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BACTERIAL EVALUATION OF READY-TO-EAT AFRICAN CASSAVA SALAD (ABACHA) SOLD AT DIFFERENT RESTAURANTS IN ABAKALIKI METROPOLIS

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Abstract:
This study investigated the bacterial evaluation of Ready-To-Eat (RTE) African cassava salad sold in Abakaliki metropolis. A total of 100 samples were randomly purchased from four different locations in Abakaliki metropolis. Isolation enumeration and identification of bacterial isolates were done using standard microbiological methods. The bacterial isolates were subjected to antibiotic susceptibility test using Kirby-Bauer disc diffusion technique. The multiple antibiotic resistance index (MARI) was deduced from the antibiogram. The results on morphological and biochemical characteristics revealed that four bacteria genera which include Escherichia coli, Salmonella spp, Staphylococcus aureus, and Klebsiella spp were identified. The percentage occurrence of these bacterial isolates according to locations showed highest occurrence rate of 37 (27%) in samples from College of Agricultural Science (CAS), followed by 36 (26.3%) from College of Health Sciences (Presco) while lowest occurrence rate of 30 (21.9%) was from Ahiaofuru market. The overall percentage frequency of distribution also showed that Salmonella species had highest percentage frequency of 48 (35%) while Klebsiella species had the lowest percentage frequency of 15 (11%). The isolates were most susceptible to Ciprofloxacin (84.6%) and least susceptible to Tetracycline (38.6%). The average MARI value of the isolates ranged from 0.33-0.55. From this study it was discovered that consumers of RTE African salad in Abakaliki metropolis are at high risk of food poisoning.

Key words: *Ready-To-Eat, Antibiotic, African salad, Contamination, Consumers*

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INTRODUCTION:

African salad (cassava – tapioca porridge (Abacha) is derived from cassava tubers (Manihot esculentacrantz), a basic food for estimated 500 million farmers, countless processors and traders in Nigeria being the largest producers (of cassava) in the world (Levin *et al.*, 1999). Abacha/ tapioca porridge as a ready-to-eat food (RTE) salad, is generally processed from single or mixed raw ingredients such as vegetables, fish, fruits, spices, potash, palm oil, cooked meat, garden eggs, grains, nuts etc, with sauce or salad dressing (Makinde *et al.*, 2018). African salad is consumed by a variety of people; school – age children and the fast – paced working class with little or no time to prepare meals as well as a side dish to various Nigerian recipes. It's economic and social advantage hinges on ease of production, availability, affordability, serviceability, and palatability, but usually prone to microbial contamination (Al Mamum *et al.*, 2013). Processing of African salad needs boiling/ cooking and fermentation by steeping in potable water for 24 hours or more to reduce starch and poisonous compounds (e.g hydrogen cyanide) to improve organoleptic properties prior to consumption. Researchers have reported poor personal hygiene, lack of education, ingredients, water and sanitary status of the environment as fundamental predisposing factors of microbial contamination of foods (Salamandane*et al.*, 2021). A variety of the microbes (e.g., bacteria, fungi and viruses) reported in RTE foods not only accentuate public health risks but also produce potential toxigenic compounds (Akande, 2017). Cross/post-contamination may also be associated with insect vectors (Ewoenam, 2014). Consequently, these contaminants in African salad may result in unwholesomeness which may be linked with outbreaks of food borne diseases (Aluko, 2014). In spite of the long history of vended RTE foods as a source of livelihood to many households, there is paucity of data regarding their contribution to the nutritional value of the diet (Mbae, 2018) as well as microbiological safety due to variation in fortification by processors. The quality of food can be assessed by the amount of available nutrients and microbiological quality of the final products. Therefore, deliberate efforts to know the quantity and quality of the nutritional contents of vended RTE foods, their contribution to the overall energy and available nutrient intake is pertinent. Additionally, adoption of more proactive approaches in identifying and reducing food safety risks by bridging the information gap with regard to consistent surveillance, monitoring and evaluation of nutritional and microbiological status of vended RTE foods such as African salad is crucial. Though, they have been reported to be a cheap source of energy and protein intake than pre-packaged processed foods (Becquey, 2010).For years now, it has been observed that people especially students living along campus area suffer from stomach upset from consuming fast food, most especially African salad (Abacha). Since bacteria have been identified as being prevalent in foods prepared by food vendors and this is as a result of contamination from different sources. During preparation, certain factors are disregarded due to ignorance and some of these traditional Nigerian food delicacies are produced and prepared in an unhygienic environment, thereby leading to contamination. Therefore, this study was carried out to investigate the bacteria quality of African salad (Abacha), sold within Abakaliki metropolis.

MATERIALS AND METHODS:

Sample Size

The total sample size used for this research study was 100 and the error margin for the study was determined to be ±0.05% using the formula Stated below;

$N = [Z\alpha/2 \times \sigma]2/E$

Where N is sample size; $Z\alpha/2$ is value of normal variables at α level of significance; E is error margin allowable and σ is the standard deviation in the population.

Note that Range = 4 σ; thus σ = 0.25 and $Z\alpha/2$ at 95% level of confidence is 1.96

Sample Collection

Freshly prepared garnished African cassava salad (Abacha) samples were purchased at four (4) stationary locations from different vendors in Ahiaofuru , kpirikpiri, Cas, and presco campuses of Ebonyi state university all in Abakaliki. A total of one hundred (100) samples were obtained. Five (5) samples each of 20g African cassava salad were obtained in the afternoon respectively from the four different locations aseptically in sterile sealable containers, packaged in polyethylene bags and transported to the Microbiology laboratory unit of Ebonyi State University, Abakaliki.

Microbiological Analysis of Samples

Ten gram (10g) samples was serially diluted in 90ml sterile peptone water and aliquot 0.1 ml volume of each sample was inoculated by spread plate method onto freshly prepared nutrient broth and incubated at 37 °C for 24 24hrs. 5ml of broth was streaked onto Nutrient agar, Mannitol salt agar, *Salmonella/Shigella* and MacConkey agar and incubated at 37 °C for 24hours. Identification of isolates was done by morphological characterization and biochemical tests (Cheesebough, 2006).

Morphological characterization
The colony appearance of the isolates on culture media, color and shape were observed and recorded accordingly.

Antibiotic susceptibility testing
Antibiotic susceptibility test of isolates was done by using Kirby-Bauer disc diffusion Method according to Clinical and Laboratory Standards Institute (CLSI, (2007).

Determination of Multiple Antibiotic Resistance (MAR) index Multiple antibiotic resistance (MAR) index was determined using the formula MAR=a/b, where **a** is the number of antibiotics to which test isolate displayed resistance and **b** is the total number of antibiotics to which the test organism has been evaluated for sensitivity (Christopher *et al*., 2013; Ariom *et al*., 2019).

Statistical analysis
The obtained data in this research were exposed to version 21.0 of SPSS statistical package. Descriptive statistics was employed to both determine the level of contamination as well as the susceptibility profile of obtained isolates.

RESULTS:

Morphology and Biochemical characteristics of bacteria isolated from ready-to-eat African cassava salad (Abacha) sold in Abakaliki Metropolis

The morphology and biochemical characteristics of bacteria isolated from ready to ea t African cassava salad (Abacha) sold in Abakaliki metropolis is shown in Table 1. It revealed the colour, shape, Gram staining reaction as well as the biochemical characteristics of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia* and *Salmonella* species as the suspected bacteria isolated.

Percentage occurrence of bacterial isolates from ready-to-eat African cassava salad (Abacha) from various locations in Abakaliki metropolis
The percentage occurrence of bacterial isolates from ready to eat Abacha from various locations in Abakaliki revealed that Abacha sold in Cas had the highest number of bacterial isolates with a percentage of 27%, Presco had a percentage of 26.3%, Kpiri-Kpiri had a percentage of 24.8% and Ahiaofuru market had the lowest contamination with a percentage of 21.9% as shown in Table 2.

Overall Frequency distribution of isolates from ready-to-eat African cassava salad (Abacha) sold within Abakaliki metropolis
The overall distribution of bacterial isolates from ready to eat African cassava salad sold in Abakaliki metropolis revealed that *Salmonella* species was the highest occurring bacteria with a percentage frequency distribution of 48(35%) followed by *Escherichia coli* with a frequency of 45(32.8%) *Staphylococcus aureus* had a percentage frequency of 29(21.2) while *Klebsiella pneumonia* was the least occurring bacteria with a percentage frequency of 15 (11%)as shown in Table 3.

Antibiotic susceptibility pattern of bacteria isolated from ready-to-eat African cassava salad sold within Abakaliki Metropolis
The antibiotic susceptibility pattern of isolated bacteria from ready-to-eat African cassava salad is shown in Table 4. *Staphylococcus aureus* showed varying degree of susceptibility to the test antibiotics. It was 86% susceptible to Gentamicin, 75.9% to Penicillin, 69%to Ciprofloxacin, 58.6% and 51.7% to Amoxicillin and Ampicillin respectively. *Klebsiella pneumonia* also showed varying degree of susceptibility to test antibiotics. It was 80% susceptible to both Gentamicin and Ciprofloxacin, 73.3% to Amoxicillin and 53.3% to Penicillin. *Escherichia coli* was 95.6% susceptible to Ciprofloxacin, 84.4% to Cefotaxim, 75.6% to Penicillin 57.7% to Gentamicin and 55.6% susceptible to both Ampicillin and Cefuroxime. *Salmonella* species was 100% susceptible to Penicillin, 97.1% to Gentamicin, 93.8% to Ciprofloxacin 83.3% to Ampicillin and 77.1% susceptible to both Cefotaxime and Tetracycline.

Determination of Multiple Antibiotic resistance index (MARI) exhibited by bacteria isolated from ready-to-eat African cassava salad sold within Abakaliki metropolis
The multiple antibiotic resistance to three and more antimicrobial agents within one and other class of drug was observed among the isolates with MARI value of 0.44, 0.55, and 0.33 as shown in Table 5

Table 1: Morphological, Microscopic and Biochemical Characteristics of Bacterial Isolates from ready-to-eat African cassava salad sold within Abakaliki Metropolis

Morphological characteristics			**Microscopic characteristics**		**Biochemical tests**				**Suspected organisms**
Shape	Colour	Media	Gram RXN	Motility test	OX	IN	CAT	CIT	
Rod	Pink	MacConkey agar	-	+	-	-	+	-	*Escherichia coli*
Rod	Black	Salmonella-Shigella agar	-	+	-	-	+	+	*Salmonella spp.*
Cocci	Yellow	Mannitol salt agar	+	-	-	-	+	+	*Staphylococcus aureus*
Rod	Pink-red	MacConkey agar	-	-	+	-	+	+	*Klebsiella pneumonia*

Key: OX= Oxidase, IND= Indole, CAT= Catalase, CIT= Citrate

Table 2: Percentage occurrence of Bacteria in African cassava salad sold within Abakaliki metropolis

Isolates	Ebonyi state University Campuses	Presco	Ahiaofuru market	Kpiri-Kpiri market
Escherichia coli	9	11	10	15
Salmonella species	15	15	8	10
Staphylococcus aureus	8	7	5	9
Klebsiellapneumonia	5	3	7	-
Total	**37** (27%)	**36 (26.3%)**	**30 (21.9%)**	**34 (24.8%)**

Table 3: Overall percentage distribution of bacterial isolates from ready-to-eat African cassava salad sold within Abakaliki metropolis

S/N	**Isolates**	**Percentage frequency distribution**
1	*Escherichia coli*	**45 (32.8)**
2	*Salmonella species*	**48 (35)**
3	*Staphylococcus aureus*	**29 (21.2)**
4	*Klebsiella pneumonia*	**15 (11)**
	Total	**137 (100%)**

Table 4: Percentage Antibiotic susceptibility pattern of isolated bacteria from ready-to-eat African cassava salad (Abacha)

Isolates	**CIP**	**AMP**	**CFM**	**T**	**CXM**	**PEN**	**GN**	**AML**	**CX**
***Staphylococcus aureus* (29)**	69.0	51.7	27.6	17.2	34.5	75.9	86.0	58.6	41.4
***Klebsiella pneumonia* (15)**	80.0	46.7	33.3	26.7	33.3	53.3	80.0	73.3	46.7
***Escherichia coli* (45)**	95.6	55.6	84.4	33.3	55.6	75.6	57.7	44.4	46.7
***Salmonella* species (48)**	93.8	83.3	77.1	77.1	45.8	100.0	97.1	43.8	45.8
Total (%)	**84.6**	**59.3**	**55.6**	**38.6**	**42.3**	**76.2**	**80.2**	**50.0**	**45.0**

KEY: CIP = Ciprofloxacin, AMP = Ampicillin, CFM = Cefotaxim, CXM = Cefuroxime, T = Tetracycline, GN = Gentamicin, PEN = Penicillin, CX = Ceftriaxone, AML = Amoxicillin

Table 5: Multiple antibiotic resistance index (MARI) exhibited by bacterial isolates from ready to eat Abacha sold in Abakaliki metropolis

Isolates	Antibiotics resistance patterns	INDEX
Staphylococcus aureus	CFM, CXM, CX, T	0.44
Klebsiella pneumonia	AMP, CFM, T, CXM, CX	0.55
Escherichia coli	AML, T, CX	0.33
***Salmonella* species**	AML, CXM, CX	0.33
Average MARI		0.41

KEY: CIP = Ciprofloxacin, AMP = Ampicillin, CFM = Cefotaxim, CXM = Cefuroxime, T = Tetracycline, GN = Gentamicin, PEN = Penicillin, CX = Ceftriaxone, AML = Amoxicillin

DISCUSSION:

The isolated organisms in this study were identified to be *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus* and *Klebsiella pneumonia* (Table 1). Research frontiers have reported similar findings from their studies on street vended foods in some African and low-income countries (Mensah et al., 2002; Afolabi et al., 2012; Igbinosa and Beshiru, 2019; Beshiru et al., 2020; Igbinosa et al., 2021).

The isolation of *E. coli*, *Klebsiella* spp., and *Salmonella* spp., from the RTE African cassava salad, suggests that most food borne enteric infection and diseases occur because of unhygienic handling of the foods and or the poor sanitary condition of the environment during preparation/processing of the food. It is also indicative of possible faecal contamination from the water used in food processing, improper washing of the vegetables used or from the fertilizer/manure used in cultivating the vegetables used. Corresponding reports were made by Wogu and Iwezua (2013)and Igbinosa et al. (2020).

More so, the presence of *Staphylococcus aureus* is suggestive of contamination by food vendors, as it has been reported that food handlers are the main source of food contamination in *S. aureus* food poisoning. *S. aureus* contamination can also arise from infected wounds, stroking hair, scalp, burns and dirty fomites of food handlers (Yeboah-Manu et al., 2010; Afolabi et al., 2012).

The percentage occurrence of these bacterial isolates according to locations showed highest occurrence rate of 37 (27%) in samples from College of Agricultural Science (CAS), followed by 36 (26.3%) from College of Health Sciences (Presco) while lowest occurrence rate of 30 (21.9%) was from Ahiaofuru market. *Salmonella* species was the most prevalent organism in African cassava salad from CAS and Presco locations, *Escherichia coli* was the most prevalent organism in African cassava salad from Ahiaofuru and Kpiri-Kpiri market.

The antibiotic susceptibility profiling of the isolates recovered from the RTE African cassava salads revealed that *Staphylococcus aureus* showed varying degree of susceptibility to the test antibioticsrespectively. *Klebsiella pneumonia* also showed varying degree of susceptibility to test antibiotics. However, in 2007, Van et al. reported 83.3% of the *E. coli* recovered from RTE retailed foods was resistant to at least one antibiotic used in their study. The MARI index according to Chitanand*et al.*, (2010) showed the organisms importance as a public health threat (Table 5). This agreed with the work of (Eluu*et al.*, 2018) who reported Bacteriological Quality, Consumption Value and Antibiotics Susceptibility Pattern of Ready-To-Eat African Salad sold and consumed in Ebonyi State University, Abakaliki, Nigeria.

CONCLUSION:

This study has provided evidence that the RTE African cassava salads sold by vendors in the sampled parts of Abakaliki metropolis pose high-risk to its consumers as they are potential reservoirs for drug resistant *Klebsiella pneumonia* and *S. aureus. Salmonella* species was the highest isolated organism from this study and has been known to be a major cause of food borne diseases. Good hygiene practices and the use of potable water and clean kitchen utensils is therefore encouraged amongst food handlers.

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