Occurrence of food borne pathogens in ready-to-eat foods sold in restaurant at Dutsin-Ma Town, Katsina State, North-Western Nigeria.

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Abstract

The bacteriological quality of forty-five ready to eat food samples sold at various restaurants in Dutsin-Ma Restaurants was analyzed for the presence of food borne pathogens. The food samples analyzed were Rice, Beans, Pounded Yam, Stew and Akara from three different Restaurants at Dutsin-Ma metropolis. The highest mean bacterial count was 4.30 x 105 cfu/g, while the highest mean total coliform count was 7.6 x 103 cfu/g. Coagulase negative *Staphylococcus aureus* has the highest prevalence 40(64.5%) while *Shigella* has the least 1(1.60%). The findings in this study revealed high bacterial load in the sample analyzed s the presence of these food pathogens at high limit pose great danger to consumers.

Keywords: Food microbiology, *Escherichia coli*, *Staphylococcus aureus*, Food borne pathogens, Food handling, Restaurants.

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Introduction

Street vended foods mean ready to eat foods and beverages that are prepared and sold especially in streets or similar public places by the street vendors or merchants for consumption at the location or later without any further preparation. The street vended foods are prepared under unhygienic conditions and displayed openly to a high degree of contamination. Street foods are sometimes stored at improper temperatures and sold from vending sites which includes kiosks, make-shift accommodation, and push carts as well as other temporary structures. In most cases running water is not available at vending sites, washing of hands and crockery are done in bowls or buckets and sometimes without soap. Thus from the health point of view, selling foods in the street is very controversial. The street food industry plays an important role in developing countries particularly following the food demands of urban dwellers. Street foods feed millions of people daily with a wide variety of foods that are relatively cheap and easily accessible. Street foods are sources of nutrition for many low-income groups at affordable prices in large urban areas. These street foods could be main vehicles for the transmission of severe food borne infections and fatal disease that could be life-threatening [1].

Food borne illness caused by microbial contamination of foods is an important international public health problem and is known to be a major cause of diarrhoeal diseases especially in developing countries [2,3]. In these developing countries a major source of ready- to-eat foods are prepared and or sold at public places such as schools, market places and along the streets, all together termed Street Foods (SFs). The SFs offer food at relatively cheaper cost and at easily accessible places. Furthermore, it offers the traditional meals and preparations of a number of them are quite laborious and time consuming. Thus, with the increase in the number of hours spent at work places by parents (especially mothers) and schools; the importance of SFs in the provision of nutritional requirements is increasingly becoming very important among all socio-economic groups [4]. However, a number of observational studies have shown that these foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings [5].

In addition, the vendors practice poor personal hygiene and reports of food vendors being carriers and therefore could serve as a potential source of transmission of enteric fevers are many. Most of the vendors have had either no formal education or few years of schooling and therefore, lack knowledge on proper food handling and their role in the transmission of pathogens. At the same time, most of the people who patronize these foods are more interested in its convenience than the question of its bacteriological quality and hygiene. The bacteriological quality of food indicates the amount of bacteria contaminants it has; a high level of contamination indicates low quality and more likely to transmit infection and the reverse is true. Thus

concerns have been raised by the Food and Agricultural Organization (FAO) and others about these foods serving as a potential source of food poisoning outbreaks [6,7].

In addition to SFs, the consumption of locally made Fast -Foods (FFs) like in developing economies is increasingly gaining popularity among the people of Dutsin-ma especially within the students. The FFs are foods that the preparations do not take much time compared to the typical location dishes that take long time to cook. These chains usually provide foods such as the corrupted Asian nasi goring locally termed fried rice, fried chicken and salads. These foods are usually sold from enclosed buildings and to the best of my knowledge there are little or no studies that have analyzed their microbial quality.

Food is any substance that people or animal eat or drink or that plants absorb to maintain life and growth. Food is any substance consumed for nutritional support for the body; it is usually of plant or animal origin. Food consists of chemical compounds which heterophilic living thing consumes in order to carry out metabolic processed. They are also substances which when introduced to the digestive system under normal circumstances contribute to growth, repair and production of energy. Foods are classified into six essential nutrients known as protein, carbohydrate, vitamin mineral, fat and oil, water.

Materials and Methods

In Dutsin-ma, hawking of cooked ready-to-eat street foods is very common. These food vendors enjoy huge patronage from different societal classes especially from students. Unfortunately, none of these food vendors is licensed or monitored by relevant agencies saddled with the responsibility of ensuring the safety of foods. Thus, owing to the manner and conditions these vendors operate, there is likelihood that some of the cooked ready-to-eat street foods may be contaminated by foodborne pathogens. Although, there is information about bacteriological quality of KununAya sold in Dutsin-ma metropolis, no information is available regarding the bacteriologiological quality of cooked ready-to-eat street foods sold in Dutsin-ma. Hence, the present study was undertaken to determine the bacteriological assessment of cooked ready-to-eat street foods sold in Dutsin-ma metropolis for the presence of *Bacillus cereus* and *Staphylococcus aureus*. The work will be of immense benefit to the unsuspecting consumers, government health agencies and the vendors on any health risk such food(s) might pose.

A lot of works had been published in related to this research topic in the past and the present but however very low turnout is recorded in Katsina state.The level of awareness and understanding of the dangers of Ready to Eat Foods among the people of Dutsin-Ma is very low.

Contamination and growth of pathogens such as *Staphylococcus aureus, Salmonella species, Shigella, Bacillus species, Pseudomonas aeruginosa, Clostridium species, Vibrio cholerae* and *Escherischia coli* can result in perceptible changes in quality of food [8].

Food borne diseases remain an important public health problem worldwide which has been reported associated with Ready to Eat Foods. This gives basis to the vulnerability for the bacteriological assessment of Ready to Eat Foods sold in Dutsin-Ma metropolis.

This research is aimed at assessing the bacteriological quality of ready to eat foods sold in Dutsin-Ma metropolis of Katsina state.

Sample collection

A total of forty-five RTE and vended food samples (9 samples each of 5 different types of Pounded Yam, Rice, Beans, Stew and Moimoi) three samples at once were purchased from different food vending sites and cafeterias in and around the Federal University Dutsin-ma, (FUDMA), within a period of five weeks. Food samples purchased were appropriately labeled, inserted into a sterile polythene bag and transferred to the laboratory for immediate analysis.

Sample processing

Five grams of food samples were homogenized in 45ml of buffered peptone water and shaken vigorously using vortex to dislodge adhered bacteria [9]. The homogenate sample gave 1:10 dilution from which further dilution was made by adding 1ml of homogenate into 9ml of buffered peptone water. Depending on the level of contamination, serial dilutions of 10^{-2} and up to 10^{-5} were also prepared for Total AerobicMesophilic Bacteria Count (TAC), Coliform Count, Enumeration and Identification of Coliforms using isolation of pathogens (MPN) [10].

Total viable counts (TVC)

Total Viable Counts (TVC) as a Microbiological analysis was carried out using the serial dilution technique with pour plating unto Nutrient agar (NA) [11,12].

Presumptive test for escherichia coli

Culture media (Eosine Methylene Blue Agar) was prepared according to manufacturer's specification and sterilization of materials was done in an autoclave at 121°C for 15 minutes. Presumptive analysis for E. coli was done using serial dilution technique with spread plating unto MacConkey Agar, a selective/differential isolation media, for assessment of lactose fermenting bacteria from non-lactose fermenters. The fourth dilution was used for plating unto this medium. This was incubated for 24-48 hrs for growth [13].

Confirmatory test for escherichia coli

Indole test: The test organism was inoculated in a bijou bottle containing 3 ml of sterile peptone water. It was incubated at 35-37 °C for up to 48hours. Half a milliliter (0.5 ml) of

Kovac's reagent was added to test for indole and the contents was gently shaked. It was examined for a red colour in the surface layer [12].

Methyl red test: A small quantity of the organism was inoculated into the sterile glucose phosphate peptone water medium and incubated at 37°C for 48 hours. Then five drops of the methyl red reagent was added into the culture after incubation, mixed and read immediately [12].

Voge's-Proskauer test: Two milliliters (2 ml) of sterile glucose phosphate peptone was inoculated with the test organism then incubated at 37°C for 48 hours. A milliliter (1 ml) of 40% potassium hydroxide and 3 ml of 5% solution of alphanaphtol in absolute alcohol was added after incubation. The tube was shaked at interval to ensure maximum aeration [12].

Citrate utilization test: Simon's citrate agar medium was used. The sterile medium was inoculated from a saline suspension of the test organism and incubated for 96 hours at 37°C [12].

Presumptive test for staphylococcus aureus

Culture media (Mannitol Salt Agar) was prepared according to manufacturer's specification and sterilization of materials was done in an autoclave at 121°C for 15 minutes. Presumptive analysis for *Staphylococcus aureus* was done using serial dilution technique with spread plating unto Mannitol Salt Agar. This was incubated for 24-48hrs for growth [14].

Confirmatory test for S. aureus

Catalase test: A drop of the hydrogen peroxide solution was placed on a glass slide while a loop full of the test organism was picked and immersed into the glass slide containing the hydrogen peroxide [9,12].

Coagulase test: A drop of physiological saline was placed on a clean microscope slide. Then a colony of the test organism was emulsified in the drop of saline to form a smooth milky suspension. A drop of plasma was added to the suspension and was gently mixed [9,12].

Test for salmonellaspp and shigellaspp

Samples were poured into the *Salmonella Shigella* agar plates and incubated overnight. Then streaked with sterile loops (sub-cultured) on the the Triple Sugar Iron Agar and incubated in aerobic condition at 37ºC for 18-24 hrs for selective growth of the organisms [9].

Confirmatory tests for salmonellaspp and shigellaspp

Oxidase test: A piece of filter paper was placed in a clean petridish and 2 or 3 drops of freshly prepared oxidase reagent added as a piece of stick or glass rod was used to remove a colony of the test organism and smeared on the filter paper. It was observed for the development of a blue-purple colour within a few seconds.

Urease test: The medium Christensen's medium, was prepared and autoclaved at 121ºC for 30 minutes, pH 6.8 glucose and urea sterilized by steaming at 100ºC for 15 minutes were added and mixed, then poured into tubes as deep slopes. The test organism was inoculated on the agar slopes and incubated at 37ºC for 4 hours and then overnight.

Statistical analysis

The values obtained for total aerobic plate count, *Escherichia coli* and *Staphylococcus aureus* counts were subjected to analysis of variance.

Results

Poor handling of food pre- and post-preparation should be minimized by creating awareness on the need for personal hygiene and care in preparation, storage and dispensing of ready to eat foods to reduce the health hazards. It is also necessary that governmental agencies responsible for maintaining the food standard in Nigeria to set up systems to ensure that food handlers remain aware of all procedures necessary to maintain the safety and suitability of food. Basic training in food hygiene is recommended to ensure that food vendors follow the required rules for proper hygiene and sanitation. Training on hygiene and sanitation, provision of continuous food safety education, screening of food handlers on regular basis for carriers, the establishment of code of conducts for the street food industry and provision of basic water and waste management utilities are recommended to diminish the gap between knowledge and practices of safe ready to eat foods [15-18].

Finally, it is hereby recommended that no interested student should be averted from further analyses on this same topic. Only that, the student should be inspired, encouraged and well guided especially to include the Antibacterial susceptibility test which is excluded in this research due to time factor [19-23].

Table 1. *Total mean coliform counts of the ready-to-eat foods sold in dutsin-ma metropolis (cfu/ml).*

Samples/sample site	A	B	C
Rice	3.20×10^3	6.70×10^3	1.80×10^3
Beans	2.40×10^3	6.10×10^3	3.00×10^3
Pounded yam	1.70×10^3	2.80×10^3	1.30×10^3
Stew	6.70×10^3	3.90×10^3	4.60×10^3
Akara	4.00×10^3	7.40×10^3	5.20×10^3

Table 2. *Mean total aerobic bacterial counts of the ready-to-eat sold in durtsin-ma.*

Samples/sample site	A	B	C
Rice	3.20×10^5	2.80×10^5	4.30×10^5
Beans	2.40×10^5	1.90×10^5	3.60×10^5
Pounded yam	1.70×10^5	2.90×10^5	2.00×10^5
Stew	3.70×10^5	3.00×10^5	3.40×10^5
Akara	1.90×10^5	2.70×10^5	2.40×10^5

Table 3. *Mean bacterial counts of ready-to-eat foods sold in dutsin-ma metropolis (cfu/g).*

Sample site	Food type	Aerobic Plate Counts (APC)	*E. coli counts*	*S. aureuscounts*
	Rice	3.20×10^5	3.00×10^5	2.00×10^5
	Beans	2.40×10^5	1.80×10^5	2.80×10^5
A	Pounded yam	1.70×10^5	1.30×10^5	4.50×10^5
	Stew	3.70×10^5	ND	2.20×10^5
	Akara	1.90×10^5	2.8×10^5	5.80×10^5
	Rice	2.80×10^5	2.00×10^5	2.90×10^5
	Beans	1.90×10^5	1.80×10^5	3.90×10^5
B	Pounded yam	2.90×10^5	1.30×10^5	3.80×10^5
	Stew	3.00×10^5	2.4×10^5	3.20×10^5
	Akara	2.70×10^5	2.90×10^5	5.40×10^5
	Rice	4.30×10^5	2.20×10^5	4.20×10^5
	Beans	3.60×10^5	1.70×10^5	4.00×10^5
C	Pounded yam	2.00×10^5	1.20×10^5	2.40×10^5
	Stew	3.40×10^5	2.20×10^5	1.50×10^5
	Akara	2.40×10^5	2.80×10^5	2.40×10^5

Table 4. *Bacteria isolated from ready-to-eat foods sold in dutsin-ma metropolis.*

Sample site	Food type	*E. coli counts*	*S. aureuscounts*	*Salmonella counts*	*Shigellaspp*
	Rice	+	+	-	-
	Beans		+	-	-
A	Pounded yam	+	+	-	-
	Stew	+	+	-	
	Akara	+	+	+	-
	Rice	+	+	-	-
	Beans	+	+	-	+
B	Pounded yam	+	+	-	-
	Stew	+	+	-	-
	Akara	+	+	+	-
	Rice	+	+	-	-
	Beans	+	+	+	-
C	Pounded yam	+	+	-	-
	Stew	+	+	-	-
	Akara	+	+	-	+

Source: +=Present -=Absent

Table 5. *Morphological appearance and biochemical characteristics of the bacteria isolates.*

Parameters	Staphylococcus aureus	E. coli	Salmonella spp	Shigellaspp
Gram reaction	+	-	-	-
Catalase test	+	+	N/A	
Citrate test	-	+	+	+
Coagulase test	+	-	N/A	N/A
Indole test	-	+	N/A	N/A
Urease test	+	-	-	+
Motility test	-	-	+	-
Cellular morphology	Cocci	Short Straight rods	Short rods	Rods
Growth in macconkey	N/A	Pinkish red	Colorless	Colorless

Growth in mannitol salt agar	Bright yellow	N/A	N/A	N/A
Growth in eosine Methylene blue agar	N/A	Green metallic sheen with dark center	Colorless N/A	Colorless N/A
Reaction on TSI	N/A	N/A	Alkaline/acid+H_2S	Alkaline/Acid+no gas production

Source: +=Growth - =No growth N/A=Not Applicable

Table 6. *Prevalence of the bacterial isolates from ready to eat foods sold in dutsin-ma metropolis.*

Isolate	Number of sample	Prevalence	Percentage (%)
S. aureus	45	35	56.45
Coagulase negative *Staphylococcci*	45	5	8.06
E. coli	45	17	27.42
Salmonella spp.	45	4	6.45
Shigela spp.	45	1	1.61
Total	45	72	100

Table 7. *Anova.*

Samples/sample site	Groups	Sum of squares	df	Mean square	F	Sig.
	Between groups	1.21E+10	2	6.03E+09	.	.
Rice	Within groups	0	0	.		
	Total	1.21E+10	2			
	Between groups	1.53E+10	2	7.63E+09	.	.
Beans	Within groups	0	0	.		
	Total	1.53E+10	2			
	Between groups	7.80E+09	2	3.90E+09	.	.
Poundedyam	Within groups	0	0	.		
	Total	7.80E+09	2			
	Between groups	2.47E+09	2	1.23E+09	.	.
Stew	Within groups	0	0	.		
	Total	2.47E+09	2			
	Between groups	3.27E+09	2	1.63E+09	.	.
Akara	Within groups	0	0	.		
	Total	3.27E+09	2			

Discussion

Pathogenic bacteria are the most common known causes of food contamination and food borne illnesses. Out of the 45 randomly selected food samples examined, 41 (91.11%) showed bacterial growth, from which a total of five bacterial species were identified. Four (8.89%) of the 45 samples showed no bacterial growth, 40(97.56%) of the 41 (91.11%) contaminated, yielded Gram Positive organisms while 10(24.39%) were Gram Negative which when tested statistically was significantly different ($P<0.05$). *Salmonella* and *Shigella* were also detected in the analysis. The bacterial genera in decreasing order of occurrence are Staphylococcus 40(64.52%), Escherichia 17(27.42%), *Salmonella* 4(6.45%) and *Shigella* 1(1.60%). Among the bacteria isolated, the Genus Staphylococcus ranked highest with (64.52%). This result shows correlation with the report given by Chinedumet [8] when worked on ready to eat food sold in Enugu metropolis, Nigeria where they isolated (61%) Staphylococcus spp. this associationbetween these two results may be due to the similarity in the environmental conditions, ignorance and unhygienic condition of most of the restaurant owners and hawkers. *Staphylococcus aureus* and Coagulase negative *Staphylococci* are most often found on soil and on the mucous membrane. Contamination with *Staphylococcus aureus* might is direct during sales or unhygienic handling of the food during processing or due to contamination from the skin, mouth, or nose of the handlers which can be introduced directly into foods. *Staphylococcus aureus* are responsible for many pyogenic infections of man [24-30]. *Staphylococcusis* a common inhabitant of the skin and mucous membranes, and it accounts for a considerable proportion of human infections. Most of the strains have been shown to be multiple drugs resistant, they are responsible for about 13% of the 2 million hospital infections reported each year. They are implicated in nearly 80,000 deaths nationwide and should not be neglected when isolated in food. *Escherichial* isolated in the food samples takes (27.42%). This disagrees with the work reported by Chinedumet [8] where they reported 14 (9.3%) in 104 food samples of cooked ready-to-eat street sold in Enugu metropolis. The presence of *Escherichia coli* in foods reflects secondary contamination, as *Escherichia coli* is known to

be associated with gastrointestinal tract of warm blooded animals, and not known to be present in the environment as a natural flora [11]. However, contamination could be through the use of non-portable water. *Escherichia coli* has been reported by, 5 (10.0%) of 50 food handlers in three small-scale food industries in Kano Metropolis investigated carried E. coli on their hands [31-33]. This percentage could easily cross-contaminate a whole production batch unnoticed. Ironically, most food handlers do not practice good personal hygiene and do not follow good manufacturing practices, which could reduce the occurrence of such bacteria in foods [34]. Nonetheless, the disagreement between these food samples may be attributed to the high level of environmental contamination as the town has only one water source.

The isolation of *salmonella* and *Shigellaspp* are however agreed with the report by Temesgenet when conducted a Bacteriological quality of Street foods in Hawassa Ethopia where they reported 9(12.70%) *Salmonella* isolates from 72 food samples, however no *shigella* was obtained. This difference in the *Shigella* report may be as a result of different food samples used for analysis [35,36].

In this study, the Mean Total Coliform Count ranged from 1.30×10^3 to 7.6×10^3 cfu/g which is much lesser compare to 5.0×10^4 cfu/g (yam) to 1.0×10^7 cfu/g (suya) reported by Maduekeet [12] in Lokoja, Nigeria. However, this finding was in line with 2.3×10^1 - 3.8×10^7 cfu/g as documented in Onitsha-Owerri Highway by Oranusi and Braide [11]. Similarly, the MeanAerobic Bacterial Count of: 1.7×10^5 to 4.30×10^5 cfu/ml was also recorded.

Conclusion

The findings of this study of some cooked ready-to-eat foods samples vended in Dutsin-Ma metropolis revealed high bacterial load in all the food samples and showed that the total aerobic counts, *Escherichia coli*, and *Staphylococcus aureus* counts were above the acceptable limit. The presence of these organisms, *Salmonella* and *Shigellaspp* inclusive in the cooked ready-to-eat foods poses great danger to the consumers and the entire inhabitants of Dutsin-Ma as shielding of these organisms may continue.

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