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Effect of Post Treatment with Brine and Steam on Phenotypic Resistant *Salmonella* Isolates from *Pentaclethra macrophylla*

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Abstract: Food contaminated with antibiotic resistant bacteria pathogens is a major threat to public health. Apart from infecting man they serve as reservoirs of genes for antimicrobial resistance and they easily transfer the resistant genes to both related and unrelated bacterial species; hence the aim of the present study. The prevalence, antibiotic sensitivity pattern and plasmid profile of *Salmonella* spp isolates from ready-to-eat ugba (*Pentaclethra macrophylla*) samples vended in various markets in Enugu State, Nigeria was investigated. The samples were further examined to determine the effect of post treatment with brine and steam respectively. A total of 40ugba (*P. macrophylla*) samples were obtained from different food vendors in the selected markets in Enugu metropolis, Enugu state, Nigeria. Cultures were done on Salmonella-shigella agar and characterized by standard microbiological methods. Post treatment with brine involved rinsing 5g of *P. macrophyla* samples in three changes of brine solution while post treatment using steam was done by boiling5g of *P. macrophyla* samples for 10 mins to an internal temperature of 30°C, 60°C, 75°C and 90°C and subsequently cultured on Salmonella-Shigella agar by pour plate method. Antibiogram of the isolates were determined by using disc diffusion method and plasmid DNA was extracted using plasmid alkaline lysis method and separated by agarose gel electrophoresis. *Salmonella* spp. was recorded at high prevalence of 95%. The isolates showed varied resistance to different antibiotics; amoxicillin (50%), augmentin (100%), streptomycin (61%), septin (95%), gentamycin (45%) and chloramphenicol (68%). Multiple antibiotic resistant was observed in 26% of the isolates. Agarose gel electrophoresis showed that all the ten selected isolates had single plasmid bands of 20kb size. The results revealed relative similarities between the *Salmonella* isolates but possibility of five clones among the *Salmonella* isolates. At $p<0.05$ steam reduced the microbial load of isolates in *P. macrophyla* than in brine. The results from this study show that *Salmonella* spp. contamination of *P. macrophyla* was high in the study area and isolates were multidrug resistant; therefore food handlers should be properly educated and monitored to ensure compliance to proper food hygiene guidelines and *P. macrophyla* should be steamed prior to consumption to reduce the rate of infection.

Keywords: *Pentactethra macrophyla*, Antibiotic Sensitivity Pattern, Plasmid Profile, *Salmonella* Spp, Multidrug Resistance

1. Introduction

The African oil bean (*Pentactethra macrophyla*) is a native of tropical Africa and belongs to the family Leguminosea. It is popular in Nigeria where it is known by several names such as Apara in Yoruba, Ukpaka or Ugbain Igbo andUkana in Efik [11]. The mesocarpof *P.macrophylla* seed serve as food, eaten as snack or dessert or used as condiment. It is prepared by first boiling for about 12 hours, dehulled, sliced, wrapped in plantain leaves and allowed to ferment for a period of 2-4 days at ambient temperatures. It may also be mixed with palm oil, spiced and eaten with cooked cassava chips (African salad) or with roasted yam. *P.macrophylla* has been reported to be rich in protein (48%) and fatty acids [14]. There is mass production of ugba (*Pentactethra macrophyla*) in various parts of the country especially in Eastern states,

Nigeria. Consumption rates are high due to its nutritional qualities but no effective steps are taken to monitor the quality of the ready to eat ugba (*P. macrophyla*) and no guidelines have been prescribed for its processing in retail markets.

Salmonella is a Gram-negative bacterium belonging to the family Enterobacteriaceae, and known as "enteric" bacteria. *Salmonella* is a ubiquitous bacterium capable of surviving in dry and wet environments. While a few serotypes are host specific and can reside in only one or few animal species, others have a wide range of hosts. *Salmonella* have been considered as one of the most important food-borne pathogens around the world [1, 15].

Salmonellosis is often associated with gastroenteritis; characterized by acute onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting. The organism can slip into the blood stream causing bacteremia and typhoid fever. Salmonellosis is one of the most common and widely distributed foodborne diseases, with tens of millions of human cases occurring worldwide per annum [1]. According to World Health Organization (2015), *Salmonella* is one of the major four global causes of the diarrheal diseases and causes about 1.2 million illnesses. *Salmonella* is most often transmitted to humans through the food chain, with 95% of Salmonellosis cases attributed to the consumption of undercooked or mishandled food [12].

Antibiotics are used to treat infection caused by Salmonellosis but as the pharmaceutical industry is making progress with development of new antibiotics, the *Salmonella* spp. is also making advancement by developing multiple antibiotic resistance, turning it into a superbug. One of the proposed mechanisms for development of resistance is by way of transfer of genes through plasmids; these plasmids are extra chromosomal genetic material of bacteria which are capable of independent replication. These *Salmonella* plasmids may be classified into three groups based on their molecular weights as serovar specific virulence plasmids, higher molecular weight plasmids and low molecular weight plasmids as per [23]. The role of plasmid profiling is that it helps in understanding molecular epidemiology of different outbreaks caused by same bacteria as reported by Orji et al [22] and Rychlik et al [23] A variety of antimicrobial agents have been used to treat salmonellosis. An increasing rate of antimicrobial resistance in *Salmonella* has been reported in many developing and developed countries [24]. Briggs and Fratamsco [8] reported that the frequency of resistance is presumably due to extensive use of antimicrobial agents in human and veterinary medicine. Furthermore, resistance to combinations of several classes of antimicrobials has led to the emergence of Multi-Drug Resistant (MDR) strains that may pass from food to humans [25]. The genes involved in resistance in *Salmonella* are often plasmid-born and therefore potentially transmissible to other pathogenic enteric microorganism with genetic factors which control antibiotic resistance [25].

1.1. Statement of Problem

Salmonellosis is one of the most common and widely distributed foodborne diseases with tens of millions of human cases occuring worldwide per annum [1] with 95% of *Salmonella* cases attributed to the consumption of undercooked or mishandled food. *Pentaclethra macrophylla*is a highly nutritional food often eaten raw in Nigeria and no effective steps are taken to monitor the quality of the ready-to-eat *P. macrophylla,* hence the burden of this work to help deteremine if *P. macrophyla* is a source of Salmonellosis infection in Enugu State, Nigeria.

1.2. Aims of the Study

The present study was undertaken to determine the prevalence, antibiotic resistance pattern and the plasmid profile of *Salmonella* spp isolated from ready-to-eat ugba samples and determine the reduction in the microbial load of *Salmonella* spp in ugba samples after treatment with brine and steam respectively.

2. Materials and Methods

2.1. Media and Reagents

Media that was used include nutrient agar, Muller-hinton agar, nutrient broth, salmonella-shigella agar and peptone water and was purchased from oxoid and prepared according to manufacturer's instruction.

2.2. Sample Collection

A total of forty (40) ready to-eat ugba samples were randomly collected from vendors in Abakpa, Eke, Gariki and Ogbete main markets Enugu. 10 ugba samples each were sampled from each markets and were transferred in sterile bags and taken to Applied Microbiology Laboratory in Enugu State University of Science and Technology for analysis.

2.3. Isolation of Test Organisms

A total of 5g of the ready to-eat ugba samples were taken from each pack, labelled and transferred into a sterile blender and blended with 45mlof distilled water. An aliquot of 0.1ml of the mixed sample was transferred unto Salmonella-Shigella agar by streaking method. Colonies presenting with black colonies on Salmonella-Shigella agar suggest the presence of *Salmonella* spp colonies. The resultant *Salmonella* spp colonies were purified by picking a loopful with sterile wireloop and transferred to Salmonella-Shigella agar and incubated aerobically at 37°C for 24h. *Salmonella* colonies were stored on nutrient agar slants at 4°C for further use. A loopful of each of the *Salmonella* isolates were transferred into sterile 5ml nutrient broth in a test tube and incubated at 37°C for 24hrs. Each of the cultures was then adjusted to 0.5 MacFarland's standard.

2.4. Antibiotic Susceptibility Test

Salmonella isolates were screened for antibiotic susceptibility using the disc diffusion method on Mueller hinton agar plates and incubated at 37°C for 24 h. 10 common antimicrobial drugs were used: septin (SXT) (30µg); chloranphenicol (CH) (30µg); sparfloxacin (SP) (10µg); ciprofloxacin (CPX) (10µg); amoxicillin (AM) (30µg); augmentin (AU) (30µg); gentamycin (CN) (10µg); perfloxacin (PEF) (30µg); tarivid (OFX) (10µg); streptomycin (S) (30µg), (Maxicare medical laboratory). The size of the area of suppressed growth (zone of inhibition) was determined by the concentration of the antibiotics present in the area, therefore, the diameter of the inhibition zones denotes, the relative susceptibility to a particular antibiotic. Inhibition zone diameters were measured after 24h of incubation at 37°C and were interpreted using the guidelines of Clinical and Laboratory Standard Institute (CLSI) (2012).

2.5. Plasmid DNA Extraction

The plasmid DNA from all tested isolates was obtained using Alkaline Lysis method as per [26] with slight modifications. After the confirmatory test has been carried out on the *Salmonella* isolates, aloopful of the *Salmonella* spp. isolated was inoculated into a test tube containing sterile buffered peptone water (BPW). The sample was incubated for 60mins at room temperature. After this pre-enrichment step, 1ml from the homogenate was transferred into 9ml of nutrient broth and incubated at 37°C for 24h for plasmid extraction. The bacterial culture was spinned down at 4500 rpm for 5 mins at 4°C, the supernant was discarded leaving about 100µl with the pellet. The cells were resuspendedin 300µl solution A (25 mMTris, 10 mM EDTA, 0.1NaOH, and 0.5% SDS), and incubated at room temperature for 5 mins. 150µl of Solution B (3.0M Sodium acetate solution) was added, vortexed to mix and incubated at room temperature for 10 mins and spinned at 20,000 rpm for 10mins at 4°C to pellet cell debris and chromosomal DNA. The supernatant was transferred to another eppendorf tube and added 900µl of ice-cold absolute ethanol (solution C), centrifuged at high speed (10,000-12,000 rpm) for 10mins to pellet the DNA, the supernanat was aspirated. 1ml of cold 70% ethanol was added to wash the DNA pellet, the supernantant was aspirated from the DNA pellet by centrifuging at high speed for 10mins. The DNA pellet was air dried for 10mins.

2.6. Agarose Gel Electropheresis of Plasmid

Electrophoresis was carried out in a horizontal gel apparatus. The method followed for agarose gel electrophoresis was as described by [27].

The gel apparatus was set up according to the product manual. 0.5g of agarose was melted into 50ml of 1xTBE (Tris/borate/EDTA) solution to form 1.0% gel, the agarose was cooled to 55°C before pouring. 2µl of a 10mg/ml EtBr (Ethidium Bromide) solution (per 50ml gel) to the melted agarose after it has cooled and mixed. The mixture was poured into the gel tray and allowed to solidify completely for about 15-20min, then the comb was removed from the gel and the gel tray was placed into the electrophoresis tank, 250-300ml of 1x TBE solution was used to fill the electrophoresis box until the gel was submerged. With the micropipette, 10µl of each plasmid DNA sample were added into a separate eppendorf tube, changing tips between samples. Molecular weight standard of 3µl was placed (Lamda DNA digest) into an eppendorf tub. Loading buffer of 3µl was added to each tube (samples and control). The loading dye was mixed with DNA samples. The samples were then loaded into the gel wells, one sample per well. Current (90V) was applied on top of the gel box. DNA is negatively charged and always migrates toward the positive (red) electrode, so the gel is positioned with the loaded wells at the negative electrode (black). The gel was run until the bromphenol blue (BPB) from the sample dye is near the end of the gel. The DNA is visualized under ultraviolet light.

2.7. Post Treatment of Ugba by Brine

Post treatment of ugba using brine was carried out to determine reduction of *Salmonella* isolates on ugba prior to use, 0.85g of NaCl was mixed with 100ml of distilled water. 5g of ready to-eat ugba samples were rinsed in 10ml of brine and transferred into a sterile blender; homogenized and mixed with 45mls of distilled water. This was carried out in three changes of brine solution. 0.1ml of the serially diluted samples (10^1 - 10^5) was transferred onto Salmonella-Shigella agar and cultured by pour plate method.

2.8. Post Treatment of Ugba by Steaming

Post treatment of ugba using steam was carried out to determine the reduction of *Salmonella* isolates on ugba prior to use, 5g of ready to-eat ugba samples were boiled in water at 10 mins to an internal temperature of 30°C, 60°C, 75°C and 90°C and transferred into a sterile blender; homogenized and mixed with 45mls of distilled water. An aliquot of 0.1ml of the serially diluted samples (10^1 - 10^5) was transferred onto Salmonella-Shigella agar and cultured by pour plate method.

2.9. Statistical Analysis

T-test was used in the statistical analysis to evaluate the data. Correlation factors were calculated based on the average of replication and treatment. The p-value at 5% level was calculated based on the standard errors comparisons. The method was followed according to the procedure described by Gomez and Gomez (1984).

3. Results and Discussion

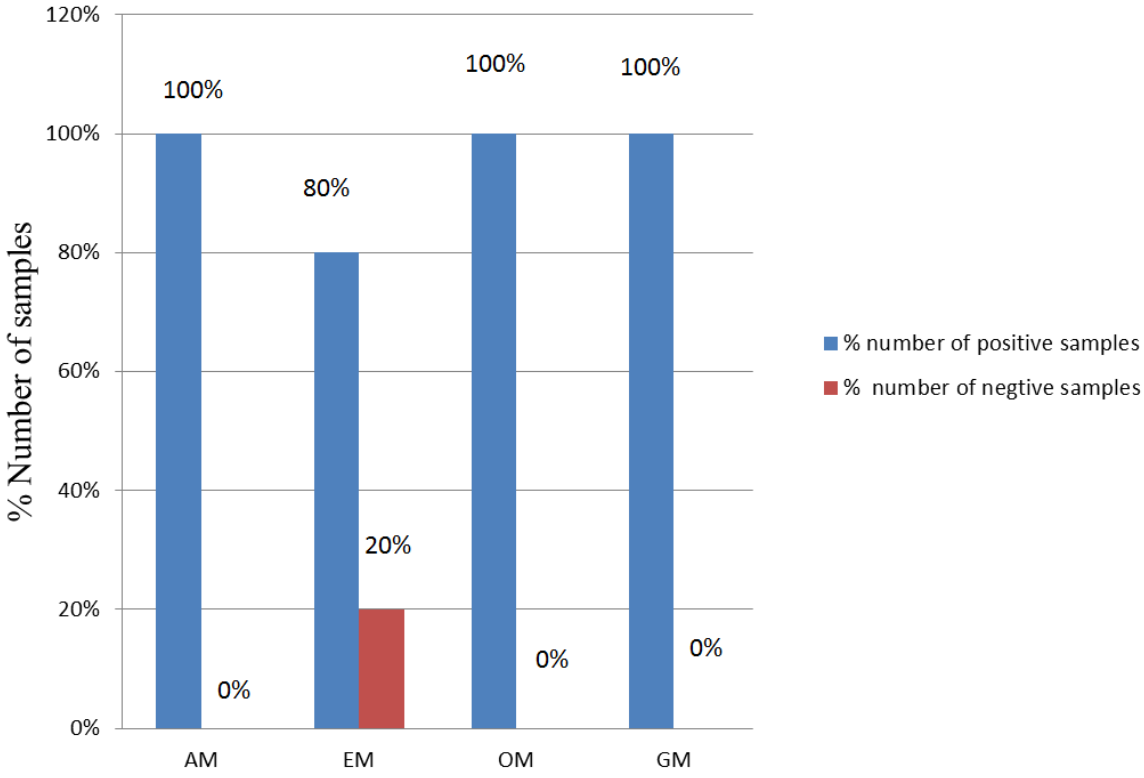

Figure 1. *Prevalence of Salmonella spp. from Different Markets.*

Where:
AM: Abakpa market
EM: Eke market
OM: Ogbete main market
GM: Gariki market

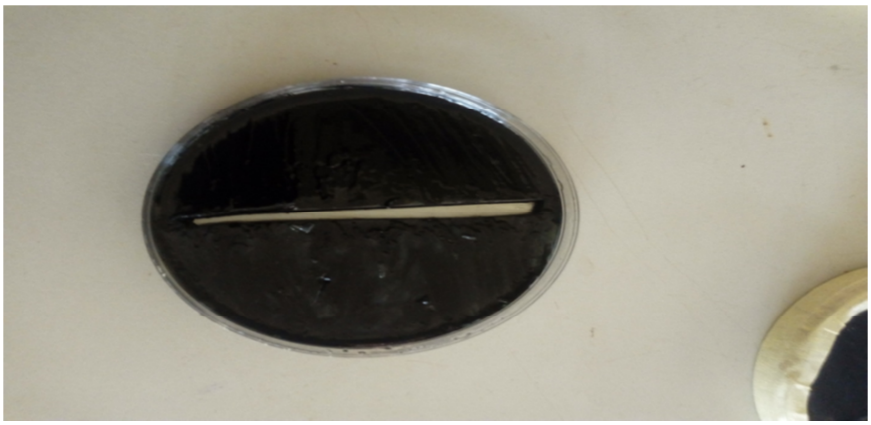

Figure 2. *Shows black colonies characteristic of Salmonella spp. on Salmonella-Shigella agar.*

3.1. Prevalence of Salmonella spp. from Ready to-Eat Ugba from Different Markets

There was high prevalence of *Salmonella* spp. in all the markets with Abakpa, Gariki and Ogbete main markets having the highest prevalence at 100% (Figure 1). Bacteria gains entry into food as a result of inadequate preparation, poor storage conditions or unhygienic handling and preparation [3, 7]. *Salmonella* infection ranks among the leading food-borne infection globally [12]. Similar high incidence of *Salmonella spp* have been reported in various food samples and is suggestive of unclean hands of the vendors, contact with sewage and contaminated water as possible sources of transmission. [9, 10, 20, 21]. This implies that the ugba samples could serve as a vehicle in the transmission of these pathogens to the consumers of these contaminated foods.

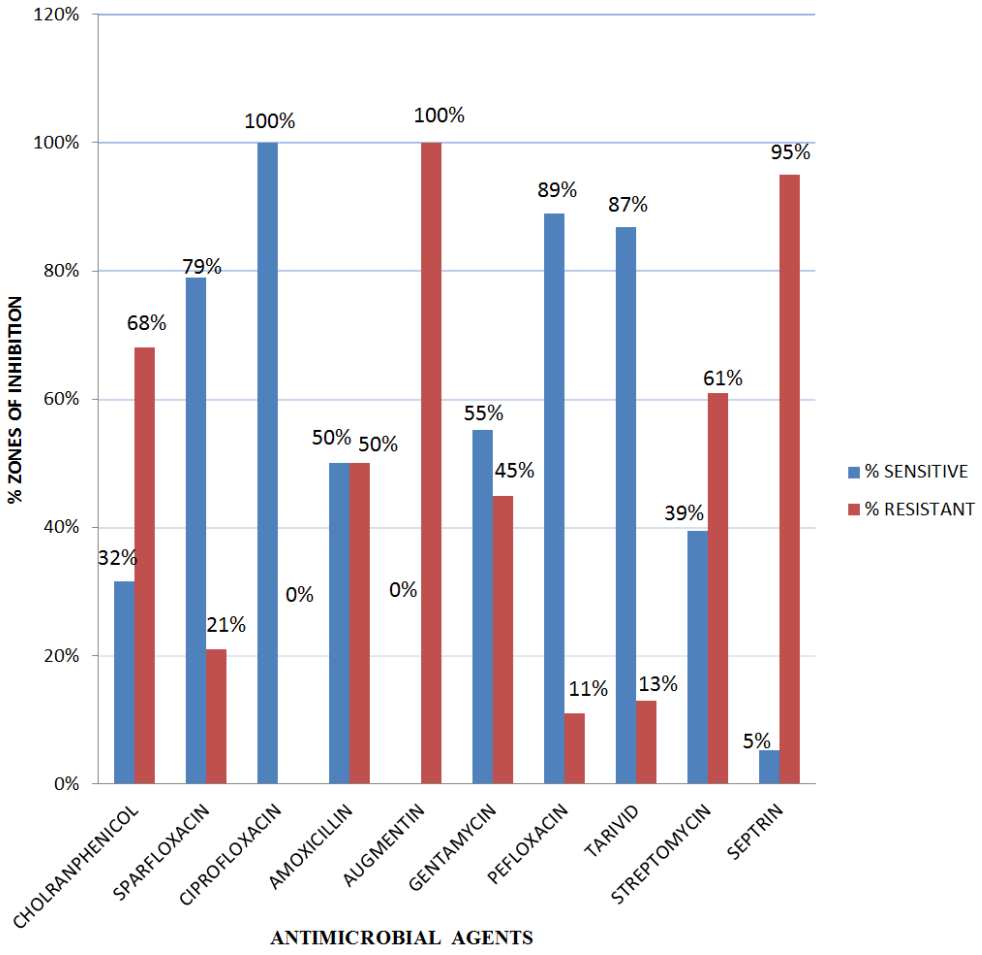

Figure 3. *Resistance and Sensitivity reactions of Salmonella spp. to antimicrobial agents.*

3.2. Antibiotics Susceptibility for Salmonella spp. Isolates from Ready to-Eat Ugba

In the study, the isolated *Salmonella* spp were highly sensitive to ciprofloxacin (100%), pefloxacin (89%), tarivid (87%) and sparfloxacin (75%), moderately sensitive to gentamycin (55%), amoxicillin (50%) and less sensitive to chloramphenicol (32%) and streptomycin (39%). The isolated *Salmonella* spp were completely resistant to augmentin (100%) and septrin (95%). The sensitive patterns to ciprofloxacin and moderately sensitivity to chloramphenicol correlates with [2, 17]. The resistance might be due to routine indiscriminate use of these antibacterial agents in human medicine [18].

Table 1. *Prevalence of Multidrug Resistance among Salmonella spp Isolates.*

Types of Isolates	Resistant to 2 Agents	Resistant to 3 Agents	Resistant to 4 Agents	Resistant to 5 Agents	Resistant to 6 Agents	Resistant to 7 Agents	Resistant to 8 Agents	Resistant to 9 Agents
Salmonella spp. (n=38)	2 (5%)	5 (13%)	10 (26%)	9 (24%)	8 (21%)	1 (3%)	2 (5%)	1 (3%)

3.3. Prevalence of Multidrug Resistance Among Salmonella spp. Isolates

Salmonella isolates showed multidrug resistance at 10 (26%) to 4 agents (Table 1). In the study, multidrug resistance of *Salmonella* to antibacterial agents was determined by *Salmonella* isolates being resistance to four and more antibacterial agent. *Salmonella* isolates showed resistance to 4, 5 and 6 agents concurrently at 26%, 24% and 21% respectively. Antibiotic resistance of *Salmonella* isolates in food samples to some antibiotics such as B-lactam, tetracycline, chloranphenicol and trimethoprim-sulfamethoxazole rising with increasing frequency [6, 10]. This is an important public health problem which may lead to therapeutic failure [10].

3.4. Plasmid DNA Profile of Salmonella spp. Isolates

10 *Salmonella* isolates were characterized by plasmid

DNA profiling. Single plasmid bands which migrated away from the wells were observed. *Salmonella* isolates (strains 1-10) had observable plasmid bands with similar molecular weights while Lane C served as control (Figure 4). In the study, the resistant phenotype of selected *Salmonella* isolates had a plasmid size of 23130bp. The study has similar results with the works of [10] who isolated *Salmonella* with plasmid size of 23130bp size.

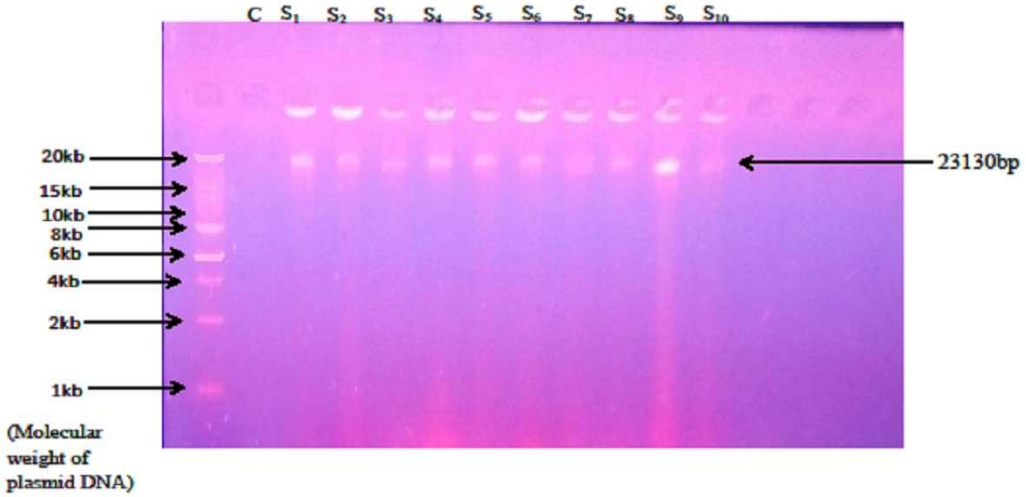

Figure 4. *Plasmd DNA profiling lanes of Sllmonella Spp isolates.*

Key
C: Control Sample
S: Salmonella Spp.

Table 2. *Resistant phenotypic Salmonella isolates and their plasmid carriage.*

Isolates of *Salmonella* spp	Resistance to antibiotics	Number of plasmid bands	Plasmid band size in kb	Plasmid band size in bp
S_1	5 (CH, SP, AM, AU, SXT)	1	20	23130
S_2	2 (AU, SXT)	1	20	23130
S_3	6 (SP, AM, AU, CN, S, SXT)	1	20	23130
S_4	6 (CH, SP, AM, AU, S, SXT)	1	20	23130
S_5	6 (CH, AM, AU, CN, S, SXT)	1	20	23130
S_6	4 (CH, AU, S, SXT)	1	20	23130
S_7	4 (AM, AU, S, SXT)	1	20	23130
S_8	5 (CH, AU, CN, S, SXT)	1	20	23130
S_9	7 (AU,CH,CN,S,SXT,AM,T)	1	20	23130
S_{10}	4 (CH, AU, S, SXT)	1	20	23130

Key:
CH: Chloranphenicol S: Streptomycin SP: Sparfloxacin SXT: Septrin
AM: Amoxicillin AU: Augumentin CN: Gentamycin T: Tarvid.

Plasmids in most cases carry antibiotic resistance genes and the genes are generally transferable to other pathogens [19]. In the study, all the selected isolates had plasmid but varied in their resistance pattern to the antibiotics tested (Table 2). S_3, S_4 and S_5 were resistant to CH,SP, AM, AU,SXT and SP; S_1 were resistant to CH,SP,AM,AU and SXT; S_6 were resistant to CH,AU,S and SXT, S_{10} were resistant to SXT and AU while S_9 were resistant to AU, CH, CN, S, SXT, AM, SP, SEP and T. In the study, all the selected isolates were completely resistant to augmentin and septrin at 100% and 95% respectively (Table 3). According to [16] the plasmid may have likely coded for the resistance of amoxicillin/clavulanic acid among other antibiotics and association between qnrS1A, Tn3-like bla TEM-1-containing transposon has frequently been detected on a conjugative plasmid isolated from *Salmonella*spp isolates.[4] also purported that the presence of plasmid in the *Salmonellaspp* confers resistance to quinolones. [13] have also reported that the development of quinolone resistance has been described not only in some clones of the widespread *Salmonella* enteric serovar Typhimurium definitive phage type 104 but also in some *Salmonella* strains isolated from travelers returning from india and other areas. This is also in line with the work of [10] who isolated resistant phenotype of selected *Salmonella typhi.* The isolates also showed multiple drug resistance to leftazidin, amoxicillin/clavulanic acid, ampicillian, gentamycin, nitrofurantoin and ofloxacin. The occurrences of isolated *Salmonella* strains showing resistance to one or more antibacterial agent have steadily increased probably due to continuous antibiotic pressure [6]. These afore-mentioned reasons maybe related to therapeutic failure. From the study, ciprofloxacin maybe drug of choice in the study area as 100% of the resistant *Salmonella* isolates where completely sensitive to ciprofloxacin at 100%.

Table 3. *Distribution of phenotypic resistant Salmonella isolates in the various markets.*

No of Strains	Phenotypic resistant isolates	Markets isolated from
5	S_2, S_4, S_7, S_9, S_{10}	Abakpa, Ogbete and Eke
2	S_3, S_5	Gariki, Ogbete
1	S_1	Gariki
1	S_8	Eke
1	S_6	Eke

Key:
S: Salmonellaspp.

3.5. Distribution of Phenotypic Resistant Salmonella Isolates in the Study Area

In the study, the resistant phenotypes of selected *Salmonella* isolates varied in the different markets within Enugu metropolis, Enugu state, Nigeria. Resistant *Salmonella* isolates comprising of S_2, S_4, S_7, S_9 and S_{10} with plasmid size of 23130bp were most prevalent in Abakpa, Ogbete and Eke markets; S_3 and S_5 were found in Gariki and Ogbete markets; S_6 and S_8 were found in Eke markets while S_1 was found in Gariki market (Table 3). These markets are the major markets in Enugu metropolis and are patronized by large number of persons making the spread of these resistant *Salmonella* isolates an important public health problem since plasmids are transferrable and may confer resistance to common antimicrobial drugs which may lead to high therapeutic failure. In Nigerian, the lack of economic resources does not allow for wide antibacterial armamentarium and other basal illness such as malaria favour the activities of systemic *Salmonella* infections.

Table 4. *Post Treatment of P. macrophylla (ugba) Samples with brine.*

Ugba Samples	Prior to treatment with brinecfu/ml	Microbial load of ugba samples with brine		
		1st rinsing with brine	2nd rinsing with brine	rinsing with brine
U_1	2.74×10^5	2.47×10^5cfu/ml	1.38×10^4cfu/ml	0.07×10^2cfu/ml
U_2	2.43×10^5	2.50×10^4cfu/ml	2.02×10^2cfu/ml	1.11×10^1cfu/ml
U_3	1.34×10^5	1.89×10^5cfu/ml	1.23×10^4cfu/ml	0.02×10^2cfu/ml
U_4	3.08×10^5	2.95×10^4cfu/ml	1.32×10^2cfu/ml	0.01×10^2cfu/ml
U_5	2.91×10^5	3.05×10^5cfu/ml	2.98×10^3cfu/ml	1.20×10^3cfu/ml

Key
Cfu: Colony forming unit.

Post treatment of P. macrophylla samples using brine.

There was statistical difference at 5% level of significance in the *Salmonella* load between the 1st and 3rd changes (Table 4).

Table 5. *Post Treatment of P. macrophylla (ugba)Samples after Steaming.*

Ugba Samples	Prior to Steaming (cfu/ml)	Microbial Load of Ugba Samples after 10 mins of steaming (cfu/ml)			
		at 30°C	at 60°C	at 75°C	at 90°C
U_1	2.74×10^5	2.20×10^3	1.19×10^3	0.01×10^3	0
U_2	2.43×10^5	2.01×10^4	1.76×10^4	0.13×10^3	0.01×10^2
U_3	1.34×10^5	1.14×10^3	1.45×10^2	0.11×10^1	0
U_4	3.08×10^5	2.99×10^4	1.65×10^4	1.20×10^3	0.01×10^1
U_5	2.91×10^5	2.45×10^5	2.23×10^5	1.39×10^4	0.02×10^2

Key
Cfu: Colony forming unit.

Post treatment of *P. macrophylla* samples using steaming. At 90°C, the *Salmonella* load reduced and there was statistical difference at 5% level of significance after 10 mins of steaming at different temperatures (Table 5).

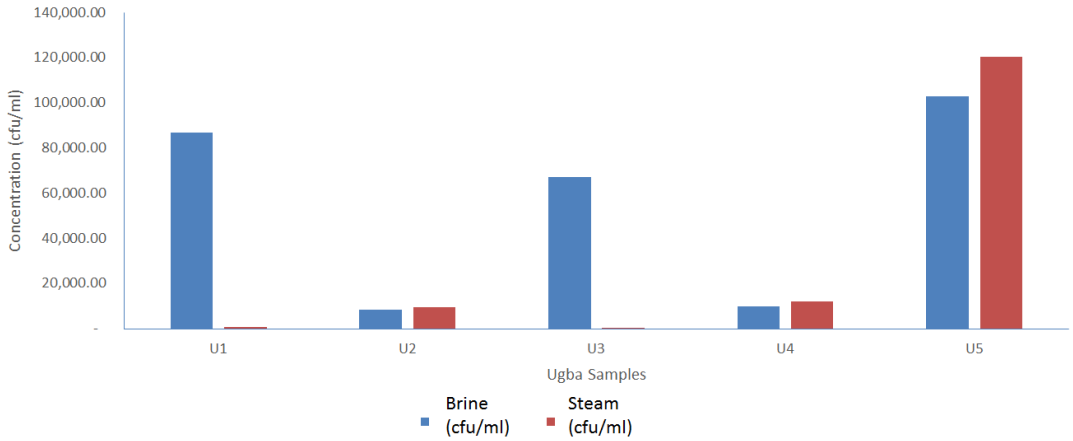

Figure 5. *Statistical effect of post treatments of ugba (P. macrophylla) with brine and steam at 5% level of significance.*

4. Conclusion

It is of significant public health concern that multidrug resistant *Salmonella* strains may constitute a potential reservoir of resistant plasmids. In the study, it can be observed that *Salmonella* spp transmitted through ugba samples were multidrug resistant. The study advocates for prior steaming of ready-to-eat ugba before consumption to help reduce microbial contamination and also proper training of food handlers on both personal and environmental hygiene to also help in reducing contamination.

References

[1] N. P. Achaand B. Szyfres (2003). Zoonoses and communicable disease common to man and animal, 3rd ed., Pan American Health Organization. Washington, DC, Pp. 40-66.

[2] S. J. Adnan (2015). Antimicrobial resistance pattern and plasmid profile of some *salmonella spp*. Isolated from clinical samples in riyadh area. *European Scientific Journal,* 11 (6). Retrieved from https://eujournal.org/index.php/esj/article/view/5131.

[3] Al-Khatib, R. Giacaman, A. Husseini, A. Ramlawi, I. Atiyya and I. Salem (2004). Microbiological quality of food samples from restaurants and sweet shops in developing countries: a case study from the occupied Palestinian Territory. *International Journal of Environmental Health Research*, 14: 443-52.

[4] M. D. Avsaroglu, R. Helmuth, E. Juner, S. Hertwig, A. Schroeter, M. Akcelik, F. Bozoglu, and B. Guerra (2007). Plasmid mediated quinolone resistance conferred by qnrs1 in *Salmonella entericaserovarvirchow* isolated from Turkish food of avian origin. Journal of Antimicrobial Chemotherapy, 60: 1146-1150. Doi: 10.1093/jac/dkm352. Epub 2007 Sep 19. PMID: 17881633.

[5] D. L. Baggase, J. E. Olsen and M. Bisgaard (1992). Plasmid profiles and phage types of *Salmonella typhimurium* isolated from successive flocks of chickens on three parent stock farms. Avian Pathol, 21: 569-579.

[6] A. Brisabois, I. Cazin, J. Breuil, E. Collatz (1997). Surveillance de la sensibilité aux antibiotiquesdes *Salmonella*. Euro Surveill. 2 (3): pii=181. Https://doi.org/10.2807/esm.02.03.00181-fr.

[7] C. K. Nkere, C. I. Ihezie and E. N. A. Mbanaso, (2011). Micropropagation of Yam (Dioscorearotundata): Assessment of Performance in Cassava Starch-gelled Medium. Biotechnology, 10: 551-553. DOI: 10.3923/biotech.2011.551.553.

[8] C. E. Briggs and P. M. Fratamico (1999). Molecular Characterization of an Antibiotic Resistance Gene Cluster of *Salmonella typhimurium*dt104. *ASM Journals*/Antimicrobial Agents and Chemotherapy/ Vol. 43, No. 4. DOI: https://doi.org/10.1128/AAC.43.4.846.

[9] S. Y. Daniyan, and C. Q. Ajibo (2011). Microbiological examination of selected fruit sold in Minna metropolis. *International Research Journal of Pharmacy*, 2 (7), pp 124-129.

[10] O. M. David and A. O. Oluyege (2015). Antibiotic resistance and plasmid carriage among *Salmonella tyhi* isolated from food and hands of food handlers in a Nigerian University. *International Journal of Current Microbiology and Applied Sciences*, 4 (3): 906-914. Http://www.ijcmas.com.

[11] V. N. Enujiugha, C. T. Akanbi (2005). Compositional changes of African oil bean *Pentaclethra macrophylla Benth* seeds during thermal processing. *Pak. J. Nutr.* 4 (1): 27-31.

[12] C. Foley, A. F. Smeaton and H. Lee. (2006). Synchronous collaborative information retrieval with relevance feedback. In collaboratecom 2006 - 2nd International Conference on Collaborative Computing: Networking, Applications and Worksharing, pages 1-4., Adelaide, Australia, IEEE Computer Society.

[13] E. Giraud, A. Cloeckaert, S. Baucherum, C. Mouline, and E. Chaslus-Danda (2003). Fitness cost of fluoroquinlone resistance in *Salmonella enteric serovartyphrimurium*. *Journal of medical microbiology*, 52: 697-703.

[14] C. O. Ikediobi (1981). Amino and fatty acid composition of*pentacleth ramacrophylla* and *treculiaafricana seeds*. *J Am Oil ChemSoc*58, 30–31. Https://doi.org/10.1007/BF02666050.

[15] L. D. Gillespie, W. J. Gillespie, M. C. Robertson, S. E. Lamb, R. G. Cumming, B. H. Rowe (2003). Interventions for preventing falls in eldery people. National library of medicine. Cochrane Database Syst Rev. 2003; (4): CD000340. Doi: 10.1002/14651858.CD000340.

[16] C. Kehrenber, S. Friederichs, A. De Jong, G. B. Michealand S. Schwarz (2006). Identification of the plasmid borne quinolone resistance gene qnrs1 in *Salmonella entericaserovarinfantis, Journal of Antimicrobial Chemotherapy*, 58: 18-22. Https://doi.org/10.1093/jac/dkl213.

[17] M. F. R. Khan, M. B. Rahman, M. S. R. Khan, K. H. M. N. H. Nazir, and M. Rahman (2005). Antibiogram and plasmid profile analysis of isolated poultry *Salmonella* of Bangladesh. *Pakistan Journal of Biological Science*, 8 (11): 1614-1619. DOI: 10.3923/pjbs.2005.1614.1619. Https://scialert.net/abstract/?Doi=pjbs.2005.1614.1619.

[18] National Committee for Clinical Laboratory Standards (NCCL) (1993). Methods for Dilution in Antimicrobial Susceptibility Tests: Approved Standard. Villanova (P. A). NCCLS: M2-A5.

[19] P. Nordmann, and L. Poirel (2005). Emergency of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, 56: 463-469. Https://doi.org/10.1093/jac/dki245.

[20] M. B. Odebisi-Omokanye (2015). Microbiological Quality and Safety of pre-cut fruit retailed in Ilorin, Kwara State, Nigeria. *Fountain Journal of Natural and Applied Sciences*, 4 (1). Retrieved from https://www.fountainjournals.com/index.php/FUJNAS/article/view/52.

[21] O. O. Oluwatoyin, I. O. Afam, J. D. Beswa. Composition and functionality of wheat bran and its application in some cereal food products. First published: 14 November 2015. Https://doi.org/10.1111/ijfs.12935|.

[22] O. Orji, O. E. orinyachinedu, C. Uzoh, U. Ekuma and I. Ama (2016). The microbial contamination of ready-to-eat vended fruits in Abakpa Main Market, Abakaliki, Ebonyi State, Nigeria. *Journal of Pharmaceutical Biology and Science*, 11: 71–80. E-ISSN: 2278-3008, p-ISSN: 2319-7676. DOI: 10.9790/3008-1106017180.

[23] I. Rychlik, D. Gregorova and D. Hradecka. Distribution and function of plasmids in *Salmonella enetrica*. Vet Microbiol, 112: 1-10, (2006).

[24] E. J. Threlfall, B. Rowe, and L. R. Ward (1993). "A Comparison of Multiple Drug Resistance in Salmonella sp from Humans and Food Animals in England and Wales, 1981 and 1990." *Epidemiology and Infection* 111, no. 2: 189-97. Accessed June 23, 2021. Http://www.jstor.org/stable/3863790.

[25] D. J. White, W. A. Take, M. D. Bolton and S. E. Munauchen (2001). A deformation measurement system for geotechnical testing based on digital imaging, close-range photogrammetry, and PIV image analysis. ID: 424679, http://eprints.soton.ac.uk/id/eprint/424679.

[26] H. C. Birnboim (1983). A rapid alkaline extraction method for the isolation of plasmid DNA. *Meth. Enzymology,* 100: 243-56.

[27] T. Maniatis, E. T. Fritsch and J. Sambrook (1982). Molecular cloning: A laboratory manual. Cold spring Harbor laboratory. Cold spring, Harbor, New York.