ENVIRONMENTAL HEALTH | RESEARCH ARTICLE

Incidence and antibiotic susceptibility of *Staphylococcus aureus* isolated from ready-to-eat meats in the environs of Bolgatanga Municipality of Ghana

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**Abstract:** *Staphylococcus aureus* is one of the major foodborne pathogens in ready-to-eat foods exposed to the environment. This study investigated the microbial load, incidence and antibiotic susceptibility of *Staphylococcus aureus* in ready-to-eat meats in Bolgatanga Municipality, Ghana. A total of 200 swabs of ready-to-eat meats (50 each of grilled chevon, mutton, pork and guinea fowl) were examined. Analyses for microbial load and *Staphylococcus aureus* were done using a modified method of the USA-FDA Bacteriological Analytical Manual. Antibiotic susceptibility test was performed using the disc diffusion method. The microbial load of the ready-to-eat meats ranged from 4.02 to 4.85 log cfu/cm² and averagely 34.0% were positive for *Staphylococcus aureus*. The highest incidence was seen in grilled guinea fowl (46%) and the lowest was found in grilled chevon and pork (24% each). Of the 16 *Staphylococcus aureus* examined against 9 different antibiotics, 44.44% 5.56% and 50.00% exhibited susceptibility, intermediate resistance and resistance,

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**PUBLIC INTEREST STATEMENT**

This research was performed to investigate the occurrence and antibiotic resistance of *Staphylococcus aureus* in ready-to-eat meats. In Ghana, Ready-to-eat meats (grilled meats) are mostly consumed directly after purchased without cooking or pre-heating. While effective grilling destroys all bacteria, past grilling handling and exposing to the environment make it possible for ready-to-eat meats to be cross-contaminated by various foodborne pathogens including *Staphylococcus aureus* making it a significant public health hazard. The study revealed that consumers are exposed to *Staphylococcus aureus* infection from the consumption of ready-to-eat meats in the Bolgatanga Municipality of Ghana. Of major concern is the multidrug resistance that occurred among most of the *Staphylococcus aureus* isolates as this will make infections and illnesses caused by *Staphylococcus aureus* to be difficult to treat. This study creates awareness about the existence of bacteria and *Staphylococcus aureus* (and their resistance to antibiotics) in ready-to-eat meats, and the need to take the necessary precaution prior to consumption.
respectively. Higher resistances were observed for ceftriaxone (81.25%) and teicoplanin (75.00%). Susceptibility was high for sulfamethoxazole/trimethoprim (87.5%) and gentamicin (81.25%). Resistance to 3 or more different antibiotics occurred for 14 (87.5%) *Staphylococcus aureus*. Some ready-to-eat meats in the environs of Bolgatanga are contaminated with *Staphylococcus aureus* which are resistant to antibiotics, posing a health risk to consumers.

**Subjects:** Environmental Sciences; Agriculture and Food; Food Additives & Ingredients; Food Laws & Regulations; Ultrasonics; Solvent Extraction

**Keywords:** antibiotics; contamination; grilled meat; ready-to-eat meats; *Staphylococcus aureus*

### 1. Introduction

*Staphylococcus aureus* is a Gram-positive bacteria that often harbour the skin and mucous membranes of animals and humans (Taylor & Unakal, 2019). It grows within a temperature range of 15°C to 45°C and in a sodium chloride concentration as high as 15% (Behling et al., 2010; Taylor & Unakal, 2019). It also has the ability to multiply quickly at room temperature to produce toxins that cause illness (Behling et al., 2010). This bacteria can cause countless infections, which range from self-limiting skin infections to life-threatening pneumonia, bacteremia endocarditis, deep abscesses, osteomyelitis and bloodstream infections (David & Daum, 2010; Rasigade & Vandenesch, 2014; Taylor & Unakal, 2019). Von Eiff et al. (2001) reported that *Staphylococcus aureus* is an opportunistic pathogen carried asymptptomatically by healthy individuals; 20% of this pathogen is constantly found in human population, however, 60% is found intermittently. Sanaa et al. (2013) reported that this pathogen is the most frequently isolated bacteria from clinical specimens from hospitalized patients and ranks second among clinical isolates from outpatients in Ghana. Li and Webster (2018) and Schmidt et al. (2015) stated that *Staphylococcus aureus* is part of the bacterial species that are known with increasing drug resistance, morbidity, invasive disease, and mortality in humans as well as animals.

In Ghana, ready-to-eat meats are sold by meat vendors or kebab sellers often in an open space, roadside and/or streets exposed to the environment. Most of these vendors normally operate in the evening until night or day break and do not adhere to recommended meat safety practices (Adzitey et al., 2020; Sulleyman et al., 2018). While grilling kills all bacteria due to the heat applied during preparation, operation of the meat vendors in an open space makes it possible for contaminations by physical, chemical or biological hazards from the environment (Adzitey, 2016). Biological hazards include microorganisms such as *Staphylococcus aureus*.

Studies have shown that *Staphylococcus aureus* can be isolated from raw and ready-to-eat meat products (Abdalrahman et al., 2015; Adugna et al., 2018; Adzitey et al., 2019; Haskell et al., 2018). However, little is known about the association and occurrence of *Staphylococcus aureus* in ready-to-eat meat products in the Bolgatanga Municipality of Ghana. Therefore, this study was carried out to investigate the incidence and antibiotic susceptibility of *Staphylococcus aureus* isolated from ready-to-eat chevon, guinea fowl, mutton and pork sold in the Bolgatanga Municipality of Ghana. Moreover, this study assessed the microbial load of the afore-mentioned ready-to-eat meat products.
2. Materials and methods

2.1. Collection of samples and analysis
A total of 200 swabs of ready-to-eat meats (grilled meats) which included 50 each of chevon, guinea fowl mutton and pork were collected from the environs of Bolgatanga Municipality in the Upper East Region of Ghana from August to December 2019. About 10 cm² area of the meat surfaces were swabbed and transported to the Spanish Laboratory of University for Development Studies Nyankpala Campus on ice for microbiological analyses for aerobic plate count and Staphylococcus aureus. The analyses were carried out immediately on arrival at the laboratory by following the procedures of Adzitey (2020), Adzitey et al. (2019), and Tallent et al. (2016). For the aerobic plate count, swabs were soaked in 10 ml of 1% Buffered Peptone Water (Oxoid, Basingstoke, UK) and serial dilutions from 10⁻¹ to 10⁻⁵ were made using 1 ml of 1% Buffered Peptone Water. The solution was then spread plated (0.1 ml) onto plate count agar and incubated at 37°C for 24 h. After that the colonies were counted and expressed in colony forming units. With regards to Staphylococcus aureus, the swabs were soaked in 10 ml Buffered Peptone Water and incubated at 37°C for 18–24 h. The aliquot was streaked onto Mannitol Salt Agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h. Yellowish colonies with yellowish zones on Mannitol Salt Agar (Oxoid, Basingstoke, UK) were picked as presumptive Staphylococcus aureus. These colonies were purified on Trypticase Soy Agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h. Gram staining and Staphylase test (Oxoid, Basingstoke, UK) were used to confirm the purified Staphylococcus aureus colonies.

2.2. Antibiotic susceptibility testing
Sixteen pure colonies of Staphylococcus aureus isolates were subjected to antimicrobial susceptibility test by following the disc diffusion method (Bauer et al., 1966). The following antibiotics were used: amoxycillin/clavulanic acid (30 μg), azithromycin (15 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), sulfamethoxazole/trimethoprim (22 μg), teicoplanin (30 μg) and tetracycline (30 μg). The Staphylococcus aureus colonies were cultured in Trypticase Soy Broth (Oxoid, Basingstoke, UK), incubated at 37°C for 18 h and the turbidity was adjusted to 0.5 McFarland Standard. This aliquot (0.1 ml) was seeded on Mueller Hinton Agar (Oxoid, Basingstoke, UK) using sterile spreader. A sterilized forceps were used to place five and four antibiotic discs on the surface of Muller Hinton Agar (Oxoid, Basingstoke, UK) at a distance to avoid overlapping of inhibition zones and incubated at 37°C for 24 h. Inhibition zones were measured after incubation and the results were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2019). The multiple antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman (1983) using the formula: a/b, where “a” represents the number of antibiotics to which a particular isolate was resistant and “b” the total number of antibiotics tested.

2.3. Data analysis
The data obtained from microbial load were subjected to analysis of variance using GenStat 12.1 Software. Generalized linear model of Statistical Package for the Social Sciences (SPSS) Version 21 was used to analyze incidence data. The means were separated at 5% significant level.

3. Results and discussion

3.1. Aerobic bacteria count of ready-to-eat meat in Bolgatanga, Ghana
The microbial load of the ready-to-eat meats obtained from the environs of Bolgatanga Municipality is shown in Table 1. From Table 1, the microbial load was 4.85, 4.17, 4.06 and 4.02 log cfu/cm² for ready-to-eat chevon, mutton, guinea fowl and pork, respectively. There were no significant differences (P > 0.05) among ready-to-eat guinea fowl, mutton and pork. Similarly, there were no significant differences (P > 0.05) among ready-to-eat pork, guinea fowl, and mutton. Microbial load of ready-to-eat chevon was significantly (P < 0.05) higher than that of ready-to-eat pork.
The microbial contamination of the various ready-to-eat meats ranged from 4.02 to 4.85 log cfu/cm². Ready-to-eat chevon recorded the highest contamination whiles ready-to-eat chicken recorded the least. Microbial contamination of ready-to-eat meat samples may occur mostly as a result of how these products are handled after grilling. Thus, after grilling the products may be safe for consumption, but may be re-contaminated base on the hygienic status of the vendor and his/her environment. The sale of ready-to-eat meats on streets, open space and by road sides to attract consumbers makes microbial contamination unavoidable. Dust from the environment, wind, sellers and buyers play significant roles in the contamination of ready-to-eat meats by microorganisms. The result of this study agree with that of Agbodaze et al. (2005) who had similer microbial load in kebab, and related it to the environment and the handling procedures employed by individual kebab vendor. In addition, the result of this study shows variation in microbial contamination for each ready-to-eat meat type; this could be associated to the fact that vendors of each meat type operated in different location and therefore had different environmental challenges. Also, how each meat type was processed and packaged for grilling differed from one location to the other, especially the sizes and the grilling temperatures for meat type. In this study, chicken and guinea fowl meats were grilled whole, whiles others were cut into different sizes before grilling. Center for Food Safety (2014) and Public Health Laboratory Service (2002) stated that when the total microbial load in food samples is <5.0 log cfu/g, that food is safe for consumption or considered satisfactory, thus the ready-to-eat meat samples could be consumed, but with some caution. Microbial contamination of grilled guinea fowl (3.63 to 5.25 log cfu/cm²) (Adzitey et al., 2015) and smoked pork (4.33 to 4.85 log cfu/cm²) (Anachinaba et al., 2015) in the Bolgatanga Municipality of Ghana has also been reported.

### 3.2. Incidence of *Staphylococcus aureus* in ready-to-eat meats

Table 2 shows the distribution of *Staphylococcus aureus* in the various ready-to-eat meats collected from the environs of Bolgatanga Municipality, Ghana. Ready-to-eat chevon (24%), guinea fowl (46%), pork (24%) and mutton (42%) were contaminated by *Staphylococcus aureus*. There were no significant differences (P > 0.05) among ready-to-eat mutton, pork and chevon (Table 3). However, the occurrence of *Staphylococcus aureus* in ready-to-eat guinea fowl was significantly (P < 0.05) higher compared to ready-to-eat mutton, pork and chevon.

| Sources                  | No. tested | No. positive | % prevalence |
|--------------------------|------------|--------------|--------------|
| Grilled chevon           | 50         | 12           | 24           |
| Grilled guinea fowl      | 50         | 23           | 46           |
| Grilled pork             | 50         | 12           | 24           |
| Grilled mutton           | 50         | 21           | 42           |
| Overall                  | 200        | 68           | 34           |

The data is presented in Table 2.

**Table 1. Microbial load of ready-to-eat meats in Bolgatanga, Ghana**

| Sources   | Bacteria load (log cfu/cm²) |
|-----------|----------------------------|
| Chevon    | 4.85<sup>b</sup>           |
| Guinea fowl | 4.06<sup>ab</sup>        |
| Mutton    | 4.17<sup>ab</sup>         |
| Pork      | 4.02<sup>a</sup>          |
| S.e.d.    | 0.293                     |
| P-value   | 0.033                     |

S.e.d. = standard error of difference; Values in the same column with different superscripts are significantly different (P < 0.05).
The overall incidence of *Staphylococcus aureus* in this study was 34.0%, which was similar to the 35% reported by Wu et al. (2018) in retail meat and meat products obtained from China. A lower (27.9%) and higher (68.00%) incidences of *Staphylococcus aureus* were reported by Ge et al. (2017) in the USA and Tang et al. (2017) in Denmark, respectively, in retail meats. The prevalence of *Staphylococcus aureus* in ready-to-eat pork and ready-to-eat chevon collected from Enugu State, Southeast Nigeria was 4.2% and was 5.9%, respectively (Okoli et al., 2018), which were lower than the 24% each observed for ready-to-eat pork and ready-to-eat chevon in this study. Also, the prevalence of *Staphylococcus aureus* in ready-to-eat pork was 12.7% and that of ready-to-eat mutton was 0% in China (Wu et al., 2018) which were lower as compared to this study.

In the present study ready-to-eat guinea fowl was the most contaminated source by *Staphylococcus aureus* followed by ready-to-eat mutton, and ready-to-eat chevon and ready-to-eat pork. The presence of *Staphylococcus aureus* in the ready-to-eat meats reveals incidence of cross contamination from improper handling and environmental sources after grilling. *Staphylococcus* spp. harbour the nose, skin and the atmosphere and if personal and environmental hygiene are not observed they can easily contaminate meats after grilling prior to consumption (Tallent et al., 2016; Taylor & Unakal, 2019; Rasigade & Vandenesch, 2014). Environmental contaminants such as dust from streets can be carried by wind or vehicles onto meats which are mostly sold by meat sellers by roadsides, streets or open markets. Such dusts could contain microorganisms and favour the contamination of ready-to-eat meats by *Staphylococcus aureus* (Okoli et al., 2018). Adzitey et al. (2015) and Anachinaba et al. (2015) also found *Staphylococcus aureus* in smoked pork and grilled guinea fowl samples from Bolgatanga.

### 3.3. Antibiotic susceptibility of *Staphylococcus aureus*

Out of the 16 *Staphylococcus aureus* isolates examined against 9 different antibiotics, 44.44% were susceptible, 5.56% were intermediate resistant and 50.00% were resistant to the various antibiotics studied (Table 4). The highest resistance was observed in cefotaxime (81.25%), followed by teicoplanin (75%), amoxicillin/clavulanic acid (62.5%), tetracycline (56.25%) and chloramphenicol (43.75%). Susceptibility was highest for sulfamethoxazole/trimethoprim (87.5%), followed by gentamicin (81.25%), ciprofloxacin (62.5%) and chloramphenicol (56.25%).

The antibiotic resistance profile and multiple antibiotic resistance index of individual *Staphylococcus aureus* obtained from ready-to-eat meats in Bolgatanga, Ghana is shown in Table 5.
Two (2) *Staphylococcus aureus* isolates were resistant to two antibiotics, three were resistant to three antibiotics, two were resistant to four antibiotics, four were resistant to five antibiotics, four were resistant to six antibiotics and one was resistant to seven antibiotics. The highest resistant *Staphylococcus aureus* was isolated from grilled chevon, and it was resistant to seven different antibiotics (Cip-Amc-Azm-Tec-Cn-Cro-Sxt). Furthermore, resistant to six different antibiotics was found in *Staphylococcus aureus* isolated from ready-to-eat chevon (Amc-Azm-Tec-Te-Cro), guinea fowl (Cip-Amc-Azm-Tec-Cro-Sxt), guinea fowl (Cip-Amc-Azm-Tec-Te-Cro) and mutton (Amc-Azm-Tec-Cn-Te-C). This study revealed that 14 out of 16 *Staphylococcus aureus* isolates were resistant to 3 or more antibiotics, thus multidrug resistance was as high as 87.5%.

The *Staphylococcus aureus* exhibited high susceptibility (>50%) to chloramphenicol, ciprofloxacin, gentamicin and sulfamethoxazole/trimethoprim. This means that, the afore-mentioned antibiotics

### Table 4. Antibiotic susceptibility patterns of *Staphylococcus aureus* from ready-to-eat meats from Bolgatanga, Ghana

| Antimicrobial                                | R%  | I%  | S%  |
|----------------------------------------------|-----|-----|-----|
| Amoxicillin/clavulanic acid 30 µg (Amc)      | 62.50 | 0.00 | 37.50 |
| Azithromycin 15 µg (Azm)                     | 0.00  | 12.50 | 12.50 |
| Ceftriaxone 30 µg (Cro)                      | 81.25 | 6.25 | 12.50 |
| Chloramphenicol 30 µg (C)                    | 43.75 | 0.00 | 56.25 |
| Ciprofloxacin 5 µg (Cip)                     | 31.25 | 6.25 | 62.50 |
| Gentamicin 10 µg (Cn)                        | 12.50 | 6.25 | 81.25 |
| Teicoplanin 30 µg (Tec)                      | 75.00 | 0.00 | 25.00 |
| Tetracycline 30 µg (Te)                      | 56.25 | 18.75 | 25.00 |
| Sulfamethoxazole/trimethoprim (Sxt)          | 12.50 | 0.00 | 87.50 |
| Overall                                      | 50.00 | 5.56 | 44.44 |

### Table 5. Antibiotic resistance profile and multiple antibiotic resistance index of individual *Staphylococcus aureus* obtained from ready-to-eat meats in Bolgatanga, Ghana

| Codes | Source     | No. of Antibiotics | Antibiotic resistant profile | MAR index |
|-------|------------|--------------------|-------------------------------|-----------|
| G06   | Chevon     | 5                  | Cip-Tec-Te-Cro                | 0.56      |
| G016  | Chevon     | 7                  | Cip-Amc-Azm-Tec-Cn-Cro-Sxt    | 0.78      |
| G022  | Chevon     | 4                  | Amc-Azm-C-Cro                 | 0.44      |
| G024  | Chevon     | 6                  | Amc-Azm-Tec-Te-Cro            | 0.67      |
| G025  | Chevon     | 2                  | Azm-Tec                       | 0.22      |
| G048  | Chevon     | 5                  | Amc-Tec-Te-Cro                | 0.56      |
| G7    | Guinea fowl | 2                  | Te-C                          | 0.22      |
| G12   | Guinea fowl | 3                  | Amc-Tec                       | 0.33      |
| G15   | Guinea fowl | 3                  | Azm-Tec-Cro                   | 0.33      |
| G19   | Guinea fowl | 3                  | Azm-Te-Cro                    | 0.33      |
| G26   | Guinea fowl | 6                  | Cip-Amc-Azm-Tec-Cro-Sxt       | 0.67      |
| G37   | Guinea fowl | 6                  | Cip-Amc-Azm-Tec-Te-Cro        | 0.67      |
| S11   | Mutton     | 5                  | Cip-Azm-Tec-Te-Cro            | 0.56      |
| S15   | Mutton     | 5                  | Amc-Azm-Te-C-Cro              | 0.56      |
| S16   | Mutton     | 6                  | Amc-Azm-Tec-Cn-Te-C          | 0.67      |
| S25   | Mutton     | 4                  | Amc-Azm-Tec-Cro              | 0.44      |
will be effective in controlling Staphylococcus aureus infections resulting from the consumption of contaminated ready-to-eat meats in the Bolgatanga Municipality. Similarly, Okoli et al. (2018) found that Staphylococcus aureus of ready-to-eat meats were susceptible to sulfamethoxazole/trimethoprim (91.7%), gentamicin (95.8%), chloramphenicol (100%) and ciprofloxacin (100%). In this study, the Staphylococcus aureus exhibited high resistance (>50%) to ceftriaxone, teicoplanin, amoxicillin/clavulanic acid and tetracycline. Contrarily to this study, Okoli et al. (2018) reported 100% susceptibility of Staphylococcus aureus isolated from ready-to-eat meats to teicoplanin and tetracycline. In China, Wu et al. (2018) reported that Staphylococcus aureus isolated from ready-to-eat meats were resistant to amoxicillin/clavulanic acid (11.1%), chloramphenicol (9.1%), ciprofloxacin (8.1%), gentamicin (26.3%), teicoplanin (0.0%), tetracycline (32.3%) and trimethoprim/sulphamethoxazole (4.0%); these resistances were lower than of this study except for gentamicin. In Ghana, Adzitey et al. (2019) reported that none of the isolated Staphylococcus aureus from grilled beef was resistant to any of the antibiotics (amoxicillin/clavulanic acid, azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, sulfamethoxazole/trimethoprim, teicoplanin and tetracycline) examined, which disagrees with the current study. Interestingly, all the Staphylococcus aureus isolates exhibited different resistance patterns; this means that, they were phenotypically diverse. The multiple antibiotic resistance ranged from 0.22 (resistant to 2 antibiotics) to 0.78 (resistant to 7 antibiotics). Okoli et al. (2018) and Wu et al. (2018), also reported multidrug resistant Staphylococcus aureus isolates from ready-to-eat meats.

4. Conclusions and recommendation

Ready-to-eat meats in the Bolgatanga Municipality were contaminated by various microbes (4.0–0.24–4.85 log cfu/cm²) but were within acceptable limit. Staphylococcus aureus were found in some of the ready-to-eat meat (24–46%) samples. Personal and environmental hygiene are responsible for the cross contamination of the ready-to-eat meat samples. The Staphylococcus aureus were highly resistant (>70%) to cephraxone and teicoplanin, but highly susceptible (>80%) to sulphamethoxazole/trimethoprim and gentamicin. The Staphylococcus aureus exhibited a high multidrug resistant which is a threat to public health. Further research should investigate the presence of resistance genes and genetic characterization of the Staphylococcus aureus isolates.

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Competing interests

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Authors contribution

Frederick Adzitey is the main financial of this work, involved in the design and carrying out of the experiment and edited the manuscript. Rejoice Eki was involved in data collection, analysis and drafting of this manuscript. Martin Aduah was involved in data collection and proof read this article.

Availability of data

This research investigated the incidence and antibiotic resistance of Staphylococcus aureus in ready-to-eat meats in Bolgatanga Municipality of Ghana. Available data have been presented in tables.

Citation information

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