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Occurrence of Virulent and Antibiotic Resistant *Staphylococcus aureus* in Selected Ready-To-Eat Foods in Obio/Akpor, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author ECO designed the study, handled the laboratory work and wrote the draft. Author OCE Modified and approved the design, supervised the research; read and approved the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Ready-to-eat (RTE) foods contaminated with *Staphylococcus aureus* or its heat-stable toxins have been implicated in food-borne illnesses lately and are now a public health concern. This study is aimed at determining the microbial safety of RTE snacks (meat-pie, Egg-roll, Doughnut, Burns and Puff-puff) sold in Obio/Akpor LGA, Rivers State.
Study Design: This work is based on a completely randomized design with two replications and the average values calculated for the mean comparison.
Place and Duration of Study: Imadavistic Laboratory, Osaks House, East-West Road Port Harcourt, Nigeria, between December 2021 and November, 2022.

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Methodology: A total of 100 samples of RTE food Snacks were randomly purchased and examined for proximate composition, microbial quality, and occurrence of antibiotic-resistant and virulent genes using standard conventional and molecular methods.

Results: Puff-puff had the highest moisture, fiber and ash content while egg-roll had the highest crude fat, also meat-pie had the highest carbohydrate and protein content. Microbial counts ranged from 3.5×10^3 to 1.5×10^6cfu/g with 44 samples unsatisfactory and 39 at borderline by food regulatory standard. Presumptive *S. aureus* count ranged from 1.4×10^3 to 4.6×10^6 cfu/g, with 5 classified as potentially hazardous, 14 as unsatisfactory and 49 as borderline, 16 as satisfactory, and 16 with no detection. The 34 confirmed *S. aureus* showed varying resistance to cloxacillin 31(91.18%), cefuroxime 28 (82.35%), ceftazidime and erythromycin 25 (73.53%), gentamicin 18 (52.94%) and augmentin 13 (38.24%). Multi-drug resistance ranged from 3 to 5 antibiotic classes. Nine isolates produced the expected band of 250bp with *SEA* while 3 produced the band of 400bp with *SEB*.

Conclusion: There is a correlation of statistically significant difference (p<0.5) between the four types of RTE food and enterotoxin A, therefore confirming that RTE foods serve as a reservoir of antibiotic resistant and virulence gene bearing *S. aureus*.

Keywords: Snacks; Staphylococcus aureus; virulence genes.

1. INTRODUCTION

The term ready-to-eat (RTE) foods refers to prepared food products that are sold for immediate consumption, raw or cooked, hot or cold, without any further heat treatment or washing, most often at the point of sale, usually at bus stops, industrials sites, marketplaces, interstate highways, streets and other similar public places [1, 2, 3]. Other terms used to describe this class of foods include convenient, ready, instant and fast foods. The popularity of RTE foods varies from culture to culture within a country and depends on the food type and the staple diets of the population [4]. Examples of such ready-to-eat foods in Nigeria include but not limited to meat pie, egg rolls, ‘moimoi’, vegetable salad, fried meat, fried chicken, plantain, potato and yam chips, puff-puff, doughnut, burns, milk and milk products, nuts, beverages. Convenience, diverse food source, affordability, palatability, availability and available alternatives to home cooking are some of the appealing factors that make RTE foods very popular and cherished by people of all age groups and particularly workers in urban areas with busy schedules [5, 6].

The increasing consumption of ready-to-eat (RTE) foods, in places other than the home, in recent years may pose a food safety problem if RTE foods are prepared improperly since they have suitable internal factors for the growth of spoilage, pathogenic and toxin-producing bacteria [7].

Foodborne diseases resulting from unsanitary food processing methods are becoming more common [8]. Most microbial etiologic agents of foodborne diseases have developed antibiotic resistance [9]. The spread of antibiotic resistance genes to other pathogenic organisms has contributed to food poisoning, increased morbidity, and mortality rates [10]. The current high incidence of *S. aureus* in ready-to-eat food may be linked to its tolerance to heat, drying, and radiation following its ability to produce heat stable and powerful enterotoxin [11, 12]. Food poisoning caused by staphylococcal enterotoxins produced in food by enterotoxigenic strains of coagulase-positive staphylococci, primarily *S. aureus*, is one of the most common food-borne disorders globally [13]. Due to the thermal stability of staphylococcal enterotoxins, they may be present in food when *S. aureus* is not present [14]. Enterotoxins are proteins generated by some staphylococci strains that if allowed to develop in foods, can produce enough enterotoxin to cause illness when the contaminated food is eaten. The enterotoxins, among the many metabolites produced by staphylococci, pose the biggest threat to consumers health [15, 16]. There is no effective long-term decolonization therapy for *S. aureus* carriers, but can be eradicated from the nose in a few weeks, with antibiotics, and relapses are likely within a few months [17].

The present study aimed to assess the quality and nutritive values of RTE snacks food as well as the presence of virulence bearing and antibiotic resistant *Staphylococcus aureus*.

2. MATERIALS AND METHODS

2.1 Study Area

The Obio/Akpor LGA, Rivers State is located between latitudes 4° 50' 08.24" N and 4° 52' 20.49" N and longitudes 7° 02' 18.48" E and 7° 06' 05.20" E as illustrated in Fig.1. The Local Government Area which is mainly constituted by the people of Ikwerre Ethnic nationality covers 260 km^2 and at the 2006 Census held a population of 464,789 [18].

2.2 Sample Collection

A total of 100 samples of ready-to-eat snacks food comprising of meat pie, egg roll, burns, puff-puff, and dough-nut were randomly purchased from vendors in different wards in Obio/Akpor L.G.A (Rumuolumeni, Ogbogoro, Eliozu, Choba, Rumuokoro, Rumuigbo, Rumuokwuta, Agip, Rumuola, Rumumasi), Rivers State. Ten samples of each distinct ready-to-eat snacks food were purchased from different wards. They were placed in sterile Ziploc bag and transported in the pack to the laboratory for analysis within 3 h.

2.3 Proximate Analysis

The percentage moisture content, crude fibre, crude fat, ash content, protein content and carbohydrate content were examined using the methods described by AOAC [19].

2.4 Microbiological Analysis

2.4.1 Sample preparation and isolation of *S. aureus*

Ten grams (10g) of each sample was weighed aseptically into a sterile stomacher bag containing 90 ml of sterile 0.1% peptone. The contents were homogenized using a stomacher (M A 106402, France, 450 to 640 strokes per minute) for 2 minutes (min). The mixture was allowed to stand for 5 min at room temperature (29±2°C). The contents were transferred into sterile flasks and thoroughly mixed by shaking before a ten-fold serial dilution [20]. Aliquots (0.1 ml) from 10^{-2} and 10^{-3} dilutions were spread on duplicated plates of Nutrient agar and Mannitol salt agar that were cooled to approximately 40-45°C after which the plates were incubated at 29±2°C for 24-48 h. Plates with distinct white to yellow glistening, smooth circular colonies which indicated fermentation of mannitol by an organism were purified by sub-culturing on freshly prepared Nutrient agar plates and Mannitol salt agar to obtain pure colonies [21, 22]. The resulting pure colonies were stored in nutrient agar slants and kept in a refrigerator pending confirmation.

2.4.2 Confirmation of isolates

Identification of the isolates was based on cultural morphology, physiological (Gram's stain) and biochemical (Catalase, coagulase) characteristics [23,24,25].

2.5 Deoxyribonucleic acid (DNA) Extraction

DNA was extracted using the boiling method described by Oliveira et al. [26], with slight modifications. 150 µL Phosphate buffered saline (PBS) was pipetted into a sterile 1.5ml Eppendorf tube. A loopful of bacterial colonies suspended from an overnight culture was suspended in 150 µL of phosphate buffered saline which was vortexed at 14000rpm for 10 seconds (s). The bacterial solution was heated in a water bath at 100° C for 15 min; chilled in ice for 15 min and allowed to thaw at 37°C. The Tubes were again centrifuged at 14000 rpm for 5 min and the supernatants were pipetted into another labeled tube and used as a template DNA in PCR.

2.6 Detection of *SEA* and *SEB* Virulence Genes using Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction assay was used to detect virulence genes that encode the production of *SEA* and *SEB* from the extracted DNA of *S. aureus* [27]. Detection of the primers used for the detection of virulence gene *SEA* forward primer was F 5´-TGCAGGG AACAGCTTTAGGC- 3´ and R 5´ - GTGTACCACCCGCACATTGA -3´, *SEB* F 5´-ATTCTATTAAGGACACTAAGTTAGGG - 3´, and R-5´ATCCCGTTTCATAAGGCGAGT- 3´ with a yield of 250bp and 400bp SEA and SEB, respectively [28, 29]. All PCR amplifications were performed in 25 µL which contained cocktail mix buffer(10X) 2.5 (µl), 25mM Mgcl2 1.0 (µl), 5pMol forward primer, 5pMol reverse primer, 1.0 (µl) each, DMSO 1.0 (µl), 2.5Mm dNTPs 2.0 Vol (µl), Taq 5u/ul, 0.1 (µl), 10ng/µl DNA, 3.0 (µl), and H_2O 13.4 (µl). PCR cycling conditions were performed as follows: Initial denaturation at 94˚C for 5 min, second DNA denaturation at 94˚C for 15 sec, annealing at 65˚C for 20 sec, extension at 72˚C for 30 s at 9 cycles, for 35 cycles denaturation at 94˚C for 15 s, annealing at 55˚C for 20 s, extension at 72˚C for 30 s, and final extension at 72˚C for 7 min. The resulting amplicons were resolved by gel electrophoresis using a Portable Gel hood built in Blue LED (470nm) by Royal Biotech/Biolympics in a 1.5% (w/v) agarose gel run at 100 volts for 40 minutes in a 0.5X TAE buffer. The gels were visualized by Ethidium bromide staining and photographed under ultraviolet light. The ladder used is a 100 kb base pair ladder from Thermo Scientific.

2.7 Antibiotics Sensitivity Test

The Clinical and Laboratory Standard Institute [30] disc diffusion method was used for the antibiotic sensitivity test. The inoculum was prepared from an 18 h old broth culture of each isolate and their absorbance was adjusted to 0.5 McFarland standard. Inoculum size (0.1mL) was spread on Mueller-Hinton agar and the antibiotic disc (µg) comprising ceftazidime (30), cefuroxime (30), gentamycin (10), ceftriaxone (30), erythromycin (5), cloxacillin (5), ofloxacin (5), augmentin (30) (Abtek Biological Ltd, UK) was aseptically placed at equidistance of the plates. Plates were incubated at 29±2°C for 18 to 24 h. The clear zones that developed around each disc were measured using a scale rule and results were recorded and compared with the zones of inhibition on the basis of CLSI [30]. The Multiple Antibiotic Resistance (MAR) index of the isolates was calculated as a/b, where "a" represents the number of antibiotics to which the particular isolate was resistant and "b" represents the number of antibiotics to which the isolate was exposed.

2.8 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) ver. 25.0 (IBM, New York, USA) program was used for statistical analysis. Standard error of the mean was used to determine the difference of mean among the different ready–to-eat snacks food, determine the correlations amongst the RTE food types and enterotoxin A and B using Pearson chi-squared(R) test to test the differences with significance of $p < 0.05$.

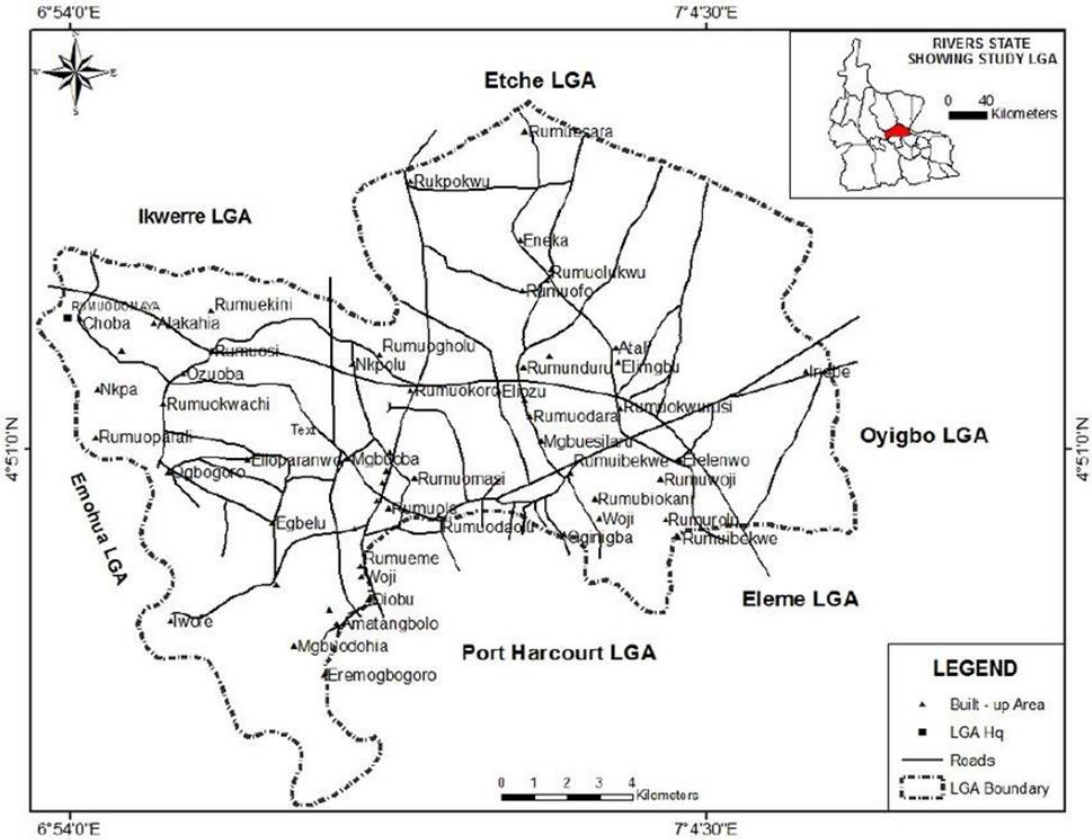

Fig. 1. Map of Obio/Akpor Local Government Area, Rivers State, Nigeria [18]

3. RESULTS AND DISCUSSION

3.1 Percentage Proximate Composition of Ready-To-Eat Snacks

The proximate compositions of the ready-to-eat (RTE) snacks are presented in Table 1. The percentage moisture content was highest in puff-puff at 20.30 and lowest in egg-roll at 8.04. The percentage of crude fiber was highest in puff-puff at 12.0 and lowest in egg-roll at 4.58. The percentage crude fat content was highest in egg rolls 21.06 and lowest in doughnuts at 5.92. The percentage ash content was highest in egg-roll, 20.85 and lowest in buns 2.37. The percentage protein content was highest in meat pies at 4.18 and lowest in buns at 3.14. The percentage carbohydrate content was highest in meat–pie 70.73 and lowest in puff-puff at 4.10.

3.2 Heterotrophic Bacteria Count and Conformity with the Microbiological Guidelines for Food (For ready-To-Eat Food in General and Specific Food Items)

The mean heterotrophic bacteria count on nutrient agar from the locations sampled are shown in Table 2. The results revealed the following ranges (cfu/g) for meat-pie (3.7 x10^4 to1.5 x 10^6), egg roll (4.8x10^3 to 8.4 x 10^5), doughnut (3.5x10^3 to 9.4 x 10^5), puff-puff (6.4x10^3 to 8.8 x 10^5), and buns (4.2x10^3 to 9.1 x 10^5). Meat-pie had the highest upper count (1.5 x 106 cfu/g), followed by doughnut (9.4 x 10^5 cfu/g) while the least was doughnut (3.5x10^3 cfu/g). The conformity of the count with the Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items) is presented in Table 3. Seventeen percent (17%) of the samples were satisfactory, 39% were at the borderlines and 44% were unsatisfactory.

3.3 *Staphylococcus* count

Table 4 Shows the mean presumptive *Staphylococcus* count on Mannitol salt agar. The results revealed the following counts (cfu/g) ranges for meat-pie (1.8 x 10^3 to 4.6 x10^5), egg-roll (1.3 x 10^3 to 3.3 x10^5), doughnut (1.6 x 10^3 to 4.8 x10^4), puff-puff (1.4 x 10^3 to 3.8 x10^5) and buns (1.4 x 10^3 to 6.8 x 10^4). The highest mean presumptive *Staphylococcus* count was observed in meat-pie 4.6 x10^5 (cfu/g), followed by puff-puff 3.8 x10^5 (cfu/g), and was lowest in buns and puff-puff 1.4 x 10^3 (cfu/g), respectively. The conformity with the Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items) for presumptive *S. aureus* counts are presented in Table 5. Among 100 samples, 32% were satisfactory, 49% were borderline, 14% were unsatisfactory and 5% were at potentially hazardous level.

3.4 Antimicrobial Susceptibility

The antimicrobial susceptibility patterns of *S. aureus* from ready-to-eat snacks are presented in Table 6. Resistant was highest in cloxacillin (Penicillin), 31(91.18%), followed by cefuroxime (Cephalosporins), 28(82.35%), ceftazidime (Cephalosporins), and erythromycin (Macrolides), 25 (73.53%) while susceptibility was highest in gentamicin (Aminoglycosides), 18(52.94%) and augmentin ((Penicillin), 13(38.24%).

3.5 Multi-Drug Resistant Pattern

The Multi-drug index patterns of *S. aureus* from the ready-to-eat snacks food are presented in Fig. 1. Out of the 34 isolates 1 (2.9%) was phenotypically resistant to 1 class with MDR index of 0.13, 6 (17.65%) were resistant to 2 class with MDR (0.25), 12(0.35) to 3 class with MDR (0.38),13 to 4 class with MDR (0.50), and 2 to 5 class with MDR (0.65).

Table 1. Percentage proximate composition of ready-to-eat snacks

Food Type	Parameters (Mean ±SEM)					
	Moisture Content	Crude fibres	Crude fat	Ash content	Protein content	Carbohydrate
Puff-puff	20.30 ± 0.12	12.0 ± 0.25	12.57 ± 0.26	14.18 ± 0.23	4.09 ± 0.06	4.10 ± 0.06
Doughnut	11.20 ± 0.16	11.10 ± 0.22	5.92 ± 0.08	10.99 ± 0.22	4.16 ± 0.25	55.23 ± 0.29
Buns	7.12 ± 0.25	11.29 ± 0.26	7.27 ± 0.14	2.37 ± 0.29	3.14 ± 0.10	67.39 ± 0.26
Meat-pie	5.40 ± 0.12	10.58 ± 0.09	6.71 ± 0.11	2.43 ± 0.09	4.18 ± 0.04	70.73 ± 0.15
Egg-roll	8.04 ± 0.10	4.58 ± 0.11	14.06 ± 0.15	20.85 ± 0.14	3.17 ± 0.10	49.35 ± 0.18

Table 2. Total heterotrophic bacteria count (cfug-1) in ready- to- eat snacks food from different ward in Obio/Akpor L.G. A

	A		**B**		**C**		**D**		**E**		**F**		**G**		**H**		**I**		**J**	
SAMPLE	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2
Meat Pie	$6.6x10^4$	TFT	$9.4x10^5$	$9.7x10^5$	$3.7x10^4$	$5.5x10^4$	$3.5x10^5$	TFTC	TFTC	$3.0X10^5$	TFTC	$6.0X10^5$	TFTC	$5.4X10^5$	$5.9X10^5$	$3.1X10^5$	3.9X05	$4.7X10^5$	$9.2X10^5$	$8.6X10^5$
Egg-Roll	$6.0X10^5$	$9.2X10^4$	$5.2X10^3$	$4.8X10^3$	$8.7X10^4$	$6.4X10^4$	TFTC	$5.5X10^4$	TFTC	TFTC	$4.0X10^5$	$9.6X10^4$	$4.4X10^5$	$1.0X10^5$	$6.4X10^4$	$5.6X10^5$	$6.4X10^4$	$8.4X10^5$	$8.1X10^5$	$6.8X10^5$
Dough Nut	$9.4X10^5$	$6.6X10^5$	$3.7X10^3$	$3.5X10^3$	$3.9X10^4$	$6.4X10^3$	$3.1X10^4$	TFTC	$4.1X10^4$	$5.4X10^3$	$8.0X10^5$	$7.3X10^5$	$8.9X10^5$	$6.1X10^5$	$8.1X10^4$	$4.5X10^4$	$8.1X10^4$	$7.4X10^4$	$7.9X10^4$	$8.4X10^5$
Puff-Puff	$8.0X10^4$	$4.9X10^4$	TFTC	$4.7X10^4$	$1.0X10^5$	$9.2X10^4$	$4.0X10^4$	$3.6X10^4$	$4.7X10^4$	$6.4X10^3$	TFTC	$8.8X10^5$	TFTC	TFTC	$8.7X10^5$	TFTC	$8.7X10^5$	$9.2X10^5$	$7.7X10^5$	$8.4X10^4$
Burns	$5.3X10^5$	$3.0X10^5$	TFTC	$6.9X10^4$	TFTC	$8.4X10^4$	$2.3X10^5$	$4.2X10^3$	TFTC	TFTC	$1.5X10^6$	$4.4X10^5$	$8.8X10^5$	$7.2X10^5$	$8.2X10^4$	$9.9X10^5$	8.2X1	$8.6X10^3$	$1.1X10^4$	$9.4X10^5$

A: Rumuolumeni, B: Ogbogoro, C: Eliozu, D: Choba, E: Rumuokoro, F: Rumuigbo, G: Rumuokwuta, H: Rumuokwurusi, I: Agip, J: Rumumasi. W1 means ward 1, W2 means ward 2, TFTC-Too Few to count

Table 3. Microbiological guideline for classification of ready-to-eat food by heterotrophic count

RTE Food	**Satisfactory$<10^3$**	**Borderline $10^3 \leq 10^5$**	**Unsatisfactory$\geq 10^5$**	**Total Number**
Meat	5(25%)	1(5%)	14(70%)	20
Egg roll	3(15%)	9(45%)	8(40%)	20
Doughnut	1(5%)	12(60%)	7(35%)	20
Puff-puff	5(25%)	10(50%)	5(25%)	20
Burns	3(15%)	7(35%)	10(50%)	20
Total	17	39	44	100

TFTC= Too few to count

Table 4. Total Staphylococcus aureus count (cfug-1) on Mannitol Salt Agar

	A		B		C		D		E		F		G		H		I		J	
SAMPLE	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2	W_1	W_2
Meatpie	$4.2x10^3$	$3.7x10^3$	$1.8x10^3$	$3.2x10^3$	$1.1x10^4$	$8.4x10^3$	$3.5x10^5$	$5.0x10^3$	$1.6x10^4$	$4.6x10^4$	$5.9x10^3$	$6.6x10^3$	$5.0x10^3$	$5.9x10^3$	$5.1x10^3$	$4.2x10^3$	$2.6x10^3$	$2.4x10^3$	$3.4x10^3$	$2.9x10^3$
Egg-roll	$4.1x10^3$	$3.1x10^3$	$6.4x10^3$	$3.7x10^3$	$5.6x10^3$	$2.8x10^3$	$2.4x10^4$	$5.5x10^4$	$1.3x10^3$	$4.0x10^3$	$2.2x10^3$	$2.9x10^3$	$2.5x10^3$	$3.5x10^3$	$3.1x10^3$	$3.3x10^5$	$2.9x10^3$	$1.8x10^3$	$2.2x10^3$	$3.1x10^3$
Dough nut	$2.9x10^3$	$3.5x10^3$	$2.3x10^3$	$4.0x10^3$	$3.1x10^4$	$4.8x10^4$	3.1 X104	$2.1x10^4$	$2.1x10^3$	$3.4x10^3$	$2.1x10^3$	$3.6x10^3$	$5.9x10^3$	$2.1x10^3$	$3.5x10^3$	$3.9x10^3$	$1.7x10^3$	$1.6x10^3$	$2.7x10^3$	$2.5x10^3$
Puff-puff	$2.8x10^3$	$2.0x10^3$	$1.4x10^3$	$1.9x10^3$	$2.1x10^4$	$3.2x10^4$	4.0 X104	$3.7x10^5$	$1.6x10^3$	$1.7x10^3$	$1.6x10^3$	$1.6x10^3$	$1.8x10^3$	$1.9x10^3$	$2.5x10^3$	$1.8x10^3$	$2.1x10^3$	$2.4x10^3$	$1.7x10^3$	$2.1x10^3$
Burns	$1.6x10^3$	$2.5x10^3$	$2.7x10^4$	$3.8x10^3$	$3.3x10^3$	$6.8x10^4$	$3.3x10^3$	$5.6x10^3$	$2.0x10^3$	$5.3x10^3$	$2.0x10^3$	$2.6x10^3$	$2.1x10^3$	$4.9x10^3$	$2.1x10^3$	$1.9x10^3$	$1.4x10^3$	$1.5x10^3$	$2.5x10^3$	$1.8x10^3$

Table 5. Microbiological guideline for classification of ready-to-eat food by *Staphylococcus aureus* count

RTE Food	Satisfactory $<10^2$	Borderline 10^2-10^3	Unsatisfactory $10^3 \leq 10^4$	Potential hazardous$\geq 10^4$	Total Number
Meat pie	6(30%)	10(50%)	3(15%)	1(5%)	20
Egg-roll	4(20%)	11(55%)	2(10%)	3 (15%)	20
Dough nut	7(35%)	9(45%)	4(20%)	-	20
Puff	8(40%)	8(40%)	3(15%)	1(5%)	20
Burns	7(35%)	11(55%)	2(10%)	-	20
Total	**32**	**49**	**14**	**5**	**100**

Table 6. Susceptibility patterns of *Staphylococcus aureus* from ready to-eat foods from Obio/Akpor L.G.A

Antimicrobial group/antibiotics	Resistant	Intermediate	Susceptible
Penicillin (β-lactam)			
Cloxacillin	31(91.18%)	0(0.00%)	3(8.82%)
Augmentin	21(61.76%)	0(0.00%)	13(38.24%)
Cephalosporins (β-lactam)			
Cefuroxime	28(82.35%)	2(5.88%)	4(11.77%)
Ceftazidime	25(73.53%)	3(8.82%)	6(17.65%)
Ceftriaxone	19(55.88%)	6(17.65%)	9(26.47%)
Macrolides			
Erythromycin	25(73.53%)	7(20.59%)	2(5.88%)
Aminoglycosides			
Gentamicin	15(44.12%)	1(2.94%)	18(52.94%)
Fluroquinolones			
Ofloxacin	20(58.82%)	3(8.82%)	11(32.35%)

3.6 *Staphylococcus aureus* Virulent Gene for *SEA* an *SEB* from RTE (Snacks) Food

Three isolates produced the band of 400bp with *SEB* while 9 isolates produced the expected band of 250bp with *SEA*, as shown in Plate 1 and 2.

3.7 Prevalence of *S. aureus* in Ready-To-Eat Food with Respect to *SEA* and *SEB*

The five different RTE snack foods had *S. aureus* prevalence with respect to enterotoxin highest in meat-pie 11(32.35%) and lowest in puff-puff 3(8.82%) with SEA number 3 and 2 respectively as seen in Table 7. The correlation between the samples according to enterotoxin A gene using Chi-squared test is shown in Table 8.

The results of the proximate composition of ready-to-eat food sourced from Obio/Akpo presented the nutrient content of food with respect to moisture, ash, fat, crude fibre, protein and carbohydrate content. The determination of the percentage moisture content showed that puff-puff had the highest moisture content of 20.30 among the snack foods examined, while meat-pie had the least (5.40). The percentage moisture content for puff-puff is within the 15.40 and 26.60 previously reported by Israel and Samuel [31] and Pikuda and Ileleboye [32], respectively. The moisture content of meat-pie in this present study is less than the 8.18% reported by Adeyeye and Ayoola [33]. The higher the percentage of moisture contents of RTE snacks food, the shorter the shelf-life stability and vis-visa [34, 35]. Moisture is important in the human diet because it provides body fluids and helps to regulate body temperature [32]. The percentage of crude fiber was also highest in puff-puff (12.00) and lowest in egg roll (4.58). The value for puff-puff in this study is higher than the 0.66% reported in similar work by Israel and Samuel [31]; whereas the meat-pie percentage crude fiber of 4.58 is higher than the 0.76, 3.50, and 0.51% previously reported [31, 33, 36]. The crude fiber contents are advantageous to adults with colon diseases, while it also prevents constipation [37]; it lowers cholesterol levels in the blood and reduces the risk of cancers [38]. The determination of the percentage crude fat content showed that egg-roll had the highest percentage of crude fat of 12.57 while doughnuts had the lowest percentage of crude fat content of 5.92. The value for egg-roll was less than 16.88, 21.50, and 22.09% previously reported [32, 33, 36]. The high percentage of crude fat content in egg roll could be attributed to their being fried in vegetable oil and that egg yolk is highly rich in cholesterol and minerals, therefore frying the egg roll leads to an increase in the cholesterol content [32]. Apart from being a concentrated source of energy, fats and oils also provide essential fatty acids necessary for the proper functioning of the human blood, but too much of them may lead to health problems such as overweight, obesity, high blood pressure and heart disease [39].

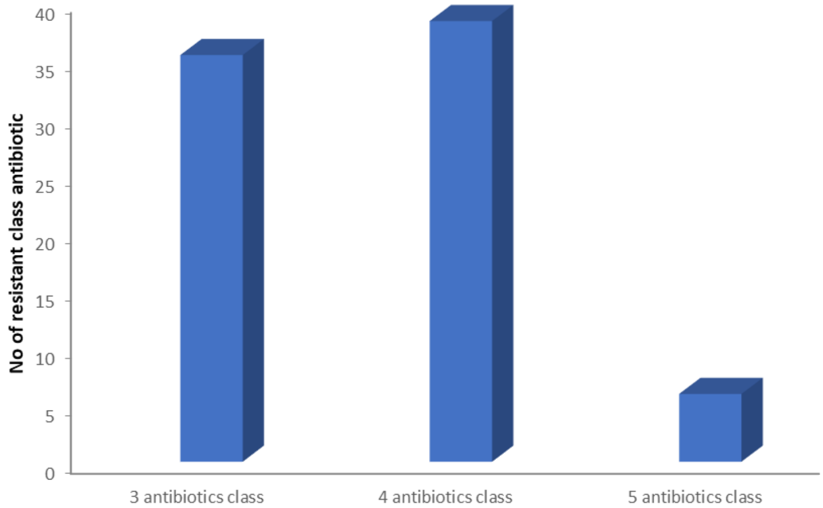

Fig. 2. Number of antibiotics

Table 7. Prevalence of *Staphylococcus aureus* in ready-to-eat food with respect to classical enterotoxin A and B

RTE food type	Total sample	Prevalence number of *S. aureus* (%)	Number of RTE food with Enterotoxin	
			SE(A)	SE(B)
Meat-pie	(n=20)	11(32.35%)	3	-
Egg-roll	(n=20)	8(23.53%)	1	2
Dough nut	(n=20)	5(14.71%)	2	1
Puff-puff	(n=20)	3(8.82%)	1	-
Burns	(n=20)	7(20.59%)	2	-
Total	100	34 (100%)	9	3

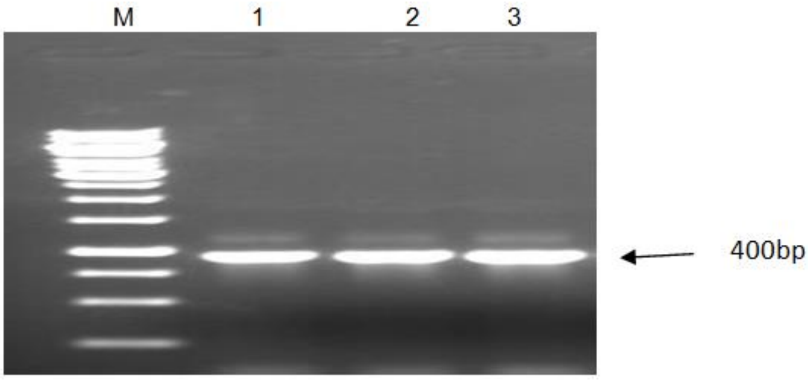

Plate. 1. *Staphylococcus* enterotoxin B (*seb*) virulence gene from ready -to - eat snacks. M is 100bp DNA ladder. Lanes 1 to 3 are positive isolates

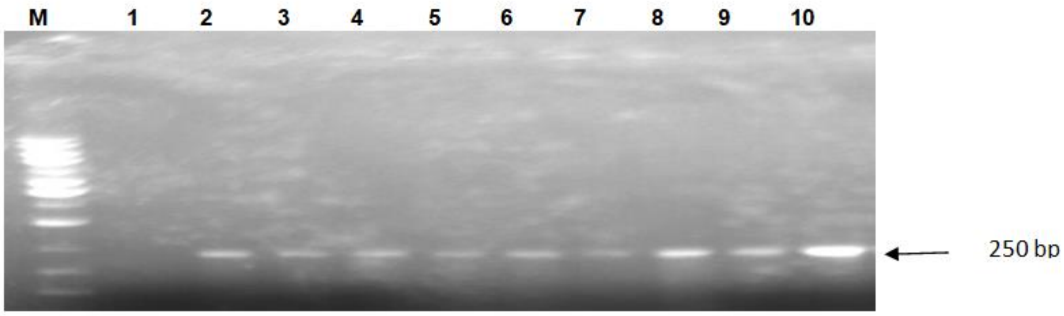

Plate. 2. *Staphylococcus* enterotoxin A (*sea*) virulence gene from ready -to - eat snacks. M is 100bp DNA ladder. Lanes 2 to 10 are positive isolates

Table 8. Correlation between samples according to enterotoxin A gene expression using chi-square test

	Value	Df	Asymp. Sig. (2-sided)
Pearson chi-square	10.000[a]	8	0.27
Likelihood Ratio	10.549	8	0.27
Linear-by-Linear Association	1.795	1	0.18
N of Valid Cases	5		

a. 15 cells (100.0%) have an expected count of less than 5. The minimum expected count is 0.20.
df = Difference; asymp. Sig = Asymptomatic Significance

Carbohydrates are the main source of energy, the energy needs of the individual are the amount of food energy required to compensate for energy expenditure when the size, body composition and level of physical activity are compatible with a lasting state of good health and the maintenance of physical activity that is economically necessary and socially desirable [40]. The percentage carbohydrate content was highest in meat-pie (70.73%) and lowest in puff-puff (4.10%). The value for meat-pie in this study is higher than 55.39 and 60.53% reported by Eke and Elechi [40] and Israel and Samuel [31], respectively. The carbohydrate content of puff-puff (4.10%) is lower than the 65.14% previously reported by Pikuda and Ileleboye [32]. The high content of carbohydrates found in meat- pie could be due to the filling with potatoes as part of the sauce, since potatoes are high in carbohydrates. The percentage ash content of a feedstuff is the inorganic residue remaining after the organic matter has been destroyed by combustion in the muffle furnace [41]. Egg rolls had the highest ash content of 14.06%, while buns had the lowest ash content of 2.37%. The ash content for egg-roll is also higher than 2.00 and 2.60% reported by Chinaza et al. [36] and Israel and Samuel [31], respectively. This shows that ready-to-eat egg-roll is rich in essential minerals, as the ash content of food is simply a measure of its mineral content, it is not a surprise that egg-roll has the highest ash content because of the egg yolk which is rich in vitamins and minerals [42]. The percentage of crude protein content was highest in meat-pie (4.18%) and lowest in buns (3.14%). The percentage of crude protein content for meat-pie in this study was lower than 10.02, 14.28 and 16.77% previously reported [31, 33,40]. This could be a result of the high cost of meat recently which could lead to a reduced quantity of meat in the sauce and an increased quantity of Irish potatoes. The crude protein content in this study was also lower than the 8.69% reported by Adeyeye and Ayoola [33]. It is pertinent to note that buns had the lowest percentage of crude protein as in this study. The differences in the values obtained for the parameters in the study and previous studies could be due to biological materials and products' compositions which change from location to location, batch to batch, and from time to time, these differences may be linked to the kind and source of the raw materials employed as well as the manufacturing method [33, 43].

On the basis of total heterotrophic microbial count, 17% of the snacks were satisfactory, 39% were at the borderlines and 44% of the samples examined were unsatisfactory according to the Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items) [44]. This showed that the microbial quality of a good number of the snacks sold in these various locations are not fit for human consumption since they are above the acceptable microbial limit [45, 46]. Total heterotrophic bacteria count showed that meat-pie had the highest count of 1.50 x 10^6 cfu/g which was higher than 1.08 x 10^6 cfu/g in a previous report by Ogidi et al. [12] but agrees with the 1.50 x 10^6 cfu/g in a previous report by Okeke et al. [47]. The microbiological analysis revealed that the number of bacteria counts had a similar trend to the report by Adolf and Azis [48]; with varying index of 10^3 to 10^6. Meat-pie count may be attributed to the meat or vegetables inside the pie and also the nutritional content which provides a good nutrient media that supports microbial growth [49]. The high counts also observed in doughnuts could be attributed to factors such as improper handling processing, use of contaminated water, cross-contamination from other raw materials, or use of unclean utensils like knives and trays [50, 51, 52].

Staphylococcus count revealed that 5(5%) were classified as potentially hazardous, 14(14%) as unsatisfactory, 49(49%) as borderline and 16(16%) had as satisfactory detection, which means these RTE Snacks food are potentially unhealthy to consume because at a range of 10^6 cfu/g. *Staphylococcus* enterotoxin found in them can cause a foodborne outbreak amongst dwellers since they carry virulence genes also pertinent to note is that enterotoxins can also be formed at a satisfactory level or marginal level because temperatures used for shallow frying and baking may not be enough to eradicate SE'S even after the microbe has been eradicated [11, 12, 53]. Presumptive *Staphylococcus aureus* occurrence of 82% was higher than the 23.3% reported in similar work by Ogidi et al. [12], 38.9% reported by Obande et al. [45] and 46% reported by Esemu et al. [54]. Meat-pie had the highest count of 3.50 x 10^6 cfu/g, while egg-roll had the lowest count of 1.3 x 10^6 cfu/g which was higher than 4.10 x 10^6 cfu/g in a previous similar work by Nwachukwu, and Nwaigwe, [55]. The difference in the occurrence rate and count range reported in this study could be due to sample size, improper handling, cross- contamination from unclean equipment during production, and diagnostic methods used for identification [12,20]. The significance of the *Staphylococcus aureus* count indicates these RTE food snacks have been contaminated [12, 20]. *Staphylococcus. aureus* has been one of the most isolated in previous work by Oje et al. [3].

Staphylococcus aureus showed the highest resistance to the antibiotics used at 91.18% which is comparable to 91.60% in a previous report by Ogidi et al. [12] but differs from the 57 and 100% reported by Obande et al. [46] and Awanye, and Uwah [45], respectively. The difference in resistance reported could be a result of the concentration of antibiotics used in a locality, the number of samples analyzed [56] and compliance with the prescription. Resistance of *S. aureus* to targeted antibiotics was highest against cloxacillin (Penicillin) (91.18%), followed by cefuroxime (Cephalosporins, β-lactam) (82.35%) and the least was against gentamicin (Aminoglycosides) (44.12%) which are all bactericidal. A number of authors have reported varying antibiograms of *S. aureus* isolated from similar foods in China, Nigeria and Ethiopia [12,45, 46, 55, 57, 58]. The resistance against cloxacillin in this study is comparable to the 87.1 and 100% reported by Obande et al. [46] and Temesgen et al. [57], respectively but higher than the 50.0% reported by Nwachukwu and Nwaigwe [55]. The resistance against augmentin is lower than the 77.1 and 83.3% by Obande et al. [46] and Ogidi et al. [12]. The 44.12% resistance against gentamycin in this study is higher than the 0, 0.8 and 14.3% reported by Nwachukwu and Nwaigwe [55] and Xing et al. [56], Temesgen et al. [57], Obande et al. [46], respectively. The value is however, agrees with the 41.7% reported by Ogidi et al. [11] but lower than the 82.0% reported by Awanye and Uwah [45]. The 73.53% resistance against erythromycin in this present study is comparable to the 70.0 and 78.1% reported by Obande et al. [46] and Xing et al. [56], respectively. The value is however, higher than the 0, 28.6 and 62.5 % reported by Nwachukwu and Nwaigwe [55], Temesgen et al. [57] and Ogidi et al. [12], respectively. The 59.1% resistance of *S. aureus* against ceftazidime is less than the 73.53% obtained in this study. It is believed that more than 80% of staphylococcal isolates now produce penicillinase regardless of their origin [58]. This may be attributed to the misuse of antibiotics towards illness and poses a health concern to consumers, also food products and the environment could be directly or indirectly involved in the transfer of resistant genes due to several human activities such as the use of antibiotics in farming [10]. This class of antibiotics used was able to penetrate the cell membrane and damage the nucleic acid of *S. aureus* [59] and could be effective in treating *S. aureus* in the area of study because gentamicin comes in injection form and is hardly abused also most person has a phobia for injection.

The multiple drug resistance index is a very helpful tool in checking health risks and also the degree of antibiotic resistance. Isolated S. aureus showed different types of resistance patterns which connotes they are phenotypically diversified, which ranged from 0.13 (resistant to 1 antibiotic) to 0.65 (resistant to 5 antibiotics), which was lower than 0.25-0.87 multiple drug resistance index in a similar work by Ogidi et al. [12]. The difference could be a result of the location and class of antibiotics commonly used in the area. Multi-drug resistant index (MARI) ≥ 0.3 indicated that the isolates originated from an environment where antibiotics were frequently used.

In this study *S. aureus* isolates showed the presence of virulent genes for *SEA* (enterotoxin A) and *SEB* (enterotoxin B). Nine isolates produced the expected band of 250bp with *SEA* while 3 produced the band of 400bp with *SEB* out of 34 isolates. *SEA* (enterotoxin A) was the most prevalent enterotoxin in this study. This agrees with the previous work of Jassim and Kandala [59] who out of 49 isolates of *S. aureus* from different food sources detected 9 positives to *SEA* and 7 *SEB*. It has been documented in most countries of food-borne outbreaks that *SEA* is the most prevalent enterotoxin isolated. The prevalence of *S. aureus* and its virulent gene was highest in RTE (Snacks) meat-pie and eggroll which agrees with previous findings that RTE foods of animal origin do have a higher prevalence of *SEA* over *SEB* most times [60] .The correlation of the selected food type to enterotoxin A showed a difference with the significance of $p < 0.05$ using the Pearson chi-squared (R) test which also agrees with the previous work of Jassim and Kandala [59] and connotes that RTE Snacks food are vehicles for transmission of resistant *S. aureus* which also harbor virulence genes.

4. CONCLUSION

Microbiologically, ready-to-eat snacks food from the different locations in Obio/Akpor L.G.A. were majorly at an unsatisfactory level as classified by the International Commission on Microbiological Specifications on RTE Snacks and *S. aureus*, the superbug was isolated and characterized from ready-to-eat snacks food obtained which calls for great concern to public health. *S. aureus* isolates showed greater a degree of resistance to β-lactam antibiotics like cloxacillin, and a greater degree of susceptibility to aminoglycosides like gentamicin and most were multi-drug resistant. Enterotoxin A and B were detected from the S. aureus isolated from the RTE snacks food samples meaning that they served as a harbour and vehicle in transmission of multi-drug resistant *S. aureus* with enterotoxin A and B virulent genes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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