Isolation of Microorganisms from Food Handler (Bread Sector) In Geidam Local Government Area, Yobe State, Nigeria

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Abstract

Food handlers has become an important public health issue due to widespread of food-borne diseases and food vendors play an all-important role due to lack of adequate food safety measures. This study aims to assess microorganisms associated with food vendors in one of the most popular bread industries in Geidam LGA, Yobe state which is known as "Al heri bread industry". Descriptive cross-sectional study and multistage sampling technique was adopted in this research, and a total of 64 food vendors were sampled. These food vendors were sampled using sterile swab sticks by aseptically swabbing their hands. The swabs were tested for bacterial and fungal contaminants. Among 64 food vendors (distributed into three group base on the kind of work the food handler is doing in the industry, i.e. 5 bakers, 8 loaders and 12 packagers.), a total of 376 bacteria and 59 fungi were isolated. The bacteria isolate from this study were S. aureus (21.01%), E. coli (23.14%), Salmonella enterica (13.03%), Pseudomonas aeruginosa (17.02%), B. cereus (11.70%), Klebsiella spp (10.90%) and Serratia marcescens (3.2%). The fungi isolates were Aspergillus spp (37.29%), Microsporium canis (15.25%), Mucor spp (11.86%), Penicillium spp (10.17%), and Candida spp (25.42%). The questionnaire and observatory study adopted in this research showed poor personal hygiene and sanitary practices among food vendors. The findings of this study emphasized the importance of food vendors as potential vehicles for transmitting food-borne diseases and thus the need to adopt food safety measures geared towards maximum food safety is required.

Keywords: Food vendors also known as Food handlers, Food-borne disease, Hygiene, Sanitary practices.

Introduction

Food handlers also known as food vendors are vital components in the interaction between the cooking environment and the food which is being prepared or served. WHO (1989) defined food handlers as those who, in the course of their normal routine work, handle food or items that may come into contact with food, such as eating and drinking utensils not meant for their personal usage. During food handling and preparation, microorganisms on raw foods can be transferred to the hands of a food worker and subsequently to other surfaces (such as water faucet handles) contacted by contaminated hands Agbodaze et al., (2005). Food handlers with poor personal hygiene working in a food service establishment could be potential sources of infection due to microorganisms Egbuim T.C. and Umeh S.Ogonna (2020). This study reports the microorganisms isolated from the palms of food handlers at Al heri bread industry in Geidam LGA in Yobe state.

Several reports have suggested that infected food handlers may play important role in food contamination and food-borne disease outbreaks. Purchasing ready-to-eat foods and ingredients from food handlers possess a considerable risk to public health, especially due to the observed poor hygienic practices of some food handlers. Lack of basic facilities like water and toilet also affect the safety of ready-to-eat foods handled by food handlers. Where food vendors lack water, they seldom wash their hands even when they visit the toilet or handle money. Most vendors are known to wash their hands only when the hands are visibly dirty Musab et al (2020). These practices tend to subject foods and ingredients to repeated contamination. Failure to perform appropriate hand hygiene has been recognized as a significant contributor to outbreaks of diseases. It has also been established that lack of adequate hand washing by food handlers who prepare, process and handle food in the retail food system can transmit pathogens especially fecal pathogens to food products after a food worker uses the toilet. When consumed in food, these pathogens can cause illness and disease (FDA, 1997).

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The Nigeria Centers for Disease Control and Prevention (NCDC) reported that approximately 20% of food-related infections are due to poor hygiene practices by food handlers (Michaels et al., 2004). Diarrhea diseases, mostly caused by food-borne microbial pathogens are leading causes of illness and death in developing countries; killing an estimated 1.9 million people annually at the global level (Adewunmi et al., 2014), Okare O.T. and Erhahon O.O (2015), although Nigeria has no official foodborne disease surveillance system. Sharmila (2011) reported that food vendors are carriers of food-borne pathogens like *Escherichia coli, Salmonella, Shigella, Campylobacter and Staphylococcus aureus* which they eventually transfer as food-borne hazards to the consumers. *Escherichia coli and Staphylococcus aureus* were recovered in a significant proportion of the food, water, hands and surface swabs tested in Harare (Gitahi et al., 2012).

The overall aim of this work is to study the presumptive microorganisms associated with food vendors and the relationship between their occurrence and the hygiene practices in Al Heri bread industry in Geidam LGA Yobe state.

Al Heri bread industry is one of the most popular bread industries in Geidam LGA, Yobe State, which probably has the highest product sales per day in the community. This study was undertaken to check for possible microorganisms (pathogens) present in their workers hands to ensure that their product does get contaminated and its won't cause' illness when consumed by people living in Geidam

The questionnaire and observatory study adopted in this research showed poor personal hygiene and sanitary practices among food vendors in the industry. The findings of this study emphasized the importance of food vendors as potential vehicles for transmitting food-borne diseases and thus the need to adopt food safety measures geared towards maximum food safety is required.

Statement of Problem

Observation shows that most of the food handlers within the industry premises lack personal hygiene, which is one of the contributing factors of food borne illness and poor hand washing, is a significant contributory factor.

Another aspect of food service that frequently causes comment is the way food handlers within the industry premises prepares the food, takes the money for the purchase returns change to the customer and then prepares food for the next customer, this is also a means of transferring microorganisms since anything that gets on money gets on the hands.

Aims and Objective of the Study

The objective of the project is to isolate and determine microorganisms from the hands of food handlers in Al Heri bread industry.

In addition, to bring to the awareness of the food handlers the importance of personal hygiene.

Hypothesis

H1. Microorganisms were isolated from hands of handlers in Al Heri bread industry in Geidam LGA Yobe State.

H2. Microorganisms were not isolated from the hands of food handlers in Al Heri bread industry in Geidam LGA Yobe State.

Significance of Study

This study is very timely especially today that there is increased awareness on the importance of healthy living and personal hygiene. The study will be of immense benefit to the general public, institutions and organized food handlers and retailers because safe food practices is imperative given to the potential for widespread outbreaks of food borne illness.

Scope of The Study

This research work covers only the food handlers in Al Heri bread industry

Limitation of The Study

This work was limited to isolation of very few microorganisms from the hands of the food handlers within the industry, this is because microorganisms are very sensitive to the environment especially temperature. Lack of proper temperature due to lack of power supply for the growth of microorganisms was a limiting factor.

Materials and Method

Equipment

The following Equipment materials were used or this research.

- Electric thermostatic incubator
- Autoclave
- Microscope
- Refrigerator
- Electronic scale

Materials

The following materials were used for this research.

- Nutrient Agar
- MacConkey Agar
- Salmonella, shigella Agar (SSA)
- Mannitol Salt Agar
- Cetrimide Agar
- Kovac's reagent
- Crystal violet stain
- Acetone
- Safranin
- Hydrogen peroxide

Study Area

The study area for this research were only al heri bread industry Geidam Yobe sate, Nigeria known for their high population and dependence on vended foods. The selected workers sector used were, beakers; loaders and packagers

Sample/Swab Collection

A total of two hundred and sixteen (216) swabs were aseptically collected in duplicates from hands, plates, spoons and aprons of various food vendors (64 vendors) from the three selected sector of the industry. Each sterile swab stick was dipped into normal saline and aseptically used to swab the surface of hands, plates, spoons and aprons of each food vendor. After collection, the swab sticks were placed in sterile bags and conveyed to the laboratory for analysis.

Preparation of Culture Media

All culture media used were prepared according to the manufacturer's instructions. They were sterilized by autoclaving at 121°C, 15psi and for 15 minutes while Salmonella-Shigella agar was prepared by boiling in a water bath at temperature of 100°C.

Isolation of Microorganisms

Bacterial Isolation

Each swab stick was aseptically rinsed into freshly prepared Nutrient Broth in test tubes (5ml per test tube and plugged); the test tubes were incubated at 37°C for 24 hours for growth which is detected through turbidity. After incubation, a loop full of each broth was streaked progressively to obtain discrete colonies on different culture media (Nutrient Agar, Columbia Blood Agar, MacConkey Agar, Mannitol Salt Agar, Salmonella-Shigella Agar, Cetrimide Agar). The plates were incubated at 37°C for 24 hours and then observed at the end of the incubation time for the kind of growth present on each agar.

Fungal Isolation

Each swab stick was aseptically rinsed into freshly prepared Sabouraud Dextrose Broth in test tubes (5ml per test tube and plugged), the test tubes were incubated at 25°C for 48 hours for growth. After incubation, a loop full of each test tube was streaked progressively to obtain discrete colonies on fortified Sabouraud Dextrose Agar

(chloramphenicol fortified to suppress bacterial growth). The plates were incubated at 25°C for 5 days and were observed daily for the kind of growth present on each plate.

Identification of the Isolates

Bacterial and fungal isolates were identified using various biochemical tests, microscopy, culture morphology and cross match of fungal isolates was done using Fungal Atlas for their easy identification. The isolation and identification follow similar practices and in global practices, Edeh N. A. (2012).

Morphology and biochemical tests carried out to identify the isolates

The following tests were carried out:

- Gram staining
- Triple Sugar Iron Agar
- Catalase Test
- Oxidase Test
- Coagulase Test
- Sulphide Indole Motility Test
- Citrate Test

Gram staining reaction

The procedure was carried out as follows:

A thin smear film of the organism (a24-houroldbacterial culture) was prepared one sterile clean glass slide, air-dried and heat-fixed by passing it horizontally over the Bunsen flame. The dried smear was stained with Crystal violet stain for 60 seconds after which it was rinsed with tap water. The resulting smear was then stained with Lugol's iodine for 60 seconds and was rinsed with tap water. The smear was decolorized with 95% ethanol until the slide appears free of the crystal violet stain. The slide was rinsed under tap water, counter–stained with safranin for 1minute and was finally rinsed with tap water. The prepared slide was allowed to dry and it was examined under the microscope using the x40 objective lens and x100 (oil immersion objective lens). The organisms that retained the crystal violet stain (purple in colour), indicated Gram positive organisms, while the organisms that appeared pinkish or reddish indicated Gram negative organisms.

Sulphide indole motility test

This test was carried out to detect the motility, sulphide and indole production of each isolate. The medium, sulphide indole-motility (SIM) medium is a semi-solid medium. The isolates were stab-inoculated aseptically and were incubated at 37oC for 24 hours. Motility is indicated by the spreading of the organism outside the line of stab, indole production is by the presence of a red-pink ring at the interphase after Kovac's reagent has been added; sulphide production is by the presence of a black colour in the medium.

Citrate test

The citrate test was carried out in order to determine the ability of the isolates to utilize citrate as their sole source of carbon and ammonia as the only source of nitrogen. Simmon citrate agar was used for this test; the agar was prepared in test tubes and was inoculated with a 24-hours old culture of each of the isolates aseptically. This was theninoculatedat37oCfor24-hours. A colour change from green to deep blue indicates positive citrate utilization while the absence of a colour change indicates negative citrate utilization.

Triple sugar iron test

This test was used to detect the fermentation of lactose (slope) and glucose (butt) due to the production of acid, the production of gas (CO_2) and the release of H2S (hydrogen sulphide) which is a four in one test. The Triple Sugar Iron was inoculated with each isolate from the pure cultures on Nutrient agar using a straight wire to stab the butt and then streaking the slope in zig-zag pattern and it was incubated at 37oC for 24 hours. A yellow butt (acid production) and red-pink slope indicates the fermentation of glucose only; cracks and bubbles in the medium indicate gas production from glucose fermentation; a yellow slope and a yellow butt indicates the fermentation of lactose; a red-pink slope and butt indicates no fermentation of glucose or lactose; blackening along the stab line or throughout the medium indicates hydrogen sulphide production.

The reaction is:

$Fe_2 + 2H_2S \rightarrow 2FeS$ (Black) $+ 2H_2$

Catalase test

Most aerobic microorganisms are capable of producing the enzyme catalase although of different extents. The principle of this is that when organisms containing catalase enzyme are mixed, Hydrogen peroxide (H_2O_2) and gaseous oxygen is released. A suspension of 18-24 hours old culture of the test organisms was placed on a clean glass microscope slide. A few drops of H_2O_2 were added using a syringe. The evolution of gas bubbles caused by the liberation of free oxygen indicated the presence catalase enzyme which shows that the reaction is positive; the absence of bubbles indicates a negative reaction.

Coagulase test

This test is used to identify Staphylococcus aureus which produces the enzyme coagulase. A drop of distilled water is placed on each end of a slide or on two separate slide. The colony of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of freshly collected plasma was added to one of the suspensions and was gently mixed. Observe for clumping of the organisms within10seconds.Ifthereisclumpingwithin10seconds, it is Staphylococcus aureus; if there is no clumping within 10 seconds, it is coagulase negative.

Antibiotic susceptibility test

This test was carried out to determine the antibiotic susceptibility pattern of the different isolates Tiruneh MDM (2013). Nutrient agar plates were inoculated with isolates from stock cultures. The Kirby-Bauer disc-diffusion test which conforms to the recommended standard of the Clinical and Laboratory Standards Institute.

Turbidity of the inoculum of various isolates of enteric bacteria is compared with 0.5 McFarland standard and each of the isolates was inoculated onto the surface of Mueller Hinton Agar plates using a sterile swab in order to ensure even distribution of the inoculum, the plates were allowed to dry for not more than 15 minutes and the antibiotic discs with different concentration were placed on the surface of the agar plates. After 30 minutes of applying the discs, the plateswereinvertedandincubatedfor24hoursat30oC. The clear zone that developed around each disc were measured as the zone of inhibition from underneath each plate with the aid of a ruler in centimeter (cm) and converted to millimeter (mm). The antimicrobial discs used include the following: Ofloxacin (5 µ g), Ciprofloxacin (5 µ g), Augmentin (30 µ g), Cefuroxime (30 µ g), Gentamicin (10 µ g), Nitrofurantoin (300 µ g), Cefixime (5 µ g), and Ceftazidime (30 µ g).

Result

Potential Microorganisms Isolated and Prevalence (%) Tables 1 and 2 shows the various potential food-borne microorganisms isolated from the food vendors while tables 3 and 4 show their prevalence

Table 1: Morphology and Biochemical Test Characteristics of Bacterial Isolates.

S/No	Colony Morphology	Gram Stain	Catalase	Coagulase	Motility	Indole	Urease	V-P	Methyl Red	Citrate	Oxidase	Starch test	Spore test	Glucose uti.	Lactose uti	Maltose uti.	Mannitol uti.	Most Probable Organism
1	Circular, and creamy	+ Cocci	+	+	-	-	V	+	+	+	-	-	-	+	+	+	+	*Staphylococcus aureus*
2	Oval, and Pinkish	- Rods	+	-	+	+	-	-	+	-	-	-	-	+	+	-	+	*Escherichia coli*
3	Black centered on SS Agar	- Rods	+	-	+	-	-	-	+	-	-	-	-	+	-	+	+	*Salmonella enterica*
4	Bluish-Greenish	- Rods	+	-	+	-	-	-	-	+	+	-	-	-	-	-	+	*Pseudomonas aeruginosa*
5	Creamy, Flat	+ Rods	+	-	+	-	-	+	-	V	-	+	+	+	-	+	-	*Bacillus cereus*
6	Pinkish-red, mucoid	- Rods	+	-	-	-	+	+	-	+	-	-	-	+	+	+	+	*Klebsiella spp*
7	Red	- Rods	+	-	+	-	-	+	-	+	-	-	-	+	-	+	+	*Serratia marcescens*

Key: + = Positive, V-P = Voges-Proskauer, V = varied, - = Negative, Uti. = Utilization.

Table 2: The Colony Morphologies and Microscopic Features of Fungal Isolates

Isolates	**Colony Description**	**Microscopic Features**	**Suspected Organism**
1a	Cottony and culture turned Brown to black with aging. Reverse: Pale yellow	Septate, hyaline hyphae. Conidiophores are long with spherical vesicles at the apex. Conidia are globose and have rough surface.	*Aspergillus niger*
1b	Cottony and powdery, turned yellow-green during Maturation. Reverse: Pale yellow	Septate, hyaline hyphae. Conidiophores are long with spherical/elongate vesicles at the apex.	*Aspergillus flavus*
2	Cottony and white Reverse : deep yellow	They have septate hyphae that produce numerous macroconidia. They are truncated, thick-walled and spindle shaped with snout.	*Microsporium canis*
3	Cottony/woolly and white, turned greyish-brown with aging Reverse: pale white	They have non-septate hyphae called the sporangiophores.	*Mucor spp*
4	Cottony and grey-green Reverse: yellowish-grey	The entire structure, the conidiophores and extending conidia resemble a "brush".	*Penicillum spp*
5	Creamy/glabrous and white Reverse : white	Shows spherical to sub-spherical budding blastoconidia. Some were germ tube test positive detecting *Candida albicans*	*Candida spp*

Table 3: Prevalence of Bacterial Isolates from the swab samples collected

Isolates	**Frequency**	**Percentage prevalence (%)**
Staphylococcus aureus	79	21.01
Escherichia coli	87	23.14
Salmonella enterica	49	13.03
Pseudomonas aeruginosa	64	17.02
Bacillus cereus	44	11.70
Klebsiella spp	41	10.90
Serratia marcescens	12	3.20
Total	376	100

Table 4: Prevalence of Fungal Isolates from the swab samples collected

Isolates	**Frequency**	**Percentage prevalence (%)**
Aspergillus spp	22	37.29
Microsporium canis	9	15.25
Mucor spp	7	11.86
Penicillium spp	6	10.18
Candida spp	15	25.42
Total	59	100

Table 5: Percentage Number of Respondents to Variables used in the Study Questionnaire

S/N	Variables	Yes N = %	No N = %
1	Food vendor with surrounding environment free of potential contaminants	19 = 29.69	45 = 70.31
2	Food vendor with food handlers medical certificate	4 = 6.25	60 = 93.75
3	Food vendor wearing protective clothing apron	24 = 37.50	40 = 62.50
4	Food vendor with clean protective clothing apron	10 = 15.63	54 = 84.38
5	Food vendor with protective hair covering/cover	29 = 45.31	35 = 54.69
6	Food vendor with any training on food hygiene	11 = 17.19	53 = 82.81
7	Food vendor who have been invited by the Government or NGOs for training	9 = 14.06	55 = 85.94
8	Food vendor that have seen the Government agencies come to inspect their premises	13 = 20.31	51 = 79.69
9	Food vendor that serve pure/treated water for drinking	49 = 76.60	15 = 23.40
10	Food vendor who encounter pests and rodents in their vending facility	52= 81.25	12= 18.75
11	Food vendor that have access to sanitary facilities	11 = 17.19	53 = 82.81
12	Total number of vendors	64	

Discussion

From the results in Table 1 and 2, potential microorganisms were isolated from the food vendors which indicate that food vendors are carriers of microorganisms, and this agrees with Isara and Isah (2009) that food vendors play an important role in transmission and prevention of food borne disease. The bacteria isolated from this study and their percentage prevalence were S. aureus (21.01%), E. coli (23.14%), Salmonella enterica (13.03%), Pseudomonas aeruginosa (17.02%), B. cereus (11.70%), Klebsiella spp (10.90%) and Serratia marcescens (3.2%). The fungi isolates were Aspergillus spp (37.29%), Microsporium canis (15.25%), Mucor spp (11.86%), Penicillium spp (10.17%), and Candida spp (25.42%). Escherichia coli and

Aspergillus spp showed the highest prevalence rates while Serracia marcescens and Penicillium spp has the least percentage prevalence for both bacteria and fungi isolates respectively. Similar types of microbial contaminants were identified in previous studies in Benin City, Ogun State and Ondo State, Nigeria (Okareh and Erhahon, 2015; Bankole et al., 2009; Ibrahim et al., 2013). The percentage number of respondents to the hygiene practices survey questionnaire used during the course of this research was shown in Table 5, and from the survey it was observed that only 17.97% of the food vendors had access to sanitary facilities. As high as 81.25% of the vendors' encounter rodents in their vending facilities and only 20.31% of the food vendors have seen government agencies come to inspect their vending premises. As few as 14.06% of the food vendors have been invited by the government or NGOs for training and only 17.19% of the food vendors have had any training on food hygiene which is very poor as studies also conducted in Nigeria by Chukuezi (2010), Omemu and Aderoju (2008) reported that only 4.76% and 12% respectively of food vendors had been exposed to formal training. Furthermore, from this study it was observed that 54.69% and 62.5% of the food vendors had no protective hair cover and apron respectively which is similar to the findings by Chukuezi (2010), Muinde and Kuria (2005). Also, only 6.25% of the food vendors studied had medical certificate while only 29.69% of the food vendors had environments free of potential contaminants. During the course of this research, most of the food vendors (76.60%) claimed to serve pure/treated water to their customers explaining that customers no longer drink water served in mugs/jugs, the remaining 23.40% of the food vendors don't serve water to their customers because they were mostly hawkers. From this result, the general hygiene practices/regulation among the food vendors were below average and generally must be regarded as poor.

Conclusion

This study has shown that most food vendors within the industry are carriers of wide variety of potentially microorganisms and could be a source of infection to their customers. The isolation of bacterial and fungal pathogens from food vendors reflects bad hygienic standards and necessitates their regular inspection by regulatory agencies. Despite the positive contributions of food vendors to the society, they also incorporate detrimental public health effects. This study therefore calls for caution in patronizing food vendors. The public health implication of the findings of this work is that it revealed that pathogenic isolates from food vendors can aggravate the ill health of the consumers if proper care and caution is not taken.

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