Enumeration, Identification And Occurrence Of Staphylococci In Cooked Foods And Hand Rinsed-Water   From Eleven Local Government Areas Of Ibadan

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Abstract

This project aimed at isolating, identifying and determining the level of occurrence of staphylococci in cooked foods,. "Amala", "fufu", "rice", "beans", "ewedu", stew and hand-rinse water were collected from restaurants in the eleven Local Government Areas (LGA) of Ibadan. Staphylococci were isolated, enumerated and characterized using standard pour plating, culturing and biochemical test methods. Nine different species of *Staphylococcus* were identified as *S. aureus, S. hyicus, S. epidermidis, S. intermidius, S. saprophyticus, S. arrieta, S. carnosi, S. haemolyticus* and *S. schleifer*. In all the samples, *Staphylococcusaureus* occurred in 32 times, while *S. Schleifer,S. hyicus, S. epidermidis, S. intermidius, S. saprophyticus, S. arrieta, S. carnosi* and *S. haemolyticus* occurred in 1, 11, 14, 9, 4, 4, 2 and 8 times respectively. Among the food samples, staphylococcal load was highest (9.8×10^2 cfu/ml) in "fufu" from Akinyele LGA. In contrast, "ewedu" from Akinyele LGA showed the lowest population (1.2×10^2 cfu/ml) of staphylococci.

KEYWORDS: Ibadan Local Government Areas, Staphylococci, Population, Occurrence and Identification.

INTRODUCTION

Staphylococci species of which *Staphylococcus aureus* is one of the most dangerous species of staphylococci already identified. It is the major cause of bacteremia, pneumonia, myocarditis, acute endocarditis, pericarditis, osteomyelitis, encephalitis, meningitis, chorioamnionitis, mastitis, and many other skin infections.(P. R. Murray *et al*, 2003). Human morbidity and mortality in hospital settings are largely caused by staphylococcal bacteremia (R. M. Klevens *et al*, 2007). The disease causing ability of Staphylococcus aureus is dependent on its to produce exoproteins and toxins. The species is identified on the basis of a variety of conventional physiological or biochemical characters. The key characters for *Staphylococcus aureus* are colony pigment, free coagulase, clumping factor, protein A, heat-stable nuclease, lipase, and acid production from mannitol (. P. R. Murray *et al*, 2003). PCR has been used to identify Staphylococcus aureus and 16S rRNA gene is reported to be the most useful and extensively investigated taxonomic marker. (Becker *et al*, 2004) The organism becomes drug resistant because of uncontrolled andexcessive use of antibiotics leaving few therapeutic options for the treatment on it(DeLeo *et al*,2010) The spring up of drug resistant Staphylococci in hospital-acquired (HA) infections dispose the organisms as a potential pathogen that is able to cope with the antimicrobial agent (Bouche *et al* 2008).

Material and Methods

Sample collection

The sample for this research work were food samples 'Amala' 'Eba', 'fufu', 'Rice', 'stew' and Handrised water. The samples were collected from three restaurats in each of Eleven Local Government Areas (LGA) in Ibadan. LGA are Akinyele, Ibadan North,. Ibadan North East,Ibadan South West, Ibadan South East, Lagelu, Oluyole, Egbeda, Ona-ara, Ido and Ibadan North West Local Govenrment Area. Samples were collected in sterile vacuums tube covered and packed on an ice and transported to Microbiology Laboratory of Federal University of Technology Akure.

1gram of sample was ground with mortal and pestle and suspended in saline solution (10ML). Serial dilution was done using 9ML saline solution in five tubes sealed with aluminiun foil, cellotaping and antoclaving at 121^0C for15 minutes.

Pour Plate Culture The diluted food sample was dropped in petrildish and cool molten mannitol salt agar was added and the plates was swirled gently and was allowed to set.Incubation was carried out at 370c.Colonies that grew on the medium were counted and expressed as colony form per unit cfu/mL .

Subculturing of the individual grown colonies was done using the 4 – corner streaking method.

Pure isolates were transfer to Mannitol salt agar(MSA) (Fawole and Osho,2007.)

Preparation of stock culture

Each of the purified bacterial isolates was streaked on the solidified slant surface double strength NA. Then, they were incubated at 37°C for 24 hours. As soon as there was visible growth, the cultures are stored in a refregerator at 4°C.

TESTS FOR IDENTIFICATION OF BACTERIA ISOLATED FROM FOOD

The cultural characteristics of the purified isolates were determined by Dubey and Mahesh Warc (1999), Features examined include elevation of colonies, pigmentation, types of margin form, surface structure and shape. Gram staining was carried out on the colcony as described below and examined under light microscope

(i) Gram's Staining Technique

A little part of each grown bacterial conony was picked with sterile inoculating loop, transferred into a drop of water on a clean slide, smeared properly and heat fixed. The glass slide was placed on a staining rack over a sink and the smear on it was flooded with crystal violet for I minute. It was briefly washed with tap water. The smear was flooded with Gram's iodine (mordant) for 1 minute before excess mordant was washed off under a running tap water. The stain was then decolourized with 95%(V/V) ethanol for about 20 seconds. As soon as the colour stopped coming off the smear. It was rinsed with tap water. The smear was counter stained with safranin for 20 seconds. Then excess dye was washed off with tap water and allowed to air dry. Immersion oil was added to the smear and examined under oil immersion lens at x 100 using a light microscope. Gram positive bacterial appeared purple while Gram negative cells appeared pink in colour. (Fawole and Osho,2007.)

(ii) Catalase Test

Catalase test was used to differentiate bacteria that produce the enzyme catalase. Two millilitres of hydrogen peroxide (3%V/V) was transfered into a test tube. Sterile glass rod was then used to pick colonies from a 24 hours old test bacterial culture and mixed with the hydrogen peroxide solution in the test tube. The production of the air bubbles was observed. Isolates that produced bubbles were referred to as catalase positive while those bacteria that did not produce bubbles were referred to as catalase negative

(iii) Coagulase Test

A drop of sterile water was placed on a clean glass slide and mixed with a colony of test bacterium.Thereafter clumping of the cells was checked after 10 seconds. Clumping of cells meant positive coagulase test and lack of clumping indicated negative coagulase test. (Fawole and Osho,2007.)

(iv) Oxidase Test

Using plate method, the culture of test bacterium grown on a nutrient agar, was flooded with a freshly prepared 1%(V/W) solution of tetrametyl-p-phenylenediamine hydrochloride and observed for purple colour development within 10 minutes. Colony that rapidly developed a purple colour was able to produce oxidase while no blue-purple colour was termed oxidase negative bacterium

(v) Test for Starch hydrolysis

A test bacterial isolate was aseptically streaked onto starch agar (SA) and incubated at 37°C for 72 hours. The SA was flooded with Gram's iodine and observed for clear region surrounding each colony, Where there was a clear region around the streaked bacterium, it meant that the organism hydrolysed the starch and the test is positive, otherwise it was negative. (Fawole and Osho,2007)

(vi) Test for Sugar Fermentation

Bacteria produce acidic products when they ferment certain carbohydrates. The carbohydrate utilization tests are designed to detect the change in pH which would occur if fermentation of a given carbohydrate occurs. Acids lower the pH of the medium which will cause the pH indicator (phenol red) to turn yellow from red. If the bacterium does not ferment the carbohydrate then there will be no acid and gas formation (Etok *et al.,* 2005). The test was carried out by using peptone water (10 mL) containing %(w/v) of glucose, sucrose, lactose, galactose, maltose, mannitol, fructose, phenol red and durham tube with 0.1 mL of 24 hours old grown test bacterial isolate. Another inoculation was made with peptone lacking any carbohydrate to serve as control. Incubation was carried out at 37°C for 24-48 hours. Fermented sugar turned the medium to yellow colour indicating positive test while the medium containing unfermented sugar retained the original (red) colour hence

the test was noted as negative. Acid production in some cases was accompanied by CO_2 evolvement which was made visible in the closed part of Durham's tube as vacuum or space due to the displacement of the medium in the inverted Durham's tube. (Fawole and Osho,2007.)

(vi) Test for Citrate Utilisation

This test shows the ability of a microorganism to utilize citrate as the sole carbon and energy source. This ability depends on the presence of a citrate permease that facilitates transport of citrate into the bacterium. The commonly used medium is citrate agar (CA). The medium was inoculated with a test bacterium. It was incubated at 37^0C for 96 hours. Colonies of citrate-utilization organism would develop a blue colour while no blue colour meant citrate negative test (green colour).(Fawole and Osho,2007.)

RESULTS AND DISCUSSIONS

AKINYELE LOCALGOVERNMENT

TABLE 1

SAMPLE	COLONIAL POPULATION(cfu/ml)		
	LOCATIONS		
	MONIYA	SASA	OJOO
Amala	3.6×10^2	5.0×10^2	3.06×10^2
Fufu	9.8×10^2	5.5×10^2	6.23×10^2
Eba	3.6×10^2	2.8×10^2	2.7×10^2
Rice	3.0×10^2	2.43×10^2	1.76×10^2
Beans	3.2×10^2	2.4×10^2	3.6×10^2
Stew	5.2×102	4.0×10^2	3.4×10^2
Ewedu	1.05×10^3	4.9×10^2	6.56×10^2
Meat	5.3×10^2	3.0×10^2	5.83×10^2
Water	5.1×10^2	4.13×10^2	6.16×10^2

EGBEDA LOCAL GOVERNMENT

TABLE 2

COLONIAL POPULATION(cfu/ml)		
SAMPLE	**LOCATIONS**		
	EGBEDA VILLAGE	**ADEGBAYI**	**ALAKIA**
Amala	3.23×10^2	2.06×10^4	1.03×10^6
Fufu	3.8×10^2	2.2×10^4	1.23×10^6
Eba	3.4×10^2	3.4×10^4	3.6×10^6
Rice	1.66×10^3	1.0×10^4	6.6×10^5
Beans	3.2×10^2	3.1×10^4	3.5×10^6
Stew	2.3×10^2	1.5×10^4	8.6×10^5
Ewedu	1.33×10^2	7.3×10^3	3.0×10^5
Meat	2.13×10^2	1.03×10^4	2.0×10^5
Water	3.2×10^2	1.76×10^4	1.13×10^6

TABLE 3: IBADAN NORTH LOCAL GOVERNMENT

COLONIAL POPULATION (Cfu/ml)		
SAMPLE	LOCATIONS	
	BODIJA	UNIVERSITY OF IBADAN	THE POLYTECHNIC IBADAN
Amala	1.93×10^2	3.1×10^2	2.9×10^2
Fufu	3.07×10^2	3.9×10^2	3.7×10^2
Eba	4.1×10^2	3.8×10^2	4.3×10^2
Rice	1.9×10^2	1.93×10^2	1.5×10^5
Beans	3.5×10	3.5×10^2	3.0×10^2
Stew	1.23×10^2	1.7×10^2	1.9×10^2
Ewedu	2.4×10^2	6.0×10^2	3.8×10^2
Meat	1.77×10^2	1.67×10^2	1.9×10^2
Water	2.2×10^2	2.67×10^2	2.87×10^2

IDDO LOCAL GOVERNMENT

TABLE 4

COLONIAL POPULATION(cfu/ml)			
SAMPLE	**LOCATIONS**		
	IDDO TOWN	ELEYELE	APETE
Amala	2.7×10^2	2.0×10^2	5.7×10^2
Fufu	3.63×10^2	2.5×10^2	6.0×10^2
Beans	3.2×10^2	3.1×10^2	3.8×10^2
Rice	1.7×10^2	1.4×10^2	4.0×10^2
Eba	3.2×10^2	2.2×10^2	2.81×10^2
Stew	1.8×10^2	1.23×10^2	1.23×10^{62}
Ewedu	5.8×10^2	2.37×10^2	4.2×10^2
Meat	1.8×10^2	1.57×10^2	1.50×10^2
Water	2.6×10^2	2.07×10^2	2.1×10^2

IBADAN SOUTH EAST LOCAL GOVERNMENT

TABLE 5

COLONIAL POPULATION(cfu/ml)			
SAMPLE	**LOCATIONS**		
	MAPO	**OJA-OBA**	**ELEKURO**
Amala	2.7×10^2	2.7×10^2	2.4×10^2
Fufu	2.23×10^2	3.0×10^2	2.1×10^2
Eba	3.0×10^2	2.5×10^2	2.2×10^2
Rice	1.8×10^2	1.5×10^2	2.1×10^2
Beans	2.8×10^2	2.9×10^2	3.2×10^2
Stew	1.4×10^2	1.7×10^3	2.3×10^3
Ewedu	3.4×10^3	2.7×10^2	2.13×10^2
Meat	1.9×10^2	1.8×10^2	2.4×10^2
Water	2.53×10^2	2.33×10^2	2.3×10^2

Table 6

IBADAN SOUTH WEST LOCAL GOVERNMENT

COLONIAL POPULATION (cfu/ml)		
SAMPLE	LOCATIONS	
	RING ROAD	ALESINLOYE	OLUYOLE EXTENSION
Amala	3.1×10^2	7.17×10^2	3.1×10^2
Eba	6.07×10^2	3.07×10^2	3.43×10^2
Fufu	6.53×10^2	5.1×10^2	6.1×10^2
Beans	4.2×10^2	4.0×10^2	3.5×10^2
Rice	3.00×10^2	4.73×10^2	5.17×10^2
Stew	3.00×10^2	5.2×10^2	2.87×10^2
Ewedu	3.00×10^2	4.2×10^2	3.2×10^2
Meat	1.45×10^2	1.53×10^2	2.57×10^2
Water	3.1×10^2	5.0×10^2	3.2×10^2

LAGELU LOCAL GOVERNMENT

TABLE 7

COLONIAL POPULATION(cfu/ml)			
SAMPLE	LOCATIONS		
	LALUPON	MONOTAN	OLODO
Amala	2.47×10^2	6.3×10^2	5.8×10^2
Fufu	2.23×10^2	4.8×10^2	5.2×10^2
Rice	2.4×10^2	3.7×10^2	4.2×10^2
Beans	3.6×10^2	3.7×10^2	3.0×10^2
Eba	2.1×10^2	3.0×10^2	3.8×10^2
Stew	2.23×10^2	4.0×10^2	4.2×10^2
Ewedu	2.1×10^2	3.0×10^2	3.8×10^2
Meat	2.3×10^2	3.2×10^2	2.8×10^2
Water	2.2×10^2	3.0×10^2	3.80×10^2

IDDO LOCAL GOVERNMENT

TABLE 8

COLONIAL POPULATION(cfu/ml)		
SAMPLE	**LOCATIONS**	
	IDDO TOWN	ELEYELE	APETE
Amala	2.7×10^2	2.0×10^2	5.7×10^2
Fufu	3.63×10^2	2.5×10^2	6.0×10^2
Beans	3.2×10^2	3.1×10^2	3.8×10^2
Rice	1.7×10^2	1.4×10^2	4.0×10^2
Eba	3.2×10^2	2.2×10^2	2.81×10^2
Stew	1.8×10^2	1.23×10^2	1.23×10^{62}
Ewedu	5.8×10^2	2.37×10^2	4.2×10^2
Meat	1.8×10^2	1.57×10^2	1.50×10^2
Water	2.6×10^2	2.07×10^2	2.1×10^2

OLUYOLE LOCAL GOVERNMENT

TABLE 9

COLONIAL POPULATION(cfu/ml)			
SAMPLE	LOCATIONS		
	IDI AYUNRE	CHALLENGE	OLOMI
Amala	2.4×10^2	2.13×10^2	2.93×10^2
Fufu	2.3×10^2	3.07×10^2	3.33×10^2
Beans	3.0×10^2	2.8×10^2	3.8×10^2
Rice	2.3×10^2	1.87×10^2	2.4×10^2
EBA	$2.5 X 10^2$	2.4×10^2	2.77×10^2
Stew	1.7×10^2	1.67×10^2	1.87×10^2
Ewedu	4.13×10^2	1.37×10^2	2.5×10^2
Meat	1.63×10^2	1.87×10^2	2.07×10^2
Water	1.77×10^2	2.3×10^2	2.1×10^2

ONA-ARA LOCAL GOVERNMENT AREA

TABLE 10

COLONIAL POPULATION(cfu/ml)		
SAMPLE	LOCATIONS	
	OLORUNSOGO	AKANRAN	ONA-ARA VILLAGE
Amala	2.43×10^2	3.1×10^2	2.9×10^2
Fufu	2.87×10^2	3.9×10^2	3.7×10^2
Beans	3.3×10^2	3.2×10^2	2.9×10^2
Rice	2.27×10^2	1.93×10^2	1.5×10^2
Eba	$2.7 X 10^2$	1.6×10^2	1.8×10^2
Beans	2.0×10^2	4.73×10^2	3.9×10^2
Stew	1.17×10^2	1.7×10^2	1.9×10^2
Ewedu	4.8×10^2	6.0×10^2	5.4×10^2
Meat	1.87×10^2	1.67×10^2	2.93×10^2
Water	2.5×10^2	2.6×10^2	2.3×10^2

IBADAN NORTH WEST GOVERNMENT

TABLE 11

SAMPLE	COLONIAL POPULATION(cfu/ml)		
	LOCATIONS		
	DUGBE	ONIREKE	ORITAMERIN
Amala	2.93×10^2	3.07×10^2	5.07×10^2
Fufu	3.2×10^2	6.03×10^2	6.37×10^2
Rice	1.57×10^2	6.3×10^2	6.03×10^2
Stew	1.7×10^2	4.3×10^2	5.00×10^2
Ewedu	5.8×10^3	4.73×10^2	6.13×10^2
Meat	1.87×10^2	5.63×10^2	6.07×10^2
Water	2.67×10^2	7.13×10^2	6.63×10^2

DISCUSSION

Biochemical and cultural characteristic of staphylococci isolates from restaurants, in eleven Local Government Areas of Ibadan was determined. Further biochemical tests were conducted on the Staphylococci isolated. All of

the isolates tested positive to catalase test, with the resultant production of bubbles, an indication that the organism produced the catalase enzyme which is able to break down hydrogen peroxide (H_20_2) to produce water and oxygen released as bubbles.. The microbial population of *Staphylococus* species varies from one location to the others. Considering areas in Akinyele Local Government Areas (Table 1), "Ewedu" from a restaurant at Ojoo market has highest microbial load of 5.6x 10^6(cfu/ml)   with lowest microbial load of 3.0 x 10^2(cfu/ml) in Rice at Moniya.

The highest microbial load at Egbeda Local Government areas was in stew with 8.6 x 10^5 (cfu/ml) in Alakia and lowest was recorded in"Ewedu"   with 1.33 x 10^4cfu/ml in Egbeda village. Ibadan North local Government Areas   has highest microbial load in"Ewedu"   from University of Ibadan 6.0 x 10^2cfu/ml and the lowest at Bodija was from stew with $1.23x10^2$. Ibadan North East Local Government Areas (Table 5) has the highest microbial load of 6.8 x 10^2cfu/ml in "Ewedu" from gate and lowest was 1.63 x 10^2(cfu/ml) in Rice. From Oje market. The Staphylococci population from Ibadan South East Local Government Areas (Table 6) was highest in "Ewedu"   with $3.4x10^2$(cfu/ml)   from Mapo and lowest microbial load from Rice in Oja-oba with $1.5x10^2$(cfu/ml) .The microbial population of the foods collected at Ibadan South West Local Government has highest Staphylococci load in Amala with7.7x102 cfu/ml from Alesinloye while meat is the lowert at ring road with $1.45x10^2$(cfu/ml) .Idddo Local Government Areas(Table 8) has highest load of staphylococci in Ewedu with $5.8x10^2$(cfu/ml)   and lowest in meat also

The exposure of marcerating broom in "Ewedu" preparation to dirt and uncleanless attitude on the part of handler contributed high level of microbial contamination in "ewedu". Marcerating broom should washed after every use and be kept in clean place before next use, this could control   microbal contaminations. The restaurants that are located where there is intense population tends to have hight mineral load as shown in sasa, Gate, Alesinloye, Bodija and Oja-oba. Those areas where there is less development also recorded high microbial load, Moniya ,Apete, Egbeda village, Ojoo, Oje and Adegbayi were not similar interm of result collected as in Oluyole, Dugbe, Ring- Road and Alesinloye. In the less developed areas drainage are seen running across the road people step on it and enter into restaurant this can contribute to cross contamination, raw foods may not contain any harmful organisms or pose risks to health of human race, cross contamination by food processors, (health carries, can pose health risk most importantly when good manufacturing practices have not been strictly adhered to. Thus handling of food items, sneezing, talking, clothing changing involving carriers can lead to food contamination. The normal flora of the nose, mouth, skin and hands of carriers who are processors can also contaminate food. (Adegoke 2004). Iddo local Government Areas has highest load of staphylococci in "Ewedu" with 5.8 $x10^2$cfu/ml.   and lowest also in meat at Apete with 1.5 $x10^2$cfu/ml. Table (10) Lagelu Local Government has highest microbial load in Amala with 6.3 $x10^2$ cfu/ml. Monatan area of Ibadan with the lowest in that Local Government at Lalupon areas is 2.1 $x10^2$ cfu/ml. .Table 9. shows highest staphylococci count in "Ewedu" at idi-ayunre with 4.0 x 10^2 cfu/ml. and 1.37 x 10^2 cfu/ml. as lowest in that Local Government Areas. Ona-Ara Local Government Areas shows highest microbial court of 6.0x10^2 in Ewedu at Akanran and lowest microbial load was in Rice at Ona-ara village with 1.5 x10^2."Ewedu" occurs frequently with highest microbial load because the method of preparation is similar to every restaurants.

CONCLUSION.

In conclusion there is strong evidence that contamination of food in restaurants by staphylococci are through cross contamination by food handlers. However this can be brought to lowest minimal with good hygien personal and in environments.

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