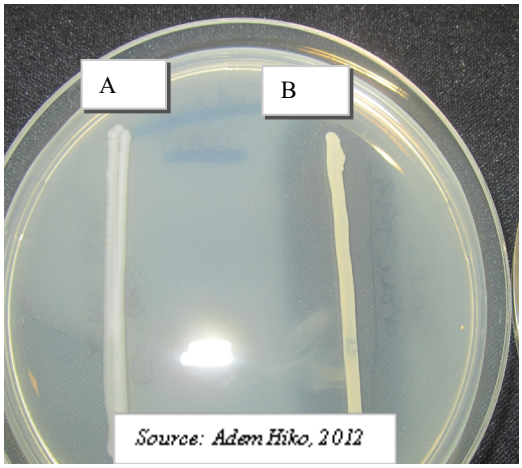

Figure 1: DNase test reaction of isolated *Staphylococcus*: A) DNase negative, B) DNase positive (Clear zone of DNA digestion).

Table 4: DNase-cross-Coagulase test reactions of *Staphylococcal* isolates from the Addis Ababa Abattoir Enterprise beef abattoir line

Diagnostic test		№ of tube coagulase		Total № (%)
		Positive	Negative	
№ of DNase	Positive	21*	2**	23 (38.3)
	Negative	2***	35****	37 (61.7)
Total № (%)		23 (38.3)	37 (61.7)	60 (100)

Note: True positives* = 21; False positives** = 2; False negatives***= 2; True negatives****=35; Almost perfect agreement (Kappa= 0. 86 (95% CI = 0.73-0.99)); Sensitivity = 91.3%; Specificity = 94.6%;

Antimicrobial Resistance test

The 60 Staphylococcus isolates were subjected to 8 types of antibiotics for antimicrobial resistance test. Out of the total isolates, 35.0% were resistance to trimethoprim, 33.7% for polymyxin B, 31.7 % for oxytetracycline, 20.0% for trimethoprim-sulfamethoxazole, 8.3% for chloramphenicol, 6.7% for oxacillin, 5.0% for gentamycin and no resistance was observed for tetracycline (Table 5).

Table 5: Drug resistance profiles of the Staphylococcal isolates from the Addis Ababa Abattoir Enterprise beef abattoir line.

Drugs used	S. aureus (n = 23)			CNS (n = 37)			Total (N = 60)		
	S. № (%)	I. № (%)	R. № (%)	S. № (%)	I. № (%)	R. № (%)	S. № (%)	I. № (%)	R. № (%)
TE	23 (100)	0	0	37 (100)	0	0	60 (100)	0	0
CN	20 (86.9)	1 (4.3)	2 (8.7)	36 (97.3)	0	1 (2.7)	56 (93.3)	1 (1.7)	3 (5.0)
OX	20 (86.9)	0	3 (13.1)	32 (86.5)	4 (10.8)	1 (2.7)	52 (86.6)	4 (6.7)	4 (6.7)
C	19 (80.6)	1 (4.3)	3 (13.1)	35 (94.6)	0	2 (5.4)	54 (90.0)	1 (1.7)	5 (8.3)
SXT	12 ()52.2	1 (4.3)	10 (43.5)	34 (91.9)	1 (2.7)	2 (5.4)	46 (76.7)	2 (3.3)	12 (20.0)
OT	10 (43.5)	0	13 (56.5)	30 (81.1)	1 (2.7)	6 (16.2)	40 (66.7)	1 (1.7)	19 (31.7)
PB	6 (26.1)	0	17 (73.9)	34 (91.9)	0	3 (8.1)	40 (66.7)	0	20 (33.7)
W	7 (30.4)	2 (8.7)	14 (60.9)	26 (70.3)	4 (10.8)	7 (18.9)	33 (55.0)	6 (10.0)	21 (35.0)

Note: C = Chloramphenicol; CN = Gentamycin; TE = Tetracycline; OT = Oxytetracycline; OX = Oxacillin; PB = Polymyxin B; SXT = Trimethoprim-Sulfamethoxazole; W = Trimethoprim

Antimicrobial resistance at different sampling sites

Two (5.9%) to 12 (35.3%) resistant *Staphylococcus* isolates from abattoir environmental samples was observed against all drugs used in this study. Similarly, resistant isolates to drugs used in this study were distributed on beef at abattoir and butchery. Exceptions are oxacillin resistant isolates were not observed on beef from abattoir and butchery while gentamycin resistant isolates were not observed in beef at abattoir (Table 6).

Table 6: Distribution of resistant *Staphylococcus* isolates by origin of sample at Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample	№ test	№ (%) resistant Staphylococcus isolates*						
		CN	OX	C	SXT	OT	PB	W
Environment**	34	2 (5.9)	4 (11.8)	12 (35.5)	7 (20.6)	11 (32.4)	11 (32.4)	12 (35.3)
Beef at abattoir	8	0	0	3 (37.5)	3 (37.5)	2 (25.0)	5 (62.5)	3 (37.5)
Beef at butcher	18	1 (5.6)	0	6 (33.3)	2 (11.1)	6 (33.3)	4 (22.2)	3 (37.5)
Total	60	3 (5.0)	4 (6.7)	21 (35.0)	12 (20.0)	19 (31.7)	20 (33.3)	21 (35.0)

Note: C = Chloramphenicol; CN = Gentamycin; TE = OT = Oxytetracycline; OX = Oxacillin; PB = Polymyxin B; SXT = Trimethoprim-Sulfamethoxazole; W = Trimethoprim; * = Tetracycline resistant isolates were not observed; ** = Abattoir Environment*

Higher resistant *S. aureus* the CNS were observed among the used drugs (Table 7). Two (15.4%) to ten (76.9%) resistant *S. aureus* isolates to chloramphenicol, gentamycin, oxytetracycline, oxacillin, polymyxin B, trimethoprim-sulfamethoxazole and trimethoprim were observed at abattoir environment (Table 7). On the other hand, resistant *Staphylococcus* isolates to chloramphenicol, gentamycin and oxacillin was not observed on beef both at abattoir and butchery. However, regardless of the number, high percentages of resistant *Staphylococcus* isolates to oxytetracycline, polymyxin B, trimethoprim-sulfamethoxazole and trimethoprim were observed on beef at butchery than at abattoir. With regards to CNS, variable types of resistant isolates to the used drugs were observed at each sample origin.

Table 7: Distribution of resistant *Staphylococcus strain* isolates by origin of sample at Addis Ababa Abattoir Enterprise beef abattoir line.

Staphylo-coccus strain	Origin of sample	№ test	№ (%) resistant Staphylococcus isolates*						
			CN	OX	C	SXT	OT	PB	W
S. aureus	Environment**	13	2 (15.4)	3 (23.1)	3 (23.1)	7 (53.9)	8 (61.5)	10 (76.9)	10 (76.9)
	Beef at abattoir	3	0	0	0	2 (66.7)	2 (66.7)	3 (100)	2 (66.7)
	Beef at butcher	7	0	0	0	1 (14.2)	3 (42.9)	4 (57.1)	2 (28.6)
	Total	23	2(8.7)	3 (13.1)	3 (13.0)	10 (43.5)	13 (56.5)	17 (73.9)	14 (60.9)
CNS	Environment**	21	0	1 (4.8)	0	0	3 (14.3)	1 (4.8)	2 (9.5)
	Beef at abattoir	5	0	0	1 (20.0)	1 (20.0)	0	2 (40.0)	1 (20.0)
	Beef at butcher	11	1 (9.1)	0	1 (9.1)	1 (9.1)	3 (27.3)	0	4 (36.4)4
	Total	37	1 (2.7)	1(2.7)	2 (5.4)	2 (5.4)	6 (16.2)	3 (8.1)	7 (18.9)

Note: C = Chloramphenicol; CN = Gentamycin; OT = Oxytetracycline; OX = Oxacillin; PB = Polymyxin B; SXT = Trimethoprim-Sulfamethoxazole; W = Trimethoprim; * = TE resistant isolates were not observed; ** = Abattoir Environment*

Resistance pattern

Out of the tested 60 isolates, 31 (51.7%) were resistant to one drug to multiple of six drugs (Table 8). Ten isolates for single drug, four for two drugs but the remaining 17 for multiple of three to six drugs resistance (MDR) were observed. Higher isolates of 5 were found resistant to four dug combination of oxytetracycline-polymyxin B-trimethoprim-sulfamethoxazole-trimethoprim (OT-PB-SXT-W).

Table 8: Single to multiple of six drug resistant patterns of the isolates at Addis Ababa Abattoir Enterprise beef abattoir line.

Resistant isolates pattern	Drugs of resistant	No. of *S. aureus*	No. of CNS	No. of Total
Single drug resistant	C	0	1	1
	OT	0	3	3
	OX	0	1	1
	PB	2	1	3
	W	0	2	2
	Subtotal* № *(%)	***2 (11.1)***	***8 (61.5)***	***10 (32.3)***
Two drugs resistant	C-W	0	1	1
	OT-PB	2	0	2
	PB-W	1	0	1
	Subtotal* № *(%)	***3 (16.7)***	***1 (7.7)***	***4 (12.9)***
Three drugs resistant	C-OT-W	0	1	1
	OT-SXT-W	0	1	1
	OT-PB-W	2	1	3
	PB-SXT-W	1	1	2
	Subtotal* № *(%)	3 (16.7)	4 (30.8)	7 (22.6)
Four drugs resistant	OT-PB-SXT-W	5	0	5
	OX-PB-SXT-W	1	0	1
	***Subtotal* №. (%)**	***6 (33.3)***	***0***	***6 (19.4)***
Five drugs resistant	C-CN-OT-SXT-W	1	0	1
	OT-OX-PB-SXT-W	1	0	1
	C-OT-PB-SXT-W	1	0	1
	Subtotal* № *(%)	***3 (16.7)***	***0***	***3 (9.7)***
Six drugs resistant	C-CN-OT-OX-PB-W	***1 (5.6)***	***0***	***1 (3.2)***
Total № (%)	31	18 (100)	13 (100)	31 (100)

Discussion

Prevalence of *Staphylococcus*

Meat is an important vehicle for the transfer of antibiotic resistances from animals to humans, and antimicrobial resistance has always been a major concern for nosocomial infections in hospital environments. Such transfer can occur in three ways: by means of antibiotic residues in food, through the transfer of resistant food borne pathogens, or through the ingestion of resistant parts

of the original food microflora and resistance transfer to pathogenic microorganisms (Kruse and Sorum, 1994; Klein, 1999; Teuber, 1999; Mayrhofer *et al*., 2004). The present overall prevalent of 35.5% of *Staphylococcus* is slightly lower than the 42.8% total *Staphylococcal* reported by Gizaw (2014). However, there is similarity in prevalence of *S. aureus* at hooks (18.2%) and personnel hand swab (46.2%). The raising *Staphylococcal* load from 23.5% at ready for distribution at abattoir to 52.9% butchers house indicated the high risk of beef contamination by *S. aureus* carriers human being and from the beef handling environment like transporting trucks (45.5%) along the meat value chain (Gudeta, 2012). Asymptomatic nasal carriers of *S. aureus* (Kluytmans *et al.,* 1997; Gorwitz *et al.,* 2008; Gizaw, 2014) as a normal flora with human an important source of spread of *S. aureus* to food and food production and processing chain were also indicated by Miller and Diep (2008), Kazakova *et al.* (2005) and Muto *et al.* (2003). This was supported by 46.2% *Staphylococcal* isolation on personnel's hands swabs observed from the current study.

The present findings of *S. aureus* (8.8%) and CNS (14.7%) were lower than the *S. aureus* prevalence 38.5% from carcass swab (Gizaw, 2014), 28.57% from poultry and 29.41% from beef, 23.86% *S. aureus* from raw meat (Pesavento *et al.,* 2007) but similar was 15.15% from pork (Pesavento *et al.,* 2007). But the prevalence on ready for distribution and the 20.6% *S. aureus* and 32.4% CNS were similar with reports of Gizaw (2014). This variation could due to difference in study area and sources of sample. Moreover, the present concomitant raising of *S. aureus* (8.8%) and CNS (14.7%) at ready for distribution to raise to 20.6% and 32.4% respectively at post-delivery at public supply could be due to further contamination of the beef product at postharvest stages followed by growth of microbial. These indicate the increasing in rate of contamination and multiplication of *Staphylococcal* on the carcasses and along the beef handling stage. Rani *et al.* (2017) suggested meat distribution stage as the most critical period where the quality can easily be compromised. During distribution, pathogenic and spoilage micro-organisms may grow (Rani *et al*., 2017).

DNase and Coagulase test

Coagulase testing was used as gold standard for differentia between S. aureus and coagulase negative Staphylococcus (CNS) (Winn et al., 2006). Clinical S. aureus isolation requires a cost effective and better tests in resource limited developing countries (Kateete et al., 2010) where tube plasma coagulase test is confirmatory. However, as an alternative diagnostic tool, DNase test can assist

or replace it. The present finding showed almost perfect agreement (Kappa = 0.86, 95%CI = 0.73-0.99) between the DNase and tube plasma coagulase tests in screening S. aureus from the CNS. According to Viera and Garrett (2005), almost perfect test agreement ranges from 0.81-0.99.

The present 91.3% sensitivity was found in agreement and lower than 94-100% previous findings reported by other investigators (Tager **et al.,** 1948, Orth **et al.,** 1971; Oranusi and Umoh, 2006) but higher than the 75-76% sensitivity reported by Kateete **et al. (**2010) using tri-combination test (human or sheep plasma/Mannitol salt agar/DNase). The differences could be due to difference in the test method where by Kanep agar and rabbit plasma were used in this study. The present 94.6% specificity is found lower than the 100% reported by Kateete **et al.**, (2010) whose used tri-combination test both by (human plasma/Mannitol salt agar/DNase). "The two DNase negative **S. aureus**" in the context of tube coagulases the gold standard, DNase negative but tube coagulase**s** positive are false negatives isolates. They may probably be MRSA isolates which are reported to react weakly. Presence of such rare coagulase negative **S. aureus** strains has been reported by Koneman **et al.** (1997) which could weakly coagulase reacting strain termed as MRSA or **Staphylococcus schleiferi subsp coagulans.** (Kateete **et al.,** 2010). Again, Rao **et al**. (2002) also reported DNase negative **S. aureus.** On the other hand, the present two isolates with DNase positive but tube coagulase negative (i.e. false positive isolates) were presume **Staphylococcus schleiferi subsp. coagulans.** Kateete **et al. (**2010) also observed isolates with such property with similar assumption in Uganda.

Drug resistance profile of Staphylococcal species

The rapidly increasing in the number of antimicrobial resistant bacteria agents could be due to the extended use and misuse of antibiotics in livestock farming and human diseases treatment). *Staphylococcal* species (Kitai *et al*., 2005; Becker *et al.,* 2014), have shown a considerable increase in resistance against most antibiotics (Valsangiacomo *et al*., 2000) with the consequence of prolonged hospital stay and increased costs of treatment (Kitara *et al*., 2011). Tetracycline resistant isolate was not observed in this study but 62.5% were reported from milk (Gizaw, 2014). Again, unlike the present finding, 19.0% *S. aureus* were resistant to tetracycline with 8.3%, 20.0% and 25.0% from poultry, beef and pork were reported (Pesavento *et al*., 2007). This could be due to the difference among the sources of isolates and geographical location. On

the other hand, the 31.7% resistant isolate to oxytetracycline observed in this study could be from frequently use of this in Ethiopia in animal's treatment (DACA, 2009) which might develop drug resistance. Present finding showed higher resistant *S. aureus* (43.5%) than CNS (5.4%) to co-trimoxazole indicating greater risk of resistance development of the pathogenic strain than the less pathogenic group to commonly used drug. The current 20.0% resistance to co-trimoxazole was lower than the resistant *S. aureus* from outpatients (53.6%) and inpatients (44.7%) (Kitara *et al.*, 2011). The overall 6.7% oxacillin resistant *Staphylococci* with *S. aureus* (13.1%) and CNS (2.7%) were lower than the 35.7% resistant *S. aureus* isolates (Pesavento *et al.,* 2007). This finding is still lower than the 66.7% from poultry and 30.0% from pork oxacillin resistant *S. aureus*. But the present oxacillin resistant *S. aureus* was similar with the 10.0% reports from beef meat items (Pesavento *et al.,* 2007). The current over all 5.0% resistance to gentamycin was similar with a pooled 9.5% resistant *S. aureus* reported by Pesavento *et al.* (2007) but lower than the 16.7%, 10.0% and 5.0% from poultry, beef and pork (Pesavento *et al.* (2007) respectively.

The present 15.4% to 76.9% resistant *S. aureus* to chloramphenicol, gentamycin, oxytetracycline, oxacillin, polymyxin B, trimethoprim-sulfamethoxazole and trimethoprim were observed at abattoir environment could be due to contamination of the abattoir environment from animals, or from carrier humans particularly from abattoir workers harboring resistant strain. Evidence that transfer of antimicrobial resistance from food-producing animals to humans directly via the food chain as route of spread were documented (Wooldridge, 2012). The absence of resistant *Staphylococcus* isolates to chloramphenicol, gentamycin and oxacillin on beef both at abattoir and butchery could be due to the fact that these drugs are not used in animal treatment in Ethiopia where no development of resistant strain. The occurrence of one or more resistant almost along the studied beef line location indicates the contamination of environmental sites which concerned as one routes of resistant isolate circulation via contact. Contaminated environmental sites were suggested as pathway for the transfer of resistant isolate in meat (Wooldridge, 2012; Bengtsson-Palme *et al.,* 2018). Moreover, transfer of resistant genetic elements between bacteria in mixed populations as a cross resistance to drugs of similar generic groups could make complex potential routes of resistant isolate spread. Similar suggestions were given (Wooldridge, 2012; Bengtsson-Palme *et al.,* 2018).

Total of 51.7% resistant isolates to one drug to multiple of six drugs consisting as multiple drugs resistance (MDR) were observed. A large proportion

(89.3%) from food line (Gizaw, 2014), 53.7% from hospital cases (Gizachew *et al.,* 2015) were showed MDR in Ethiopia. The presences of nasal carriage of resistance staphylococcus were also reported by Tewodros and Gedebou (1984) in Ethiopia. Like the present 51.7% MDR isolation, Pesavento *et al.* (2007) also reported 30.95% resistant isolates to at least three antibiotics in indicating significance resistance among these pathogens.

Conclusion and recommendation

The present finding indicates increased contamination of meat product along handling line with both by *S. aureus* and CNS with more than half percent of the isolates being resistant to single to multiple drug resistant. Thus, this finding warrants the application of good hygienic practices along the beef abattoir line with control of drug resistance. The DNase test showed strong agreement with tube coagulase test with low false positive and negative isolates indicated the possible alternative use of these diagnostic tests for screening staphylococcal isolates in resource-limited settings like Ethiopia.

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Conflict of interest

There is no conflict of interest to declare

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