FULL LENGTH RESEARCH ARTICLE

ENTEROTOXICITY OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BEANS PUDDING

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ABSTRACT
36 samples of beans pudding from selected sources were analysed for *Staphylococcus aureus* and *Bacillus cereus* using standard protocols aimed at assessing its bacteriological quality. Samples obtained from restaurant showed slightly lower value for total plate count (1.3×10^4 - 1.6×10^6 cfu/gm) compared to samples from cafeteria (2.7×10^4 - 2.6×10^6 cfu/gm) and hawkers (3.1×10^4 - 3.2×10^6 cfu/gm). Count of *S. aureus* from samples obtained from hawkers varied between 1.2×10^3 - 2.0×10^6 cfu/gm while those of cafeteria and restaurant showed counts varying between 1.3×10^3 - 1.0×10^5 cfu/gm and 2.2×10^3 - 1.1×10^5 cfu/gm respectively. The counts for *B. cereus* from samples obtained from hawker ranged from 1.0×10^2 - 1.3×10^4 cfu/gm, while those from cafeteria and restaurant ranged from 1.0×10^2 - 2.10×10^4 cfu/gm and 1.0×10^2 - 1.0×10^4 cfu/gm respectively. 53% of the 13 isolates of *S. aureus* produced enterotoxin A but non produced enterotoxins C and D. The results from this study showed that *S. aureus* is common in vended beans capable of producing pathogenic enterotoxin A.

Keywords: *Staphylococcus aureus,* moin-moin, enterotoxin, enterotoxicity, cellophane

INTRODUCTION
The steamed beans pudding or moin-moin made from cowpea (*Vigna unguiculata (L) Walp)* is a popular meal in Nigeria and other West African Countries (Anthonio & Isoun 1982). It is common in home diets, restaurants and ceremonial occasions. Its consumption cuts across tribe and creed by virtue of its popularity among the few indigenous foods that are now being projected at public eating-places (Okoli 2002).

The microbiological quality of food item is determined by microbiological examinations including total aerobic plate count in order to ensure that they meet the required microbiological standards specified by FAO (1979).

Bacillus cereus is a pathogen of public health importance because of its ability to produce toxins that cause intoxicative food poisoning (Geopfert *et al.* 1972). The two major toxins produced by *Bacillus cereus* are diarrhea toxin and emetic toxin (Geopfert *et al.* 1972; Dinges *et al.* 2000)

Staphylococcal food poisoning is of major concern in public health programs worldwide (Adekeye & Adesiyun 1984). The predictive models for *S. aureus* growth and Staphylococcal enterotoxin (SE) production would be powerful tools that have great potential in microbial risk assessment, especially in the food industries (Huang & Bergdoll 1970; Schmitt & Spero 1983; Huang *et al*. 1987). This study was carried out to assess the bacteriological quality of local vended beans pudding in Kaduna town, Nigeria.

MATERIALS AND METHODS
36 samples of beans pudding were collected from different food selling out-lets in Kaduna town Nigeria, 12 each from Hawkers, Cafetaria and Restaurants respectively. Samples collected were wrapped aseptically in a clean polythene bag and carried to the laboratory immediately for analysis. The number of samples required for the study was calculated from an estimated variability of level of *S. aureus* in 16 ad-hoc samples of beans pudding examined in preliminary experiments which indicated that at the 95% confidence level, 36 samples will be required to give a sampling error of less than 1% with a variability of $\pm$ 2.8.

Food homogenate was prepared by weighing 10 g of moin-moin into 90 cm^3 sterile peptone water. This was homogenized in a sterile blender and serial dilutions made from the method described by FAO (1979).

Total aerobic plate count: Total plate count was prepared by pipetting 0.1cm^3 of appropriate dilution (10^3, 10^7) into marked solidified standard plate count agar. The inoculum was spread on the plate and incubated at 37$\pm$2°C for 24-48 hr. Colonies that developed were counted and gram stained. The total aerobic plate counts were computed by multiplying the number of colonies by the dilution factor.

Enumeration of *B. cereus*: 0.1cm^3 of diluted homogenate was spread onto the egg yolk with polymisin B Sulphate medium (oxide) and incubated at 37$\pm$2°C for 24-48 hr. Colonies with dense halo of precipitate (leathinase activity) were counted and the total number of *B. cereus* in the original samples was computed. Colonies were further confirmed by biochemical tests using standard protocols (Geopfert *et al.* 1972)

Enumeration of *S. aureus*: On to sterile plate of Manitol salt agar was transferred 0.1cm^3 of the dilution (1:10, 1:100 and 1:1000) onto the surface of dried agar plate. The inoculum was then spread with an alcohol flamed bent glass rod.

The plates were incubated at 37 °C for 24-48 hr. Yellow colonies appearing after 24hr with evidence of mannitol fermentation were counted. Representative colonies of *S. aureus* from mannitol salt were gram stained and gram positive *cocci* in clustered forms were purified by streaking on nutrient agar plate and incubated for another 24 hr at 37 °C. The isolates were preserved on nutrient agar slants for identification by biochemical tests which includes catalase test, carbohydrate fermentation, coagulate test, Dnase production, gelatinase production, β haemolysis on blood agar, lecithinase test and nitrate reduction test.

Production of *S. aureus* enterotoxin: The cellophane over agar method of Robbin *et al*. (1984) was used with a 5 cm diameter filter paper. Cellophane discs were cut from 3¼ inch dialysis tubing and placed alternatively with filter papers moistened with distilled water to eliminate wrinkling of the cellophane discs.

The petri-dish containing the disc was sterilised at 121 ^{o}C for 15 min. BHI agar (15-20 cm^3) in plates and BHI broth (5cm^3 in screw capped tubes) were prepared. Sterile cellophane was aseptically placed onto the BHI agar plates and 0.1cm^3 of BHI culture of the test strains transferred on to the surface of thin cellophane disc and spread evenly with sterile bent glass rod. After 24 hr of incubation, the resulting growth was harvested using 2.5 cm^3 of 0.1mol/dm^3 disodium hydrogen phosphate (Na_2HPO_4) and was centrifuged at 2000 rpm. The supernatant fluid was then decanted into clean sterile Bijou bottles and analysed for enterotoxin. Where the analysis was delayed, it was frozen until when required.

Enterotoxin assay: The supernatant was tested for enterotoxins A, C and D by double gel diffusion technique of Casman & Benhete (1965) using standard antiserum and enterotoxin.

Taping and pre-coating: Electrician insulating tape was wrapped twice round each end of clean dust free slides, leaving 2 cm space between two ends. The surfaces between the tapes were pre-coated with 0.2 % Bacteriological agar.

Preparation of microslide: 2 cm^3 of 1.2 % melted agarose was placed on the pre-coated slide. The lubricated template was immediately layered on the agar.

Sterile Pasteur pipette was used to drop antiserum into the centre wall of the template. The standard enterotoxin was placed on top well, while unknown samples were placed in the other wells. The tests were carried out in triplicate and the slides were incubated at 25 ^{o}C for 48 hr in humid chambers. After incubation period, the slides were immersed directly into thiazine red solution for 5min and then transferred to 1% Trichloro acetic acid (to enhance the precipitated line for easy reading).

RESULTS

All the 36 samples of beans pudding collected from three different sources indicated the presence of *S. aureus* (Table 1). The mean microbial counts were 5.3 x 10^4cfu/gm for Hawkers, 3.9 x 10^4cfu/gm for Cafeteria and 1.8 x 10^4cfu/gm for samples obtained from Restaurants.

Table 2 showed the results of enterotoxin production from 24 randomly selected isolates, with 13(53 %) of the isolates producing enterotoxin A while none produced enterotoxin C and D. Enterotoxins B and E were not tested for due to non-availability of the specific antisera.

TABLE 1. LEVEL OF CONTAMINATION OF BEANS PUDDING WITH *STAPHYLOCOCCUS AUREUS*

SAMPLE SOURCE	RANGE OF COUNTS (cfu/gm)	MEAN COUNTS (cfu/gm)
Hawkers	1.2 x 10^3 - 2.0 x 10^5	5.3 x 10^4
Cafeteria	1.3 x 10^3 - 2.0 x 10^5	3.9 x 10^4
Restaurant	2.2 x 10^2 - 1.1 x 10^5	1.8 x 10^4

TABLE 2. ENTEROTOXIN PRODUCTION PATTERN BY ISOLATES OF *STAPHYLOCOCCUS AUREUS*

ENTEROTOXIN	NO. ISOLATES TESTED	NO. (%) ISOLATES POSITIVE FOR ENTEROTOXIN
A	24	13(53.0)
C	24	0(0.0)
D	24	0(0.0)

DISCUSSIONS

After preparation, Moin-moin meals are mostly kept at ambient temperature until they are consumed, a situation that encourages the proliferation of pathogenic microbe species that may lead to food intoxication (Bryan 1988). The results from this investigation revealed higher *S. aureus* count in samples from Hawkers compared to those from Cafeteria and Restaurant. High level of exposure to microbial contamination resulting from poor handling may be responsible for this observation because hawkers carry their wares from place to place and exposed looking for buyers.

Enterotoxin A is most often implicated in cases of *Staphylococcal* food poisoning in man (Notermans & Heuvelman 1984). This could be attributed to several reasons including the ability of *S. aureus* to grow in a wide range of temperature between 7-48.5 ^{o}C (Schmitt *et al.* 1990) and pH of 4.2-9.3 (Bergdoll 1989). These conditions support the growth of the bacteria in a wide variety of foods (Bergdoll 1989).

The fact that 53% of the tested isolates produced enterotoxin A, but non produced entrotoxin C and D may be an indication of contamina

-tion by handling after processing (Bergdoll 1989) since enterotoxin A is predominantly associated with humans (Notermans & Heuvelman 1984). Adekeye & Adesiyun (1984) reported the isolation of enterotoxigenic agent of *Staphylococci* from the milk of a nursing mother in Kaduna, Nigeria, and found that Enterotoxin A dominated the enterotoxins produced. *Staphylococcal* enterotoxins are prototype superantigens with the ability to bind to major molecules and activate a large fraction of T-lymphocytes (Schad *et al*. 1995; Dinges *et al*. 2000).

Among the bacterial toxins, enterotoxins are unique because they are heat resistant. This account for their ability to tolerate the heat treatment given to beans pudding before consumption following storage. Enzymes produced by *S. aureus* that showed association with enterotoxigenecity are β-heamolysis, lecithinase and Dnase: The association of coagulase with enterotoxin production as found in this study, agreed with the observations of Bergdoll (1983).

The detection of pathogenic organism such as *S. aureus* in vended beans pudding that is mostly patronized by low income people portends great danger to the population. Measures such as refrigeration and wholesome packaging should be encouraged to reduce excessive microbial contamination of the product. In addition, heat treatment of stored beans pudding should be carried out at elevated temperature before consumption.

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