IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 11 Ver. I (Nov. 2016), PP 116-124
www.iosrjournals.org

Antibiotic Susceptibility Patterns Of Bacterial Pathogens Isolated From Filled Baked Products Sold In Some Retail Outlets In Abakaliki Metropolis, Ebonyi State, Nigeria.

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Abstract: *This study was conducted to evaluate the antibiotic susceptibility patterns of pathogens isolated from filled baked products sold in some retail outlets in Abakaliki Metropolis. A total of 30 baked food samples were collected and they include; meat pie, egg roll and fish roll from five different selling points located at Abakpa Market, Azuiyiokwu, kpirikpiri, CAS and PRESCO Campus of Ebonyi State University Abakaliki, Nigeria. One gram each of the baked food sample was ground on a sterile mortar and was inoculated on nutrient agar by pour plate method. Suspected bacteria colonies were further characterized using biochemical testing method, Gram staining, culturing and other standard microbiology techniques. Antibiotic susceptibility pattern of isolated bacteria was determined using Disc Diffusion Method of Kirby and Bauer Method using the following antibiotics cefoxitin (30µg), ampicillin (10µg), cefuroxime (30µg), sulphamethoxazole/trimthroprim (30ug) and clindanycin (2µg). The results showed that Streptococcus and Staphylococcus species were hundred percent (100%) susceptible to sulphamethoxazole and clindamycin respectively, while Pseudomonas, E.coli and Streptococcus species were also hundred percent (100%), ninety percent (90%) and thirty-three percent (33%) susceptible to sulphamethoxazole. Klebsiella sand Salmonella species were completely resistance to all the antibiotics tested.*

Keywords: *Bacterial pathogens, Baked production, Abakaliki metropolis and Antibiotic.*

I. Introduction

Food preservation is a challenging issue where proteins such as meat, eggs and fish products are regarded generally as high risk food to infection and oxidation [1]. These food borne diseases and their consequent illness are some of the major global challenges that result to high mortality and economic shortage [2]. Food borne diseases are diseases resulting from consumption of bacteria, toxins as well as cells produced by microorganisms present in food [3]. The intensity of the signs and symptoms some times vary with the amount of contaminated food consumed and susceptibility of the persons to the toxins. Hygienic conditions are poor especially when foods are not processed industrially, mainly due to lack of monitoring during food processing. These contaminated foods will go along way infecting children, immune-suppressed persons and the elderly who are highly susceptible [4].

Meat pies, egg rolls, fish rolls are mainly used in the production of filled baked foods which are ready-to-eat foods. In all these ready-to-eat food products, the bakery products offer convenience and balanced diet at the same time for the busy and health conscious customers [5]. Microorganisms play important role in the flavoring of baked products, but microbes also play major role in the spoilage of the baked products due to inadequate preparatory steps (cooling, slicing and transport) [6].

In addition to bacteria, other microbes like molds also play major role in the spoilage of the baked products. The fresh bakery products are sterile but soon become contaminated when it comes in contact with the air and other environmental materials. Improper handling also introduces contamination as bakery workers on their own are major source of contaminants [7]. Foods are served after passing through a long chain of steps involved in production, processing, distribution and marketing. Man stands as the ultimate consumer at the head of many food chains and because of biomagnifications; they may be exposed to diverse harmful agents in increasing concentrations [8].

Contamination can come from raw materials and equipment, additional processing and unhygienic conditions contribute substantially to the entry of bacterial pathogens [9].

Lack of awareness about food safety and hygiene among vendors also result in food contamination [10] . Street food vendors are usually poorly educated, untrained, unlicensed, in food hygiene and they carry out their work in an uncondusive manner, also in unclean environment with little or no understanding of the effect it will have in food poisoning or any other food borne illness [11].

Some of the foods are not covered well to prevent flies, which may carry food borne diseases. Normal temperatures for food safety are not always applied to foods on the street. Pathogens implicated in the contamination of foods that are health risks include; *Salmonella typhi*, *Pseudomonas* species, *E. coli* and *Proteus* or *Staphylococcus* species during processing: preparation and other handling stages [12].

Filled baked products contain meat, fish, egg and other savory ingredients. In facts, filled baked foods are delicate foods with a short shelf-life after production and so need special attention and care.

These products can get spoilt easily, may be as a result of its fillings, which include; meat, fish, egg as well as other ingredients. Because of the delicious nature of meat pie, egg roll and fish roll, microorganisms do thrive easily in the products [13].

Baked foods are preferred eaten warm and to keep it at steady warm temperature, electricity with high voltage bulb is often used, and the tendencies of spoiling these products by thermophilic bacteria like *Clostridium perfrigens* are not rare. Since the bacterium can thrive very well when not in competition with other microorganisms and under aerobic conditions, temperatures of $55^{O}C$ can support the growth of this microorganism [2]. Filled baked products have chances of contamination by microbes due to several unhygienic conditions, environmental factors and source of raw materials.

Aim And Objectives

Aim: The aim of this research work is to evaluate the microbial contamination of unbranded filled baked products sold within Abakaliki Metropolis.

Specific Objectives

- To isolate, characterized and determine the bacterial load on the filled baked products at different time of the day (morning and late afternoon).
- To determine the percentage frequency of occurrence of bacterial pathogens.
- To determine the antibiotics susceptibility patterns of isolated bacterial pathogens

II. Materials And Methods

Materials

The materials used includes the following: disposable Petri-dishes, beaker, glass test tubes, measuring cylinder, forceps, glass slides, conical flasks, spatula, meter rule, pasture pipettes, swab stick, Bijou bottles, wire loops, masking tapes, wooden stick, aluminum foil, markers and cotton wool.

Media

Media used for this work includes:-

Nutrient agar (Oxoid, UK) Manitol salt agar (Oxoid CM 085 UK), *Salmonella-shigella* agar, MacConkey agar (McA) (Antex, UK), peptone water (CM 0087) and Mueller Hinton agar (Oxiod, UK). All culture media used were prepared in line with the manufacturer's instructions.

Methods

Area of Study

Samples were collected from five different locations within Abakaliki metropolis, Ebonyi State, Nigeria. Abakaliki is located between latitude $6^{0}2'0N$ and longitude $8^{0}06'E$ the pattern of rainfal is biomodal (April-July). September November with a short spell some times in August. The annual rainfall is between 1000 mm-1500 mm. the vegetation of the area is predominantly derived Savannah. The mean annual temperature is $24^{0}C$ and the relative humidity is between 60-80 % [14]. Abakaliki has a population of about one hundred and thirteen thousand one hundred and thirty (113,130) people [15].

The major occupations of people in Abakaliki are farming and trading, there are also civil servants and students and all these people engage in a busy activity of life.

Sample Collections

A number of 30 samples including; meat pie, egg roll and fish roll were aseptically collected using sterilized polythene bags, 10 samples each from five point locations at different times of the day-morning and late afternoon from different retail outlets in Abakaliki metropolis.

The samples collected were carefully labeled, each of the 5 samples in the morning and late afternoon, immediately conveyed to the Applied Microbiology Laboratory unit, Ebonyi State University, Abakaliki in sterile polythene bags and they were analysed within 1 hour according to standard microbiological procedures.

Sample Analyses

Each baked food products was macerated using a sterile marble mortar [16]. One gram (Ig) of each sample was measured using electronic weighing balance and suspended into 9ml of phosphate buffered saline (PH-8.9). The samples were serially diluted in the phosphate buffered saline (PH-8.9). One ml from the dilutions was plated on nutrient agar and MacConkey agar respectively by pour plate technique. The plates were incubated at 37^0C for 24 hours. The colony count was performed with conventional plate count method [17]. Suspected growths of *E. coli* and *Klebseilla* spp were sub-cultured on Eosin methylene blue (EMB) agar for exact identification and differentiation respectively [16].

In the detection of the presence of *Staphylococcus aureus* in the samples, suspected colonies of *Staphylococcus aureus* was sub-cultured on mannitol salt agar while *Salmonella-Shigella* agar was used for isolation of *Salmonella* spp. Every suspected growths of *E. coli*, *Klebsiella*, *Salmonella*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* species were sub- cultured on nutrient agar slants from which Gram staining and biochemical tests were carried out.

Identification and Characterization of Isolates

Gram staining

Gram staining was carried out on smears prepared from all samples in different culture plates. The smear preparation was made on a clean grease-free glass slide. It was air dried and heat fixed by gentle heating on a bursen burner. The heat fixed smears were flooded with crystal violet stain for 30 secs and washed off under a slow running, tap. The smears were covered with lugols' iodine for 60 sec and washed off with a slow running tap. They were decolourized rapidly using acetone-alcohol and washed off within some seconds with clean water. The smears were covered with safranine for 30 secs and washed off with slow running tap. The smears were blotted dry and arranged on a slide racks. The slides were examined using x100 objective lens of microscope with oil immersion and the observation was recorded [18].

Biochemical Tests

Coagulase Test

A drop of sterile water was added on two separate glass slides and a growth of the test bacteria was used to emulsify in each of the drops to obtain a suspension that is thick. Loopful of sheep blood plasma was placed on one of the suspension and observed for clumping within 10 seconds [18].

Oxidase Test

Piece of filter paper was placed on a clean glass Petri-dish and 2-3 drops of newly prepared oxidase reagent was added. A piece of stick was used to collect bacteria growth and smear was made on the filter paper. It was allowed to stand and observed for appearance of a blue-purple color after a few seconds.

Principle of Oxidase Test

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organisms is then smeared on the filter paper. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour [18].

Indole Test

The test organisms were inoculated in Bijour bottle containing 5ml of sterile peptone water. It was allowed in the incubator at 37^0C for 72 hours and 0.5ml of Kovac's reagent was added, shaken gently and observed for change of colour to red within 10 mins.

Principle of Indole Test

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by kova's or Ehrlich's reagent which contains 4(P)- dimethylaminobenzaldehyde. This reacts with the indole to produce a red coloured compound [18].

Catalase Test

Several colonies of the test organism were picked with a sterile wire loop and emulsified in a drop of normal saline on a slide. A drop of 3 % hydrogen peroxide was added and observed for immediate air bubbles.

Principle of Catalase Test

Catalase acts a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24hrs old [18].

Preparation of 0.5 McFarland Turbidity Equivalent Standards

Turbidity standard equivalent of 0.5 McFarland standards was prepared by adding 1.0 ml of concentrated tetraoxosulphate (vi) acid (H_2S0_4) to 99ml of distilled water. Then 0.5g of dehydrated barium chloride ($BaCl_2\,H_2O$) was dissolved in 50ml of distilled water in a separate flask.

A volume of 0.6ml of barium chloride solution was introduced into tetraoxosulphate (vi) acid solution (99.4ml) in a separate test tube. It was mixed well to obtain 0.5 McFarland turbidity equivalent standards. Some portions of the mixed solutions were transferred to test tube and stored at room temperature (28^0C). This was used to compare the turbidity of the test organisms [18].

Standardization of Test Bacteria

All the test bacteria were standardized before use by inoculating 5 ml normal saline in sterile test tubes with loopful of a 24hrs culture of the test organism from a nutrient agar slant. Then dilutions using loopful of the test organism and sterile water were carried out in order to obtain microbial population of 10^5 colony forming, unit per milliliter (cfu/ml) by comparing it with 0.5 McFarland turbidity standards [19].

Antibiotic Susceptibility Pattern

The susceptibility and resistance patterns of the isolates were determined by the Kirby-Bauer susceptibility test method as recommended by the Clinical and Laboratory Standard Institute [20]. The following antibiotics were used for this study; cefoxitin (30µg), Ampicillin (10µg), cefuroxime 30µg), sulphamethoxazole/ thrimthroprim (30µg) and clindanycin (2µg).

Mueller Hinton agar was prepared according to manufacturer's instructions and 0.5 McFarland equivalent standard of the test organisms was inoculated on the surface of the agar using a sterile cotton swab sticks. Test antibiotics listed above were aseptically placed on the inoculated agar plates and incubated at 37^0C for 18-24hrs. Inhibition zone diameters were measured and the organisms were identified as either susceptible or resistance based on Clinical and Laboratory Standard Institute Standard [20].

III. Results

Table 1: Distribution of Bacteria loads on filled Baked Food Samples from the five different locations both in the morning and late afternoon period.

	Morning				Afternoon			
	Samples	**No of Colonies**	**Location**	**(cfu) m/l**	**Samples**	**No of colonies**	**Location**	**(cfu/m/l)**
1	Meat Pie	15	Presco	$1.5x10^7$	Meat Pie	45	Presco	$4.5x10^7$
	Egg Roll	12	Presco	$1.2x10^7$	Egg Roll	55	Presco	$5.5x10^7$
	Fish Roll	20	Presco	$2.0x10^7$	Fish Roll	92	Presco	$9.2x10^7$
2	Meat Pie	12	CAS	$1.2x10^7$	Meat Pie	60	CAS	$6.0x10^5$
	Egg Roll	20	CAS	$2.0x10^7$	Egg Roll	52	CAS	$5.2x10^7$
	Fish Roll	20	CAS	$2.0x10^7$	Fish Roll	70	CAS	$7.4x10^5$
3	Meat Pie	15	Azuiyiokwu	$1.5x10^7$	Meat Pie	102	Azuiyiokwu	$10.2x10^8$
	Egg Roll	17	Azuiyiokwu	$1.7x10^7$	Egg Roll	108	Azuiyiokwu	$10.8x10^8$
	Fish Roll	20	Azuiyiokwu	$2.0x10^7$	Fish Roll	85	Azuiyiokwu	$8.5x10^7$
4	Meat Pie	20	Kpirikpiri	$2.0x10^7$	Meat Pie	68	Kpirikpiri	$6.8x10^7$
	Egg Roll	22	Kpirikpiri	$2.2x10^7$	Egg Roll	85	Kpirikpiri	$8.5x10^7$
	Fish Roll	18	Kpirikpiri	$1.8x10^7$	Fish Roll	64	Kpirikpiri	$6.4x10^7$
5	Meat Pie	12	Abakpa	$1.2x10^7$	Meat Pie	110	Abakpa	$11.0x10^5$
	Egg Roll	15	Abakpa	$1.5x10^7$	Egg Roll	96	Abakpa	$9.6x10^7$
	Fish Roll	20	Abakpa	$2.0x10^5$	Fish Roll	127	Abakpa	$12.7x10^8$

Key:

Cfu = colony forming units per millimeter, CAS = College of Agricultural Science.

Table 2: Shows the morphology and biochemical Characterization of the bacteria isolates using Gram- staining, catalase test, oxidase test, coagulase test and indole test.

	Morphological characterization		**Biochemical characterization**					
	Colour	**Consistency/texture**	**Gram staining**	**Catalase test**	**Oxidase test**	**Coagulase test**	**Indole test**	**Suspected bacteria**
1	Black	Small rough surface	-	-	-	+	+	*Salmonella* spp
2	Pink	Little smooth surface	-	-	-	+	+	*E. coli*
3	Creamy/white	Raised/smooth edge	+	+	+	-	-	*Staphylococcus* spp
4	Green	Oily surface	-	-	+	+	+	*Pseudomonas* spp
5	Light yellow	Mucoid	-	-	-	+	-	*Klebsiella* spp

6	Creamy	Rough surface	+	+	+	-	-	*Streptococcus* spp

Key: + = Positive, - = Negative

Table 3: Shows the Percentage Distribution of Bacteria isolated from all the Baked Food Samples Based on Period (Morning or Afternoon).

Organisms	Morning (%)	Organisms	Afternoon (%)
Pseudomonas spp	3 (21 %)	*Pseudomonas* spp	8 (24 %)
E. coli	2 (14 %)	*E. coli*	3 (9 %)
Klebseilla spp	2 (14 %)	*Klebseilla* spp	2 (6 %)
Staphylococcus spp	3 (21 %)	*Staphylococcus* spp	6 (18 %)
Streptococcus spp	2 (14 %)	*Streptococcus* spp	7 (21 %)
Salmonella spp	2 (14 %)	*Salmonella* spp	7 (21 %)
Total	14		33

Table 4: Antibiotics Susceptibility Patterns of Bacteria isolated from Baked Food Samples from five different locations in Abakaliki Metropolis

S/No	Isolates	Antibiotics used				
			FOX	CXM	SXT	AMP
1	*Pseudomonas* spp	Meat pie	S	R	S	R
2	*Pseudomonas* spp	Meat pie	S	R	S	R
3	*Pseudomonas* spp	Meat pie	S	R	S	R
4	*Pseudomonas* spp	Meat pie	R	R	S	R
5	*E. coli* spp	Meat pie	R	R	S	S
6	*E.coli* spp	Meat pie	S	S	R	S
7	*Salmonella* spp	Meat pie	R	R	R	R
8	*Salmonella* spp	Meat pie	S	R	S	S
9	*Salmonella* spp	Meat pie	S	R	S	R
10	*Staphylococcus* spp	Meat pie	R	R	S	R
11	*Staphylococcus* spp	Meat pie	S	R	S	R
12	*Staphylococcus* spp	Meat pie	S	R	S	S
13	*E. coli spp*	Meat pie	R	R	S	R
14	*Streptococcus* spp	Meat pie	R	R	R	R
15	*Streptococcus* spp	Meat pie	S	R	S	R
16	*Streptococcus* spp	Meat pie	S	R	S	R
17	*Pseudomonas* spp	Fish roll	S	R	S	R
18	*Pseudomonas* spp	Fish roll	R	R	S	R
19	*Pseudomonas* spp	Fish roll	R	R	S	R
20	*E. coli* spp	Fish roll	S	R	S	R
21	*E. coli* spp	Fish roll	R	R	S	R
22	*Salmonella* spp	Fish roll	R	R	R	R
23	*Salmonella* spp	Fish roll	R	R	S	R
24	*Salmonella* spp	Fish roll	S	R	S	S
25	*Staphylococcus* spp	Fish roll	S	R	S	S
26	*Staphylococcus* spp	Fish roll	R	R	S	S
27	*Staphylococcus* spp	Fish roll	S	R	S	R
28	*Klebsiella* spp	Fish roll	R	R	R	R
29	*Streptococcus* spp	Fish roll	R	R	S	R
30	*Streptococcus* spp	Fish roll	R	R	S	R
31	*Streptococcus* spp	Fish roll	S	R	S	R
32	*Pseudomonas* spp	Egg roll	S	R	S	R
33	*Pseudomonas* spp	Egg roll	S	R	S	R
34	*Pseudomonas* spp	Egg roll	S	R	S	R
35	*Pseudomonas* spp	Egg roll	S	R	S	R
36	*Salmonella* spp	Egg roll	R	R	R	R
37	*Salmonella* spp	Egg roll	R	R	R	R
38	*Salmonella* spp	Egg roll	S	R	R	S
39	*Salmonella* spp	Egg roll	R	R	S	R
40	*Staphylococcus* spp	Egg roll	S	R	S	S
41	*Staphylococcus* spp	Egg roll	S	R	S	R
42	*Staphylococcus* spp	Egg roll	S	R	S	R
43	*Klebsiella* spp	Egg roll	R	R	R	R
44	*Klebsiella* spp	Egg roll	R	R	R	R
45	*Klebsiella* spp	Egg roll	R	R	R	R
46	*Streptococcus* spp	Egg roll	R	R	R	R
47	*Streptococcus* spp	Egg roll	S	R	S	R

Key:

Fox = Cefoxitin, CXM = Cefuroxime, SXT = Sulphamethoxazole, AMP = Ampicillin, S = Susceptibility, R = Resistance

Table 5: Susceptibility Patterns of *Pseudomonas* spp Isolated from Filled Baked Food to Antibiotics

***Pseudomonas* spp**				
	FOX	**CXM**	**SXT**	**AMP**
1	R	R	S	R
2	R	R	S	R
3	R	R	S	R
4	R	R	S	R
5	R	R	S	R
6	R	R	S	R
7	R	R	S	R
8	R	R	S	R
9	R	R	S	R
10	R	R	S	R
11	R	R	S	R

Key:
Fox = Cefoxitin**,** CXM = Cefuroxime SXT = Sulphamethoxazol AMP = Ampicillin R = Resistance, S = Susceptible.

Table 6: Susceptibility Patterns of *E. coli* Isolated from Filled Baked Food to Antibiotics

E. coli				
1	**FOX**	**CXM**	**SXT**	**AMP**
2	R	R	S	R
3	R	R	S	R
4	R	R	S	R
5	R	R	S	R
6	R	R	S	R

Key:
Fox = Cefoxitin**,** CXM = Cefuroxime SXT = Sulphamethoxazol AMP = Ampicillin R = Resistance, S = Susceptible.

Table 7: Susceptibility Patterns of *Salmonella* spp Isolated from Filled Baked Food to Antibiotics

***Salmonella* spp**				
	FOX	**CXM**	**SXT**	**AMP**
1	R	R	R	R
2	R	R	R	R
3	R	R	R	R
4	R	R	R	R
5	R	R	R	R
6	R	R	R	R
7	R	R	R	R
8	R	R	R	R
9	R	R	R	R

Key:
Fox = Cefoxitin**,** CXM = Cefuroxime SXT = Sulphamethoxazol AMP = Ampicillin R = Resistance, S = Susceptible.

Table 8: Susceptibility Patterns of *Staphylococcus* spp Isolated from Filled Baked Food to Antibiotics

***Staphylococcus* spp**					
	FOX	**DA**	**CXM**	**SXT**	**AMP**
1	R	R	R	R	R
2	R	R	R	R	R
3	R	R	R	R	R
4	R	R	R	R	R
5	R	R	R	S	R
6	R	R	R	S	R
7	R	R	R	S	R
8	R	R	R	R	R
9	R	R	R	R	R

Key:
Fox = Cefoxitin**,** DA = Clindamycin, CXM = Cefuroxime SXT = Sulphamethoxazol AMP = Ampicillin R = Resistance, S = Susceptible

Table 9: Susceptibility Patterns of *Klebsiella* spp Isolated from Filled Baked Food to Antibiotics

***Klebsiella* spp**				
	FOX	**CXM**	**SXT**	**AMP**
1	R	R	R	R

2	R	R	R	R
3	R	R	R	R
4	R	R	R	R

Key:
Fox = Cefoxitin**,** CXM = Cefuroxime SXT = Sulphamethoxazol AMP = Ampicillin R = Resistance, S = Susceptible.

Table 10: Susceptibility Patterns of *Streptococcus* spp Isolated from Filled Baked Food to Antibiotics

***Streptococcus* spp**					
	FOX	**DA**	**CXM**	**SXT**	**AMP**
1	R	S	R	R	R
2	R	S	R	R	R
3	R	S	R	R	R
4	R	S	R	R	R
5	R	S	R	S	R
6	R	S	R	S	R
7	R	S	R	S	R
8	R	S	R	R	R
9	R	S	R	R	R

Key:
Fox = Cefoxitin**,** DA = Clindamycin, CXM = Cefuroxime SXT = Sulphamethoxazol AMP = Ampicillin R = Resistance, S = Susceptible.

Table 11: Percentage (%) Susceptibility of Antibiotics tested on all the bacteria isolated from the filled baked food products.

Organisms	**Antibiotics (%)**				
	SXT	**CXM**	**FOX**	**DA**	**AMP**
Pseudomonas spp	90	0	0	0	0
E. coli	100	0	0	0	0
Klebsiella spp	0	0	0	0	0
Salmonella spp	0	0	0	0	0
Staphylococcus spp	100	0	0	0	0
Streptococcus spp	33	0	0	100	0

Key:
Fox = Cefoxitin, DA = Clindamycin, CXM = Cefuroxime, SXT = Sulphamethoxazole, AMP = Ampicillin.

IV. Discussion

The distribution of bacteria pathogen on the filled baked food samples from different locations are shown in Table 1. The result revealed that bacteria growth load was high in samples collected from Abakpa and Azuiyokwu while that of Kpirikpiri, PRESCO and CAS Campus of Ebonyi State University, Abakaliki was low. It was also observed that the growth of bacteria increases with time. Hence, the number of colonies recorded in the afternoon period was higher than that of morning period.

The morphology and biochemical characteristics of bacteria isolated from the filled baked food products are shown in Table 2. The result revealed that six bacterial isolates were isolated and they include: *Salmonella* species, *Escherichia coli,Staphylococcus species Pseudomonas species, Klebsiella* species *and Streptococcus* species. Table 3. Shows percentage (%) distributions of bacteria isolated from filled baked food samples based on period (morning and Afternoon).

The result revealed that *Pseudomonas* spp has the highest occurrence with the total of eleven isolates representing (45%) of the total bacteria isolated, followed by *Staphylococcus, Salmonella* and *Streptococcus* spp that has a total of nine isolates each while *Escherichia coli* and *Klebsiella* spp has a total of five and four bacteria isolates respectively. The number of all bacteria isolated in the morning was fourteen while the bacteria isolated in late-afternoon were thirty three. This shows that the bacteria load increases as long as the baked food samples last in the shop.

The antibiotics susceptibility patterns of bacteria isolated from filled baked food samples are shown in Table 4. The result of the antibiotics susceptibility patterns revealed that *Escherichia coli, Salmonella, Staphylococcus* and *Streptococcus* spp was susceptible to four out of five antibiotics used. These include; cefoxitin (FOX), sulphamethoxazole (SXT), Ampicillin (AMP) and clindamycin (DA). While all the bacteria isolated were resistant to cefuroxime (cxm). It was also observed that sulphamethoxazole inhibited all the bacteria isolated, followed by cefoxitin, which inhibited all bacteria isolated except *Klebsiella* spp. Ampicillin inhibited *Escherichia coli, Salmonella* and *Staphylococcus* spp while clindamycin inhibited *Streptococcus* spp and *Staphylococcus* spp respectively. Table 5; shows the result of antimicrobial susceptibility studies of the bacteria isolated from meat pie, egg roll and fish roll. It revealed that *Klebsiella and Salmonella* spp were resistant to all the antibiotics tested, *Escherichia coli was* susceptible to sulphamethoxazole, while

Streptococcus and *Staphylococcus* spp were susceptible to sulphamethoxazole and clindamycin respectively. *Pseudomonas* and *Streptococcus* spp were susceptible to sulphamethoxazole only. This result conformed to the work done by [16], who reported that presence of *E. coli* in food impose a serious health problems.

The sample from Azuiyiokwu and Abakpa Area within Abakaliki metropolis recorded the highest bacteria loads both in the morning and late- afternoon while the samples from Kpirikpiri, PRESCO and CAS Campus of EBSU, Abakaliki has comparatively low bacteria load. This might be attributed to the cleanliness of the environment and since the activities of people out there is such that does not generate environmental pollution.

The Local Government Health Inspection Officers together with the State Ministry of Health and other regulatory bodies should be proactive in the check of foods which are not processed in the industry routinely. Those individuals' bakeries or the food handlers should be given adequate training as it concerns food borne diseases like diarrhea or fever as well as good personal hygiene and environmental cleanliness. If such practices are strictly followed it will go a long way to ensure that quality foods and food products are sold to the general public.

V. Conclusion

The present study revealed the presence of bacteria pathogens in filled baked products. However, the presence of *E. coli, Salmonella, Klebsiella, Pseudomonas, Streptococcus* and *Staphylococcus* species in meat pie, egg roll and fish roll indicated an alarming situation for the consumers within this area. *Salmonella* in food may cause severe enteric problems like diarrhea, dysentery, abdominal pain and vomiting.

The bakery food handlers have to improve on the hygienic status of production process beginning from raw materials to final products finishing. However, the regulatory agencies, like State Ministry of Health, Local Government Health Inspection officers should design a frame work to monitor and evaluate the activities of bakery foods that are not industrially processed from time to time in order to reduce bacterial contamination in food to the barest minimum, thereby curbing the risk of disease outbreak in Abakaliki Ebonyi State Nigeria.

Lack of good sanitary environment, portable water and raw materials used in the mixing of the flour may have contributed to the high frequency of pathogenic bacteria in samples analysed.

Studies has shown that multidrug resistance *E. coli* strains from food samples were significantly higher than those from clinical samples and this has been attributed to the feacal source of the pathogenic organisms [21]. The frequency of bacteria from the baked food products and their high resistance to the antibiotics tested in this present work is a thing of serious concern to the general public.

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