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PLASMID BORNE RESISTANCE AMONG BACTERIA ISOLATED FROM AFRICAN SALADS ("ABACHA")

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors EOD and FNO designed the study, wrote the protocol and interpreted the data. Author OAU anchored the field study, gathered the initial data and performed preliminary data analysis. Authors AAD and HOO managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

The present study aimed at studying the bacteriological quality of African Salads and antibiotics resistance pattern of isolates before and after plasmid curing to ascertain if the resistance to multiple antimicrobials was plasmid mediated. The results showed mean Salmonella/Shigella count of the African salad samples ranged from 25× 10^5cfu/g to 201× 10^5cfu/g, Staphylococcal count ranged from 39× 10^3 cfu/g to 215× 10^5cfu/g, coliform count ranged from 27× 10^3 cfu/g to 215× 10^5 cfu/g and Listeria count ranged from 6× 10^3cfu/g to 113× 10^5cfu/g. The prevalent bacteria were *Listeria sp* having a 60% occurrence while *Escherichia coli* had a 30% occurrence. The species of bacteria isolated were identified as *Listeria sp, E. coli, Klebsiella sp, Proteus sp, Salmonella sp, Staphylococcus aureus* and *Shigella sp.* All isolate were susceptible to gentamycin and ofloxacin. Resistance was observed with erythromycin (90.0%), cloaxacilin (70.2%), augmentin (60.5%), ceftazidime (34.7%) and cefuroxime (22.2%). Plasmid analysis showed that most of the isolates carried plasmids greater than 10kbp and after plasmid curing the isolates were cured of its resistance to cloxacillin and ceftazidime but some isolates still retained resistance to erythromycin (25.8%), augmentin (16.9%) and cefuroxime (19.7%).

Keywords: Plasmid curing; African salads; resistance; antibiotics; bacteria.

1. INTRODUCTION

Abacha is a cassava product that is popular and relished by the Eastern and Southern Nigerians. It contains raw vegetables and other ingredients that are consumed without further heating. The preparation of African Salad takes great efforts and the ingredients needed to prepare African salad vary according to ones taste and availability. They may include; Ugba (*Pentaclethra macrophylla*), palm oil, potash, onions, nutmeg, crayfish, salt, pepper, maggi, ogiri (*Ricinus communis*), garden egg, garden egg leaves, Utazi leaves (*Gongronema latifolium*), Okazi (Ukazi) leaves (*Gnetum africana*), Ozeza (Uzeza) leaves (*Piper guineense*), cooked kpomo (cow skin), meat and stockfish/fish [1]. These ingredients are mixed thoroughly with the shredded cassava to give this delicacy and can be eaten on its own or in combination with other snacks like coconut, palm kernel and groundnut. African salad is known to be nutritious and a rich source of protein, carbohydrate, vitamins, and minerals.

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African salads can be contaminated from the different ingredients during preparation. A major ingredient which could serve as a major source of contamination to Abacha is the raw vegetables; vegetables have been associated with outbreaks of food borne disease in many countries [2]. Raw vegetables harbor many microorganisms through contact with soil, dust, water, handling at harvest or during processing and these microorganisms may be found in the final product [3]. Street- vended foods such as African salads are frequently associated with diarrhea diseases due to the presence of pathogenic bacteria, environmental contaminants, and disregard for good hygiene practices (GHPs). These vendors of these foods are often poorly educated and untrained in food hygienic processes and they work under crude unsanitary conditions with little or no knowledge about the causes of food borne disease. Food-borne illnesses of microbial origin are a major health problem associated with street vended foods. In addition, resistance of food-borne microorganisms to antibiotics makes food safety more vulnerable. The present study aimed at studying the bacteriological quality of African salads and antibiotics resistance pattern of isolates before and after plasmid curing to ascertain if the resistance to multiple antimicrobials was plasmid mediated.

2. MATERIALS AND METHODS

2.1 Sample Collection

Forty (40) samples of African salad were purchased from different vendors around Oba Market and New Benin market in Benin City, Edo state, Nigeria. The sample were collected in sterile specimen containers and transported in cold pack to the Laboratory for analysis within one hour.

2.2 Microbial Analysis of Samples

Ten fold serial dilutions was carried out on all samples after which 0.1ml of aliquot was platted on Listeria selective agar (Oxford formulation)(Oxoid), Mannitol salt agar (LabM), Eosin Methylene Blue agar (Biomark), MacConkey agar (LabM) and Samonella Shigella agar (LabM) using spread plate method. Plating was done in duplicate and incubated for 24-48 hours at 37°C.

2.3 Identification of Species

For species identification, conventional methods were used including Gram staining, catalase, hemolysis on blood agar, oxidase, indole, urease, citrate utilization, hydrogen sulfide formation, and fermentation of different sugars.

2.4 Antibiotics Susceptibility Test

Antibiotics susceptibility test was performed using the disc diffusion method. The antibiotics multidisc (Abtek Biologicals Ltd) used contained Ceftazidime (Caz) 30 ug, Cefuroxime (Crx) 30 ug, Gentamicin (Gen), 10 ug, Ceftriaxone (Ctr) 30 ug, Erythromycin (Ery) 5 ug, Ofloxacin (Ofl) 5 ug, Augmentin (Aug) 30 ug. Respective colonies were on forceps and incubated at 37°C for 24 hours. The zones of inhibition was recorded in millimeter (mm) and classified as resistant or sensitive based on the interpretative chart of Clinical laboratory standard (CLS).

2.5 Plasmid Isolation

Plasmids were isolated using Akaline lysis method as described by [4], after which they were ran at 100v for 40 mins on 0.8% agarose gel electrophoresis containing two drops of ethidium bromide then the bands were visualized in a UV trans illuminator.

2.6 Plasmid Curing

Plasmid curing was carried out using sub-inhibitory concentration of 10% of sodium dodecyl sulphate (SDS) as described by Sheikh et al. [5] with slight modification. Antibiotic resistant isolates were grown on tryptone soy broth containing 10% SDS at 37°C for 48hrs. After 48hrs, the broth was agitated to homogenize the content and a loopful subcultured onto Mueller Hinton agar (MHA) plates. The plates were incubated at 37ºC for 24 hours after which colonies were screened for antibiotic resistance by the disk diffusion method. Cured markers were determined by comparison between the pre- and post-curing antibiograms of isolates. Loss of resistance after the plasmid curing was indicative of plasmid mediated resistance [6].

3. RESULTS AND DISCUSSION

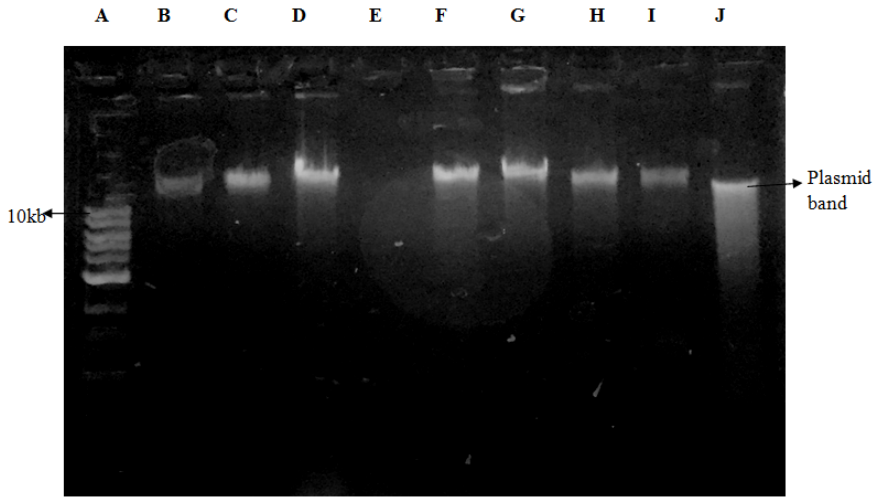
The range of bacteria obtained in the different African salad samples from sampling locations is shown in Table 1. All the samples had the presence of *Listeria* sp, *Staphylococcus aureus*, *Salmonella* sp, *Coliform* and *Shigella* sp. Staphlococcal counts ranged from $39.00x10^3$ to $112.00x\ 10^5$ from samples around Oba market. Counts of $6.00x10^3$ – $88.00x\ 10^5$, 37.20–$80.00\ x\ 10^5$ and $30.00x10^5$– $215.00x\ 10^5$ represented range of Listeria counts, Salmonella counts, and coliform counts respectively. The high microbial count recorded in this study reflects the high level of contamination of this food product and can be attributed to the unhygienic nature of preparation by

the personnel. Contamination of African salads can also come from the utensils/equipments, water used for preparation and the processing environment.

The acceptable limit of coliforms in foods in developed countries is 10^6 colony forming unit per gram [7]. Meanwhile, the bacterial counts obtained in this study were far higher than the above standard. High microbial count in foods such as salad has been reported in Nigeria and other parts of Africa [8]. Another reason that could be attributed to the high microbial count recorded in this study could be due to the fact that African salad is a product of raw vegetables with no heat treatment to reduce microbial load. This finding agrees with the result of Ifediora et al. [9] who reported that food preparations pattern can affect the microbial load of the food product. The addition of fermented products like 'Ugba' and 'Ogiri' could also have contributed to the microbial load recorded in this study. 'Ugba' and 'Ogiri' has been reported to have high microbial load [10].

The prevalence of bacteria isolated from African salads is shown in Table 2 with *Listeria* sp having the highest occurrence of 50% while *Proteus* sp and *Klebsiella* sp had the lowest frequency of occurrence of 3%. The presence of *Escherichia coli* in this study implies possible faecal contamination and the possibility of food borne illness due to enteric pathogens. Its presence in these foods indicates a diarrheal risk for consumers of the contaminated salads. Similar observation was made by [10] who stated that the standard of personal and environmental hygiene could be a factor that determines the presence of *Escherichia coli* in foods. The presence of *Listeria* sp in Abacha samples in this study indicates that Abacha can be a vehicle for the transmission of listeriosis. Listeria_*sp* is thought to occur on the vegetables that are added during the preparation of African salads because of their ubiquitous nature. Although, contaminated wash water may also play a role. This is supported by Eni et al. [2] and Bello [11] who reported a strong association between *Listeria* sp and vegetable foods and attributed their presence to high levels of fermentable sugars which can be readily utilized by *Listeria* spp.

The antibiotics resistance pattern of the bacteria isolated from African salads is shown in Table 2. Majority of the organisms showed multiple drug resistance. From the resistance pattern it was found that all the isolates were susceptible to ofloxacin and gentamycin. 47% *Listeria* sp were resistant to erythromycin while 48.50% were resistance to augumetin. Resistance to antibiotics has been ascribed in most instances to the presence of plasmids. In a similar study, Poorna and Randhir, [12] and Udoh and Okpokwasili [13] reported the antimicrobial resistance profile of potential human pathogens isolated from fresh vegetables. These antibiotic resistant properties of a bacterium could be its inherent properties or occur due to chromosomal mutation(s) or by acquiring extra-chromosomal DNA plasmid [14].



Picture 1. Plasmid band on agarose gel

Key: A – Ladder (1kb); B, C and D - Listeria sp.; E and F – Staphlococcus aureus; G and H – Salmonella sp; I and J– Shigella sp

Table 1. Range of microbial counts cfu/g of African salad sold around Oba and New Benin markeets

Sample site	Listeria count	Staphylococcal count	Salmonella count	Shigella count	Coliform count
ObaM	$10.40x\ 10^3 – 113.00x\ 10^5$	$39.00\ x10^3 – 112.00\ x\ 10^5$	$25.50\ -50.00\ x\ 10^5$	$66.00\ -201.00\ x\ 10^5$	$27.00x10^3 – 200.00x\ 10^5$
NewM	$6.00x10^3 – 88.00x\ 10^5$	$50.00\ x10^3\ -\ 215.00\ x10^5$	$37.20 – 80.00\ x\ 10^5$	$40.30\ -201.00\ x\ 10^5$	$30.00x10^5 – 215.00x\ 10^5$

Table 2. Frequency of occurrence of bacteria isolated from African salads

Bacteria	No of Isolates (% occurrence)
Listeria sp	100 (50)
E. coli	60 (30)
Klebsiella sp	6 (3)
Proteus sp	6 (3)
Salmonella sp	8 (4)
Shiglla sp	10 (5)
Staphylococcus aureus	10 (5)
Total	200 (100)

Table 3. Antibiotic resistance pattern of bacteria isolated from African salads

	CAZ	CRX	GEN	ERY	CXC	OFL	AUG
Listeria sp	37 (18.50)	35 (17.50)	NIL	94 (47.00)	90 (45.00)	NIL	97 (48.50)
E. coli	13 (6.50)	5 (2.50)	NIL	55 (27.50)	30 (15.00)	NIL	10 (5.00)
Klebsiella sp	3 (1.50)	NIL	NIL	49 (2.00)	4 (2.00)	NIL	2 (1.00)
Proteus sp	4 (2.00)	NIL	NIL	5 (2.50)	3 (1.50)	NIL	2 (0.50)
Salmonella sp	3 (1.50)	2 (1.00)	NIL	8 (4.00)	3 (1.50)	NIL	3 (1.50)
Shiglla sp	6 (3.00)	2 (1.00)	NIL	6 (3.00)	5 (2.50)	NIL	6 (3.00)
Staphylococcus aureus	3 (1.50)	NIL	NIL	8 (4.00)	5 (2.50)	NIL	1 (0.50)
Total	69 (34.7%)	44 (22%)	NIL	180 (90%)	140 (70.2%)	NIL	121 (60.5%)

Key: GEN: Gentamycin, OFL: Ofloxacin, ERY: Erythromycin, CXC: Cloaxacilin, AUG: Augmentin (60.5%), CAZ: Ceftazidime, CRX: Cefuroxime

Table 4. Antibiotic resistance pattern of bacteria after plamid curing

	No of isolates	CAZ	CRX	GEN	ERY	CXC	OFL	AUG
Listeria sp	100	100 (50.00)	69 (34.50)	100 (50.00)	20 (10.00)	100 (50.00)	100 (50.00)	25 (12.50)
E. coli	60	60 (30.00)	54 (27.00)	60 (30.00)	25 (12.50)	60 (30.00)	60 (30.00)	4 (2.00)
Klebsiella sp	6	6 (3.00)	6 (3.00)	6 (3.00)	4 (2.00)	6 (3.00)	6 (3.00)	NIL
Proteus sp	6	6 (3.00)	6 (3.00)	6 (3.00)	NIL	6 (3.00)	6 (3.00)	NIL
Salmonella sp	8	8 (4.00)	8 (4.00)	8 (4.00)	NIL	8 (4.00)	8 (4.00)	NIL
Shiglla sp	10	10 (5.00)	8 (4.00)	10 (5.00)	NIL	10 (5.00)	10 (5.00)	1 (0.50)
Staphylococcus aureus	10	10 (5.00)	10 (5.00)	10 (5.00)	2 (1.00)	10 (5.00)	10 (5.00)	1 (0.50)
	200	200 (100)	39 (19.70)	200 (100)	51 (25.80)	200 (100)	200 (100)	34 (16.90)

4. CONCLUSION

Proper washing of vegetables, use of clean utensils and practice of good hygienic and sanitary conditions can reduce the contamination of Abacha. Also, education of food handlers and the general public on food safety measures, effective Hazard Analysis Critical Control Point application and Good Manufacturing Practices (GMP) implementation is imperative.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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