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Dangers of bacterial contamination: An overview of the Antibiogram of bacterial species from ready to eat abacha (African salad) sold in Abakaliki, metropolis, Ebonyi state Nigeria

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Abstract

Four different locations where ready-to-eat abacha is sold within Abakaliki were randomly selected for the purposes of sample collection. Three samples of ready-to-eat abacha were collected each from the following locations: Presco market, meat market, kpirikpiri market and Spera-in-deo. *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The Total Aerobic Bacterial Counts of Abacha Sold at Different Locations within Abakaliki was determined. The counts range from 1.8 x 10^6 (the highest) and 1.0x10^6 (the lowest) count. The highest bacterial count was obtained from Meat market while the lowest bacterial count was obtained from Spera-in-deo market. The bacterial isolates with their respective percentage occurrence frequencies include; *Escherichia coli* (53.33%), *Staphylococcus aureus* (22. 67%), *Klebsiella pneumoniae* (14%) and *Shigella* spp (10%). *Escheriachia coli* was the most predominant isolate followed by *Staphylococcus aureus* while the least term of occurrence was *Shigella spp* in abacha sold within Abakaliki. The Results of Antibiotics Susceptibility Pattern of Bacterial Isolates on Some Tested Antibiotics showed that the bacterial isolated from ready–to- eat abacha had high resistance to the antibiotics tested especially *Escherichia coli*. The result obtained from the total percentage antibiotics susceptibility pattern of bacterial isolate from ready to eat abacha indicate that all the organisms were susceptible to gentamycin whereas 50% of the organisms were susceptible to ciprofloxacin. The Result of Multiple Drug Resistance Value of Each Organism Isolated from Ready to Eat Abacha Sold within Abakaliki showed that the values obtained from *Escherichia coli* (0.71) indicates less resistance to the antibiotics tested while *Klebsiella pneumonia* (0.39) had high resistance to the tested antibiotics.

Keywords: Bacterial isolates; Bacterial count; Antibiotics susceptibility; Resistance; Bacterial occurrence; Antibiotics

1. Introduction

Food borne diseases are an important cause of morbidity and high mortality worldwide in known that food borne illness are caused by bacteria viruses parasites and chemicals (Abdullahi and yakubu, 2013; Adebayo,2010;Bamidele etal.,2010). Some food borne bacterial infection causes self limiting diarrhea though sometimes systemic infection can occur especially in vulnerable individuals (elderly people, immune compromised people, infant and young children (Adeneye,1991; Ajmair and Akhtar,2012; Amao et al.,2006; Emodi and Madukwe,2011). It is reported that bacteria alone accounts for 70% deaths associated with food borne illness Bacteria food poisoning is the most common type of food poisoning and it is caused as a result of the presence of harmful bacteria or poisonous substances produced by the organism. Pathogenic bacteria find their way with food in different ways such as unhygienic and inappropriate handling and processing practices by humans (Adams and Moss, 1999) pathogens can be carried by humans and passed on to others by individuals who are carriers of these pathogen such carriers may have recently suffered an attack of food poisoning and still labor the organism in their body. In most cases food that has high risk of being infected by these organisms are food intended to be eaten raw or light cooking example vegetables fruits, salads, fruit drink, etc. Most

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locally prepared food if not handled well has been reported to harbor bacteria pathogens (Ganguly, 1995; Gbadamosi, 2007; He and mukherjee, 2007; Hemmington and king,2000). The growing resistance of pathogens isolated from locally prepared raw food samples and ready to eat food is a public health concern in both develop and developing countries. Bacteria resistance has caused lots of setback in treatment of infections in the health care system limiting therapeutic options.

2. Methodology

2.1. Study Area

The study area is Abakaliki town in Ebonyi State located in south eastern part of Nigeria. It is located 64 kilometers southeast of Enugu. Abakaliki is situated on latitude $6^0$20'N and longitude $8^0$06E (NPC, 2010).

2.2. Sample Collection

Four different locations where ready-to-eat abacha is sold within Abakaliki were randomly selected for the purposes of sample collection. Three samples of ready-to-eat abacha were collected each from the following locations: Presco market, meat market, kpirikpiri market and Spera-in-deo. The samples were transported to the microbiology laboratory of EBSU, Abakaliki in transport media where they were analyzed following standard techniques in Microbiology.

2.3. Analysis of Samples

Samples gotten from each location were used for the test to represent the four locations of sample collection. Each of the samples obtained from the four points were macerated using a sterile mortar. Each of the samples macerated was dissolved with di methyl sulfoxide (DMSO) which was used as a diluent to dissolve the oil content in the abacha samples. Five gram (5g) each of the samples was dissolved into 25ml of distilled water contained in a test tube labeled according to the locations of collection. A tenfold serial dilution was carried out on each of the labeled samples. Exactly 0.5ml of the suspension was collected each from 10^{-1} to 10^{-5} serial dilution of the different samples were inoculated into a nutrient agar plate using pour plate method. They were all incubated for 18 to 24 hours at 37 ℃.

2.4. Determination of Aerobic Plate Count

Standard plate count method proposed by Cheesebrough (2002) was used to determine the total aerobic colony count of the samples. Only plates with moderate growth were counted. The average microbial loads of the samples obtained from the different location were expressed as colony forming units per Milliliter (Cfu/ml) of abacha.

2.5. Isolation and Identification of Bacteria Isolated from Abacha Samples.

Mac Conkey agar, Eosin methylene blue agar, mannitol salt and salmonella – shigella agar were employed for the isolation of bacteria for the purpose of identification. Mac Conkey agar was used to isolate lactose fermenting gram negative bacteria, Eosin methylene blue agar was used for the selective isolation of enteric coliforms, mannitol salt agar was used for the selective isolation of salt-tolerant bacteria and salmonella –shigella agar was used for the isolation of enteric bacilli particularly *Salmonella and Shigella* species. All plates were incubated at 37 ℃ for 24 hours. Identification of bacteria isolates was based on the standard culture, morphological and biochemical methods (Cheesbrough, 2002).

2.6. Antibiotic Susceptibility Test.

The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disk diffusion method (Cheesbrough, 2002). A suspension of each isolate was prepared in peptone water to match 0.5 Mcfarland turbidity standards in order to standardize the inoculums. The standardized inoculums of each isolate were inoculated on to the surface of plain Mueller- Hinton agar plates. The tested antibiotics include; Erythromycin (15 μg), Ciprofloxacin (5 μg), Ampicilin (10 μ g), Clindamycin (2 μ g), Gentamycin (30 μ g), Sulfamethoxazole (25 μ g), Tetracycline (30 μ g), Nitrofuratin (200μg), Vancomycin (30μg) Cefoxitin (30μg) discs were placed and incubated at 37 ℃ for24 hours. The zones of inhibition were measured with meter rule and compared with the clinical and laboratory standards institute (CLSI) guidelines (Kim etal.,2011).

3. Result

3.1. The Biochemical Characteristics of the Bacterial Isolates

Table 1 Morphological and biochemical characteristics of bacterial isolates from ready to eat abacha sold within abakaliki

S/N	Sample Code	Gram Reaction	Shape	Catalas	Citrate	Idole	Oxidase	Coagulase	Methylene Red	vp	Suspected Organism
1	S.S Meat Market	-	Rod	+	-	+	-		+	-	*Shigella sp*
2	EMB	-	Rod	+	-	+	-		+	-	*E.coli*
3	Macconkey Meat Market	-	Rod	+	+	-	-		-	+	*Klebsiella*
4	Mannitol salt meat market	-	Cocci	+	+	-	-		+	+	*pneumonia*
5	Macconkey kpirikpiri	-	Rod	+	-	+	-		+	-	*E.coli*
6	EMB kpirikpiri	-	Rod	+	+	-	-		-	+	*Klebsiella*
7	Mannitol kpirikpiri salt	+	Cocci	+	+	-	-		+	+	*Staphylococcus aureus*
8	S.S kpirikpiri	-	Rod	+	-	+	-		+	-	*Shigella sp*
9	Mannitol salt presco	+	Cocci	+	-	+	-		+	+	*Staphylococcus aureus*
10	EMB presco	-	Rod	+	+	-	-		-	+	*Klebsiella pneumonia*
11	Macconkey presco	-	Rod	+	-	+	-		+	-	*Escherichia coli*
12	S.S Presco	-	Rod	+	-	+	-		+	-	*Shigella sp*
13	Mannitol salt spera-in-deo	+	Cocci	+	+	-	-		+	+	*Staphylococcus aureus*
14	Macconkey spera-in-deo	-	Rod	+	-	+	-		+	_	*Escherichia coli*
15	S.S Spera-in										
	deo	_	Rod	+	-	+	-	-	+	-	*Shigella sp*

Four bacteria isolates were identified namely: *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Coagulase test was carried out to identify *Staphylococcus aureus*, a gram positive organism while others are gram negative rods as shown in Table 1 above.

3.1.1. The Total Aerobic Bacterial Counts of Abacha Sold at Different Locations within Abakaliki.

After the preliminary analysis, 10^{-4} was found to have definite colonies thus; it was used as the dilution factor. The counts range from 1.8×10^6 (the highest) and 1.0×10^6 (the lowest) count. The highest bacterial count was obtained from Meat market while the lowest bacterial count was obtained from Spera-in-deo market as shown in Table 2 below.

3.1.2. The percentage frequency of Bacterial Species Isolated from the Samples of Abacha Sold at Different Locations within Abakaliki.

The bacterial isolates with their respective percentage occurrence frequencies include; *Escherichia coli* (53.33%), *Staphylococcus aureus* (22. 67%), *Klebsiella pneumoniae* (14%) and *Shigella* spp (10%). *Escheriachia coli* was the most predominant isolate followed by *Staphylococcus aureus* while the least term of occurrence was *Shigella spp* in abacha sold within Abakaliki as shown in table 4 below.

3.1.3. ***The Results of Antibiotics Susceptibility Pattern of Bacterial Isolates on Some Tested Antibiotics.***

The bacterial isolated from ready–to- eat abacha had high resistance to the antibiotics tested especially *Escherichia coli* on the antibiotics tested as shown in Table 4 below.

3.1.4. Total Percentage Antibiotics Susceptibility Pattern of Bacterial Isolate from Ready to Eat Abacha Sold within Abakaliki.

The result obtained from the total percentage antibiotics susceptibility pattern of bacterial isolate from ready to eat abacha indicate that all the organisms were susceptible to gentamycin whereas 50% of the organisms were susceptible to ciprofloxacin as shown inTtable 5 below.

3.1.5. The Result of Multiple Drug Resistance Value of Each Organism Isolated from Ready to Eat Abacha Sold within Abakaliki.

The values obtained indicate that *Escherichia coli* (0.71) had less resistance to the antibiotics tested while *Klebsiella pneumonia* (0.39) had high resistance to the tested antibiotics as shown in table 6 below.

Table 2 Total bacterial counts (cfu/ml) of ready-to-eat abacha sold in different locations within abakaliki

S/N	SAMPLE CODE	NUMBER OF COLONY	CFU/ML	DILUTION FACTORS
1	Kprikpri Market	60	1.2×10^6	10^{-4}
2	Presco Market	70	1.4×10^6	10^{-4}
3	Spera-in-deo	50	1.0×10^6	10^{-4}
4	Meat Market	90	1.8×10^6	10^{-4}

Table 3 Percentage frequency of bacterial isolate from abacha sold in different locations

Bacterial isolates	Locations				Total	Occurrence
	K	M	P	S		FREQUENCY (%)
Escherichia coli	25	30	15	10	80	53.33
Staphylococcus aureus	11	9	9	5	34	22.67
Klebsiella pneumonia	7	3	5	6	21	14
Shigella sp	5	3	5	2	15	10

Key: K=kpirikpiri Market; M=Meat Market; P= presco Market; S= Spera-in-deo

Table 4 Antibiotics susceptibility pattern of bacterial isolate from ready to eat abacha sold within abakaliki

S/N	E	CIP	AMP	DA	CN	RL	TE	F	VA	FOX	SUSPECTED ORGANISMS
1.	R	S	ND	ND	S	R	R	ND	ND	S	*Shigella sp*
2.	R	S	ND	ND	S	R	R	ND	ND	R	*Klebsiella pneumoniae*
3.	R	R	ND	ND	S	R	R	ND	ND	R	*Escherichia coli*
4	S	S	R	R	S	R	R	R	R	S	*Staphylococcus aureus*
5.	R	S	ND	ND	S	R	S	ND	ND	R	*Escherichia coli*
6.	R	S	ND	ND	S	S	S	ND	ND	S	*Klebsiella pneumoniae*
7.	S	S	R	R	S	S	R	R	R	S	*Staphylococcus aureus*
8	R	S	ND	ND	S	S	S	ND	ND	S	*Shigella sp*
9	S	S	R	R	S	S	S	R	R	S	*Staphylococcus aureus*
10.	R	S	ND	ND	S	R	S	ND	ND	S	*Klebsiella pneumoniae*
11.	R	S	ND	ND	S	R	R	ND	ND	R	*Escherichia coli*
12.	R	R	ND	ND	S	R	S	ND	ND	R	*Shigella sp*
13	R	S	R	R	S	R	S	R	S	R	*Staphylococcus aureus*
14.	R	R	ND	ND	S	R	R	ND	ND	R	*Escherichia coli*
15.	R	R	ND	ND	S	R	S	ND	ND	R	*Shigella sp*

Keyword: R = Resistance, S = Susceptible, ND = Not determined

Antibiotics Tested

E = Erythromycin, CIP = Ciprofloxacin, AMP = Ampicilin, DA = Clindamycin, CN = Gentamycin, RL = Sulfamethozole, TE = Tetracycline, F = Nitrofuratin, VA = Vancomycin; FOX = Cefoxitin.

Table 5 Total percentage antibiotics susceptibility pattern of bacterial isolate from ready to eat abacha sold within abakaliki

Antibiotics	**Susceptible/ Resistance**	**Bacterial isolates in percentage (%)**					
		Escherichia coli	***Klebsiella pneumonia***	***Shigella sp***	***Staphylococcus aureus***		
Erythromycin (E)	Susceptible Resistance	0.0 100.0	0.0 100.0	0.0 100.0	75.0 25.0		
Ciprofloxacin (CIP)	Susceptible Resistance	50.0 50.0	100.0 0.0	50.0 50.0	100.0 0.0		
Ampicillin (AMP)	Susceptible Resistance	ND	ND	ND	0.0 100.0		
Clindamycin (DA)	Susceptible Resistance	ND	ND	ND	0.0 100.0		
Gentamycin (CN)	Susceptible Resistance	100.0 0.0	100.0 0.0	100.0 0.0	100.0 0.0		
Sulfamethozole (RL)	Susceptible Resistance	0.0 100.0	33.3 66.7	25.0 75.0	50.0 50.0		

Tetracycline (TE)	Susceptible Resistance	25.0 75.0	66.7 33.3	75.0 25.0	50.0 50.0
Nitrofuratin (F)	Susceptible Resistance	ND	ND	ND	0.0 100.0
Vancomycin (VA)	Susceptible Resistance	ND	ND	ND	25.0 75.0
Cefoxitin (FOX)	Susceptible Resistance	0.0 100.0	66.7 33.3	50.0 50.0	75.0 25.0

Keyword: ND= Not determined

Table 6 The result of multiple drug resistance value of each organisms isolate from ready to eat abacha sold within abakaliki

Organisms	**MARI values**
Escherichia coli	0.71
Klebsiella pneumonia	0.39
Staphylococcus aureus	0.51
Shigella spp	0.5

Keyword: MAR 1 = Multiple Antibiotics Resistance Index

4. Discussion

Enteric organism; *Staphylococcus aureus* are responsible for many of the global cases of food poisoning (Emodi and Madukwe,2011; Flake and Nzeka,2009; Guiurati,2004). This is not far from the truth owing to the total number of bacteria Isolated from the food sample (Abacha) in this study (Table 2). The percentage occurrence of *E. coli, Staphylococcus aureus, Klebsiella poeumoniae* and *Shigella* spp are pointer to the fact that ready-to-eat Abacha sold at different locations within Abakaliki are contaminated with potentially pathogenic bacteria and this may be as a result of the type of water used for domestic purposes or the human handling during processing and sales of the product respectively. This is in agreement with Jabbar and Domenico; Jansen,1992. Nwachukwu etal., (2011) who reported earlier that water used for production coupled with the crude method of production and packaging under improper sanitary conditions pre-disposes drinks and foods to microbial contamination by an array of both gram negative and gram positive bacteria. There is therefore need for surveillance by public health officials to ensure safety of the ready-to-eat Abacha being sold within Abakaliki for public consumption. There is need to also ensure that the water used for the preparation and processing of Abacha is safe and free from microbial contaminants (Dibb and simkin,1997;Galguera etal.,2006). The sources of contamination may also have come from the spices used additive (Melesse and Beyene, 2009; Mullins *etal*., 1994;Onyemauwa,2010;Philip etal.,2013;Pindyck and Rubin,1998).

The antimicrobial susceptibility studies of the bacteria isolates from ready to –it-eat Abacha in this work on some selected antibiotics showed that ten (10) antibiotics were used. Gram positive, organism, *Staphylococcus aureus* was tested with all the antibiotics while gram-negative organisms were tested with (6) antibiotics out of the ten used. *Escherichia coli* had high resistance to erythromycin (100%), sulfamethozole (100%), cefoxitin (100%), tetracycline (75%), ciprofioxacin (50%) and sensitive to only gentamycin (100 %), *Klebsiella pneumonia* had high resistance to erythromycin (100 %), sulfamethozole (66.7 %), and sensitive to ciprofloxacin (100 %), gentamycin %), tetracycline (66,7 %) and cefoxitin (66.7%). *Shigella* sp had high resistance to erythromycin (100%), sulfamethozole (75 %) and sensitive to ciprofloxacin (50%), gentamycin (100%), tetracycline (75%), cefoxitin (50%), *Staphylococcus aureus* had high resistance to ampliclin (100%), clindamycin (100%), nitrofuratin (100%), vancomycin (75%), and sensitive to ciprofloxacin (100%), gentamycin (100%), sulfamethozole (50%), tstracycline (50%) cefoxitin (75%). Gram negative organisms were resistance to erythromycin (100%) while all the bacteria isolates were sensitive to gentamycin (100%). The sensitivity of these isolates to the antibiotics used is compared to earlier reports (Popkin etal.,1989 Ruel etal., 2005;Kobe and Valentine,2001;Wojcicki and Heyman, 2010;Ade-omowaye etal., 2006). The prevalence of resistant strains of Escherichia coli enteric bacteria such as (*Klebsiella pneumonia and Shigella* sp) and *Staphylococcus aureus* in ready-to-eat foods such as Abacha, zobo drinks, soy bean milk etc is a reflection of the use and misuse of the antibiotics

in the society. This is not surprising because outside the hospital environment, the global population have access to various kinds of antibiotics at any drug store with or without any prescription from a medical practitioner.

Multi-drug resistance values in (table 6) indicate that *Klebsiella pneumonia* (0.39) had more resistance to the antibiotic tested while *Escherichia coli* (0.71) had less resistance to the tested antibiotics. This table contradicts the recent report which states that multi drug resistance strain of *Escherichia coli* from food origin was significantly higher than those of clinical origin and this has been associated to the fecal source of the pathogen (Ayo and madaki,2005; Baker et al ,1995;Collis and Hussey ,2003;Dana,1999).

Finally, this study revealed the presence of enteric and non-enteric organism including. *E.coli, Klebsiella pneumonia, Shigella spp* and *Staphylococcus aureus* in the ready-to-eat Abacha sold at different locations within Abakaliki were resistant to some available drug. Regular monitoring of the quality of foods and drinks sold to students and other un-suspected members of the public in this region is required to forestall any imminent health danger. The public health implication of this study is that antimicrobial resistant strains of pathogenic bacteria may colonize the human population through consumption of contaminated ready-to-eat Abacha and this would lead to chemotherapeutic failures among the human consumers of this ready-to-eat Abacha within Abakaliki. Food handlers should also be educated and be observant to current public health guidelines in their profession so as to minimize food-borne related illnesses.

5. Conclusion

The presence of enteric and non-enteric organism in the ready-to-eat Abacha sold at different locations within Abakaliki was significantly high and the isolates exhibited considerable resistance to some tested antibiotics. The public health implication is that antimicrobial resistant strains of pathogenic bacteria may spread amongst the populace through consumption of such contaminated ready-to-eat Abacha and this would lead to chemotherapeutic failures and resistance to antibiotics among the human consumers of this ready-to-eat Abacha within Abakaliki. Food handlers must be properly informed and guided to minimize such imminent health danger.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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