Inyang et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (2): 240 - 246 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Apr 2026; Vol.27 No.2
AJCEM/2423. https://www.ajol.info/index.php/ajcem

Short Communication **Open Access**

Evaluation of microbial quality of grilled foods sold at roadside in Calabar metropolis, Cross River State, Nigeria

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Abstract:

Background: Roadside foods are central parts of culinary custom in many African countries. In addition to their dietary importance, they contribute immensely to the national and economic values of a nation. However, the growing incidence of food-borne illnesses in relation to the roadside foods is a great concern. Hence, this study assessed the microbial quality of grilled foods sold at roadside in Calabar metropolis, Cross River State, Nigeria.
Methodology: A total of thirty grilled food (roasted ripe or unripe plantain with grilled mackerel fish) samples were collected from the three busiest areas (Marian, Etagbor and Murtala Muhammed Highway) in Calabar metropolis. Isolation and enumeration of bacteria and fungi were done using standard techniques to obtain colony counts that were correspondingly employed in the determination of total bacteria count (TBC) and total fungi count (TFC).
Results: Grilled ripe plantain from Marian axis recorded the highest TBC ($4.0x10^6$ CFU/g) while grilled unripe plantain from Etagbor location had the least ($8.0x10^3$ CFU/g) TBC. *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were isolated from the samples, with *E. coli* as the most predominant. Grilled food from Marian location had the highest ($2.8x10^4$ CFU/g) TFC while samples from Etagbor axis had the lowest ($3.6x10^3$ CFU/g) TFC, with *Aspergillus flavus* and *Penicillium digitatum* as the most predominant fungal isolates.
Conclusion: The level of microbial loads detected was above the safe limit, thus signifying lack of proper food handling and sanitary practices among the grilled food vendors.

Keywords: Grilled foods, Microbial quality, Roadside, Vendor, Calabar metropolis

Received Sep 29, 2025; Revised Jan 23, 2026; Accepted Jan 24, 2026

Évaluation de la qualité microbiologique des aliments grillés vendus en bord de route dans la métropole de Calabar, État de Cross River, Nigéria

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Résumé:

Contexte: La restauration de rue occupe une place centrale dans les traditions culinaires de nombreux pays africains. Outre son importance nutritionnelle, elle contribue largement au patrimoine national et économique. Cependant, l'incidence croissante des maladies d'origine alimentaire liées à cette restauration est très préoccupante. Cette étude a donc évalué la qualité microbiologique des aliments grillés vendus en bord de route dans l'agglomération de Calabar, dans l'État de Cross River, au Nigéria.
Méthodologie: Trente échantillons d'aliments grillés (bananes plantains mûres ou vertes rôties accompagnées de maquereau grillé) ont été prélevés dans les trois zones les plus fréquentées de l'agglomération de Calabar (Marian, Etagbor et l'autoroute Murtala Muhammed). L'isolement et le dénombrement des bactéries et des

champignons ont été réalisés selon des techniques standard afin d'obtenir le nombre de colonies, lequel a ensuite été utilisé pour déterminer le nombre total de bactéries (NTB) et le nombre total de champignons (NTC).
Résultats: Les bananes plantains mûres grillées de la zone de Marian présentaient le NTB le plus élevé ($4,0\times10^6$ UFC/g), tandis que les bananes plantains vertes grillées d'Etagbor présentaient le NTB le plus faible ($8,0\times10^3$ UFC/g). Des *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli* et *Bacillus subtilis* ont été isolés des échantillons, *E. coli* étant l'espèce prédominante. Les aliments grillés provenant de la zone de Marian présentaient la charge microbienne totale la plus élevée ($2,8x10^4$ UFC/g), tandis que ceux provenant de l'axe Etagbor affichaient la plus faible ($3,6x10^3$ UFC/g). *Aspergillus flavus* et *Penicillium digitatum* étaient les isolats fongiques les plus prédominants.
Conclusion: Le niveau de charge microbienne détecté était supérieur au seuil de sécurité, ce qui indique un manque de bonnes pratiques de manipulation et d'hygiène des aliments chez les vendeurs d'aliments grillés.

Mots-clés: Aliments grillés, Qualité microbiologique, Bord de route, Vendeur, Métropole de Calabar

Introduction:

The availability and consumption of roadsides foods have tremendously increased recently in many parts of the globe, serving both as a source of livelihood and dietary nutrients to the growing populace. In Nigeria, roadside foods fall under the category of ready-to-eat street foods, as they are always consumed immediately after purchased without further processing, thereby posing substantial public health threats as the food can be easily contaminated (1).

According to Food and Agriculture Organization (FAO), street foods are defined as ready-to-eat foods and drinks made and/ or sold by food merchants especially in an open space along major roads and public places (2). These roadside ready-to-eat foods including, but not limited to suya and pork, roasted plantains, grilled meats, fishes and chicken gratify indigenous cravings, in addition to posing exceptional ethnic values for citizens and tourists. Grilled foods are meals cooked using dry-heat cooking methods, mostly over a heat source, such as charcoal, wood, gas, hot plate or electric grill. Meat (e.g. steak, chicken, pork, sausages, and burgers), seafood (e. g. fish, shrimps, and oysters), vegetables (e. g. corn, peppers, zucchini, and onions), fruits (e.g. pineapple, peaches, and strawberries), pizza, flatbreads, and others are among the various types of grilled foods

Recently, food hawking has grown into a norm in the metropolitan of Cross River State, with a good number of residents and tourists eating meals from food sellers, citing time-saving, lower costs, and easy accessibility as major reasons for such practices (3). An overall scrutiny of the social order in Calabar metropolis has placed the area as a centre for tourism, with urban settlement, travelling workforces, and less household focused activities, leading to complete dependent on the ready-to-eat roadside food by high number of the populace. These have shifted the burden of food hygiene and handling practices from households to the food sellers who may not adequately comply with the necessary food processing principles (4).

The quality and safety of these roadside foods is therefore a universal challenge, as these foods are basically prepared in open-air surroundings, behind dirty and smelly drainages, with limited access to adequate cooking utensils, thereby suggesting possibility of microbial contamination at the various processing and packing points (5).

Previous studies on microbiological assessment of roadside foods in different countries have reported microbial contamination and high incidence of food-borne bacterial pathogens (6). For instance, in India, Rath and Patra (7), and Suneetha et al., (8) reported high loads of bacterial pathogens on common street foods in various areas of the country. In Africa, El-Shenawy et al., (9) reported high loads of *Bacillus cereus, Staphylococcus aureus, Shigella sonnei, Escherichia coli,* and *Salmonella arizonae* in roadside foods in Egypt. In Nigeria, Ahmad et al., (10) reported various antibiotics bacterial pathogens in roadside foods sold in Katsina metropolis. Also, Oranusi and Braide (11) reported the presence of *Salmonella* spp*, S. aureus, E. coli, B. cereus, Shigella* spp*, Enterococcus, Pseudomonas* and *Aspergillus niger* in grilled foods sold in Onitsha-Owerri metropolis. In addition, Oyet et al., (5) reported the presence of microbial pathogens in grilled foods in Port-Harcourt metropolis, Rivers State, Nig eria. Unfortunately, most of these pathogens recovered from grilled foods have been implicated in food poisoning, intoxication as well as foodborne diseases (4).

Foodborne infections are described as one of the utmost prevalent diseases, bothering the healthcare system, responsible for the preponderance of infections, mortality and morbidity across all categories of individuals globally, mostly in the developing countries (12). Although numerous risk factors of foodborne illnesses, including polluted water and environment, inadequate and non-compliance to food safety regulations, poverty and ignorance, have been reported; poor knowledge, attitude and practices of food safety by food dealers have been regarded as the paramount factor in the progress of the disease (13). This may be due to the fact that most

of these food vendors are not licensed and their activities are not regulated by appropriate food safety bodies. More worrying is the great demand and consumption of roadside grilled foods in Calabar, Cross River State, as many consumers are ignorant of the health implication of eating these foods.

Although there are published studies in the literature on nutritional composition and microbial assessment of some roadside fruits and fruit salads, soups, and foods in Calabar metropolis, there is currently no information on the microbial evaluation of grilled foods (roasted ripe or unripe plantain with sausage, commonly called "bole") in this setting despite their high demand and consumption. Therefore, the present study was aimed at evaluating the microbial quality of roadside grilled foods sold in Calabar metropolis, Cross River State, Nigeria.

Materials and method:

Study setting and sample collection:

Grilled plantain (ripe and unripe) with mackerel fish were collected randomly from three busiest locations (Marian, Murtala Mohammed Highway and Etagbor) in Calabar metropolis, Cross River State, Nigeria. Five samples of each of roasted ripe and unripe plantain with grilled mackerel fish were purchased directly from roadside vendors in 3 randomly selected locations, making a total of 30 samples. The samples were put in different sterile aluminum foils, and immediately transported to the laboratory in the Department of Biochemistry, University of Calabar for analysis.

Sample preparation and microbial analysis:

Ten grams of each grilled sample were homogenized by blending in 90 ml of nutrient broth (Biotec, UK) and serial dilutions of each grilled sample homogenate were made to 10^{-5} dilutions. Precisely, 0.1 ml of the appropriate dilutions were spread using pour plate methods onto duplicate sterile nutrient agar and MacConkey agar, and Sabouraud Dextrose agar (SDA) plates for bacterial and fungal isolation respectively.

The nutrient and MacConkey agar plates were incubated for 24 hours at 37° C, while the SDA plate was incubated for 96 hours at 30° C. The agar plates were examined after incubation for growth of pathogens and the colonies were counted using colony counter (Gallenkamp, England) and expressed as colony forming unit/gram (CFU/g) of sample homogenate.

Identification of isolates:

Pure colonies of each isolate were kept on agar slants at 4° C for subsequent identification by the methods described by Oranusi et al., (14) and Fawole and Oso (15) for bacterial and fungal isolates respectively. However, the identities of other bacterial isolates were done by comparing their appearances with the descriptions in the Bergey's Manual of Determinative Bacteriology (16).

Statistical analysis:

Data obtained for total bacterial and fungal counts were calculated and all CFU/g values were converted to log10. Foods were then classified according to the values obtained after conversion with reference to International Commission on Microbiological Specifications.

Results:

Table 1 shows the total bacterial counts of grilled foods sold at roadside in 3 major locations (Etagbor, Marian and Murtala Mohammed Highway) in Calabar metropolis, Cross River State, Nigeria. The result indicated that, the highest total bacterial counts were found in the roasted ripe plantain grilled mackerel fish from Marian axis with $4.0x10^6$ CFU/g, while the lowest value was found the in roasted unripe plantain with grilled mackerel fish from Etagbor axis with $8.0x10^3$ CFU/g. The results of bacterial identification revealed the presence of *Streptococcus* spp, *S. aureus, E. coli* and *B. subtilis* in the samples, with *E. coli* as the most prevalent.

Table 2 showed the total fungi counts of the grilled foods sold at roadside from 3 major locations in Calabar metropolis, Cross River State, Nigeria. The result indicates that the highest total fungal counts were found in roasted unripe plantain with grilled mackerel fish from Marian axis, with 2.8×10^4 CFU/g, while lowest value was found in roasted ripe plantain with grilled mackerel fish from Etagbor axis with $3.6x10^3$ CFU/g. The results of fungal identification revealed the presence of *Aspergillus* spp*, Penicillium* spp*, Rhizopus* spp and *Saccharomyces cerevisiae* in the samples with *Aspergillus* spp and *Penicillium* spp as most abundant fungi isolates in the samples.

Table 1: Bacterial load of grilled foods sold at roadside in Calabar metropolis, Cross River State, Nigeria

Samples/location	Total number of samples	Total bacterial count (CFU/g)	Identified bacterial species
Roasted ripe plantain with grilled mackerel fish from Etagbor	5	1.0×10^4	*Streptococcus* spp, *Escherichia coli,* and *Staphylococcus aureus*
Roasted ripe plantain with grilled mackerel fish from Marian	5	4.0×10^6	*Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus*
Roasted ripe plantain with grilled mackerel fish from Highway	5	1.0×10^4	*Bacillus subtilis, Streptococcus* spp, *Escherichia coli* and *Staphylococcus aureus*
Roasted unripe plantain with grilled mackerel fish from Etagbor	5	8.0×10^3	*Streptococcus* spp*, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*
Roasted unripe plantain with grilled mackerel fish from Marian	5	4.4×10^4	*Bacillus subtilis, Streptococcus* spp and *Escherichia coli*
Roasted unripe plantain with grilled mackerel fish from Highway	5	3.5×10^4	*Escherichia coli, Streptococcus* spp*, Staphylococcus aureus.* and *Bacillus subtilis*

Table 2: Fungal load of grilled foods sold at roadside in Calabar Metropolis, Cross River State, Nigeria

Samples/location	Total number samples	Total fungal count (CFU/g)	Identified fungal species
Roasted ripe plantain with grilled mackerel fish from Etagbor	5	3.6×10^3	*Rhizopus* spp*., Penicillium* spp. and *Mucor* spp*.*
Roasted ripe plantain with grilled mackerel fish from Marian	5	2.1×10^4	*Aspergillus* spp*., Penicillium* spp. and *Rhizopus* spp.
Roasted ripe plantain with grilled mackerel fish from Highway	5	4.6×10^3	*Penicillium* spp*., Aspergillus* spp. *and Saccharomyces cerevisiae*
Roasted unripe plantain with grilled mackerel fish from Etagbor	5	2.4×10^4	*Penicillium* spp*., Aspergillus* spp. and *Rhizopus* spp.
Roasted unripe plantain with grilled mackerel fish from Marian	5	2.8×10^4	*Penicillium* spp*., Aspergillus* spp. and *Rhizopus* spp*.*
Roasted unripe plantain with grilled mackerel fish from Highway	5	4.1×10^3	*Aspergillus* spp*., Penicillium* spp. and *Rhizopus* spp.

Discussion:

The comparative assessment of the microbial load of grilled food from roadside vendors in three busiest locations in Calabar metropolis, Cross River State was carried out in order to compare the safety of this highly demanded roasted ripe or unripe plantain with grilled mackerel fish commonly referred to as "bole" to ascertain which vendor complies with the appropriate hygienic and food safety practices. The microflora of the roasted ripe and unripe plantain is an assessment index of the quality of the grilled food, the hygienic conditions of the food handler, environment and the cooking utensils employed in the preparation of the food (17).

From the present study, the total bacterial counts ranged from 8.0×10^3 to 4.0×10^6 CFU/g, and the total fungal load ranged from 3.6×10^3 to 2.8×10^4 CFU/g. These values, mostly the bacterial loads, were above the safe limit stated by the International Commission on Microbiological Specifications for grilled food, with $0\text{-}10^3$ as safe, $10^4\text{-}10^5$ as bearable and $>10^6$ as unacceptable (18). The high microbial load of the grilled food from roadside vendors implicates their consumption as a risk factor of food borne illnesses, as most of the isolated pathogens have been

linked to the illness.

However, the total bacterial counts recorded in the present study were lower than those reported by Henry, et al., (4), Brooks (19) and Ekpenyong et al., (1) for ready-to-eat foods, fruits salads and soups respectively, sold in the streets of Calabar metropolis. The variation in the total bacterial counts observed between the present study and previous findings may be attributed to handling and processing methods employed in the preparation of the various foods, as the present study employed roasting, where the pathogens must have been subjected to a very high temperature, compared to the cooking process used in the previous studies. This therefore underscore the recent reports of studies (20-23) that independently revealed that heating/roasting reduced the level of harmful compounds in food, thereby promoting the therapeutic potentials of processed foods as they have been implicated in the fight against cardio-metabolic disorders (24).

The present study revealed variation in the total bacterial counts among the three samples location, with Marian samples having the highest bacterial load. The high microbial load from the Marian location suggests unhygienic conditions and preparation, use of unclean water and utensils, exposure of the grilled food to air, as well as unhygienic behavior of the food vendors. Marian route can be referred to as unclean location due to high industrial activities, and the heavy presence of street children (urchins), locally called "scolombo", who are known for poor hygienic practices, going days without having a bath or change of clothes, sleeping in the street at nights, defecating and urinating in open places, thereby contaminating the location with faecal matters. Unfortunately, grilled food vendors usually prepare and sell these foods in this vicinity with complete exposure to air and dust, resulting in poor food handling safety practices, leading to high microbial loads in the area.

Our analysis further revealed *Streptococcus* spp, *S. aureus*, *E. coli* and *B. subtilis* as the bacterial pathogens present in the grilled food, with *E. coli* having the highest frequency of occurrence. The isolation of *E. coli* could be linked to processing contamination, faecal contamination and poor sanitary practice of food handlers. The fecal contamination may also be attributed to the high existence of water activity (fluid) in the fish, following contamination of the rivers/ponds where the mackerel fish were obtained. The results of the present study agree with previous findings of Lambu et al., (25) and Brooks (19), who separately reported the presence of *E. coli* in foods sold in the metropolis of Kano and Calabar, respectively. *Staphylococcus aureus* and *Streptococcus* spp were also among the most frequent pathogens present, and this finding is in line with the report of Ire and Imuh (26), who reported the presence of these isolates in ready-to-eat food in Port-Harcourt, Nigeria. The presence of these pathogens in food may be linked to the untidiness and dusty vending locations, hand contact during processing and vending, drops of saliva from both the vendor and customer's mouths, contact with skin and superficial wounds, as well as cross contamination after roasting. While, some of the isolates are beneficial, their continuous and high concentration when ingested have been implicated in food poisoning, as well as foodborne diseases (27). Hence, their presence in roadside grilled foods is a subject of concern, thereby posing a serious health risk to ignorant consumers.

In addition, *Bacillus subtilis* was also isolated, predominately from Marian and Highway samples. The presence of *Bacillus* spp in the grilled foods could be attributed to the fact that it is regarded as ubiquitous lipase-producing bacteria related to pathogenicity, with high presence in water, soil, and the environment. The spores producing nature of the *Bacillus* spp, makes them extremely resilient to the high temperature of roasting, heating and ultraviolet radiation (4). Their presence in ready-to-eat soup in Calabar metropolis has been recently reported by Ekpenyong et al., (1). However, the presence of *E. coli*, *S. aureus, Streptococcus* spp and *B. subtilis* in roadside grilled foods indicates a possible health threat as these bacterial are disease-causing microbes, which have been linked to progress of foodborne infections (11).

In addition to the bacteria isolates, five different fungal pathogens (*Penicillium digitatum, Aspergillus flavus, Rhizopus stolonifer*, *Saccharomyces cerevisiae* and *Mucor* spp) were isolated from the samples and roasted unripe plantain with grilled mackerel fish from Marian route recorded the highest total fungi counts, with *P. digitatum, A. flavus* and *R. stolonifer* being the most prevalent. Our findings also revealed that the total fungal count was higher in the ripe plantain samples, compared to the unripe plantain samples. The reason for the pathogen variation may be attributed to the sugar content in the samples, as the ripe plantain tends to contain more sugar from the carbohydrate hydrolysis during the ripening process, which may in turn favor microbial growth. The results of our findings substantiate the reports of Ahmad et al., (10) and Oranusi and Braide (11), who reported the presence of these fungal pathogens in foods sold in the metropolis of Katsina State and Onitsha-Owerri southeast, Nigeria respectively. The presence of these fungi species in grilled foods is worrying, as most of these isolates have been

implicated in various diseases condition, ranging from food poison to cancer. For instance, mycotoxins of *Aspergillus* spp are known to be carcinogenic and have also been linked to various forms of human and animal infections, including respiratory, local and superficial fungal infections (17,28). The spore-producing nature of mold (*Aspergillus and Mucor spp*) makes them heat resistant to the high temperature of roasting, heating and ultraviolet radiation, thereby favoring their growth in grilled foods (29,30).

From the results of this study, the presence of the isolated pathogens is a public concern, as they have been implicated in food borne diseases, among other devastating illnesses. The most disturbing situation is the high loads of the isolated pathogens, mostly above the safe limit as reported by the ICMSF (18) and the high patronage these vendors receive on daily basis by the ever-growing populace of Calabar metropolis. Consequently, this condition is detrimental not only to the consumers of the roadside grilled foods, but also to the fragile Nigerian healthcare system, which may not be capable of combating any potential foodborne disease outbreaks as a result of continuous consumption of the roadside grilled foods, which has become a culture in the city of Calabar, among other cities in Nigeria.

There is therefore a need for public health guidelines and enactment of dietary hygiene practices regarding the preparation, handling and vending of roadside grilled foods. Also, roadside grilled food vendors should be properly educated, monitored and possibly licensed by the food regulatory agencies before serving the public, in order to safeguard the lives of the teaming consumers of grilled foods and that of the generation yet unborn.

Conclusion:

The results of this study showed that roadside grilled ripe or unripe plantain with mackerel fish sold at Marian, Etagbor and Murtala Mohammed high way in Calabar metropolis were contaminated with high bacterial and fungal count of $4.0x10^6$ CFU/g and $2.8x10^4$ CFU/g respectively, with ripe samples sold along Marian routes recording the highest microbial loads. Poor food handling and unhealthy sanitary practices by the vendors are among the possible causes of pathogens contamination of these roadside grilled foods. Unfortunately, these pathogens have been implicated in foodborne diseases and other devastating health conditions. Hence, public health regulation and enactment of dietary hygiene practices regarding the preparation, handling and vending of roadside grilled foods are among the measures capable of reducing the microbial contamination of grilled foods.

Acknowledgements:

The authors are grateful to the management of the University of Calabar for providing enabling environment for this study.

Contributions of authors:

MAA and ANI conceptualized and designed the study; MAA, ANI, EDE, EOO and JEM were involved in acquisition, analysis, and interpretation of data; and MAA, ANI, EDE, OOA, JEM and EOO were involved in manuscript drafting, revision, and provision of resource materials. All authors read and approved the submitted for publication.

Source of funding:

No funding was received for the study

Conflict of interests:

Authors declare no conflict of interest

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