BACTERIOLOGICAL EVALUATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF ISOLATES FROM READY- TO- EAT FRIED FISH.

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Abstract

The study was carried out to ascertain the level of bacterial contamination of ready-to-eat fried fish hawked in Lapai metropolis of Niger state, Nigeria. Fried fish samples were collected from two different markets in Lapai. The bacterial load of the samples was determined using the pour plate technique. Bacteria growth was sub-cultured on MacConkey agar and Mannitol salt agar. Identification and characterization of various isolates were based on Gram staining technique and conventional biochemical tests. Results indicated that the mean bacteria population across the two markets after sampling for two (2) months ranged from 7.0 x 10^4 to 8.4 x 10^6 cfu/mg. The lowest bacteria population of 7.0 x10^4 cfu/mg occurred in the samples from the main market, while the highest bacteria population of 8.4 x 10^6 cfu/mg occurred in the samples from Badeggi market. Pseudomonas aeruginosa isolates had the highest distribution of 4 (44.5%), while Streptococcus spp isolate had the least distribution of 1 (11.1%). Bacillus subtillis and Staphylococcus aureus isolates had equal distribution of 2 (22.2%). Antibiotic susceptibility test was conducted on the isolates, and the result showed that S. aureus was 100% resistant to all the antibiotics used in the research study. Bacillus subtilis showed resistance to all the antibiotics, except Levofloxacin and Rifampicin to which the isolates were susceptible by 50%. Streptococcus spp was 100% resistant to Ampiclox, Erythromycin, Chloramphenicol, Norfloxacin, Amoxil and Rifampicin, but showed 100% susceptibility to Gentamicin, Levofloxacin, Ciproflox and Streptomycin. The isolates of P.aeruginosa were moderately susceptible to all the antibiotics used, but resisted Ampiclox and Norfloxacin. The findings of this research indicated that ready- to- eat foods are potential vehicles for transmitting food borne illnesses, therefore, there is the need to develop practical strategies that would prevent microbial contamination of street hawked foods.

Keywords: Contamination, hawked, ready- to- eat, fried fish

Introduction

One of the most important sources of food worldwide is fish. It is a sea food whose value is recognized for its high nutritional content (Abisoye*et al*., 2011). Twenty-percent (20%) of animal protein is derived from fin fish or shell fish. Fish is an essential source of protein throughout recorded human history. FAO(2008) assert that about 35% of all fishes are eaten fresh, chilled or frozen. In Nigeria, 20-25% average animal consumption is been attributed to fish alone and could extend to 80% in coastal and riverine areas. A reduction in fish availability will have a harmful effect on the nutritional status of people living in areas where fish contribute essentially to protein consumption (Eyo, 2001).Fish and other marine product can be put to use industrially for perfume, varnish soap, margarine and lubricant production. Normal growth specifically for blood vessel and nerves is enhanced by Omega-3-fatty acid derived from fish. Omega-3-fatty acid also keeps the skin and other tissues of the body fresh and young.

Ready-to-eat food can be described as the status of food being ready for instant intake at the point of sale. Such food could be raw, cooked or undercooked, hot or frozen. They are eaten without further subjecting them to heat (Tsang, 2002). Terms such as fast food, pastries, meat pie, sausage roll, burger, salad, fried meat, fried chicken, fried fish, and milk product such as cheese e.t.c. have been used to describe ready-to-eat food (Patience *et al*., 2002). Myriad of itinerary workers, reduced home based activities and increased transportation is typical of our society today, and this has led to more ready-to-eat food consumed outside home(Musa and Akande, 2002).Hawking of ready-to-eat fried fish is a common practice in Nigeria. These foods are hawked most especially in metropolitan areas due to high demand. However, fried fish is particularly common in Northern Nigeria. In order to meet the increasing fish food demand, contamination during processing, preparation and packaging of the fish is certain. The fish may contact bacteria which may render it unsuitable for consumption. The surrounding environment, vendors and fish contact surfaces could be the source of the bacteria. Also, while processing the food, contamination could result from vendor due to poor hygiene (Sapers*et al*., 2005; Peariso, 2005). In fact some of the vendors rarely take their bath.

Lack of hygiene and sanitation in outlets where the food is been processed, prepared, packaged and sold is obvious and as such there is a serious health consequences after ingesting these foods ranging from allergic reactions, stomach and intestinal cancerous growth, a general degradation of peripheral cellular tissue to gradual breakdown of the digestive and excretive system (Edema *et al*., 2005). Therefore hawking of fried fish has become an important public health issue which is of great concern to everybody due to widespread food borne diseases. Food borne illnesses are a major problem associated to ready-to-eat food. In addition, resistance of food borne microbes to multi drugs calls for great concern.

Bacteria are group of microorganism all of which lack a distinct nuclear membrane and hence are considered more primitive than animal and plant cells and most of which have a cell wall of unique composition. Most bacteria are unicellular; generally, they range in size between 0.5 and 5um (Elizabeth and Martin, 2003). Pathogenic bacteria found to be associated with ready-to- eat fried fish Include *Salmonella* spp, *Shigella* spp, *Escherichia coli*, *Bacillus* spp,

Staphylococcus aureus e.t.c. *Bacillus*spp, *S. aureus* and similar bacteria are capable of producing toxins on food and if ingested food poisoning can result. Other bacteria species such as *Salmonella* spp, *Shigella* spp, *Escherichia coli* e.t.c. have the potential of triggering food infections.

Methodology Study Area

Lapai is a Local Government Area in Niger State, Nigeria adjoining the Federal capital Territory. Its headquarters is in the town of Lapai on the A124 highway in the west of the area 9.05000^{o}N 6.56667^{o}E. It has an area of 3,051km^{2} and an average population of 110,127 at the 2006 census. This research study was conducted in two popular markets in Lapai metropolis (that is, Badeggi market and the main market).

Sample Collection

Ready-to-eat-fried fish samples were purchased from different selling locations in Lapai metropolis which included kiosk and hawkers from the two markets (Badeggi and Main market) in Lapai metropolis. The samples were aseptically collected separately with the aid of sterile sample bags and labeled accordingly. A total of thirty (30) samples were collected at a time at intervals of two weeks for two (2) months duration.

Total Bacteria Count

After sample collection, the fried fish samples were transported to the microbiology laboratory, Ibrahim Badamasi Babangida University, Lapai, and 1g each was homogenized in 9ml of peptone water which make up the stock for serial dilution. Five-fold dilution (10^{-1}to10^{-5}) of the homogenate was made. 1ml of 10^{-4} and 10^{-5} dilutions was plated on Nutrient agar in duplicate (for each sample) using the pour plate method. The plates were incubated at 37^{0}C for 24 hours. After incubation, bacteria colonies arising from each sample were counted using illuminated colony counter. The average count was expressed in colony forming unit per mg (Cfu/mg) (Cheesbrough, 2006).

Isolation of Bacteria

Colonies observed from each plate were was aseptically sub- cultured using sterile wire loop onto Manitol salt agar and MacConkey agar via streaking method and incubated at 37^{0}C for 24 hours. More so, all Bacteria detected to have grown in all cultured plate were picked and sub- cultured onto slant culture of Nutrient agar and preserved in the refrigerator for further biochemical tests, characterization and eventual identification (Cheesbrough, 2006).

Identification of Bacteria

Identification was carried out by subjecting the isolates to Gram stain, and then series of biochemical tests, such as catalase, coagulase, Indole, Methyl-red, Oxidase, Citrate utilization, Voges-Praskauer and sugar fermentation tests were carried out. The isolates were identified based on their reaction to the tests mentioned above (Cheesbrough, 2006).

Antibiotics Sensitivity Test

This test was carried out to determine the susceptibility profile of the isolates to the antibiotics. Suspensions of the isolates were prepared to conform to 0.5McFarland standards. Mueller-Hinton (molten) agar was prepared and poured into sterile petri dishes. The suspensions of the isolates wereinoculated onto the petri dishes containing the solidified Mueller-Hinton agar with the aid of sterile swab sticks by spreading evenly and allowed to dry. Multiple antibiotics discs were picked using forceps and placed onto the solidified Mueller-Hinton agar. The agar plates were incubated at 37^0C for 24 hours, after which the zones of inhibition were measured to the nearest millimeter (mm) with the aid of a ruler (CLSI, 2012).

Results

Bacterial colony count of ready-to-eat fried fish from the markets in Table 1 below indicated that samples from Badeggi market have higher bacteria load than that of the main market. The mean bacterial population across the two markets for the two (2) months duration ranged from 7.0×10^4 to 8.4×10^5cfu/mg.

Table 1: Bacteria Count from Different Markets in Lapai Metropolis

Market	Dilution Factor	Number of colony	Population (cfu/mg)
Main	10^{-4}	7	7.0×10^4
	10^{-5}	5	5.0×10^5
Badeggi	10^{-4}	80	8.0×10^5
	10^{-5}	84	8.4×10^6

Table 2 indicated that *P. aeruginosa* had the highest prevalence of 44.5% while *Bacillus subtilis* and *S. aureus* had the same percentage distribution of 22.2%. *Streptococcus* spp had the least prevalence with 11.1% percentage distribution. The table also revealed that the bacteria species above were found to be predominantly associated with ready-to-eat fried fish hawked in Lapai metropolis, most especially *P.aeruginosa.*

Table 2: Incidence of Bacteria from Hawked Ready-to-Eat Fried fish Hawked in Lapai Metropolis

Bacteria	Colony	Percentage Distribution (%)
Bacillus subtilis	2	22.2
Pseudomonas aeruginosa	4	44.5
Staphylococcus aureus	2	22.2
Streptococcus spp	1	11.1
Total	**9**	**100.0**

Table 3 revealed that *S. aureus* was 100% resistant to all the antibiotics used. *B. subtilis* showed resistance to nearly all the antibiotics used except, Levofloxacin and Rifampicin where the isolates were susceptible by 50%. *Streptococcus* spp was 100% resistance to Ampiclox, Erythromycin, Chloramphenicol, Norfloxacin, Amoxil and Rifampicin showed 100% susceptibility to the remaining antibiotics. The isolates of *P. aeruginosa* were moderately susceptible to all the antibiotics, but resisted Ampiclox and Norfloxacin.

Table 3: Percentage Susceptibility of Bacteria to Antibiotics

Antibiotics	**Susceptibility (%)**			
	B. subtilis(n=2)	*P. aeruginosa* (n=4)	*S. aureus* (n=2)	*Streptococcus* Spp(n=1)
Ampiclox (20mcg)	0(100)	25(75)	0(100)	0(100)
Gentamycin (10mcg)	0(100)	50(50)	0(100)	100(0)
Levofloxacin (20mcg)	50(50)	50(50)	0(100)	100(0)
Erythromycin (30mcg)	0(100)	50(50)	0(100)	0(100)
Ciproflox (10mcg)	0(100)	50(50)	0(100)	100(0)
Chloramphenicol(30mcg)	0(100)	50(50)	0(100)	0(100)
Norfloxacin(10mcg)	0(100)	25(75)	0(100)	0(100)
Streptomycin (30mcg)	0(100)	50(50)	0(100)	100(0)
Amoxil (20mcg)	0(100)	50(50)	0(100)	0(100)
Rifampicin (20mcg)	50(50)	50(50)	0(100)	0(100)

NB: The values outside the parenthesis represent the percentage susceptibility while those inside the parenthesis represent the percentage resistance.

Discussion

From the study carried out, it was revealed that the mean bacterial count of ready-to-eat fried fish hawked across the two markets (that is, Main market and Badeggi market) in Lapai metropolis ranged from 7.0×10^4 to 5.0×10^6 cfu/mg. *P. aeruginosa*had the highest prevalence of 44.5%, while *Streptococcus*spp had the least prevalence of 11.1%. *B. subtilis*and *S. aureus*had the same prevalence rate of (22.2%).

The variation in bacterial load between Main market and Badeggi market could be due to sanitary condition of the location where the vendors sell their products.The presence of Bacteria in these foods could be due to contamination during food processing from infected food handlers as poor hygiene is a factor (Saper*et al*., 2005; Peariso, 2005). Also, the presence of *Streptococcus* spp,*B. subtilis* and *P. aeruginosa* is suggestive of the fact that these foods were contaminated by soil, water or dust due to the unwholesome practices of the vendors during post-frying and pre-frying processes. *Staphylococcus* spp are normal flora of human body and are found on the skin cavities, mucous membranes and also in the air. Based on these reports, it is not

surprising that the fish samples screened, tested positive for *S. aureus*. Handling and aerial microbial load could therefore be the major sources of contamination in hawked foods (Chukwu*et al*., 2013). The findings of Oluwafemi and Simi-saye (2005) and Okonko*et al.* (2009) support this study, since similar organisms were isolated. In a similar study carried out by Chukwu*et al*. (2013), *Bacillus* spp, *S. aureus*, *Pseudomonas* and *Streptococcus faecalis* were found to be associated with fried fish. The reports of Soliman and Shalby (2001) and Salim (2008) are also in agreement with this result, since *Escherichia coli* was not isolated. However, Ahmed and Anwar (2007) and Abd Allah (2010) reported that the fried fish samples were free from coagulase positive *Staphyloccus*spp which negates this study.High incidence of pathogenic bacteria in ready-to-eat food has been implicated in the studies of Chukwu*et al*. (2006), Chukwu*et al*. (2009) and Okonko*et al*., (2009).

However, the result of this study gives an overall picture of the level of contamination of fried fish hawked in Lapai metropolis and the sanitary condition of its preparation and handling. The result of this study suggests that these products are prepared and sold under condition that will permit the survival and propagation of various bacteria.

In the course of this study, antibiotics sensitivity test was carried out which revealed that *S. aureus* was 100% resistant to all the antibiotics (multi drug resistance). The resistance of most of the isolates to more than one antibiotics could be attributed to inappropriate practices such as misuse and abuse of antibiotics, self-medication, expired antibiotics e.t.c (Chikere*et al*., 2008; Prescott *et al.,* 2008). In a country such as Nigeria, self-medication is a common practice and that could probably be the major cause of antibiotics resistance. In the report of Achi*et al.* (2007),*S. aureus* was found to be multi drug resistance which is in agreement with this report. In another research carried out by Nkang*et al.* (2009), *S. aureus* was reported to be susceptible to Gentamicin, Erythromycin and Ciprofloxacin which negate the result of this study. Also, in the same research,*Streptococcus*spp was found to be predominantly resistance to erythromycin which supports this study. In a similar research carried out by Kirechi and Kareem (2014), *P. aeruginosa* was susceptible to Gentamicin and Ciprofloxacin which support this report. The findings of Balakrishman*et al*. (2003) negate this study, since *Bacillus* spp was reported to be susceptible to chloramphenicol.

However, infections involving multi-drug resistant bacteria are a major concern for hospitals and health care facilities, since they contribute to morbidity and mortality compared to the underlying diseases alone. They also impact length of stay and related healthcare cost (Laxminarayan and Melani, 2007).

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