# Meat Hygienic Practice, Staphylococcus aureus Isolation and its Antimicrobial Susceptibility in Beef at Municipal Abattoir and Retail Beef Shops in Assosa Town, Western Ethiopia

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**Abstract:** The meat is potentially subjected to contamination from a range of sources within and outside animal during the slaughter of animal, processing, transportation and at retail shops. A cross-sectional study was conducted from September 2024 to March 2025 to assess meat hygienic handling practices at the Assosa abattoir and retail beef shops and to determine the prevalence and antimicrobial susceptibility of *S. aureus* from beef of the Assosa abattoir and retail beef shops in Assosa town. 240 meat swab samples were taken from the abattoir and retail beef shops randomly. A structured questionnaire survey was used to assess meat hygienic handling at the municipal abattoir and retail shops. The antimicrobial susceptibility test was conducted on 30 randomly selected isolates of *S. aureus* using the disc diffusion susceptibility method. From total 240 swab samples taken from the abattoir and retail shops, 73 (30.42%) were positive for *S. aureus*. Depending on the source of sample, contamination of retail beef shop with *S. aureus* was 2 times more likely to occur compared to abattoir (OR=2; CI=1.169 - 3.824; p value= 0.013), showing statistically significant variation in the prevalence of *S. aureus* among sample sources. Similarly, depending on the types of sample, contamination of utensil swab sample with *S. aureus* was 4.2 times more likely to occur compared to carcass (OR=4.2; CI=1.95-9.05; p value = 0.000), which was statistically highly significant, and contamination of hand swab sample with S. *aureus* was 2.5 times more likely to occur compared to carcass (OR= 2.5; CI=1.159 - 5.383; p-value = 0.020), which was statistically significant. It was noted that factors such as lack of training, wearing inappropriate clothing, inappropriate and inadequate hand, equipment and surface washing as well as not using sterilizing material might have contributed to contamination with *S. aureus*. The large proportions of *S. aureus* isolates were resistant to Cefoxitin, Penicillin G, Chloramphenicol and Nalidixic acid. *S. Aureus* was a major meat contaminant in abattoir and butcher shops due to inadequate food safety knowledge and low level of hygienic practices. Therefore, improving the food safety knowledge and handling practice of meat handlers is important to prevent *S. aureus* foodborne intoxications.
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**Keywords:** Abattoir; Butcher shops; Contamination; Prevalence; hygienic handling; S. aureus

## 1. INTRODUCTION

### 1.1. Background of the Study

In developing countries, food-borne infections constitute the major cause of sickness and death. Food-related illnesses are caused by changes in eating patterns, mass catering, and improper food handling in developing countries like Ethiopia, where the production of meat and meat products often occurs under unsanitary conditions and the consumption of raw meat is common.

Meat is the most valuable livestock product and for many people serves as their first choice from animal protein which provides all the essential amino acids and various micronutrients in proper proportion to the human beings. This includes all processed or manufactured products prepared from animal tissues (Sebsibe *et al*., 2007). However, apart from its nutritional and health benefits, meat can be a source of both chronic (Wyness, 2016) and infectious diseases (Battaglia *et al*., 2015). Foodborne diseases occur in developing countries because of the poor food handling and sanitation problems. Although animal's tissue is sterile, during slaughtering, microorganisms could contaminate the tissue primarily from the exterior or the interior environments (Goja *et al*., 2013); (Beyene *et al*., 2017). Even though modern technologies are advanced for safe meat production, the safety of meat processing in developing countries including Ethiopia is still a concern. Except in the big cities of Ethiopia, the animals are slaughtered locally in open areas without any hygienic prerequisites (Gutema *et al*., 2021). As a result, the chance of the meat being contaminated with pathogenic microbes is exceedingly high. The contamination of meat occurs during the removal of hides, evisceration, processing, packaging and storage, and distribution at slaughterhouses and retail outlets (Lonergan *et al*., 2019).

*Staphylococcus aureus* is a Gram-positive, facultative anaerobic bacterium with size 0.5- 1.0μm in diameter which grows individually, in pairs, short chains or grape-like clusters (Schleifer and Bell, 2009). The bacterium is catalase and coagulase positive, oxidase-negative, non-motile microorganism that does not form spores. On agar medium it creates smooth, convex, glistening, circular colonies with entire margins reaching a size of 6-8mm in diameter (Le Loir *et al*., 2003); (Medveďová and Valik, 2012). They are resistant to desiccation and can survive on different surfaces, and resist sanitation, forming biofilms (Moretro, 2017). *S. aureus* is able to tolerate pH ranges from 4.5 to 9.0 and NaCl concentrations up to 9% and can grow and express virulence in a wide range of environmental conditions (Le Loir *et al*., 2003).

*Staphylococcus aureus* is one of the most common foodborne pathogens causing intoxication. Staphylococci can be introduced in the environment of food processing installations through various routes, such as raw materials, food handlers, or poor hygiene in food processing equipment. It is one of the food borne diseases transmitted from the contaminated animal source foodstaffs ( Nouichi and Hamdi, 2009). It produces heat stable and proteolytic enzyme resistant enterotoxins that cause food poisoning in humans leading to vomiting, abdominal pain and diarrhea (Busani *et al*., 2006). It is found in 30% nonclinical nasal carrier population (Levinson, 2008).This could be the sole source of contamination in abattoir and butcher workers for those who do not have enough awareness on the nature of the disease. Billions of people are at risk every year and thousands die as a result of consuming unsafe food. Many outbreaks of FBD are due to cross contamination that occurs during food preparation within food service establishments (Sibanyoni *et al*., 2017).

*Staphylococcus aureus* is a foodborne pathogen which is responsible for contamination of different food products and results food spoilage, reduction of food safety and shelf life and cause foodborne poisoning via production of deadly enterotoxins. It 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 (Kwon *et al*., 2006). In the last few decades, staphylococcal food poisoning has been reported as the third cause of foodborne illnesses in the world (Zadoks *et al*., 2000).

The prevalence and burden of S. aureus is growing worldwide, and it is a major concern of public health programs (Kadariya *et al*., 2014). However, the true incidence of staphylococcal food poisoning is underestimated due to misdiagnosis, under-reporting, improper sample collection and laboratory examination (Argudín *et al*., 2010). The global prevalence of S. aureus ranges from 23.3% to 73% (Reygaert, 2013). In Africa, there is scarce data regarding to the public health impact and burden of S. aureus.

Considering the poor hygienic conditions during food production and processing, coupled with poor cooling facilities (Tarekgne *et al*.,2015), there is a likely of high impact of staphylococcal food poisoning in African countries like Ethiopia. The prevalence varies from 16.0% in Tunisia to 52.0% in Egypt (Falagas *et al*., 2013) and up to 57.8% in Ethiopia (Alebachew *et al*., 2012).In addition, the prevalence in Ethiopia from meat reaches up to 40% reported form butcher shops of Mekelle city (Endale, 2013).Ethiopian raw beef consumption habit is the potential cause of foodborne illnesses ( Beyene *et al*., 2017); ( Girmay et al., 2015).Raw meat is available in open-air local butchers without the cold-chain process and purchased by consumers. Meat processing at butchers is likely to contribute for the contamination of minced beef meat as compared to the carcasses (Haimanot *et al*., 2010).

**1.2. Statement of the Problem**

In developing countries like Ethiopia food-borne diseases is higher compared to developed country and occur because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment hygiene and lack of education for food-handlers (WHO, 2004). The increased demand for meat is as a result of increased urbanization, higher disposable income and the human desire for a greater variety in their diets (Sofos, 2008). A large proportion of the people living in towns and urban centers consume beef (Zhao *et al*., 2001). However, the full value chain of meat supply from abattoirs, distribution, butchery shops to final consumer are not properly handled to ensure the soundness, wholesomeness and hygiene despite the presence of many studies on *S. aureus* in different parts of Ethiopia (Adugna *et al*., 2018; Thomas *et al*., 2015; Beyene *et al*., 2017).

There is little information about *S. aureus* related to the region or Asosa town. In addition to this the antimicrobial susceptibility of *S. aureus* is variable from time to time. These factors could hinder government and other stockholders to accurately apply measures on the impact of food contamination problems on public health. The final consumer has also limited information of the meat consumed regularly. Therefore, information should be gathered to develop an effective strategy to reduce the foodborne illness and antimicrobial resistance.

### 1.3. Significance of the Study

*Staphylococcal* Food Poisoning (SFP) is the most common Food borne diseases (FBD) with major concern in public health programs globally (Hennekinne *et al*., 2012). Staphylococcal infections are frequent but are usually contained by immune mechanisms to the site of entry. The highest incidence of disease usually occurs in people with poor personal hygiene, overcrowding and in children (Rho and Schaffner, 2007). In developing countries, the surveillance system of FBD hardly exists therefore, difficult to estimate the real magnitude of the problem (Boschi-Pinto, 2008). Even in countries where surveillance services are very efficient, the precise incidence of food poisoning is not known, as outbreaks are often not reported to public health authorities. Food borne diseases are a serious and growing problem

in the world. Among FBD, SFP is of major concern in global public health programs.

*Staphylococcal* organisms alone have found to cause hospitalization rates as high as 14% (Baron, 2007). Although not considered especially lethal, death can ensue if large amounts of SE are ingested: fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immune compromised persons, elderly persons and children (Kerouanton, 2007). Hence, the significance of this study are of public health and economic improvement from the proper handling of the meat at any point in time with main outcome of sustainable hygienic production, handling and supply.

### 1.4. Objectives of the Study

#### 1.4.1. General Objective

- To assess meat hygienic practices using *S. aureus* isolation from beef at abattoir and retail shops in Assosa town, Assosa zone.

#### 1.4.2. Specific Objectives

- To assess prevalence of *S. aureus* contamination from beef meat in Assosa town
- To evaluate antimicrobial susceptibility of *S. aureus* isolates
- To assess the major location of meat contamination, meat hygienic practices at Assosa Municipal Abattoir and the retail meat shops

## 2. MATERIALS AND METHOD

### a. Description of the Study Area

The study was conducted in Assosa town. Assosa is the capital of Benishangul Gumuz Regional State. It is located at 8°30′and 40°27′ N latitude and 34°21′ and 39°1′ E longitude, 687 km western of Addis Ababa according to the CSA (CSA, 2015). The altitude of Assosa ranges from 580 to over 1,560 m above sea level. The area is characterized by low land plane agro-ecology which has ‘kola’ micro climate with land coverage of 2,317 $km^2$ areas. According to national meteorological service agency/ NMSA/ the average annual rainfall of the town is 850–1316 mm with unimodal type of rainfall that occurs between April and October(NMSA, 2014). Its mean annual temperature ranges between 16.75°C and 36°C. The estimated total human population of the town was 66,062 of whom 33,947 are men and 32,115 are women (CSA, 2021). Cattle population of the area was 1,300(Source: Assosa town Agricultural Directive, 2023).

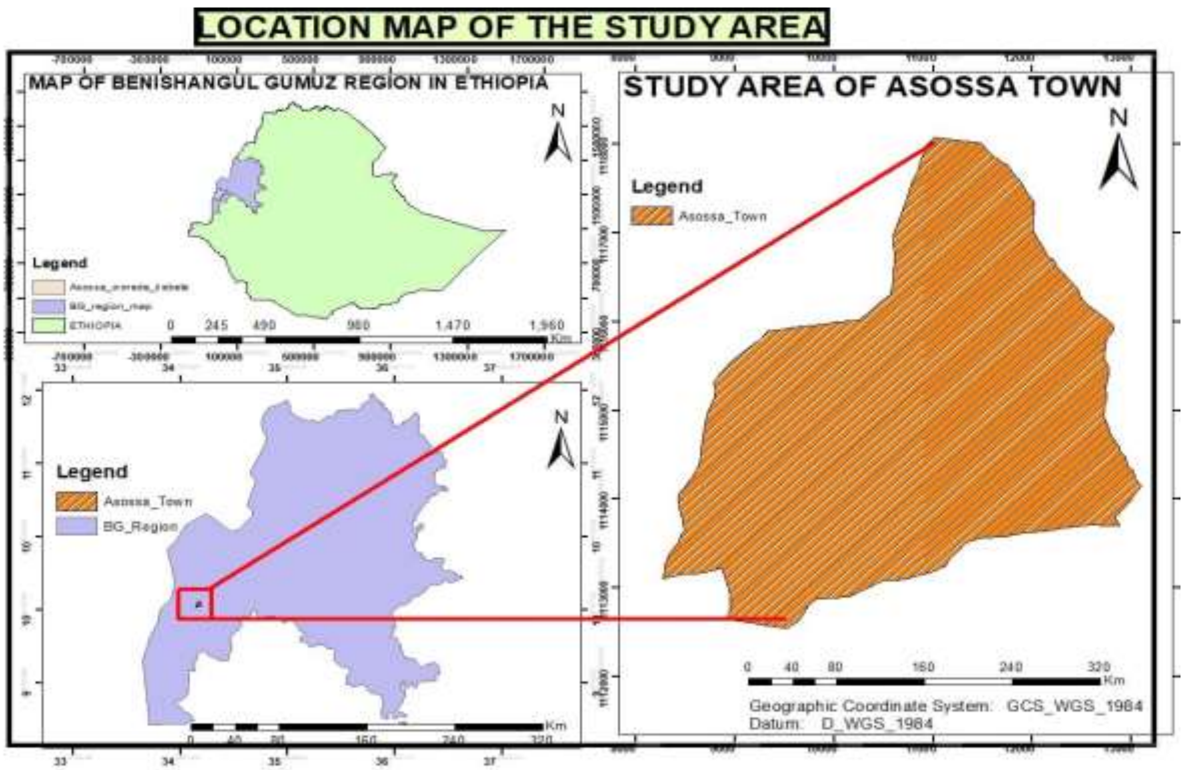


**Figure 2**:Location map of the study area of Assosa town

**b. Study Design**

A cross - sectional study design was conducted at the slaughter house and retail meat shops in Assosa town, Assosa Zone from September 2024 to April 2025 to assess the hygienic meat handling practice with *Staphylococcus aureus* isolation and identification from beef in Assosa town, Assosa zone.

**c. Study Population**

The study population represents beef meat from Assosa municipal abattoir and retail shops. Cattle presented to the abattoir originated from Bambasi and Asossa districts of Assosa zone and its surroundings. In addition, meat handlers, utensil, hall and floor were included to assess the hygiene practices at abattoir and retail shops.

**d. Sample Size Determination and Sampling Procedures**

The sample size was determined using the formula described by (Thrusfield, 2005) by assuming 95% confidence interval, 5% desired level of precision and 15 % expected prevalence of S. aureus that was done by (Adugna *et al*., 2018) at Addis Ababa City slaughterhouse and retail meat shops as follows:

$$n = \frac{(1.96)^2 x\ P\ (1\text{-}P)}{d^2}$$

Where: n = the total sample size, P = expected prevalence (15%), d = desired absolute precision (5%), (0.05) at 95% CI

Based on the above given formula, the minimum sample size, 196 was to be collected from slaughter house and retail meat shops. However, to increase the precision of the study a total of 240 microbiological samples were collected and tested. The sample distribution was 60 from abattoir, 60 from butcher shops (carcass/meat swab samples) and 120 pooled environmental swab samples (from abattoir and butcher shops) (Abdulle, 2023).

For questionnaire survey sample size was calculated by using the formula given by Arsham (2002). $N = 0.25/SE^2 = 100$. Where: N= sample size, SE (standard error) = 5%). The sample size required for the questionnaire survey, based on the above formula, was 100 but overall 52 respondents from abattoir and retail meat shops were purposively included based on availability and their voluntary consent to participate in this study and interviewed.

### e. Sampling Technique

Simple random sampling was employed to select study animals at slaughter house and samples were also taken from retail meat shops. Purposive sampling was also employed to collect pool environmental samples from both abattoir and meat retail shops. The samples were processed, according to the protocol described by (ISO/TS 6888-2, (2005).

### f. Sample Collection Procedure and Transportation

240 samples were collected from one municipal abattoir and twelve randomly selected butcher shops in Assosa town. The selected carcasses were swabbed using the method described in ISO 6888-2 (NCCLS, 2012) 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(NCCLS, 2012). On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube.

Swab samples were taken from hands of abattoir workers and butchers, utensils (knives, cutting boards, hooks, etc.) and surfaces (floor and hall of the abattoir and butcher shops). For convenience, the swabs acquired from utensils, hands, floor and hall were considered as samples from direct working and slaughter environment. Finally, all samples were transported to the Assosa Regional Veterinary Laboratory for analysis in an ice box on ice packs and were analyzed within 24 h of sampling (ISO/TS 6888-2, 2005)

3.6.1. Beef swab sample

During each abattoir visit, four different sites of the carcass (The abdomen, thorax, crutch and breast) sites were swab sampled according to ISO 68882 (CLSI, 2012). During retail meat shop visit beef swab sample were taken simply from different location under aseptic conditions using sterile cotton and sterile containers

3.6.2. Environmental sample

At each abattoir and retail shop visit, three types of environmental samples were collected from utensil, floor & hall, and hand by swabbing the workers' hand, utensil as well as floor and hall.

### g. Study Methodology

i. Questionnaire survey and observation

Personal observation and structured questionnaire survey was conducted on butchers and abattoir workers to assess the hygienic practices. Information was collected on source and species of animal slaughtered, means of transporting meat, frequency of health checkup for abattoir workers and butchers, hygienic status of abattoir and retail meat outlets, training taken. The field observation was guided by a checklist on items and facilities required for good hygienic practices in the handling of raw beef at abattoir and retail meat shop. Main items on the checklist included educational level, experience, health certificate, training taken, washing (clean water and soap), proper clothing (gown, aprons and head cover) and general handling practices. The questionnaire was pre-tested before the formal study began. The questionnaire was filled by face to face interview with 52 beef handlers.

ii. Isolation and identification of *Staphylococcus aureus*

Aseptically the beef samples were taken for the bacteriological examination. Briefly A loop full of beef swab sample streaked on blood agar base (Oxoid, UK) enriched with 5-10% defebrinated sheep blood, and then α and β hemolytic coccal isolates were sub-cultured on selective media mannitol salt agar. Gram-positive, catalase-positive, were presumptively identified as Staphylococci and subjected to coagulase test using rabbit plasma at four hours for grit of coagulation property of organism then Purple agar base (PAB) with the addition of 1 % maltose was used to differentiate the pathogenic staphylococci, particularly the coagulase-positive isolates. (Quinn *et al*., 2011).

### *h.* Antimicrobial Susceptibility Test

Antibacterial susceptibility testing of 30 randomly selected *S. aureus* isolates was evaluated against ten different antibiotics (cefoxitin, gentamycin, vancomycin, penicillin-G, tetracycline, streptomycin, chloramphenicol, nalidixic acid, amoxicillin, Sulphamethoxazole-trimethoprim) by using Kibry-Bauer disk diffusion method following Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). Colonies isolated from pure culture were transferred into tubes containing 5 ml of normal saline or Tryptone soya broth. The broth culture was incubated at 37ºC for 8 hrs until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of Muller-Hinton agar plate with in a sterile safety cabinet. The plates were kept at room temperature for 15 minutes to allow drying. Antibiotic discs, with known concentration of antimicrobials, cefoxitin (30µg), gentamycin (10 µg), vancomycin (5 µg), penicillin G(10IU), tetracycline (10 µg), streptomycin (10 µg), chloramphenicol (10µg), Nalidixin acid (30µg), amoxicillin (30µg) , Sulphamethoxazole-trimethoprim (25µg) were placed and the plates were incubated for 24 hrs at 37ºC. Following incubation, the diameters of zone of inhibition were recorded to nearest millimeters for each disc used and then were classified as resistant, intermediate, and susceptible according to published interpretive chart of CLSI (2018).

### i. Data Management and Statistical Analysis

Processing of data was done by computer software. Data was coded and entered to MS Excel spreadsheet and checked for accuracy. After validation, it was transferred and processed using computer software STATA version 13 for analysis. Percentages, proportions, and frequency distributions were applied to compute some of the data. Logistic regression tests were used when appropriate to analyze the proportions of categorical data. Odd ratio and 95% CI were computed, the 95% confidence level was used, and results were considered significant at (P < 0.05).

## 3. RESUT

### a. Prevalence of *Staphylococcus Aureus*

After 240 swab samples subjected to culturing and biochemical isolation, 73(30.42%) were positive for *S. aureus*. The prevalence varied between sample sources and among sample types. Depending on the source of sample, contamination of butcher shop with *S. aureus* was 2 times more likely to occur compared to abattoir (OR=2; CI=1.169 - 3.824; p value= 0.013), showing statistically significant variation in the prevalence of *S. aureus* among sample sources. Similarly, depending on the types of sample, contamination of utensil swab sample with *S. aureus* was 4.2 times more likely to occur compared to carcass (OR=4.2;CI=1.95-9.05; p value = 0.000), which was statistically highly significant, contamination of hand swab sample with *S. aureus* was 2.5 times more likely to occur compared to carcass (OR= 2.5;CI=1.159 - 5.383; p-value = 0.020), which was statistically significant, and the bacteria were less likely to be present in floor and hall swab as compared to carcass swab (OR = 0.573;CI=0.216 - 1.519; P-value = 0.263), which was statistically not significant.

Table 3: Prevalence of *S.aureus* by sample source and type

| Sample source and type | | Sample analyzed | No of Positives (%) | OR | 95%CI | P- value |
|---|---|---|---|---|---|---|
| Sample Source | Abattoir | 120 | 28 (23.3%) | Ref | - | - |
| | Butcher shop | 120 | 45 (37.50%) | 2.114 | [1.169 - 3.824] | 0.013* |
| Sample Type | Carcass swab | 120 | 28 (23.3%) | Ref | - | - |
| | Utensil swab | 40 | 22 (55.0%) | 4.198 | [1.947 - 9.051] | 0.000** |
| | Hand swab | 40 | 17(42.50%) | 2.494 | [1.159 - 5.383] | 0.020* |
| | Floor and hall swab | 40 | 6 (15.0%) | 0.573 | [0.216 - 1.519] | 0.263 |
| Total | | 240 | 73 (30.42%) | | | 0.000 |

N.B. * = significant, ** = highly significant, CI = Confidence Interval, OR = odds ratio

### b. Socio-demographic Characteristics of Respondents

A total of 52 individuals were interviewed during the study of which 16 were abattoir workers and 36 were butchers and all of the respondents were males. 50% of respondents in abattoir and retail meat shops were found within the age range between 18-30years. In this study 16 abattoir workers were interviewed, out of which 44% were illiterate and 56% were literate. Similarly in retail meat outlets, 41.7% were illiterate and 58.3% were literate. Among 36 abattoir workers, 64% had work experience of less than five years and 36 % had above 5 years. It was also observed that 68.7% of abattoir workers had work experience of less than five years and 31.3% had above 5years.

Table 4: Socio demographic characteristics of respondents

| No. | Variable | Category | Abattoir workers (N=16%) | Butchers Shop workers (N=36, %) |
|---|---|---|---|---|
| 1 | Age | 18-30yrs | 8 (50) | 18 (50) |
| | | >30yrs | 8 (50) | 18 (50) |
| 2 | Sex | Female | 0 (0.0) | 0 (0.0) |
| | | Male | 16 (100) | 36(100) |
| 3 | Experience | <5yrs | 11 (68.7) | 23 (64) |
| | | >5yrs | 5 (31.3) | 13 (36) |
| 4. | Education | Illiterate | 7 (44) | 15 (41.7) |
| | | Literate | 9 (56) | 21 (58.3) |

**c. Assessment on Hygienic Handling Practice of Workers**

The study showed among 36 butcher shop workers interviewed to assess their hygienic practices, 78% had not obtained training, 75% did not wear apron, and 58.3% did not wear hair cover. Similarly, out of 16 abattoir workers, 94% had not obtained training, 37.5% did not wear apron, and 62.5 % did not wear hair cover. It was noted that out of 36 retail shop workers interviewed, 83% had no health certificate, 56% washed their hands improperly, 75% did not use sterilizing material, 67% collected and handled money by themselves, 53% did not use detergent for washing, 78% wore jewelry, and 78% cleaned their houses once a day.

Similarly, it was observed that 94% abattoir workers did not have health certificate, 75% did not use detergent, 100% cleaned house once a day, 75% wore jewelry, 75% did not use sterilizing material, and 87.5% washed hand improperly. This study also revealed that 62.5% and 75% of the meat cutting blocks at the abattoir and butcher shops respectively were found to be unclean.

**Table 5: Hygienic handling practice of workers**

| **No** | **Variable** | **Category** | **Abattoir workers (N=16, %)** | **Butchers shop (n=36)** |
|---|---|---|---|---|
| 1 | Use detergent | Yes | 4 (25) | 17 (47) |
| | | No | 12 (75) | 19 (53) |
| 2 | House clean Frequency | Twice a day | 0 (0.0) | 8 (22) |
| | | Once a day | 16 (100) | 28 (78) |
| 3 | Cutting board condition | Clean | 6 (37.5) | 9 (25) |
| | | Unclean | 10 (62.5) | 27 (75) |
| 4 | Apron/white coat | wore | 10 (62.5) | 9 (25) |
| | | Did not wear | 6 (37.5) | 27 (75) |
| 5 | Hair cover | covered | 6 (37.5) | 15 (41.7) |
| | | Did not cover | 10 (62.5) | 21 (58.3) |
| 6 | Health certificate | Had | 1 (6) | 6 (17) |
| | | Did not have | 15 (94) | 30 (83) |
| 7 | Training | Yes | 1 (6) | 8 (22) |

| | | | | |
|---|---|---|---|---|
| | | No | 15 (94) | 28 (78) |
| 8 | Jewelry | Yes | 12 (75) | 28 (78) |
| | | No | 4 (25) | 8 (22) |
| 9 | Sterilizing material | Yes | 4 (25) | 9 (25) |
| | | No | 12 (75) | 27 (75) |
| 10 | Hand washing | Proper | 2 (12.5) | 16 (44) |
| | | Improper | 14 (87.5) | 20 (56) |
| 11 | Handling money | cashier | | 12 (33) |
| | | Butcher with Bare hand | | 24 (67) |

### d. Antimicrobial Susceptibility

Due to shortage of discs, 30 *S. aureus* isolates were randomly selected and tested to ten antimicrobials, using the disc diffusion technique and their susceptibility varied among the ten antimicrobials. The isolates were susceptible to Amoxicillin (60%), Sulphamethoxazole-trimethoprim (56%), Gentamycin (50%), Tetracycline (46.7%) and Streptomycin (33%). On the contrary, isolates were found to be resistant to Cefoxitin (100%), Penicillin-G, (97%), Chloramphenicol (83%), Nalidixic acid (77%), Tetracycline (53.3%), Amoxicillin (40 %), Gentamycin (36.7%), Streptomycin (27%) and Sulphamethoxazole-trimethoprim (26.6%). Intermediate susceptibility was observed in Vancomycin (90%), Streptomycin (40%), Nalidixic acid (20%), Sulphamethoxazole-trimethoprim (16.6%), and Gentamycin (13.3%). No intermediate susceptibility was demonstrated in Cefoxitin, Penicillin-G, Tetracycline, Chloramphenicol, and Amoxicillin.

Table 6: Antimicrobial susceptibilities of the selected isolates of *S. aureus*.

| Drug type | Susceptible no.% | I no.% | R no.% |
|---|---|---|---|
| Cefoxitin 30 µg | 0 (0.0) | 0 (0.0) | 30 (100) |
| Gentamycin 10 µg | 15(50) | 4 (13.3) | 11 (36.7) |
| Vancomycin 5 µg | 1(3) | 27 (90) | 2 (7) |
| Penicillin-G 10 IU | 1(3) | 0 (0.0) | 29 (97) |
| Tetracycline 10 µg | 14 (46.7) | 0 (0.0) | 16 (53.3) |
| Chloramphenicol10 µg | 5(17) | 0 (0.0) | 25 (83) |
| Nalidixic acid 30 µg | 1(3) | 6 (20) | 23 (77) |
| Amoxicillin 30 µg | 18 (60) | 0(0.0) | 12 (40) |
| Sulphamethoxazole-trimethoprim 25 µg | 17(56.6) | 5 (16.6) | 8 (26.6) |
| Streptomycin 10 µg | 10 (33) | 12(40) | 8 (27) |

N.B. I=Intermediate, R=Resistant

## 4. DISCUSION

Present finding justifies significant presence of *S. aureus* as a zoonotic contaminate at abattoir and butcher shop in Asosa town which could be due to poor sanitation and poor hygienic handling process. The overall prevalence of the present study was 30.4%, with 23.3% observed in abattoir and 37.50% in butcher shops. The high prevalence was noted from butcher shops and abattoir due to the high probability of contamination during loading, transporting and unloading of meat. This finding was nearly comparable with that reported by Tesema and Tsegaye ( 2017) in Alage (Ziway; 28.2%) , Girmay *et al.* ( 2020) in Shire (Tigray; 29.09%), Tesfaye *et al.* (2021) in Adama town (30.6%) , Olatoye *et al.* (2018) in Oyo State ( Nigeria; 31.5%), Matallah *et al.* (2019) in Algeria (31.56%), and Hassen *et al.* (2018) in Asella town (34.3%) , but the finding was greater than 9.3% from abattoir, 19.5% from retail shops, and 17.5% from equipment of retail shops and abattoir (Adugna, 2018) in Addis Ababa Abattoir and retail shops, Ethiopia, and 20.3% obtained by (Thomas *et al.*, 2015) from abattoir and retail shops from Wolita Soddo. This variation could arise due to the differences in sample type, degrees of environmental hygiene, slaughtering procedures and hygiene, cleanliness of equipment, hygienic conditions during handling, and transportation among areas. In this study, the prevalence from hand swab and utensil swab was in line with that reported by Endale and Hailay (2013) in Mekelle city (40%) and Hassan *et al*. (2018) in Asella town (50%) respectively.

The present result was nearly comparable with prevalence rate reported from retail houses by Tefera and Jermen, ( 2021) in Debrebirhan town (37.5%), Pekana and Green (2018) in South Africa from abattoir of the Eastern Cape ( 20.4%), and (Abdulle, 2023) 20% from abattoir in Chiro town. The result was consistent with the previous findings (Senait and Moorty, 2016) 36.5% and (Adem *et al*., 2018) 36.4%. On the other hand, it was different from prevalence reported by (Birhan *et al*. 2020) 54.45%, (Weldeselassie *et al*., 2020) 68.75.%, and ( Abebe E *et al*.,2024) 50%.This difference was due to the type of samples taken and the relatively lower cleaning exercise could be attributed to the higher prevalence in their areas.

Ahmad and coworker of Egypt, isolated higher prevalence in a beef outlet (70%) than beef abattoirs (55%) (Ahmed *et al*., 2013).This accords with present result in that higher prevalence of contamination is observed in the butcher shops than the abattoir because of the continuous contamination through the transportation, loading and unloading process. In this study the high contamination of S. aureus at meat retail may be due to unhygienic carcass transportation, improper loading and unloading, unhygienic meat shop equipment and personnel. The variation in the prevalence of S. aureus from the different studies might be due to differences in sample size and type, isolation techniques, handling practices, awareness and skill of the worker, animal health delivery systems, and geographic region of the sampled area.

In recent years, the widespread use of antibiotics to treat bacterial infections has led to the emergence of multidrug-resistant (MDR) bacteria, which constitute a great hazard to public health For example, *S. aureus* could adapt to adverse environmental conditions, leading to the emergence of many strains resistant to different antibiotic classes (Mccallum. *al* 2010). In the current study, the large proportions of the *S. aureus* isolates were phenotypically resistant to Cefoxitin (100.0%), Penicillin G (97%), Chloramphenicol (83%) and Nalidixic acid (77%).The 100% resistance of *S. aureus* to Cefoxitin was consistent with the previous findings of Ayele *et al*. (2017) in Sebeta town, Aliyu *et al*. (2019) in Nasarawa State (Nigeria) and Yakubu *et al*. (2020) in Nasarawa State (Nigeria) who reported similar 100.0% resistance to Cefoxitin. However, Mekonnen *et al*. (2018) reported 100% sensitive *S. aureus* isolates to Cefoxitin in North-Western Ethiopia and Sudhanthiramani *et al*. (2015) reported low magnitude of Cefoxitin resistance (4.65%) in Tirupathi, India. The high resistance pattern of the isolates to Penicillin G (97%) was in agreement with the previous studies reported by Ayele *et al*. (2017) (98.85%) in Sebeta town, Pati and Mukherjee (2016) (96.0%) in Northern Plains of India, Daka *et al*. (2012) (100.0%) in Hawassa area, Girmay et al. (2020) (100.0%) in Shire town, Derib *et al*. (2017) (100%) in Wolaita Sodo town, Ayana *et al*. (2017) (100%) in Bishoftu town, and Mathenge *et al*. (2017) (99.6%) in Nairobi, Kenya. It was also noted that the *S. aureus* isolated from meat and meat environment samples had high prevalence of resistance against Chloramphenicol (83%), and this is in agreement with the previous study reported by (Abdulle, 2023) 80%. However, (Mohammed *et al*., 2016) detected 100% resistance against Chloramphenicol in Egypt. The proportion resistance to Nalidixic acid obtained in the present study was consistent with the report of Abebe *et al*. (2024) who reported 74.8% resistance to Nalidixic acid in Dessie and Kombolcha towns.

The antimicrobial sensitivity testing result indicated that 53.3% of the isolates showed resistance to Tetracycline which was consistent with Mekonnen *et al*. (2018) who reported 54.0% resistance to Tetracycline in North-Western Ethiopia. However, 100.0% susceptibility to Tetracycline was reported by Sori *et al*. (2011) in Jimma town. In the current study, 40% of the isolates were also resistant to Amoxicillin/Clavulanic acid which was consistent with the report of Pati and Mukherjee (2016) (37.0%) in Northern Plains of India. Furthermore, high proportions of *S. aureus* isolates (90%) showed intermediate susceptibility to Vancomycin. However, Massawe *et al*. (2019) and Pati and Mukherjee (2016) reported *S. aureus* isolates which were 100.0% susceptible to Vancomycin in Mbeya Region (Tanzania) and Northern Plains of India, respectively.

In this study, all of respondents in abattoir and retail meat shop were males with age ranging between 18-30 years (50%). This result was in accord with the result reported by (Ntanga, 2013) found 50% of workers had an average age 18-30 years, 31-40 years (30%), 10% below 18 years and 41-60 years (10%). This result was in harmony with the reports that males were more likely to be involved in meat processing than females (Tegegne *et al* 2017),`(Adzitey *et al*. 2011b) reported that the butchering activity is more dominated by the youth and middle aged men who are more energetic as the butchering business requires much physical strength. Finding from this study was different from what was reported by (Adzitey *et al*. 2011b) that found 45% of the abattoir workers were within the ages of 41-50 years, followed by 31-40 (23%), 51-60 (16%) and 21-30 (13%).

From 52 interviewed respondents, 42.3% of them were illiterate. This result was consistent with that of (Gurmu and Gebretinsae, 2013) who reported out of 24 butcher shop workers that were interviewed for their knowledge on meat hygiene, and 41.7% of them found to be illiterate and also (Adugna, 2018) reported that 41.7% of respondents were illiterate, but different from the result of survey conducted in Makelle City, Ethiopia by (Haileselassie *et al*. 2013) that reported out of 26 abattoir workers interviewed, 7.7% of them were illiterate. (Bhandare *et al.*, 2009) reported that workers working in the abattoir in most cases in developing countries are untrained and thus, they pay no attention to the hygienic standards and as a result contribute immensely to bacterial contamination. Meat handlers can serve as a vehicle of cross contamination and spread of foodborne pathogens (Wambui *et al*., 2017). The educational level of food handlers about the basic concept and requirements of personal hygiene and its environment plays an important part in safeguarding the safety of products to consumers. During this study, it was revealed that the abattoir and beef retail shop workers had low level of education and this could make difficult in the acceptability of modern slaughtering practices as well as adherence to strict hygienic and standard slaughtering practices that contribute to microbial contamination.

In this study out of 52 abattoir and retail shop workers, 65% had less than 5 years of experience which was comparable with report of (Birhan *et al*. 2020) that reported 68.3.2% of the food handlers had experience less than 5 years and (Gutema *et al*. 2021) reported (57.1% )of the food handlers had experience 1-5 years.

The practice of wearing protective clothes helps to reduce the burden of contaminants in meat. Regarding this, the Ethiopian Ministry of Agriculture (MoA, 2010) recommends that personal clothing can carry microorganisms (germs) that have been gathered from a wide variety of sources in to the meat or meat handling facility. Therefore, to protect meat and meat handling facilities from contamination because of personal clothing, protective overalls or hair cover should be worn at all times when handling meat. The overalls should be light in color so that contamination can be easily identified and the overalls cleaned easily. This study shows that 37.5 of abattoir works did not wear apron and 62.5% did not wear hair cover, this report was inconstant with 48.4% of abattoir workers did not cover their hair, (29%) did not use apron, which reported by Abebe *et al*. (2019), but this finding is in agreement with (Haileselassie *et al*., 2013) where (61.6%) of abattoir workers did not cover their hair. Similar to the abattoir, protective cloth is important in the butcher shops to reduce the chance of contamination. In order to protect both food products and meat handlers from cross contamination, the abattoir and butcher shop workers should wear protective clothes while working. In this study, 58.3.% of butcher shop workers did not wear hair cover and 75 % did not wear apron, which is comparable with study conducted in Addis Ababa, central Ethiopia by (Adugna, 2018) who reported 58.5% of butcher shop workers did not cover their hair and 75% did not wear apron.

In this study, (67%) of the butchers collected and handled money bare hand while working with meat. This report was in agreement with (66.7%) and (65%) reported by (Abebe *et al*., 2019) and (Adugna, 2018) respectively, but this report was different from the result reported by (Kyayesimira *et al*. 2019) (93.5%) of meat handlers doubled as cashiers and this means that they handled money and meat concurrently. (Nervy *et al.* 2011) reported that handling of carcasses and money with the same unwashed hands could be good sources of contamination. This makes it as a prime transmission medium for various microorganisms and it may pose a major health hazard particularly, when meat and money were grasped together without washing hands (Girma *et al* 2014).

This study revealed that (77%) of the workers had worn jewelry during butchering process. This finding was in agreement with the result of the study conducted by (JyotiPawan, 2019) that reported (77.8%) of the meat handlers wore jewelries like watches, earrings and rings which was against the principle that jewelry should not be worn in food service area because bacteria and food could gather within them and the area underneath the jewelry warms up thus further encourage the growth and spread of bacteria (FSSAI, 2010; Marriott and Gravani 2006)). But this result was different from the result reported by (Birhan *et al.* 2020) that found (59.41%) of the workers were wearing jewelry during butchering operation and (Haileselassie *et al*., 2013) found (78.9%) of the butcher shop workers wore jewelry during butchering operation. Jewelry during butchering operation can become favorable breeding site for microorganisms and in turn source of contamination of meat.

In this study out of 16 abattoir worker interviewed, only the inspector had training on meat handling and the rest did not. This result was similar with the result reported by (Henok *et al.* 2017) that found out none of respondents had attended training related to food safety except one meat inspector working in the municipality. Several studies mentioned that food safety trainings should be provided to improve knowledge, attitude and safety practices of food handlers (Akabanda *et al*., 2017). In this study from 52 abattoir and retail shop workers, (82.3%) did not receive training about food safety and meat hygiene, and this result was in line with the result reported by (Alam *et al*. 2020) that found the majority (85%) of meat handlers in slaughter houses and meat selling centers had not taken any form of training concerning food safety and meat hygiene.

A regular health checkup of butcher shops workers is important since it helps in the control and prevention of transmission of food borne diseases to the consumer. However, in this study out of 36 butcher shop workers, (83%) of them never had any health check-up. The finding from this study was consistent with (JyotiPawan *et al.*, 2019) who reported that (83.3%) of workers never had any healthy checkup, but different from that of (Ntanga, 2013) who reported that all workers in retail meat outlets of Morogoro municipality, Tanzania, had a routine medical examination and regularly inspected by Health Officers. The high percentage of butcher shop workers who never had any heath checkup might be attributed to the lack of knowledge, poor economic background and lack of visit by the health officers. This report was also not in line with (Gutema *et al*. 2021) reported 98% of the respondents at retail shops confirmed having had a medical check-up.

Most of surveyed retail meat outlets had poor hygienic condition despite daily cleaning of their shops with water and soap. (Ali *et al*. 2010) reported that butcher men lack knowledge of disinfecting and sanitizing, they clean their shops once in 24 hours with detergent and water which is not enough to maintain the hygienic environments in the butcher. Regular cleaning and disinfecting the beef retail outlets is important since it helps to reduce microbial contamination. In this study 78% of them cleaned their shops once a day. Based on findings from this study, the sources of meat contamination originated from the slaughter process in the abattoir, poor hygienic environmental condition and unhygienic handling of meat to the retail meat outlets.

Meat wood cutting blocks are commonly used in most of the butcher shops even though it harbors microorganisms due to absorptive nature. In the present study, 75% of the meat wood cutting blocks used in the selected butcher shops were in poor hygienic condition. This finding was in line with the report of (Ntanga ,2013) who reported 73.70% of the wood cutting blocks present in the shops of Tanzania were in poor hygienic condition and (JyotiPawan *et al*., 2019) who reported that 77.78% of the meat wood cutting blocks used in the butcher shops were in poor hygienic condition.
In this study 53% of butcher shop workers did not used detergent for cleaning, this result was in line with that reported by (Adugna *et al*., 2018) 41.1% of the butcher shop workers used only water for cleaning and also our report was comparable with the result of (Kyayesimira *et al*., 2019) that reported (60%) workers didn’t use detergent.

In general, the observed unhygienic practices at the slaughterhouses and retail shops can be linked with lack or inadequate knowledge of basic hygienic practices, lack of infrastructure or facilities and poor compliance to standards of good handling practices of food. Moreover, the insufficient implementation of the government control systems and ensuing timely corrective actions by the food regulatory bodies, which is common in most developing countries including Ethiopia, might contribute to sustaining such unhygienic practices leading to a higher risk for human infection necessitating urgent interventions (Gutema *et al* 2021).

## 5. CONCLUSION AND RECOMMENDATIONS

This study identified poor hygienic practices at both slaughterhouses and retail shops that can predispose the public to meat borne infections and intoxication. This was supported by several factors such as lack of training, not wearing appropriate clothing, inadequate equipment, hand, and surface washing as well as absence of sterilizing and low educational level of the workers. From these results, it was figured out that contamination existed at the abattoir and retail shops where meat was processed and sold before it got into hands of the consumers. According to the findings from this study utensil, human hand, hall and floor at abattoir and retail shops were sources of contamination of animal meat with bacteria *Staphylococcus aureus* and their contamination was indicator of poor hygienic practices. Large proportions of *S. aureus* isolates developed resistance to the antimicrobial agents such as Cefoxitin, Penicillin G, Chloramphenicol, and Nalidixic acid. The development of antimicrobial resistance might be as a result of repeated therapeutic and/or indiscriminate use of them in veterinary medicine. Based on the above conclusion, the following recommendations have been given:-

- It is important to create awareness about hygiene and sanitation of meat both at abattoir and butcher shop, and appropriate control method of the problems should be designed and implemented
- The government authorities should renovate the abattoir in Asosa municipality.
- Awareness should be raised on responsible and discriminate use of antibiotics as well as handling, and storage. Strict regulation on their handling, storage, and usage must be made.
- Regular quality control on risk factors associated with hygienic handling practices at abattoir and retail shop should exist
- Drug resistant S. aureus have a wide distribution in meat and meat environment therefore care should be taken to reduce contamination with the microorganisms to avoid the risk of human infection
- Further study should be carried out to isolate and characterize *S. aureus*
- in meat in the area

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