International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 4, Issue 1, pp: (220-227), Month: April 2016 - September 2016, Available at: www.researchpublish.com

CULTURE-DEPENDENT MICROBIAL ANALYSIS OF HAWKED STREET FOODS IN UYO METROPOLIS, SOUTHERN NIGERIA

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Abstract: **Background: Street foods have been associated with health problems to their consumers with increased risk of mortality and reduced quality of life due to poor food handling and sanitary practices.**

Objectives: To determine the occurrence of microbial agents in street foods through laboratory analysis and testing the response/resistance of pathogenic agents to anti-microbial drugs.

Materials and Methods: This was a microbial analysis of 150 samples of cooked street food chosen from rice and stew, beans, cakes, peppersoup, moi moi and fried meat selected through systematic random sampling technique.

Results: The highest occurrence of bacterial isolate was Vibrio sp with 60%, followed by Escherichia coli 53%, Streptococcus pneumoniae 49%, Aerococcus sp 48%, while Actinomyces and Shigella dysenteriae occurred at 36% frequency each. The most occurring mycological species were Saccharomyces sp 44%, Verticillum sp 37% and Aspergillus niger 35%. On microbial sensitivity testing, Vibrio sp showed resistance to all antibiotics, E. coli showed moderate response to Erythromycin (30mm) and Shigella dysenteriae also showed moderate response to Chloramphenicol (31mm).

Conclusions: Hawked street foods in Uyo Metropolis have shown to be of poor microbial quality.

Keywords: **hawked foods, microbial, food contamination.**

I. INTRODUCTION

Food is defined as any substance composed of carbohydrates, protein, fats and water, that is either eaten or drunk by any animal including humans for nutrition or pleasure [1]. In addition to nutrients, food conveys emotional satisfaction, and hormonal stimuli that contribute to health [2]. Food is a true "ecosystem" and it's high nutrient environment is capable of sustaining many microbial lives.

Street foods are foods and beverages prepared and/or sold by vendors, especially in streets and other similar public places [3]. Street foods are accessible, cheap, and diverse. They are considered unsafe due to poor handling practices [4] . They are prepared and distributed in places that lack the facilities to ensure safe food preparation and maintenance [5]. There has been a remarkable increase in the number of street food vendors due to the relative ease of starting and maintaining the business. It requires low capital and the profits are highly rewarding [6].

Microbial assessment of foods in developing countries have been demonstrated to be problematic [7]. Foodborne illness is a growing public health problem in developing as well as developed countries, causing morbidity and mortality in the general population, especially in vulnerable groups, such as infants, young children, elderly and the immunocompromised [8]. In developing countries, an estimated 70% of diarrheal episodes are associated with the ingestion of contaminated foods [9] . Approximately 10 to 20% of food-borne disease outbreaks result from contamination of foods by the food handler [10] . In African region, several devastating outbreaks of food borne diseases have been reported, including acute aflatoxicosis in Kenya in 2004 that was attributed to maize [11] .

In Uyo Metropolis, street food is prepared and sold in close proximity to waste bins, waste water channels in unhygienic conditions with high patronage. This served as a basis for this study to perform a microbial survey of street food, especially cooked foods in the metropolis.

II. MATERIALS AND METHODS

A. Study Area:

This study was conducted in Uyo metropolis, located in the North Eastern part of Akwa Ibom State . Akwa Ibom State is a state in Nigeria located on the southern part of the country lying between latitudes 4°32'N and 5°33'N, and longitudes 7°25'E and 8°25'E with a population of over 5 million people.

B. Sample collection :

One hundred and fifty (150) samples of six(6) types of foods(rice and stew, beans, pepper soup, cakes, *moi moi* and roasted meat) were collected in sterile polythene bags and bowls directly from the vendors from five (5) locations (Aka Road, Abak Road, Nwaniba Road, Ikot Ekpene Road, and Oron Road) during the rainy season over a period of four months. The sample was collected in the daytime between 7a.m and 6pm.

Thirty(30) of the different types of food listed above were collected per location in several schedules and transported immediately to the laboratory for analysis, irrespective of whether it was solid-based(rice and stew, cake, roasted meat) or semi-solid(beans, *moi moi).*

The sampling technique used was systematic random sampling. The first sample was selected using a simple random sampling by ballot method after which a sampling interval of 5 was calculated. Thereafter, every fifth vendor was selected for the study.

C. Preparation of samples and serial dilution:

One gram of each sample was weighed aseptically and transferred into 9 mls of sterile distilled water. This was the aliquot (10^{-1}). 1ml of the solution from 10^{-1} was pipetted and diluted in another 9ml of sterile distilled water in a test tube. The serial dilution continued down the dilution gradient to 10^{-6}.

D. Microbial Analysis:

Nutrient agar (NA), MacConkey agar(MA), Sabouraud Dextrose Agar (SDA) and Eosin Methylene Blue Agar (EMBA) were used. All media were prepared according to manufacturer's specification. 100 micro ml of Cycloheximide (Nystatin) was incorporated into the nutrient agar to prevent fungal growth.

1ml of each of these diluent from 10^{-6} was used to plate out on the media for each purpose. The Stokes/Kirby -Bauer method[12] was adopted to estimate the bacteriological and fungal quality of the samples initially and every 48 hours.

The inoculated plates were incubated aerobically and anaerobically at 37°C for 1 day to 5 days.

E. Isolation of Pure Cultures:

To get pure cultures, colonies from pure plate cultures were sub-cultured on freshly prepared and solidified media using streak plate technique such that pure cultures were obtained at the end of the streaking lines [13] . The pure cultures were maintained as stock on agar slants until required for characterization and identification.

F. Characterization and identification of isolates:

Bacterial isolates were characterized based on colonial, cultural, morphological, microscopic examination and biochemical tests such as catalase test, coagulase test, oxidase test, nitrate reduction test, urase production test, indole fermentation test, nitrate utilization test, acid production, Voges Proskauer test, starch hydrolysis test, hydrogen sulphide production test, sugar fermentation, haemolysis, motility test, carbohydrate utilization, and spore formation test as described by Holt et al. [14]

Identification of the isolates was done by comparing their characteristics with those of known taxa as described in Bergey's Manual for Determinative Bacteriology. [14]

The characterization and identification of fungal isolates was carried out as described by Barnett and Hunter. [15] The identification was based on their cultural and morphological as well as vegetative and reproductive structures.

G. Antibiotic Sensitivity Testing:

The method of Nester et al [16] was adopted. Prepared commercial antibiotics were transferred aseptically onto the lawns of freshly inoculated agar medium of the isolates. The culture was incubated for 24 hours and thereafter, the clearing zones (zones of inhibition) were measured in millimeters (mm) and reported.

III. RESULTS

Table 1 shows the total bacteriological count in food samples. Bacterial count ranged from 0.14 x 10^8 – 1.58 x 10^8 in pepper soup, 0.17 x 10^8 - 2.50 x 10^8 in rice and stew, 0.23 x 10^8 – 1.68 x 10^8 in *moi moi*, 0.04 x 10^8 – 0.58 x 10^8, 0.14 x 10^8 – 1.08 x 10^8 in fried meat and 0.17 x 10^8 – 2.01 x 10^8 in beans.

Table 2 shows the mycological count in the food samples. Mycological count ranged from 0.07 x 10^8 – 1.09 x10^8 in pepper soup, 0.03 x 10^8 – 2.02 x 10^8 in rice and stew, 0.10 x 10^8 – 0.50 x 10^8 in *moi moi*, 0.03 x 10^8 – 0.33 x 10^8 in cake, 0.04 x 10^8 – 0.31 x 10^8 in fried meat and 0.02 x 10^8 – 0.40 x 10^8 in beans.

Table 3 shows the anti-microbial sensitivity testing of bacterial isolates.

Figure 1 shows the frequency of occurrence of bacterial species isolated from the food samples. *Vibrio cholerae* had the highest frequency of occurrence in all the food samples of 60%, followed by *Escherichia coli* (53%), *Streptococcus pneumoniae* (49%) and *Aerococcus* (48%). *Actinomyces* and *Shigella dysenteriae* shared a frequency of 36%.

Figure 2 shows the frequency of occurrence of fungal isolates from the food samples. *Saccharomyces sp* had the highest frequency of 44% , followed by *Verticillum sp* (37%) and *Aspergillus niger* (35%).

Tables and Figures:

Table.1: Total Bacteriological Count in Food Samples

Samples	Total Bacterial Count(cfu/g)	Total Pathogenic Bacterial Count (cfu/g) *
P1	0.35 x 10^8	-
P2	1.20 x 10^8	0.49 x 10^8
P3	0.17 x 10^8	0.09 x 10^8
P4	1.58 x 10^8	0.18 x 10^8
P5	0.14 x 10^8	0.09 x 10^8
R1	0.17 x 10^8	0.15 x 10^8
R2	0.34 x 10^8	0.12 x 10^8
R3	2.50 x 10^8	1.28 x 10^8
R4	2.50 x 10^8	0.25 x 10^8
R5	1.00 x 10^8	1.00 x 10^8
M1	0.48 x 10^8	0.31 x 10^8
M2	1.68 x 10^8	-
M3	0.23 x 10^8	0.11 x 10^8
M4	1.04 x 10^8	1.00 x 10^8
M5	0.92 x 10^8	0.19 x 10^8
C1	0.40 x 10^8	0.19 x 10^8
C2	0.04 x 10^8	0.01 x 10^8
C3	0.43 x 10^8	0.27 x 10^8
C4	0.58 x 10^8	0.22 x 10^8
C5	0.14 x 10^8	0.10 x 10^8
F1	0.25 x 10^8	0.03 x 10^8
F2	0.92 x 10^8	0.09 x 10^8
F3	0.17 x 10^8	0.04 x 10^8
F4	1.08 x 10^8	0.18 x 10^8
F5	0.14 x 10^8	0.04 x 10^8
B1	0.17 x 10^8	0.15 x 10^8
B2	0.34 x 10^8	0.02 x 10^8
B3	2.01 x 10^8	1.28 x 10^8
B4	1.58 x 10^8	0.25 x 10^8
B5	1.02 x 10^8	0.80 x 10^8

P= Peppersoup; R= Rice and Stew; M= Moi Moi; C= Cake; F= Fried meat; B= Beans

1= Mbiabong Motor Park; 2= Itam Junction Market; 3= Urua Akpan Andem Market; 4=Aka-Etinan Road; 5= State Secretariat.

Pathogenic bacteria = *Salmonella*, *Shigella*, *Escherichia coli*, *Vibrio cholera*

Table.2: Total Mycological Count in Food samples

Samples	Cell count (cfu/g)
P1	0.07×10^8
P2	1.09×10^8
P3	0.31×10^8
P4	0.04×10^8
P5	0.11×10^8
R1	0.16×10^8
R2	0.03×10^8
R3	0.03×10^8
R4	2.02×10^8
R5	0.51×10^8
M1	0.10×10^8
M2	0.20×10^8
M3	0.47×10^8
M4	0.46×10^8
M5	0.50×10^8
C1	0.33×10^8
C2	0.03×10^8
C3	0.07×10^8
C4	0.11×10^8
C5	0.14×10^8
F1	0.31×10^8
F2	0.10×10^8
F3	0.04×10^8
F4	0.10×10^8
F5	0.12×10^8
B1	0.02×10^8
B2	0.03×10^8
B3	0.21×10^8
B4	0.04×10^8
B5	0.40×10^8

P= Peppersoup; R= Rice and Stew; M= Moi Moi; C= Cake; F= Fried meat; B= Beans
1= Mbiabong Motor Park; 2= Itam Junction Market; 3= Urua Akpan Andem Market; 4=Aka-Etinan Road; 5= State Secretariat.

Table.3: Sensitivity Testing of Bacterial Isolates (mm)

Isolate	**CPX**	**NB**	**GN**	**LC**	**S**	**RD**	**E**	**CH**	**APX**	**FLX**
Yersinia pseudo-tuberculosis	R	20	17	23	10	R	15	25	R	R
Aerococcus viridans	10	18	20	20	27	18	20	30	10	15
L. acidophillus	10	15	23	25	25	10	15	30	23	10
S.choleresius	R	R	10	R	18	R	21	18	R	R

P. mirabilis	30	11	21	R	17	22	25	15	18	15
S. epididermis	41	14	18	22	18	18	22	35	10	14
S. salivarius	38	21	20	24	13	11	28	35	25	21
Actinomyces sp	20	25	20	30	28	30	32	40	10	10
E. coli	R	17	10	15	11	14	30	10	9	18
Micrococcus virans	18	20	23	17	10	18	22	32	R	15
S. pneumoniae	R	12	18	20	25	20	22	28	18	10
C. septicum	R	R	10	R	9	12	10	10	R	R
B. polymiza	10	12	18	22	20	28	20	R	10	13
S. aureus	R	15	18	20	27	26	21	18	20	R
Vibrio sp	R	R	10	10	R	R	R	10	R	R
B. subilis	19	11	20	28	21	18	16	25	20	17
B. cereus	20	23	18	25	20	15	18	25	20	17
Shigella dysenteriae	R	10	18	R	23	25	R	31	11	R
Salmonella typhi	R	17	22	R	28	11	R	28	R	R

Key: CPX – Ciprofloxacin; RD – Rifampicin , NB – Norfloxacin; E – Erythromycin, GN – Gentamicin; CH – Chloramphenicol, LC – Lincocin; APX – Ampiclox , S – Streptomycin; FLX – Floxapen , R – Resistant

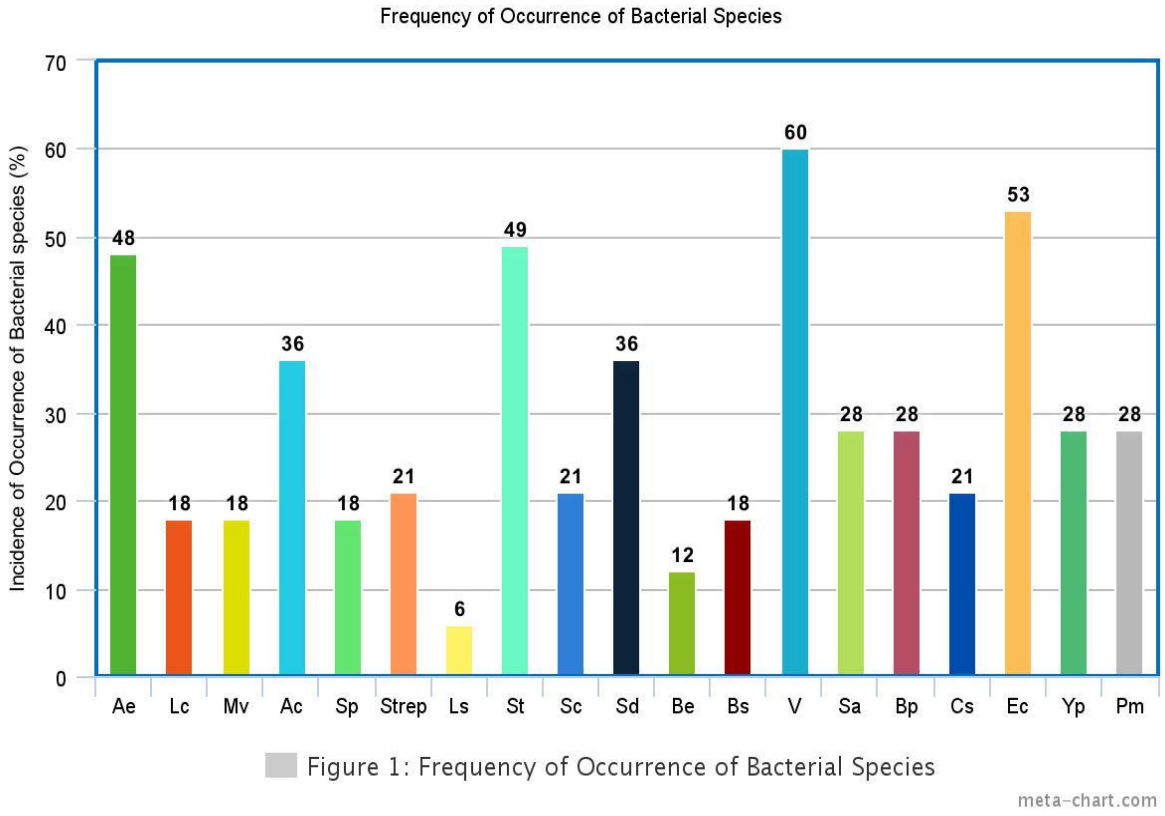

Figure.1:

Key: Ae= Aerococcus; Lc= L. acidophillus; Mv= Micrococcus; Ac= Actinomyces ; Sp= Staphylococcus epididermis; Strep= Streptococcus pneumoniae ; Ls= Lactobacillus salivarius ; St= Salmonella typhi; Sc= Salmonella choleriaesius ; Sd= Shigella dysenteriae; Be= Bacillus aereus; Bs= Bacillus subtilis; V= Vibrio sp; Sa= Staphylococcus aureus; Bp= B. polymiza; Cs= Clostridium septicem; Ec= E. coli; Yp= Yersinia pseudo-tuberculosis; Pm= Proteus mirabilis

Figure 2: Frequency of Occurrence of Mycological Species

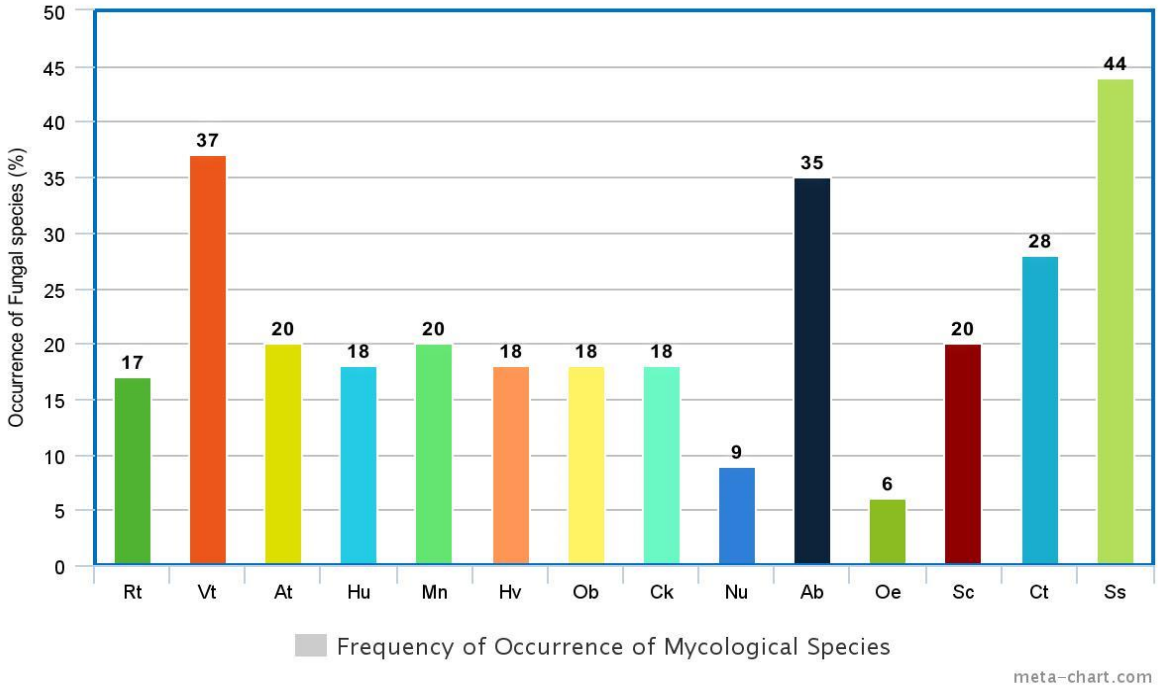

Figure.2: Frequency of Occurrence of Mycological species

Key: Rt = Rhizopus stolonfer, Vt = Verticillum sp, At = Aspergillus terreus, Hu= Humicola sp, Mn= Moniaua sp, Hv= Helminthosporium velutinum, Ob= Penicillium notatum, Ck= Closdiosporium sp, Mi = Microsporium sp, Ab= Aspergillus niger, Oe= Penicillium expansium, Sc= Saccharomyces cerevisae, Ct= Candida tropicalis, Ss= Saccharomyces sp.

IV. DISCUSSION

A total of 19 species of bacteria and 14 species of fungi was isolated from 150 hawked food samples analyzed. The Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items) recommended total bacterial plate counts of bacteria for foods cooked immediately prior to sales is in the range of less than 10^3 cfu/g [17]. It was observed that almost all the foods examined had bacterial counts above the acceptable limit and is therefore microbiologically unacceptable.

It was observed that food samples from Mbiabong Motor Park(P1, R1,M1,C1,F1,B1) were the least contaminated. This could be due to the lower population density of this location compared with other locations. The park is located away from the centre of the city with better sanitary conditions. Overcrowding increases air pollution which will inevitably increase the risk of food contamination.

Cake and fried meat samples were also the least contaminated of the hawked food sales. This may be attributed to their low water content which reduces microbial population. On the other hand, rice and stew and beans samples which had the highest water content were the most contaminated as they favour conditions for microbial growth [18]

There was a high incidence of enteric bacteria observed in the hawked food samples. These bacteria include *Salmonella*, *Shigella*, *E.coli and Vibrio sp.* 72% of these samples were contaminated by at least one of these bacteria having serious health implications. This agrees with Madueke et al [19] who reported that laboratory analysis of samples of certain hawked food had shown high levels of coliform and pathogenic bacteria to include *Salmonella sp*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Vibrio cholerae*.

Also, organisms isolated in this study might have been introduced into these foods from feacally polluted water used for washing utensils (e.g. knives, trays, and pans), wrapping materials and the exposure of these products to low temperature [20]. It may also be as a result of the failure of food handlers to observe basic sanitary rules [21].

Lactobacillus salivarius is a normal flora in the human mouth [22]. This bacteria was isolated from the hawked food samples. Contamination of the food samples from this agent may have resulted from talking and tasting during food preparation as well as through sharing of cutleries. Its presence also indicates careless handling of food after cooking.

Mycological counts were reported in high densities in this study. The highest count was observed in rice and stew samples from Aka Etinan Road and the lowest count was found on samples from Mbiabong Motor Park. Pathogenicity of a microbial agent depends on its concentration and virulence. *Aspergillus niger* ,a virulent aflatoxin-and-spore-producing fungus was isolated from the hawked food samples. They should not be found in food. It is the aetiologic agent of Aspergillosis.

V. CONCLUSION

The present study shows that hawked street food contains pathogenic and non-pathogenic micro-organisms in dense concentrations. It also reveals a high concentration of enteric bacteria suggesting possible faecal contamination of street food or water used in cooking them. Isolation of *Lactobacillus salivarius* suggests possible salivary contamination of food by oral flora. All these are pointers to poor food handling and sanitary practices among street food hawkers in Uyo Metropolis, Southern Nigeria. This study also agrees with the reports of poor sanitary and handling practices among street food hawkers, especially that reported by Madueke et al [19] and Adesetan et al [23].

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

Author's Contributions:

Authors' contributions	EIA	EIF
Research concept and design	✓	✓
Collection of/and Assembly of data	✓	✓
Data analysis and interpretation	✓	--
Writing the Article	✓	✓
Critical Revision of the Article	✓	--
Final Approval of the Article	✓	✓
Statistical Analysis	✓	--

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