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

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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 samples (T1), 18 grilled beef samples tested immediately after grilling (T2) and 18 grilled beef samples tested 1 h 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 1 h 30 min (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 sulfamethoxazole/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.

Subjects: Microbiology; Biotechnology; Food Additives & Ingredients

Keywords: Antibiotic susceptibility; grilled meat; Staphylococcus aureus; raw beef

1. Introduction

Staphylococcus aureus is Gram-positive bacterium that is often present in the skin and nasal passages of some humans and animals (Bush, 2019; Taylor & Unakal, 2019). Foster and Geoghegan (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 Disease Control and 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-PVF- lukF-PV) and toxic shock syndrome toxin-1 (TSST-1 gene (tst-1) have been reported (Castro, Santos, Meireles, Silva, & Teixeira, 2016; Miller et al., 2011; Sdougkos et al., 2008). Nonetheless, high rates of pvl greater than 10% colonizing Staphylococcus aureus have been reported in some African studies (Hogan et al., 2016; Ouedraogo et al., 2016).

Treatment of Staphylococcus aureus infections involves the use of antibiotics. However, usage of antibiotics for the 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 (Adugna, Pal, & Girmay, 2018; Haskell et al., 2018; Hori et al., 2012; Jackson, Davis, & Barrett, 2013; 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, Möki-Petäys, and Korkeala (2000) reported that Staphylococcus aureus in meat is as a result of improper hygienic practices at the point of handling by 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 (Adzitey, 2015a, 2015b; Anachinaba, Adzitey, & Teye, 2015; Danikuu, 2004; Soyiiri, Agbogli, & Dongdem, 2008). 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 1 h 30 min after grilling (T3) were randomly collected from butchers and kebab sellers in Nyankpala Community. Approximately 10 cm² of 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 10 ml of 1% Buffered Peptone Water (Oxoid, Basingstoke, UK). Serial dilutions from $10^{-1}$ to $10^{-5}$ were performed using 1 ml of 1% Buffered Peptone Water (Oxoid, Basingstoke, UK) from each dilution. Approximately 100 ul of the aliquots were spread plated onto Plate Count Agar (Oxoid, Basingstoke, UK). The Plate Count Agar were incubated at 37°C for 24 h and counted. The colony-forming unit was obtained from the count using the formula:

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

where $N$ = Number of colonies per cm²

$\Sigma C$ = 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 10 ml Buffered Peptone Water (Oxoid, Basingstoke, UK) and incubated at a temperature of 37°C for 18–24 h. It was then streaked onto Mannitol Salt Agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h. 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) and incubated at 37°C for 18–24 h. 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, Sherris, & Turk, 1966) against the following antibiotics amoxycillin/clavulanic acid (30 μg), azithromycin (15 μg), ceftriaxone (30μg), chloramphenical (30ug), ciprofloxacin (5ug), gentamicin (10ug), sulfamethoxazole/trimethoprim (22ug), teicoplanin (30 μg) and tetracycline (30ug). The Staphylococcus aureus were grown in Trypticase Soy Broth (Oxoid, Basingstoke, UK) and incubated at 37°C for 18 h. 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 h.
After incubation, the inhibition zones were measured and the results interpreted using the Clinical and Laboratory Standards Institute (2008).

2.5. Statistical analysis
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, and 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. Koffi-Nevry, Koussemon, and 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 <10^6 is considered satisfactory, between 10^5–10^6 is considered to be on the borderline and ≥10^6 is considered unsatisfactory (Center for Food Safety, 2014). From this study, the aerobic 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 (10^5–10^7) and foods that have received 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 1 h 30 min after grilling (16.67%, T3) were positive for Staphylococcus aureus. Thus, the absence of Staphylococcus aureus in the raw beef samples indicates that Staphylococcus aureus was significantly (P < 0.05) in the grilled beef.

| Sample                     | Log cfu/cm² |
|----------------------------|-------------|
| Raw beef (T1)              | 3.59^a      |
| Grilled beef (0h, T2)      | 2.94^b      |
| Grilled beef (1h 30min, T3) | 2.83^b      |
| SEM                       | 0.42        |
| P value                   | 0.00        |

SEM = Standard Error of Means. Different superscripts a & b signifies different (P<0.05) and vice versa.
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 and 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 beef 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).

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

| Source of meat          | Number of samples tested | Number of positive/negative samples | %Prevalence |
|-------------------------|--------------------------|------------------------------------|-------------|
| Raw beef (T1)           | 18.00                    | 0                                  | 0.00        |
| Grilled beef (0h, T2)   | 18.00                    | 6                                  | 33.33       |
| Grilled beef (1h 30min, T3) | 18.00                  | 3                                  | 16.67       |
| Overall                 | 54.00                    | 9                                  | 16.67       |

### Table 3. Percentage antibiotic resistance of *Staphylococcus aureus* isolated from grilled beef samples collected from Nyankpala

| Antimicrobial                        | R (%) | I (%) | S (%) |
|--------------------------------------|-------|-------|-------|
| Amoxycillin/clavulanic acid (Amc) 30 µg | 0.00  | 0.00  | 100.00|
| Azithromycin (Amz) 15 µg             | 0.00  | 66.67 | 33.33 |
| Chloramphenicol (C) 30 µg            | 0.00  | 0.00  | 100.00|
| Ciprofloxacin (Cip) 5 µg             | 0.00  | 0.00  | 100.00|
| Ceftriaxone (Cro) 30 µg              | 0.00  | 0.00  | 100.00|
| Gentamicin (Cn) 10 µg                | 0.00  | 0.00  | 100.00|
| Tetracycline (Te) 30 µg              | 0.00  | 33.33 | 66.67 |
| Teicoplanin (Tec) 30 µg              | 0.00  | 33.33 | 66.67 |
| Suphamethoxazole/trimethoprim (Sxt) 25 µg | 0.00  | 0.00  | 100.00|
| Overall prevalence                  | 0.00  | 14.81 | 85.19 |

S, susceptible; I, Intermediate; R, resistant.
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 amoxicillin/clavulanic acid, chloramphenicol, ciprofloxacin, ceftriaxone, gentamicin and sulfamethoxazole/trimethoprim. None of the isolates was resistant to any of the antibiotics examined. However, intermediate resistant occurred for azithromycin (66.67%), tetracycline (33.33%) and teicoplanin (33.33%). The intermediate resistant isolates were neither resistant or susceptible. Such isolates have the tendency to become resistant and 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, Pekana and Green (2018) found 39.2%, 24.5%, 9.8% and 2.7% resistance to tetracycline, sulfamethoxazole/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 sulfamethoxazole/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 sulphonamide/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 Pekana and Green (2018) contradict this study. Furthermore, Abdalrahman et al. (2015) found the 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/sulfamethoxazole (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 the handling and processing of beef. Staphylococcus aureus of grilled beef origin were mostly susceptible to the antibiotics examined.

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Competing interests
Authors declare no competing interests

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Availability of data
This research investigated the prevalence and antibiotic resistance of Staphylococcus aureus of beef origin in Nyankpala 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 after the first draft and proofread as well.
Rejoice Ekli was involved in data collection and drafting of this manuscript.
Alexander Abu supported this work financially and proofread this manuscript.

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Not applicable

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