Special Bacteria Pathogens Journal
(*Spec. Bact. Pathog. j.*); © *Special Pathogens Research Network Ltd, Bushenyi Uganda.* Km 58 Mbarara Fort-portal Rd, Box 324, **RC No: 144926.**
sbpj@spparenet.us ; Tel: +256782101486; ISSN = 2413-600X

Bacterial and fungal burden of local packing papers used for roasted meat in Kano Nigeria

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Citation: Ahmad HB, Bai HMS, Alkali B. Bacterial and fungal burden of local packing papers used for roasted meat in Kano State Nigeria. Spec Bact Pathog J 2016; Vol 2, No 1 and 2: 01-08

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ABSTRACT

Background: The appetite for fast food in developing countries continue to rise although plans to regulate how these food are safely delivered to the consumers are not common if they exist.

Objective: To determine the Bacterial and fungal burdens of local packing papers used for roasted meat in Kano State Nigeria.

Materials and Methods: Standard microbiological methods were used to determine the bacterial and fungal loads of 108 locally improvised packaging papers for fast food roasted meats in strategic locations of the Kano metropolis. The packaging materials were obtained from consenting roasted meat vendors in the Kano metropolis. Appropriate aseptic precautions were adopted to ensure a good, reliable, and reproducible result. **Result:** The mean aerobic bacterial count observed ranged between 9.9 x 10^4 and 2.47 x 10^5 –3.09 x 10^2 cfu/ml and 1, 24 to 3.09 x 10^2 cfu/ml for fungi respectively. High counts of both bacteria and fungi were found in samples of meat wrapping papers. *B. cereus* and *E. coli* were isolated from 55% and 100% of samples of meat wrapping papers respectively. The roasted meat wrapping papers included old newspapers (80%), used exercise books (8%), and disposed of office printed stationeries (12%). The most probable bacterial number MPN showed that the papers for meat packaging were more exposed to contamination (75MPN/ml) while the paper plate had (7MPN/ml).

Conclusion: The papers used for roasted meat fast food were found to be unsafe and unfit for packaging purposes as they can easily be a source of disease epidemic due to the confirmed loads of bacterial and fungal pathogens. The factory sterilized paper plates are also not free from microbial contamination but might be due to exposure to unsanitary storage and marketing conditions during delivery to end-users.

INTRODUCTION

The spread of communicable diseases by personnel who handle ready-to-eat food and also the use of certain food packaging materials constitute a possible health hazard. This phenomenon appears more likely in third-world countries like Nigeria, where public health awareness is poor and where most food vendors are not certified medically by

appropriate food handling authorities (1). One of the most serious and widespread risks from foods is the occurrence of potentially pathogenic microorganisms. The Food and Drug Administration (FDA, USA) and the National Agency for and Drug Administration and Control (NAFDAC-Nigeria), including state and local government health offices, offer strict requirements for the handling of foods to reduce microbial hazard leading to disease outbreaks(2). Mishandling of precooked, frozen, refrigerated, catered, or vended foods may give rise to contamination with potential pathogens, which can cause disease outbreaks. Contamination may come from unsanitary food distributors, poor health practices, such as failure to wash hands thoroughly or have open wounds. Mishandling is probably the most common problem in many food-borne disease outbreaks (1).

The basic materials for food packages and wrapping today include paper board, cellophane, steel, and aluminum. Aluminum foils, cellophane, and other papers meant for packaging are costly and beyond the reach of ordinary street vendors who serve the general public with irresistible roadside ready-to-eat fast food. This may explain why packaging and wrappings are done with improvised dilapidated old newspapers and exercise books destined for recycling. Another list of improvised packaging materials in the study area include but not limited to: Cement bags, disposable papers, old aluminum plates/cups,

Conventional packages forms include wraps, bags, pouches, cartons, set-up boxes, cans, bottles, pails, drums, barrels, and bulk containers (3). These items though manufactured under strict aseptic conditions are later exposed in shops, supermarkets, and other retail outlets, and therefore may be liable to contamination. Food may be contaminated from utensil surfaces and pieces of equipment. Bacteria, including pathogenic organisms, might be left in food particles or moisture on perfectly washed crockery and utensils. It is unsafe to use contaminated utensils and containers particularly for cooked foods not intended for immediate use (4). Outbreaks of food poisonings frequently occur as a result of improper food preparation in which cross-contamination with inadequate storage or cooking was implicated in many instances (5). Dishcloth and sponges meant for cleaning eating utensils were recognized as a potential source of spreading micro-organism, and it was observed that bacteria persisted on these vehicles (6 and 7).

Micro-organisms have a wide distribution and are often found in a wide variety of habitats. A significant number of microbes can survive and use the air as a means of dispersal. There is a possibility of these microbes to be deposited on the eating materials such as paper plates or improvised packing materials such as newspapers, cement bags, milk bags, polythene bags, and many others. In some instances molds may grow on such papers which are ready to use, raising the possibility of packaging mold and fungal spores together with the meat unintentionally. Since it may difficult to prevent or dissuade people from patronizing the roadside fast-food vendors that are likely to use these improvised packaging materials, one way the ignorant and innocent general populace can be assisted may be by determining how safe these packaging materials may be to design effective intervention against potential disease outbreaks. The above reason calls for an investigation into the possible microbial carriage pattern of such wrapping papers and disposable plates. This study was therefore designed to investigate the microbiological safety of disposable roasted meat wrapping papers and plates used in serving and preservation of fast food in homes and cafeterias in Kano. Thus, safety considerations in this study will entail determining bacterial and fungal load, detecting bacterial identities, and using this result to confirm the safety of different packing papers used to wrap meat in the Kano metropolis.

MATERIALS AND METHODS

A total of 108 pieces of paper plates and meat wrapping paper (representing a significant population of the meat vendors in the study area) were collected from three different locations in

each of the six metropolitan areas of Kano State. The samples comprised of 54 samples of each of locally improvised wrapping papers and 54 paper packaging plates respectively.

Analyses of the Samples for bacterial isolation and count

Inoculation of samples was carried out using swab-rinse and standard plate count methods (8). Each of the samples was soaked in 100ml aliquots of sterile buffered (0.1% w/v) peptone water (Oxoid) for 20 minutes at ambient temperature. The resultant washed water was used to carry out serial dilution up to the fifth dilution (2). One milliliter of each dilution was taken using a sterile syringe and placed into each of the appropriately marked duplicate dishes of 15ml PCA (kept at 45^0C in a water bath). Within 15 minutes of the time of original dilution, media were poured into each of the Petri dishes, sample dilution, and agar medium was thoroughly and uniformly mixed and allowed to solidify. The prepared media were inverted and incubated at 30^0C for 72hrs.

Three tubes each containing 9ml MacConkey broth with an inverted Durham tube were each inoculated with 1.0ml of the sample washed in sterile physiological saline to give a 1 in 10 dilutions. These were repeated with 1 in10^2 and 1in10^3 dilutions. All tubes were incubated at 37^0C for 24hrs (2). Tubes that show gas production was recorded. A loopful of broth from each gas positive test tube and the presumptive test tube was inoculated into a separate tube containing brilliant green lactose bile broth (BGLB) and the tubes incubated at 37^0C for 48hours. The formation of gas confirmed the presence of coliform bacteria. The numbers of positive tubes were recorded and the most probable number of the coliforms determined from and most probable number table (2)

Simultaneously with the confirmatory test, transfer was made from positive presumptive tubes to other tubes containing enrichment medium, and the tubes incubated at 37^0C for 24hours. Gas and acid formation was recorded and the bacteria density was estimated from the most probable number table. Gas and acid production confirmed the presence of fecal coliform (2). These tests were carried out by the procedure described by Odoki et al, (9). A loopful from each positive tube from the presumptive test was transferred to a spate tube of *E. coli* broth re-incubated at 37^0C for 48hours. Gas and acid production were recorded. Levine's eosin methylene blue agar (L-EMB) plates were incubated from each positive EC tube and incubated at 35^0C for 18-24hrs. The production of dark purple colonies with metallic sheen confirmed the presence of *E. coli*. Gram staining of the cultures was carried out and the presence of *E. coli* was confirmed by IMViC test (2).

The procedure adopted from FAO (2) and Jean (10) were employed. These tests were carried out to confirm the presence of *E. coli* based on their biochemical characters. These are Indole, methyl red, Voges Proskauer, and Citrate utilization tests (IMViC). The presence of indole is detected by the addition of a reagent called KOVAC'S reagents. The test indicates the presence of indole as a byproduct of the breakdown of the tryptophan radical by some bacteria. One % solution of tryptone was inoculated and incubated overnight at 37^0C. About 0.5ml of KOVAC'S reagent was added and agitated slightly. A dark brown layer indicates the presence of an indole or positive test, while a negative result produces no color change. Positive confirms the presence of *E. coli*. The test indicates the pH of a broth culture in MR-VP medium after two days of incubation at 37^0C. Two to three drops of methyl red solution would be added. A red color indicates a positive reaction while a yellow color shows a negative reaction. MR – VP broth was inoculated and incubated for 48hours at 37^0C. Twelve drops of 5% solution of alpha-naphthol and four drops f 40% potassium hydroxide (KOH) were added. The mixture was agitated vigorously and left to stand. Positive test results in a red-pink color. Simmon's citrate agar slants were streaked on the surface and incubated at 37^0C for 48hrs. Color change from green to blue indicates positive reactive cultures produce no color changes.

Enumeration and Identification of Fungi

Serially diluted 1.0ml aliquots of the sample wash water were poured into sterile Petri-dishes and 1.5ml of potato dextrose agar (PDA) to which 0.5mg streptomycin/liter has been incorporated. These were poured into plates. Plates were allowed to solidify, inverted, and incubated at room temperature for 5days. Isolates were counted, cultural characters studied and microscopic examination carried out and documented. Isolates were identified by comparison with standards reference keys and color atlases (17).

RESULTS

The result of the enumeration of samples for bacterial and fungal counts is as shown in Tables 1. The paper plates samples from Dala local government gave the highest mean bacterial count of 9.9 x 10^4 followed by the mean count of 1.35 x10^5 count for samples from Kano Municipal Council (KMC), the mean count of 1.30 x10^5 count for samples from Gwale local government, mean count of 1.27 x10^5 count for samples from NAS and Tarauni local government respectively. In Table 2 which has details of bacterial count from roasted meat wrapping papers, the samples obtained from Dala local government with a mean bacterial count of 2.47 X 10^5 was the most contaminated followed by; mean count of 2.37 X 10^5 from Nasarawa local government, and mean bacterial count of 2.26 X 10^5 from Tarauni local government.

Again, fungal plates from Dala local government had the highest contamination with a mean bacterial count of 1.35 X10^2 followed by 1.31 X10^2 fungal count from Gwala and KMC while 1.30 X10^2 fungal count were obtained from Tarauni local government Table 3. Mean fungal count from roasted meat wrapping papers obtained from Tarauni local government were the most contaminated followed by mean count of 2.80 X 10^2 from KMC; 2.73 x10^2 from Gwale and 2.60 X 10^2 from Dala local government respectively Table 4.

Table 1: Mean Mesophilic Aerobic Bacterial Count/+ Standard Deviation in Paper Plates from 3 locations

	Location 1	Location 1	Location 1	Mean count
LGA	Cfu/ml			
Dala	0.89 X 10^5 ± 0.16	1.05 X 10^5 ± 0.12	1.00 X 10^5 + 0.22	9.9 X 10^4 ± 0.13
Fagge	1.06 X 10^5 ± 0.16	1.02 X 10^5 ± 0.12	1.1 X 10^5 + 0.22	1.09 X 10^5 ± 0.13
Gwale	1.28 X 10^5 ± 0.16	1.29 X 10^5 ± 0.12	1.32 X 10^5 + 0.22	1.30 X 10^5 ± 0.13
KMC	1.28 X 10^5 ± 0.16	1.33 X 10^5 ± 0.12	1.34 X 10^5 + 0.22	1.35 X 10^5 ± 0.13
NAS	1.26 X 10^5 ± 0.16	1.26 X 10^5 ± 0.12	1.30 X 10^5 + 0.22	1.27 X 10^5 ± 0.13
Tarauni	1.30 X 10^5 ± 0.16	1.17 X 10^5 ± 0.12	1.18 X 10^5 + 0.22	1.27 X 10^5 ± 0.13

Key: KMC-Kano Municipal council, NAS-Nassarawa and TRN-Tarauni

Table 2: Mean Mesophilic Bacterial Count in Paper for Meat Wrapping from 3 different locations

LGA	Location 1	Location 1	Location 1	Mean count Cfu/ml
Dala	$2.5 \times 10^5 \pm 0.18$	$2.42 \times 10^5 \pm 0.18$	$2.46 \times 10^5 + 0.19$	$2.47 \times 10^5 \pm 0.18$
Fagge	$2.09 \times 10^5 \pm 0.18$	$2.02 \times 10^5 \pm 0.18$	$2.08 \times 10^5 + 0.19$	$2.06 \times 10^5 \pm 0.18$
Gwale	$2.20 \times 10^5 \pm 0.18$	$2.18 \times 10^5 \pm 0.18$	$2.30 \times 10^5 + 0.19$	$2.23 \times 10^5 \pm 0.18$
KMC	$2.19 \times 10^5 \pm 0.18$	$2.44 \times 10^5 \pm 0.18$	$2.48 \times 10^5 + 0.19$	$2.37 \times 10^5 \pm 0.18$
NAS	$2.12 \times 10^5 \pm 0.18$	$2.08 \times 10^5 \pm 0.18$	$2.00 \times 10^5 + 0.19$	$2.06 \times 10^5 \pm 0.18$
Tarauni	$2.00 \times 10^5 + 0.18$	$2.42 \times 10^5 + 0.18$	$2.36 \times 10^5 + 0.19$	$2.26 \times 10^5 + 0.18$

Key: KMC-Kano Municipal, NAS-Nassarawa and TRN-Tarauni

Table 3: Mean Fungal Count for Paper Plates obtained from different locations in the metropolis

LGA	Location 1	Location 1	Location 1	Mean count Cfu/ml
Dala	$1.26 \times 10^2 \pm 0.46 \times 10^2$	$1.33 \times 10^2 \pm 0.31 \times 10^2$	$1.46 \times 10^2 \pm 0.21 \times 10^2$	$1.35 \times 10^2 \pm 0.33 \times 10^2$
Fagge	$1.26 \times 10^2 \pm 0.46 \times 10^2$	$1.20 \times 10^2 \pm 0.31 \times 10^2$	$1.26 \times 10^2 \pm 0.21 \times 10^2$	$1.24 \times 10^2 \pm 0.33 \times 10^2$
Gwale	$1.33 \times 10^2 \pm 0.46 \times 10^2$	$1.26 \times 10^2 \pm 0.31 \times 10^2$	$1.33 \times 10^2 \pm 0.21 \times 10^2$	$1.31 \times 10^2 \pm 0.33 \times 10^2$
KMC	$2.40 \times 10^2 \pm 0.46 \times 10^2$	$1.42 \times 10^2 \pm 0.31 \times 10^2$	$1.40 \times 10^2 \pm 0.21 \times 10^2$	$1.31 \times 10^2 \pm 0.33 \times 10^2$
NAS	$1.20 \times 10^2 \pm 0.46 \times 10^2$	$1.30 \times 10^2 \pm 0.31 \times 10^2$	$1.20 \times 10^2 \pm 0.21 \times 10^2$	$1.27 \times 10^2 \pm 0.33 \times 10^2$
Tarauni	$1.30 \times 10^2 \pm 0.46 \times 10^2$	$1.26 \times 10^2 \pm 0.31 \times 10^2$	$1.33 \times 10^2 \pm 0.21 \times 10^2$	$1.30 \times 10^2 \pm 0.33 \times 10^2$

Key: KMC-Kano Municipal, NAS-Nassarawa and TRN-Tarauni

Table 4: Mean Fungal Count for ± Standard Deviation for Paper for Meat Wrapping

LGA	Location 1	Location 1	Location 1	Mean count Cfu/ml
Dala	$2.93 \times 10^2 \pm 0.41 \times 10^2$	$2.47 \times 10^2 \pm 0.45 \times 10^2$	$2.40 \times 10^2 \pm 0.31 \times 10^2$	$2.60 \times 10^2 \pm 0.36 \times 10^2$
Fagge	$2.07 \times 10^2 \pm 0.41 \times 10^2$	$2.20 \times 10^2 \pm 0.45 \times 10^2$	$2.13 \times 10^2 \pm 0.31 \times 10^2$	$2.14 \times 10^2 \pm 0.36 \times 10^2$
Gwale	$2.47 \times 10^2 \pm 0.41 \times 10^2$	$2.93 \times 10^2 \pm 0.45 \times 10^2$	$2.80 \times 10^2 \pm 0.31 \times 10^2$	$2.73 \times 10^2 \pm 0.36 \times 10^2$
KMC	$2.87 \times 10^2 \pm 0.41 \times 10^2$	$2.93 \times 10^2 \pm 0.45 \times 10^2$	$2.60 \times 10^2 \pm 0.31 \times 10^2$	$2.80 \times 10^2 \pm 0.36 \times 10^2$
NAS	$2.27 \times 10^2 \pm 0.41 \times 10^2$	$2.07 \times 10^2 \pm 0.45 \times 10^2$	$2.40 \times 10^2 \pm 0.31 \times 10^2$	$2.25 \times 10^2 \pm 0.36 \times 10^2$
Tarauni	$3.13 \times 10^2 \pm 0.41 \times 10^2$	$3.13 \times 10^2 \pm 0.45 \times 10^2$	$3.00 \times 10^2 \pm 0.31 \times 10^2$	3.09×10^2

Key: KMC-Kano Municipal, NAS-Nassarawa and TRN-Tarauni

DISCUSSION

Among the two varieties of samples analyzed highest mean bacterial count of 2.47 x 10^5 cfu/ml was obtained from wrapping papers for roasted meat. The highest microbial count obtained from paper packing plates was 1.35 x 10^5 cfu/ml as shown in table 1 and 2. High count obtained from locally improvised wrapping papers compared to professionally design packaging plates maybe because locally improvised wrapping papers are waste papers due for recycling and obtained from dust bins, warehouses, kitchen/office storerooms, and exercise books abandoned by kindergarten and primary school children after being completely mutilated. Both packing paper plates and locally improvised wrapping papers are not sterilized in any way before use neither is there plan by constituted authorities, to sterilize or regulate sterilization before use. The above scenario complicates the already complex public health challenge of controlling disease epidemics in resource-poor settings because the public appetite for such roadside food continues to increase with the increasing potential danger posed by such food to the populace.

Although we did not investigate what fuels the appetite for such roadside roasted meat, with potential health risk, it appears cost, ready-to-eat nature of the food, availability when needed and poor time management by customers may be playing a significant role in the patronage of roadside meat vendors. This is different when it is compared to expensive, impatience to procedural hygiene protocol for food preparation common at big hotels and restaurants, the need to place a food order and wait for a specified time before a portion of such meat is served, thus making people patronize the fast food vendors more than the standard food centers.

Again, the handlers and the poor aseptic environment where such meat is packaged are also issues to be considered in understanding why the observed microbial burden exists in the first instance. Future studies should be able to outline the microbial burden of the wrapping papers vis-à-vis the microbial burden of the food handlers themselves to be able to design and implement an effective intervention during the associated epidemic (11-13).

High bacterial counts obtained in this study also corroborates with the work of Aboloma (14), Fansani *et al.,* (15), Nwachukwu *et al.,* (16) and Adegunloye, (17) who also found high mean bacterial counts values of 2.78 x 10^5 12.26 x 10^5 and 2.68 x 10^5 cfu/ml from table scrapping from meat stalls, polyethylene packed sliced watermelon and banana leaves used as food wrap respectively. It may be true that the use of shopping bags is recommended to receive and convey any material from place to place, this practice is not yet accepted by the general populace as the questions which have no clear answer remains who will pay for the bags. Again, use of shopping bags does not preclude the fact that such ready-to-eat food should be wrapped before being packed.

Coliforms isolated from the two varieties of samples in low numbers indicate fecal contamination and may also be an indication of poor unhygienic handling of the packs (2). Statistical analyses of results revealed that there is a significant difference ($p<0.05$) between the different varieties of samples from locally improvised wrapping papers and paper plates involved in this study. This implies that underlying factors control the colonization of roasted meat wrapping papers/paper plates by potentially pathogenic bacteria/fungi.

Among the population who patronize the roadside meat vendors, it is not common to see anybody washing hands thoroughly before eating the meat, because most of the meat purchased by the roadside are also consumed by the roadside thus making it possible for most if not all the roasted meat to be consumed completely before getting

home. It, therefore, implies that a clear understanding of the real threat caused by the bacterial potential pathogens may be possible if the baseline evaluation of the bacterial load of the meat handlers, the meat itself as well as the customers is carried out.

Finally, results of the fungal counts from paper plates and meat wrapping papers are shown in tables 3 and 4, above and the fungal species isolated morphologically and biochemically resembled *Mucor, Rhizopus* and *Penicillium, Fusarium,* and *Aspergillus***.** The isolation of these organisms gives serious cause for concern because Aspergillus species is specifically known to produce mycotoxins (18), which causes food intoxication in humans and other animals. It is also in line with Okegbue (19) who reported that some strains of toxin-producing *Aspergillus Flavius* are increasingly prevalent as an opportunistic agent of tropical infection. Aflatoxin which are mycotoxins produced by such strains is undesirable in foods because they are potential health hazards and cause of epidemic and great mortality due to disease outbreaks in resource-limited settings.

In conclusion: The papers used for roasted meant fast food were found to be unsafe and unfit for packaging purposes as they can easily be a source of disease caused by bacterial and fungal agents of diseases epidemic. Improved diagnostic and identification methods to improve recovery of isolates, appropriate sterilization process, and associated awareness campaign to prevent associated disease epidemic are highly recommended

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