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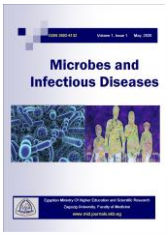

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Original article

Microbiological assessment of dispensed foods in primary schools in Bokkos L.G.A. of Plateau State, Nigeria

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ABSTRACT

Background: The microbiological assessment of food safety is of paramount importance in ensuring the well-being of populations, particularly in regions where foodborne illnesses are prevalent. This study aimed to assess microbiological quality of food dispensed in primary schools within Bokkos L.G.A of Plateau State, Nigeria. **Methods:** A cross-sectional study was conducted. A total of 20 food samples were collected over a specified period of time. Assessments included temperature monitoring and compliance with food safety and hygiene standards. Food samples were collected aseptically from the selected schools and transported to a microbiology laboratory. Various standard microbiological techniques were employed to analyze the samples for the presence of harmful microorganisms, including bacteria and fungi. Microbiological data were analyzed to determine the prevalence of pathogenic microorganisms in the dispensed foods. **Results:** Microbiological analysis demonstrated the presence of pathogenic bacteria and fungi; *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species (spp), *Bacillus cereus*, *Enterobacter* spp, *Pseudomonas* spp, *Proteus* spp, *Streptococcus* spp, *Shigella*, *Lactobacillus* spp, *Enterococcus* spp, *Candida* spp, *Saccharomyces cerevisiae*, *Aspergillus* spp, *Fusarium* spp, *Mucor* spp and *Penicillium* spp. Eighty percent (80%) of food samples were above tolerable limit (unacceptable) and the highest plate count could be due to inadequate handling and processing by vendors, contamination caused by storage facilities, or poor hygiene/poor quality of grains and water used. **Conclusion:** The presence of pathogenic microorganisms in dispensed foods underscores the importance of implementing stringent food safety protocols and promoting hygiene education among food handlers in school kitchens.

Introduction

Human food is any substance which can be ingested by human to protect life and enhance growth of the body [1]. Food is of great importance for the maintenance and sustenance of human health. Nonetheless, unintentional contamination sets in via food preparations [2]. Foods are classified as either safe or non-safe. Non safe foods are foods that have been previously exposed to contamination and are thus, unfit for human consumption. Safe food on the other hand, is defined as a food that contains no biological contaminants, no chemical

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contaminating agents and other substances which could be harmful or lay open human health [3]. Food displays a relevant role in human's lives-therefore immense level of food safety is needed to guard humans against food-borne diseases or dangers associated with foods [3]. Safe foods are fundamental human right although many foods are mostly adulterated with freely occurring disease causing microbes which cannot be seen, smelled or tasted but are capable of causing ailment ranging from mild to severe, and even death when considerable heights of contamination of microbes is attain [4]. Food is a basic need of human being which is nutritive only when pure, fresh and free from hazardous matter such as pathogenic bacteria and sub-standard food colour [5].

Dispensed foods are vended foods- foods prepared at home and are been consumed in schools without further preparation [6]. Vended foods serve as source of affordable food for students but often at times, vended foods don't meet proper hygienic standard as a result of inadequate food safety laws, lack of financial resources to invest in safer equipment, and lack of education enlighten for food-handlers. Food handlers are very essential set of people when considering food safety. Their hygiene practices affect vast population of people who solemnly depends on them for their meals [6].

Ready to eat foods may also be referred to as ready to consume foods. These are foods ingested in likewise manner they are sold. Our consumption of ready-to-eat meals that can satisfy any gourmet needs and are consumed more frequently is alarming [7]. Some read to consume foods enhances the growth of disease-causing organisms and as a result, are referred to as potentially in-secured. In other to reduce the growth of any disease-causing organism that may exist in food or limit toxins formation in foods, such foods must be conserved at specific temperatures and surroundings. Ready to eat foods most a time include a bunch of food additives which may or may not be cooked. Furthermore, these foods are mostly susceptible to contamination/cross contamination from water, air, topmost layer of the earth crust, food handlers/vendors, conservation/dissemination facilities or environment due to the structure of these foods and their means of preparation which include thorough handling [8].

Ready to eat foods can be classified as either street vended foods or dispensed foods. Street foods are defined as ready to eat foods and beverages prepared and/or sold by street vendors and handlers especially along the streets and other similar places for instant or later consumption without further preparation or processing. Even though street foods are relatively cheap and found almost everywhere, it is most a time associated with health problems [9]. Dispensed foods on the other hand, are ready to eat foods that are sold or serve in places such as schools and hospitals. Since Ready to eat foods does not usually undergo further processing, it can serve as a good vehicle for food-borne microbial organism transmission into the body, if it is not handled properly [9]. There are lots of reasons why people eat away from home of which include absence from home while travelling, studying, while at work or need for a change both in terms of food type and the location [9].

Some importance of ready to eat foods include source of inexpensive, convenient and often nutritious food; a major source of income for a vast number of persons particularly women; and a chance for self-employment and the opportunity to develop business skills with low capital investment. Despite these numerous advantages of ready to eat foods, there are also multiple health hazards associated with it [10].

The microbial analysis of food is very vital so as to ensure safe products for the consumers since preventing food contact with microorganisms is nearly impossible as food from vendors are exposed to a wide range of microbes. The carry-over of potential pathogens from food vendors is also influence by many environmental factors including the microbial load present in the water used for cooking, type of food and a host of other single or interacting factors [10].

Food-borne pathogens are defined as disease causing microorganisms which are found in food as a result of contamination which can be either by exposure or improper hygiene. Ingested food-borne pathogens give rise to food-borne diseases. Such pathogens range from bacteria; *Salmonella* spp, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* spp, *Clostridium botulinum*, *Listeria monocytogenes*, *Pseudomonas* spp, *Clostridium perfringes*, *Campylobacter jejuni*, *Enterococcus* spp, *Klebsiella* spp, *and Proteus* spp [10,11], Viruses; hepatitis A and E, Norovirus, Parasites; Entamoeba histolytica, Taenia solum, Taenia

saginata, Algae; Lyngbya majuscule, moulds, fungi and yeasts [8].

Food-borne diseases are major problem worldwide capable of causing considerable morbidity and mortality annually. World Health Organization (WHO) reported that daily, more than 5000 children die globally due to consumption of contaminated food and water. Food-borne illnesses are prevalent in all parts of the world and their toll on human well-being is enormous which lead to major economic loss. The incidence rate of food-borne diseases is also rising up. Therefore, food-borne diseases are diseases resulting from the ingestion of bacteria, toxins and cells produced by microorganisms present in food. Once the bacteria have produced toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Many of their toxin are gene-based that is carried on plasmids [9]. Symptoms of food-borne diseases most at times differs amongst pathogens but some general symptoms of the pathogens may include; diarrhoea, nausea, vomiting, fever and abdominal cramps. Some can cause organ failure.

Microbial agents get into foods from both internal and external sources. The amount and type of microbial agent present in food relies on the caution used during manufacturing, processing and storage. Food contamination may be as a result of in-aseptic practices in handling food substances, dirty food service environment, contaminated water, additives, equipment used in food preparation as mentioned earlier. In addition, microorganisms such as bacteria are ushered into foods via aerosols-which are obtainable from vendor-customer interaction at the point of sale. Bacteria present in the released aerosols from saliva lodge on the food and transported without the consent of the buyer to site of infection. In some instances, insects and flies cannot be totally avoided from coming in contact with the food. This could result to cross contamination [11].

This study evaluated the presence of microorganisms in dispensed foods obtained from different primary schools in Bokkos Local Government Area (LGA) of Plateau state.

Materials and methods

Collection of samples

This is a cross-sectional study, which allowed for the collection of data at a single point in time. This design is suitable for assessing the current status of the quality of dispensed foods in primary schools. A stratified random sampling technique was employed to select primary schools in Bokkos L.G.A. Stratification was based on the geographical location of the schools within the L.G.A. This ensured that both urban and rural schools were included in the study. A total of 20 food samples were collected from four different primary schools in Bokkos LGA of Plateau State. The schools and food samples include:

1. Precious Pearl International Primary School
Moimoi, Local ice cream, Buns, Awara, and Masa.

2. Rev. Week's Nursery & Primary School
Gwote, Rice and Beans, Zobo, Kunun zaki, and Jellof rice

3. Nha'akam Christian Academy
Jellof rice 1, Jellof rice 2, Buns, Chips, and Awara

4. Shalom Nursery & Primary School
Beans, Masa, Buns, Rice, and Chips

The food samples were randomly collected from the school registered food vendors within the school premises while other foods were collected from the school's served foods. Samples were placed aseptically into a sterile specimen container and transported to the laboratory for microbiological analysis within an hour of collection. The various samples were labeled at the point of collection and the data collected were recorded in the field note book including the vendor interview. The samples were collected shortly after preparation at about 11am-12noon consecutively for over a period of two weeks in June and July 2017.

Ethical considerations

The study adhered to ethical guidelines, including obtaining informed consent from school authorities and ensuring the anonymity of the participating schools.

Sample processing

One (1) gram analytical unit of each sample collected was weighed out and transferred into a sterile porcelain crucible and homogenized. Afterward, mashed samples were transferred into flat bottom flask and mixed with 9ml of sterile distilled water to give a suitable food homogenate. Using a sterile pipette, 1ml of the food homogenate was pipetted and transferred into sterile test-tube containing 9ml of sterile distilled water and mixed thoroughly to obtain 102 dilutions of sample. 1ml of the 102 dilution of sample was taken into another sterile test tube containing 9ml of sterile distilled water to obtain further dilution of 103. The

procedure was repeated until a higher dilution of 106 was obtained.

Sample analysis

The streak plate technique was used for plating out the appropriate dilution (103). One ml of the dilution was pipetted and transferred into a well labeled sterile petri-dishes containing the molten media. The inoculated agar plates were incubated at 37oc for 24 to 48 hours for Nutrient agar (for total viable count), 37oc for 24 to 48 hours for Mac Conkey agar plates (for coliform/*enterobacteriacaea*) and 25^{o}c for Sabouraud dextrose agar plates. The cultured plates were examined after incubation and colonies per plates were counted and recorded for estimation of colony forming unit per grams (cfu/g) of the original sample.

Purification of isolates

After incubation for 24 to 48 hours, developing colonies on plates were randomly picked and sub-cultured until pure cultures were obtained on corresponding agar plates for purification.

Identification of isolates

Bacteria isolated from the different food samples were characterized to generic level and where possible to the species level on the basis of Gram stain and their cultural features (which include shape, size, texture, elevation, appearance, pigmentation, margin, etc), morphological features (such as motility, gram reaction and cell shape) and biochemical features. Moulds and yeasts were culturally and morphologically characterized. For morphological characterization of moulds, lacto phenol cotton blue was used as stain while Gram stain was used for yeast. The results obtained were then compared with standard references for proper identification for isolates. Identified yeast cells were further inoculated on CHROM agar and incubated at 37oc for 24 to 48 hours and the colour change was observed for 3 consecutive days to identify the species level of yeast cells. Atlas of medical bacteriology and atlas of medical mycology were used for the identification of bacteria and fungi respectively.

Table 1. Cultural and morphological features of bacterial isolates.
+ Positive

Cultural features							**Morphological features**			
Bacterial code	**Size**	**Shape**	**Elevation**	**Texture**	**Appearance**	**Margin**	**Gram reaction**	**Cell shape**	**Motility**	**Suspected organisms**
B1	Punctiform	Circular	Convex	Smooth	Pale yellow	Entire	+	Cocci in cluster	-	*Staphylococcus aureus*
B2	Small	Circular	Raised	Smooth	Cream	Entire	-	Rods singly	+	*Escherichia coli*
B3	Large	Irregular	Umbonate	Rough	White	Undulate	+	Rods singly	+	*Bacillus cereus*
B4	Moderate	Oval	Umbonate	Smooth	Dark green	Undulate	-	Rods singly	+	*Enterobacter* spp
B5	Small	Circular	Convex	Smooth	Tan	Entire	-	Rods singly		*Pseudomonas* spp
B6			Convex	Smooth		Entire	-	Rods singly	+	*Proteus* spp
B7							-	Rods singly	-	*Salmonella* spp
B8	Small	Circular	Convex	Smooth	Pale yellow	Entire	-	Rods singly	-	*Streptococcus* spp
B9	Punctiform	Circular	Convex	Smooth	White	Entire	+	Rods singly	-	*Shigella* spp
B10	Large	Circular	Umbonate	Smooth	White	Undulate	+	Rods singly		*Lactobacillus* spp

- Negative

Table 2. Cultural and morphological features of mould isolated.

Mould codes/features	**Septation**	**Colour**	**Type of spore**	**Colour of spore**	**Appearance of spore head**	**Suspected organism**
M1	Septate	White	Conidiospore	Black	Rough	*Aspergillus niger*
M2	Septate	Cream	Conidiospore	Deep brown	Rough	*Aspergillus flavus*
M3	Septate	-	Conidia in chains	Green	Rough	*Aspergillus fumigatus*
M4	Non-septate	Colourless	Conidiospore	White	Rough	*Pennicillum* spp

Table 3. Total Plate Count (cfu/ml)

S/N	**Food Samples**	**TFC (cfu/ml)**	**TAC (cfu/ml)**	**TEC (cfu/ml)**	**TPC (cfu/ml)**
1	Moimoi	3.2×10^5	7.5×10^5	4.2×10^5	1.5×10^6
2.	Local ice-cream	2.0×10^4	2.4×10^6	5.3×10^5	3.0×10^6
3.	Buns	4.0×10^4	8.6×10^5	7.7×10^5	1.7×10^6
4.	Awara	7.1×10^5	1.3×10^6	6.4×10^5	2.7×10^6
5.	Masa	1.9×10^5	2.1×10^6	9.4×10^5	3.2×10^6
6.	Gwote	9.0×10^4	1.2×10^6	2.7×10^5	1.6×10^6
7.	Rice & Beans	2.2×10^5	2.5×10^6	5.6×10^5	3.3×10^6
8.	Zobo	1.7×10^5	6.6×10^5	4.8×10^5	1.3×10^6
9.	Kunun zaki	1.9×10^5	1.0×10^6	6.5×10^5	1.8×10^6
10.	Jellof Rice 1	3.0×10^4	6.7×10^5	7.5×10^5	1.5×10^6
11.	Jellof Rice 2	4.0×10^4	2.0×10^5	1.3×10^6	1.5×10^6
12.	Jellof Rice 3	3.0×10^4	1.4×10^6	1.9×10^5	1.6×10^6
13.	Buns	2.0×10^4	1.0×10^6	5.8×10^5	1.6×10^6
14.	Chips	3.0×10^4	5.6×10^5	1.7×10^5	7.6×10^5
15.	Awara	2.0×10^4	1.2×10^6	2.2×10^5	1.4×10^6
16.	Beans	4.1×10^5	5.4×10^5	1.4×10^6	2.4×10^6
17.	Masa	9.8×10^6	9.3×10^6	8.5×10^3	2.0×10^7
18.	Buns	5.0×10^3	4.5×10^4	7.8×10^4	1.3×10^5
19.	Rice	7.8×10^4	6.0×10^5	4.2×10^4	7.2×10^5
20.	Chips	3.5×10^4	2.9×10^4	3.3×10^4	9.7×10^4

Key

TFC Total Fungal Count
TAC Total Aerobic Count
TEC Total Enterobacteria Count
Cfu/ml Coliform Forming Unit per ml
TPC Total Plate Count

Table 4. Biochemical characteristics of bacterial isolates.

Bacterial Codes/ Features	Catalase	Oxidase	Coagulase	Indole	Citrate	Fructose	Sucrose	Glucose	Lactose
B1	+	-	-	-	+	NR	NR	AG	NR
B2	+	-	-	+	-	AG	AG	AG	AG
B3	+	-	-	-	+	A	A	A	A
B4	+	+	-	+	+	A	A	A	A
B5	+	+	-	+	+	AG	AG	AG	AG
B6	+	-	+	-	+	A	A	A	A
B7	+	-	NR	-	NR	NR	-	NR	-
B8	-	-	NR	NR	NR	G	A	NR	A
B9	+	-	NR	-	NR	NR	-	AG	-
B10	-	+	-	+	+	A	A	A	A

Key

+	Positive
-	Negative
NR	No Reaction
AG	Acid and Gas
A	Acid
G	Gas

Table 5. Microorganisms isolated from various food samples.

S/N	Samples	Microorganisms isolated
1.	Moimoi	*Escherichia coli, Aspergillus niger, Aspergillus fumigatus, Penicillium* spp, *Staphylococcus aureus, Bacillus cereus.*
2.	Local ice-cream	*Staphylococcus aureus, Aspergillus niger, Lactobacillus* spp, *Salmonella* spp, *Pseudomonas* spp, *Proteus* spp
3.	Buns	*Lactobacillus* spp, *Staphylococcus aureus, Strptococcus* spp, *Candida albicans, Fusarium* spp, *Aspergillus fumigatus.*
4.	Awara	*Proteus* spp, *Streptococcus* spp, *Enterobacter, Staphylococcus* spp, *Aspergillus fumigatus, Saccharomyces cerevisiae*
5.	Masa	*Enterobacter, Saphylococcus aureus, Bacillius cereus, Penicillium* spp, *Saccharomyces cerevisiae*
6.	Gwote	*Pseudomonas* spp, *Staphylococcus aureus, Mucor* spp, *Penicillium* spp, *Fusarium* spp, *Candida flavus.*
7.	Rice & Beans	*Pseudomonas* spp, *Bacillus cereus, Aspergillus flavus, Aspergillus fumigatus, Staphylococcus aureus, Fusarium* spp
8.	Zobo	*Escherichia coli, Staphylococcus aureus, Bacillus cereus, Salmonella* spp, *Aspergillus fumigatus, Candida krusei, Shigella* spp
9.	Kunun zaki	*Escherichia coli, Staphylococcus aureus, Bacillus cereus, Enterococci, Fusarium* spp, *Candida albicans, Penicillium* spp
10.	Jellof Rice 1	*Enterobacter, Staphylococcus aureus, Escherichia coli, Penicillium* spp, *Bacillus* spp
11.	Jellof Rice 2	*Bacillus cereus, Aspergillus flavus, Aspergillus fumigatus, Penicillium* spp, *Candida krusei, Staphylococcus aureus*
12.	Jellof Rice 3	*Pseudomonas* spp, *Fusarium* spp, *Bacillus cereus, Enterobacter, Staphylococcus aureus, Salmonella* spp
13.	Buns	*Pseudomonas* spp, *Shigella* spp, *Lactobacillus* spp, *Bacillus cereus, Mucor* spp, *Candida glabrata, Aspergillus fumigatus, Staphylococcus aureus*
14.	Chips	*Saccharomyces cerevisiae, Aspergillus fumigatus, Salmonella* spp, *Escherichia coli, Bacillus cereus, Mucor* spp, *Staphylococcus aureus*
15.	Awara	*Fusarium* spp, *Pseudomonas* spp, *Candida glabrata, Staphylococcus aureus, Proteus* spp, *Salmonella* spp, *Shigella* spp
16.	Beans	*Staphylococcus aureus, Bacillus cereus, Shigella* spp, *Aspergillus fumigatus, Streptococcus* spp
17.	Masa	*Pseudomonas* spp, *Staphylococcus aureus, Salmonella* spp, *Proteus* spp, *Aspergillus niger, Escherichia coli, Fusarium* spp

18.	Buns	*Lactobacillus* spp, *Pseudomonas* spp, *Bacillus cereus, Candida albicans, Escherichia coli, Saccharomyces cerevisiae, Aspergillus fumigatus*
19.	Rice	*Enterobacter* spp, *Streptococcus* spp, *Pseudomonas* spp, *Penicillium* spp, *Candida krusei, Fusarium* spp.
20.	Chips	*Bacillus cereus, Escherichia coli, Stapylococcus aureus, Salmonella* spp, *Fusarium* spp, *Aspergillus niger, Saccharomyces cerevisiae*

Table 6. Percentage of occurrence of bacterial isolates.

S/N	Bacterial Organisms	Frequency	Percentage of Occurrence (%)
1	Escherichia coli	8	10.4
2	Staphylococcus aureus	18	23.4
3	Bacillus cereus	13	16.9
4	Lactobacillus spp	4	5.2
5	Salmonella spp	7	9.1
6	Proteus spp	4	5.2
7	Pseudomonas spp	9	11.7
8	Streptococcus spp	4	5.2
9	Enterobacter spp	6	7.7
10	Shigella spp	4	5.2
	Total	77	100%

Table 7. Percentage occurrence of fungal organisms.

S/N	Bacterial Organisms	Frequency	Percentage of Occurrence (%)
1	*Aspergillus flavus*	2	4.2
2	*Aspergillus niger*	4	8.3
3	*Aspergillus fumigatus*	10	20.7
4	*Mucor* spp	3	6.3
5	*Fusarium* spp	9	18.8
6	*Penicillium*	7	14.6
7	*Saccharomyces cerevisiae*	4	8.3
8	*Candida albicans*	3	6.3
9	*Candida krusei*	4	8.3
10	*Candida glabrata*	2	4.2
	Total	48	100%

Figure 1. Percentage occurrence of fungal isolates.

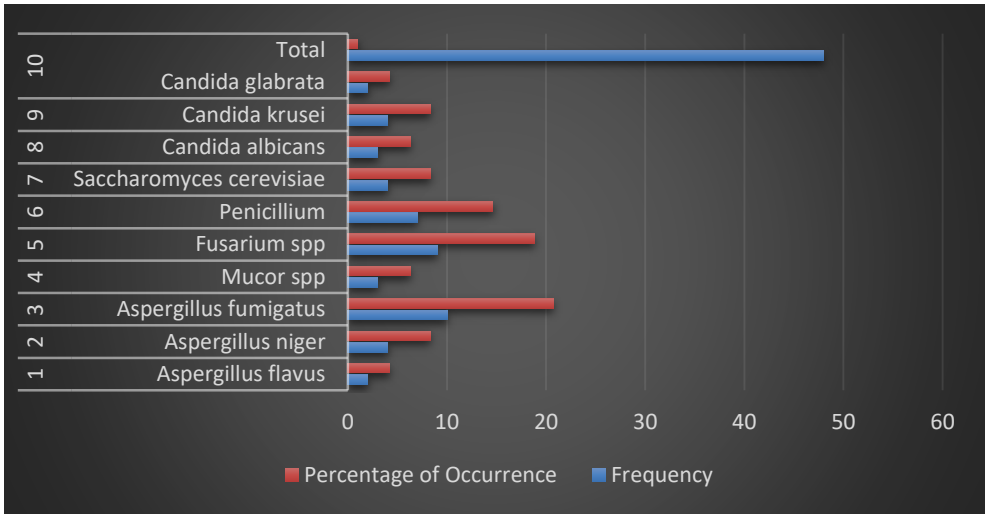


Figure 2. Bacterial isolates on Mac-conkey agar and nutrient agar.

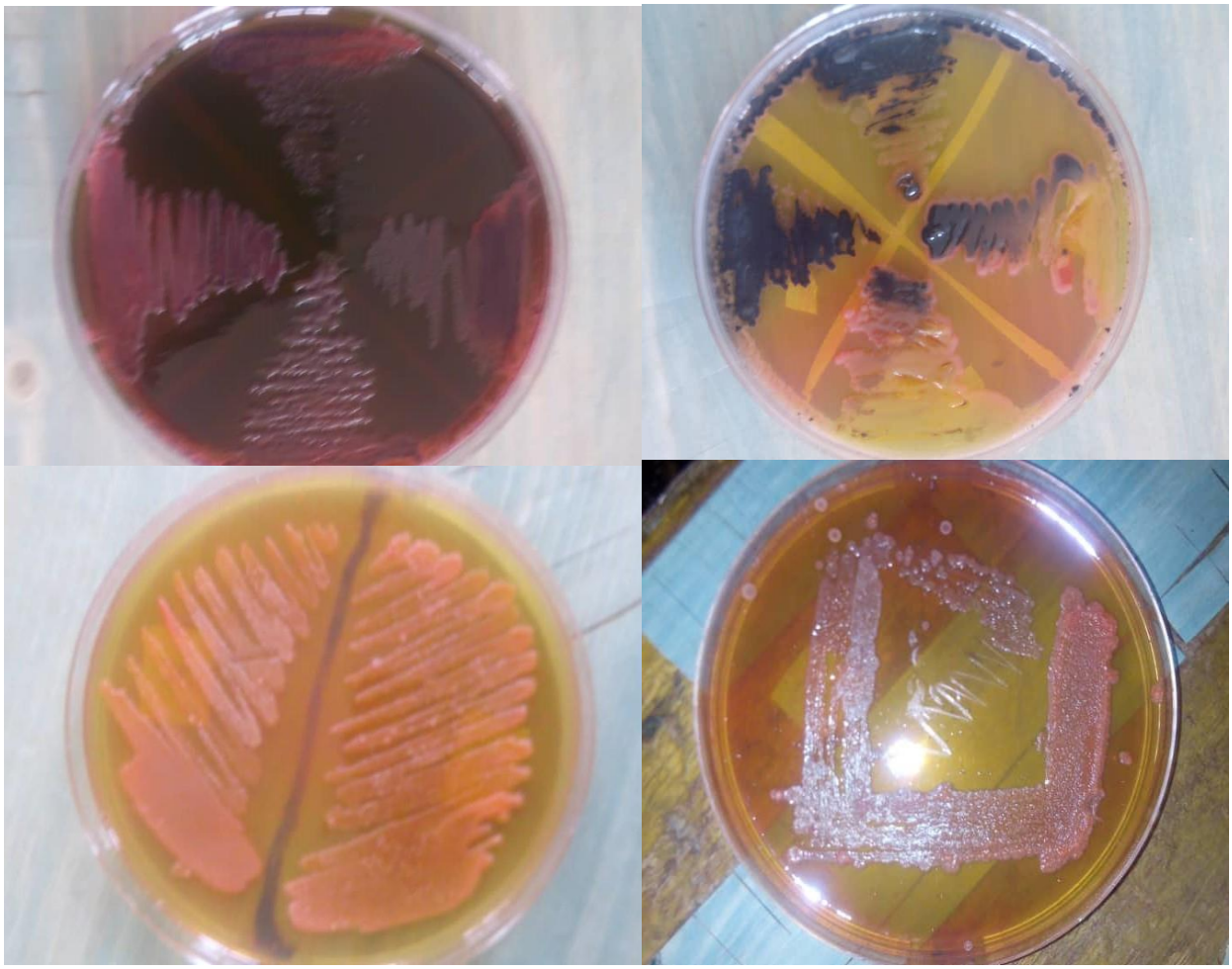
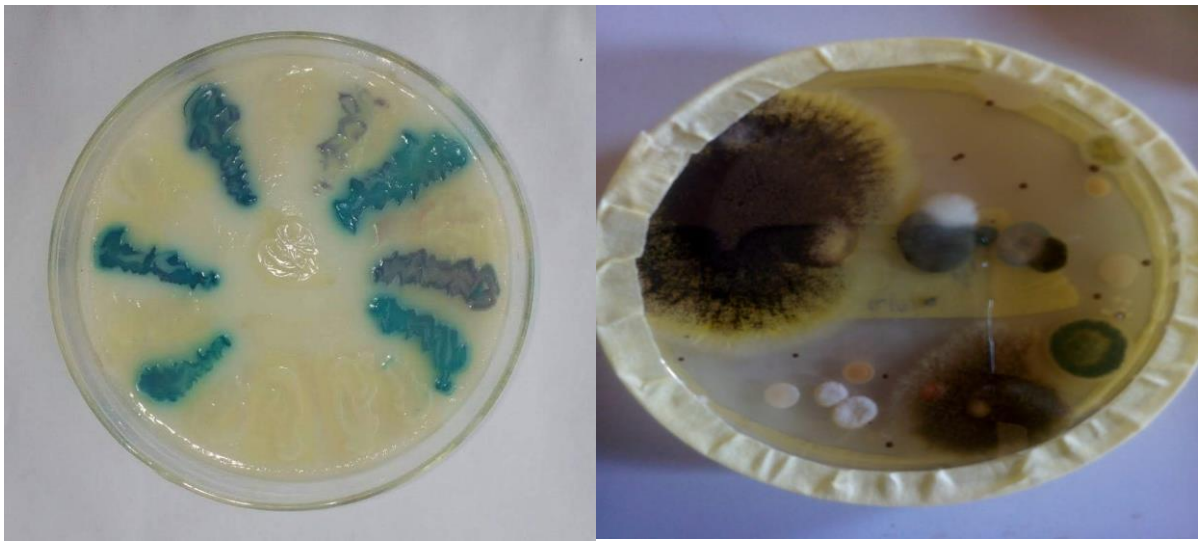


Figure 3. Fungal isolates on A: Chrom agar and B: Sabouraud dextrose agar



A B

Discussion

The foods dispensed or sold to primary school pupils by the vending sites in school compounds are of tolerable and unacceptable microbiological quality. The International Commission for Microbiological Specification for Foods (ICMSF, 1996) states that ready to eat foods with plate counts between 0-103 is acceptable, between 104- 105 is tolerable and 106 and above is unacceptable. The data obtained in this study has demonstrated the activities of both pathogenic bacteria and fungi associated with ready to eat foods and confirmed previous microbiological reports on other ready to eat foods [13-20]. The level of contamination of the total samples that was above tolerable limit (unacceptable) was 80% while 20% was tolerable. Masa (sample 17) has the highest plate count and this could be due to inadequate handling and processing by vendors, contamination caused by storage facilities, either poor hygiene or poor quality of grains and water used. Similarly, the extensive mixing during processing could have introduced contaminants via food handlers, cooking utensils and from the environment.

The presence of *Bacillus cereus, Pseudomonas* spp, *Salmonella* spp, *Shigella* spp are known to be environmental contaminants and opportunistic pathogens [21] have been implicated in foodborne diseases and are known to cause food spoilage that can lead to economic loss. *Escherichia coli, Enterobacter, Staphylococcus, Proteus* spp are of concern and further support the possibility of faecal contamination of products due to poor sanitation [21]. The most predominant bacterial contamination was *Staphylococcus aureus* with 23.4% and this could be traced to the fact that it is abundant in human body, skin, nails, hair. Similarly, Bacillus cereus showed high percentage (16%), its presence can be traced to the fact that it is abundant spore former in soil, air and water, hence can be present in these foods. This report is in agreement to reports of [22-24], they isolated similar organisms from sausages, meatpie, sea foods respectively.

The presence of *Aspergillus, Penicillium, Mucor, Fusarium, Saccharomyces cerevisiae* and *Candida* could be attributed to the surrounding air, soil and packaging materials [8]. *Aspergillus* spp are very common fungal agent of foodborne illness [8] as a result, it has the highest percentage of occurrence of fungal contaminants of 33.2%.

Finally, the result presented heralded the fact that ready to eat foods could be a source of diarrhoea and/or gastrointestinal disturbances in both children and adults if they exceed the acceptable limit. This is in agreement with the findings of other workers concerning street foods in countries with high ambient temperature condition [24-25].

The current situation of high levels of food contaminations in primary schools today is due to the absence of regulations and guidelines for food vendors/cooks in primary schools and also the political and economic situation restricting the efforts of Ministry of Health to take actions against sub-standard food production.

Conclusion

The microbiological assessment of dispensed foods in primary schools in Bokkos L.G.A. is crucial for ensuring the health and well-being of school children. This study provides a comprehensive methodology for assessing food quality and safety in school feeding programs, which can serve as a model for similar studies in other regions.

Limitations

The study acknowledged certain limitations, such as potential recall bias in the physical assessment due to the reliance on observations, and the need for regular microbiological assessments over time to establish trends.

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Not declared.

Conflict of interest

Not declared.

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