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Assessments of Contamination and Susceptibility Pattern of Bacteria Isolated from Pounded Yam Sold Along Major Roads in Makurdi Metropolis, Benue State, Nigeria

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Abstract

Pounded yam is a staple food consumed in Makurdi, Benue State. The food is sold along major streets and roads in the town, as such; it is prone to bacterial contamination from different sources. The study was aimed at assessing the contamination and susceptibility pattern of bacteria isolated from pounded yam sold along major roads in Makurdi metropolis. One hundred and forty (140) samples of pounded yam were aseptically collected from seven food vending sites (Wurukum, Wadata, High level, Naka road, Otukpo road, Gboko road and North bank) and analyzed for bacterial contamination using standard microbiological procedures. The Kirby-Bauer disk diffusion method was used for the antibacterial susceptibility testing. The antibiotics tested were Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Pefloxacin, Tarivid and Streptomycin. If a bacterial isolate proved resistant to at least three of the tested antibiotics, it was deemed to be multi-drug resistant (MDR). A total of four species of bacteria which included Staphylococcus aureus (35.66%), Escherichia coli (18.18%), Klebsiella spp (11.20%) and Proteus spp (34.97%), were isolated from the pounded yam samples. The mean total viable count of all the samples; which ranges from 2.61x10^{5}cfu/g (Wadata) to 3.25x10^{5}cfu/g (Otukpo road) are within the acceptable limits for food. Staphylococcus spp, Escherichia coli and Proteus spp were multidrug resistant to four, six and three antibiotics respectively, according to the antibacterial susceptibility test. Staphylococcus spp showed 31.37% susceptibility to Septrin, 37.25% to Streptomycin and 33.33% of the Staphylococcus isolates were sensitive to Sparfloxacin and Amoxycillin. For Escherichia coli, more than 50% of the 26 Escherichia coli isolates showed resistance to Septrin (46.16%), Sparfloxacin (42.31%), Amoxicillin (42.31%), Gentamycin (38.46%), Perfloxacin ((46.15%) and Streptomycin (23.08%). Proteus spp showed 44.00% susceptibility to Chloramphenicol, 30.00% to Sparfloxacin and 40.00% to amoxicillin. Klebsiella spp showed 25.00% susceptibility to Amoxicillin only. The study identified that pounded yam sold along major roads in Makurdi metropolis are contaminated with bacteria of public health importance. Therefore hygienic way of food preparation is of utmost importance to prevent contamination of food with these organisms resulting to food borne diseases.

Keywords: Pounded yam, Bacteria, Susceptibility pattern.

INTRODUCTION

Street foods are foods and beverages that are sold by street vendors or hawkers which could be raw or cooked. They include starchy samples of maize, cassava, rice, plantain and yam (Ameko *et al.,* 2012). The preparation and sale of street foods is an age old activity and has reached new dimensions as a result of rapid urbanization (Ike *et al.,* 2015). Many urban dwellers obtain a significant portion of their diet from street foods which has increased the demand for street foods in major cities (Pikuda *et al.,* 2009). Street food vending is a large source of employment in the cities of many developing countries (Choudhury *et al.,* 2011), thus contributing significantly to household incomes (Feglo *et al.,* 2012).

Street vendors are often poorly educated, unlicensed, untrained in food hygiene practices and they work under poor sanitary conditions with little or no knowledge about the causes of food borne diseases (Barro *et al.,* 2007). Street foods on display for sale can be contaminated easily by dust, automobile exhaust, smoke, insects, hands of processors and intending buyers and other substances in the environment (Ike *et al.,* 2015). The interaction of chemical, physical and microbial factors influences the safety and shelf life of these street foods (Oranusi *et al.,* 2013). Foods are often exposed near the road and vehicular emission could be a major source of pollution of most street vended foods (Opeolu *et al.,* 2010). Most of the foods are not protected from flies which may carry food borne pathogens like *Shigella* sp, *Staphylococcus* sp, *Escherichia coli, Clostridium* sp, *Vibrio* sp, *Campylobacter* sp, *Listeria* sp *and Salmonella* sp (Akinnibosun and Airiohuodion, 2015).

The people's lifestyle characterized by increased mobility and large itinerary workers due to urbanization has caused less family and home centered activities. This situation has resulted in more street vended and ready-to-eat (RTE) foods that are taken outside the home (Odu and Imaku, 2013). The busy and populated Makurdi metropolis is an area where street food vendors thrive in business as such there is need for continuous effort to determine the safety of vended foods for human consumption.

The spread of food-borne pathogens resistant to antibiotics is through the consumption of contaminated food (Oniciuc *et al.,* 2019) and these pathogens thrive more in food processing areas (Rodríguez-López *et al.,* 2020). The primary source of antibiotic resistant bacteria is foods of animal origin. Available data linked foods of non-animal origin with 10% of occurrence of food-borne pathogen (EFSA, 2013). One of the important routes for the spread of antibiotic resistant *Campylobacter* spp and *Salmonella typhimurium* to humans is poultry meat and pork (2 Van Boxstael *et al.,* 2012) Quinolone-resistant *Escherichia coli* have been isolated from veal, beef and dairy products (Catry *et al.,* 2016). Depoorter *et al.* (2012) implicated a meal made of chicken meat as the major cause of exposure to cephalosporin resistant *Escherichia coli*. Studies have also shown a significant concern about antibiotic use and resistance development in the aquaculture sector (Miranda *et al.*, 2003).

The consumption of road side foods is on the rise but with this comes an increase in the exposure to food-borne pathogens (de Giusti *et al.*, 2010; Zou *et al.,* 2011). Considering that human health has significantly been made better with the use of antibiotics for treating common diseases, the development of resistance by these food-borne pathogen is now of public health concern. This study therefore aimed to determine the susceptibility pattern of bacterial isolates from pounded yam sold along major roads in Makurdi metropolis to commonly used antibiotics.

MATERIALS AND METHODS

Sampling site

A total of seven food vending sites which covered the entire city and served at least thirty (30) customers per day were selected for sampling. They included: Wurukum, Wadata, Lafia road (North Bank), Gboko road (Gyado Villa, Gaadi, Fiidi), Otukpo road (Idye, Kanshio, Apir), Naka road (Ankpa Quarters, Modern Market) and High level.

Sample collection

A total number of 140 samples of pounded yam were randomly collected in sterile containers with seals and transported to the laboratory of the Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi for immediate analysis.

Microbiological analysis

Samples were prepared according to the modified method of Feglo *et al.* (2012). Twenty five (25) grams of each pounded yam sample was suspended in 225 mL of buffered peptone water and stirred to form a suspension. The colloidal liquid suspension formed the stock sample from which dilutions were made to obtain 10-fold serial dilutions. A 0.1 mL of it was transferred into a sterile Nutrient agar, Eosin Methylene Blue agar, Mannitol Salt agar, and MacConkey agar, using pour plate method and incubated at 37 ^{0}C for 24 hours (Feglo *et al.,* 2012). After 24-48 hours of incubation, growth showing colonies (if any) were counted using a digital colony counter (Cheesbrough, 2006). Bacterial counts were expressed as colony forming unit per gram of food sample analyzed (Cheesbrough, 2006).

Isolation of bacteria

The inoculated plates were examined for discrete colonies, growth characteristics and other colonial characteristics such as colour, colony shape, morphology and formation of mucoid colonies. The results were recorded. A discrete colony of each growth was picked and re-inoculated in repeated sub culturing unto fresh Nutrient agar to obtain pure cultures from which bacterial identification was done (Cheesbrough, 2006).

Biochemical tests

The first was Gram staining according to Cheesbrough (2006). Other tests carried out include Catalase, Coagulase, Indole, Citrate, Urease, Triple Sugar Iron, and Methyl Red/Voges-Proskauer (MR/VP) tests (Cheesbrough, 2006).

Antibiotic Susceptibility Test

The Clinical and Laboratory Standards Institute (CLSI, 2017) defined the Kirby-Bauer disk diffusion method as an antimicrobial susceptibility test employing drugs containing discs from Oxoid. This was the method used for the antibacterial susceptibility testing of the bacterial isolates.

The antibiotics discs and the concentration used were ampicillin 25 µg, Avicef 30 µg, Cotrimoxazole 25 µg, Collistinsulphate 25 µg, Chloramphenicol 30 µg, Oflaxacin 5 µg, Erythromycin 5 µg, Gentamycin 25 µg, Naladixic acid 30 µg, Nitofurantoin 200 µg, Penicillin 10 µg, Streptomycin 25 µg and Tetracycline 25 µg. Isolates were classified as either resistant, intermediate sensitive or sensitive based on the definition of the Clinical and Laboratory Standard Institute (CLSI, 2017) and in accordance with WHO requirements (Onanuga *et al.*, 2005). Some laboratory strains of known sensitivity like *S. aureus, E. faecalis, E. coli* and *P. aeruginosa* were used as quality control strains for the antimicrobial discs (Onanuga *et al.*, 2005, Santos *et al.*, 2010).

Resistant and intermediate isolates were grouped together for analysis in this study. An isolate was considered multi-drug resistant if it was resistant to at least three of the antibiotics tested (Santos *et al.*, 2010).

Statistical Analysis

The values obtained for total aerobic plate counts were subjected to analysis of variance (Das *et al.*, 2022) and mean values separated by Duncan's Multiple Range Test at P = 0.05.

RESULTS

The mean total viable bacterial count of samples from the seven locations studied ranges from $2.61x10^5$cfu/g (Wadata) to $3.25x10^5$cfu/g (Otukpo road) (Table 1) (p>0.05). The prevalence of bacterial isolates across study locations was presented in Table 2. Samples from Wadata accounted for the least percentage prevalence (14.00%) while samples from Northbank (Lafia road) and Naka road accounted for 16.10% each; being the highest percentage occurrence. *Staphylococcus* specie has percentage of occurrence of 35.66% followed closely by *Proteus* specie (34.97%) and the least was *Klebsiella* spp (11.20%).

The percentage susceptibility of bacterial isolates to antibiotics is presented in Table 5. The result showed Ciprofloxacin was the most active antibiotic as it had 100 percent susceptibility to three bacterial isolates (*Staphylococcus* spp, *Klebsiella* spp and *Proteus* spp) out of the four isolates identified with the fourth; *E. coli* having 92.31 percent susceptibility. Augmentin was also very active with 98.04% susceptibility in *Staphylococcus* spp, 100.00% in *Escherichia coli*, 100.00% in *Klebsiella* spp, and 86.00% in *Proteus* spp. Conversely, Amoxicillin showed the least percentage susceptibility on the isolates as it had between 25-45% susceptibility.

Table 1: Mean Total Viable Bacterial Count of samples from the various locations

Location	Mean count	Standard Deviation
High level	$2.83x10^{5b}$	$3.94x10^5$
Wadata	$2.61x10^{5b}$	$3.91x10^5$
Wurukum	$2.90x10^{5b}$	$3.97x10^5$
Northbank	$2.67x10^{5b}$	$3.88x10^5$
Otukpo	$3.25x10^{5a}$	$4.73x10^5$
Naka	$2.73x10^{5b}$	$3.94x10^5$
Gboko	$2.84x10^{5b}$	$3.86x10^5$

Mean on the same column with different superscript vary significantly using Duncan's Multiple Range Test ($P \leq 0.05$)

Table 2: Prevalence of bacterial isolates across study locations

			N(%)		
Locations	***S. aureus***	***E. coli***	***Klebsiella* spp**	***Proteus* spp**	**Total**
High level	8 (5.59)	5 (3.50)	1 (0.70)	8 (5.59)	22 (15.40)
Otukpo	10 (7.00)	4 (2.80)	0 (0.00)	5 (3.50)	19 (13.30)
Wurukum	6 (4.20)	5 (3.50)	3 (2.10)	4 (2.80)	18 (12.60)
Wadata	5 (3.50)	3 (2.10)	1 (0.70)	11 (7.69)	20 (14.00)
Gboko	5 (3.50)	0 (0.00)	4 (2.80)	7 (6.30)	18 (12.60)
N/bank	8 (5.59)	5 (3.50)	2 (1.40)	8 (5.59)	23 (16.10)
Naka	9 (6.30)	4 (2.80)	5 (3.50)	5 (3.50)	3 (16.10)
Total	**51 (35.66)**	**26 (18.18)**	**16 (11.20)**	**50 (34.97)**	**143 (100.00)**

Table 3: Percentage susceptibility of bacterial isolates to the tested antibiotics

Antibiotics	***Staphylococcus* spp N=51**	***Escherichia coli* N=26**	***Klebsiella* spp N=16**	***Proteus* spp N=50**
Septrin	16 (31.37)	12 (46.16)	13 (81.25)	28 (56.00)

Chloramphenicol	46 (90.20)	22 (84.62)	12 (75.00)	22 (44.00)
Sparfloxacin	17 (33.33)	11 (42.31)	8 (50.00)	15 (30.00)
Ciprofloxacin	51 (100.00)	24 (92.31)	16 (100.00)	50 (100.00)
Amoxicillin	17 (33.33)	11 (42.31)	4 (25.00)	20 (40.00)
Augmentin	50 (98.04)	26 (100.00)	16 (100.00)	43 (86.00)
Gentamycin	39 (76.47)	10 (38.46)	12 (75.00)	41 (82.00)
Pefloxacin	34 (66.67)	12 (46.15)	11 (68.75)	26 (52.00)
Tarivid	28 (54.90)	21 (80.77)	13 (81.25)	38 (76.00)
Streptomycin	19 (37.25)	6 (23.08)	14 (87.50)	26 (52.00)

N= Number of samples.

The antibiotic resistance patterns and multidrug resistance of the bacterial isolates as presented in Table 4 revealed that three of the four bacterial isolates were multidrug resistant. *Escherichia coli* showed the most resistance to six antibiotics; Septrin, Sparfloxacin, Amoxicillin, Gentamycin, Pefloxacin and Streptomycin followed by *Staphylococcus aureus* to four antibiotics; Septrin, Sparfloxacin, Amoxicillin and Streptomycin. *Proteus* spp was resistant to Chloramphenicol, Sparfloxacin and Amoxicillin while *Klebsiella* spp was resistant to only Amoxicillin.

Table 4: The antibiotic resistance patterns and multidrug resistance of the bacterial isolates

	Resistance patterns			
Antibiotics tested	***S. aureus***	***E. coli***	***Klebsiella* spp**	***Proteus* spp**
Septrin	+	+	-	-
Chloramphenicol	-	-	-	+
Sparfloxacin	+	+	-	+
Ciprofloxacin	-	-	-	-
Amoxicillin	+	+	+	+
Augmentin	-	-	-	-
Gentamycin	-	+	-	-
Pefloxacin	-	+	-	-
Tarivid	-	-	-	-
Streptomycin	+	+	-	-
Multidrug resistance	**YES**	**YES**	**NO**	**YES**

+ = Resistant, - = Susceptible

DISCUSSION

The morphological and biochemical characteristics of the bacterial isolates show four genera of bacteria were implicated as contaminants of pounded yam. Out of the four, only *Staphylococcus* specie was gram positive, the other three were gram negative bacteria namely; *Escherichia coli, Klebsiella and Proteus* species. The organisms implicated in this study shows pounded yam can be contaminated by pathogenic organisms prior to its consumption and this can be detrimental to health. Similar bacteria have been implicated in the contamination of garri, cassava, foo foo and other foods (Oranusi *et al.*, 2013; Kabiru *et al.*, 2013).

The presence of these organisms is of concern because *Escherichia coli* is an indicator organism for faecal contamination and it may have come in through the water they use in washing or cooking the yam, or from plates. Allam *et al.* (2016) reported that there is generally poor hygienic practice by those involved in handling of food as such; the handlers may also be the route of entry. *Staphylococcus* and *Klebsiella* species are air-borne which means their route of entry may be due to the exposure of the pounded yam to the air or environment. Those who patronize these eateries may release some of these organisms through breathing (Oniciuc *et al.*, 2019).

In general, the mean total viable count of all the samples was within the range of 10^5cfu/g which is the borderline for food as prescribed by the Food and Agriculture Organization of the United States of America (FAO, 2001). The fact that the microbial load of these food samples is on the borderline of tolerable limits raises public health concerns, particularly given that pounded yam is a staple food in Benue State and is consumed widely by the locals. As a result, even though the food's microbial load is within the acceptable range, food authorities always advise that these foods be closed until a follow-up study deems them safe. The high bacteria count in this food may be attributed to its rich nutritional composition. Pounded yam is rich in carbohydrate, minerals and vitamins, hence a good base for growth and proliferation of bacteria. The total viable count of 10^5cfu/g is consistent with earlier investigations proving the microbial load of some roadside foods is unsafe for consumption (Wogu *et al.,* 2010; Kabiru *et al.,* 2013).

The prevalence of bacterial isolates across locations shows that *Staphylococcus* specie was most prevalent, accounting for 35.66% of isolates in this study followed closely by *Proteus* specie (34.97%). Conversely, *Klebsiella* specie had the least prevalence (11.20%) followed by *Escherichia coli* with 18.10% prevalence. On the effect of location on prevalence of bacterial isolates, Northbank (Lafia road) and Naka road samples had the highest bacterial contamination accounting for 16.10% each while Wurukum and Gboko roads had the least contamination of 12.60% each. This implies that the pounded yam from Wurukum and Gboko roads were prepared under better hygiene when compared to other locations.

Pounded yam samples from Otukpo road accounted for the highest contamination with *Staphylococcus* species (7.00%) confirming the unhygienic state of its preparation. *Escherichia coli* were highest in High level, Wurukum and Northbank. The presence of *Escherichia coli* in 26 out of the 143 samples studied in this research was high as presence of *Escherichia coli* can cause enteric infection. The variation in the levels of contamination by the different bacterial species underscores the biodiversities across locations in Benue State, Nigeria.

The antibacterial susceptibility of bacterial isolates shows wide spread resistance of the bacteria to antibiotics. *Staphylococcus* spp, *Escherichia coli* and *Proteus* spp are multidrug resistant to Septrin, Sparfloxacin, Amoxicillin, Gentamycin, Perfloxacin and Streptomycin. This implies that these drugs have little or no effect on these organisms and if used for treatment purposes would yield no clear cut result (Kabiru *et al.* (2013).

This wide spread resistance of food pathogens is of public health concern because any disease outbreak by food pathogens may pose disastrous consequences on the populace, as health workers may find it difficult to control the outbreak using readily available antibacterial agents.

CONCLUSION

Street vended pounded yam sold along major roads in Makurdi metropolis, Benue State, Nigeria has varying levels of bacterial contamination, thus making the food unsafe for consumption. These foods were found to harbor multidrug resistant bacteria namely; *Staphylococcus* spp and *Escherichia coli*.

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Conflict of interest

The authors declare that there have no conflicting interests.

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