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RESEARCH ARTICLE

STUDIES ON BACTERIAL CONTAMINANTS ON NAILS OF FOOD VENDORS IN SELECTED MARKETS OF GBOKO LOCAL GOVERNMENT AREA, BENUE STATE, NIGERIA

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ABSTRACT

The aim of this study was to assess the occurrence of bacterial contaminants in fingernails of food vendors in three selected markets of Gboko Local Government Area, Benue State, Nigeria. A total of thirty (30) nail samples were collected from food vendors, 10 samples from each market using new razor blade for each subject. Standard laboratory procedures were carried out on nail samples including inoculation, cultural characterization, microbial count and biochemical identification of bacteria. Data collected were analysed on the Minitab (17.0) software for appropriate descriptive and inferential statistical tools at 95% level of confidence ($P \leq 0.05$). Six species of bacterial contaminants isolated from fingernails of food vendors were: *Proteus* spp., *Klebsiella* spp., *Bacillus* spp., *Staphylococcus* spp., *Salmonella* spp. and *Shigella* spp. Total occurrence of contaminants (TOC) at Ortese, Gboko main and Tsekucha markets was 20 (33.3%), 26 (43.3%) and 16 (26.7%) respectively. The highest occurring bacteria were *Klebsiella* spp. (60%) and *Bacillus* spp. (60%) isolated from Gboko main market and Ortese market vendors respectively. *Bacillus* spp had the highest prevalence of 53.3% followed by *Proteus* and *Klebsiella* species (43.3% each). *Staphylococcus* and *Shigella* species had prevalence rates of 26.7% each while and *Salmonella* spp. had the lowest prevalence (13.3%). Nail contamination was significantly associated with the type of bacteria species ($\chi^2 = 28.4$, $P<0.05$). Gboko main market had the highest level (43.3%) of bacterial contaminants followed by Ortese market (33.3%) and Tsekucha market (26.75). Tsekucha had the highest mean TVC (Total Viable Count) of 174.2±9.63 closely followed by Ortese market (173.4±12.8) and Gboko main market (163.0±9.9). The highest TVC recorded was observed in a sample from Ortese market (261 cfu/ml). Result showed that fingernails of food vendors in three markets were highly contaminated based on all parameters reported above. The information provided is important to stakeholders in the public health sector saddled with the responsibility of disease prevention and control.

INTRODUCTION

The Nigerian food sector is confronted with challenges such as inadequate supervision and proper monitoring by food safety officers; weak enforcement of food hygiene regulation; lack of training in food safety; poor hygiene practices among food handlers (Adesegun *et al.,* 2020). Hence street foods are at risk of contamination often in all stages of handling. Food contamination refers to the presence of harmful chemicals, objects and microorganisms in food which can cause consumer illness that are capable of causing harm in human when consumed. Microbial contamination occurs when bacteria or other harmful microorganisms contaminate food; it is a common cause of food poisoning and food spoilage.

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Food poisoning can happen when disease-causing bacteria or other germs, also called 'pathogens', spread to food and are consumed (Feglo and Sakyi, 2012; Mir *et al.,* 2018).WHO (2014) reported that, 10 out of every 40 persons in Africa suffers from food borne illness annually. Some of the foodborne pathogens that account for these foodborne diseases include *Staphylococcus aureus, Listeria, Salmonella, Bacillus and Escherichia coli*. Diarheogenic bacteria are leading causes of diarrhoeal diseases or bacterial gastroenteritis. They are attributed to species of *Salmonella*, *ShigellaEscherichia, Campylobacter*, *Clostridium* and *Vibrio* (Jacob and Cohen, 2016). The role of the human body or parts of the body in food contamination cannot be over emphasized. All parts of the human body including the fingernail harbor both harmless and harmful bacteria and fungi. Some opportunistic are normal floral of the skin. However, they cause infections when there are opportunities such as poor hygiene, compromised immunity and synergistic interactions with other pathogens (Jamal *et al.,* 2018). The hand serves as a major vehicle of transmission of various microbes, including the enteric species (Oniya *et al.,* 2006; Prescott *et al.,* 2005).

The nail is often considered an impermeable barrier, but this is not true. In fact, it is much more permeable than the skin, and the composition of the nail includes 7–12% water. This permeability has implications for penetration by substances and microorganisms (Hedderwick *et al.,* 2000). Some pathogenic organisms such as *S.aureus,* Gram negative bacilli and yeast are associated with nails that are implicated in the outbreaks of food borne disease outbreaks (Lau *et al.,* 2000). Food is considered hygienic when there is no dangerous substance that can be injurious to the health of human or animal (Ababio and Adi, 2012). The practice or observation of food and personal hygiene are keen to the prevention of many food-borne diseases. Hygiene practices among food vendors and catering services have been reported to be below acceptable standards. Feglo and Sakyi (2012) in their study on food vendors found that the vendors had little education on food handling, processing and food hygienic practices. Ensuring food hygiene practice among vendors is very challenging however there is the need for vendors to adhere to high standards of food safety and hygienic practices (Monney *et al.,* 2014).

A typical Nigerian market is characterized by overcrowding and lack of space, hence level of hygiene is considered low. Food items and goods are displayed openly on bare and the environment is best described as contaminated. Nevertheless, microbial contamination of food could be reduced if food vendors adhere to certain hygienic practices in order to control outbreak of diseases. There are ready-to-eat food sold in market places, therefore a continuous assessment of level of hygienic practices is necessary among food vendors. Cases of food borne infection are high in the study area. These include diarrhoea, typhoid, dysentery, cholera and others. They are often caused by diverse species of bacteria such as *E. coli, Salmonella, Vibrio, Shillegga* and other pathogenic bacteria. Food borne infections have been linked to poor hygienic practices of food vendors. Raw or prepared food samples are often contaminated due to cross contamination of their fingernails. The aim of this study was to assess the occurrence of bacterial contaminants in fingernails of food vendors in selected markets of Gboko Local Government Area, Benue State, Nigeria

MATERIALS AND METHODS

Study Area: Benue is one of the North Central States in Nigeria with a population of about 4,253,641 in 2006 census. Benue State is named after the Benue River and was formed from the former Benue-plateau State in 1976 along with Igala and some parts of Kwara State, (Govt. of Benue State, 2020). Gboko on the other hand is a fast-growing Town in Benue State. The name Gboko also refers to a Local Government in Benue State. The population of the town is over 500, 000 and mostly Tiv people. Gboko people are mostly farmers or petty traders. The business mostly carried out by Gboko people is Food vending.

Ethical Consideration: The study was carried out with the consent of the participants before sample collection.

Sample Size and Collection: Ten (10) nail samples were collected among the food vendors from each of three selected markets (Ortese, Gboko main and Tsekucha markets), all located within Gboko Local Government, Benue State of Nigeria.

A total of thirty (30) nail samples were collected from food vendors in the study area, 10 samples per market.Finger nails were collected from adult food vendors from 18 years and above within the period of August and September, 2021. Sample collection was done using razor blades (Tiger brand) and each subject was provided with a new blade to avoid spread of transmissible infection. The nails were collected in sterile plastic envelope (pharmaceutical grade) and transported to the laboratory for analysis using screw-capped tubes (Hedderwick *et al.,* 2010).

Sample Inoculation and Cultural Characterization: About 1g of the nails were weighed for each subject and suspended in 9ml of sterile normal saline and allowed to stand for 2 hours. Exactly 1ml of the suspension was inoculated on nutrient agar, MacConkey agar and *Salmonella-Shigella* agar (SSA). Incubation was done at 37^0C for 24hours (Abdullahi *et al.,* 2010; Hedderwick *et al.,* 2010).Morphological observations were recorded on the culture media. These include the colour, shape and outline of the colony as well as shape of each bacterium. Motility test was done by adding a drop of peptone water on a glass slide containing bacterial colony covered with a slip and viewed under the microscope with high power objective lens (Cheesbrough, 2006).

Bacterial Count: One milliliter of the suspension was transferred into 9ml of normal saline followed by a tenfold serially dilution up to 10^{-5}. One milliliter aliquot from 10^{-2} and 10^{-4} was inoculated on sterile media plates using pour plate method (Cheesebrough, 2006). The plates were incubated at 35^oC-37^oC for 24 hours. Observations were made after incubation for development of colonies. Visible colonies on the plates were counted using Colony Counter. Total Viable Counts (TVC) were recorded for each the samples in cful/ml (colonu forming unit per mililitre (Hedderwick*et al.,* 2010). Discreet colonies were sub-cultured on Nutrient agar plate for biochemical test (Hedderwick *et al.,* 2010).

Biochemical Tests: Biochemical tests carried out were: Gram staining, catalase, citrate, urease, indole and hydrogen sulphide tests using protocol given by Cheesbrough (2006). All plate incubation was done at 37^oC for 24-48 hours. Gram staining reaction was carried out by preparing a thin smear of the test isolate on a clean slide followed by the application of .crystal violet (the primary stain), Lugol's iodine as a mordant and 95% alcohol and safranin. The smear preparation was observed under the light microscope using the oil immersion (x 100) objective. Catalase test was carried out by using three drops of 3% hydrogen peroxide added to the smear on the slide for the observation of effervescence**.** Citrate utilization test was doneby inoculating the isolate on Simmon's citrate medium. Urease test was carried out by streaking the isolate on the surface of a urea agar slant followed by the observation in colour change of pH indicator. Indole test was done by inoculating the test organism into bijou bottles containing 5ml of sterile peptone water and 0.5 ml of Kovac's reagent (Cheesbrough, 2006).

Data Analysis: Data collected were analysed on the Minitab (17.0) software for descriptive statistical tools such as relative frequency, percntages, mean, standard error, minimum and maximum values, coefficient of variation (CV) and bar charts. Inferences were made using the Chi-Square and One-Way ANOVA (Analysis of Variance) at 95% level of confidence ($P \leq 0.05$).

RESULTS AND DISCUSSION

Cultural characteristics of bacterial isolates distinguished them into six unique groups (Table 1). Colonies had different colours such as pale, mucoid pink, white or cream with either circular or irregular shape outline. Only one of the six groups was of the cocci type (ball shapped), the remaining five groups were rod shaped. Three of the groups had flagellated bacteria (motile), others were aflagellated (non-motile). Identification of the six groups of bacterial isolates (Group 1-6) using biochemical characteristics (Table 2) confirmed the presence of six species of bacteria Positive reactions to catalase, citrate, urease and hydrogen sulphide tests confirmed the presence of *Proteus* spp. Positive reactions to catalase, citrate and urease tests only confirmed the presence of *Klebsiella*spp. Positive reactions to Gram's staining, catalase, citrate and indole tests confirmed the presence of *Bacillus*spp. The group that showed positive reaction to Gram's staining, catalase and citrate tests confirmed the activity of *Staphylococcus* spp. whereas *Salmonella* spp. reacted positively to catalase, citrate and hydrogen sulphide production. *Shigella*spp, were identified by their positive reaction in catalase test only while other reactions were negative. Therefore, the six species of bacterial contaminants present in the fingernails of food vendors in the study area were: *Proteus* spp., *Klebsiella* spp., *Bacillus* spp., *Staphylococcus* spp., *Salmonella* spp. and *Shigella* spp.Results are consistent with previous studies on fingernails thought to provide conducive environment for different types of microorganisms to survive and multiply especially in the presence of food particles underneath the nail plate (Jacob and Cohen, 2016; Mir *et al.,* 2018; Wachukwu*et al.,* 2007). The present outcome also corroborates other studies on hand contamination as it serves as a major vehicle of transmission of various microbes, including the enteric species (Oniya*et al.,* 2006; Prescott *et al.,* 2005).

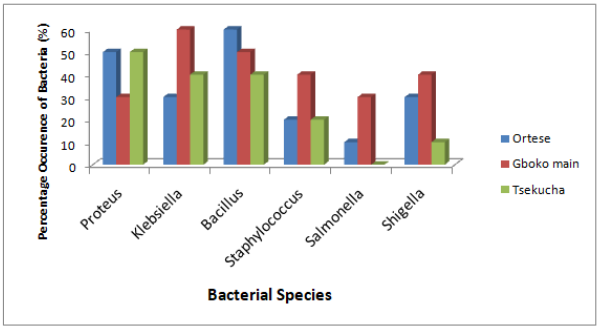
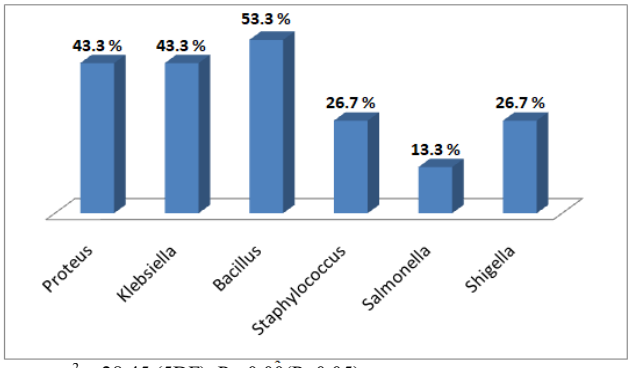
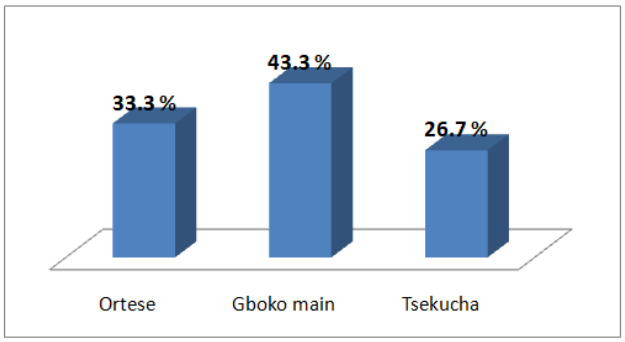


Figure 1: Distribution of Five Bacterial Contaminants among Food Vendors in the three Markets



χ^2 = 28.45 (5DF), P= 0.00(P<0.05)

Figure 2. Prevalence of Bacterial Species Present in Vendors' Fingernail



χ^2 = 4.057 (2DF), P= 0.132 (P>0.05)

Figure 3. Prevalent of Contaminants in Vendors Fingernail Based on Market Type

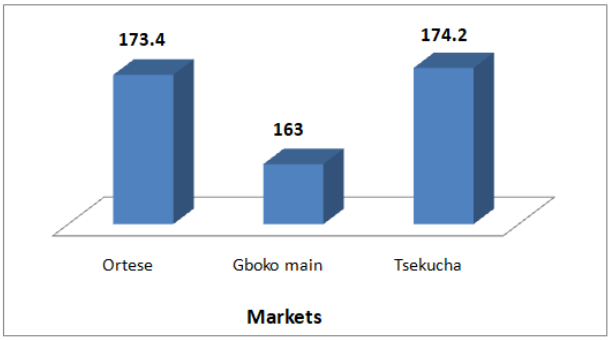


Figure 4. Average Viable Count of Bacterial Contaminants Based on Market types

These bacteria are clinically important from the public health point of view. Each of them had been implicated in many disease outbreaks as food borne pathogens. They are notorious drug resistant pathogens causing many diseases such as gastroenteritis, diarrhoea, food poisoning, typhoid fever and other food related diseases (Jacob and Cohen, 2016; Feglo and Sakyi, 2012; Mir *et al.,* 2018). This work agreed with the outcome of a study carried out by Lau *et al.* (2000) where *S. aureus,* Gram negative bacilli and yeast associated with nails are implicated in the outbreaks of food borne disease outbreaks (Lau *et al.,* 2000).The outcome of this study aligns with other reports in other places in terms of the functional type of bacterial contaminants. Some workers earlier reported some of the highlighted bacteria as part of that the most predominant bacteria in African foods especially *Bacillus, Proteus, Salmonella*, *Shigella*and *Escherichia* (Odonkor*et al.,* 2011; Ofosu*et al.,* 2014; Okojie*et al.,* 2014) although the latter organsims was not found as part of the contaminants reported in the present work. Table 3 gives the occurrence and distribution of bacterial contaminants in fingernails of ten food vendors at Ortese market. Number of contaminants ranged from 1 to 4 bacteria per nail sample. *Bacillus* spp. had the highest occurrence (60%) followed by *Proteus* spp. (50%). The lowest occurring contaminant was *Salmonella* spp. (10%). Total occurrence of contaminant (TOC) in this location was 20 (33.3%). Table 4 gives the occurrence and distribution of bacterial contaminants in fingernails of ten food vendors at Gboko main market. Number of contaminants ranged from 1 to 5 bacteria per nail sample. *Klesiella*spp. and *Bacillus* spp. had the highest occurrence (60%) followed by *Staphylococcus* spp. and *Shigella*spp. (40%). The lowest occurring contaminant was *Salmonella* spp. (10%). Total occurrence of contaminant (TOC) was at the highest figure at this location with a value of 26 (43.3%).

Table 1: Cultural Characteristics of Bacteria Isolated from Fingernail Samples

Groups of isolates	Colony colour	Colony Outline	Organism shape	Motility test	Suspected bacteria
Group 1	Pale	Circular	Rod	+	*Proteus* spp.
Group 2	Mucoid Pink	Circular	Rod	-	*Klesiella*spp.
Group 3	White	Irregular	Rod	+	*Bacillus* spp.
Group 4	Cream	Circular	Cocci	-	*Staphylococcus* spp.
Group 5	Pale	Circular	Rod	+	*Salmonella* spp.
Group 6	Pale	Circular	Rod	-	*Shigella*spp.

Table 2. Biochemical Characteristics and Identification of Bacteria Isolated from Fingernail Samples

Groups of isolates	Grams Staining	Catalase	Citrate	Urease	Indole	H_2S	Identified bacteria
Group 1	-	+	+	+	-	+	*Proteus* spp.
Group 2	-	+	+	+	-	-	*Klesiella*spp.
Group 3	+	+	+	-	+	-	*Bacillus* spp.
Group 4	+	+	+	-	-	-	*Staphylococcus* spp
Group 5	-	+	+	-	-	+	*Salmonella* spp.
Group 6	-	+	-	-	-	-	*Shigella*spp.

Table 3: Occurrence of Bacterial Contaminants in Nails of Food Vendors at Ortese Market

Nail sample code	*Proteus*	*Klebsiella*	*Bacillus*	*Staphylococcus*	*Salmonella*	*Shigella*	TOC (%)
NS1A	+	+	-	+	-	+	4
NS2A	-	+	+	+	-		3
NS3A	+	-	-	-	-		1
NS4A	-	-	+	-	-	+	2
NS5A	+	+	+	-	+		4
NS6A	-	-	+	-	-		1
NS7A	-	-	-	-	-		0
NS8A	+	-	-	-	-		1
NS9A	+	-	+	-	-	+	3
NS10A	-	-	+	-	-		1
Frequency/ Proportion	5 (50%)	3 (30%)	6 (60%)	2 (20%)	1 (10%)	3 (30%)	20 (33.3%)

N= 60 Key: + = presence of bacterial contaminant - = absence of bacterial contaminant TOC = total occurrence of contaminants NS1-10= nail sample numberA= market location A = Ortese market

Table 4: Occurrence of Bacterial Contaminants in Nails of Food Vendors at Gboko Main Market

	Proteus	*Klebsiella*	*Bacillus*	*Staphylococcus*	*Salmonella*	*Shigella*	TOC (%)
NS1B	-	-	+	-	+	+	3
NS2B	+	+	-	-	-	-	2
NS3B	+	-	+	+	+	+	5
NS4B	-	+	-	-	-	-	1
NS5B	-	-	+	+	-	+	3
NS6B	-	+	+	+	-	-	3
NS7B	+	+	-	-	-	-	2
NS8B	-	-	+	-	-	-	1
NS9B	-	+	+	-	-	+	3
NS10B	-	+	-	+	+	-	3
Frequency/ Proportion	3 (30%)	6 (60%)	6 (60%)	4 (40%)	3 (30%)	4 (40%)	26 (43.3%)

N= 60 Key: + = presence of bacterial contaminant - = absence of bacterial contaminant TOC = total occurrence of contaminants NS1-10= nail sample number B= market location B = Gboko main market

Table 6. Total Viable Count (TVC) of Bacterial Contaminants in Nails of Food Vendors in the Study Area

Market type	NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8	NS9	NS10	Average Load Count	CV %
	TVC (cfu/ml)											
Ortese TVC	261^{H}	164	156	232	132^{L}	158	152	172	159	149	173.4 ± 12.8^{a}	23.4
Gboko main TVC	146	136	128	164	172	208	212^{H}	128^{L}	148	188	163.0 ± 9.9^{a}	19.2
Tsekucha TVC	216	192	144	160	132^{L}	184	224^{H}	172	172	146	174.2 ± 9.63^{a}	17.5

F (Viable count in three markets) = 0.33, P=0.722 (P>0.05) [a]superscript letter denotes that means that share the same letters are insignificant at 95% confidence level L and H denote the lowest and highest viable counts respectively

Table 5 gives the occurrence and distribution of bacterial contaminants in fingernails of ten food vendors at Tsekucha market. Number of contaminants ranged from zero (0) to 4 bacteria per nail sample. There were no *Salmonella* contaminant at this location. *Proteus* spp. had the highest occurrence (50%) followed by *Klesiella*spp. and *Bacillus* spp. (40%). Total occurrence of contaminant (TOC) was lowest at this location with a value 16 (26.7%). The overall distribution of bacterial contaminants found in the nails of food vendors in the three markets is shown in Figure 1. The highest occurring bacteria were *Klebsiella*spp. (60%) and *Bacillus* spp. (60%) isolated from Gboko main market and Ortese market vendors respectively. This is followed by *Proteus* spp. (50%) isolated from both Ortese and Tsekucha markets vendors as well as *Bacillus* spp. (50%) isolated from Gboko main market vendors. Figure 2 gives the overall prevalence of each species of bacteria isolated from the fingernails of food vendors in the study area. *Bacillus* spp had the highest prevalence of 53.3%. *Proteus* and *Klebsiella*species had prevalence rates of 43.3% each. *Staphylococcus* and *Shigella* species had prevalence rates of 26.7% each while and *Salmonella* spp. had the lowest prevalence (13.3%). Contamination is significantly associated with the type of bacteria species ($\chi^2 = 28.4$, $P<0.05$).Figure 3 presents the overall level of contaminants in the three markets. Gboko main market had the highest level (43.3%) followed by Ortese market (33.3%) and Tsekucha market (26.75). There was no association between market type and level of bacterial contamination of finger nails ($\chi^2 = 4.057$, $P>0.05$). Table 6 gives the Total Viable Count (TVC) of bacteria in nails of food vendors in the study area.

At Ortese market, TVC ranged from 132 cfu/ml to 261 cfu/ml with an average count of 173.4±12.8. At Gboko main market, TVC value was between 126 cfu/ml and 212 cfu/ml with an average count of 163.0±9.9. At Tsekucha market, TVC was in the range of 132 cfu/ml and 224 cfu/ml with mean count of 174.2±9.63. The largest variation in TVC value among food vendors was observed at Ortesemrket (23.4%) while Tsekucha had the highest mean TVC of 174.2±9.63 (Figure 4). The observed differences in mean TVC of the three markets were insignificant ($F=0.33$, $P>0.05$). However, TVC varied from one food vendor to another. Values >200 cfu/ml were counted from some fingernail samples such as NS1 (261 cfu/ml) and NS4 (232 cfu/ml) at Ortese markets; NS6 (208 cfu/ml) and NS7 (212 cfu/ml) at Gboko main market as well as NS1 (210 cfu/ml) and NS7 (224 cfu/ml) at Tsekucha market. The highest TVC was recorded in the study area was observed in NSI sample at Ortese market (261 cfu/ml). Hygiene practices among food vendors and catering services have been reported to be below acceptable standards. Feglo and Sakyi (2012) in their study on food vendors found that the vendors had little education on food handling, processing and food hygienic practices. It appears there is need to intensify efforts to sensitize food vendors in markets on the need to practice personal hygiene. This observation was earlier suggested by Monney*et al.* (2014) on the need for vendors to adhere to high standards of food safety and hygienic practices. The apex world health regulatory body (WHO, 2020) also submitted that microbial contamination of food could be reduced if food vendors adhere to certain hygienic practices in order to control outbreak of diseases. Hand washing is a fundamental precautionary measure to protect against the spread of disease and is one of the primary practices to reduce the transfer of bacteria, whether from person to person, or from person to food contact surfaces (Mir *et al.,* 2018).

CONCLUSION

Six different bacterial contaminants were isolated from fingernails of food vendors. They were: *Proteus*, *Klebsiella*, *Bacillus*, *Staphylococcus*, *Salmonella* and *Shigella* species. The prevalence of *Bacillus*, *Proteus* and *Klebsiella* species in fingernails was high. *Staphylococcus* and *Shigella* species had medium prevalence while *Salmonella* spp. had the low prevalence. Fingernails of food vendors in the three markets are contaminated as there were up to 5 bacterial contaminants isolated from fingernails of some individual vendors. The three markets are equally vulnerable to food contamination through their vendors' fingernails although the highest level of fingernail contamination was observed in Gboko main market (43.3%). Total viable bacterial count (TVC) was high in fingernails. The lowest TVC was 126 cfu/ml while the highest was 261 cfu/ml. The three markets are equally predisposed to high bacterial load. The information provided is important to stakeholders in the public health sector saddled with the responsibility of disease prevention and control.

RECOMMENDATION

Food vendors should be educated on the sources and heath implication of food contamination through advocacy and campaign. Good hygienic practices through constant hand washing and the use of hand sanitizers from time to time to prevent and reduce contaminants are recommended. The affected markets should be decontaminated using large scale disinfectants. Government should create time for this type of exercise after creating public awareness. By extension, other markets in Benue State may benefit from this exercise**.** There is need for continuous monitoring of level of microbial contamination (bacteria and fungi) of food sold in the affected markets with appropriate control measure put in place after identifying the sources of such contamination.

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