ISOLATION AND IDENTIFICATION OF *Staphylococcus aureus* FROM EGGROLLS SOLD ON THE CAMPUS OF FEDERAL POLYTECHNIC, IDAH, KOGI STATE, NIGERIA

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ABSTRACT

Staphylococcus aureus is an important cause of food poisoning and are normally associated with the skin, skin glands and mucous membrane of warm blooded animals causing diseases like skin infections such as pimples, boils, abscesses, scalded skin syndrome, lung infection, meningitis, osteomyelitis, heart infection and toxic shock syndrome (TSS) in human This study isolated *S. aureus* from eggrolls sold on Federal Polytechnic Idah campus, and also determined the sensitivity to some common antibiotics. Isolation was carried out using mannitol salt agar and nutrient agar and was identified using cultural and simple biochemical method of identification. A sterile polythene bags were used to collect 30 egg roll samples; out of the samples collected 23 were positive for *S. aureus*. The isolate was found to be catalase, coagulase, urease, voges proskauer, citrate and methyl red positive and also negative for indole and oxidase test. *S. aureus* was gram positive for gram staining examination with grape like cluster of cocci cells. *S. aureus* is sensitive to pefloxacin, Tarivid, Gentamycin and ciprofloxacin. Most of the egg rolls sold on the campus of Federal Polytechnic Idah are contaminated with *S. aureus* and thus not fit for consumption. *S. aureus* can be found in snacks such as eggrolls, breads etc when the food

handlers fails to observe personal hygiene. Food handlers should improve in personal hygiene and use clean water when preparing food. Hand-gloves should be worn when preparing food especially egg rolls. Food handlers should not pick their nose when preparing or packaging egg rolls.

Key words: *Staphylococcus aureus,* eggrolls, Isolation, Identification, Antibiotic sensitivity

INTRODUCTION

Staphylococcus aureus belongs to the family of Staphylococcaceae, member of this genus are facultatively aerobic, non-motile, gram positive cocci that usually form irregular clusters. They are coagulase and catalase positive, oxidase negative and ferment glucose (Christopher *et al*., 2009). *S. aureus* is an important cause of food poisoning and they have been extensively studied for their biological toxicities and implication on human. They produce exotoxins that are heat-labile (Jett *et al*., 2001). Staphylococci are normally associated with the skin, skin glands and mucous membrane of warm blooded animals and they can cause diseases like minor skin infection such as pimples, boils, abscesses, scalded skin syndrome, lung infection, meningitis, osteomyelitis, heart infection, toxic shock, syndrome (TSS) etc in human (Bramer, 2007; CDC, 2009).

Food borne disease often arises from the consumption of contaminated food (Jorgensen *et al*., 2015). Transmission of *S. aureus* can be associated with a wide range of food types, and the way the food is handled (Atanassova *et al*., 2005). In recent time, the incident of food borne infection has greatly increased globally and it is estimated that nearly a quarter of the population is at risk of the infection (Oliver *et al*., 2005). *S. aureus* can contribute to the development of opportunistic infection if it senses a weak point in the body (Jin *et al*., 2004).

A conventional staphylococcal infection can be treated with antibiotics such as methicillin, penicillin, gentamicin, erythromycin, ciprofloxalin, fusidic acid and mupirocin which

may be administered orally or applied directly to the site of a skin infection in the form of a tropical cream (Adam and Holseher, 2002).

Staphylococci are responsible for many human diseases. *S. epidermidis* is a common skin resident that is sometimes responsible for endocarditis and infection of patients with lower resistance (e.g wound infections, surgical infections, urinary tract infection, body piercing (Christopher *et al*., 2009). *S. aureus* is often found in biofilms formed in a medical devices implanted in the body or on human tissue. It is commonly found with another pathogen, *Candida albicans*, forming multispecies biofilms. The latter is suspected to help *S. aure* penetrate human tissue. A higher mortality is linked with multispecies biofilms. This study was carried out to isolate *S. aureus* from eggrolls sold on thecampus of FPI. The sensitivity of the isolate to some common antibiotics was also determined.

MATERIALS AND METHODS

Study Area

The study was carried out in Idah Local Government Area. Idah, an old river port, lies on the eastern bank of the river Niger in the middle belt region of Nigeria. It is the traditional head quarter of the Igala kingdom, whose traditional ruler is known as the Attah of Igala. Idah LGA has an area of about 36km^2 around the town and a population of 79,815 as at 2006 census. It has commercial routes on the river Niger Linking Lokoja, the Kogi State Capital to the north of the country and Onitsha, Enugu State to the South, Agenebode, Edo state across the Niger to the west. It is a major food supplier of Kogi State. The Federal Polytechnic Idah, is in Idah LGA.

Idah is densely populated with various categories of people, some of which are peasant farmers, traders and artisans. Some also engage in private businesses and civil service work. Its

geographical coordinates are 7º60 North, 6º 44 East. Its warmest temperature is 39ºC and it coldest temperature 20.0ºC.

The interior part of the town is more densely populated with houses without efficient toilet facilities, forcing the occupants to visit bushes, refuse dumps and open gutters for defaecation. Few houses in the area are served by pit latrines. The central parts are occupied mostly by the middle class and upper class whose houses have water closets with soak away pits. The drainage system consists of open gutters, often littered with refuse and eroded soil.

Sample Collection

For the purpose of this work, 30 eggrolls samples were collected from 5 different shops (Convoc Square, Science Laboratory Technology (SLT), Mini marketing area, School of Environmental Area) in Federal Polytechnic Idah for 5 consecutive times. Using a sterile polythene bags, a distance of hundred m (100) from the shop and transferred to the microbiology laboratory of Department of science Laboratory Technology, Federal Polytechnic Idah for microbiological essay for isolation and identification of *Staphylococcus aureus*.

Isolation of *S. aureus* from Eggroll

S. aureus was isolated from eggrolls by first of all, roll it in the peptone water, to obtain the stock samples. Isolation was carried out following serial dilution of samples and inoculation on nutrient agar and mannitol salt agar. 10^5 serial dilution was performed from the samples and 1ml of different dilutions were aseptically pipetted unto sterile petri dishes and 20 ml of molten Nutrient agar (45ºC) and Mannitol salt agar respectively were poured, and swirled to mix, covered and allowed to gel before incubation. This was carried out in triplicates. The plates were incubated at 37ºC for 24 hours and colonies that emerged were observed

Preparation of Nutrient Agar and Peptone Water

Nutrient agar and peptone water (Oxoid, London) were prepared according to the manufacturers' instructions.

Identification of Isolates

Discrete colonies of bacteria from the respective plates were selected and sub cultured on nutrient agar and mannitol salt agar plates and incubated at 37ºC for 24h. The bacterial isolates were then identified following standard microbiological procedures based on cultural, morphological and biochemical characteristics as described by Cheesbrough (2002).

Gram Staining

Gram staining of purified colonies was carried out according to the methods described by Cheesbrough (2005), and a drop of immersion oil was placed on it and examined under the oil immersion objective (X100).

Biochemical Test

Catalase Test, Indole Test, Voges – Proskauer Test, Methyl red Test, Coagulase Test, Oxidase Test, Urease Test and Citrate Tests were carried out according to the methods of Cheesbrough, (2002) and Kanika (2014).

Sensitivity Test

This was carried out against 10 commonly used antibiotics using the instruction of Clinical and Laboratory Standards Institute guidelines ( ). Briefly, pure culture plate of one of the organisms to be test was selected, a colony from the plate was aseptically emulsified in the sterile saline solution, and thoroughly mixed to ensure that no solid material from the colony is

visible in the saline solution. It was repeated until the turbidity of the saline solution visually match that of the standard turbidity. A sterile swab was taken and dipped into he broth culture of organism, the swab was gently squeezed inside of the tube in order to remove excess fluid in the swab, a sterile Mueller-Hinton agar (MHA) plate was brought. The swab with the test organism were used to streak a MHA plate for a lawn of growth. After the streaking was completed, the plate was allow to dry for 5 minutes before putting the antibiotic discs using sterilized forceps. The discs was gently pressed onto the surface of agar using flamed sterilized forceps. The inoculated plates were carefully inverted and incubate for 24 hours at 37ºC; After the incubation, a metric ruler was used to measure the diameter of the zone of inhibition for each antibiotic used, the measurement obtained from the individual antibiotics were compared with the standard table to determine the sensitivity zone.

RESULTS

Isolation of *S. aureus* from eggroll sold on FPI campus

After the media were cultured with the eggroll samples and incubated for 24 hours at 37ºC, different colonies were observed; some were white and milky in colour having rhizoid shape and convex in elevation, golden yellow colour having circular shape, convex in elevation and entire in margin, some have purple colour. Of the 30 samples of eggrolls studied 76.7 % (23/30) were positive for *S. aureus.*

Pure Culture of *S. aureus* from eggroll sold on FPI campus

After the growth of suspected colonies were sub-cultured, it was observed that pure golden colour growth appeared having a diameter 0.8-1.0mm; circular in shape, convex in

elevation and entire in margin (Figure 1). Gram examination revealed a gram positive cocci, in grape-like cluster (Table 1).

Biochemical Reactions of the *Staphylococcus aureus* Isolates

Biochemical tests revealed that the isolates were catalase, coagulase, urease, voges proskauer, citrate and methyl red positive, but indole and oxidase negative (table 1)

Antimicrobial susceptibility of *S. aureus* isolates

S. aureus is more sensitive to pef which is 20mm followed by the following drugs: SP, CPX, CN and OFX which are 15mm and S is least effective against the isolated *S. aureus.* AM, CH, AU are not effective

Table 1: Biochemical Reactions of the *Staphylococcus aureus* Isolates

Test	A
Gram staining	+
Catalase	+
Coagulase	+
Indole	-
Urease	+
Voges proskauer	+
Citrate	+
Oxidase	-
Methyl red	+
Organism	*S. aureus*

Key + = Positive; - = Negative

ezemaTable 2: Percentage prevalence of *S. aureus* isolated from eggrolls sold on FPI campus

Batches	No. Samples	No. of positive	% prevalence
1st	6	6	100
2nd	6	6	100
3rd	6	2	33.3
4th	6	3	50
5th	6	6	100
Total	30	23	76.7

Table 3: Results of Antimicrobial Sensitivity Disc.

S/N	Antibiotics	Conc (ug)	Zone of inhibition (mm)
1	Gentamycin (CN)	10	15
2	Tarivid (OFX)	10	15
3	Septrin (SXT)	30	10
4	Ciprofloxacin (CPX)	10	15
5	Sparfloxacin (SP)	10	15
6	Pefloxacin (PEF)	30	20
7	Streptomycin (S)	30	5
8	Amoxicilin (AM)	30	0
9	Chloranphenicol (CH)	30	0
10	Augmentin (AU)	30	0

Standard resistant zone of inhibition $\leq$ 0.5mm; Standard sensitive zone of inhibition $\geq$ 30.0mm

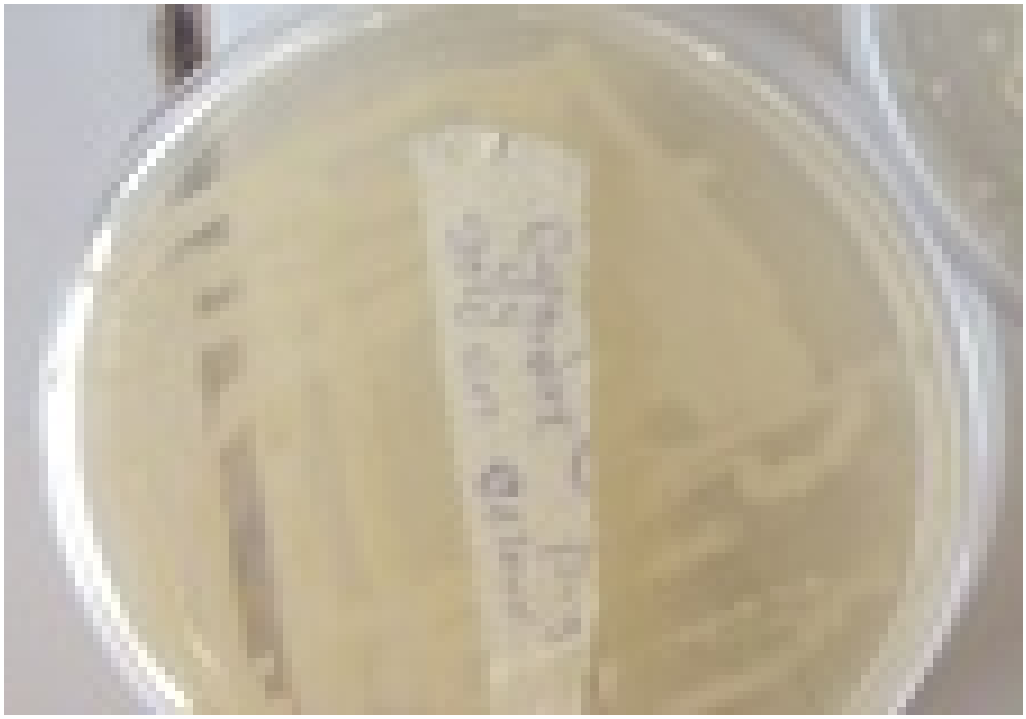

Figure 1. Pure Culture of *S. aureus* from eggroll sold on FPI campus

DISCUSSION

The growth that was observed on nutrient agar after inoculation with eggroll samples were of different colours having different shapes and margins. After sub-culturing the suspected colonies and pure colony was obtained having diameter of 0.8-1.0mm, circular in shape, convex in elevation and entire in margin, with golden yellow colour (Warnke *et al*., 2014; Alwan and Talak, 2015) on nutrient agar which was typical of *Staphylococcus aureus* and they were in grape-like clusters under the microscope (Sundararaj *et al*., 2019). The significant factor influencing the microbiological spoilage of bakery foods is water activity (aw), as the minimal aw required to support the growth of spoilage microbes in bakery foods is 0.60. Bakery foods have high moisture content (aw 0.94–0.99) which supports the growth of almost all bacteria, molds, and yeasts. Most bacteria need a high aw for growth and bakery foods provide high moisture content therefore, bacterial contaminations often occur in bakery foods, i.e. pizza, cake, pastries, soft cookies, milk products, chicken and meat products, and egg products (Sundararaj *et al*., 2019).

The isolates were identified using conventional biochemical tests and were found to be catalase, coagulase, urease, voges proskauer, citrate and methyl red positive, but indole and oxidase negative (table 1) which was indicative of *S. aureus*. These observations are similar to those of Sundararaj *et al*. (2019) who carried out a similar study on isolation and identification of enterotoxigenic *S. aureus* isolates from Indian food samples, and evaluation of in-house developed aptamer linked sandwich ELISA (ALISA) method and reported that isolates were

positive for catalase, urease, nitrate, mannitol and glucose fermentation and negative for coagulase (i.e. *S. epidermidis*) and citrate

The prevalence of *S. aureus* isolated from eggrolls sold in different shops on FPI campus were high in first, second and fifth batches while less in 3rd and 4th, batches. The less prevalence may be attributed to personal hygiene (Mark and Bill, 2011). Furthermore, the way in which food is handled may contribute to the contamination of the food. (Atanassova *et al*., 2005). It has also been reported by Sundararaj *et al*. (2019) that unhygienic methods in preparation and selling, and multiple routes of entrance have been reported as the chief factors accountable for assisting the entry of microbial pathogens, and humans harbouring *S. aureus* are a foremost cause of contamination of bakery foods throughout the preparation or post preparation processes. Post preparation contamination is also probable from surfaces, air, and cross-contamination. Constituents of bakery foods might also serve as sources of contamination of *S. aureus*.

S. aureus is more sensitive to pefloxacin which is 20mm followed by the following drugs: Sparfloxacin, Ciprofloxacin, Gentamycin and Tarivid which are 15mm and Streptomycin is least effective against the isolated *S. aureus.* Amoxicillin, Chloramphenicol and Augmentin are not effective against the isolated *S. aureus.* Hasanpour –Dehkordi *et al*. (2017) in a similar study of One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* recovered from raw meat in Iran also reported resistance to some common antibiotics especially erythromycin, penicillin G, cefazolin, ciprofloxasin, vacomycin and amoxiclave. Antibiotics have target organisms on which they act due to the presence of target site of action of the drugs. From this study, sensitivity of Pefloxacin is 20mm, which is close to another study where sensitivity to Pefloxacin is 20.45mm. Furthermore, 20.50mm sensitivity to pefloxacin; 15.30mm, to Sparfloxacin; 15.42mm to Ciprofloxacin; 15.10mm to

Gentamycin and 15.28mm to Tarivid were found in another study conducted in University of Lagos.

The bacterial sensitivity profile therefore revealed that Pefloxacin is highly effective on *S. aureus* isolate from eggroll compared to other antibiotics. Molecular characterization could not be carried out to elucidate more on the species and strains of the isolates, which is an area of consideration in subsequent research of this nature.

CONCLUSION

S. aureus was isolated in eggroll sold in FPI campus. *S. aureus* Isolated was sensitive to pefloxacin, Gentamycin, Tarivid and sparfloxacin but Resistant to Amoxacilin, Chloranphenicol and Augmentin. *S. aureus* can be found in already made food like eggrolls, breads, but it is a function of personal hygiene. This therefore means that the eggrolls sold on the campus of FPI are contaminated with normal flora of either the preparers or the handlers or vendors, and may be responsible for pockets of some fever of unknown origin been reported or experienced at the Polytechnic clinic.

From the study, it was observed that most food handler do not take proper care of themselves before the preparation of food thereby contaminating the food that is being prepared. Before preparation of food, food handlers should wash their hands and should not touch any part of their body i.e. personal hygiene should be properly observed during preparation of the food to avoid contamination.

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