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# Identification and Anti-bacterial Testing of *Staphylococcus aureus* Isolated from Jollof Rice sold at selected Cafeterias in Federal University Dutse Campus, Jigawa State.

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**Abstract**
This research was aimed at identifying *Staphylococcus aureus* isolated from jollof rice samples at selected cafeterias in Federal University Dutse campus. A total of fifteen (15) samples were collected and processed within an hour in sterile containers from five (5) different cafeterias (A, B, C, D and E). The total aerobic bacterial counts recorded from samples A, B, C, D and E were 1.6 x 105, 1.1 x 105, 1.1 x 107, 9.3 x 104, 1.3 x107 cfu/g respectively while total Staphylococcal counts recorded from samples A, B, C, D and E were 1.3 x 104, 6.5 x 103, 6.0 x 105, 4.0 x 103, 1.5 x108 cfu/g respectively. Based on staphylococcal counts recorded, sample A result is unacceptable while samples B and D are within tolerable microbiological quality while that of samples C and E are also of poor quality. *Staphylococcus aureus* isolated from the samples was higher in samples C and E than in samples A, B and D. The isolate was susceptible to Ofloxacin and Gentamicin based on susceptibility tests conducted. Closer supervision of food handlers preparing and serving the food, most be carried out routinely by relevant authorities within the campus with a view to preventing possible outbreak of food borne illness caused by *S. aureus*.
**Keywords**: *Staphylococcus aureus*, jollof rice, bacterial counts, cafeterias

## INTRODUCTION

Consumption of safe foods should be a basic human right despite the fact that numerous food items are often contaminated with naturally occurring pathogenic microorganisms. Greig *et al*. (2007) did estimate that about 2.5 billion people patronize food vendors in the world over. According to Ogunyemi *et al*. (2015), ready-to-eat food items can potentially serve as a reservoir of pathogenic microorganisms that have got the ability of transmitting diseases. It has been reported by Itoandon *et al*. (2011), that the presence of mesophilic microorganisms in food items is a suggestion that pathogenic microorganisms are probably present in such food items. Some authors (Balaban and Rasooly, 2000; Oranusi *et al*., 2006a, 2006b) have reported a number of food items locally sold in Nigeria as vastly contaminated with *Staphylococcus* sp. *Staphylococcus aureus* has been reported over the years as having the ability of causing food poisoning. This submission has been supported by the report of Monday *et al*. (2014) that the bacterium can cause food poisoning and other food-borne diseases.

In any community, *Staphylococcus aureus* has been considered as the major pathogen that has got the ability of colonising and infecting both hospitalized patients exhibiting decreased immunity and healthy immuno-competent people. According to Centre for Disease Control and Prevention (2006), this bacterium produces toxins and is found commonly on the skin, in the nose and throats of up to 25% of healthy people as a normal flora. Cases of most *S. aureus* food borne illnesses reported in the literature are mostly triggered by poor hygiene exhibited by food handlers and grossly improper food handling practices.

Jollof rice is a sweet-smelling dish that is illustrious across the sub-region of West Africa owing to its exceptional taste and subtle spiciness (Adam, 2017). This author further reported that Jollof is thought to have sprung up from the Senegambia region of West Africa amongst the indigenous Wolof people that refer to it as benachin. Due to the popularity of jollof rice for its irresistible taste and reported cases of jollof rice being prone to contamination with pathogens, this research was conducted with a view to screening the jollof rice sold on the campus of Federal University Dutse for the possible presence of *S. aureus*.

## MATERIALS AND METHODS

### Study Area

The study was conducted in designated area meant for commercial activities popularly called backside or bacteria (students' version) situated on the campus of Federal University Dutse North western, Nigeria. According to Peel *et al*. (2007), Dutse is the capital city of Jigawa state. The authors reported that Dutse lies on latitude of 11°42'8.46" N and longitude of 9°20'2.4p6" E.

**Collection of samples**

Jollof rice samples were purposively collected from five (5) cafeterias (A, B, C, D and E) on Federal University Dutse campus, Jigawa State. Fifteen (15) different samples ($A_1$, $A_2$, $A_3$, $B_1$, $B_2$, $B_3$, $C_1$, $C_2$, $C_3$, $D_1$, $D_2$, $D_3$, $E_1$, $E_2$ and $E_3$) of jollof rice comprising of three (3) each from the restaurants were collected within the period of four (4) weeks. All the samples were collected in sterile containers and transported within one (1) hour of collection to the Microbiology laboratory for onward microbiological examination.

**Media preparation**

Peptone water, Nutrient agar, Mannitol Salt agar and Muller-Hinton agar were employed in this research and prepared according to the instructions of the manufacturers.

**Viable counts**

As prescribed by Cheesbrough (2006), ten (10) grams of jollof rice for each sample was weighed aseptically using a weighing balance and was placed into a sterile blender and homogenised with 90mL Peptone water. Further ten-fold serial dilutions of the resultant homogenates were made to obtain $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$ respectively. From $10^{-4}$ and $10^{-5}$, 0.1mL was plated in replicate onto Nutrient agar using spread plate technique for total aerobic plate count. All inoculated plates were incubated at 37ºC for 48 hours. At the end of the incubation period, the different culture plates were examined for microbial growth and colonies that developed were counted using the colony counter. The count for each plate was expressed as colony forming unit per gram of the sample (cfu/g). Discrete colonies resembling *Bacillus cereus* on Nutrient agar plates were sub-cultured onto Nutrient agar slants and incubated at 37ºC for 24 hours. The slants were stored for Gram staining and biochemical characterization as prescribed by Monday *et al*. (2014).

**Staphylococcal counts**

Ten (10) grams of jollof rice for each sample was weighed aseptically using a weighing balance and was placed into a sterile blender and homogenised with 90mL Peptone water. Further ten-fold serial dilutions of the resultant homogenates were made to obtain $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$ respectively. From all the dilutions, 0.1mL was aseptically pipetted onto Mannitol Salt agar using spread plate technique for total staphylococcal count. All inoculated plates were incubated at 37ºC for 48 hrs. The procedure was carried out in duplicate plates. At the end of the incubation period, the different culture plates were examined for microbial growth and the colonies were counted using the colony counter. The count for each plate was expressed as colony forming unit per gram of the sample (cfu/g) as prescribed by Cheesbrough (2006).

**Isolation of *Staphylococcus aureus***

From the stock solution, 0.1mL was spread onto Mannitol Salt agar plates with a sterile bent glass rod. The inoculated plates were incubated at 37ºC for 48 hours. After incubation, the plates were observed for characteristic colonies of *S. aureus* on the Mannitol Salt Agar and were sub-cultured on nutrient agar slants and incubated at 37ºC for 24 hours. The slants were stored for Gram staining and biochemical characterization at 4ºC as presented by Monday *et al*. (2014).

**Gram staining**

Gram staining was done according to the procedure prescribed by Olutiola *et al*. (2000) with a view to identifying the isolate.

**Biochemical characterisation of *Staphylococcus aureus***

The choice of the various biochemical tests that were conducted with a view to identifying *Staphylococcus aureus* isolated in this study was influenced by the prescription of Barrow and Feltham (1993). Oxidase, Voges Proskauer, Coagulase, Lactose, Maltose, Mannitol, Fructose, Sucrose, Xylose, Cellobiose, Phosphatase, Mannose, Nitrate, Arginine and Protease tests were conducted according to the procedures prescribed by Cheesebrough (2006); Ochei and Kolhatkar (2008); Wilson (2012); Hemraj et al. (2013); Microbeonline (2019).

**Antibiotic susceptibility tests**

Inocula of the test organisms were prepared by taking and emulsifying distinct colonies from Nutrient agar on a sterile normal saline. Antibiotic susceptibility testing was performed on all the isolates according to the criteria of the Clinical and Laboratory Standard Institute (CLSI). A fresh sterile cotton-tipped swab was dipped into the suspension and the excess liquid was removed from the swab by pressing it against the side of the tube. The surface of the entire Muller Hinton agar plate was inoculated with the swab by streaking back and forth from edge to edge and was evenly distributed on the plate. The plates were allowed to stand for few minutes, then

multiple antibiotic disks were impregnated using sterile forceps and then evenly dispensed onto the agar surface. It was then incubated at 37°C for 24 hours. Interpretation of results was done using the zone sizes. The zone was measured in millimeter using a ruler placed at the reverse side of the plates and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately or resistant.

**Statistical Analysis**

Data generated from the total viable and staphylococcal counts were analyzed using Microsoft Excel. Descriptive statistics in form of tables were employed to summarize the means that were generated.

## RESULTS

The results of the viable and staphylococcal counts coupled with antibiotic sensitivity tests conducted in this study are depicted in Tables 1 and 2 respectively.

**Table.1: Total Viable counts of jollof rice sold in the cafeterias on Federal university Dutse campus.**

| Sample | Cafeteria | | | | |
|---|---|---|---|---|---|
| | Mean count for cafeteria A (cfu/g) | Mean count for cafeteria B (cfu/g) | Mean count for cafeteria C (cfu/g) | Mean count for cafeteria D (cfu/g) | Mean count for cafeteria E (cfu/g) |
| 1 | $1.5\times10^5$ | $1.1\times10^5$ | $1.5\times10^7$ | $8.0\times10^4$ | TNTBC |
| 2 | $2.0\times10^5$ | TNTBC | $7.5\times10^6$ | $9.0\times10^4$ | $1.1\times10^7$ |
| 3 | $1.3\times10^5$ | TNTBC | TNTBC | $1.1\times10^5$ | $1.5\times10^7$ |
| **MEAN** | $1.6\times10^5$ | $1.1\times10^5$ | $1.1\times10^7$ | $9.3\times10^4$ | $1.3\times10^7$ |

**KEY: TNTBC= Too numerous to be counted**

**Standard =** ≤$10^3$ cfu/g is acceptable; Between $10^4$ - $10^5$ cfu/g is tolerable; ≥$10^6$ cfu/g and above is unacceptable (ICMSF, 2011)

**Table 2: Total Staphylococcal count (cfu/g) of jollof rice sold in cafeteria in Federal university Dutse.**

| Sample | Cafeterias | | | | |
|---|---|---|---|---|---|
| | Mean count for cafeteria A (cfu/g) | Mean count for cafeteria B (cfu/g) | Mean count for cafeteria C (cfu/g) | Mean count for cafeteria D (cfu/g) | Mean count for cafeteria E (cfu/g) |
| 1 | NG | $5.0\times10^3$ | $6.0\times10^5$ | NG | $1.1\times10^5$ |
| 2 | $1.0\times10^4$ | $8.0\times10^3$ | NG | NG | $1.5\times10^5$ |
| 3 | $1.5\times10^4$ | NG | NG | $4.0\times10^3$ | $2.0\times10^5$ |
| **MEAN** | $1.3\times10^4$ | $6.5\times10^3$ | $6\times10^5$ | $4\times10^3$ | $1.5\times10^8$ |

**Key: NG= No growth**

**Standard =** ≤$10^2$cfu/g is satisfactory; Between $10^2$ - $10^3$ cfu/g is Marginal; $10^4$ cfu/g is unacceptable; ≥$10^5$ cfu/g is potentially hazardous (ICMSF, 2011)

**Table 3: Biochemical Characterization of the Isolate**

| Biochemical Tests | Status |
|---|---|
| Oxidase | – |
| Voges Proskauer | + |
| Coagulase | + |
| Lactose | + |
| Maltose | + |
| Mannitol | + |
| Fructose | + |
| Sucrose | + |
| Xylose | – |
| Cellobiose | – |
| Mannose | + |
| Phosphatase | + |
| Nitrate | + |
| Arginine | + |
| Protease | + |
| **Identity of possible bacterium** | ***Staphylococcus aureus*** |

**Key:** + = Positive; - = Negative

**Table 4: Antibiotic Susceptibility Pattern of *Staphylococcus aureus* isolated from jollof rice sold in cafeteria in Federal University Dutse.**

| Antibiotics | Pattern of sensitivity | | |
|---|---|---|---|
| | Sensitive | Intermediate | Resistance |
| Ofloxacin (5µg) | 15mm | – | – |
| Augmentin (30µg) | – | – | 12mm |
| Ceftazidime (30µg) | – | – | 10mm |
| Cefuroxime (30µg) | – | – | 13mm |
| Gentamicin (10µg) | 17mm | – | – |
| Ceftriaxone (30µg) | – | – | 23mm |
| Erythromycin (15µg) | – | 15mm | – |
| Cloxacillin (15µg) | – | 8mm | – |

## DISCUSSION

In this study, the mean viable plate count across all the cafeterias ranged between 1.1 x $10^5$ and 9.5 x $10^4$ cfu/g (Table 1) while mean staphylococcal count obtained ranged between 1.3 x $10^4$ and 6.5 x $10^3$ cfu/g (Table 2). As recorded in this study, a similar study conducted by Oranusi *et al.* (2013); Monday *et al.*, (2014) reported high bacterial population in fried jollof rice examined in their respective studies. The findings of this study revealed that there was staphylococcal contamination in the jollof rice sold at the cafeterias (Table 2). Viable counts of bacteria in the jollof rice from cafeteria C and E (1.1 x $10^7$ and 1.3 x $10^7$ cfu/g) were higher than that of jolly rice in cafeteria A, B and D (1.6 x $10^5$,1.1 x $10^5$ and 9.3 x $10^4$ cfu/g) respectively (Table 1).

The staphylococcal counts of jollof rice sold in cafeteria C and E (6.0 x $10^5$ and 1.5 x $10^5$ cfu/g) were higher than that of the jollof rice in cafeteria A, B and D (1.3 x $10^4$, 6.5 x $10^3$ and 1.5 x $10^5$ cfu/g) respectively (Table

2). The occurrence of *Staphylococcus aureus* in jollof rice samples sold in cafeteria C and E was higher than that of cafeteria A, B and E. These trends can be attributed to variations in hygienic practices put up by the different cafeterias during this study

The isolation of *Staphylococcus aureus* in this study is in agreement with the findings of Taulo *et al*. (2009); Oranusi *et al*. (2013) in which this bacterium was implicated in contaminating ready-to-eat foods analysed in their respective studies. The presence of *Staphylococcus aureus* in food items is basically as a result of human contact and this suggests poor hygiene practices of the vendors since the bacterium is a normal flora of the skin and nasal passage. Interestingly, Bibek (2001); Ali *et al*. (2008) have reported that poor personal hygiene and inappropriate food handling techniques are factors that influence the chances of *S. aureus* being transferred to jollof rice. Some authors (Garret, 2002; Nichols *et al.*, 2005) have reported that *Staphylococcus aureus* produces a wide variety of toxins including staphylococcal entero-toxins that have got enteric activity. These widely reported entero-toxins must have influenced the contamination of the jollof rice sampled and analysed in this study.

In the antibiotic sensitivity tests conducted in this study, *Staphylococcus aureus* was found to be susceptible to Ofloxacin and Gentamicin (Table 4). It was intermediate to Erythromycin and also resistant, while it was resistant to Augmentin, Ceftazidime, Cefuroxime, Ceftriaxone, Cloxacillin (Table 4). These results are similar to the report of a study conducted by Akpomie *et al*. (2013) in which *Staphylococcus aureus* was sensitive to Ofloxacin, Gentamicin and Ciprofloxacin but resistant to Cefuroxime, Cefixime and Ceftriazone (Cephalosporin's), Augmentin (ß-lactam inhibitor) and septrin (Sulphonamide).

## CONCLUSION AND RECOMMENDATIONS

The results obtained in this study indicate that the jollof rice consumed by members of the University community that patronize these cafeterias is not safe for human consumption as samples from majority of the food vendors recorded staphylococcal counts. This, however, implies the deplorable state of poor hygienic and sanitary practices employed in the processing and handling of foods prepared and served to the students that patronise the cafeterias. Therefore, effective sanitization of cooking areas and food serving surfaces should be carried out to prevent cross contamination from utensils and flies. Utensils and surfaces should be washed before and after use with hot, soapy water or preferably, they should be sanitized with diluted bleach. Also a closer and routine supervision of food handlers most especially those selling ready-to-eat foods, should be carried out by relevant authorities within the school campus to prevent possible outbreak of food borne illness.

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