

## Antibiotic Susceptibility of *Staphylococcus aureus* Isolated from Chicken Eggs, Eastern Ethiopia

Jelalu Kemal<sup>1\*</sup>, Wakene Beji<sup>2</sup>, and Gebregeorgis Tesfamariam<sup>2</sup>

<sup>1</sup>College of Veterinary Medicine, Haramaya University, P.O. Box, 138, Dire Dawa, Ethiopia

<sup>2</sup>College of Veterinary Medicine, Jigjiga University, Ethiopia

### Abstract

**Introduction:** *Staphylococcus aureus* is responsible for a variety of infections in humans and animals that can pose a major public health burden in many countries, including Ethiopia.

**Objectives:** This study was aimed to isolate *Staphylococcus aureus* present on the shell surfaces and in the contents of chicken eggs, and determine antibiotic susceptibility patterns.

**Material and Methods:** One hundred seventy-four (174) egg samples were obtained from open market and 161 from poultry farm. The surfaces of eggs were sampled using a sterile cotton swab. After sterilizing the shells, the egg contents were sampled. Identification of *Staphylococcus aureus* was done based on culture characteristics, and biochemical tests. The isolates were subjected to antibiotic susceptibility testing using disc diffusion method.

**Results:** A total of 93 (27.8%) *Staphylococcus aureus* samples were isolated. From these, 28 (17.4%) were from Haramaya University poultry farm while 65 (37.4%) were from market. In addition, 63 (18.8%) were from the shell while 30 (8.9%) were from the egg content. The occurrence of *Staphylococcus aureus* in the egg shell from open markets was significantly higher than the content from the egg shells obtained from farms ( $P = 0.021$ ). The level of *Staphylococcus aureus* content was also significantly higher in the market ( $P = 0.003$ ). All 76 *Staphylococcus aureus* isolates were resistant to at least one of the antimicrobials tested with the overall value 3.9–92.0% level of resistance pattern showing higher resistant to penicillin (92%) and ampicillin (89.5%). A lower level of resistance was observed to chloramphenicol, gentamycin and ciprofloxacin with complete susceptibility to vancomycin. Multiple drug resistance was detected in 86.8% of the *Staphylococcus aureus* isolates.

**Conclusion:** The study showed a significant level of *Staphylococcus aureus* with considerable antibiotic resistant pattern. Further studies are needed to better define bacterial resistance to antibiotic agents with emphasis on surveillance of multiple drug resistance.

**Keywords:** Antimicrobials; Egg shell; Egg content; Open market; Poultry farm; Resistance to antimicrobials

### 1. Introduction

Staphylococci are among the most common causative agents of food-borne outbreaks of infections worldwide and constitute a major public health burden and represent a significant cost in many countries (CDC, 2013; Yang *et al.*, 2016). Reports demonstrate that *Staphylococcus aureus* is responsible for a variety of infections in humans and animals (Petrovski *et al.*, 2006; Hata *et al.*, 2008). In humans, it is responsible for a variety of conditions ranging from superficial skin infections to life-threatening diseases, such as hemolytic pneumonia as well as endocarditis (Lindsay and Holden, 2004). The presence of the pathogen in food is one of the most common causes of staphylococcal food poisoning and toxic shock syndrome worldwide (Becker *et al.*, 2015). In animals, *S. aureus* causes mastitis, which is responsible for significant financial losses to dairy

farmers (Fitzgerald, 2012). Some studies conducted in Ethiopia found the occurrence of *S. aureus* from animal derivative food at various proportions such as 35.8% in Adama (Hailemariam Mekonnen and Tesfaye Ali, 2010), 12% in Jimma (Haimanot Tassew *et al.*, 2010) and 24% in Bishoftu (Mekonnen Addis *et al.*, 2011).

Misuse of antimicrobials in animal foods can generate genomic selective pressures that enable microbes to adapt and acquire resistance (Kohinur *et al.*, 2010) that could globally become an increasing public health issue (Michael *et al.*, 2014). Antimicrobial resistance, especially of pathogenic bacteria, has been partly attributed to the misuse of antimicrobial agents in medicine and agriculture (Michael *et al.*, 2014). Antimicrobial agents have been used widely in both human and veterinary medical practices that are widely used by the poultry industry to enhance growth and feed efficiency (Landers



*et al.*, 2012). Incorporation of these agents into poultry feed poses the emergence of some resistant bacteria either through genetic or non-genetic mechanisms (Ivanov, 2008). This and the husbandry practice such as feeding and watering used in the poultry industry made poultry a major reservoir of antimicrobial resistant pathogen (Hedman *et al.*, 2020).

The reservoir of antibiotic resistant bacteria in poultry and poultry products including eggs implies a potential risk for transfer of antibiotic resistant bacteria, or resistant genes to humans (Odwar *et al.*, 2014). High level of resistance to antibiotics in *S. aureus* isolates has been documented by several authors from countries such as Brazil (Costa *et al.* 2000), United States (De Oliveira *et al.*, 2000), Lebanese (Zouhairi *et al.*, 2010) and Portugal (Soares *et al.*, 2011). It is a common belief in Ethiopia that antimicrobials can be obtained without prescription (Serawit Deynu *et al.*, 2017). To our knowledge, the extent of *S. aureus* contamination of eggs sold at retail outlets and farms, and the antimicrobial profile of the *S. aureus* isolates has not been adequately studied. There is no information from eastern Ethiopia at all. Therefore, this study was conducted to investigate the occurrence and antimicrobial resistance patterns of *S. aureus* isolated from chicken eggs collected from Haramaya University poultry farm and nearby retail open market outlets.

## 2. Materials and Methods

### 2.1. Description of the Study Site and Study Population

The study was conducted at Haramaya University poultry farm and local market at Haramaya district. Haramaya district is located in eastern Hararge Zone of Oromia Regional State. It is found at the distance of 508 km from Addis Ababa to the easterly direction at the elevation of about 2006 meters above sea level, 9°26'N latitude and 42°3'E longitude. The mean annual minimum temperature is 8.5°C and with the maximum temperature of 24.4°C. Haramaya University poultry farm is located at 9°26'N latitude, 42°3'E longitude, and an altitude of 1980 meters above sea level and 513 km away from Addis Ababa. The annual average minimum and maximum temperature of the area are 8°C and 24°C, respectively (CSA, 2012). Haramaya University poultry farm practices intensive management system with exotic breed chickens. The farm aims to supply live chickens, eggs and three-month-old chicks to the surrounding farms, farmers, and private poultry farmers. The farm supplies antibiotics and other feed additives aimed to stimulate egg production, enhance growth performance, and for growing healthier chicks. Some of these antibiotics and additives include egg stimulant (Medion, Bandung, Indonesia), Oxytetracycline 20% power (Chengdu Qiankun Veterinary Pharmaceutical Co.,Ltd., China), Trisulpha Forte (Jordan Vet and Agr. Med. Ind., Co., Amman, Jordan), Amprolium 20% powder (Chengdu Qiankun Veterinary Pharmaceutical Co., Ltd.,

China), Aminovit (Medion, Bandung, Indonesia), Laprovit (Tours Cedex 2, France) and Vita Chicks (Medion, Bandung, Indonesia).

### 2.2. Study Design and Sample Size

A cross-sectional study was conducted from December 2017 to April 2018 which was aimed at isolating *S. aureus* present on the shell surfaces and in the internal parts of chicken eggs, and determine antibiotic susceptibility patterns. A total of 335 chicken eggs from Haramaya University poultry farm (n = 161) and local market (n = 174) in Haramaya district were collected.

### 2.3. Egg Collection and Transportation

On average, ten eggs from Haramaya University's white leghorn caged birds and similarly 10 eggs from the retail market were collected once a week using a simple random sampling technique. Each egg sample was collected separately using sterile plastic bags and transported in an ice box for analysis in the veterinary microbiology laboratory of Haramaya University within a few hours of collection.

### 2.4. Sample Processing

The sampled eggs contained in the sterile plastic bags were opened using scissors and processed. The entire surface areas of the egg shell were swabbed with sterile cotton swabs which were dipped into sterile buffered peptone water (BPW: Oxoid Ltd, Hampshire, UK; Lab M Ltd., Quest Park, UK). A test tube that contained 10 mL BPW was used to incubate the egg shell swab samples separately. The egg contents were sampled from the same eggs from which the shell samples were collected after sterilizing the egg surfaces by immersing in 70% alcohol for at least 2 minutes; the eggs were then dried with air in a sterile chamber for 10 minutes, after which they were cracked with a sterile scalpel blade. Stomacher bags containing 225 mL sterile BPW were used to homogenize the egg contents for around 1 minute in a stomacher and incubated at 37°C for 18–24 h (ISO, 2002).

The samples were then transferred onto blood agar plates containing 5% sheep blood (Oxoid Ltd, Hampshire, UK; Lab M Ltd., Quest Park, UK) and then incubated under aerobic conditions at 37°C for 24-48 h, depending on the rate of growth of the bacteria. An initial bacteriological characterization was performed by evaluating the morphology of the colonies and the presence and type of haemolysis. *S. aureus* identification was done based on Gram staining, morphology, and conventional biochemical tests, including catalase, coagulase, and mannitol fermentation tests as described by Quinn *et al.* (2002).

### 2.5. Antibiotic Susceptibility Test

The *S. aureus* isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disc agar diffusion method on Mueller-Hinton agar medium (Oxoid Ltd,

Hampshire, UK; Lab M Ltd., Quest Park, UK), according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2013). The antimicrobial discs (Oxoid Ltd., Cambridge, UK) were selected in line with the recommendation of CLSI 2013; ampicillin (10µg/disc), amoxicillin (20µg/disc), chloramphenicol (30µg/disc), penicillin G (10IU/disc), tetracycline (30µg/disc), gentamicin (10µg/disc), cefoxitin (30µg/disc), erythromycin (15µg/disc), streptomycin (10µg/disc), kanamycin (30µg/disc), ciprofloxacin (5µg/disc), and trimethoprim-sulfamethoxazole (SXT, 25µg), vancomycin (30µg/disc). The antimicrobials used were selected from the currently available and commonly used chemotherapeutic agents

for the treatment of *S. aureus* infection in humans and animals. The results were read and interpreted based on the diameter of the zone of inhibition. The strains were designated as resistant (R), intermediate resistant (I), or susceptible (S) to a particular antibiotic based on the cut-off value (Table 1). Multiple drug resistant (MDR) were recorded for isolates showing resistance to more than two antimicrobials (Rota, 1996).

## 2.6. Quality Control

All the media and reagents were subjected to quality control using standard bacteria. *Staphylococcus aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used as quality control during the test.

Table 1. Zone diameter interpretive standards chart for *Staphylococci* species (CLSI, 2013).

Antibiotic agent	Disc code	Potency (µg)	Zone diameter nearest whole mm		
			R	I	S
Amoxicillin	AML	25µg	≤19	-	≥20
Ampicillin	AMP	10	≤13	14-16	≥17
Chloramphenicol	C	30µg	≤12	13-17	≥18
Penicillin G	P	10IU	≤28	-	≥29
Tetracycline	TE	30µg	≤14	15-18	≥19
Gentamicin	CN	10µg	≤12	13-14	≥15
Cefoxitin	FOX	30µg	≤21	-	≥22
Erythromycin	E	15µg	≤13	14-22	≥23
Streptomycin	S	10µg	≤11	12-14	≥15
Kanamycin	K	30µg	≤13	14-17	≥18
Ciprofloxacin	CIP	5µg	≤15	16-20	≥21
Sulphamethoxazole trimethoprim	SXT	25µg	≤10	11-15	≥16
Vancomycin	VA	30µg	≤15	-	≥15

Note: R = resistance, I = intermediate, and S = susceptible.

## 2.7. Data Management and Analysis

The data were entered into Excel databases and analyzed using STATA version 11.0 statistical software package programs. Descriptive statistics such as percentages and frequency distribution were used to describe the nature and the characteristics of the data. Comparisons between sample source and sample type were done by Chi-square ( $\chi^2$ ). Logistic regression was used to reveal the strength of the association of the potential risk factors with positivity of the samples. In this line, the degree of association between risk factors and the prevalence of *S. aureus* was analyzed using test odds ratio (OR). In all the analysis, the level of significance was set at 5% and the 95% confidence interval.

## 3. Results

### 3.1. Occurrence of *S. aureus* Spp. in Raw Chicken Egg Shell and Egg Contents

From the total 335 chicken eggs sample examined for bacteriological status, 93 (27.8%) of the samples

harbored *S. aureus*. The occurrences of *S. aureus* varied among the sampling types and sources. Out of the 93 (27.8%) eggs that tested positive for *S. aureus*, 63 (18.8%) were from the shell while 30 (8.9%) were from the internal content of the eggs. Of the 93 positive samples, 28 (17.4%) were the ones sampled from the poultry farm while 65 (37.4%) were from the open market (retail outlets). The occurrence of *S. aureus* in the egg shell collected from the local market was significantly higher than the level of *S. aureus* in the egg shells obtained from the poultry farm (CI = 0.2904 - 0.9078;  $p = 0.021$ ). The level of *S. aureus* in the egg contents from the open market was also significantly higher than the level of *S. aureus* in the egg contents from the poultry farm (CI = 0.0962- 0.6085;  $p = 0.003$ ). Similarly, the overall proportion of *S. aureus* from the eggs sampled in the open market was significantly higher than the level of *S. aureus* from the eggs obtained from the poultry farm (CI = 0.2591- 0.7585;  $p = 0.003$ ) (Table 2).

Table 2. Occurrence of *S. aureus* in egg shell and content of raw chicken eggs from local markets and poultry farm in eastern Ethiopia.

Sample source	Poultry farm		Open market		Total		OR(95% CI)	p-value
	No. examined	No. +ve (%)	No. Examined	No. +ve (%)	Examined	Positive (%)		
Egg shell	161	22 (13.7)	174	41 (23.6)	335	63 (18.8)	0.5 (0.2904 - 0.9078)	0.021
Egg content	161	6 (3.7)	174	24 (14.9)	335	30 (8.9)	0.25 (0.0962- 0.6085)	0.003
Total	161	28 (17.4)	174	65 (37.4)	335	93 (27.8)	0.5 (0.2591- 0.7585)	0.003

Note: OR = *odds ratio*.

### 3.2. Antibiotic Susceptibility Testing

In the antimicrobial resistance trials, out of 93 *S. aureus* isolates, 76 (81.7%) were subjected to antimicrobial resistance test. All the isolates showed resistance to at least one of the antimicrobials tested. The percentage of isolates susceptible, intermediate, and resistant to each antimicrobial agent is outlined in Table 2. Overall, *S. aureus* isolates revealed 3.9–92.0% level of resistance pattern to the antimicrobials tested. A large proportion of the isolates were resistant to penicillin (92%), ampicillin (89.5%), amoxicillin (55.3%) and erythromycin (51.3%). A lower level of resistance was observed against chloramphenicol, gentamycin and ciprofloxacin with a resistance level of about 3.9% each.

All the *S. aureus* isolates were susceptible to vancomycin (100%) (Table 3).

The level of multiple resistance patterns in *S. aureus* isolates is given in Table 4. Multiple drug resistance to more than two antimicrobial agents was detected in 66 (86.8%) of the total 76 *S. aureus* isolates. Three isolates (4.5%) were resistant to ten antimicrobials tested. Fourteen isolates were resistant to 4 antimicrobials tested. Multiple drug resistance was defined as resistance exhibited to more than two antimicrobials tested. Among the *S. aureus* isolates, 19.7%, 21.2%, and 18.2% exhibited resistance to three, four, and five antimicrobials, respectively.

Table 3. Antimicrobial resistance patterns of *S. aureus* isolates (n = 76) from chicken eggs sampled from a poultry farm and open local markets in Haramaya district, eastern Ethiopia.

Antimicrobial agent	Disc potency ( $\mu\text{g}/\text{disc}$ )	Resistant N (%)	Intermediate N (%)	Susceptible N (%)
Ampicillin	10	68 (89.5)	0 (0.0)	8 (10.8)
Amoxicillin	20	42 (55.3)	5 (6.6)	29 (38.1)
Cefoxitin	30	8 (10.5)	0 (0.0)	68 (89.5)
Chloramphenicol	30	3 (3.9)	2 (2.6)	71 (93.4)
Ciprofloxacin	5	3 (3.9)	2 (2.6)	71 (93.4)
Erythromycin	15	39 (51.3)	0 (0.0)	37 (48.7)
Gentamycin	10	3 (3.9)	2 (2.6)	71 (93.4)
Kanamycin	30	16 (21)	3 (3.9)	57 (75)
Penicillin	10 IU/disc	70 (92)	2 (2.6)	4 (5.3)
Streptomycin	10	27 (35.5)	4 (5.3)	45 (59.2)
Tetracycline	30	26 (34.2)	3 (3.9)	46 (60.5)
Trimethoprim-sulfamethoxazole	25	7 (9.2)	0 (0.0)	69 (90.8)
Vancomycin	30	0 (0.0)	0 (0.0)	76 (100%)

Table 4. Resistance profiles of *S. aureus* isolates (n = 76) against 13 antimicrobial agents from chicken eggs sampled from a poultry farm and open local markets in Haramaya district, eastern Ethiopia.

Drugs developed resistance	Antimicrobial resistance pattern	Multiple drug resistance of <i>S. aureus</i>	
		Isolates with same pattern	Percentage
3	AML, AMP, P	13	19.7
4	AML, AMP, P, E	14	21.2
5	AML, AMP, P, E, FOX	12	18.2
6	AML, AMP, P, TE, S, C	7	10.6
7	AML, AMP, P, E, TE, K, SXT	6	9.9
8	AMP, AML, P, E, TE, K, FOX, S	6	9.9
9	AML, AMP, P, E, S, C, SXT, FOX, K	5	7.6
10	AML, AMP, P, E, CN, SXT, K, CIP, FOX	3	4.5
Total		66	100%

#### 4. Discussion

The presence of pathogenic bacteria in food, including table chicken eggs, may pose a serious health problem (Baumann and Sadkowska, 2011; Pyzik and Marek, 2012). Eggs are food with high nutritive values for humans. Similarly, they are an excellent source of nourishment for many pathogens. Bacteria can infect eggs through diverse means such as during development in the reproductive system, directly after hatching, during storage and transport, or even while preparing the eggs as food for consumption (Stępień *et al.*, 2009). Among the most widespread foodborne infections directly connected with egg consumption are *S. aureus* infections. Our study revealed high level of *S. aureus* contamination of table chicken eggs accounting for 27.8% of isolates. Comparable with the results of this study, Stępień *et al.* (2009) found 19.8% *S. aureus* from table eggs.

The available literature shows that while these bacteria are isolated from eggs with varying frequency depending on geographical location, they can pose a serious threat to consumer health by inducing food poisoning. In France, for instance, a fairly high percentage (11%) of cases of food poisoning in 1999–2000 resulted from eating eggs and egg products contaminated with staphylococci (Haeghebaert *et al.* 2002). In 2009, analysis of the epidemiological situation of food poisoning and foodborne infections in Poland showed that 25% of food poisoning cases were induced by *S. aureus*. This was caused by consumption of table eggs (Baumann and Sadkowska, 2011).

In our study, although most of the *S. aureus* were isolated from shells, a considerable number of the pathogen was isolated from the contents. Corroborating the results of this study, Pyzik and Marek (2012) reported a fairly higher rate of *S. aureus* isolates on the shells of the eggs (55.5%) than the contents (27.8%) from Poland. In contrast to our results, however, Stępień *et al.* (2009) reported less isolates of *S. aureus* from egg shells (10.4%) than from egg contents (35.2%)

that were collected from large- and small-scale poultry farms and eggs purchased from supermarkets. These variations might be due to different sampling techniques, areas, time, storage practice and the low isolation rate of culture methods compared to more sensitive immunological and molecular methods.

In this study, significantly higher numbers of *S. aureus* isolates were detected in the egg samples collected from the open markets than from eggs sampled from Haramaya University poultry farm. This variation might be attributed to differences in the level of care given and sanitation practiced at the two egg sample collection sources. This may imply that a higher care is given to eggs and better sanitation is practiced at the poultry farm of the University than at poultry farmers from which the farmers sell eggs in the open market. Eggs collected from hens kept in a cage system have been less likely exposed to the pathogens than those kept in litter system and retail outlets or open markets. There are several critical points that contribute to the contamination of eggs with microorganisms in the pathways of reaching the consumers such as the environment, storage condition, transport and handling practices (Stępień *et al.*, 2009). Another important point that has a serious threat to consumer health with a global concern is antimicrobial resistance of *S. aureus* isolated from eggs.

In this study, all the isolates showed resistance to at least one of the antimicrobials tested. The proportion of amoxicillin resistant isolates found in this study is supported by the report of Serawit Deynu *et al.* (2017) who reported 90.9%. Fikre Gizaw (2014) reported a comparable proportion of penicillin resistant *S. aureus* to the present study who detected 90.2%. This higher rate of resistance to penicillin could be due to their frequent use in Ethiopia (Gebretekla Gebremedhin and Mirgissa Kaba, 2016). All (100%) of the *S. aureus* isolates showed susceptibility to vancomycin. This higher susceptibility rate to vancomycin in the present study is comparable to the global estimate (Zhang *et al.*, 2015). Serawit Deyno *et al.* (2017) reported that 74.2% of *S. aureus* isolates

showed resistance against vancomycin in Ethiopia. Meseret Guta *et al.* (2014) reported high vancomycin resistant *S. aureus* which is inconsistent with our study. Can *et al.* (2017) reported a similar finding that all isolates of *S. aureus* were susceptible to vancomycin followed by chloramphenicol (97.5%), penicillin (95%) and ampicillin (92.5%). Similarly, Yang *et al.* (2016) displayed a 100% susceptibility to vancomycin and cefoxitin with a higher rate to chloramphenicol. Vancomycin has been considered the best drug for the treatment of staphylococci related infections. It has been known as the last line of defense against gram-positive cocci infections (Micek, 2007).

Susceptibility of the isolates to vancomycin and gentamicin that we found in this study is in agreement with the findings of other researchers from different countries (Gündoğan *et al.*, 2006; Normanno *et al.*, 2007; Pesavento *et al.*, 2007; Hanson *et al.*, 2011; Can and Çelik, 2012; Hu *et al.*, 2013). Consistent with the results of this study, Pyzik *et al.* (2014) reported that all *S. aureus* isolates tested were susceptible to chloramphenicol and gentamicin. Among the isolates of *S. aureus*, the most frequently observed resistance patterns were observed against amoxicillin, ampicillin, penicillin G, erythromycin and tetracycline. The higher resistance frequency against beta-lactams, penicillin, ampicillin and amoxicillin, among the isolates from chicken eggs could be attributed to the extensive and uncontrolled use of these groups of antibiotics in the agriculture sector.

In agreement with the present finding, Rasoul *et al.* (2015) reported a susceptibility rate of *S. aureus* isolates to be 94.9% and 83.7% for cefoxitin and trimethoprim-sulfamethoxazole, respectively. However, our finding is distantly related to the finding of Rasoul *et al.* (2015) who reported that 69.4% of *S. aureus* isolates were susceptible to tetracycline. In some other studies *S. aureus* showed varied resistance level against erythromycin (1.7%–100%), tetracycline (5%–84%), ciprofloxacin (0%–42%) and vancomycin (9%–46%) (Attien *et al.*, 2013; Adegoke and Okoh *et al.*, 2014; Gharsa *et al.*, 2015; Schaumburg *et al.*, 2015). High penicillin resistance of *S. aureus* isolates was also identified in other parts of the world (Gharsa *et al.*, 2015; Chairat *et al.*, 2015).

Multidrug resistance was detected in 66 (86.8%) of the total 76 *S. aureus* isolates. The most frequently observed resistance pattern was resistance to ampicillin in combination with penicillin, erythromycin and amoxicillin. Similar findings were reported by Fikru Gizaw (2014) with multiple drug resistance of 89.3% of the total isolates tested. Barena and Fetene (2003) and Chao *et al.* (2007) reported a similar rate of multi-drug resistant *S. aureus* (80%) and (79%) with the present investigation respectively. The resistance against antimicrobial observed in this study is slightly higher than reported by Sharma *et al.* (2011) who indicate that 60–70% of the *S. aureus* isolates showed multiple drug resistance. Such a high incidence of multi-drug

resistance may apparently have occurred due to indiscriminate use of antimicrobial agents which enhance the development of drug resistance (Van Den Bogaard and Stobberingh, 1999). The multiple drug resistance observed in the current study might also be mediated by genetic mobile elements such as plasmids, transposons, and integrons as seen in other studies (Macrina and Archer, 1993; Firth and Skurray, 2006; Shearer *et al.*, 2011; Li and Zhao, 2018; Partridge *et al.*, 2018).

## 5. Conclusion

The results of this study have revealed that a considerably high percentage of the chicken eggs were contaminated with *S. aureus*. Egg shells harbored a significantly considerable level of *S. aureus* compared to egg contents. A significantly higher rate of contamination was recorded for eggs sampled from the open market than those sampled from Haramaya University poultry farm. Detection of the high prevalence of *S. aureus* in this study indicates a potential risk of food poisoning. The results have also demonstrated the existence of an alarming level of resistance of *S. aureus* to antimicrobial agents commonly used in veterinary and human practices such as ampicillin, amoxicillin, penicillin and erythromycin. The majority of *S. aureus* isolates showed multiple resistances to drugs, ranging from three to nine of the antimicrobials tested. The high prevalence of *S. aureus* and isolates with multiple drug resistance is alarming because this could pose a significant risk to public health if the microorganisms are transmitted to humans through food chains. Therefore, additional research is required with continuous surveillance and monitoring of pathogens to better define this bacterial resistance to antimicrobial agents with emphasis on surveillance of multiple drug resistant *S. aureus* isolates.

## 6. Acknowledgements

We acknowledge Haramaya University for providing material supports for the research. We also thank Mr. Dereje Regassa, Haramaya University College of Veterinary Medicine, for his support during the laboratory tests.

## 7. References

- Adegoke, A.A. and Okoh, A.I. 2014. Species diversity and antibiotic resistance properties of *Staphylococcus* of farm animal origin in Nkonkobe Municipality, South Africa. *Folia Microbiologica (praha)*, 59: 133–140.
- Attien, P.S., Moussaoui, W., Dadié, T., Chabi, S.K., Djéni, T., Bankole, H.S., Kotchoni, S.O., Edoh, V., Prévost, G., Djè, M. *et al.*, 2013. Prevalence and antibiotic resistance of *Staphylococcus* strains isolated from meat products sold in

- Abidjan streets (Ivory Coast). *African Journal of Microbiology Research*, 7: 3285–3293.
- Barena, B. and Fetene, D. 2003. Nasal carriage of Methicillin Resistant *Staphylococcus aureus* strains among inpatients of Jimma hospital, South Western Ethiopia. *Ethiopian Journal of Health Science*, 13: 30–40.
- Baumann, P.A. and Sadkowska, M. 2011. Foodborne infections and intoxications in Poland in 2009. *Przegląd Epidemiologiczny*, 65: 227–234.
- Becker, K., Skov, R.L. and von Eiff, C. 2015. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci. Pp. 354–382. In: Jorgensen, J.H., Carroll, K.C. Funke, G., Pfaller, M.A. Landry, M.L. Richter, S.S. and Warnock, D.W. (eds.). *Manual of Clinical Microbiology*. 11th edition. Washington, DC: ASM Press.
- Can, H.Y, Elmali, M. and Karagöz, A. 2017. Molecular typing and antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from raw milk, cheese, minced meat, and chicken meat samples. *Korean Journal of Food Science of Animal Resources*, 37(2): 175–180.
- Can, H.Y. and Çelik, T.H. 2012. Detection of enterotoxigenic and antimicrobial resistant *S. aureus* in Turkish cheeses. *Food Control*, 24: 100–103.
- CDC (Centers for Disease Control and Prevention). 2013. Antibiotic Resistance Threats in the United States, 2013. Available at: <https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf>.
- CSA (Central Statistics Authority). 2012. Agricultural sample survey 2008-2009. Report on livestock and livestock characteristics, vol. II,” Statistical Bulletin 446, CSA, Addis Ababa, Ethiopia.
- Chairat, S., Gharsa, H., Lozano, C., Gómez-Sanz, E., Gómez, P., Zarazaga, M., Boudabous, A., Torres, C. and Ben Slama, K. 2015. Characterization of *Staphylococcus aureus* from raw meat samples in Tunisia: Detection of clonal lineage ST398 from the African Continent. *Foodborne Pathogenic Disease*, 12: 686–692.
- Chao, G., Zhou, X. and Jiao, X. 2007. Prevalence and antimicrobial resistance of food borne pathogens isolated from food products in China. *Foodborne Pathogenic Disease*, 4:277–284.
- CLSI (Clinical and Laboratory Standard Institute). 2013. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement, CLSI, document M 100-S23, Wayne, Pennsylvania, USA.
- Costa, E.O., Benites, N.R., Guerra, J.L. and Melvilla, P.A. 2000. Antimicrobial susceptibility of *Staphylococcus aureus* spp. isolated from mammary parenchymas of slaughtered dairy cows. *Journal of Veterinary Medicine Series B*, 47: 99–103.
- De Oliveira, A.P, Watts, J.L, Salmon, S.L. and Aarestrup, F.M. 2000. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States. *Journal of Dairy Science*, 83: 855–862.
- Fikru Gizaw. 2014. *Staphylococcus*: Epidemiology and its drug resistance in cattle, food chains and humans in Central Ethiopia. MSc Thesis, College of Veterinary Medicine and Agriculture, Addis Ababa University.
- Firth, N. and Skurray, R.A. 2006. The *Staphylococcus* genetics: accessory elements and genetic exchange. Pp 413–426. In: Fischetti, V.A., Novick, R.P., Ferretti, J.J., Portnoy, D.A. and Rood, J.I. (eds.). *Gram-positive pathogens*. 2nd edition. ASM Press, Washington, DC.
- Fitzgerald, J.R. 2012. Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. *Trends Microbiology*, 20: 192–198.
- Gebretekla Gebremedhin and Mirgissa Kaba. 2016. Exploration of over the counter sales of antibiotics in community pharmacies of Addis Ababa, Ethiopia: pharmacy professionals' perspective. *Antimicrobial Resistant and Infection Control*, 5: 2, DOI 10.1186/s13756-016-0101-z.
- Gharsa, H., Ben Slama, K., Gómez-Sanz, E., Lozano, C., Zarazaga, M., Messadi, L., Boudabous, A. and Torres, C. 2015. Molecular characterization of *Staphylococcus aureus* from nasal samples of healthy farm animals and pets in Tunisia. *Vector Borne Zoonotic Diseases*, 15: 109–115.
- Gündoğan, N., Citak, S. and Turan, E. 2006. Slime production, DNase activity and antibiotic resistance of *Staphylococcus aureus* isolated from raw milk, pasteurized milk and ice cream samples. *Food Control*, 17: 389–392.
- Haeghebaert, S., Le Querrec, F., Gallay, A., Bouvet, P., Gomez, M. and Vaillant, V. 2002. Les toxoinfections alimentaires collectives en France, en 1999 et 2000. *Bulletin of Epidemiology and Hebdomen*, 23: 105–109.
- Hailemariam Mekonnen and Tesfaye Ali. 2010. Prevalence and etiology of mastitis and related management factors in market oriented smallholder dairy farms in Adama, Ethiopia. *Revue de Médecine Vétérinaire*, 161(12): 574–57.
- Haimanot Tassew, Alemseged Abdisa, Getenet Beyene and Solomon Gebre Selassie. 2010. Microbial flora and food borne pathogens on minced meat and their susceptibility to antimicrobial agents. *Ethiopian Journal of Health Sciences*, 20(3), DOI:10.4314/ejhs.v20i3.69442.
- Hanson, B.M., Dressler, A.E., Harper, A.L., Scheibel, R.P., Wardyn, S.E., Roberts, L.K., Kroeger, J.S. and Smith, T.C. 2011. Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus*

- aureus (MRSA) on retail meat in Iowa. *Journal of Infection and Public Health*, 4: 169–174.
- Hata, E., Katsuda, K., Kobayashi, H., Nishimori, K., Uchida, I., Higashide, M., *et al.* 2008. Bacteriological characteristics of *Staphylococcus aureus* isolates from humans and bulk milk. *Journal of Dairy Science*, 91: 564–569.
- Hedman, H.D., Vasco, K.A. and Zhang, L. 2020. A Review of Antimicrobial Resistance in Poultry Farming within Low-Resource Settings. *Animals*, 10: 1264.
- Hu, S., Liu, S., Hu, W., Zheng, T. and Xu, J. 2013. Molecular biological characteristics of *Staphylococcus aureus* isolated from food. *European Food Research and Technology*, 236: 285–291.
- ISO (International Organization for Standardization). 2002. Microbiology of food and animal feeding stuff-horizontal method for the detection of Salmonella, 4th edition. ISO 6579. Geneva: ISO.
- Ivanov, I. 2008. Disinfection of eggs contaminated with some fungal moulds. *Trakia Journal of Science*, 6: 98–101.
- Kohinur, B., Tanvir, A.R., Margia, H., Akil, H., Kabirul, H., Shaik Nahid, H., Nargis, A., Aliza, A. and Utpal, B. 2010. Isolation, identification and antimicrobial resistance pattern of *Salmonella* spp. from chicken eggs, intestines and environmental samples. *Bangladesh Pharmaceutical Journal*, 13: 23–27.
- Landers, T.F., Cohen, B., Witlum, T.E. and Larson, E.L. 2012. A review of antibiotic use in food animals: Perspective, policy and potential. *Public Health Reports*, 127(1): 4–22.
- Lindsay, J.A. and Holden, M.T. 2004. *Staphylococcus aureus*: superbug, super genome? *Trends in Microbiology*, 12: 378–385.
- Li, L. and Zhao, X. 2018. Characterization of the resistance class 1 integrons in *Staphylococcus aureus* isolates from milk of lactating dairy cattle in Northwestern China. *BMC Veterinary Research*, 14:59, DOI.org/10.1186/s12917-018-1376-5
- Macrina, F.L. and Archer, G.L. 1993. Conjugation and broad host range plasmids in streptococci and staphylococci. Pp. 313–329. *In*: Clewell, D.B. (ed.). *Bacterial Conjugation*. Plenum Press, New York, NY.
- Mekonnen Addis, Mahindra, P. and Moses, N.K. 2011. Isolation and identification of *Staphylococcus* species from Ethiopia cottage cheese (Ayib) in Debre Zeit, Ethiopia. *Veterinary Research*, 4(1): 13–17.
- Meseret Guta, Kassaye Aragaw and Yared Merid. 2014. Bacteria from infected surgical wounds and their antimicrobial resistance in Hawassa University referral teaching hospital, southern Ethiopia. *African Journal of Microbiology Research*, 8(11): 1118–24
- Micek, S.T. 2007. Alternatives to vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. *Clinical Infectious Diseases*, 45(3): S184–90.
- Michael, C.A, Dominey-Howes, D. and Labbate, M. 2014. The antibiotic resistance crisis: Causes, consequences and management. *Front Public Health*, 2: 145.
- Normanno, G., la Salandra, G., Dambrosio, A., Quaglia, N.C., Corrente, M., Parisi, A., Santagada, G., Firinu, A., Crisetti, E. and Celano, G.V. 2007. Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *International Journal of Food Microbiology*, 115: 290–296.
- Odwar, J.A. Kikuvi, G. Kariuki, J.N. and Kariuki, S. 2014. A cross sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Research Notes*, 7(1): 627.
- Partridge, S.R., Kwong, S.M., Firth, N. and Jensen, S.O. 2018. Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews*, 31(4): 1–61.
- Pesavento, G., Ducci, B., Comodo, N. and lo Nostro, A. 2007. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). *Food Control*, 18: 196–200.
- Petrovski, K.R., Trajcev, M. and Buneski, G. 2006. A review of the factors affecting the costs of bovine mastitis. *Journal of Science of African Veterinary Association*, 77: 52–60.
- Pyzik, E. and Marek, A. 2012. Characterization of bacteria of the genus *Staphylococcus* isolated from the eggs of Japanese quail (*Coturnix coturnix japonica*). *Polish Journal of Veterinary Science*, 15: 767–772.
- Pyzik, E., Marek, A. and Hauschild, T. 2014. Characterization of *Staphylococcus aureus* and *Staphylococcus aureus* – like strains isolated from table eggs. *Bulletin of Veterinary Institute Pulawy*, 58: 57–63.
- Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. 2002. *Clinical veterinary microbiology*. Har court publishers, Virginia, USA. Pp. 331–344.
- Rasoul, Y.M., Seyed, M.H., Seyed, M.M. and Mohammad, R.A. 2015. Prevalence of enterotoxin genes and antibacterial Susceptibility pattern of *Staphylococcus aureus* strains isolated from animal originated foods in West of Iran. *Oman Medical Journal*, 30(4): 283–290.
- Rota, C., Yanguela, J., Blanco, D., Carraminana, J.J., Arino, A. and Herrera A. 1996. High prevalence of multiple resistances to antibiotics in 144



- Listeria* isolates from Spanish dairy and meat products. *Journal of Food Protection*, 59: 938–943.
- Schaumburg, F., Pauly, M., Anoh, E., Mossoun, A., Wiersma, L., Schubert, G., Flammen, A., Alabi, A.S., Muyembe-Tamfum, J.J., Grobusch, *et al.* 2015. *Staphylococcus aureus* complex from animals and humans in three remote African regions. *Clinical Microbiology Infection*, 21: 1–8.
- Serawit Deyno, Sintayehu Fekadu and Ayalew Astatkie. 2017. Resistance of *Staphylococcus aureus* to antimicrobial agents in Ethiopia: a meta-analysis. *Antimicrobial Resistance and Infection Control*, 6:85: 1–15.
- Sharma, D., Sharma, P.K. and Malik, A. 2011. Resistant *Staphylococcus aureus* in raw milk of dairy cattle. *International Research Journal of Microbiology*, 2(11): 466–470.
- Shearer, J.E., Wireman, J., Hostetler, J., Forberger, H., Borman, J., Gill, J., Sanchez, S., Mankin, A., Lamarre, J., Lindsay, J.A., Bayles, K., Nicholson, A., O'Brien, F., Jensen, S.O., Firth, N., Skurray, R.A. and Summers, A.O. 2011. Major families of multi-resistant plasmids from geographically and epidemiologically diverse staphylococci. *G3-Genes Genomes Genetics*, 1(7): 581–591.
- Soares, J.C., Marques, M.R., Tavora, F.K., Pereira, J.O., Malcata, F.X. and Pintado, M.M. 2011. Biodiversity and characterization of *Staphylococcus* species isolated from a small manufacturing dairy plant in Portugal. CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, P-4200-072, Porto, Portugal.
- Stepień, P.D., Marek, A. and Rzedzicki, J. 2009. Occurrence of bacteria of the genus *Staphylococcus* in table eggs descended from different sources. *Polish Journal of Veterinary Science*, 12: 481–484.
- Van Den Bogaard, A.E. and Stobberingh, E.E. 1999. Antibiotic usage in animals. Impact on bacterial resistance and public health, *Drugs*, 58(4): 589–607.
- Ventola, C.L. 2015. The antibiotic resistance crisis: Causes and threats. *Physical Therapy*, 40(4): 277–283.
- Yang, X., Zhang, J, Yu, S., Wu, Q., Guo, W., Huang, J. and Cai, S. 2016. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in retail ready-to-eat foods in China. *Frontiers in Microbiology*, 7(7): 816. DOI: 10.3389/fmicb.2016.00816.
- Zhang, S., Sun, X., Chang, W., Dai, Y. and Ma, X. 2015. Systematic review and meta-analysis of the epidemiology of vancomycin-intermediate and heterogeneous vancomycin-intermediate *Staphylococcus aureus* isolates. *PLoS One*, 10(8).
- Zouhairi, O., Saleh, I., Alwan, N., Toufeili, I., Barbour, E. and Harakeh, S. 2010. Antimicrobial resistance of *Staphylococcus* species isolated from Lebanese dairy based products. *Eastern Mediterranean Health Journal*, 16(12).

