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Full Length Research Paper

Bacteriological characterization of snacks sold in Rivers State University of Science and Technology, Port Harcourt, Nigeria

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Two types of pastry snacks *(Moin-moin and Buns)* were bought from four different hawkers in the Rivers State University of Science and Technology, Port Harcourt, from four locations viz. Student Residential area, Faculty of Science, Faculty of Engineering and the Old Administrative area. Ten representative bacterial isolates from each of the snacks from the locations were tentatively identified separately from the external and internal parts of the snacks. The cumulative Total Colony Forming Units (TCFU) from the snacks' exterior of the locations included, *Staphylococcus* sp. 30 (50.0)*; Bacillus and other Gram positive rods 14 (23.3); *Escherichia coli* 4 (6.7); *Proteus* sp 6 (10.0); *Enterococcus* sp.; Flavobacterium/ Xanthomonas spp. and *Pseudomonas* spp. 2 (3.3) each. While for the interior it was Staphylococcus sp. 38 (63.3); Bacillus and other Gram positive rods 4 (6.3); *E. coli* 6 (10.0); *Proteus* sp. 4 (6.7); *Enterococcus* sp.; Flavobacterium/ Xanthomonas spp. 2 (3.3) and *Pseudomonas* spp. 4 (6.7) each. The exterior of the snacks were significantly more colonised than the interior (P = <0.001) with TCFU of bacteria ranging from 5.42E+05 to 3.58E+06. Both the exterior and the interior of the snacks were however below the expected standard of 10 coliform counts per gram for pastry foods. A comparison of the bacterial load of the exterior to the interior of the snacks further indicated that there was significant variability in the TCFU according to the locations from which the snacks were purchased (P = <0.001). This was further confirmed by the observed distribution of Coagulase positive *Staphylococcus aureus* in the locations. The likely public health implication of the presence of some of the representative bacterial forms was discussed including relevant recommendations. *Numbers in parenthesis represent percentages.

Key words: Snack, *moin-moin*, *buns*, gastroenteritis, Enterobacteriaceae, Micrococcacae.

INTRODUCTION

Sale and consumption of snacks

There is an increasing tendency for the populace to patronize out-door foods also called snacks or "Take-away-foods" in the University Campuses in particular and in the society at large. The term "Snack" is used to describe "High-Energy" foods such as crisps of all types, fried fish or meat, and even African delicacies such as "*Akara*" (fried bean cakes), "*Moin-moin*" (steamed bean pastry food), oil-fried ripe plantain (or "*Dodo*") etc. Snack foods are very popular worldwide especially among children and the working class. Furthermore, snack foods are increasingly becoming choice foods as a result of general food-shortages and poverty in the Third world countries, (as a result of low income, which encourages expenditure of limited money on the often cheaper snacks); and urbanization influence which encourages more hours of work away from home. Especially in most Campuses of Nigerian Universities, eating of snacks by staff and students in the rush hours in-between lectures

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Table 1. Preparation of 0.2 M phosphate buffer (pH 7.4).

Solution A (0.2 M Na_2HPO_4 in saline)	Solution B (0.2 bMKH_2PO_4 in saline)
Na_2HPO_4 (Analar R 10248).....28.4 g	KH_2PO_4 (Analar R 10203).....27.2 g
NaCl (Analar R 10241) ..... 8.5 g	NaCl (Analar R 10241) ..... 8.5 g
Distilled water (made up to)..... 1 L	Distilled water (made up to)..... 1 L

has become a common practice.

Food contamination

Foods are usually contaminated with microorganisms as a result of inadequate preparation, unsanitary handling, ineffective storage, improper packaging and unsanitary exposure. According to Isara et al. (2010) the prevalence of food contamination in the fast food restaurants in Benin in Nigeria, was found to be 37.5%. In which *Bacillus cereus* and *Staphylococcus aureus* were the most commonly isolated bacteria, while salad, meat pie and fried rice were the most commonly contaminated foods. Foods are easily contaminated as they serve as rich substrates for most microorganisms including different pathogens which could cause gastroenteritis and food poisoning. Using the coliform standard, permissible standard differ for different food (Jay, 2005).

Food sanitary quality

The contamination of foods with pathogens had been known for over a century (Buttiaux and Mossel, 1961). Diseases such as brucellosis, typhoid fever, diphtheria and tuberculosis have from time to time, been associated with poor pasteurization of milk and meat. A much studied source of food contamination is that which arises from handling. The sanitary quality of food is usually determined by the presence or absence of pathogens or by the low microbial counts per gram of the food. In general however, the content of certain indicator organisms is used for determining the sanitary quality of foods. The indicator organisms presently used for food quality assessment include, Coliforms and the *Enterococci* (Burton, 1949). McCoy (1961) stated that in the examination of foods, the presence of *Enterobacteria* indicated the level of cleanliness while the presence of pathogens indicated the safety level of the food. A tentative standard using the Coliform (*Escherichia coli* count) which applies to some foods indicated that for a grade A milk, 10 ml^{-1}; for pre-cooked/partially cooked frozen foods 10 g^{-1}; while for crab-meat and pastry foods such as custard and probably *Buns* and *Moin-moin*, 100 g^{-1} were acceptable limits. For frozen foods in particular, the use of the *Enterococcus* index for food safety had been advocated (Jay, 2005).

In view of the increasing number of snack-hawkers around University Campuses and the associated probable health risk from such food-handlers, it become necessary to ascertain the bacteriological state of the snacks sold in the Rivers State University of Science and Technology, Port Harcourt, as a probable insight into similar practices in other universities in Nigeria.

MATERIALS AND METHODS

Snacks

Two types of snacks (*Moin-moin* and *Buns*) were bought from four different hawkers in the Rivers State University of Science and Technology campus from four locations viz. Student Residential area, Faculty of Science, Faculty of Engineering and the Old Administrative area.

Moin-moin is made from grounded beans to which had been added chipped onion, salt, pepper and crayfish after stirring, the pastry mix is wrapped in aluminium foil water-proof wrapping or put in tin-cans and then steamed until it is cooked. *Buns* on the other hand is made from wheat-flour to which has been added water, eggs, baking powder, sugar, salt etc., and made into pastry balls and deep-fried in cooking oil.

Sanitary condition of the locations

All the locations had high density of human population with many juvenile and adult hawkers. Packaging of the *Buns* was not done as they were packed in transparent plastic buckets from which buyers freely selected buns with either forks or bare and unprotected hands occasionally. *Moin-moin* was dispensed by the hawkers from metal cans using their bare and unprotected hands; in few instances though at a higher price, *Moin-moin* was well packaged in aluminium foil or in covered plates. Due to the crusted nature of fried buns, it was difficult to ascertain if they were properly cooked or if they have been left-over from the previous day (Isara et al. 2010).

Reagents and media

All media were autoclaved at 121°C for 15 min except for the sugar solutions which were autoclaved for 10 min; Culture media were prepared according to manufacturers specifications. Recovery diluents were prepared using phosphate buffer saline (PBS).

Preparation of 0.2 M phosphate buffer (pH 7.4)

Table 1, shows the composition of Solutions A and B in the preparation of 0.2 M phosphate buffer (pH 7.4). To Solution A (840 ml) was added 160 ml of Solution B, this was mixed thoroughly and stored at 5°C. The preparation of 0.02 M Phosphate buffer (pH 7.4) was carried out by mixing 0.2 M Phosphate buffer (pH 7.4) 100 ml and 7.7 g NaCl and making up the volume to 900 ml using distilled water. This was mix thoroughly and stored at 5°C. The Recovery

Diluents (0.1% Peptone in PBS) was finally prepared by adding 1 g of Bacteriological peptone (Oxoid L37) in 1 L of (0.02 M) PBS. This was mixed thoroughly and dispensed and stored at 5°C in 100 ml portions in plugged conical flasks.

Treatment of *Moin-moin* and buns samples

Surface rinsing of whole *Moin-moin* and whole *Buns* was separately carried out using 20 ml recovery diluents in separate conical flasks. The rinsate was allowed to stand at ambient temperature for 6 h to allow for the activation of organisms from their resting stages. Internal samples were extracted per sample and were collected after rinsing their exterior with 0.1% $HgCl_2$ and rinsing thrice with distilled water then using sterile forceps and scalpels 1.0 g per sample was collected. This was macerated in 5 ml of recovery diluents. All samples were treated in triplicates. 0.1 ml of rinsates in the recovery diluents was spread onto surface-dried Nutrient Agar (NA) from which TCFU was determined. Ten representative morphologically different bacterial forms were randomly isolated and stored in Agar slants at 5°C for further identification. Further tests to identify the bacterial forms included growth on Kligler Iron Agar (KIA) slants; Citrate utilization test for characterization of the Enterobateriaceae and the Urease test for the identification of the *Proteus* sp. Indole test was carried out using organisms subcultured in peptone water (Oxoid L37) and testing with Kovac's reagent (*p*-dimethylaminbenzaldehyde). Manitol Salt Agar (MSA) was used as a differential and selective medium for the isolation of *S. aureus* and the Catalase test for distinguishing Catalase and non-catalase producing bacteria. *S. aureus* isolates were further tested for bound and free Coagulase using the Tube and Slide Coagulase tests (Cheesbrough, 2006).

RESULTS

The pastry snacks viz. *Moin-moin* and *Buns* were analysed for their bacteriological characteristics (Table 2), while the TCFU were shown in Figures 1 and 2. The preponderance of coagulase positive *S. aureus* is as shown in Table 3.

For the interior of the snacks indication was that for Moin-moin bought from Student Residential (IMSR), Buns bought from Student Residential area (IBSR), Buns bought from the Science Faculty (IBSC) and Buns bought from the Old Site (IBOS); the *Staphylococcus* spp were more prevalent (with a frequency of 38% of the isolates) while *Proteus* spp and *E. coli* occurred in the IMSC and IMOS respectively.

As shown in Table 2, the tentatively identified aerobic bacterial species from the exterior of the snacks included the Micrococcacae followed by the Enterobateriaceae. The Micrococcacae that were identified include *Staphylococcus* sp 30 (50.0)* and the *Enterococcus* sp 2 (3.3). The Enterobateriaceae included *E. coli* 4 (6.7); *Proteus* sp 6 (10.0); *Flavobacterium/ Xanthomonas* spp. and *Pseudomonas* spp. 2 (3.3) each, while the *Bacillus* and other Gram positive rods were 14 (23.3). From the interior the Micrococcacae that were identified include *Staphylococcus* sp 38 (63.3) and the *Enterococcus* sp 2(3.3). The Enterobateriaceae included the *E. coli* 6 (10.0); *Proteus* sp. 4 (6.7); *Flavobacterium/ Xanthomonas* spp. 2 (3.3) and *Pseudomonas* spp. 4 (6.7) each, while the *Bacillus* and other Gram positive rods were 4 (6.7).

The EMSR was more colonized by the *Proteus* and the *Staphylococcus* spp. while the exterior of *Moin-moin* from the faculty of science (EMSC) was colonised by Bacillus and other Gram positive bacteria and *Staphylococcus* spp. The exterior *Moin-moin* Student Residential (EMSR) was colonized by the *Bacillus* and other Gram-positive rods and *Staphylococcus* sp. For the exterior *Moin-moin* Old Site (EMOS), exterior *Buns* Student Residential (EBSR) and exterior *Buns* Old Site (EBOS) it was predominantly the *Staphylococcus* sp and for the exterior *Buns* Science Faculty (EBSC) it was the *Bacillus* and other Gram positive rods. For the exterior of the snacks tentatively it was the hardy environmentally preponderant species of the *Staphylococcus* and *Bacillus* and other Gram-positive forms (ranging from 6 to 30%) that were most frequently isolated. For the interior of the snacks indicated that for interior *Moin-moin* Student Residential (IMSR), IMSC, interior *Buns* Student Residential (IBSR), interior *Buns* Science Faculty (IBSC) and interior *Buns* Old Site (IBOS), the *Staphylococcus* spp. were more prevalent (for 38% of the isolates) while *Proteus* spp and *E. coli* occurred in the IMSC and interior *Moin-moin* Old Site (IMOS) respectively.

As shown in Figures 1 and 2, the exterior of the snacks were significantly more colonised than the interior ($P = <0.001$) with TCFU of bacteria ranging from 5.42E+05 to 3.58E+06. Both the exterior and the interior of the snacks were however below the expected standard of 100 coliform counts per gram for pastry foods. A comparison of the bacterial load of the exterior to the interior of the snacks according to the locations indicated that there was significant variation in the TCFU depending on where the snacks were purchased from ($P = <0.001$).

Table 3, shows the distribution of Coagulase positive *S. aureus* on the external and interior of the snacks according to the various locations. External contamination was 69.6% compared to the interior which was 30.4%. The highest incidence of exterior contamination occurred in the *Moin-moin* bought at the Old Administrative Site (MOS) followed by the *Moin-moin* bought at the Engineering Faculty (ME) and *Buns* bought at the Residential Area (BSR). Contamination of the interior of the snacks occurred more in the *Buns* bought at the Faculty of Engineering (BE) followed by the *Buns* bought at the Residential Area (BSR).

DISCUSSION

The preponderance of the Micrococcacae (especially of *S. aureus*) and the *Bacillus* and other Gram positive rods on the external of the snacks was not unexpected as these are some of the hardy organisms that can survive in harsh environmental conditions. Nevertheless, the occurrence of nearly 70% of Coagulase positive *S.*

Table 2. Characteristics of bacteria isolated from the external and internal of snacks.

Tests and assessments	Isolates						
	I	II	III	IV	V	VI	VII
Colonial morphology	Creamy entire	Whitish/dry entire	Golden entire	Mucoid entire	Creamy entire	Mucoid entire	
Gram's reaction	+ve cocci in chains	+ve rods in chains	+ve cocci in clusters	-ve rods	+ve cocci	-ve rods	
Growth on MSA	- ve	NT	+ve	NT	NT		NT
Tube coagulase	- ve	NT	+ve	NT	NT		NT
Slide coagulase	- ve	NT	+ve	NT	NT		NT
Litmus test	+ve	NT	- ve	NT	NT		NT
Oxidase	NT	NT	NT	- ve	+ve		
Sugar utilization:	NT	NT	NT				
Lactose				- ve	- ve		
Mannitol				- ve	- ve		
Glucose				+ ve	- ve		
Sucrose				+ve	- ve		
Growth in KIA:							
-Acid/Gas				-ve		-ve	+ve
-H_2S				-ve		-ve	-ve
-Butt /Slope				R/Y	NT	R/R	Y/Y
Tentative identification	*Enterococcus* sp.	*Bacillus* sp. & other rods	*Staphylococcus* sp.	*Proteus* sp.	*Flavobact/ Xanth.* spp.	*Pseudomonas* spp.	*E. coli*
CFU g^{-1} from Snacks							
EMSR	-	2	4	4	-	-	-
EMSC	-	6	4	-	-	-	-
EMOS	-	-	8	-	-	-	2
EBSR	-	2	8	-	-	-	-
EBSC	-	4	2	-	2	-	2
EBOS	2	-	4	2	-	2	-
Cumulative CFU g^{-1} from (Exterior) Snack	2 (3.3)*	14 (23.3)	14 (23.3)	30 (50.0)	6 (10.0)	2 (3.3)	4 (6.7)
IMSR	-	2	8	-	-	-	-
IMSC	-	-	2	4	-	-	4
IMOS	2	2	-	-	2	2	2
IBSR	-	-	8	-	2	2	-
IBSC	-	-	10	-	-	-	-

Table 2. Contd.

IBOS	-	-	10	-	-	-	-
Cumulative CFU g^{-1} from (Interior) Snack	2 (3.3)	4 (6.7)	38 (63.3)	4 (6.7)	2 (3.3)	4 (6.7)	6 (10.0)
Cumulative isolation Frequency (Int. & Ext.)	4 (3.3)	18 (15.0)	68 (56.7)	10 (8.3)	4 (3.3)	6 (5.0)	10 (8.3)

SPC were averages of three plate counts; * Numbers in parenthesis represent percentages; NT: Not tested.

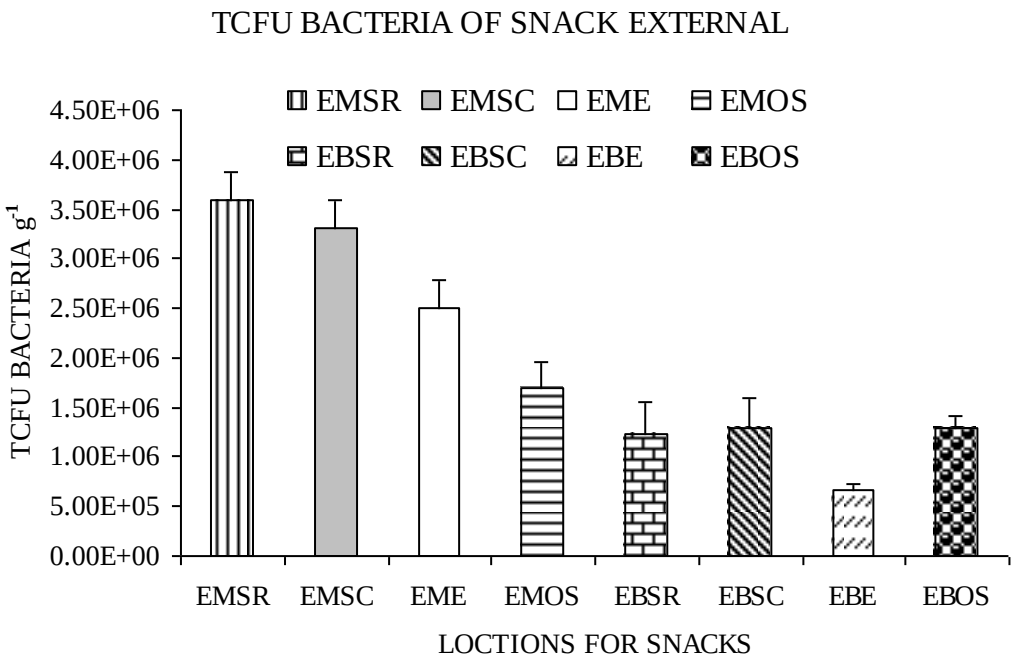

Figure 1. TCFU of bacteria from the exterior of snacks (P = <0.001). EMSR = Exterior Moin-moin Student Residential; EMSC = Exterior Moin-moin Science. EME = Exterior Moin-moin Engineering; EMOS = Exterior Moin-moin Old Site; EBSR = Exterior Buns Student Residential; EBSC = Exterior Buns Science Faculty; EBE = External Buns Engineering; EBOS = Exterior Buns Old Site (Mean n = 3 ± SE).

aureus and of the Enterobacteriaceae is of serious public health interest. While the presence of the former would imply that the snacks were

Table 3. Frequency of isolation of coagulase positive *S. aureus* from locations.

Snack locations	Exterior	Interior
MSR	2	1
MSF	1	1
MOS	4	0
ME	3	0
BSR	3	2
BSC	1	0
BE	1	3
BOS	1	0
Total	16 (69.6%)	7 (30.4%)

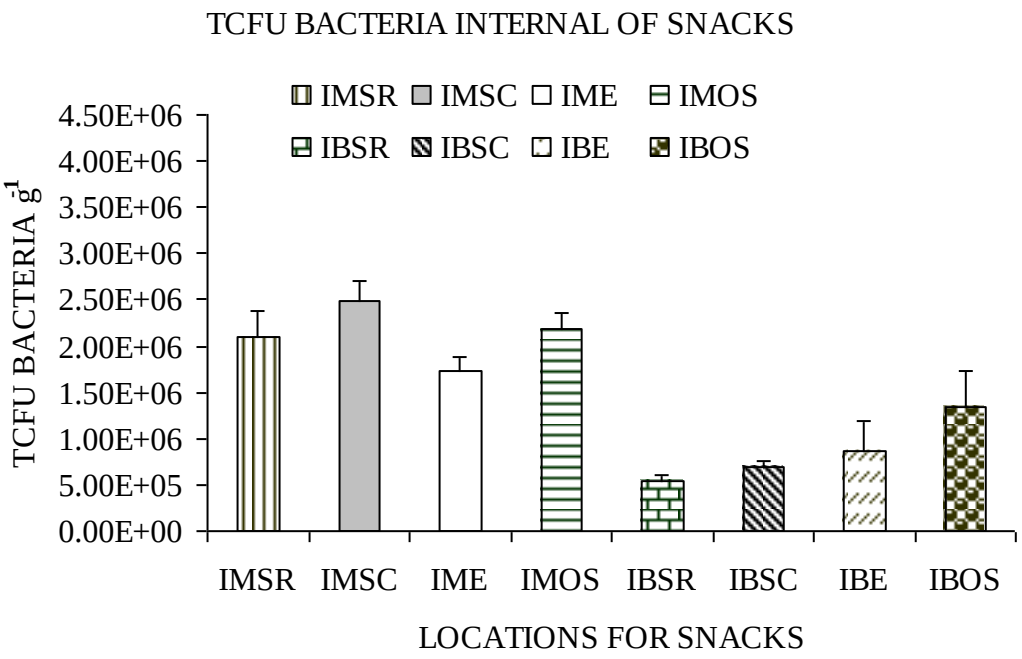

Figure 2. TCFU of bacteria from the interior of snacks (P = <0.001). IMSR = Interior Moin-moin Student Residential; IMSC = Interior Moin-moin Science; IME = Internal Moin-moin Engineering; IMOS = Interior Moin-moin Old Site; IBSR = Interior Buns Student Residential; IBSC = Interior Buns Science Faculty; IBE = Internal Buns Engineering; IBOS = Interior Moin-moin Old Site (Mean n = 3 ± SE).

much handled by persons suspected to harbour potentially pathogenic *S. aureus* the presence of the later would imply faecal contamination. Of particular concern was the occurrence of over 30% Coagulase positive *S. aureus* and over 27% Enterobacteriaceae species in the interior of the snacks. This would imply poor handling and partial cooking of the snacks (especially of the hurriedly fried buns which brown-crusted exterior could be misinterpreted to imply complete cooking) and or use of water of low quality. Ibiebele and Sokari (1989), had studied domestic water usage in Port Harcourt Metropolis and indicated that water sources of questionable microbiological quality such as well water, rain water and pipe-borne water were much used. The assessment of the locations indicated that the highest incidence of exterior contamination occurred in MOS followed by the ME and BSR. Contamination of the interior of the snacks occurred more in the BE followed by BSR. Thus, it could easily be inferred that for exterior and internal assessments of the snacks, the Engineering Faculty appeared to be more inundated by hawkers of questionable state of hygiene or that the environment of the Faculty of Engineering was more unsanitary than the other locations. This factor appear to be justified by the fact that of all the locations under study, the Faculty of Engineering lacked peripheral fencing and thus there was

no control on the sanitary state and type of hawkers that were allowed to sell snacks.

The study provided a simple method for environmental survey of probable dissemination of pathogens in the community. The first indication was that there was need to exercise control over hawkers of all types in the community. Secondly, that the handling, preparation and appropriate packaging of foods, should be given more serious consideration.

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