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Microbiological Quality of Food Sold in Different Grades of Mobile Food Vendors and Canteens in Owerri Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MON and JNA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PCO and RIAN managed the analyses of the study. Author MII managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The need to maintain proper hygiene in different grades of mobile food vendors and canteens cannot be overemphasized. The present study was conducted at Biotechnology laboratory, Federal University of Technology Owerri in August 2019 to ascertain the microbiological quality of food in different grades of mobile food vendors and canteens in Owerri Metropolis. Samples were collected from Fast food and Buka of which Two triplicates samples of rice, soup and moimoi were collected from two mobile food vendors in Owerri metropolis. Microbial count was carried out on each food sample using nutrient agar (NA) for bacteria count and sabouraud dextrose agar (SDA) for fungi count. Colonies were also identified using standard procedure and biochemical test up to

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genera level. The result showed that the organism isolated from canteen (Buker) is higher than those of fast food. Organisms generally isolated includes *staphylococcus aureus 0(0.0), Bacillius* sp. 59(9.5), *Pseudomonas aeruginosa* 162(25.6), *Proteus* 0(0.0), in fast food while in canteen (Buka) the organism isolated includes *Staphylococcus aureus* 160(25.0), Bacillius sp. 0(0.0%), *Pseudomonas aeruginosa* 16(2.6) and Proteus 227(36.4) and the organisms generally identified includes Penicillium sp. 20(6.4), Saccharomyces 26(8.7), Geotrichum 0(0.0%) and mucor sp. 0(0.0%) in fast food while in canteen(Buker) the organisms generally identified includes Penicillium sp. 0(0.0%), saccharomyces 45(15.0), Geotrichum 4(0.3) and Mucor sp. 205(68.3) and some of the genera that are of public health concern. The study suggests the need for continuous monitoring of the food vendors to forestall any form of infection.

Keywords: Microbiology; food; hygiene; pathogen; canteen.

1. INTRODUCTION

Mobile food vendors are people who engage in the sales of ready-to-eat food known as street foods, especially on the streets and other similar public places [1]. Street foods are inexpensive and readily available, that in many countries, these form an integral part of the diet, because they are consumed with regularity and consistency across all income groups, but particularly among the urban poor and in some countries, children; the business is also profitable [2]. For these reasons and more, street food hawkers have noticeably increased in number of food hawkers can be attributed to the dwindling economy, high rates of unemployment and the changing food demand by the urban dwellers who need cheaper food in the face of a harsh economy [3].

In terms of modes of selling, hawkers can be broadly classified into "stationary and ambulatory", with stationary hawkers being the most predominant type in most countries [4]. While the stationary hawkers sell their wares (i.e foods) from small stalls, kiosks, pushcarts and so forth [5], the ambulatory hawkers sell theirs from mobile, carts, baskets, trays and balance poles, [6]. Most of the street hawkers run their own business, which they find quite profitable and family members in some way or the other help out; but these hawkers do not understand the value of hygiene and sanitation [7]. This may be due to the fact that, they usually have very low literacy level, hence, about 25-30 percent of them keep unclean nails and hair, while about 20 percent wear filthy clothes [8].

In spite of the advantages of cheaper cost and consistency in availability offered by street foods, there is several health hazards associated with their hawkers [6]. Multiple lines of evidence have revealed that foods exposed for sale on roadsides may become contaminated by spoilage or pathogenic microorganisms, most of which exist on the skins and other parts of the hawkers [9,10,11]. By ingesting these foods, which have been contaminated by these pathogens, Gastroenteritis results [12]. Many of the pathogens responsible for food poisoning include species of the following genus: *Staphylococcus, Bacillus, Clostridium, Escherichia, Vibrio, Shigella, Salmonella, and Campylobacter* [13]. However, staphylococcal food poisoning is the most common type in the world today and it is caused by ingestion of food that contains one or more enterotoxins which are produced by only some staphylococcal species and strains, a good example being, *staphylococcus aureus* [14].

Staphylococci can be divided into pathogenic and relatively non-pathogenic strains based on the synthesis of the enzyme coagulase; these include the coagulase-positive strains (such as *S. aureus)* and coagulase-negative staphylococci (CoNS), such as *S. epidermidis* which do not produce coagulase [15]. But enterotoxin production is believed generally to be associated with *S. aureus* strain that produces coagulase and thermonuclease (Tnase) [15]. Among the coagulase-positive staphylococci spp. *S. aureus, S. intermedium* and *S. hyicus* have been generally associated with enterotixin production, while *S. delphini, S. simulans* and *S. schleiferi subsp. Coagulans* have not been reported to produce enterotoxins [16]. *Staphylococcus intermedius* and *S. hyicus* are rarely found in humans and only some. *S. hyicus* produce enterotoxins, but *S. aureus* strains are believed to be highly associated with enterotoxins production found in human [14].

S. aureus (a grain-positive coccus) is very resistant to heat, drying and radiation and is found in the nasal passages and on the skins of

human and other mammals worldwide [13]. As a source from humans, especially food handlers (i.e food hawkers), the microbiota on the hands and outer garments of handlers generally reflect the environment and habit of individuals and additional important sources are those that are common in nasal cavities, the mouth and on the skin that may enter food through poor personal hygiene practice [17]. It is therefore important that proper personal hygiene and sanitation amongst food hawkers be practiced.

Street foods are the cause of several types of food borne disease, safety of street foods is questionable as in most cases they are prepared under unsanitary conditions. The illiterate by having poor personal hygiene contaminate the food and increase greatly due to extremely poor environmental condition in which they are prepared and served [18].

Moreover, use of artificial colours, like metanil yellow, are cause of serous health hazards. Proper garbage removal facilities are also not available, thus leading to poor environmental condition [19]. Studies on street food in food were responsible for serious food poisoning outbreak as these foods are sometimes found to be contaminated with pathogens. The main objective of this study was to determine the microbiological quality of food sold in different grades of mobile food vendors and canteens in Owerri Metropolis.

2. MATERIALS AND METHODS

The study area is the Ekeonunwa Market square Owerri, Imo State Nigeria. It lies between latitudes 5.4850° N, and longitude 7.0350° E. The study area is populated with the market men and women of Owerri Town, with about 2 million individuals who deal on already-to-eat food, different kinds of goods and Agricultural products such as grains and vegetables. The Eke onunwa market is situated at Douglas road and it is very large markets where you can buy anything you want.

2.1 Source of Sample

The specimens examined in this investigation were collected at Eke onunwa market from market women that sell already-to-eat food or cook food along the road side of the market and was also collected from fast-food along Douglas Road in Oweerri Metropolis.

2.2 Sample Collection

Six (6) samples, three (3) each were bought from two different food hawkers in Owerri metropolis for study, the foods include; Rice, moimoi and soup. The samples were aseptically collected in a container and taken to the laboratory for immediate microbial examination and identification and the food samples were surveyed within the temperature range of 45°-50°C.

2.3 Culture Media Preparation and Sterilizations

Nutrient Agar (NA) and Sabouraud dextrose agar (SDA) were used. In preparing the media 28 g of NA 62 g of SDA were dispersed in one litre of distilled water but 250 ml each was needed, 7 g of NA and 15.5 g of SDA were used. The weighed media were mixed with distilled water in a sterile conical flask, autoclaved for 15 min at a temperature of 121°C and pressure of 15 psi (pounds per square inch) in an autoclave for sterilization and kept to cool to 40°C. The top of the conical flasks were wrapped with foil to prevent contamination and leakage [2]. The area of the bench where the work was done was properly swabbed with disinfectant soup and deionized water. Wire loop was flamed before and after each use and all glass wares were sterilized in an oven at 160°C for 2 hrs before use.

2.4 Serial Dilution

A tenfold serial dilution was prepared. For sample, 6 test tubes were filled with 9ml of deionised sterile water and covered with 1g of each sample (Rice, moimoi and soup) were transferred into the test tube (making it 10 ml) and thoroughly mixed by shaking, further more sequential dilution were made by taking/pipetting 1ml of mixture to the other test tubes and all were labelled 10^{-1}- 10^{-6}.

2.5 Culturing, Incubating, Colony Counting and Identification

After the serial dilution 1 ml of each sample was taken the 4^{th} and 5^{th} dilution were transferred to a petri dishes that has been appropriately labelled with marker and tape, the pour plate method was used for culturing [20] the petri dishes were shaken in an anticlockwise and clockwise direction gently to enable the agar that was poured into it to set and spread out evenly.

The plates for bacteria count were kept in the incubator at 37°C for 24 hrs at room while that of fungi were kept on the bench for 4 hrs at room temperature (25°C). All the Petri dishes were incubated upside down [21]. After 24 hours of incubation, the total bacteria count was carried out colonies appeared as discrete and each plate was counted, observed from colony characteristics and result was recorded, same was done for fungi after 48 hrs of incubation. Isolates identified were taken and sub cultured in a slope [22]. Identification was carried out on the incubated isolates using standard procedure and biochemical tests. The test done were gram staining for bacteria, catalase test, motility test, coagulase test, citrate utilization test and lactophenol cotton blue test for fungi and catalase test, according to [23,24,25].

2.6 Gram Staining Test

A smear was made by suspending a 24 hrs old culture in small drops of distilled water at one end of a clean and grease-free slide. The smear was heat-fixed, then examined with crystal violet (Bema) for one minute, then it was washed under gentle running tap water and flooded with logol's iodine solution for 30 minutes, this was blotted dry, then decolourized with 95% alcohol (ethanol) until the solvent flowed colourlessly and flooded with Safranin (BEMA) for two minutes. It was blotted dry again, then examines under the microscope using oil immersion objective x100 [26].

2.7 Catalase Test

This test aids in the determination of microorganisms that contain catalase enzyme are capable of releasing oxygen gas when mixed with hydrogen peroxide (H_2O_2). A drop of 6% H_2O_2 was placed on a clean and grease-free slide, a loopful of a 24 hrs old culture was placed on the drop and was instantly examined for the appearance of bubbles the evolution of bubbles indicated a positive reaction.

2.8 Motility Test

The method reported by [27] was adopted using wet mount method, fresh nutrient broth cultures of microorganisms were used. The broth culture was mixed well. Several loopfull of a pure microbial isolate. Cultures were placed in the middle of a clean, scratch free glass slide and then covered with a clean cover slip by lowering it over the drop as though it were hinged at one side avoiding bubbles. The preparation was examined under microscope first under x 40 objective lens. The motile and non-motile organisms were identified. The slides were cleaned up with alcohol first (because it had live bacteria on it), followed by soap and water and cover slip was discarded.

2.9 Coagulase Test

This was used to differentiate the organism that are coagulase positive from the ones that are coagulase negative. For instance, *Staphylococcus aureus* is coagulase positive. Whereas *S. epidermidis, S. saprophyticus and Escherichia coli* are coagulase negative [24].

This test was done using slide test technique. An inoculum of the test organisms was emulsified in a drop of physiological saline on a glass slide to make a thin suspension. A drop of serum was added to the suspension and mixed gently. A clumping of organisms within 10 seconds shows positive test but absence of clumping within 10 seconds indicates negative test [25].

2.10 Citrate Utilization Test (Gyrate Test)

This was used for the identification of bacteria that can utilize citrate as its sole carbon source and ammonia as its only source of nitrogen, it was carried out by using a sterile straight niddle to lick a loopful test organisms and streaked on the surface of the fresh prepared Simon's citrate agar media. Then, incubated at 37°C for 4 days which resulted in an alkaline reaction that changes the colour the indicator (bromothymol blue) from green to royal blue with growth along the line of streaking which indicates positive test whereas absence of growth and colour change indicates negative [25].

2.11 Sugar Fermentation Test

This test was used for the identification of organisms that can ferment sugar with or without production of acid and gas, the sugars used were glucose and lactose 1.0 g of each of the sugar was weighed out and dissolved into 100 mls of distilled water in different test tubes and autoclaved at 121°C for 10 minutes. 0.5 ml of each medium was then dispensed aseptically into different test tubes containing 5 ml of peptone water in which already 12.5 ml were autoclaved in the presence of the Durham's tube/ the tubes were allowed to cool. Thereafter inoculated with the test organism and incubated for 24-48 hours at 37°C. Acid production was

indicated by change in the colour of the medium from red to yellow while gas production was shown by air space in the inverted Durham's tubes. Also the bubbles at the top layer of the medium were also an indication of gas production [28].

2.12 Fungi Staining

The method used was a described by [25] to large drop of lacto-phenol cotton blue was placed on a grease free slide portion of the growth picked with wire loop and teased out in the drop of lacto-phenol blue stain on the slide. Covered with a clean cover slip talking care to exclude air bubbles, left for 10-15 min, examined using x 40 (high power dry) objective for closer examination of selected field.

3. IDENTIFICATION OF FUNGAL ISOLATES

3.1 Microscopic Identification

Fungal isolates were macroscopically identified base on their morphology and colour of the front and back surface of the colonies on the plates. References were made to the fungal identification guide line described by [29] and [30].

3.2 Mould Isolates

Wet mount of the isolates was prepared using lactophenol cotton. A drop of the lactophenol blue was placed on a sterile grease free slide. With a sterile dissecting needle. Small portion of the culture was collected and teased on the slide [24] and was covered with a cover slip and then examined hyphae cells and spores under the microscope using (x 40) objectives lens with the iris sufficiently closed to give a good contrast.

3.3 Yeast Isolates

Wet mount of the yeast isolates were prepared using physiological normal saline. A drop of the normal saline was placed on a sterile grease free slide. With the aid of a sterile needle, small portion of the culture was collected and teased on the slide [26]. This was covered with a cover slip and then examined for yeast cells under the microscope using (x 40) objectives lens with the iris sufficiently closed to give a good contrast.

3.4 Statistical Analysisis

Data obtained were computed using scientific calculator and subjected to percentages.

4. RESULTS AND DISCUSSION

4.1 Bacteria and Fungal Isolate from Fast Food and Canteens (Buker)

From this study a total of 624 (100%) was isolated. Out of 624 (100%) *pseudomonas* had 178 (28.5%), *Bacillus* sp had 59 (9.5%), Staphylococcus *aureus* had 160 (25.6%) and *proteus* had 227 (36.4%) bacteria respectively. In the fast-food sample, organisms isolated form moimoi (102) that gave 16.3%, Rice (59) that gave 9.5% and soup (60) that gave 9.6% in fast food while in canteen the fungi isolate from moimoi (160) that gave 25.6%, Rice (16) that gave 2.6% and soup (227) that gave 36.4% respectively. The result on bacterial isolates from the two vendors shows that fast food had a bacteria isolate of 221 (35.4%) while the canteen had 403 (64.6%) of bacteria isolate, which means that the bacteria isolated from canteen was higher than those isolated from fast food (Table 1).

Table 1. Bacterial and fungal isolates from fast food and buka

Food vendors	*Pseudomonas aeruginosa*	*Bacillus* species	*Staphylococcus aureus*	*Proteus*	Total
Fast food					
Moimoi	102(6.3)	0(0.0)	0(0.0)	0 (0.0)	102 (16.3)
Rice	0(0.0)	59(9.5)	0(0.0)	0.(0.0)	59(9.5)
Soup	60(9.6)	0.(0.0)	0(0.0)	0.(0.0)	60(9.6)
Total (%)	162(25.6)	59(9.5)	0(0.0)	0.(0.0)	221(35.4)
Canteen (Bukar)					
Moimoi	0(0.0)	0(0.0)	160(25.6)	0 (0.0)	160(25.6)
Rice	16(2.6)	0(0.0)	0(0.0)	0.(0.0)	16(2.6)
Soup	0(0.0)	0(0.0)	0(0.0)	227(36.4)	227(36.4)
Total (%)	16(2.6)	0(0.0)	160(25.6)	227(36.4)	403(64.6)
Overall (%)	178(28.5)	59(9.5)	160(25.5)	227(36.4)	624(100)

Table 2. Fungal isolates from fast food and bukar

Food vendors	*Pencillium* sp	*Saccharomyces*	*Geotrichum* sp	*Mucor* sp	Total
Fast food					
Moimoi	10(3.3)	0(0.0)	0(0.0)	0 (0.0)	10 (3.3)
Rice	0(0.0)	13(4.3)	0(0.0)	0.(0.0)	13(4.3)
Soup	10(3.3)	13(4.3)	0(0.0)	0.(0.0)	23(7.7)
Total (%)	20(6.7)	26(8.7)	0(0.0)	0.(0.0)	46(15.3)
Canteen (Bukar)					
Moimoi	0(0.0)	42(14.0)	0(0.0)	0 (0.0)	42(14.0)
Rice	0(0.0)	3(1.0)	4(0.3)	0.(0.0)	7(2.3)
Soup	0(0.0)	0(0.0)	0(0.0)	205(68.3)	205(68.3)
Total (%)	0(0.0)	45(15.0)	4(0.3)	205(68.3)	254(84.7)
Overall (%)	20(6.7)	72(24.0)	4(0.3)	205(68.3)	300(100)

Table 3. Bacteria Identification of fast food and buka

Isolate	Morphological characteristics	Colonical characteristics	Gram staining test	Catalase test	motility test	Coagulatase test	Citrate test	Sugar fermentation test	Probale organism
	Vendor A								
Fast food rice	Budded spores	Moderate, round film, opaque colour, smooth	+	+	-	+	-	+	*Bacillius* specie
Fast Food soup	Rods	Green Glossy, mucoid, Raised colonies, Round film	-	+	+	+	+	-	*Pseudomonas aeruginosa*
Fast food moimoi	Rods	Irregular, raised colonies, glossy mucoid, orange	-	+	-	+	+	+	*Pseudomonas aeruginosa*
	Vendor B								
Buka Rice	Rods in pairs	Green, Glossy, Mucoid, Raised colours, round film	-	+	+	-	+	-	*Pseudomonas aeruginosa*
Buka soup	Long Rod	Cream, soft, smooth, raised circular colonies	-	+	+	-	-	-	*Proteus*
Buka Moimoi	Cocci	Yellow, small, smooth, circular colonies	-	+	-	+	+	+	*Staphylococcus aureus*

Table 4. Fungal identification

Isolate	Morphological characteristics	Colonical characteristics	Gram staining test	Lacto-phenol cotton blue	Probable organisms
Vendor A					
Fast food Rice	Single conidio-spore	Short aerial mycelium, green, brown and white surface and light yellow base, round films	+	-	*Penicillum sp*
Fast food soup	Unicellularr budded spore	Round film	-	+	*Saccharomyces*
Fast food moimoi	Single conidiospores	Green, brown and white surface, short aerial mycelium, yellow base, round film	-	+	*Penicillum* sp
Buka Rice	Arthrospores	White and yellow surface, short aerial mycelium yellow bases	-	-	*Geotrichum* sp
Buka soup	Sporangiospore	White surface, black spores, white base and long arial mucelium	-	+	*Mucor* sp
Buka moimoi	Budded spore	Round film	-	-	*Sacharomyces*

For fungi; a total fungal isolate was 300 (100%), out of 300 (100%) penicillium had 20 (6.7%), *saccharomyces* has 72 (27.0%) *geotrichum* had 4(0.3%) and mucor sp had 205 (68.3%) respectively. From the fastfood sample, organisms isolated from moimoi (10) that gave 3.3%, Rice (13) that gave 4.3% and soup (23) that gave 7.7% in fast food while in canteen the organisms isolate from moimoi (42) that gave 14.0%, rice (7) that gave 2.3% and soup (205) that gave 68.3% respectively. The result on fungal isolate from the two vendors show that fast food had a fungal isolate of 46 (15.3%) while the canteen had 254 (84.7) of fungal isolate. Which means that the fungal isolated from canteen was higher than those isolated from fast food (Table 2).

4.2 Bacteria and Fungal Identification

In the result of identification, the species obtained included *Bacillus* species and *Pseudomonas aeruginosa*, from fast food sample while *Proteus*, *Staphylococcus aureus* was in Buker sample. The fast food sample was predominated by *Penicillium* sp. in the rice, mucor sp. in the soup and saccharomyces being the probable species in the moimoi sample.

Geotrichum is a genus of fungi found worldwide in soil, water, air and sewage, as well as in plants, cereals, and dairy products, it is also commonly found in normal human flora and is isolated from food, sputum and faeces [31].

Finally, saccharomyces and penicillum are fungi (yeast) obtained from the samples. These fungi appear positive after staining and are found majorly as microflora usually associated with mostly cereals and legumes but they are transferred to food also by improper cooking handling technique [32].

4.3 Discussion

Generally the result on the bacterial isolate from the two vendors (fast-food and canteen) shows that fast-food had a bacterial isolate of 221(35.4%) while the canteen had 403(67.6%) of bacterial isolate and in fungal isolate from the two vendors it shows that fast-food had a fungal isolate of 46(15.3) while the canteen had 254(84.7%) of fungal isolate. In the result of bacterial identification the organisms obtained includes *Bacillius* sp., *Pseudomonas aureginosa*, *proteus* and *staphylococcus aureus* while in fungal identification the organisms obtain includes *saccharomyces*, *geotrichum* and *Mucor* species and these agrees with the work of [33] which stated that food safety and hygienic practices of street food vendors in owerri, Nigeria and [34] that worked on the fast food business in Nigeria [35].

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study shows that environmental micro flora, raw, materials, processing conditions and handlers hygiene can be a significant source of food contamination with microbes, the totality of all these possible gross malpractice in good services can be summarized and determined by subjecting the food which is the final product to microbiological examination to determine if the appropriate technique was applied during processing.

5.2 Recommendation

It is therefore suggested that preventive measure should be recommended to the food handlers and street food hawkers in general and there is need to advocate for good manufacturing practices for food processors and handlers.

These are the possible preventive measures recommended;

1. Food should be prepared on a hygienic environment.
2. Food should be prepared and stored at the appropriate temperature.
3. Food handlers and street food hawkers should be educated on the importance of keeping to good hygienic.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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