Antibiotic Resistance and Susceptibility Patterns of Staphylococcus Aureus isolates in Raw Chicken Gizzards from Selected Retailers in Katabi Sub-county, Wakiso District, Uganda. A cross-sectional Study.

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Abstract

Introduction:

Compromised public health structures, poor disease surveillance, antimicrobial resistance, and enterotoxin production, poor educational program approaches, local law enforcement in addition to other factors have led to food-borne illness as a great public health concern and these are commonly underreported. This study examines raw chicken gizzards.

Methodology:

A cross-sectional study was done on the fresh raw chicken gizzards randomly selected from different supermarkets and fresh poultry products outlets which were then transported at 4°C to the microbiology laboratory for culture and antibiotic susceptibility testing.

Results:

A total of 39 raw chicken gizzards randomly selected from different fresh chicken outlets were used in the study to test for the presence of Staphylococcus aureus. Findings showed that 10 samples were positive for Staphylococcus aureus. The results show that out of the 10 samples found positive in the Coagulase slide test for staphylococcus aureus, 6/10 (60%) were resistant to **Penicillin (P)**, 9/10 (90%) were resistant to **vancomycin(Va)**, 9/10 (90%) were resistant to **Ampicillin(Amp)**,4/10 (40%) were resistant to **Cefoxitin(FOX)** and 10/10 (100%) were resistant to **Tetracycline(TE)**.

Conclusion:

This study demonstrated that the Staphylococcus species have entered the food chain.

Recommendation:

As the amounts were low, the pathogen is not likely to cause disease, especially if chicken gizzards are properly prepared before consumption.

Keywords: Staphylococcus aureus, Fresh raw chicken gizzards, Antibiotic resistance, Katabi,
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1. Background of study

A report by WHO showed that over 420,000 people die while 600 million were reported ill as a result of contaminated food consumption annually. There are various predisposing factors as by Faour-klingbel and Todd (2019) who showed that compromised public health structures, poor disease surveillance, antimicrobial resistance, and enterotoxin production, poor educational program approaches, local law enforcement in addition to other factors leading to food-borne illness as a great public health concern and these

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are commonly under-reported.

The world bank also concluded that these foodborne hazards' effects on the health status were directly proportional to the economic hand of the affected especially low and middle-income countries, where productivity losses were estimated to be US$ 95.2 per year. With the increasing world population, food security becomes a great threat to the planet yet to sustain life and promote good health sufficient amount of safe and nutrition is vital (Xu *et al.*, 2016), further still; Ali et al (2017), in their review highlighted that the quality of food has greatly been affected by bacterial food hazards indicating *staphylococcus aureus* as the most common pathogen both in animal and plant food sources with its relation to causing food poisoning.

Staphylococcus aureus carriers usually contaminate food if they don't wash their hands before handling or touching it. If food is contaminated with *staphylococcus aureus,* the bacteria multiply in the food and produce toxins that make people ill. *Staphylococcus aureus* bacteria are killed by cooking, but the toxins are not destroyed and will still be able to cause illness (Freeman-Cook, L, *et al.*, 2006). The organism is reported to cause staphylococcal food-borne disease (SFD) as a result of protein toxins, "staphylococcal enterotoxins (SE)," which are produced before the preparation of the contaminated food.

Staphylococcal food poisoning (SFP) is a common disease in our communities whose real incidence is underestimated for a number of reasons, which may include unreported minor outbreaks, misdiagnosis, improper sample collection, and improper laboratory examination. The control of the disease is due to social and economic benefits. This may represent a considerable burden in terms of loss of productivity, increased hospital expenses, and economical losses in food industries, restaurants, and catering companies. (Baron F *et al.*, 2003).

Paudyal et al (2017), produced a meta-analysis from selected African countries with Uganda inclusive and reported St*aphylococcus aureus* being contained in all foods analyzed at 42%. However, 37% (31/95) of S.aureus prevailed in chicken gizzards recorded in 2015 by Abdalrahman and Fakln in their studies on the outbreaks within US and Europe concerned with chicken gizzards and livers consumption. In their study, they asserted that S.aureus isolates from these raw chicken products were methicillin, Cefoxitin, and Oxacillin resistant due to possession of some genes, and their enterotoxin production depended on the seg and sei genes. Bortolaia et al (2016), in their survey, concluded that however, S.aureus in raw retail gizzards can be a possible consequence of meat handlers, contaminated water, towels, or working surfaces. They reported the contamination in their study to be between 17.8% to 68% for S. aureus and 0.3% to 25% for MRSA. With these factors therefore, multi-drug resistance enters toxigenic organisms like *Staphylococcus aureus*, becoming of great threat to food safety with its specific effects of causing foodborne disease that is known to cause high morbidity and mortality cases among humans (Osei-Tutu and Ando, 2016)

The risk of ingesting multi-drug-resistant S.aureus and enterotoxins becomes challenging to treat with its ability to resist a wide spectrum of antibiotics.

2. Methodology

This was a cross-sectional study employed to asses and isolate staphylococcus aureus in raw gizzards in selected fresh poultry products outlets (i.e. chicken, gizzards, chicken wings, etc.) located within Katabi Sub County in Wakiso district which was randomly selected. Such samples were transported in a cool box to the microbiology laboratory of the Faculty of Health Sciences at the University of Kisubi for further analysis of S. aureus.

2.1. ***Sample definition***

The proposed samples in the study were the fresh chicken gizzards on retail from different selected supermarkets and fresh poultry products outlets of the study.

2.2. Sample size determination

In this study the sample size shall be estimated using the formula below;

N= 4PQ

L2

Where

Is the sample size required

Is the estimated percentage of the occurrence of staphylococcus aureus 11% (0 11

Q =1-P, which is 1-0.11 =0.89

L2 is the allowable error (10%) =0.1

Since there is no data available on the occurrence of staphylococcus aureus among raw chicken gizzard an estimated percentage of 11% was used to calculate the sample size with an allowable error of L=10%.

N= 4x0.11x0.89 = 39 Samples

0.1x 0.1

Sample size of 39 samples was used

2.3. Sampling strategy and criteria

Random sampling was utilized within this study, packed chicken gizzards were randomly purchased from different supermarkets and retail hubs around Katabi Sub County.

2.4. Sample collection, transportation, and quality control

The chicken gizzards were collected following their purchase from the randomly selected outlets. These were then enclosed within a cool box containing ice packs to maintain the stability of the samples and then immediately transported to the microbiology laboratory.

In consideration of quality control aspects here; the packs were checked if there are no breakages or damages. The samples were maintained between 2°C and 8° ranges (the refrigeration temperature).

2.5. Materials used during sample collection, transportation, and sample processing.

1. Mannitol salt agar
2. Nutrient agar
3. Peptone water
4. Distilled water
5. Incubator
6. Heat source
7. Microbiology plates
8. Sterile beakers & test tubes
9. Knife
10. Cool box and ice packs
11. Inoculation wire loops
12. Forceps
13. Microscope & Microscope slides
14. Weighing balance
15. Autoclave
16. Gram stains

2.6. Sterilization of media and materials

Mannitol salt Agar (MSA), Nutrient Agar (NA), and Muller Hinton Agar (MHA) were used. Media preparation was according to the manufacturer's specifications and sterilized in the autoclave "Advantage-Lab Model AL02-11" at 121 degrees centigrade for 15 minutes. Clean and sterile materials such as media plates, glass test tubes, and wire loops were used.

2.7. Laboratory methods, susceptibility testing, and related quality control

2.7.1. Sample processing

Following the reception of samples at the microbiology laboratory, the chicken gizzards were chopped into small pieces and inoculated into a clean beaker containing 10 ml of sterile broth of peptone water as a primary culture medium. The broth medium was then incubated at 37°C overnight for the recovery of any bacteria that may be present as a gizzard contaminant.

2.7.2. Inoculation and identification of S.aureus

The presence of turbidity within the broth-gizzard medium in the primary culture was suggestive of bacterial multiplication within the broth.

Inoculation onto sterile Mannitol salt agar (MSA) medium was done followed by sub-cultivation of the Nutrient Agar to obtain a pure culture for the selective identification and isolation of S. aureus if present within the gizzard

sample being processed. This sub-cultivation was carried out as an overnight incubation of the inoculum-containing MSA medium at 37°C.

Following the MSA sub-cultivation above, a plate reading to identify the colony characteristics of the bacterial growth (if any) obtained on the MSA was done. S. aureus was expected to show characteristics of yellow colonies and yellow media which were further examined by gram staining.

Gram staining was carried out on any yellow colonies obtained on MSA for the morphological identification and confirmation of the presence of Staphylococcus species in the gizzard samples. This staining technique was fulfilled according to the procedure provided by Cheesbrough (2006).

Biochemical tests for the confirmation of S. aureus in the gizzard samples under investigation were included but not limited to; catalase and coagulase.

2.7.3. ***Antibiotic sensitivity testing (AST) for S. aureus***

The S. aureus isolates obtained above were subjected to the AST technique to determine the susceptibility and/or resistance patterns of S. aureus towards the antibiotic agents such as Vancomycin (Va) 30μg, Cefoxitin (FOX) 30μg, Ampicillin (Amp) 30μg, Tetracycline (TE) 30μg and Penicillin (P) 10μg of study choice. The Kirby Bauer disc diffusion technique using Mueller Hinton Agar (MHA) was employed and the procedures by Cheesbrough (2006) were followed. Interpretation of the drug discs was done using the CLSI antibiotic disc interpretative criteria and quality control chart.

2.7.4. ***Data collection and analysis***

Raw data collected about the gizzard samples during collection i.e. the considerations here were; markets or outlets, date, and refrigeration status were recorded. The other category of raw data obtained was in the laboratory i.e. data about morphology and staining characteristics of the bacteria obtained, during biochemical testing, and finally during the AST process.

The raw data above was collected, summarized, and arranged with the aid of the Microsoft excel computer application. The summarized and arranged data were to be analyzed with the same application to generate tables and/or graphs that were to be presented.

3. Results

A total of 39 raw chicken gizzards randomly selected from different fresh chicken outlets were used in the study to test for the presence of Staphylococcus aureus. Out of 39 samples, 16 samples were found to be present for staphylococcus species since they all presented as yellow colonies with a yellow media on MSA while the other 23 samples with cream colonies and pink colonies were non staphylococcus species.

3.1. ***Biochemical characteristics of Staphylococcus species isolated from the chicken gizzards.***

Further biochemical testing using catalase slide test and coagulase was performed on those samples that were staphylococcus species to confirm staphylococcus aureus organisms. The Coagulase slide test confirmed that out of the 16 samples, 10 samples were positive for Staphylococcus aureus. This meant that 27% of the tested samples were positive for Staphylococcus aureus.

Key:

(+) stands for positive while (-) stands for negative.

Table 1: **Mannitol salt agar colony characteristics for the gizzard samples.**

Mannitol salt Agar (MSA)		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Cream colonies	13	33.3	33.3	33.3
	yellow colonies & yellow media	16	41.0	41.0	74.4
	pink colonies	10	25.6	25.6	100.0
	Total	39	100.0	100.0	

Source: primary data.

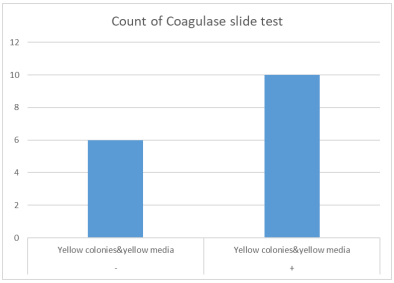

Figure 1: Coagulase test results of Staphylococcus species isolated from chicken gizzards

3.2. Antibiotic resistance and susceptibility patterns of Staphylococcus aureus isolated from chicken gizzards.

Table 2: Antimicrobial resistance and susceptibility patterns of *Staphylococcus aureus* isolated from chicken gizzards

Diameter for zone of inhibition (mm)	Frequency	Percent
Penicillin (P) 10µg		
RESISTANT	6	60.0
SUSCEPTIBLE	4	40.0
Vancomycin (Va) 30µg		
RESISTANT	9	90.0
SUSCEPTIBLE	1	10.0
Ampicillin (Amp) 30µg		
RESISTANT	9	90.0
SUSCEPTIBLE	1	10.0
Cefoxitin (FOX) 30µg		
RESISTANT SUSCEPTIBLE	4	40.0
	6	60.0
Tetracycline (TE) 30µg RESISTANT	10	100.0

Source: primary data

From table 2: The results show that out of the 10 samples found positive in the Coagulase slide test for staphylococcus aureus, 6/10 (60%) were resistant to Penicillin (P), 9/10 (90%) were resistant to vancomycin(Va), 9/10 (90%) were resistant to Ampicillin(Amp),4/10 (40%) were resistant to Cefoxitin(FOX) and 10/10 (100%) were resistant to Tetracycline(TE).

However, there was sub–optimal antibiotic susceptibility of staphylococcus aureus to Penicillin (P) 4/10(40%) and Ampicillin (Amp) 6/10 (60%). The results obtained from the study can be compared to the study conducted by (Bhediet et al., 2019). In India, from which they found S.aureus antibiotic resistance isolated from fresh chicken gizzards. For ampicillin (70.37%, 19/27), followed by tetracycline (62.96%, 17/27) methicillin (25.96%, 7/27), and vancomycin at (18.51%, 5/27). However, for new retail gizzard sample isolates in Nigeria, S.aureus resistance was shown at 76.86 for tetracycline, 60.4% for ciproflaxin,

36.6% for oxacillin and 25% for vancomycin (Onaolapo et al., 2017).

4. Discussion

Antimicrobial resistance of S. aureus isolates Antibiotic-resistant S. aureus can be transmitted by food, including contaminated meat. Also, investigators indicated that food was a good way to transmit antibiotic resistance to humans. In our study, the S. aureus strains were found highly resistant to Penicillin, Vancomycin, and Tetracycline. This is not surprising, because Penicillin, Vancomycin, and Tetracycline are broad-spectrum antibiotics that are commonly used for the treatment of infections in humans and animals. In comparison with other studies, differences in antibiotic resistance may arise from the animal population, the inappropriate use of antibiotics for each infection, and the use of antibiotics as a growth factor in animal feeding and to promote animal growth. In this study, we found that S. aureus was isolated from chicken gizzards randomly selected from different fresh chicken outlets.

For statistical analysis of the antibiotics susceptibility of S.aureus positive samples to different antibiotics, a (t) test using Pearson's chi-square test was done to determine the significance of susceptibility using SPSS (Ver.28.0.0.0).

Table 3: Statistical analysis of the antibiotic susceptibility of *Staphylococcus aureus* positive samples.

Parameter Estimates (q=0.5)[a,b]							
						95% Confidence Interval	
Parameter	Coefficient	Std. Error	t	df	Sig.	Lower Bound	Upper Bound
(Intercept)	-13.000	28.9907	-.448	5	.673	-87.523	61.523
[P=1]	11.000	26.2230	.419	5	.692	-56.408	78.408
[P=2]	0[c]	.	.	.	.	.	.
[Va=1]	31.000	26.2230	1.182	5	.290	-36.408	98.408
[Va=2]	0[c]	.	.	.	.	.	.
[Amp=1]	.000	31.5162	.000	5	1.000	-81.015	81.015
[Amp=2]	0[c]	.	.	.	.	.	.
[FOX=1]	6.000	19.5455	.307	5	.771	-44.243	56.243
[FOX=2]	0[c]	.	.	.	.	.	.
a. Dependent Variable: Sample ID							
b. Model: (Intercept), P, Va, Amp, FOX							
c. Set to zero because this parameter is redundant.							

From the statistical(t) test at a 95% confidence interval the P- value is greater than 0.05, thus the null hypothesis is rejected (there is no antibiotic resistance of S. aureus isolated from fresh chicken gizzards) and we accept the alternative (there is antibiotic resistance of S.aureus isolated from fresh chicken gizzards) and the observed statistics are not due to chance.

5. Conclusion

The presence of clonal growth of different species of Staphylococcus species on MSA media indicates cross-contamination of the fresh chicken gizzards at some point during processing.

This study demonstrates that Staphylococcus species have entered the food chain. As the amounts were low, the pathogen is not likely to cause disease, especially if chicken gizzards are properly prepared before consumption. However, contamination of food products may be a potential threat to the acquisition of MRSA by those who handle the food. Thus, for public health and hygienic chicken meat production, HACCP systems should be implemented effectively.

6. Recommendation

Further study on the enterotoxigenicity of isolated S. aureus from fresh chicken gizzards should be conducted, and comparison studies for other sites should be conducted to rule out contamination from slaughter places other than bird sources.

7. Acknowledgment

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8. List of abbreviations

MSA: Mannitol salt agar
Aureus: Staphylococcus aureus

SaCGC: Staphylococcus aureus chicken gizzard contamination

SE: Staphylococcus enterotoxins

SFD: Staphylococcus food borne disease

AMR Antimicrobial Resistance

MRSA Methicillin resistance staphylococcus aureus

W.H.O World Health Organization

U. S : United States of America

MDRSA Multi drug resistance Staphylococcus aureus

CDC: Centers for Disease Control

FBD: Food borne disease

NA: Nutrient Agar

MHA: Muller Hinton Agar

CLSI: Clinical Laboratory Standards Institute

HACCP: Hazard Analysis Critical Control Point.

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