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Prevalence and antibiotic susceptibility of *Staphylococcus aureus* isolated from raw and grilled beef in Nyankpala community in the Northern Region of Ghana

Frederick Adzitey¹,²* Rejoice Ekli ¹ and Alexander Abu¹

¹University for Development Studies, Department of Animal Science, Box TL 1882, Tamale, Ghana.

²University for Development Studies, Department of Veterinary Science, Box TL 1882, Tamale, Ghana.

*Corresponding author: Email: adzitey@yahoo.co.uk; Tel: +233249995310

**ORCID ID**
Frederick Adzitey: [https://orcid.org/0000-0002-8814-0272](https://orcid.org/0000-0002-8814-0272)
Rejoice Ekli: Not Available
Alexander Abu: [https://orcid.org/0000-0003-3946-5991](https://orcid.org/0000-0003-3946-5991)

**ABSTRACT**
*Staphylococcus aureus* is among the important pathogens contributing to foodborne illnesses. They easily cross contaminate foods from hands that comes in contact with the nose and mouth. They cause endocarditis, boils, impetigo, cellulitis and scalded skin. This study was carried out to determine the prevalence and antibiotic susceptibility of *Staphylococcus aureus* isolated from
raw and grilled beef sold in Nyankpala, Northern Region of Ghana. Isolation of *Staphylococcus aureus* was done according to the USA-FDA Bacteriological Analytical Manual. Antibiotic susceptibility test was performed using the disc diffusion method and the results interpreted using the CLSI guidelines. A total of 54 beef samples made up of 18 raw beef (T1), 18 grilled beef samples tested immediately after grilling (T2) and 18 grilled beef samples tested 1h 30 min after grilling (T3) were examined. Overall, 16.67% (9) were positive for *Staphylococcus aureus*. *Staphylococcus aureus* were isolated from grilled beef immediately (T2) after grilling (33.33%) and 1h 30min (T3) after grilling (16.67%). They were not isolated from raw beef samples (T1). Aerobic plate count was highest in T1 (3.59 log cfu/g), followed by T2 (2.94 log cfu/g) and T3 (2.83 log cfu/g). Out of 18 positive samples 85.19% were susceptible, 14.81% were intermediate resistant and none was resistant. *Staphylococcus aureus* were all (100%) susceptible to amoxicillin, chloramphenicol, ciprofloxacin, ceftriaxone, gentamicin and sulamethoxazole/trimethoprim. This study revealed that *Staphylococcus aureus* are present in grilled beef rather than the fresh samples examined. In conclusion, *Staphylococcus aureus* contamination mostly occurred after processing and were susceptible to most antibiotics.

**Keywords**: Antibiotic susceptibility, grilled meat, *Staphylococcus aureus*, raw beef

**About the Authors**

Frederick Adzitey is an Associate Professor in Meat Science and Technology. He holds a PhD in Food Safety and MSC in Meat Science and Technology. He works on the isolation, antibiotic resistance and characterization of foodborne pathogens.
Rejoice Ekli is an MPHIL student in Animal Science (Meat Science and Technology Option). She is working on antibiotic residues and prevalence of resistant Salmonella species in beef obtained from the Wa Abattoir, Ghana.

Alexander Abu holds an MPHIL in Meat Science and Technology and a Research Fellow. He works on the use of local food resources to formulate meat and meat products. The aim is to produce meat products that are healthier, less expensive and have longer shelf-life.

**Public Interest**

This research was performed to investigate the prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from raw and grilled beef. Raw and grilled beef are consumed by most Ghanaians. Grilled beef in particular are consumed without preheating after retail purchase. As such consumes are exposed to bacterial infections from the consumption of contaminated meat and meat products. This work creates awareness about the existence of bacteria and *Staphylococcus aureus* in raw and or grilled beef, and the need to take caution when one wants to consume it. It also tells the antibiotics that can be used to treat patients suffering from *Staphylococcus aureus* infections as a result of consumption of contaminated grilled beef from Nyankpala.

**1. INTRODUCTION**

*Staphylococcus aureus* is Gram positive bacterium that are often present in the skin and nasal passages of some humans and animals (Bush, 2019; Taylor, & Unakal, 2019). Foster, & Geoghean (2015) estimated that approximately 20% of humans have the nares persistently colonized by *Staphylococcus aureus*. *Staphylococcus* may colonize the skin and nasal passage without the victim showing symptoms (Rasigade, & Vandenesch, 2014). However,
*Staphylococcus aureus* involved in skin infections and food poisoning has been reported (Taylor, & Unakal, 2019). They are also involved in multiple human infections such as pneumonia, heart valve infections, bacteremia, urinary tract infections, toxic shock, meningitis, and bone infections (Centers for Diseases Control & Prevention, 2003; Taylor, & Unakal, 2019). They get to cause infection mostly through direct contact and sometimes through the bloodstream (Rasigade, & Vandenesch, 2014). Bush (2019) indicated that of all Staphylococci species, *Staphylococcus aureus* has been noted to be the most dangerous of all. *Staphylococcus aureus* bacteremia was found to be responsible for considerable human illnesses and deaths, yielding a case mortality of 20-25% (Mejer et al., 2012).

Toxins produced by *Staphylococcus aureus* in particular triggers a series of gastrointestinal problems, toxic shock syndrome among others, and consequently discomfort to humans. Hitherto, the prevalence of toxins in *Staphylococcus aureus* present in healthy individuals have been reported. A high rate of staphylococcal enterotoxins (SE) genes and a low prevalence of Panton-Valentine leucocidin (PVL) gene (*pvl, lukS-PV-lukF-PV*) and toxic shock syndrome toxin-1 TSST-1 gene (*tst-1*) have been reported (Sdougkos et al., 2008; Miller et al., 2011; Castro, Santos, Meireles, Silva, & Teixeira, 2016). Nonetheless, high rates of *pvl* greater than 10 percent in colonizing S. aureus have been reported in some African studies (Hogan et al., 2016; Ouedraogo et al., 2016).

Treatment of *Staphylococcus aureus* infections involve the use of antibiotics. However, usage of antibiotics for treatment of infections and other purposes in general have been responsible for the development of multidrug resistant bacterial isolates and a major public health issue.
Staphylococcus aureus of meat sources resistant to various antibiotic have been reported (Hiroi et al., 2012; Jackson, Davis, & Barrett, 2013; Adugna, Pal, & Girmay, 2018; Haskell et al., 2018; Pekana, & Green, 2018). For instance, Adugna et al. (2018) observed that Staphylococcus aureus isolated from beef were resistant to bacitracin (100%), methicillin (100%), neomycin (100%), tetracycline (95%), penicillin G (49.5%), vancomycin (45.5%), and cloxacillin (45%).

Hatakka, Björkroth, Asplund, Mäki-Petäys, & Korkeala (2000) reported that Staphylococcus aureus in meat is as a result of improper hygienic practices at the point of handling by the slaughter personnel during meat production. In Ghana, there are evidences that poor handling of animals and carcasses have resulted in the contamination of meat by various types of bacteria (Danikuu, 2004; Soyiri, Agbogli, & Dongdem, 2008; Adzitey, 2015a; Adzitey, 2015b; Anachinaba, Adzitey, & Teye, 2015). Meat and meat products are relished by Ghanaians. While, most Ghanaians enjoy grilled beef as a luxury, raw beef is used in the preparation of soups and stews, as a normal part of typical Ghanaian food. Among the reasons for which meat and meat products are consumed include their high protein content, and available, lipids, mineral, vitamins and savory sensation. Despite the importance of raw and grilled beef in the diet of most Ghanaians, their association with Staphylococcus aureus in Nyankpala Community of Ghana has not been reported. This study aimed to determine the prevalence and antibiotic susceptibility of Staphylococcus aureus isolated from raw and grilled beef sold in Nyankpala, Northern Region of Ghana.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation
A sum of 54 swabs consisting of eighteen (18) raw beef samples (T1), eighteen (18) grilled beef samples tested immediately after grilling (T2) and eighteen (18) grilled beef samples tested one hour thirty minutes after grilling (T3) were randomly collected from butchers and khebab sellers in Nyankpala Community. Approximately 10cm² beef surfaces were swabbed. The swabs were kept in an ice chest containing ice block and transported to the Spanish Laboratory of University for Development Studies, Nyankpala Campus for microbiological analysis for *Staphylococcus aureus*, aerobic plate count and antibiotic susceptibility test. Microbiological analysis was carried out immediately on arrival at the laboratory.

### 2.2 Enumeration of aerobic plate count

The swabs were dipped into 10ml of 1% Buffered Peptone Water (Oxoid, Basingstoke, UK). Serial dilutions from $10^{-1}$ to $10^{-5}$ were performed using 1ml of 1% Buffered Peptone Water (Oxoid, Basingstoke, UK) from each dilution. Approximately 100ul of the aliquots were spread plated onto Plate Count Agar (Oxoid, Basingstoke, UK). The Plate Count Agar were incubated at 37°C for 24 hours and counted. The colony forming unit was obtained from the count using the formula:

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)}$$

where

- $N$ = Number of colonies per cm²
- $\sum C$ = Sum of all colonies on all plates counted
- $n_1$ = Number of plates in first dilution counted
- $n_2$ = Number of plates in second dilution counted
- $d$ = Dilution from which the first counts were obtained
2.3 Isolation and confirmation of Staphylococcus aureus

The swabs were placed in 10ml Buffered Peptone Water (Oxoid, Basingstoke, UK) and incubated at a temperature of 37°C for 18-24 hours. It was then streaked onto Mannitol Salt Agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. Presumptive Staphylococcus aureus colonies produced yellow colonies surrounded by yellow zones on Mannitol Salt Agar (Oxoid, Basingstoke, UK) and such colonies were purified on Trypticase Soy Agar (Oxoid, Basingstoke, UK) incubate at 37°C for 18-24 hours. The purified colonies were confirmed using Gram staining and Staphylase test (Oxoid, Basingstoke, UK).

2.4 Antimicrobial susceptibility test

Purified Staphylococcus aureus (n=9) were subjected to antimicrobial susceptibility using the disc diffusion method (Bauer-Kirby, 1966) against the following antibiotics: amoxycillin/clavulanic acid (30μg), azithromycin (15μg), ceftriaxone (30ug), chloramphenicol (30ug), ciprofloxacin (5ug), gentamicin (10ug), suphamethoxazole/trimethoprim (22ug), teicoplanin (30μg) and tetracycline (30ug). The Staphylococcus aureus were grown in Trypticase Soy Broth (Oxoid, Basingstoke, UK) and incubated at 37°C for 18 hours. It was then adjusted to 0.5 McFarland standard using sterile Trypticase Soy Broth and spread plated on Müller Hinton Agar (Oxoid, Basingstoke, UK). Four antibiotic disks were placed on the surface of the Müller Hinton Agar (Oxoid, Basingstoke, UK) at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 24 hours. After incubation, the inhibition zones were measured and the results interpreted using the Clinical Laboratory Standard Institute (2008).

2.5 Statistical analysis

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The data (aerobic plate count) obtained was analyzed using ANOVA of Genstat Edition Version 12 and means were separated at 5% significant level.

3. RESULT AND DISCUSSION

3.1 Total aerobic plate count of raw and grilled beef samples

The aerobic plate count is shown in Table 1. Raw beef samples (3.59 log cfu/cm²) had the highest bacterial load, which was also significantly higher (P < 0.05) than that of the grilled beef samples (2.94 log cfu/cm² for T2 and 2.83 log cfu/cm² for T3). The grilled beef samples did not differ (P>0.05) from each other, although T2 was numerically higher than T3. Adzitey, Abdul-Aziz, & Moses (2014) reported the presence of aerobic plate count in beef samples collected from Yendi Municipality in Ghana. They reported an average bacterial load of 5.74 log cfu/cm², which was higher than that found in this study. They also showed that the bacteria load of the beef samples varied among different locations. Similarly, the bacteria load varied among the raw and grilled beef samples in this study. In Abidjan, Cote d’ivoire, Koffi-Narvry, Koussemon, & Coulibaly (2011) found aerobic plate count of log 4.93 log cfu/g in beef samples collected from the main abattoir meant for retail sale; which was also higher than the aerobic plate count reported in this study. Contrarily, aerobic plate count of 1.62 log cfu/g was reported for beef samples collected from slaughterhouses in East Java, Indonesia (Soepranianondo, Wareham, Budiarto, & Diyantoro, 2019). A much higher aerobic plate count of 5.40-8.35 log cfu/g were observed for raw beef sold in different markets of Sylhet Sadar in Bangladesh (Jahan, Mahbub-E-Elahi, & Siddique, 2015).

Cooked meat products display for sale at ambient temperature for a limited period of time containing aerobic plate count of <10⁶ is considered satisfactory, between 10⁵ – 10⁶ is considered
to be on the borderline and $\geq 10^6$ is considered unsatisfactory (Center for Food Safety, 2014). From this study, the aeropbic plate count for raw beef was within the satisfactory limit. Food Standards Australia New Zealand (2016) report indicated that aerobic plate count in raw commodities including raw beef is likely to be high ($10^6$-$10^7$) and foods that have receive heat treatment should have low aerobic plate count levels ($<10^3$-$10^4$). The grilled beef samples in this study also met the criteria of the Food Standards Australia New Zealand and therefore, relatively safe for consumption.

### 3.2 Prevalence of Staphylococcus aureus in raw and grilled beef samples

The prevalence of *Staphylococcus aureus* in raw and grilled beef samples collected from Nyankpala is shown in Table 2. *Staphylococcus aureus* were not isolated from raw beef (T1). Grilled beef samples immediately after grilling (33.33%, T2) and one hour thirty minutes after grilling (16.67%, T3) were positive for *Staphylococcus aureus*. Thus grilled beef samples were contaminated with *Staphylococcus aureus*. The absence of *Staphylococcus aureus* in the raw beef samples indicates that *Staphylococcus aureus* was significantly ($P<0.05$) in the grilled beef samples, and the contamination was not by chance. However, there was no significant difference ($P>0.00$) between the grilled beef samples. Contamination of grilled beef samples could have resulted from cross contamination from the hands, skin or nose of processors. Food Standards Australia New Zealand (2016) reported that foods associated with Staphylococcal food poisoning are those that often require considerable handling during preparation. In Eastern Cape, South Africa, Pekana, & Green (2018) found that 20.4% of beef samples obtained from the abattoir were contaminated with *Staphylococcus aureus*. The prevalence of *Staphylococcus aureus* from beef products in Georgia was 63% (Jackson et al., 2013). Haskell et al. (2018) reported that 2.8% of beef samples collected from grocery stores/wholesale stores/ethnic markets
in various cities in Utah County, Utah were positive for *Staphylococcus aureus*. Hiroi *et al.* (2012) also observed in Shizuoka Prefecture, Japan that 44% of meats collected from the were contaminated with *Staphylococcus aureus*. A prevalence of 36% was reported for beef carcasses obtained from Addis Ababa, Ethiopia (Adugna *et al.*, 2018). Comparable to this study, *Staphylococcus aureus* was not found in fresh beef samples. This study detected lower *Staphylococcus aureus* in grilled beef samples as compared to studies by Hiroi *et al.* (2012), Jackson *et al.* (2013) and Adugna *et al.* (2018), but higher than that of Haskell *et al.* (2018). The overall prevalence of *Staphylococcus aureus* in beef samples was found to be 65.6%, while it was 80% and 50% for beef livers and other beef cuts, respectively in the USA (Abdalrahman, Wells, & Fakhr, 2015).

### 3.3 Antibiotic susceptibility of Staphylococcus aureus isolated from grilled beef samples

The antimicrobial susceptibility of the isolated *Staphylococcus aureus* is shown in Table 3. The isolates were all susceptible (100%) to amoxycillin/clavulanic acid, chloramphenicol, ciprofloxacin, ceftriaxone, gentamicin and suphamethoxazole/trimethoprim. None of the isolates was resistant to any of the antibiotics examined. However, intermediate resistant occurred for azithromycin (66.67%), tetracyline (33.33%) and teicoplanin (33.33%). The intermediate resistant isolates were neither resistant or susceptible. Such isolates have the tendency to become resistant and are pose a challenge when it comes to treatment when they are involved in infections (Adzitey, Nsoah, & Teye, 2015). Adugna *et al.* (2018) also observed intermediate susceptibility for vancomycin (54%), erythromycin (27%), amoxicillin (13%), norfloxacin (13%) and tetracycline (4.5%) in *Staphylococcus aureus* isolated from beef in Ethiopia.
In South Africa, Pekena, & Green (2018) found 39.2%, 24.5%, 9.8% and 2.7% resistance to tetracycline, suphathoxazole/trimethoprim, gentamicin and chloramphenicol, respectively in *Staphylococcus aureus* isolated from beef. Jackson *et al.* (2013) reported resistance to ciprofloxacin (100%), ceftriaxone (75%), and tetracycline (25%) but, no resistance was found for suphathoxazole/trimethoprim and gentamicin in beef from Georgia. *Staphylococcus aureus* isolated from beef in Japan was observed to be resistant to gentamicin (11.4%), tetracycline (6.8%) and chloramphenicol (6.8%) (Hiroi *et al.*, 2012). The 100% susceptibility to suphathoxazole/trimethoprim and gentamicin as found by Jackson *et al.* (2013) was consistent with that of this study. However, the resistances to the various antibiotics found by Hiroi *et al.* (2012), Jackson *et al.* (2013) and Pekena, & Green (2018) contradict this study. Furthermore, Abdalrahman, Wells, & Fakhr (2015) found resistance of *Staphylococcus aureus* (isolated from beef livers and cuts in the USA) to tetracycline (24.7%), azithromycin 16.4, ciprofloxacin (7.3%), gentamicin (13.7%) and trimethoprim/sulfamethazole (1.4%). This study found no resistances to the afore-mentioned antibiotics.

4. CONCLUSION

This is the first report on the prevalence and antibiotic resistance of *Staphylococcus aureus* of beef origin in Nyankpala Community of Ghana. Raw beef samples had high bacterial load as compared to grilled beef samples, but they all met recommended standards. Grilled beef samples from Nyankpala are contaminated with *Staphylococcus aureus* rather than the raw meat. *Staphylococcus aureus* contamination occurred during handling and processing of beef. *Staphylococcus aureus* of grilled beef origin were mostly susceptible to the antibiotics examined.

**Funding**
This work was funded by the authors.

**Competing interests**

Authors declare no competing interests

**Availability of data**

This research has been investigating the prevalence and antibiotic resistance of *Staphylococcus aureus* of beef origin in Nyanpkala Community of Ghana. Available data has been presented in tables.

**Authors contribution**

Frederick Adzitey is the main financial of this work, involved in the design and carrying out of the experiment, wrote most part of the manuscript and proof read.

Rejoice Ekli was mostly involved in data collection and drafting of this manuscript.

Alexander Abu supported this work financial and proof read this article.

**Ethics approval and consent to participate**

Not applicable

**Consent for publication All authors**

All authors read and approved the final manuscript.

Frederick Adzitey: adzitey@yahoo.co.uk

Alexander Abu: alexable2012@yahoo.com
References


Table 1: Total aerobic bacteria count in raw and grilled beef collected from Nyankpala

<table>
<thead>
<tr>
<th>Sample</th>
<th>Log cfu/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw beef (T1)</td>
<td>3.59⁹</td>
</tr>
<tr>
<td>Grilled beef (0h, T2)</td>
<td>2.94⁻⁹</td>
</tr>
<tr>
<td>Grilled beef (1h 30min, T3)</td>
<td>2.83⁻⁹</td>
</tr>
<tr>
<td>SEM</td>
<td>0.42</td>
</tr>
<tr>
<td>P value</td>
<td>0.00</td>
</tr>
</tbody>
</table>

SEM= Standard Error of Means

Table 2: Prevalence of *Staphylococcus aureus* in raw and grilled beef collected from Nyankpala

<table>
<thead>
<tr>
<th>Source of meat</th>
<th>Number of samples tested</th>
<th>Number of positive/ negative samples</th>
<th>%Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw beef (T1)</td>
<td>18.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Grilled beef (0h, T2)</td>
<td>18.00</td>
<td>6</td>
<td>33.33</td>
</tr>
<tr>
<td>Grilled beef (1h 30min, T3)</td>
<td>18.00</td>
<td>3</td>
<td>16.67</td>
</tr>
<tr>
<td>Overall</td>
<td>54.00</td>
<td>9</td>
<td>16.67</td>
</tr>
</tbody>
</table>
Table 3: Percentage antibiotic resistance of *Staphylococcus aureus* isolated from grilled beef samples collected from Nyankpala

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>R (%)</th>
<th>I (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin/clavulanic acid (Amc) 30 µg</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Azithromycin (Amz) 15 µg</td>
<td>0.00</td>
<td>66.67</td>
<td>33.33</td>
</tr>
<tr>
<td>Chloramphenicol (C) 30 µg</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Ciprofloxacin (Cip) 5 µg</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Ceftriaxone (Cro) 30 µg</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Gentamicin (Cn) 10 µg</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Tetracycline (Te) 30 µg</td>
<td>0.00</td>
<td>33.33</td>
<td>66.67</td>
</tr>
<tr>
<td>Teicoplanin (Tec) 30 µg</td>
<td>0.00</td>
<td>33.33</td>
<td>66.67</td>
</tr>
<tr>
<td>Suphamethoxazole/trimethoprim (Sxt) 25 µg</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Overall prevalence</td>
<td>0.00</td>
<td>14.81</td>
<td>85.19</td>
</tr>
</tbody>
</table>
S, susceptible; I, Intermediate; R, resistant