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DIVERSITY, ANTIBIOGRAM AND PLASMID PROFILE OF MICROBIAL CONTAMINANTS OF SOME SELECTED VEGETABLES SOLD IN BAYELSA NIGERIA

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Abstract: *This study identified 12 microorganisms, including eight bacteria and four fungi associated with different vegetables; cabbage (Brassica oleracea), ugu leaf (Telfairia occidentalis), scent leaf (Ocimum gratissimum), okra (Abelmoschus esculentus), waterleaf (Talinum fruticosum), uziza leaf (Piper guineense), and bitter leaf (Vernonia amygdalina) sold in Otuoke market located in southern Nigeria. Cultural methods were used to quantitatively and qualitatively elucidate fungi and bacteria contaminants in the samples. The average bacteria concentration across all the vegetables was 3.88 X 10^9cfu/g. Abelmoschus esculentus had the highest bacterial concentration of 9.03 (10^9) cfu/g, while Brassica oleracea has the least bacterial load of 1.67 (10^9) cfu/g. The coliform count ranged from 0.5 to 2.63 (10^9) cfu/g for Vernonia amygdalina and Ocimum gratissimum respectively. Mean coliform count was 8.43(10^9) cfu/g. The fungi count varied from 0.5 to 1.5 (10^3) cfu/g for Ocimum gratissimum and Vernonia amygdalina respectively. Biochemical and morphological characterization identified Enterobacter sp., Escherichia coli, Klebsiella sp., Salmonella Sp., Serratia marcescens, Micrococcus sp., Proteus mirabilis and Staphylococcus sp. as key bacterial contaminants of the vegetables. Fungi species isolated from these vegetables include Aspergillus niger, Alternaria sp., Rhizopus sp. and Fusarium sp. The antibiotic susceptibility testing revealed that al the organism isolated were resistant to two or more antibiotics, including a 100% resistance to the penicillin family of antibiotics. Furthermore, all the isolates contained plasmids with a range of 100-200kbp except Salmonella sp., and the fungi; Rhizopus, Fusarium and Alternaria.*

Keywords: *Foodborne infection; Food Safety; Public health; Antibiotics resistance; Food intoxication*

1. Introduction

Antimicrobial drug resistance is a global health challenge that sabotages the attainment of the sustainable development goal of good health [1]. This occurs when bacteria, viruses, fungi or parasites develop mechanisms that make antimicrobials ineffective over time. Currently, the rate at which microorganisms acquire new resistance mechanisms outweighs the development of new drugs [2,3]. Multidrug resistant microorganisms are widespread and have been isolated from all parts of the globe. In Nigeria, several reports have highlighted a high prevalence of antimicrobial-resistant bacteria and this corresponds to the high burden of infectious diseases, including tuberculosis, diarrheal diseases, respiratory infections, urinary tract infections, zoonotic and nosocomial infections [4,5]. In Nigeria, food and water pose a major contributing factor to disease burden, with the country

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ranked amongst those with the highest burden of foodborne illness with its concurrent negative effect on Disability-adjusted life years (DALYs) [6]. Various microbial agents are implicated in foodborne illnesses in the country, with the bacteria genera of *Salmonella, Escherichia* sp., *Shigella* sp., and *Staphylococcus aureus* being the most commonly encountered [7]. Different food products can be contaminated with pathogenic microbial species and serve as a mode of entrance of such pathogens into the population, causing foodborne disease outbreaks. Of importance are vegetables which receive minimal processing and are mainly consumed fresh.

Vegetables are an essential food group consumed daily by a large number of individuals. It may exclude foods derived from some plants such as nuts, and cereal grains but include savoury fruits such as tomatoes, flowers such as broccoli, and seeds such as pulses [8]. Vegetables remain one of the essential parts of a balanced diet because of the many nutritional benefits associated to their consumption. They are rich sources of micronutrients, minerals, vitamins, and most importantly, antioxidants fibre. They are vital to human health, well-being and disease prevention [9]. Recently, the level of public awareness with regards to the benefits of healthy eating habits has increased, prompting an increasing demand for and incorporation of fresh vegetables into diets [10]. The Food and Agriculture Organization and the World Health Organization strongly recommend a daily intake of >400 g/day for fruits and vegetables in diets to promote good health [11]. Despite the health benefits derived from consuming fresh vegetables, the risk of microbiological contamination in vegetables is of public concern due to the possibility of pathogenic microorganisms coming in contact with the fresh food products along the food chain, beginning from the vegetable farm to the dinner table [8]. Microbiological contamination of vegetables can occur directly or indirectly, firstly through contact with soil, dust, and water, and secondly through punctures and open cuts of tissues of vegetables; thus, contaminations of vegetables may occur internally or externally during cultivation, harvest, packaging, and storage, transporting and marketing [9,12,13]. Vegetables are rich in nutrients and serve as a suitable substrate for the growth of invading microorganisms, supporting their growth, leading to spoilage and unwholesomeness of the food. Furthermore, because most vegetables are eaten fresh (not cooked) or slightly cooked, the risks of foodborne infections and intoxication increases as washing may not guarantee decontamination of pathogenic microbial contaminants which can easily enter the alimentary canal through the food. There have been many reported cases of foodborne disease outbreaks caused by consuming fresh vegetables. The most commonly implicated microorganisms are; *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp [14,15]. Foodborne disease outbreaks lead to various illnesses, hospitalizations, deaths, and even food recalls, in all parts of the world [16,17]. Of these, there are increasing cases of antimicrobial drug-resistant foodborne pathogens with far-reaching impacts on public health. A variety of factors influence the development of antibiotic resistance in bacteria. These include the use of antibiotics as growth promoters in farm animals, antibiotics abuse in humans and in food fraud cases where unapproved antibiotics are used in food preservation [18]. Of paramount importance is the possession of plasmids [19]. Plasmids are non-essential extrachromosomal DNA

molecules located in the cell's cytoplasm and can replicate independently of the chromosomal DNA [20]. Because they can be passed to bacteria of the same or different genera through conjugation or transduction and are potential carriers of resistant genes, their rapid dissemination poses a threat to the rapid development of multidrug resistance in a population, thus necessitating the need for plasmid profiling in microbial isolates [21].

There is widespread belief and acknowledgement among most consumers that vegetables sold in the local Nigerian markets are unwholesome. This belief is because vegetables are primarily produced and handled under unhygienic conditions and practice. These unhygienic practices include the application of manure obtained from animal faecal matter to soils during the cultivation of vegetables, irrigation of vegetable farms with heavily contaminated water, use of unclean water to wash vegetables, dressing vegetables on the bare floor and concrete slabs on the farm and at the grocery, and insufficient pre and post-harvest inspections of vegetables [22]. In Otuoke Nigeria, river water is the most commonly used water source to irrigate vegetable farms. This is due to the high cost and unreliability of pipe-borne water supply. Unfortunately, these water sources are heavily polluted, especially with pathogenic and toxigenic microorganisms which may contain plasmids that confers resistance to commonly utilised antibiotics [23-25]. In addition to conducting studies to ascertain the safety of foods sold in local markets such as Otuoke based on microbial contamination, it is important to elucidate the antibiogram of isolated pathogens and analyse for the presence of plasmids which may be conferring such resistance. The present study, therefore, aimed at investigating the microbiological contaminations of vegetable samples including; cabbage (*Brassica oleracea*), pumpkin leaf (*Telfairia occidentalis*), scent leaf (*Ocimum gratissimim*), okra (*Abelmoschus esculentus*), waterleaf (*Talinum fruticosum*), uziza leaf (*Piper guineense*), and bitter leaf (*Vernonia amygdalina*), sold in Otuoke market, their antibiogram and plasmid profiles.

2. Materials and methods

2.1 Study Area and Sampling

The study area, Otuoke, is located in Bayelsa state of Nigeria. Otuoke has one major market where vegetables and other groceries are sold. The collection of the vegetable samples followed a completely randomized experimental design. To minimize experimental error, the samples were collected randomly from each sampling unit in triplicates.

A total of seven (7) vegetable species were collected randomly and in triplicates. These samples include *Brassica oleracea* (cabbage), *Telfairia occidentalis* (ugu leaf), *Ocimum gratissimum* (scent leaf), *Abelmoschus esculentus* (okra), *Talinum fruticosum* (waterleaf), *Piper guineense* (uziza leaf), and *Vernonia amygdalina* (bitter leaf). The samples were put in clean containers, labelled and immediately transported to the laboratory for microbiological analyses.

2.2 Samples preparation

Each vegetable was homogenized in phosphate buffered saline (PBS) into a working solution following the method described by Yafetto *et al*. [26].

Nutrient agar was used for total bacteria enumeration, MacConkey agar to isolate coliforms, and Potato Dextrose Agar (PDA) media was used to isolate fungi from the vegetables. All the media used were commercially purchased (Sigma-Aldrich) and prepared according to the manufacturer's instructions.

2.3 Microbiological analysis

The microbial quality of the vegetables was analysed following the methods described in the microbiological examination methods of food and water laboratory manual for aerobic mesophilic bacteria and coliforms [27]. Standard pour plates of 10-fold serial dilution were prepared in three replicates for all vegetable samples. Ninth dilutions were plated using the pour plate method for the triplicate samples of each vegetable for bacteria, while the third dilution factor was used for fungal isolation.

After 24 hours of incubation at 37^0C, predominant bacteria colonies were sub-cultured using MacConkey agar and nutrient agar plates. Identification of bacteria cultures were done using standard biochemical tests, morphological characteristics and grams' reaction test [28].

2.4 Fungal isolation and identification

The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation [29]. 1 mL aliquot (10^3) of stock solution of the leaves was dispensed into a Petri dish. 20mL of molten but cooled Potato Dextrose Agar (PDA) medium was added and evenly mixed with the stock solution using the pour plate method to identify fungi. The cultured plates were allowed to solidify and incubated at 25°C for seven days in an incubator (Plus II, Gallenkamp, England). The technique of Oyeleke and Manga [30] was also adopted to identify the isolated fungi using cotton blue in lactophenol stain. A small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with a sterile needle. A coverslip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified according to published literature [31,32].

2.5 Antimicrobial susceptibility test

Antimicrobial susceptibility of the isolates was tested using the modified Kirby-Bauer multi discs diffusion method [33]. Commercial antibiotic discs (ROSCO) containing the antibiotics were applied on Muller Hinton agar. The antibiotics evaluated for efficacy against the isolates include; pefloxacin(pef) 10ug, gentamycin(cn) 10ug, ampicillin(apx) 30ug, cefuroxime(z) 20ug, amoxicillin(am) 25ug, Ceftriaxone(r) 25ug, ciprofloxacin(cpx) 10ug, streptomycin(s) 30ug, erythromycin(e) 10ug [34].

2.6 Plasmid profile analysis

Plasmid DNA was isolated as previously described [35]. Briefly, microbial cells were pelleted, and resuspended in E buffer with 40mM Tris-hydroxide and 2mM EDTA. These were subsequently lysed, heated to 60°C for 30 minutes and digested with proteinase K. This was incubated at 37°C for 60 minutes with 1ml phenol-chloroform-isoamy alcohol (Sigma-Aldrich), followed by centrifugation at 8000g for 7 minutes. Plasmid profile analyses of the isolated strains were undertaken by electrophoresis of DNA using 0.8 % agarose gel electrophoresis, stained with ethidium bromide and visualised by a UV transilluminator as described previously [36,37]. Briefly, 1g of agarose powder was weighed out into 100ml of 1XTBE buffer and dissolved by boiling for 3-5 minutes using a magnetic stirrer and water bath. The media was allowed to cool at 50 degrees Celsius and 10ul of Ethidium bromide was added and

gently mixed by swirling. The media was poured into the tank, ensuring that the comb was in place to obtain a gel thickness of about 4.5mm; bubbles were avoided. The media was allowed to stand for 20min to solidify, and then the comb was removed, and the tray placed in the electrophoresis tank. 1XTBE buffer was poured, ensuring that it covered the surface of the gel. 15-20ul of the sample with 2ul of the loading dye was mixed and carefully poured into the well created by the comb with marker and land, followed by the control. The electrode was connected, and electrophoresis was run for 60-100V until the loading dye migrated to the end of the gel field. The electrodes were turned off, and the gel was observed on the UV-illuminator.

3. Results and discussion

3.1 Microbiological Quality of Vegetable Samples

A total of seven vegetables were screened for their microbial quality. The mean coliform, total aerobic bacteria and fungi count are presented in figure 1.

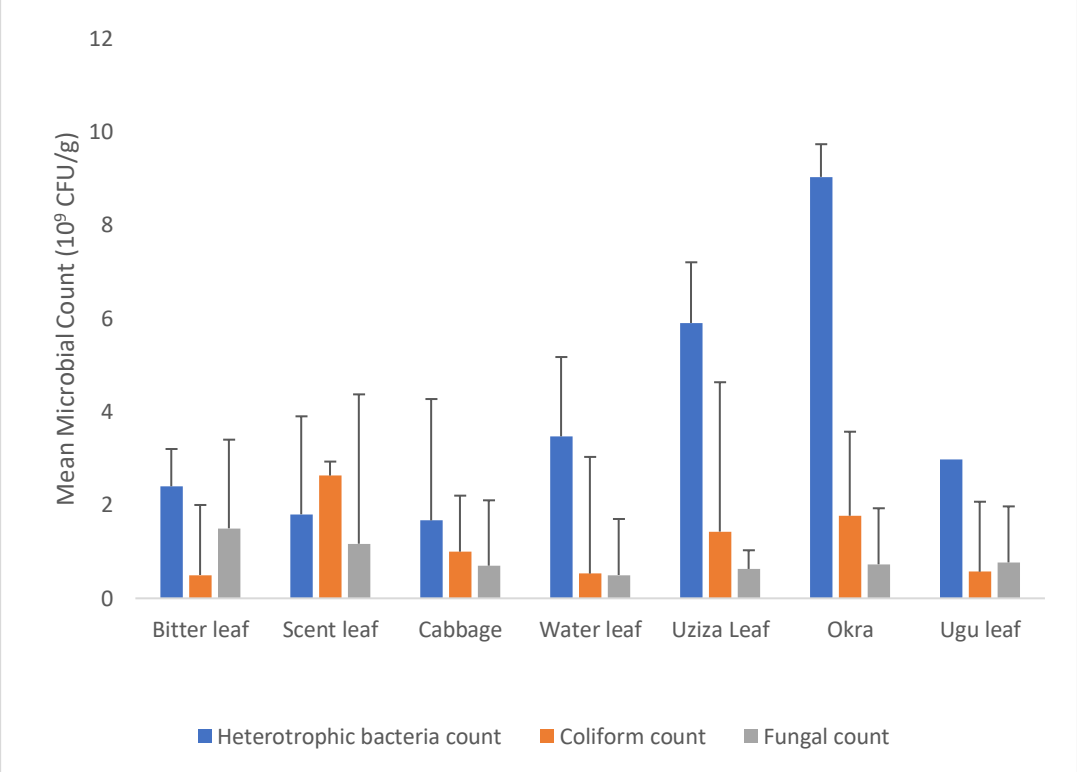

Fig. 1. Mean Microbial Count of Vagetable Samples Randomly Sourced from Otuoke Market

Figure 1 shows that cabbage had the least viable bacteria count of $1.67x10^9$ while Okra (*Abelmoschus esculentus*) had the highest total bacteria count of $2.7x10^9$. The total bacteria count from Okra (*Abelmoschus esculentus*) was higher than previous reports [38] having a total viable count of $1.0x10^5$. The total heterotrophic bacteria count recorded in this work is higher than the specified standard limit of bacteria in foods by The European Commission EC No. 2073/2005 regulation on microbiological criteria for foods [39]. The aerobic bacteria load of cabbage

(*Brassica oleracea*) was the least with a count of $1.67x10^9$, which is higher than what was reported by Obiageli, *et al.* [40] at $1.8x10^7$ in Nigeria. It was observed from this study that there was microbial contamination of all the vegetable samples, and this can be attributed to poor hygiene, soil and environmental contamination. All vegetable samples recorded a high coliform count. The highest coliform count was recorded by scent leaf (*Ocimum gratissimum*) $2.63x10^9$ while the least was observed for bitter leaf (*Vernonia amygdalina*) with a load count of $0.5x10^9$. This result is higher than the load count of $1.8x10^9$ reported in the research of Ofor *et al*. [41]. This high level of microbial load suggests the possibility of faecal contamination through untreated animal manure, poor hygiene or polluted irrigation water. It could also result from human activities such as bathing, washing, and dumping refuse in the rivers where vegetable samples are washed. Previous research [42] has recorded this level of microbial contamination in freshwater apple snails (*Pila ampullaceal*) obtained from the same locale.

Based on the mean colony counts reported for this study, it is evident that the extent of microbiological contamination of the vegetables sold in the Otuoke Daily market is higher than permissible limits and their consumption can pose a threat to public health. This suggests that most of the vegetables sold in the Otuoke community may be unwholesome and the food safety concerns among consumers in the Metropolis are understandable. The high load of bacteria pathogen indicates poor sanitary conditions of vegetables. This is a growing issue as similar high microbial loads have previously been reported from other studies within markets in the southern region of Nigeria [8,43,44]. Thus, urgent action is needed to correct this and prevent the outbreak of foodborne illness within the region.

3.2 Characterization of Bacterial Isolates

The bacteria and fungi isolates were characterized based on morphological and biochemical characteristics. The complete identification was done by comparing viewed characteristics with known taxa [45]. The predominant bacteria isolates include *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., *Serratia marcescens*, *micrococcus* sp. *Proteus mirabilis* and *Staphylococcus* sp.

The isolated bacteria contaminants are of public health significance. The presence of *Escherichia coli* which is an indicator organism, suggests the possibility of faecal contamination of the samples [46]. *Escherichia coli* has been implicated in other studies to be one of the leading causes of diarrheic infection and is one of the superbug organisms [47,48]. *Serratia marcescens*, a pink-pigmented Gram-negative bacterium of the family *Enterobacteriaceae*, was isolated from waterleaf. This organism is notorious for its pathogenicity and toxigenicity, as reported in a previous study [49]. Similarly, *Staphylococcus aureus* contamination is a major public health issue because of its ability to cause a wide range of infections, especially foodborne intoxication [50]. *Staphylococcus aureus* is a gram-positive coccus, a normal flora of the skin and nasal passages; however, when consumed with vegetables becomes opportunistic, and this contamination is through infected vegetable handlers [14]. The two bacteria; *Escherichia coli* and *Staphylococcus* sp. have been significant contaminants of vegetables [51-54]. Similarly, [49] observed the presence of *Serratia mercescens* while working with raw vegetables, with the potential to cause spoilage and gastrointestinal illness. The

presence of *Salmonella* sp. is an indication of polluted water used in washing vegetables. *Salmonella* sp. is of high clinical importance in Nigeria because many studies conducted within the country have reported the organism to be pathogenic, toxigenic, and multidrug resistant (MDR) [55-57].

3.3 Antibiotic susceptibility test of isolated bacteria groups

The antibiotic susceptibility pattern of the isolates is presented in table 1. The isolates showed some level of resistance to all assayed antibiotics. The antibiotic which was most effective in inhibiting the isolates was pefloxacin (PEF), with a sensitivity of 87.5%. Whereas the least effective antibiotics were ampicillin (AMP) and amoxicillin (AM) to which all isolates were resistant. The most resistant isolate is *Klebsiella* sp. which was resistant to 88.8% (8 out of 9) of assayed antibiotics. While the most sensitive organism is *Staphylococcus aureus* which was sensitive to 55.5% (5 out of 9) of the assayed antibiotics.

Table 1

Antibiotics sensitivity pattern of bacteria isolated from vegetables

ISOLATES	CN (10ug)	R (30ug)	AM (25ug)	CPX (5ug)	APX (30ug)	PEF (5ug)	**S** (10ug)	E (15ug)	Z (20ug)
Serratia marcescens	R(10)	R(0)	R(0)	S(42)	R(0)	S(34)	R(0)	R(0)	I(16)
Enterobacter spp	S(16)	I(15)	R(0)	S(38)	R(0)	I(22)	R(0)	R(0)	R(0)
Salmonella spp.	I(14)	R(8)	R(0)	S(40)	R(0)	S(32)	R(0)	R(0)	S(20)
Proteus mirabilis	S(16)	R(0)	R(0)	S(40)	R(0)	S(36)	I(10)	R(10)	S(22)
Escherichia coli	R(12)	R(12)	R(0)	S(36)	R(0)	S(42)	S(20)	R(10)	R(0)
Klebseilla spp.	R(12)	S(22)	R(0)	R(0)	R(0)	R(0)	R(0)	R(0)	R(0)
Staphylococcus spp.	R(0)	R(0)	R(0)	S(40)	R(0)	S(34)	S(36)	S(28)	S(28)
Micrococcus spp	R(12)	R(14)	R(0)	S(38)	R(0)	S(36)	R(0)	R(0)	I(16)

Key: pefloxacin(pef), gentamycin(cn), Ampicillin(apx), Cefuroxime(z), amoxicillin(am), Ceftriaxone(r), ciprofloxacin(cpx), streptomycin(s), erythromycin(e). Resistant (R), Sensitive (S), Intermediate (I).

Overall, it can be observed that all the gram-negative bacteria were sensitive to pefloxacin(pef) and Ciprofloxacin (cpx), except *Klebsiella* sp. A similar report of *Klebsiella* sp. resistance to quinolone group antibiotics was recorded in a recent review [58]. The gram-positive bacteria, *Micrococcus* sp. and *Staphylococcus* sp., were most sensitive to Ciprofloxacin (cpx). They were also

sensitive to pefloxacin (pef) and cefuroxime(z). Similar reports of *Staphylococcus and Micrococcus* sensitivity to Ciprofloxacin has been reported by [59,60]. All the bacteria isolates showed 100% resistance to penicillin (Amoxicillin and Ampicillin). Penicillin resistance of gram-negative bacteria obtained in this study is consistent with reports from previous studies on this growing trend [61]. The resistance of *Serratia marcescens* to multiple antibiotics utilised in this study is consistent with previous experimental results [62] which reported that *Serratia marcescens* was only sensitive to Ciprofloxacin (cpx) and pefloxacin (pef) with resistance to other commonly utilised antimicrobial agents. The result obtained from this assay shows a high prevalence of antimicrobial resistance with all isolated organisms being resistant to more than one antibiotic. This is consistent with the report on disease burden and antibiotic resistance by the Nigerian Ministry of Health which found a high incidence of multi-drug resistance bacteria in Nigeria, consequently increasing the potential mortality and morbidity associated with infectious and disease burden from these organisms [63].

3.4 Characterization of Fungal Isolates

A total of 69 fungi isolates were obtained from assayed vegetables. These were characterised based on their microscopic and macroscopic attributes on potato dextrose agar (PDA), including the colony growth pattern, conidial morphology, and pigmentation as presented in table 2. The technique of Oyeleke and Manga [30] was also adopted to identify the isolated fungi by comparing them with that of known taxa. The presence of fungi in vegetables sold in Otuoke market is an indication of possible deterioration of the vegetable. Isolated species include *Aspergillus niger* (34.78%), *Alternaria* spp. (15.94%), *Rhizopus* sp., (26.09%) and *Fusarium* spp. (23.19%).

Table 2

Macroscopic and Microscopic Characteristics of Fungal Isolates.

Isolate	Macroscopic description (PDA)	Microscopic morphological characteristics
Aspergillus niger	Colonies are initially white, becoming black with conidia production.	Hyphae are septate and hyaline. Conidial heads are radiate. Conidiophores are long, smooth, and transparent, becoming darker at the apex. The conidia are very rough.
Alternaria spp.	The texture of the colony is downy to woolly, with the colour pale-grey to olive-brown on the surface. The reverse is brown to black, and the growth rate is rapid.	Hyphae septate appears brown. Conidiophores are brown, septate, and branched. Conidiophores are scarce.
Fusarium spp.	Fluffy white growth and dark violet pigmentation on the undersurface of the plate.	Conidiophores are short, and non-septate. The conidiophores have a slightly inflated appearance as their sides aren't parallel but slightly bulge out in the middle.
Rhizopus spp.	The texture is deeply cottony, with a white colour which becomes grey-brown on the surface. The growth rate is very rapid.	Hyphae is broad and septate; rhizoid and stolon present, sporangiophores brown, sporangia round; sporangiophores ovoid.

The most isolated fungi specie is *Aspergillus niger* (34.78%) which predominantly contaminated Bitter leaf. The fungi isolated in this study are similar to previous reports [64,65] and highlight that green vegetables present suitable substrates that encourage fungi growth and food deterioration. The presence of a high fungi load in vegetables may be due to poor handling of the products and extended display in the marketplace. Furthermore, fungi can gain entrance into the foods through improper post-harvest handling leading to bruising of the vegetables. Some fungi pathogens isolated such as *Fusarium* sp. and *Aspergillus* sp. are of public health concern as they produce mycotoxins such as fumonisin, zearalenone and aflatoxin, which can cause a wide array of health challenges [66].

3.5 Plasmid DNA present in isolates

Plasmid profiles of the bacteria and fungi isolates were carried out and visualized in a gel electrophoresis field as presented in figure 2. Of the 12 isolates assayed, 8 showed the presence of plasmids, while four (4) isolates showed no visible band indicating the absence of plasmids. In contrast, the other isolates, *Enterobacter* sp, *Micrococcus* sp., *Aspergillus* sp, *Serratia* sp. and *Staphylococcus* sp. showed the presence of multiple plasmids by the visible bands. M is a 1Kb marker of standard molecular weight for calculating the molecular weight of the plasmid DNA [67]. Bacteria are known to develop drug resistance following external environmental pressure, through the direct introduction of antibiotics or resistant strains into the environment or transfer of resistance gene sequence via plasmids [68]. Thus, the results obtained in this gel electrophoresis indicates that resistance to the assayed antibiotics may be mediated by plasmids.

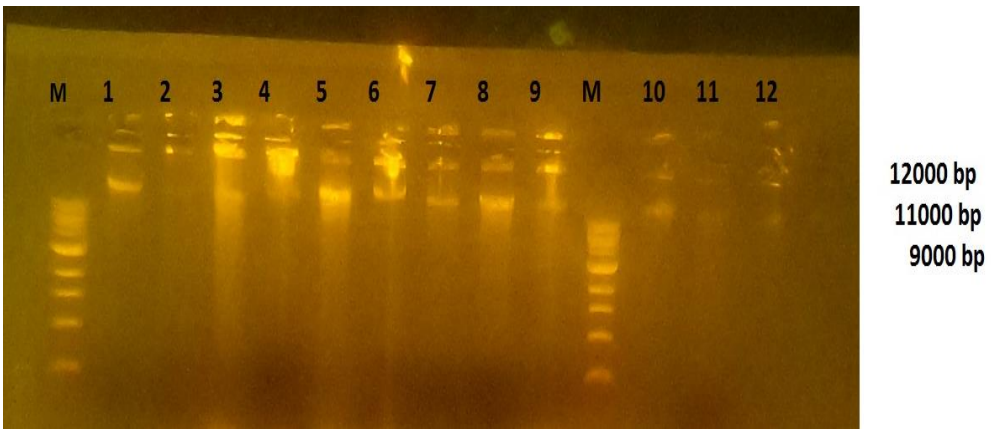

Fig. 2: Plasmid profile pattern of bacterial isolates in 0.5% agarose gel.

Plasmid profile photographic representation indicates the isolates and bands created by each isolate. Each well is represented in codes, while M is a 1Kb marker of standard molecular weight for calculation of the molecular weight of the plasmid DNA.

Codes: 1- *Enterobacter* sp. 2- *Salmonella* sp. 3- *Micrococcus* spp. 4- *Serratia* sp. 5- *Proteus* spp. 6- *Staphylococcus* spp. 7- *Escherichia coli*. 8- *Klebsiella* sp. 9- *Aspergillus niger* .10- *Alternaria* sp.11- *Rhizopus* sp.12- *Fusarium* sp.

All the isolated organisms possessed plasmids except *Salmonella* sp., *Rhizopus* sp, *Fusarium* sp. and *Alternaria* sp. Several researches have shown that bacteria acquire resistance through plasmids [68,69], with others highlighting a positive correlation between the presence of plasmids and bacterial resistance [21]. There is evidence of horizontal gene transfer in diverse bacteria genera, including *Enterobacter* isolates containing plasmids that confer resistance to antibiotics to other bacteria [70]. This is similar to studies conducted on *Serratia* sp. [71]. Additionally, the DNA carried on plasmids can be integrated into bacterial DNA, thus not only conferring the ability to resist antibiotics, but making the resistance genes fully inheritable [21]. In this study, plasmids were identified in the fungi isolates. Although the function of plasmids has not been fully understood in fungi, studies have suggested that plasmids could confer a selective survival advantage to some fungi species of agricultural importance [72].

4. Conclusion

This study highlighted the high microbial load of retail vegetables in Otuoke, Nigeria. The possible sources of these contaminations could be poor handling by sellers, extended stay in the market, use of animal manure for cultivation and washing vegetables with polluted water. The contaminating bacterial and fungal genera are of a public health concern as they are causative agents of numerous foodborne disease outbreaks and food spoilage. The presence of these organisms in retail vegetables can result in economic loss, reduced manpower, food shortages and pose serious food safety challenges, as vegetables are minimally cooked before eating. Plasmid profile study results demonstrated presence of multiple plasmids especially in *Enterobacter* sp. *Micrococcus* sp. and *Alternaria* sp. The presence of plasmids indicates the possibility of multidrug-resistant bacterial presence, as has been demonstrated by literature and in the antibiogram assay of this study in which all the isolates were shown to be resistant to two or more antibiotics. The result obtained in this study is of public health concern and highlights the need for systematic approach in the control of microbial contaminants in foods as this may be a potent source of antimicrobial drug-resistant microbial strains into the population. Similarly, the use of antimicrobials in Nigeria needs to be further regulated to curb the spread of antimicrobial resistance. Further research is required to determine all primary sources of antibiotic resistance in microbial contaminants of vegetables. The collation of data on the prevalence and type of antibiotic resistance in microorganisms isolated from vegetables may help direct public health efforts in the fight against multidrug resistant pathogens. It is recommended that vegetable farmers employ the use of pipe-borne water or chlorinated water instead of water from streams, wells, and storm drains to irrigate or wash their vegetables and observe good pre-and post-harvest handling practices for vegetables. Furthermore, vendors should practice good personal and environmental hygiene at the markets. Traders and consumers must thoroughly wash vegetables before sale and consumption, respectively. The vegetable producers (farmers) and retailers should improve storing, handling, transporting, and preserving their products to free them from pathogenic microorganisms.

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