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Antimicrobial Resistance Profiles of *Staphylococcus aureus* Isolates along Asella Municipal Beef Abattoir Line, South Eastern Ethiopia

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Abstract

Staphylococcus aureus is associated with poor food handling and sanitation practices responsible for notorious for causing human diseases. Using a cross-sectional study, it was isolated along Asella Municipal Beef Abattoir line and assessed for antimicrobial resistance profiles. A total of 470 samples consisting of 400 beef carcasses swab and 70 environmental (personal hand, apron, knife, splitting axil, hooks and meat wrapping plastic swab samples and cleaning water) samples were collected. Randomly selected isolates from each location were tested for 12 different antimicrobial agent using disk diffusion techniques. An overall 171 (36.4%) samples for which 137 (34.3%) from the carcass swab and 34 (48.6%) from Abattoir facilities were positive for *S. aureus*. The isolation was ranged from 30% in clean water sample to 60% each from personal hand swab and Hook swab sample. Although resistance was not observed for bacitracin, chloramphenicol, kanamycin and sulfamethozathole, the resistance of 77.5%, 45.0%, 36.3%, 28.8%, 26.3%, 21.3%, 20.0% and 7.5% to amoxicillin, cloxacillin, ampicillin, penicillin-G, vancomycin, erythromycin, tetracycline and doxycycline were observed in descending order. One, two, three, four and five drugs resistant isolates were observed in 14.1%, 33.3%, 29.5%, 19.2% and 3.8% of the resistant isolates. Resistant isolate to amoxicillin, cloxacillin, ampicillin and penicillin-G, were frequently observed along the majorities of sampling location. The findings indicate the risk of public in acquiring drug resistant, staphylococcal infections enter food poisoning. Implementing line based hygienic operation during beef carcass processing and transporting could minimize carcass contamination with *S. aureus* including the resistant one.

Keywords Abattoir; Antimicrobial resistance; Carcass; *Staphylococcus aureus*

Introduction

Food processing and handling is complex operation process with possible problems leading to food poisoning and infection. Foodstuffs, especially from animal products, such as meat from infected animals or carcasses can be contaminated with pathogenic bacteria [1]. In fact, tissue from healthy animals are sterile [1,2] and is an excellent source of protein for human but highly susceptible to microbial contaminations, which can cause spoilage and food borne infections [3,4] resulting in economic and health losses [3]. The microbiological contamination of carcasses occurs mainly along slaughtering chains during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments [1,5,6]. Main possible sources of contaminates are exterior of the animal and its intestinal tract, contaminated working equipment in general [6,7] and personnel [8,9].

Staphylococcus aureus is one of the most contaminates and prevalent causes of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multidrug resistance [10]. Moreover, it is one of the most common causes of foodborne infections in most of the countries around the world [11]. In the last few decades, staphylococcal food poisoning has been reported as the third cause of foodborne illnesses in the world [3]. While growing food, some strains produce toxins such as heat-stable protein that resists heating at 100°C for 30-70 min. The toxins are responsible for causing gastrointestinal disease [4]. In Ethiopia, consumption of raw beef meat is a common habit which may results in acquire food-borne diseases and intoxication [12-15], including Staphylococcal infection [12-16] which have been reported in a fragments from selected food items in Ethiopia. However, structured survey along Asella Municipal Beef Abattoir line with drug resistance test on *Staphylococcus aureus* were not yet conducted. Therefore, the study was aimed to isolate and assess antimicrobial resistance profiles of *Staphylococcus aureus* along Asella Municipal Beef Abattoir line in Ethiopia [17].

Materials and Methods

Study design

A cross-sectional study was employed to isolate *Staphylococcus aureus* and test the isolates for drug resistance along beef line at Asella Municipal Abattoir in Ethiopia.

Study area description

The study was conducted at Asella Municipally Abattoir in Asella town located in Arsi Zone, Oromia Regional Stat and South Eastern Ethiopia. Asella town, the capital of Arsi Zone, is located at about 175 km Southeast of Addis Ababa at 6° 59' to 8° 49' N and 38° 41' to 40° 44' E with an altitude of the area ranges from 2500 to 3000 meter above sea level. The maximum and minimum temperatures of the area are 25°C

and 10°C respectively. Asella has an estimated human population of 67,269 of whom 33,826 were male and 33,443 were female [18].

Study population

The study population was beef carcass slaughtered at Asella Municipal Abattoir, abattoir equipment and the water used for slaughtering and processing operation.

Sample size determination and samples sources

The sample size was calculated using 50% expected prevalence of *Staphylococcus aureus* in the area within 95% Confidence Intervals (CI) at 5% desired accuracy, according to the Thrusfield [19] formula.

Thus, $n=1.96^2 \times P_{exp}(1-P_{exp}) /d^2$

Where n=required sample size, d=desired absolute precision, P_{exp}=expected prevalence (50%).

Sample collection and management

The samples were collected twice per week from Asella Municipal Abattoir. The selected carcasses was swabbed using the method described in ISO 6888-2 [20] by placing sterile template (10 × 10 cm) on specific sites of a carcass. Using a sterile cotton tipped swab (2 × 3 cm) fitted with shaft and first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) was rubbed horizontally and then vertically several times on the carcasses. The abdomen (flank), thorax (lateral), crutch and breast (lateral) sites were sampled according to ISO 6888-2 [20]. On completion of the rubbing process, the shaft will be broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube.

Swab samples was also taken from knives, splitting axe, personal hands, apron, wrapping meat plastic and hooks while a certain volume of cleaning water were taken directly from the pipe line. For convenience, the swabs acquired from the equipment and the working environment was considered as samples from the immediate slaughter environment. Finally, all samples were kept in the cold chain (+4°C) using icebox and shipped to Asella Regional Veterinary Laboratory for analysis.

Laboratory work

Culturing: The swab samples which has been dipped into 10 ml BPW during collection were directly incubated for 24 h at 37°C. The water samples were also enriched at proportion of 1 ml water to 9 ml of BPW and incubated for 24 h at 37°C. A loop full of enrichment was streaked on 7% heparinized sheep blood based agar plate (BAP) enriched and incubated at 37°C for 24 hrs under aerobic condition. Presumptive *Staphylococcus* colonies were determined based on their morphological characteristic such as opaque, white or creamy, grayish or yellow colonies and beta hemolysis. Suspected colonies was further gram stained on these gram positive grape like colonies which were transferred to mannitol salt agar and incubated at 37°C for 24 hours to get characteristic colony. The colony was incubated on nutrient agar at 37°C for 24 hours for different biochemical tests.

Biochemical test: Biochemical tests and identification of the organism and species assignment was based on catalase test, oxidase test, coagulase test by using rabbit plasma [20], and DNase test agar to detection of DNase activity (for detection of deoxyriboneuclease enzyme) of *Staphylococcus aureus*.

Antimicrobial resistance test: Antibiotic resistance test was performed using disk diffusion techniques according to NCCLS guidelines [21] on 80 randomly selected isolates from each beef line locations. The isolates were inoculated into Brain Heart Infusion broth (Oxoid) and incubated until the turbidity of 0.5 McFarland standards was achieved. The bacterial lawns were well spread on Muller Hinton agar plats (Oxoid). Thus, 12 different drugs (Oxoid) including amoxicillin (AML 25 µg), ampicillin (AMP 10 µg), bacitracin (B 10 µg), doxycycline (Do 30 µg), chloramphenicol (C 30 µg), erythromycin (E 15 µg), kanamycin (K 30 µg), cloxacillin (OB 5 µg), penicillin G (P 10 µg), tetracycline (TTC 30 µg), trimethoprimsulphamethoxazole (SXT 1.25/25 µg), vancomycin (V 30 µg) were used. Inhibition zones were measured and interpreted as susceptible, intermediate, and resistant.

Data analysis: The data was entered in Microsoft Excel 2013© and computed for descriptive statistics was analyzed using STATA 11 version and SPSS version 20.0, 2011 software. Percentages, proportions, and frequency distributions were applied to compute some of the data. The Midd-prevalence exact were calculated and 95% Confidence Interval was used to determine the differences of in isolation of *S. aureus* between and among sample source and sample types.

Results

From total 470 samples taken from the abattoir beef line, 171 (36.4%) were positive for *S. aureus*. It was 48.6% in environmental samples and 34.3% in the carcass swab samples. The isolation ranged from 30% in clean water sample to 60% each from personal hand swab and hook swab sample. Regardless of numbers of samples, highest isolation was recorded in environmental samples (Table 1).

Sample sources	Sampling location and sample type	No of tested isolate	No (%) Positive	95% CI for Positive
Environment	Personnel hand swab	10	6 (60.0)	31.2, 83.2
A1 sample	Apron swab	10	4 (40.0)	16.8, 68.7
	Knife swab	10	5 (50.0)	23.7, 76.3
	Splitting axe swab	10	5 (50.0)	23.7, 76.3
	Hooks swab	10	6 (60.0)	31.2, 83.2
	Water sample	10	3 (30.0)	10.8, 60.3
	Meat wrapping plastic swab	10	5 (50.0)	23.7, 76.3
	Sub Total	70	34 (48.6)	37.2, 60.0
Meat sample	Carcass swab	400	137 (34.3)	27.8, 39.0
Total		470	171 (36.4)	32.3, 40.8

Table 1: *Staphylococcus aureus* along Asella Municipal Abattoir Beef line in 2016.

Of 80 randomly selected *S. aureus* from each sampling location and tested isolates, total of 78 (97.5%) were resistant to at least one drug. As shown in Table 2, resistance of 77.5%, 45.0%, 36.3%., 28.8%, 26.3, 21.3%, 20.0%, 7.5% to AML, OB, AMP, P-G, V, E, TTC and Do were

observed in descending order of resistance. But resistant isolate to B, C, K and SXT were not observed.

	Sampling Location	No of Samples	No. (%) resistant Isolates							
			AML	AMP	DO	E	OB	P	TTC	V
Sample sources	Personnel hand swab	3	2 (66.7)	1 (33.33)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.67)	0 (0.0)	0 (0.0)
	Apron swab	2	2 (100)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)
	Knife swab	3	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.33)	1 (33.33)
	Splitting Axe swab	3	2 (66.7)	1 (33.33)	0 (0.0)	1 (33.33)	2 (66.67)	0 (0.0)	0 (0.0)	1 (33.33)
	Hooks swab	3	3 (100)	1 (33.33)	0 (0.0)	1 (33.33)	0 (0.0)	1 (33.33)	1 (33.33)	0 (0.0)
	Water sample	3	1 (33.3)	2 (66.66)	0 (0.0)	0 (0.0)	3 (100.0)	2 (66.67)	1 (33.33)	1 (33.33)
	Meat wrapping plastic swab	3	2 (66.7)	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	0 (0.0)
Meat sample	Carcass swab	60	47 (78.3)	22 (36.67)	5 (8.33)	13 (21.67)	29 (48.33)	17 (28.33)	11 (18.33)	17 (28.33)
Total		80	62 (77.5)	29 (36.3)	6 (7.5)	17 (21.25)	36 (45)	23 (28.75)	16 (20)	21 (26.25)
Mean (X)% Resistant			76.6	35.9	5.5	21.4	38.2	28.6	24.6	22.7

Table 2: Antimicrobial resistance profile of *Staphylococcus aureues* isolated along Asella Municipal Beef Abattoir line. Note: AML=amoxicillin, AMP=ampicillin, Do=doxycycline, E=erythromycin, OB=cloxacillin, G=penicillin, TTC=tetracycline, V=vancomycin; *resistance to C=chloramphenicol, B=bacitracin, K=kanamycin and SXT=Trimetoprimsulphamethoxazole, were not observed.

As shown in Figure 1, single to MDR of five drugs resistance profile of the 78 resistant *S. aureus* isolates. An overall 78 (97.5%) of the tested 80 isolates showed single to multiple drug resistance. The observed result indicate that 11 (14.1%), 26 (33.3%), 23 (29.5%), 15 (19.2%) and 3 (3.8%) were showed resistance to one, two, three, four and five drugs in respectively (Figure 1).

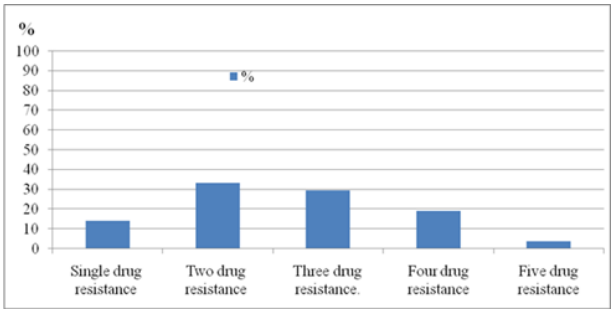

Figure 1: Single to MDR of *S. aureus* isolate from Asella Municipal Beef Abattoir line.

The distribution of resistant isolates to single to MDR isolate to specific drug of test were shown in Table 3. Higher resistance to single drugs, AML and OB were 45.5% and 18.2% respectively. Resistance for two drugs, AML-AMP, AML-OB and AML-TTC were 15.4% for each. Resistance for three drugs, AML-AMP-OB, and AML-AMP-E were 17.4% and 13.0% respectively. Resistance for four drugs, AML-AMP-PG-V and AML-E-OB-V were 13.3% for each. Resistance for five drugs, AML-AMP-E-OB-V, AML-AMP-Do-OB-V and AML-AMP-OB-TTC-V were 33.3% for each.

Types of Resistance	Type(s) of Drug*	No. (%)
Single drug resistance	AML	5 (45.5)
	OB	2 (18.2)
	V	1 (9.1)
	E	1 (9.1)
	AMP	1 (9.1)
	P-G	1 (9.1)
	Sub total	11 (100)
Two drug resistance	AML, AMP	4 (15.4)
	AML, DO	1 (3.8)
	AML, E	2 (7.7)
	AML, OB	4 (15.4)
	AML, P-G	2 (7.7)
	AML, V	3 (11.5)
	P-G, O	1 (3.8)
	DO, OB	1 (3.8)
	AMP, OB	1 (3.8)
	OB, V	1 (3.8)

	TTC, V	1 (3.8)
	AML, TTC	4 (15.4)
	Sub total	26 (100)
Three drug resistance	AML, AMP, E	3 (13.0)
	AML, E, P-G	2 (8.7)
	AML, P-G, V	1 (4.3)
	AMP, AML, V	2 (8.7)
	AMP, OB, P-G	1 (4.3)
	AMPDO, P-G	1 (4.3)
	AML, E, OB	1 (4.3)
	AML, OB, P-G	2 (8.7)
	AML, P-G, TTC	2 (8.7)
	OB, TTC, V	1 (4.3)
	AMLOB, TTC	2 (8.7)
	AML, AMP, OB	4 (17.4)
	AMP, AML, TTC	1 (4.3)
	Sub total	23 (100)
Four drug resistance	AML, AMP, P-G, V	2 (13.33)
	AMP, AML, OB, V	1 (6.67)
	AML, AMP, E, OB	1 (6.67)
	AML, AMP, OB, TTC	1 (6.67)
	AML, DO, E, P-G	1 (6.67)
	AML, E, OB, V	2 (13.33)
	AML, E, OB, P-G	1 (6.67)
	AML, AMP, OB, P-G	1 (6.67)
	AMP, OB, P-G, V	1 (6.67)
	AML, E, OB, TTC	1 (6.67)
	AML, P-G, TTC, V	1 (6.67)
	AML, OB, P-G, TTC	1 (6.67)
	E, OB, P-G, V	1 (6.67)
	Sub total	15 (100)
Five drug resistance	AML, AMP, E, OB, V	1 (33.33)
	AML, AMP, DO, OB, V	1 (33.33)
	AML, AMP, OB, TTC, V	1 (33.33)
	Sub total	3 (100)

Table 3: Single to MDR isolate of the 78 resistant *S. aureus* isolate from Asella Municipal Beef Abattoir line. Note: AML=amoxicillin, AMP=ampicillin, Do=doxycycline, E=erythromycin, OB=oxacillin, G=penicillin, TTC=tetracycline, V=vancomycin; *resistance to C=chloramphenicol, B=bacitracin, K=kanamycin and SXT=trimethoprim sulphamethoxazole, were not observed.

Discussion

Meat can be contaminated with microbes at any production and processing stage [9] and are responsible for the spoilage and food borne infections resulting in economic loss and health risk [2]. Present finding justify significant presence of *S. aureus* as a zoonotic contaminate along abattoir line at Asella Municipal Abattoir which could be due to poor sanitation and poor handling process. The present overall 36.4% *S. aureus*, with 34.3% in beef carcass 48.6% in abattoir environmental samples were lower than 68% meat samples collected from retainer in Thailand [22] due to higher probability of contamination at retail shop than at abattoir. But our finding was higher than the 26% reported from food and food environments in Italy [23], 9.3% from abattoir, 19.5% from retail shop and 17.5% from equipment of retail shop and abattoir [16] in Addis Ababa Abattoir and retail shop, Ethiopia due to difference in degrees of environmental hygiene and food safety tool application among areas.

The prevalence of antimicrobial resistance among foodborne pathogens are increasing [24] in which the present antibiotic resistance profiles of *S. aureus* isolates demonstrated higher resistance of 77.5%, 45.0%, 36.3%, 28.8%, 26.3, 21.3%, 20.0% and 7.5% to amoxicillin, oxacillin, ampicillin, vancomycin, erythromycin, tetracycline, penicillin G and doxycycline respectively in descending order of resistance. Majorities of resistance isolate to Amoxicillin, Ampicillin and penicillin G were frequently observed along the majorities of sampling isolations. Resistant *S. aureus* isolates were also reported at 90% to oxacillin, 85% to ampicillin, 65% to erythromycin, 60% to amoxicillin, 35% to streptomycin and 20% to vancomycin in Jimma, Ethiopia. Scientists also reported 49.5%, 45.5%, 45% and 13% resistant *S. aureus* strains to penicillin G, vancomycin, cloxacillin and norfloxaciln respectively. Moreover, resistant of as high as 100% to bacitracin, neomycin, methicillin, and 95% to tetracycline were reported. The presence of MDR of five drugs resistance *S. aureus* isolates in a combination in this study area also indicates the possible significant risk of the resistant strain along the studied beef line. Such resistant *S. aureus* were also reported from different parts of Ethiopia [12-16]. On the other hand, all (100%) of the isolates were sensitive to chloramphenicol, bacitracin, kanamycin and Trimethoprim sulphamethoxazole in this study. Similarly, scientists reported 100% sensitive *S. aureus* to Trimethoprim sulphamethoxazole.

Conclusion

The finding shows presence of poor hygiene and working practices of the meat handlers during the processing stage as well as lack of sterilization of equipment and working surfaces, insufficient water supply for abattoir for washing carcass, lack of safety rule in abattoir and lack of awareness of meat handlers are the major challenges along Asella Beef Abattoir line. These were demonstrated by contamination of the line with *S. aureus* whereby raw meat can act as a reservoir of antibiotic resistance bacteria that can be transferred to humans, thereby constituting a health problem. The application of hygienic practices along the chain and sagacious use of antibiotics for human and in animal husbandry in the area could control further emergence of antibiotic resistance.

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