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Egbuim T. C
Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, P.M.B 5025, Awka, Anambra State, Nigeria

Umeh S. O
Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, P.M.B 5025, Awka, Anambra State, Nigeria

Correspondence:
Egbuim T. C
Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, P.M.B 5025, Awka, Anambra State, Nigeria

Microorganisms Associated With Food Vendors in Anambra State, Nigeria

Egbuim T. C, Umeh S. O

Abstract
Food vending has become an important public health issue due to widespread of food-borne diseases and food vendors play a major role due to lack of adequate food safety measures. This study aims to assess microorganisms associated with food vendors in Anambra state. Descriptive cross-sectional study and multistage sampling technique was adopted in this research, and a total of 128 food vendors were sampled. These food vendors were sampled using sterile swab sticks by aseptically swabbing their hands, aprons, plates, and spoons. The swabs were tested for bacterial and fungal contaminants. Among 128 food vendors, a total of 752 bacteria and 118 fungi were isolated. The bacteria isolates from this study were *S. aureus* (21.01%), *E. coli* (23.27%), *Salmonella enterica* (13.03%), *Pseudomonas aeruginosa* (16.89%), *B. cereus* (11.84%), *Klebsiella spp* (10.90%) and *Serratia marcescens* (3.06%). The fungi isolates were *Aspergillus spp* (37.29%), *Microsporium canis* (14.41%), *Mucor spp* (12.71%), *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, Food-borne disease, Hygiene, Sanitary practices

Introduction
Food vending trade is currently growing from its low class image and is becoming a booming industry which involves peddling, selling or offering for sale of food products. Food vending has become an important public health issue and of great concern to the world due to widespread food-borne diseases, mostly as a result of food vendors who lack adequate understanding of the basic food safety measures (Sharmila, 2011). The risk of food getting contaminated depends largely on the health status of the food vendors, their personal hygiene, knowledge, attitude and practices (Prabhu and Shah, 2014).

Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other similar public places (Nurudeen *et al.*, 2014). Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature (Ghosh *et al.*, 2007; deSousa, 2008). In Nigeria, consumption of street foods have witnessed a phenomenal growth over the years as rapid population growth, urbanization, unemployment and poverty; occupational pressures and lifestyles changes have created a poll of mobile and transient population who depend almost entirely on these relatively low cost foods for their nutrition (Martins, 2006).

The Centers for Disease Control and Prevention (CDC) 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). Although Nigeria has no official food-borne disease surveillance system, the World Health Organization (WHO) estimates that more than 200,000 people die of food poisoning annually in Nigeria (Onyeneho and Hedberg, 2013). 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 Anambra State.

Materials and Methods

Study Area

The study areas for this research were some selected towns in Anambra state, Nigeria known for their high population and dependence on vended foods. The selected towns used were Agulu (Anaocha Local Government Area), Nnewi (Nnewi-North Local Government Area), Oba (Idemili-South Local Government Area); Awka, Mbaukwu and Nise (Awka-South Local Government Area).

Study Design

In this study, Descriptive cross-sectional design was used. The study design utilized a quantitative method to describe the hygiene practices of the food vendors under study.

Study Population

The target population were all food vendors who cooked and sold ready to eat foods within the selected study area. The inclusion criteria was food vendors who sell cooked/prepared food on streets, markets, restaurants, fast-food joints and hawkers, while the exclusion criteria were food vendors who were not selling cooked foods, but snacks, fruits and vegetables.

Sample Size Determination

The sample size (n) was determined using standard Fischer's *et al.* (1998) formula, used when the study population is not known as elaborated by Muhonja and Kimathi (2014). The formula states as follows: $n = \frac{Z^2pq}{d^2}$

n = sample size, Z = Standard deviation which corresponds to confidence interval (1.96).

p = Proportion of the study population having the characteristics, q = (1 - p).

d = degree of precision i.e. the margin of error that is acceptable (0.05).

The proportion of the population (p) used in this study was drawn from previous research by Okareh and Erhahon (2015). If assuming p was unknown, p = 0.5 is used which assumes maximum heterogeneity (i.e. a 50/50 split). From the previous research by Okareh and Erhahon (2015), p was 0.92 while q (1 - p) is 0.08.

Substituting, Z = 1.96, p = 0.92, q = 0.08 and d = 0.05 in the equation above:

$n = \frac{Z^2pq}{d^2} = \frac{1.96^2 x\ 0.92\ x\ 0.08}{0.05^2} = 113.\ 0967 \approx 113$

Therefore, the minimum number of food vendors to be included in this study was 113 food vendors. In this study, 128 food vendors were selected and used.

Sampling Technique

This study employed multi-stage sampling technique which includes random sampling, stratified sampling and cluster sampling techniques. The selected towns used in this study were randomly selected from the 177 towns in Anambra state, Nigeria. Each of the selected towns was considered a stratum, and the strata were clustered into groups pending on the availability and location of the food vendors. Proportionate sampling based on the number of food vendors in each cluster was done to get the number of respondents.

Questionnaire Study

A descriptive study was conducted in the selected towns in Anambra state, Nigeria through structured questionnaire and observation adopted from Githaiga (2012).

Swab Collection

A total of four hundred and thirty two (432) swabs were aseptically collected in duplicates from hands, plates, spoons and aprons of various food vendors (128 vendors) from the six selected towns. 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.

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	158	21.01
Escherichia coli	175	23.27
Salmonella enterica	98	13.03
Pseudomonas aeruginosa	127	16.89
Bacillus cereus	89	11.84
Klebsiella spp	82	10.90
Serratia marcescens	23	3.06

Total number of isolates = 752

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

Isolates	Frequency	Percentage prevalence (%)
Aspergillus spp	44	37.29
Microsporium canis	17	14.41
Mucor spp	15	12.71
Penicillium spp	12	10.17
Candida spp	30	25.42

Total number of isolates = 118

Questionnaire Study

Table 5 below shows percentage number of respondents to Variables used in the Study

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

S/N	Variables	Yes N = %	No N = %
1	F.v with surrounding environment free of potential contaminants	38 = 29.69	90 = 70.31
2	F.v with food handlers medical certificate	8 = 6.25	120 = 93.75
3	F.v wearing protective clothing apron	48 = 37.50	80 = 62.50
4	F.v with clean protective clothing apron	20 = 15.63	108 = 84.38
5	F.v with protective hair covering/cover	58 = 45.31	70 = 54.69
6	F.v with any training on food hygiene	22 = 17.19	106 = 82.81

7	F.v who have been invited by the Govt or NGOs for training	18 = 14.06	110 = 85.94
8	F.v that have seen the Govt agencies come to inspect their premises	26 = 20.31	102 = 79.69
9	F.v that serve pure/treated water for drinking	98 = 76.60	30 = 23.40
10	F.v who encounter pests and rodents in their vending facility	104= 81.25	24 = 18.75
11	F.v that have access to sanitary facilities	23 = 17.97	105 = 82.03

F.v = Food vendors, N = number, % = Percentage, Total number of vendors = 128

Discussion

From the results in Table 1 and 2, potential pathogenic microorganisms were isolated from the food vendors which indicate that food vendors are carriers of pathogenic 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 isolates from this study and their percentage prevalence were *S. aureus* (21.01%), *E. coli (*23.27%), *S. enterica* (13.03%), *P. aeruginosa* (16.89%), *Bacillus cereus* (11.84%), *Klebsiella spp* (10.90%) and *Serratia marcescens* (3.06%). The fungi isolates and their percentage prevalence were *Aspergillus spp* (37.29%), *Microsporium canis* (14.41%), *Mucor spp* (12.71%), *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 sampled locality in Anambra state are carriers of wide variety of potentially pathogenic 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.

Recommendations

Good hygiene practices, use of good quality raw materials in processing of food and strict personal hygiene should adopted to ensure safety in the food industry, as this will help reduce the prevalence of pathogenic microorganisms.

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