Isolation, Identification and Antimicrobial Susceptibility Profiles of *Staphylococcus Aureus* from Slaughtered Swine in Bishoftu Town, Ethiopia

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Abstract

Bacteria's of the Staphylococcus species are major public health crisis which causes a number of human and animal diseases. A cross-sectional study was conducted from November 2017 to May 2018 to determine the prevalence of *Staphylococcus aureus* in Bishoftu slaughter house from carcasses and lung swab and antibiotic resistance profiles of the isolates found in the swine. In the present study a total of 150 swine were examined. From which the swab samples were obtained from carcass (n=67) and lung (n=83). Carcass was the most contaminated with *S. aureus* with a prevalence of 19.4% (13/67) and the prevalence from the lung swab was 15.7% (13/83). Consequently, there was no statistically significant association (P=0.547) observed between carcass and lung swabs. The prevalence of *S. aureus* was statistically significant difference (P=0.029) between the age of swine. Body conditions has statistically significant association (P=0.037) with the *S. aureus*. Swine at the age of ≤ 2 years are more susceptible to *S. aureus* infection.

Antimicrobial susceptibility test was also conducted on 9 isolates of *S. aureus*, using the disc diffusion susceptibility method. In this study, varying level of resistance of *S. aureus* was recorded against Amoxicillin, Penicillin G, and Ampicillin, and 88.9% to Nitrofurantoin and Sulphamethoxazoletrimethoprim. Based on bacteriological culture result, and classic antimicrobial susceptibility test it is concluded that pork can be source of staphylococcus to human and routine inspections should be conducted.

Keywords: *Antimicrobial susceptibility tes; Coagulase positive; Ethiopia; Prevalence; Staphylococcus aureu; Swine*

1. Introduction

Coagulase positive staphylococci (CPS) are often used as indicators for which are world wide the most important cause of Foodborne diseases (FBD). FBD are universal public health problems (Kerouanton *et al*., 2007; Pal, 2013) and occur commonly in developing countries, particularly in Africa because of the prevailing poor food handling and sanitation practices, weak regulatory system, lack of financial resources to invest in safer equipment and lack of education for food handlers (Haileselassie *et al*., 2013). It often follows the consumption of contaminated foodstuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria as Salmonella species, *Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejiuni,* and *Escherichia col*i O157: H7 (Nouichi and Hamdi, 2009).

Staphylococcal food poisoning (SFP) is considered to be the second or third most common pathogen causing outbreaks of food poisoning only outnumbered by Salmonella species, and in competition with *Clostridium perfringen* (Aycicek *et al*., 2005). It has been reported that, one of the important pathogens often transmitted via food contaminated by infected food handlers is *Staphylococcus aureus* (Arse *et al.,* 2013). Staphylococcosis, an infectious bacterial zoonosis of global significance, is caused by *Staphylococcus aureus*, Gram-positive cocci which can be isolated, paired and most often aggregated, forming unmoving grapelike structures (Pal, 2007).

They can be divided into two groups, coagulase positive Staphylococcus (CPS) and coagulase negative staphylococci (CNS), according to production of coagulase enzyme, which is capable of coagulating blood plasma (Bergeron *et al*., 2011). An important characteristic that differentiates *S. aureus* from most staphylococcal species is its ability to produce coagulase, an enzyme that clots blood plasma. Other coagulase positive species such as *S. hycus* have been also identified (Shah, 2003).

Pork is considered an important source of proteins, essential vitamins, minerals and amino acid which is fit for normal wellbeing (an overall health) of individuals. Due to this rich composition, it offers a highly favorable environment for the growth of pathogenic bacteria. The microbiological contamination of pork occurs mainly during processing and manipulation, such as skinning (shaving), evisceration, storage and distribution at slaughter houses and retail establishments (Abdalla *et al.*, 2009). The presence of even small numbers of pathogens in pork and edible offal may lead to heavy contamination of minced pork when it is cut into pieces; as more microorganisms are added to the surfaces of exposed tissue (Abuna *et al.*, 2013).

Antimicrobial resistance is a major public health problem in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food (Normanno *et al.*, 2007). Staphylococcus is a genus of worldwide distributed bacteria correlated to several infectious diseases of different sites in human and animals. Its importance is not only because of its distribution and pathogenicity but especially due to its ability to overcome antimicrobial effects (Souza *et al.*, 2012).

In spite of the aforementioned prevailing situation and the presence of a number of public health problems due to FBDs resulting from the consumption of different food items including pork products, in Ethiopia, there is none of well-documented information on the occurrence of Staphylococcus in the swine. Although *S. aureus* is a commensal of several mammalian species, until recently animals were considered of negligible significance as reservoirs for human *S. aureus* infections. Therefore the objectives of this study were; to isolate and identify the Staphylococci and to evaluate the antimicrobial susceptibility patterns of the isolates *Staphylococcus aureus* derived from swine slaughtered at ALEMA Abattoir PLC in Bishoftu, Ethiopia.

2. Material and Methods

2.1. Sample Collection and Transportation

Simple random sampling method was employed to select swine presented for slaughter regardless of age, sex and body condition scores. Carcass and lung swabs were taken from slaughtered pigs. For convenience, the swabs were considered as samples from the immediate slaughtered swine. Each of the sterilized test tubes was labeled by mentioning sample number, type of samples (carcass and lung swabs) and date of collection. Then, they were transported in ice box packed with solid packs to keep the cold chain until they reached Addis Ababa University College of Veterinary Medicine Microbiology laboratory for analysis. Samples was kept at $+4^{O}C$ when processed within two days and refrigerated at $-20^{0}C$ for a greater than two days for later processing (OIE, 2009).

2.2. Bacteriological Examination

Bacteriological examinations of the collected samples were conducted according to standard methods recommended by Woldehiwot (1996) and Quinn *et al.* (1999). The samples from abattoir were cultured on blood agar plates and incubated at 37°C for 24-48 hours aerobically. After 24hours of incubation, colonial morphology, color, presence or absence of hemolysis and growth on blood agar plates were recorded. From culture positive plates representative colonies were further sub-cultured to nutrient agar to get pure colonies for further tests. Pure culture isolates were subjected to tests that were used as primary identification (Gram stain, catalase test and O-F test, oxidase) and secondary biochemical test (coagulase test, MSA, PAB) were carried out according to technique recommended (Quinn *et al*, 1999; Carter, 1999).

2.3. Isolation and Identification of Staphylococci

Final identification of Staphylococci organisms and species was done based on culture characteristics, Gram staining, series of biochemical tests like catalase test, coagulase test and sugar fermentation as described by (Quinn *et al.*, 2002) (figure 1-6).

2.3.1 Culturing and colony appearance

All the samples was directly streaked onto 5% sheep blood agar and incubated aerobically at $37^{0}C$ for 24–48 hours. The bacteriological media used was prepared according to the manufacturer's recommendations. The plates were examined for the presence of Staphylococcus colonies. Isolates supposed to belong to Staphylococcus species on the basis of their morphological aspects (round, smooth and white or yellow colonies) and hemolytic pattern on the surface of blood agar plates (BAP) were collected. Presumed staphylococcal colonies were then sub-cultured on nutrient agar plates (NAP) and incubated at $37^{0}C$ for 24-48 hours to get a pure culture (clone of cells derived from a single cell).

After growth, presumptive colonies were identified by using conventional bacteriological techniques on the basis of colony characteristics, pigment production and hemolysis. Final identification of the organisms and species was done based on Gram staining, catalase test, sugar fermentation and coagulase test (by using rabbit

plasma). Pure cultures of a single colony type from the NAP were inoculated into tryptone soya broth and incubated at 37^0C for 24-48 hours under aerobic culture conditions. The pure isolates in the tryptone soya brothwere preserved and maintained at 4^0C for further need (Quinn *et al*., 2002).

2.3.2 Gram's staining

All suspected cultures of Staphylococci species were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, shape, and cell arrangements. The Gram stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive staphylococcus species (Quinn *et al*., 2002).

2.3.3 Catalase test

The center of an 18-24hour pure colony of the isolates were picked using a sterile loop form the nutrient agar plate and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism positive, bubbles of oxygen liberated within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as Staphylococci (Quinn *et al*., 2002).

2.3.4 Oxidase test

The test was used to detect the production of cytochrome-c-oxidase by microorganisms. Colonies assumed to be staphylococci were streaked firmly across a piece of filter paper moistened with 1% aqueous solution of tetramethyl-p-phenylenediaminedihydrochloride with a sterile plastic stick in a Petri-dish. The development of dark purple color along the streaked line within 10 seconds was taken as positive reaction (Gebrewahid *et al*., 2012).

2.3.5 Oxidation-Fermentation (O-F) test

The convectional O-F medium is most suitable for non-fastidious Gram negative bacteria. The modification of the O-F medium which is applied for the identification of Staphylococcus and Micrococcu was used (Quinn *et al., 2002*).

2.3.6 Mannitol salt agar

The colonies that were identified by Gram-staining reaction and catalase test as Staphylococcus were streaked on Mannitol salt agar (MSA) plates and incubated at 37^0C and examined after 24-48 hours for growth and change in the colour of the medium. The presence of growth and change of pH in the media (red to yellow colour) were regarded as confirmative identification of Staphylococci. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discolouration of the medium. Colonies that develop weak or delayed yellow colour after 24 hours of incubation were taken as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus* and CNS (Quinn *et al*., 2002).

2.3.7 Coagulase test

The tube coagulase test was performed in sterile tubes by adding 0.5 ml of selected isolates of Staphylococcus grown on tryptone soya broth (TSB) at 37^0C for 24 hours to 0.5 ml of fresh rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37^0C along with a negative control tube containing a mixture of 0.5 ml of sterile TSB and 0.5 ml of rabbit plasma. Clotting was evaluated at 30 minutes intervals for the first 4 hours of the test and then after 24 hours incubation. The reaction was considered positive, if any degree of clotting from a loose clot to a solid clot that is immovable when the tube is inverted (tilted) was visible within the tube and no degree of clotting would be taken as negative (Quinn *et al*., 2002).

2.3.8 Purple agar base

Purple agar base (PAB) with the addition of 1% maltose was used to differentiate the pathogenic staphylococci, particularly the coagulase-positive isolates. The suspected culture was inoculated on PAB media plate with 1% of maltose and incubated at 37^0C for 24-48 hours. The identification was based on the fact that *S. aureus* rapidly ferment maltose and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. *S. hicus* did not ferment maltose but attacks the peptone in the medium producing an alkaline reaction (a deeper purple) around the colonies (Quinn *et al*., 2002).

2.4 Antimicrobial Susceptibility Testing

Positively identified Staphylococci strains were tested for their susceptibility to different antimicrobials using the disk diffusion method with incubation at 37^0C overnight (Wikler, 2008). Ten different antimicrobials drugs were used: Amoxycillin (AML-2μg), Streptomycine(S-25μg) Chloramphenicol (C-30μg), Ampcillin (AMP-

10μg), Bacitracin (B-10μg), Nitrofurantoin (F 50μg), Streptomycin (S-10μg), Sulphamethoxazoletrimethoprim (SXT-25μg), Vancomycin(VA-30μg), Penicillin G (P-10 μg) and Oxytetracycline (OT-30μg). Well isolated colonies of the same morphological type were selected from a non-selective agar plate and suspension was made in sterile saline. The turbidity of the suspension was adjusted by comparison with a 0.5ml McFarland turbidity standard. A sterile swab was dipped into the standardized suspension of bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube. The swab was streaked in three directions and continuously brushed over the Mueller Hinton agar (MHA) and inoculated plates were allowed to stand for 3-5 minutes. The discs were placed onto the agar surface using sterile forceps and gently pressed with the point of a sterile forceps to ensure complete contact with the agar surface and the plates were incubated aerobically at 37°C for 18–24 hours (CLSI, 2015; CLSI, 2012). The inhibition zone was reported as the diameter of the zone of surrounding the individual disk which bacterial growth was absent. Based on this, the isolates were defined as resistant, intermediate and susceptible according to the guide lines of the manual manufacturer and clinical laboratory standard institute (CLSI) (CLSI, 2012; CLSI, 2015) (table 3).

2.4 Data Analysis

All collected row data was enter and coded using Microsoft Excel W 2010 and analyzed using the software package STATA 13 (Stata Corp, Texas, USA) and IBM SPSS (Statistical Package for Social Science) version 20. Prevalence was calculated as the proportion of suspected swab positive animals from the total number of animals sampled (Thrusfield, 2007). Many attribute data was imported to database system, includes, age, sex, body condition and laboratory result. Variation of infection prevalence between age, sex, body condition, and sample type were determined by using a chi-square (x^2) statistics. A p-value of less than 0.05% at 95% confidence interval was considered as statistically significant.

3. Results

3.1 Prevalence and Distribution of Staphylococcus Species

Presence of Staphylococci was detected in 42.00% (63/150) from analyzed samples of swine. The results of biochemical characterization of these isolates showed that *S. aureus* was the most frequently isolated species among two different sample types. From 63 Staphylococci isolates were comprised of 41.27% (26/63) *S. aureus*, 30.16% (19/63) *S. hycius* and 28.57 (18/63) Coagulase-Negative Staphylococcus (CNS). The identification results showed a predominance of Coagulase-Psitive Staphylococcus (CPS) (i.e. *S. aureus* and *hycius*) with a total of 71.43% (45/63) isolates of the population studied.

The frequency of isolated *S. aureus* were the same between two different sample types (carcass swab=13/67 and lung swabs=13/83), but the frequency of isolation of *S. aureus* varied between age (≤2 years=13/44, 3-4 years=9/61, and ≥5 years=4/45), sex (male=11/56 and female=15/94) and body conditions (medium=7/21 and fat=19/129) of the slaughtered swine.

There were no statically significant difference between sample types (P=0.547) and sex (P= 0.65) of the animal. Carcass was the most contaminated with *S. aureus* with a prevalence of 19.4% (13/67) than lung swabs 15.7% (13/83) and female slaughtered swine was the most contaminated with *S. aureus* with prevalence of 19.6% (11/56) than male slaughtered swine 16.0% (15/94).

Prevalence of Staphylococci were statistically significant (P=0.029) between the age of swine with prevalence (3 month-2 years=29.5%, 3-4 years=14.8% and 5-6 years=8.9% %). Body conditions was also statistically significant (P=0.037) with the prevalence of *S. aureus* (table 1).

A total of 9 *S. aureus* isolates were tested to various antimicrobial agents by disc diffusion technique. The resistant pattern varied among ten drugs. In general, all strains of *S. aureus* were (100%) susceptible to chloramphenicol and vancomycin. On the other hand, all isolates strains were found to be (100%) resistant to Amoxicillin, Penicillin G, and Ampicillin, and 88.9% to Nitrofurantoin and Sulphamethoxazole trimethoprim. It was noticed that the strains of *S. aureus* were resistant to (33.3%) Streptomycin, while 44.4% of the strains were susceptible to Bacitracin. Intermediate susceptibility was observed in Nitrofurantoin (11.1%), Streptomycin (44.4%) and Bacitracin (55.6%) (table 2).

4. Discussion

The present study assessed the prevalence of *S. aureus* and antibiotic susceptibly test in swine slaughtered at ALEMA abattoir PLC. The Genus *Staphylococcus* consists of a variety of opportunistic pathogens of variable relevance in veterinary medicine. The most clinically relevant *Staphylococcus* in veterinary medicine is the coagulase positive *Staphylococcus aureus*. A noted property of staphylococci is their ability to become resistant to antimicrobials (Weese & Duijkeren, 2009; Brown *et al*., 2005). *Staphylococcus aureus* is an important cause of food poisoning, pneumonia, wound and nosocomial bacteremia. It is one of the natural components of microflora and may exist in environment, on skin and in mucus membrane. Most animals may be colonized with *S. aureus*, but only recently MRSA strains were isolated from several food production animals, including swine, cattle, chicken and other animals (Boucher *et al*., 2010; Weese & Duijkeren, 2009). Although all this constraints were exists between *S. aureus* and food producing animal species there was no previous recorded valid data indicating *S. aureus* in swine in Ethiopia.

In the present study, 42.00% (63/150) staphylococci from analyzed samples of swine were detected. The prevalence of *staphylococcus aureus* was 41.27% (26/63), which is in agreement with findings of Bedada & Hiko, (2011), Workineh *et al*. (2002), Dego & Tareke, (2003), Huseain *et al*. (2013) and Stefania & Antonio (2000) who reported 39.1%, 39.2, 40.3%, 41.1% and 43.3% *S. aureus* isolates in bovine mastitis in Ethiopia, respectively. But this finding is higher than the findings observed in Taiwan reported 11.3% by Jyhshiun *et al*., (2009) that isolated *S. aureus* strains from pork carcass and by Bitaw *et al*. (2010) who reported 20.3% *S. aureus* isolated from dairy farms in Bahir Dar town and its environment.. The current result was in agreement with the study carried out in Ethiopia, Abunna *et al*. (2017) reported 41% of the isolation rate of *S. aureus* from samples that were taken from dairy farm as well as abattoir of beef cattle.

The present study showed 41.27% positive for *S. aureus* is almost four times higher than the findings of Bishi (1998) and Hussein *et al*. (1997) who reported 9% and 10% prevalence in Addis Ababa respectively from lactating dairy cows, swabs of materials and personnel in the farm. Such dissimilarities could arise from the difference in sample type, study animal and isolation procedure employed.

From Athens Georgia Charlene *et al*. (2013) also reported that 65% of beef product from retail meat was contaminated by *S. aureus* which is in disagreement result with the result of present study. The probable reason for differences in prevalence rate could be explained by the different techniques used differences in sample type or by geographical differences. The high isolation rate of *S. aureus* in this study indicate poor hygiene and working practices of the pork handlers during the processing stage as well as lack of sterilization of equipment and working surfaces.

Prevalence of *S. aureus* was statistically significant between the age of the animal and Body conditions were also statistically significant with the prevalence of *S. aureus.* This was in agreement with the report of Smith *et al*. (2009) and kuller *et al*. (2004). Significant difference was not observed in this study in relation to infection rate between male and female as well as sample types. This was in agreement with the report of Okunlola & Ayandele (2015) that isolates MRSA *S. aureus* from swine farm.

In the present study, result of antibiogram showed that *S. aureus* isolates demonstrated higher susceptibility (100%) to chloramphincol and vancomycin. On the other hand, all isolates strains were found to be highly resistant (100%) to Amoxicillin, Penicillin G, and Ampicillin, and 88.9% to Nitrofurantoin and Sulphamethoxazole. The results of present study are almost comparable with the work of Abera *et al*. (2012) and Iroha & co-workers (2011), which the strains of *S. aureus* were susceptible to chloramphincol and disagreement with the recorded of the same authors on relatively lower susceptibility 11.33% to tetracycline and 22.2% to streptomycin. Thus the resistance figures from different countries can considerably vary, from very low to very high. This may probably reflect the uses of effective antimicrobials in those countries.

The results of this study indicated that the resistance of *S. aureus* to Amoxicillin, Penicillin G, and Ampicillin was higher than the findings reported previously in Ethiopia 100% by Spohia (2011) and Bedada & Hiko, (2011). This study presented the contamination status of pork in Bishoftu abattoir, its surrounding environment as well as demonstrated the role of raw pork as a reservoir of antibiotic resistance bacteria that can be transferred to humans, thereby constituting a health problem.

Conclusion and Recommendations

Staphylococcus species were prevalent in municipal abattoir of ALEMA, Bishoftu Ethiopia. This study highlighted the prevalence and drug resistance of *Staphylococcus aureus* isolated from slaughtered swine. *Staphylococcus aureus* was proportionally higher when compared to another Staphylococcal species. Over all, the

presence of pathogenic Staphylococcus poses a growing problem of concern, worldwide since the bacteria can be easily circulated in the environment. According to the findings of the present study from the antimicrobial susceptibility test higher multi-drug resistance of *S. aureus*, except (100%) sensitive to vancomycin (30µg) and chloramphenicol (30µg) was recorded. Food contamination with antibiotic-resistant bacteria can be a major threat to public health. To the best of our knowledge, this is the first report on the occurrence of *S. aureus* in pork from Ethiopia, the results warrant further investigations to elucidate the public health significance, and the enterotoxigenecity of the isolates in pork.

Therefore, based on the above conclusive remarks, the following recommendations are forwarded:-
- ➤ Abattoir workers and pork handlers should be educated and advocated on the adverse effects of lack of proper personal, environmental hygiene and sanitation; safeguard the public against the risks of food borne bacterial infections, practicing good sanitation and pork handling techniques in the abattoir
- ➤ The occurrence of multidrug resistance Staphylococcus particularly *S. aureus* should be under consideration during selection of antimicrobials for the treatment.
- ➤ Multiple drug resistant *Staphylococcus aureus* have a wide distribution in pork and therefore care should be taken in to account during processing to destroy the micro-organisms to avoid the risk of human infection.

Acknowledgments

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