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Isolation and Identification of Microorganisms from Street Vended Ready-to-Eat Foods in Gombe Metropolis, Nigeria

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Abstract
The consumption of ready-to-eat foods vended in streets have raised public health concern in terms of food safety implying their microbiological quality status could be questionable; consequently, this study therefore aimed at investigating consortium of microbes present in ready-to-eat foods vended in five streets of Gombe metropolis. Traditional culture method was adopted for the isolation of microorganisms via pour plating method, then identification by colonial morphology, Gram staining and microscopy, and further biochemical analysis for confirmation of microbes. Findings revealed the presence of sixteen diverse microorganisms of bacteria and fungi lineage with varied percentage of occurrences. Microorganisms isolated ranges from spoilage group (P. aeruginosa (11.5%), Rhizopus spp (4%)), Coliform (E. coli (34.5%), K. pneumoniae (16.1%)), pathogenic (S. typhi (15%), Shigella spp (2.30%), S. aureus (12.6%), P. aeruginosa (11.5%), Aspergillus niger (26%), Aspergillus flavus (18%), Fusarium oxysporum (4%)) and opportunistic pathogens (Aspergillus fumigates (14%), Penicillium spp. (4%)) - where the pathogenic microbes are known to cause food-borne diseases and fungal poisoning. Accordingly, the presence of these pathogenic microbes suggests significant public health hazards. Therefore, stringent enforcement of standard and food safety measures is advised to curtail future outbreak of food-borne diseases.
***Keywords**: Microorganisms, microbiological quality, ready-to-eat foods, coliform group, faecal contamination.*

INTRODUCTION

Occupied work schedules and activities of majority of people, convenience in accessibility and affordability have necessitated food consumers to resort to street vended foods for survival in their day to day activities. These street foods are often ready-to-eat (RTE) varieties of food sold in streets or other public places such as markets and from a portable food booth or truck by hawkers or vendors (Simopoulos *et al.*, 2011). Falola *et al.*, (2011) also stated that Street food are ready-to-eat food and beverages prepared and sold by vendors and handlers especially in streets and other related public places for immediate consumption or consumption at extended period without further processing. However, some street foods are regional while many are not; having spread beyond their region of origin. Suneetha *et al.*, (2011) have related that most street foods are classed as both finger food and fast food which are mostly cheaper on average than restaurant meals. Others may purchase street food for few numbers of reasons, such as to obtain reasonably priced and flavourful food in a sociable setting, to experience diverse ethnic cuisines and for nostalgia.

Extensive street vending of foods in Nigeria have been attributed to several factors such as deterioration of rural living conditions, urban migration, urban congestion, long commuting distances between the workplace and home, unemployment, lack of cooking knowledge, changes in family cohesion and a shortage of establishments that serve reasonably priced food close to the workplace (Suneetha *et al.*, 2011). Street-vending of food provide a major source of income for a vast number of persons, particularly women involved in the business, thus importantly served as a self-employment opportunity specifically for low capital investors. Besides, it provides affordable and most accessible means of obtaining a nutritionally balanced meal outside the home for many low-income people (Rane, 2011). Despite the economic, nutritional and other benefits attached to street foods, many food

safety and healthcare personnel have raised concern over the consumption of these roadside foods by advising on the potentially increased risk of food borne illness, as street foods are readily contaminated from different sources (Tambekar *et al.*, 2011). The sources of microbial contamination of ready-to-eat food are unhygienic handling and display of food (Clarence *et al.*, 2009), poor hygiene practices that could lead to introduction of bacterial strains of faecal origin such as *Enterococcus faecalis* (Ibrahim *et al.*, 2020), and body flora microbes such as *S. aureus* (Nichols *et al.*, 1999) and contamination from kitchen formites like utensils (Moshood *et al.*, 2012). The health implication of consumption of an unsafe food has labelled street food vending as an important public health issue of great concern due to widespread foodborne diseases. The health issues are often caused or aggravated since most food vendors lack adequate knowledge and understanding of basic food safety rules and standard measure for handling foods. These valid insinuations have prompted this study targeted at isolation and identification of microbes contained in street vended ready-to-eat food principally to assess the microbial safety and quality of foods sold in the streets of Gombe Metropolis. This study is significant in enlightening on quality status of foods sold by street vendors aside from divulging the types of microbes associated with theseready-to-eat foods and what their presence signifies.

MATERIALS AND METHODS

Sample collection

Triplicates of each three (3) varied ready-to-eat vended foods (Rice and Beans, Bean porridge and Moi-moi) were purchased from five (5) different vending points namely Tashan Dukku, Jekadafari Street, Gombe State University (GSU), Tashan Bauchi and Gombe main market. The purchased food samples were collected in a new and sterile pre-labelled sample bags then immediately transported to the laboratory at 4 ^{o}C in an ice cooler for microbiological analysis. In total, 45 vended food samples were aseptically collected for analysis.

Sample processing and serial dilution

A Portion from the food sample was uniformly homogenized by blending in a sterilized mortar and pestle. One (1) gram from the blended paste of food was transferred into a sterile test tube containing 9 mL of distilled water then thoroughly vortexed to obtain a stock homogenate solution for serial dilution. Afterwards, ten-fold serial dilution was achieved up to 10^{-5} dilutions by taking 1 mL from the prepared stock homogenate then dispensed into 9 mL distilled water for the first dilution (10^{-1}). Dilution was continued until 10^{5} dilutions was reached according to the standard serial dilution protocol to obtain discrete colonies (Marshal *et al.*, 1995).

Sample inoculation

Pour plating technique was adopted for culturing of microorganisms in solid culture media such as Nutrient, MacConkey and Potato Dextrose Agar. Here, 1 mL from the 10^{-2} and 10^{4} dilutions (diluted sample inoculum) were initially dispensed in sterile Petri dishes before subsequent addition of 20 mL of prepared culture media cooled to ~45°C then gently rocked on the bench to mix uniformly. The inoculated plates were allowed to set firmly for at least five minutes then inverted before incubation. For the isolation of bacteria, culturing was done on Nutrient agar (NA) and MacConkey agar (MCA) and inoculated plates were incubated at 37°C for 24 hours. Contrary to bacteria culturing, culturing for the isolation of fungi was achieved using Potato Dextrose Agar (PDA) where inoculated plates were incubated at room temperature (25°C) for 5 to 7 days. At the end of the incubation period, viable bacterial (aerobic mesophilic bacterial plate count) was established by expressing results of counts generated using colony counter in CFU/g based on standard calculation.

Identification and confirmation of Microorganisms

Colonial morphology also termed macroscopic examination for the identification of bacterial isolates based on their colony morphology or cultural characteristics (colour, shapes, elevation, margin and pigment formation on the colonies) was done using experimental microbiology manual by Aneja, (2003). Also, the fungi were initially identified based on their colonial morphology using mycology atlas by Ellis *et al.*, (2007).

Gram Staining and Microscopic examination

The microscopic investigation included were determination of the bacterial cell wall's gram reaction status (either Gram-positive or -negative) after gram staining aside from examining the cell morphology under the microscope (microscopic morphology). Prior to the bacterial cell observation under the microscope, gram staining was performed according to the standard gram staining protocol as described by Cheesbrough, (2006). Afterwards, bacterial gram reaction and cell morphology were observed under the microscope at x100 objective lens. On the other hand, the cellular morphology of fungi

isolates was determined using lacto phenol blue staining technique described by Cooper (1995).

Confirmatory Biochemical Analysis

Further confirmation of bacteria isolates was achieved by biochemical tests that include Catalase, Coagulase, Indole, Citrate Utilization, Motility and Urease tests to enable genuine confirmation of the identified bacteria isolated from the various food samples. Protocols for biochemical analysis were carried out as described by Cheesbourgh (2005).

RESULTS

The results of the microbiological analysis carried out on the three (3) selected street vended ready-to-eat foods (Rice and beans, Bean porridge and Moi-moi) from five different vending areas (GSU, Tashan Dukku, Gombe main market, Jekadafari streets and Tashan Bauchi) are as follows:

Table 1 relate the microscopic morphology and biochemical characteristics of eight (8) bacteria genera/species isolated from the three (3) types of ready-to-eat food vended in the five different selected areas within Gombe metropolis. These bacteria isolates were identified and confirmed as *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Proteus mirabilis, Salmonella typhi*, and *Shigella* spp.

Table 2 shows the mean of total aerobic mesophilic bacteria plate count in CFU/g across the five different vending or sampling areas (GSU, Tashan Dukku, Gombe main market, Jekadafari streets and Tashan Bauchi) for the combined three (3) different ready-to-eat food samples analyzed. Extract from result portrayed Gombe main market has the highest mean of viable plate count of 9.9 $x10^7$CFU/g proceeded by Tashan Dukku with 9.3$x10^7$CFU/g while the least mean viable count was observed in GSU with 2.1$x10^7$ CFU/g.

Table 3 shows the average of the total aerobic mesophilic bacteria plate count recorded for the three (3) ready-to-eat food samples such as rice and beans, beans porridge and moi-moi sampled from all the five (5) different vending areas within Gombe metropolis. The result from Table 3 revealed that rice and beans recorded the highest viable bacteria count of 2.33 x 10^7CFU/g, followed by moi-moi with 2.16 x 10^7CFU/g while beans porridge had the lowest viable count of 1.97 x 10^7 CFU/g.

Table 1: Microscopic morphology and biochemical characteristics of bacteria isolated from the three (3) ready-to-eat street vended foods.

Microorganisms	Biochemical tests										
	GR	Morp.	Ur	Cat	Co	Cit	Mot	Ind	MR	Ox	H_2S
E. coli	-	srs	-	+	-	-	d+	+	+	-	-
S. aureus	+	cc	-	+	+	-	-	-	+	-	-
Enterococcus faecalis	+	*cpsc*	-	-	nd	-	-	-	-	-	-
Proteus mirabilis	-	*rps*	+	+	nd	+	+	-	+	-	+
Salmonella typhi	-	srs	-	+	nd	-	+	-	+	-	d+
Shigella spp.	-	srs	-	+	nd	-	-	d+	+	-	-
Pseudomonas aeruginosa	-	srs	d+	+	-	+	+	-	-	+	-
Klebsiella pneumoniae	-	*lrs*	+	+	-	+	-	-	+	-	-

Table 1 shows the microscopic morphology and biochemical tests results that confirm bacteria isolated from the three (3) different ready-to-eat foods vended within Gombe metropolis. Key: Positive sign + signifies positive reaction, d+ signifies 16-84% strains are positive (mostly positive), Negative sign - signifies negative reaction, nd = Not determined, Ur = urease test, Cat = catalase, Co = coagulase test, Cit = Simmons' citrate test, Mot = motility test, Ind = indole test, MR = Methyl red test, Ox = Oxidase test, Morp: microscopic morphology represented as cc = cocci in cluster, cpsc = cocci in pairs and short chain, srs = short rods in singles, rps = rods in pairs and singles, lrs = long rods in singles. Biochemical test results were checked with Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham,1993) for convincing confirmation of bacterial isolates.

Table 2: Mean of total aerobic mesophilic bacteria plate count (TAMBPC) across the five (5) different vending outlets for all food samples.

Sample area	Mean of TAMBPC (CFU/g)
GSU	$2.1x10^7$
Tashan Dukku	$9.3x10^7$
Gombe main market	$9.9x10^7$
Jekadafari Street	$7.3x10^7$
Tashan Bauchi	$4.8x10^7$

Table 3: Mean of total aerobic mesophilic bacteria plate count (TAMBPC) for the three (3) food samples collected from five (5) different locations.

Food samples	Mean of TAMBPC (CFU/g)
Rice and beans	$2.33x10^7$
Beans porridge	$1.97x10^7$
Moi-moi	$2.16x10^7$

Table 4 shows the frequency of occurrence of different bacteria in all the food samples collected from the entire five (5) different sampling areas (vending points) within Gombe metropolis – where this is in no reference to particular sampling area but overall occurrence in all food samples analysed. An excerpt from result contained in the table 4 revealed *E. coli* has the highest occurrence frequency and percentage with 30(34.5%), then proceeded by *Klebsiella pneumoniae* with 14(16.1%) while *Proteus mirabilis* with 1(1.15%) had the least occurrence in food samples from all vending points in this study.

Also, table 5 shows the occurrence of the various fungal isolates in all food samples collected from the five (5) sampling areas. The result showed that *Aspergillus niger* have the highest occurrence 13(26%), followed by *Aspergillus flavus* with 9(18%), while *Penicillium* spp recorded the least occurrence with 2(4%).

Table 4: Occurrence of bacteria isolates in food samples from the five (5) sampling areas.

Bacteria	Frequency of occurrence	Percentage (%)
E. coli	30	34.5
Klebsiella pneumoniae	14	16.1
Salmonella typhi	13	15.0
S. aureus	11	12.6
Pseudomonas aeruginosa	10	11.5
Enterococcus faecalis	6	6.89
Shigella spp.	2	2.30
Proteus mirabilis	1	1.15
Total	**87**	**100**

Table 5: Occurrence of fungal isolates in food samples from five (5) study areas

Fungi	Frequency of occurrence	Percentage (%)
Aspergillus niger	13	26
Aspergillus flavus	9	18
Mucor spp.	8	16
Aspergillus fumigatus	7	14
Microsporum audouinii	7	14
Fusarium oxysporum	2	4.0
Penicillium spp.	2	4.0
Rhizopus spp.	2	4.0
Total	**50**	**100**

DISCUSSION

This study aimed at isolation and identification of microorganisms from street vended ready-to-eat food, sixteen (16) diverse microorganisms (eight (8) bacteria and eight (8) fungi) were isolated, identified and confirmed from the three kinds of foods vended in five different vending outlets sampled. This is regardless of the overall sample size applied in this study as it should be reminded that the food samples analysed are cooked and ready-to-eat thus ought to be devoid of any pathogenic microorganism (vegetative cells) due to the varied heat treatment (mostly above 100°C) usually applied during preparation. Relative to the insinuation of heat-treated food samples analysed, the undeserved diversity of microbes isolated from these ready-to-eat foods could meaningfully implicate the safety of the foods vended in those outlets. This diversity of microbes isolated may be justifiable especially with realization of the traditional culture technique adopted in this study for the isolation and identification of microorganism from the ready-to-eat food samples. Ibrahim, (2020) reviewed that an alternative culture independent approach such as metagenomics would unravel complete and unbiased microbial communities in any sample as compared with culture technique which has failed to capture the full spectrum of microbial diversity. Aside from diversity, it is paramount to note the varied occurrence statistics of these microorganisms in the variety of food samples collected from the combined sample sites-where the presence of the highly frequent bacteria (mostly *E. coli*, then *K. pneumoniae*, *S. typhi*, *S. aureus* and *P. aeruginosa*) and fungi (*Aspergillus* spp.) admissibly commensurate with the physical attributes of the vending outlets and possible ways of contamination as would be discussed later on.

Noteworthy to this study, the presence of microbial pathogens and opportunistic pathogens (e.g., *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella* spp, *Enterococcus faecalis* and others) in the ready-to-eat food samples analysed have been recognised, and this portrays a significant public health hazard as these microbes in the contaminated street vended foods are potential causes of several food-borne illnesses. The spoilage strains (e.g., *P. aeruginosa*, *Rhizopus* spp) could causes spoilage and objectionable characteristics in food rendering it unpalatable while fungal contaminants could produce assortments of mycotoxins in food that could lead to fatal food poisoning which is another typed food-borne illness. This result corroborated with the work of Oranusi *et al.*, (2013) whose finding reveal the presence of *Salmonella* spp.,*S. aureus*, *E. coli*, *B. cereus*, *Shigella* spp., *Enterococci*, *A. niger* and *Pseudomonas spp* in ready-to-eat foods and snacks. Besides, *Pseudomonas* have been reported as dominant food spoilage organisms (Borch *et al.*, 1996). Several other studies (e.g., Rath and Patra, 2012; Suneetha *et al.*, 2011; and Arijit *et al.*, 2010) have also revealed the presence of bacterial pathogens on popular street foods especially in many developing countries.

Moshood *et al.* (2012) also reported the presence of microbial pathogens identified in this study (*S. typhi, Shigella* spp, *S. aureus* and others) in ready-to-eat food (meat product) sold in the street of Bauchi metropolis, and elucidated that these pathogens could cause range of mild to severe food-borne diseases such as enteric fever, dysentery, travellers' diarrhoea, abdominal disorder and pains, sore throats, staphyloenterotoxemia, salmonellosis, mild fever and many others. However, the infectious loads of ready-to-eat food products may be deliberated by others. The presence of Coliform (*Escherichia coli,* and *Klebsiella pneumoniae*) in the ready-to-eat food samples analysed (Table 4) is mostly attributed to contamination from fecal sources as a result of poor hygiene practices during handling of foods. Study of Ibrahim *et al.* (2020) that reported Coliform group depict indicators of microbial pathogens from enteric sources reasonably agree with this.

It is only judicious to state that microbial contamination of these ready-to-eat foods could be attributed to loads of sources. Primarily, these are ready-to-eat foods sold or vended on the street and Motor Parks thus it is mostly typical for the microbial contaminants to arise from the immediate environment as bacterial and fungal aerial spores are readily carried in the air and dropped in exposed food. For instance, the occurrence of *Mucor* spp and *Aspergillus* spp (Table 5) is associated to the fact that they are spore formers thus their spores could be easily deposited in food vended in such peculiar environment. Oranusi *et al.* (2013) support that microbial spore mostly contaminate food from dusty environment and heat-resistant spores could survive temperature that can destruct vegetative cells. Clarence *et al.*,(2009) also reported that other major sources of microbial contamination of ready-to-eat food arefrom improper food handling and display due to poor hygiene practices.

This study revealed an inequality in the mean of total TAMBPC for the five (5) different vending points studied within Gombe metropolis (Table 2). This dissimilarity in bacterial load of the street vended foods in the five retail outlets within Gombe isrationally expected as these vending sites have their peculiar features and activities that could necessarily influence the bacterial loads in any ready-to-eat food items sold in these places. Even though, unique attribute entails varieties of ready-to-eat foods are vended in open places and streets. In this regard, the location or situation of vending point especially in parks and markets is a crucial factor that could greatly contribute to the increase in the bacterial load in street vended foods. This is obvious in the mean count for Gombe main market with the highest count and Tahsan Dukku (Motor Park) as high counts may arise from microbes carried in the air or dust as spores that could later germinate to vegetative cells in food under favourable conditions. Barro *et al.,* (2006) also reported high bacterial population in busy streets and commercial centres such as main markets in towns and cities while Mashood *et al.* (2012) reported significantly high bacterial loads in Railway Park and Mudalawal market. Consequently, this justify the lowest count recorded in GSU outlet, reasonably attributed to the fact that it's a non-market environment known for good sanitation contrary to a distinctive market place. To buttress this,similar works by Nichols *et al.,* (1999) also reported low bacterial count from school environment having low commercial activities thus corroborating earlier assertion.

The mean of TAMBPC of the three different food samples revealed that Rice and beans had the highest count (2.33 x 10^7CFU/g), followed by Moi-moi (2.16 x 10^7CFU/g) and Beans porridge with the least occurrence (1.97 x 10^7CFU/g) as shown in Table 3. It is simply justifiable to relate the differences in microbial count of these different foods to unhygienic handling of food in various vending outlets and the exposure of the food types via utensils as corroborated by Tambekar *et al.* (2011). However, it should be reminded that these food samples constitute differed nutritional composition which could reasonable influence the proliferation of microbial contaminants while in food consequently affecting the overall count. Nonetheless, it must be acknowledged that this is an average of total count thus is in no reference to a particular site which could translate a different meaning. Regardless, it can be established that these average counts recorded in the three ready-to-eat foods is higher and above the acceptable limit (≤ 10^3 CFU/mL or CFU/g) set by the International Commission on Microbiological Specifications for Foods (ICMSF). Carry (1996) reported that the ICMSF limit for total aerobic bacterial and fungal counts in the order of ≤ $10^3$is regarded acceptable while 10^4 to $10^5$is tolerable for ready to eat foods. This basically implies that counts beyond these specified limits are unacceptable.

CONCLUSION

This study revealed diverse microbial group in the ready-to-eat foods analysed. The presence

of pathogenic strains and the higher bacterial counts suggest that these foods vended in public streets are grossly contaminated with multiplicity of microorganisms above the acceptable limit. Consequently, rendered the Rice and beans, Moi-moi and Beans porridge unacceptable and unsafe for consumption and depict its poor microbial quality. It is therefore, recommended that good hygiene practices, standard rules for food handling and good sanitation measures should be sternly applied by the vendors.

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