# Bacterial Aetiological Agents of Vended Foods in Vom, Plateau State, Nigeria

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**Abstract:**
***Aims:*** *Foodborne diseases are multifactorial in origin and are major cause of death worldwide. This study was aimed at detecting the presence of bacterial pathogens in already prepared vended foods in Vom.*
***Methodology and results:*** *Two hundred (200) cooked food (ready-to-eat) samples were subjected to bacteriological examinations using differential, selective and enriched culture media. A total of 228 bacterial isolates were obtained. These includes Aeromonas hydrophila (3)1.5%, Bacillus species (32)16%, Citrobacter freundii (18)9%, Citrobacter braekii (9)4.5%, Citrobacter youngae (1)0.5%, Chryseomonas luteola (1)0.5%, Enterobacter cloacae (28)14%, Escherichia coli (14)7%, Klebsiella  pneumoniae (6)3%, Kluyvera species (1)0.5%, Morganella morganii (3)1.5%, Providencia species (4)2%, Pseudomonas aeruginosa (5)2.5%, Proteus mirabilis (2)1%, Salmonella species (12)6%, Staphylococcus aureus (34)17%, coagulase negative Staphylococcus (49)24.5%, Streptococcus faecalis (5)2.5% and Vibrio hollisae (1)0.5%, twenty one (21) samples had  no bacterial growth. The identification of the Gram-negative organisms were confirmed using API 20E. These isolates were further subjected to antimicrobial sensitivity testing using the Abtex commercial disc. Most isolates were resistant to Amoxycillin, Cloxacillin, Cotrimoxazole and Erythromycin. Ciprofloxacin had about 99% activity against all the isolates.*
***Conclusion:*** *The isolation of bacterial pathogens is indicative of bacterial contamination in vended foods in Vom within the period of the study.*
***Keywords:*** *Bacteria -Vended Food -Vom, Nigeria*

## I.    Introduction

Foodborne diseases are major cause of illness and death worldwide (Adak *et al.,* 2002). An estimated 76 million cases of foodborne illness occur each year in the United States, costing between $6.5 and $34.9 billion in medical care and lost of productivity ( Mead *et al*., 199). In the developing world, food-borne infections leads to the deaths of many children and the resulting diarrhoea disease can have long term effects on children's growth as well as their physical and cognitive development (Guerrant *et al.,* 1999). Foodborne illnesses continue to be a major threat to the health of people in Africa, especially vulnerable groups such as children, the elderly and people with underlying diseases such as HIV/AIDS (Mensah *et al*., 2012). In the industrialized world, food-borne infections cause considerable illnesses, heavily affecting health care systems (Adak *et al.,* 2002). Bacteria are the causative agents of foodborne illness in 60% cases requiring hospitalization (Mead *et al*., 1999). The international impact of foodborne illness is difficult to estimate. However, due to the diarrhoeal related illnesses annually, it is suspected that food or water is a vehicle for many of these illnesses (WHO, 2002).

In the United states, incidence of foodborne illness is documented through food Net, a reporting system used by public health agencies that captures foodborne illness in over 13% of the population (IFT, 2004). Of the 10 pathogens tracked by foodnet, *Salmonella, Campylobacter* and *Shigella* are responsible for most cases of foodborne illness, *Salmonella* causes 31% of food related deaths followed by *Listeria* (28%), *Campylobacter* (5%) and *Escherichia coli* 0157:H7 (35%) (Mead *et al*., 1999., Mensah *et al*., 2012).

Contaminated food continues to cause numerous devastating outbreaks in the African Region. In Africa, a large proportion of ready-to-eat foods are sold by the informal sector, especially as street foods. The hygienic aspects of vending operations and the safety of these foods are problematic for food safety regulators. The global food crisis has worsened an already precarious food situation because when food is in short supply people are more concerned about satisfying hunger than the safety of the food (Mensah *et al*.,   2012). The

presence of potentially life threatening pathogens in our environment, the ability of some of them to survive and /or proliferate under refrigeration indicate the seriousness of the potential hazards with which we are faced.

This study was aimed at determining the prevalence of bacterial contaminants in foods (ready-to-eat) vended or served in Vom and its environs, Jos-South Local Government Area of Plateau State,  Nigeria

## II.    Materials And Methods

**Sample Collection**

Two hundred samples of cooked food ready-to-eat (RTE) which includes rice, fermented milk (nono), moimoi, soup and other cooked foods which includes cassava, plantain, yam, potatoes and tuwo were collected at very low temperature from restaurants, hawkers  or food vendors and residential homes in Vom and its environs by convenient sampling method as documented by Danladi *et al.,* 2014.

The food samples were collected into a sterile universal bottle after being dished. These samples were transported to the laboratory as soon as possible and processed within 2 hours.

**Total Viable Count**

The modified surface drop count method of Miles and Misra as described by Ochei and Kolhatkar (2000) was used for the enumeration of each samples.

**Bacterial Isolation**

About 1g of food sample was inoculated into 10ml of sterile nutrient broth, alkaline peptone water, selenite faeces (SF) and phosphate buffer saline (pH 7.2) (Fluka, Sigma, Aldrich Chemie, GmBH, Germany) and  incubated for 18-24 hours at $37^0$C. The phosphate buffer saline was incubated for 21days in the refrigerator at $4^0$C. From the nutrient broth, blood agar and MacConkey agar plates were aseptically inoculated using  sterile wire loop. Cultures were incubated at $37^0$C aerobically and anaerobically for 18-24 hours according to the method of Cheesbrough (2002). The SF broth were subcultured aseptically onto Deoxycholate agar plate (DCA)( Oxoid ,UK) and incubated at $37^0$C aerobically for 18-24 hours. Samples in alkaline peptone water were inoculated into thiosulphate citrate bile salt sucrose (TCBS) medium and incubated aerobically at $37^0$C.  The phosphate buffer saline (PBS) of the primary cultures were similarly subcultured onto selective media (cefsoludin irgasan novobiocin (CIN) for the isolation of Yersinia species and incubated at room temperature ($25^0$C) for 24-48 hours (FDA/CFSAN, 2001).

**Bacterial Identification**

The culture plates were all read macroscopically, microscopically, enzymatically and characterized biochemically using API-20E (Sharma *et al.,* 1990., Okwori *et al.,* 2007).

**ANTIBACTERIAL SUSCEPTIBILITY TEST**

The sensitivity spectrum of each of the isolates to seven different antibiotics was determined by standardized diffusion method (NCCLS, 1995., NCCLS, 2002). The antimicrobial agents used were Ciprofloxacin (10μg/ml), Tetracycline (10μg/ml), Gentamycin (10μg/ml), Cloxacillin (5μg/ml), Erythromycin (5μg/ml), Cotrimoxazole (10μg/ml), and Amoxicillin (20μg/ml) (Abtek biologicals Ltd, Liverpool, UK). The diameters of the zones of inhibition around each antibiotic disc were measured in millimetres.

**Statistical Analysis**

Descriptive statistical analysis was done using chi-square data management analysis. Differences between proportions were assessed. Statistical significance was set at 0.05.

## III.    Results

Two hundred (200) cooked food (ready-to-eat) samples (rice, moimoi, fermented milk (nono), soup and other cooked foods) were examined for a spectrum of bacteria. A total of 228 bacterial isolates were obtained. Twenty one (21) of the food samples showed no bacterial growth.

The number of individual organism isolated and percentage occurrence is represented in Table 1. Three samples contained *Aeromonas hydrophila*   (3)1.5%, *Bacillus* species (32) 16%, *Citrobacter freundii* (18) 9%, *Citrobacter braekii*, (9)4.5%, *Citrobacter youngae* 0.5%, *Enterobacter cloacae* (28)14%,  *Escherichia coli* (14)7%,  *Chryseomonas luteola* (1)0.5%, *Klebsiella pneumonia* (6)3%, *Kluyvera* species (1)0.5%, *Morganella morganii* (3)1.5%, *Providencia* Species (4)2%, *Pseudomonas aeruginosa* (5)2.5%, *Proteus mirabilis* (2)1%, *Salmonella* species (12)6%,  *Staphylococcus aureus* (34)17%,  *Coagulase negative Staphylococcus* (49)24.5%, *Streptococcus faecalis* (5)2.5%, *Vibrio hollisae* (1) 0.5%.

Each of the isolates was also subjected to antimicrobial sensitivity testing. Ciprofloxacin had a high antimicrobial activity against most of the isolates evident by a large area of inhibition elicited followed by Gentamycin (Table 3).

**Table 1: Percentage spectrum of bacterial isolates**

| Isolated organisms | Number of isolates | % |
|---|---|---|
| *Aeromonas hydrophila* | 3 | 1.5 |
| *Bacillus species* | 32 | 16 |
| *Citrobacter freundii* | 18 | 9 |
| *Citrobacter braekii* | 9 | 4.5 |
| *Chryseomonas luteola* | 1 | 0.5 |
| *Citrobacter youngae* | 1 | 0.5 |
| *Enterobacter cloacae* | 28 | 14 |
| *Escherichia coli* | 14 | 7 |
| *Klebsiella pneumonia* | 6 | 3 |
| *Kluyvera specie* | 1 | 0.5 |
| *Morganella morganii* | 3 | 1.5 |
| *Providencia species* | 4 | 2 |
| *Pseudomonas aeruginosa* | 5 | 2.5 |
| *Proteus mirabilis* | 2 | 1 |
| *Salmonella species* | 12 | 6 |
| *Staphylococcus aureus* | 34 | 17 |
| *Staphylococcus albus* | 49 | 24.5 |
| *Streptococcus faecalis* | 5 | 2.5 |
| *Vibrio hollisae* | 1 | 0.5 |
| Total | 228 | 89.5 |

**Table 2:  Distribution of bacterial isolates in foods screened**

| Isolates | Rice | Moimoi | Soup | Nono | Others |
|---|---|---|---|---|---|
| Aeromonas hydrophila | 1 | 1 | Nil | Nil | 1 |
| *Bacillus species* | 15 | 3 | 5 | 3 | 6 |
| *Citrobacter freundii* | 7 | 2 | 4 | 2 | 3 |
| *Citrobacter braekii* | 2 | 1 | 2 | 1 | 3 |
| *Citrobacter youngae* | Nil | 1 | Nil | Nil | Nil |
| *Enterobacter cloacae* | 7 | 5 | 6 | Nil | 10 |
| *Escherichia coli* | 4 | 2 | 5 | Nil | 3 |
| *Chryseomonas luteola* | Nil | Nil | 1 | Nil | Nil |
| *Klebsiella pneumonia* | 3 | Nil | 2 | Nil | 1 |
| *Kluyvera species* | 1 | Nil | Nil | Nil | Nil |
| *Morganella morganii* | 2 | Nil | 1 | Nil | Nil |
| *Providencia species* | 1 | Nil | 1 | Nil | 1 |
| *Pseudomonas aeruginosa* | Nil | 1 | 3 | Nil | Nil |
| *Proteus mirabilis* | 1 | Nil | Nil | Nil | 1 |
| *Salmonella species* | 2 | Nil | 7 | Nil | 3 |
| *Staphylococcus aureus* | 5 | 5 | 3 | 17 | 4 |
| *Staphylococcus albus* | 7 | 2 | 8 | 24 | 8 |
| *Streptococcus faecalis* | 1 | 2 | Nil | Nil | 1 |
| *Vibrio hollisae* | 1 | Nil | Nil | Nil | Nil |
| Total | 60 | 25 | 48 | 47 | 48 |

**Table 3: Resistance pattern of bacterial isolates to antimicrobial agents.**

| Organism | Amoxycillin | Cloxacillin | Gentamycin | Cotrimoxazole | Ciprofloxacin | Erythromycin | Tetracycline |
|---|---|---|---|---|---|---|---|
| | % (*) | % (*) | %(*) | % (*) | % (*) | % (*) | %(*) |
| *Aeromonas hydrophila* | 0 (0) | 100 (3) | 100 (5) | 100 (3) | 0 (0) | 100 (3) | 0 (0) |
| *Bacillus species* | 34.4 (11) | 100 (32) | 65.6 (21) | 100 (32) | 0 (0) | 65.6 (21) | 100 (32) |
| *Citrobacter species* | 50 (14) | 100 (28) | 25(7) | 89.3 (25) | 0 (0) | 100 (28) | 75 (21) |
| *Escherichia coli* | 36 (5) | 85.7 (12) | 50 (7) | 100 (14) | 0 (0) | 92.9 (13) | 78.6 (11) |
| *Enterobacter species* | 42.9 (12) | 85.7 (24) | 28.6 (8) | 100 (28) | 0 (0) | 85.7 (24) | 85.6 (11) |
| *Klebsiella pneumonia* | 0 (0) | 100 (6) | 0 (0) | 100 (6) | 0 (0) | 100 (6) | 100 (6) |
| *Kluyvera species* | 100 (1) | 100 (1) | 100 (1) | 100 (1) | 0 (0) | 100 (1) | 100 (1) |
| *Proteus mirabilis* | 100 (2) | 100 (2) | 0 (0) | 0 (0) | 0 (0) | 100 (2) | 0 (0) |
| *P. aeruginosa* | 40 (2) | 60 (3) | 20 (1) | 100 (5) | 0 (0) | 100 (5) | 100 (5) |
| *Salmonella species* | 66.7 (8) | 100 (12) | 50 (6) | 100 (12) | 8.3 (1) | 83.3 (10) | 83.3 (10) |
| *Staphylococcus aureus* | 64.7 (22) | 100 (34) | 29.4 (2) | 100 (34) | 0 (0) | 100 (34) | 20.5 (7) |

Key:
(*)=Number of resistant isolates

# IV. Discussion

Unexpectedly, most foods still contain some micro organisms even after cooking. Consequently cooking could not be seen as sterilization as thermophilic organisms survived cooking temperature. However other sources of micro organisms in cooked meals may include, utensils, water and carrier status of some food handlers. Studies have also implicated cross contamination as a source of microbial contamination of cooked foods (Zhao *et al.*, 2001., Beach *et al.*, 2002).

Our study has shown high incidence of *Bacillus* species supporting the fact that they are aerobic spore bearers that could easily contaminate foods (Shingawa *et al* (1980). Its presence in various food types further buttresses the fact that this microorganism can readily be isolated from a variety of foods as previously documented by Shingawa *et al* (1980). Notably, Bacillus species has the ability to form heat resistant spores and thus possessing great preponderance to cause foodborne diseases. *Staphylococcus aureus* and coagulase negative *Staphylococcus* isolated in our study is similar to the findings of Bergdoll (1979) and Mohapatra *et al* (2002). *Staphylococcus aureus* normally inhabits the nose, throat, skin etc. of about 50% of healthy individuals thus increasing its chances of contaminating foods. Outbreaks of *Staphylococcus aureus,* Bacillus species and proteus species food poisons after eating in a restaurant have been reported by many researchers (Noah, 2009., Fry *et al.,* 2005., Norinaga *et al.,* 2000., Vijay *et al.,* 2007., Lorraine *et al.,* 2008., Yon *et al.,* 2010., Mensah *et al.,* 2012). Food borne intoxication is very common as documented by Mead *et al* (1999) and IFT (2004) owing to the ability of *Staphylococcus aureus* to quickly produce toxins that are heat stable causing illness even after the cells have been destroyed by cooking or reheating. Nevertheless, it remains a major cause of food borne disease because it can contaminate food products during preparation and processing (Yves *et al.,* 2003).

This study has also shown a high incidence of *Salmonella* species which is in consistent with report of IFT (2004) and FDA/CFSAN (2001) implicating *Salmonella* as one of the leading causes of food borne illness. This is of great clinical significance since *Salmonella* is known to have a very low infective dose and high virulence. The presence of *E. coli* indicates faecal contamination as reported by Warburton (2000) and Josefa *et al* (2005). Amongst these *E. coli* isolated could exist the highly pathogenic *E.coli 0157:H7* even though it was not searched for. The isolation of other organisms such as *Aeromonas hydrophila, Chryseomonas luteola, Kluyvera* species, *Morganella morganii* which are not regularly reported as foodborne pathogens further buttressed the reports of Mead *et al* (1999) that says the organisms carried by food over the few past years are gradually changing due to changes in methods of cooking, food handling and probably national or geographical variability.

*Bacillus* species had the highest distribution in rice thus supporting the report of Gilbert *et al* (1974) and Shingawa *et al* (1980). The presence of *Salmonella* species could be traced probably to the meat or vegetable in the soup, cross contamination or the handlers of the food as documented by Zhao *et al* (2001) and Beach *et al* (2002). The findings of this study has revealed the danger to which this community is exposed, thus requiring that more attention be paid to food hygiene. However, the cases studied here are not established cases of food borne disease in Vom metropolis. It is also important to state that, it is not routine that consumption of such foods so contaminated with these organisms would certainly result in a disease condition as highlighted by IFT (2004), rather, according to FDA/CFSAN (2003), outcome is dependent on the dose of organism consumed in the food, the virulence of the contaminating organism and the immune status of the consumer of such food.

Refrigeration at $4^0$C has been reported by Mead *et al* (1999) to be effective in retarding the growth of organisms in food (even though there are certain organisms that grows or multiply at $4^0$C) thus an effective method of food storage. But with the erratic nature of power supply experienced today in Nigeria, this method is gradually going into extinction.

The relatively high level of resistance to antimicrobial agents may be reflection of misuse or abuse of these agents in the environment.

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