World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

MOLECULAR CHARACTERIZATION OF BACTERIA FROM EDIBLE VEGETABLES SOLD IN NNEWI MARKETS, ANAMBRA STATE

Nwachukwu Anthonia Chinwe*[1], Ekejindu Ifeoma M.[2], Ochiabuto Ogochukwu M. Barbara[2], Okwelogu Izunna Somadina[3] and Okpara Vivian Chinenye[4]

[1]Department of Medical Laboratory Science, Chukwuemeka Odumegwu-Ojukwu University, Amaku, Awka, Anambra State.
[2]Department of medical Laboratory Science, Nnamdi Azikiwe University, Awka, Nnewi Campus, Anambra State.
[3]Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka Campus, Anambra State.
[4]Medical Centre, Nnamdi Azikiwe University, Awka Campus, Anambra State.

***Corresponding Author: Nwachukwu Anthonia Chinwe**
Department of Medical Laboratory Science, Chukwuemeka Odumegwu-Ojukwu University, Amaku, Awka, Anambra State.

Article Received on 20/01/2020 **Article Revised on 10/02/2020** **Article Accepted on 01/03/2020**

ABSTRACT

Vegetables are dietary source of nutrient, vitamins and fiber for human growth and development cultivated with irrigation water and sewages which can cause contamination. A cross sectional study on molecular characterization of bacteria from edible vegetables and tubers sold in Nnewi markets in Anambra State was conducted. A total of 360 vegetable samples obtained from 8 kinds of vegetables from two markets (Nkwo-edo and Orie- agbor) were used. Bacterial contamination was accessed by serial dilution- pour plate method, identified and significant count done. Some bacteria were further identified molecularly with Proteinase K enzymatic method, sequenced according to NCBI blast nucleotide sequence, and blasted with program selection optimized for highly similar sequences (Megablast). Phylogenetic tree was constructed using Neighborjoining method in the Geneious package (version 9.0.5). Statistical analysis was done using SPSS version 21.Results revealed that out of 360 vegetables sampled, 17 (9.2%) were contaminated with bacteria which include *Escherichia coli 4(4.1%), Pseudomonas species 5(1.4%), Enterococcus gallinarum 4 (1.1%), Proteus species and Klebsiella pneumonia 3(0.8%), Enterobacter species 2 (0.5%), Alcaligenes feacalis, Enterococcus casseliflavus, Sporosarcina contaminans, and Ochrobacterum anthropi 1(0.3%).* Highest occurrence of bacteria in vegetables from Orie-agbor market and Nkwo-edo markets were *Proteus sp.* 1(6.6%) and *Pseudomonas sp.* 3(1.6%), while most contaminated vegetable were *Daucus carota* (carrot) 3 (13.6%) and *Latuca sativa* (Lettuce) 4 (17.4%). Phylogenic analysis of bacteria based on nucleotide sequence of part of the 16srRNA showed numbers of occurrences of repetitive groups with T1_907-R(C02.8.abI , T2_907-R(D02.11.abI;T3_907-R(E02.14.abI;T4_907-R(F02.1`7.abI, T6_907-R(G02.20.abI, T7_907-R(H08.23. abI, T8_907-R(H02.23.abI ,T9_907-R(A09.03.abI, and T10_907-R(AO3.abI isolates having similar sequence with some stored isolates in the gene bank with several accession numbers.Contaminated vegetables from Nnewi markets contain pathogenic bacteria isolates which can serve as possible source of gastroenteritis. It is necessary that preventive awareness should be created in the area.

KEYWORDS: Molecular characterization, bacteria, edible vegetables, Nnewi Markets.

INTRODUCTION

Access to sufficient amount of safe and nutritious food is the key to sustaining life and promoting good health. Vegetable gives human body the necessary vitamins, fats and oil, minerals in the right proportion for human growth and development (Aminu and Ali, 2017). A hazard-free pleasant environment that promotes healthy living is a fundamental right to every human being (Ahmed, 2018). Vegetables are in contact with soil, polluted water, animal manure and even stool. They can therefore easily become contaminated with bacteria. In many countries, such leafy plants are eaten raw or slightly cooked to preserve the taste. Ready to Eat (RTE) leafy vegetables are minimally processed products considered as foods that can be consumed immediately at the point of sale without further preparation or treatment (Tatsika *et al.*, 2019).

Vegetables are among the food groups implicated with greater frequency in recent years as causative agents of enteric diseases. Generally, vegetable microbiome is recognized as a reservoir of several opportunistic pathogens (Berg *et al.*, 2014). They are colonized by a variety of bacteria and recent outbreaks of human disease

associated with fresh products have shown their vulnerability to colonization by food-borne pathogens. Susceptibility of vegetables is largely due to differential chemical composition such as pH and moisture contents. The higher pH and moisture contents are associated with their greater predisposition to bacterial activities. However, Bekele *et al*., (2017) believed that prevention of contamination remains the most effective way of reducing vegetable borne infections and this can be achieved by proper washing of vegetables, improved hygienic practices of vegetable handlers and improvements in sanitation standards.

Despite new developments in research in the past few decades, it is not too common in N igeria to see researches that used molecular characterization in identification of isolates due to high cost. The necessity and importance for that surpasses the high cost disadvantage. Prior knowledge of the molecular strains of contaminating bacteria would be very advantageous in guiding Food and Agricultural Organization (FAO) and other related bodies in the prevention of emerging diseases.

Between 2000 and 2018, many prevalence studies on bacterial and parasitic infections associated with contaminated vegetables were carried out in Nigeria, particularly in the South East (Ikpeze and Chima, 2017; Ejike *et al.*, 2018). From these studies, it appeared that consumption of contaminated vegetable is widespread in Nigeria. However, there is paucity of data on molecular characterization of these edible vegetables especially, in Anambra State, hence this study.

The aim of the study is to characterize bacterial isolates from vegetables sold in Nnewi markets using molecular technique.

MATERIALS AND METHODS

Research Design

This is a cross- sectional study to isolate, characterize bacteria by molecular techniques from vegetables from markets located in Nnewi. This study was conducted between June and September 2018.

Study Area

This research was carried out in two markets in Nnewi, Anambra State.

Study Population

The study population consists of 8 different edible raw vegetables (tuber, leaves and pod) which include Irish potato (*Solanum tuberosum*), Garden egg (*Solanum* L), Carrot (*Daucus Carota* L), Pumpkin leaves (*Telfairi occidentallis*), Spinach (*Celosta argentea*), Utazi leaf (*Gongronema latifolium*s), Cabbage (*Brassica oleracea*), Lettuce (*Latuca sativa*). A total of 360 listed vegetables above were collected from 20 different stalls selling the listed vegetables. Each of the vegetables were bought from each stall in the two markets in Nnewi (Orie Agbor and Nkwo-Edo Nnewi).

Sample Collection

A total of eight different kinds of vegetables leaves and tubers were procured from rural farmers that brought their vegetables to the selected markets in Nnewi. The samples were collected twice monthly between June, 2017 and September 2017 from wholesellers and retailers between 8 – 10am. One hundred and eighty samples (180) were collected from each selected markets. The specimens were collected into sterile, labeled polythene bags and transported to the post graduate Laboratory, Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus for bacteriological examinations and processed immediately in batches.

Sample Size Determination

The minimum sample size determination was obtained using:

$N = Z^2pq/d^2$ (Naing, 2006).

n = Minimum sample size,

Z = Standard normal confidence interval, usually set at 1.96.

P = proportion in the target population.

Using prevalence of 25.8% (Ikpeze and Chima, 2017).

q = 1.0 – p (that is, 1-0.258) = 0.742,

d = degree of accuracy, usually set at 0.05

Therefore: $n = \frac{(1.96)^2 \times 0.258 \times 0.742}{(0.05)^2}$

Sample size = 294. Approximately, 360 selected vegetables were collected during the study.

Ethical Consideration

Ethical approval was obtained from the Ethics Committee, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State.

Sample Processing

Vegetable homogenates were cultured using surface streaking as described by Jay(2005) as described below:

Prior to culturing, Ten (10g) of each vegetables and tubers were washed and pre-weighed. The Vegetables and tubers were crushed in a sterile plastic mortar. Sterility of the mortars were confirmed after hot-oven sterilization by culturing a portion of mortar, incubating and assessing it for any growth. The weighed crushed vegetables and tubers were homogenized with 90ml of sterile Phosphate buffered saline (PBS) to make a concentration of 1:10 dilution (ten-fold serial dilution).Finally, 0.1ml of the dilution was inoculated onto MacConkey agar (NEOGEN, UK), chocolate agar, blood agar, EMB agar, Bile Esculin Agar (Himedia) and TSAagar (OXOID, USA) in duplicate platesusing a standard wire loop of 0.02µl and incubated for 48hours at 37^{o}C. After incubation, the positive growths were

counted and results recorded as Colony forming unit per miles (cfu/ml).

Total Plate Count of Bacteria (CFU/ml)
Microbial load in each vegetable sample was determined as CFU/ml and was calculated using formula described by Prescott *et al. (*2002).
Cfu/ml = {(No. of colonies X dilution factor) / volume of inocula

Isolate Identification
1. Olonial morphology: Cultural morphologies of each isolate was assessed visually and the species tentatively identified by their physical colonial appearances.
2. G Ram staining technique (Cheesbrough 2010).
3. B Iochemical tests (Cheesbrough 2010).
4. DNA extraction (Miniprep plus kit/genomic).
5. PCR (polymerase chain reaction).
6. Sequencing.

Gram stainig technique
The Gram staining reaction was used to differentiate the isolates as Gram positive or Gram negative bacteria.

Biochemical Tests
Catalase test
The test was used to establish the catalase activity of pathogenic bacteria

METHODS

Two or three ml of the hydrogen peroxide solution was poured in a test tube. A sterile wooden stick was used to remove some colonies of the test organism and immersed in the hydrogen peroxide solution. Care was taken not to take the agar alongside the organism. Bubbles were checked for immediately.

Oxidase test
Method
The strip was moistened with a drop of sterile water. Using an applicator stick, a colony of the test organism was rubbed on the strip**.** Red purple colour was checked for within 20 seconds

Urease Test:
Method Using Christensens (Modifid) Urea Broth
The test organism was inoculated in a bijou bottle containing 3ml sterile Christensen modified urea broth**.** It was incubated at 35 to 37^{o}C for 24 hours**.** A pink colour was checked.

Kligers Iron Agar (Kia) /Tripple Sugar Iron (Tsi)
This was used for the differentiation of enterobacteriaceae and other gram negative rods.

Method
A small amount of growth was harvested with a sterile (1ul) needle; the surface of the agar slant was lightly inoculated. A single stab was made into the butt of the tube. Tubes were incubated under aerobic conditions at 36 ±10c with caps loosened. Tubes were examined and results recorded at 24hrs, 48hrs for 5-7 days (unless H_2S production occurred sooner).

Procedure of Voges–Proskauer (VP) Test
Prior to inoculation,the medium was allowed to equilibrate to room temperature. Using organisms taken from an 18-24 hour pure culture, themedium was lightly inoculated. Incubated aerobically at 37^{0}c for 24 hours.Following 24 hours of incubation, 2 ml aliquot of the broth was added to a clean test tube, the remaining broth wasre-incubated for an additional 24 hours, six (6) drop of alpha-naphthol was added and mixed well to aerate, two (2) drops of 40% potassium hydroxide, was added and mixed well.The tubes were checked vigorously during the 30-minutes period. . pink-red color was observed at the surface within 30minutes,

Molecular Study
The Molecular study was done in Inqaba Biotechnical Industries Ltd, South Africa.

DNA Extraction
Two hundred (200µl) sample was added to a micro centrifuge tube. 200µl Biofluid (Lyses the cell to release the nucleic acid) and cell buffer were added. Twenty (20µl) Proteinase K (Digests the other cells in sample leaving only DNA) was also added, thoroughly mixed and incubated in a tube at 55ºC for 10 minutes. One volume of Genomic Binding Buffer was added to digest the sample. It was vortexed /mixed again thoroughly (equal volume of genomic binding buffer and digested sample).

The mixture was transferred to a Zymo-Spin ™ IIC-XL column in a collection tube, Centrifuged at 12,000 xg rpm for 1 minute. Collection tube was discarded with the flow through. Four hundred (400µl) DNA pre-wash buffer was added to the column in a new collection tube and centrifuged for 1 minute, then collection tube also emptied. (Pre-wash buffer contained 95% alcohol since DNA is not soluble in it).Seven hundred (700)µl g-DNA wash buffer was added again and spun for 1 minute, emptying the collection tube. Another 200µl g-DNA wash buffer was again added, spun for 1 minute, and then collection tube discarded with the flow through.

To elude the DNA, (rate limiting step), fluid was transferred to a clean 1.5 ml micro centrifuge tube. Greater than or equal to(≥)50µl DNA Elution Buffer was added, incubated for 5 minutes at room temperature, and then spun for 1 minute.

Polymerase Chain Reaction
12.5µl of One Taq Quick-Load 2X Master was mixed with Standard Buffer (NewEnglandBiolabs Inc.); 0.5µl each of forward and reverse primers,Universal primers (27F;5'-AGAGTTTGATCMTGGCTCAG -3' and 5'-1492R TACGGTTACCTTGTTACGACTT -3); 8.5µl

of Nuclease free water and 3µl of DNA template was used to prepare 25µl reaction volume of the PCR cocktail.

The reaction was gently mixed and transferred to a preheated thermalcycler.

PCR Amplification

Amplification conditions for the PCR was as follows: 2 min at 95oC to denature the DNA, followed by 30 cycles of denaturation at 95Oc for 30secs, primer annealing at 60oC for 30secs and strand extension at 72^{o}C for 1min on an Eppendorf nexus gradient Mastercycler (Germany). PCR products were separated on a 2% agarose gel and DNA bands were visualized wit h syber gold.

Agarose Gel Electrophoresis

Ten microliter (10µL) of the PCR product was analyzed using 1% agarose gel electrophoresis and stained with ethidium bromide. 1% Agar rose gel was prepared by measuring 100ml of TBE buffer into a flask. 1g of agarose gel powder was added to the buffer (TBE buffer), heated for 3 minutes in a microwave to dissolve the powder. It was allowed to cool to 56^{o}c. 5µl of ethidium bromide was added to it to stain it, and the molted gel solution mixture poured in a gel mold in which the gel comb has been appropriately inserted. Agarose was allowed to solidify for 45 minutes at room temperature.

Electrophoresis was carried out by loading 10µl of the ready-to-use DNA ladder (molecular marker) in the first well of the solidified gel immersed in TBE buffer in gel electrophoresis chamber. It was run at 90 Volts for 60 minutes and then viewed under gel documentation system with UV Tran illuminator.

PCR Product Purification

PCR product was purified by adding 20 ul of absolute ethanol to the PCR product, followed by incubation at room temperature for 15 minutes. Centrifugation was done at 10000 rpm for 15 minutes. The supernatant was decanted. Centrifugation was repeated again at 10000rpm for 15 minutes followed by addition of 40 ul of 70% ethanol. The resultant supernatant was decanted, filtrate air dried. Finally, 10 ul of ultrapure water was added. The product was used to prepare the sequencing reaction.

Sequence Analysis

Nine (9) bacterial isolates were selected for sequencing.

PCR products were cleaned using ExoSAP Protocol as follows

Exo/SAP master mix was prepared by adding the following to a 0.6ml micro-centrifuge tube:
a. Exonuclease I (Catalogue No. NEB M0293L) 20 U/ul 50 µl
b. Shrimp Alkaline Phosphatase (Catalogue No. NEB M0371) 1 U/ul 200 µl

The following reaction mixture was prepared
Amplified PCR Product 10 µl
ExoSAP Mix (step 1) 2.5 µl
It was mixed well and incubated at 37°C for 15 min
The reaction was stoped by heating the mixture at 80°C for 15 min

Fragments were sequenced using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions:
https://www.nimagen.com/products/Sequencing/Capillary-Electrophoresis/BrilliantDye-Terminator-Cycle-Sequencing-Kit/
The labelled products are then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053):
http://www.zymoresearch.com/downloads/dl/file/id/52/d4052i.pdf
The cleaned products are injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50cm array, using POP7:
https://www.thermofisher.com/order/catalog/product/4406016
Sequence chromatogram analysis is performed using FinchTV analysis software:
https://www.softpedia.com/get/Science-CAD/FinchTV.shtml.Comparism of aligned nucleotide sequences was made with those available in NCBI GenBank database using BLAST with program selection optimized for highly similar sequences (Megablast).

Statistical Analysis

The data obtained was organized and subjected to Chi-square statistical analysis using statistical package for the social sciences (SPSS).

RESULTS

Out of 360 vegetable samples examined, twenty-six (26) bacterial isolates were recovered thus giving rise to overall prevalence of 7. 2%.

Molecular identification of selected bacterial isolates from the test vegetables indicated the presence of six (6) species of bacteria isolated; namely; *Alcaligenes faecalis, Enterococcus casseliflavus, Enterococcus gallinarum, Klebsiella pneumoniae, Sporosarcina contaminans Ochrobacterum anthropi*(Fig 1-9).

Table 1: Bacterial isolates from edible vegetables sold in Nnewi markets.

Markets	No. Examined	% Frequency	*Escherichia coli*	*Enterobacter species*	*Alcaligenes feacalis*	*Enterococcus gallinarum*	*Enterococcus casseliflavus*	*Klebsiella pneumoniae*	*Sporosarcina contaminans*	*Ochrobcterum anthropi*	*Proteus species*	*Pseudomonas specie*	Total %
Orie Agbor	176	100	1(0.6)	1(0.6)	0(0.0)	2(1.1)	0(0.0)	2(1.1)	0(0.0)	0(0.0)	1(0.6)	2(1.1)	9(5.1)
Nkwo Edo	184	100	3(1.6)	1(0.5)	1(0.5)	2(1.1)	1(0.5)	2(1.1)	1(0.5)	1(0.5)	2(1.1)	3(1.6)	17(9.2)
Total	360	100	4(4.1)	2(0.5)	1(0.3)	4(1.1)	1(0. 3)	3(0.8)	1(0.3)	1(0. 3)	3(0.8)	5(1.4)	26(7.2)

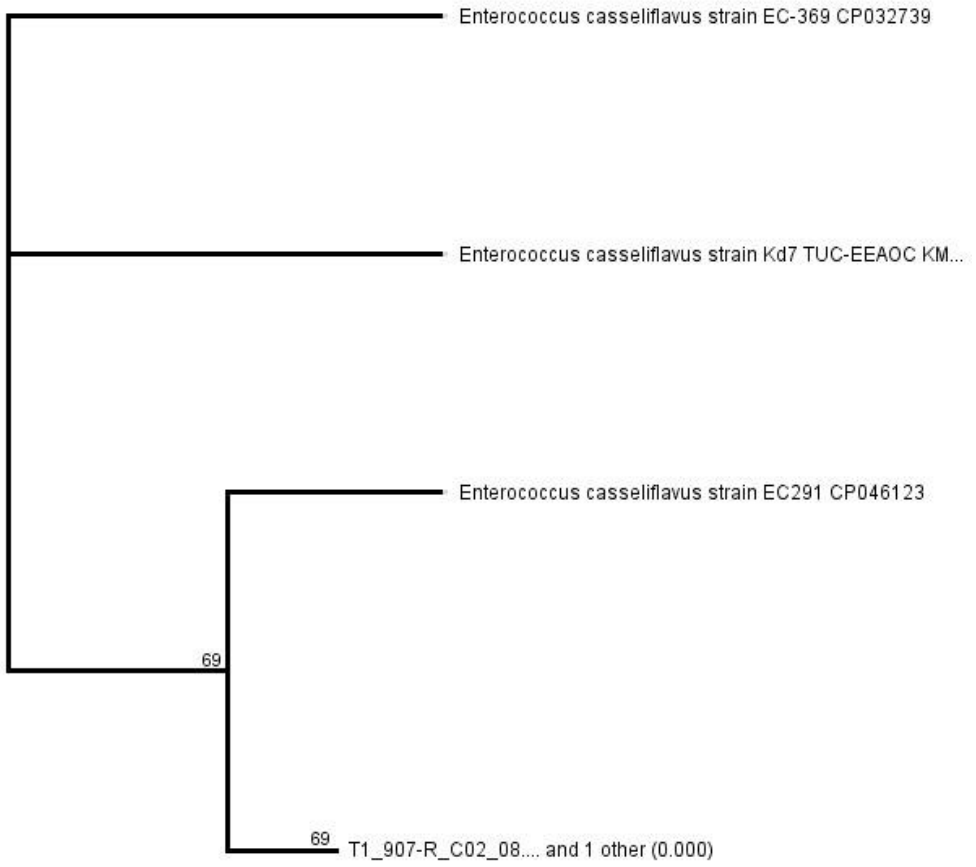


Figure 1: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers of occurrences of the repetitive groups. T1_907-R(C02.8.abIisolates has similar sequence with *Enterococcus casseflavus* strain EC 291with accession number Cp046123.

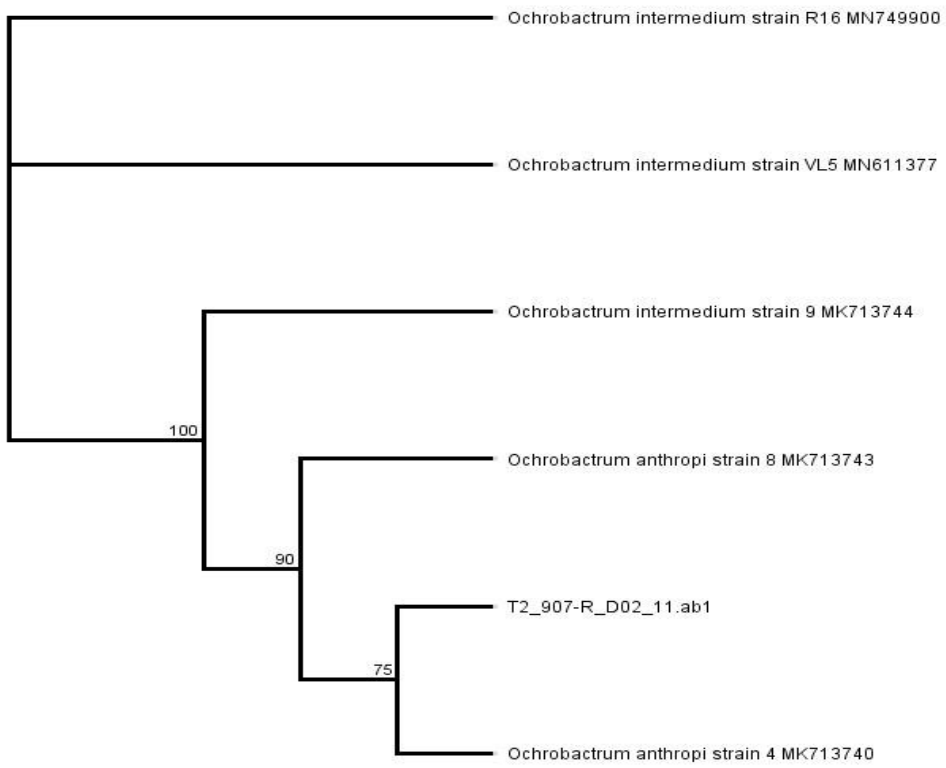


Figure 2: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers of occurrences of the repetitive groups. T2_907-R(D02.11.abI isolates has similar sequence with *Ochrobacterum anthropi* strain 8 with accession number MK713743.

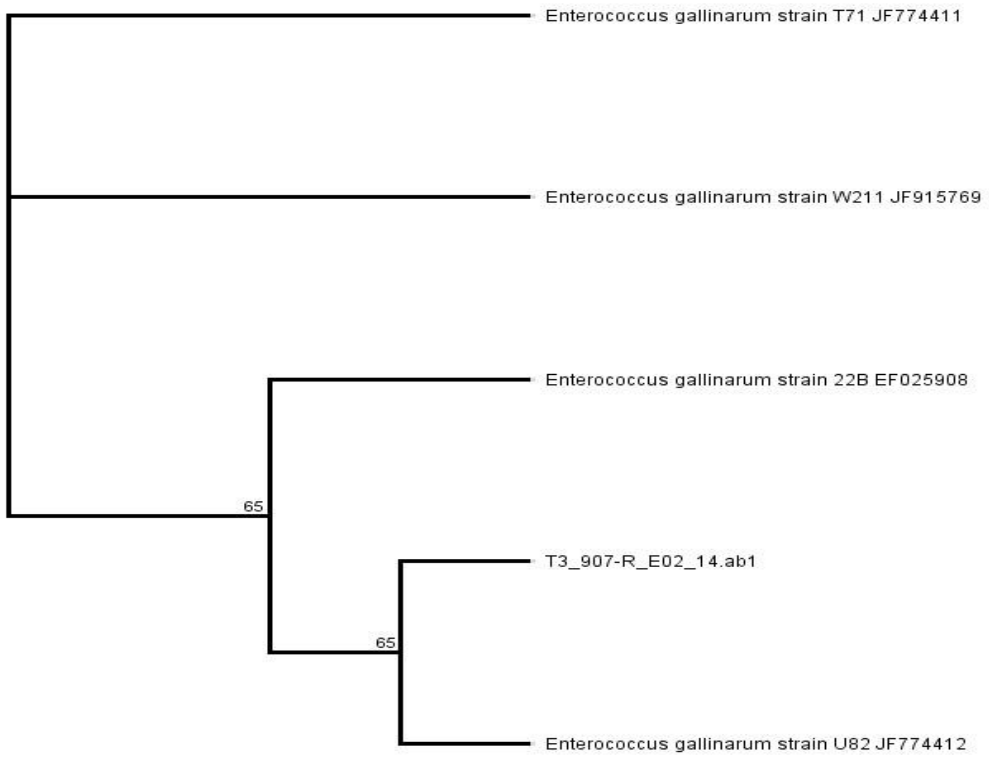


Figure 4: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers of occurrences of the repetitive groups. T4_907-R(F02.1`7.abI isolates has similar sequence with *Enterococcus gallinarum* strain 22Bwith accession number EF025908.

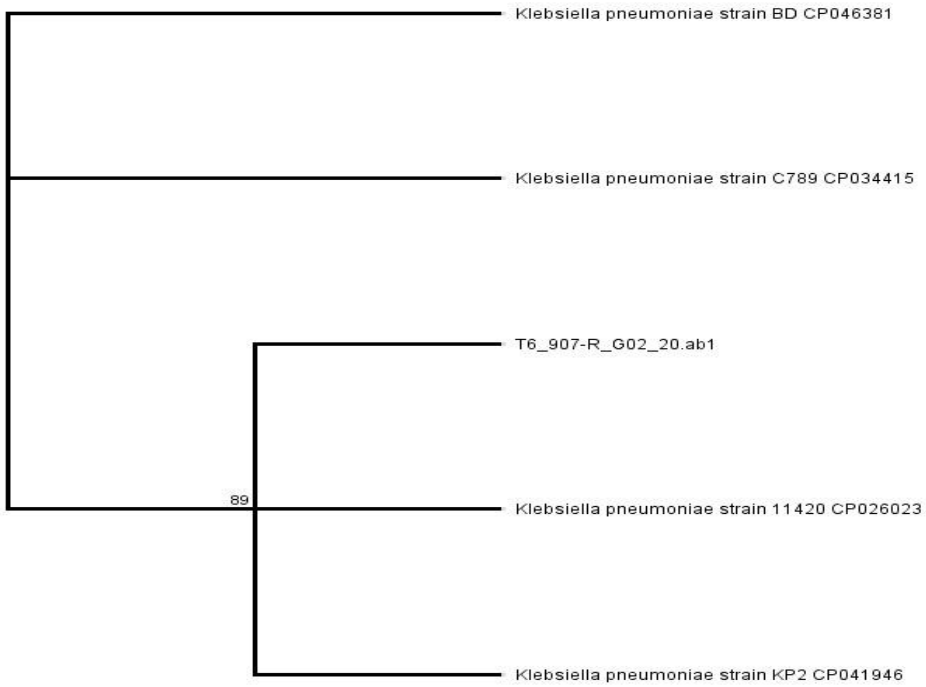
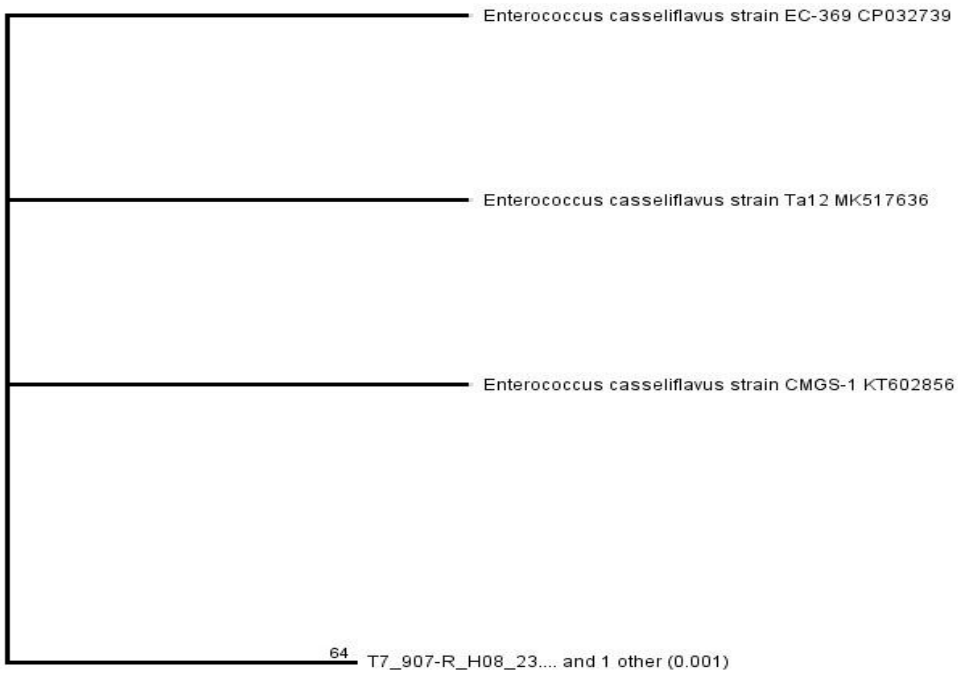


Figure 5: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers of occurrences of the repetitive groups. T6_907-R(G02.20.abI isolates has similar sequence with *Klebsiella pneumonia* strain C789 with accession number CP034415.



Figureb 6: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers of occurrences of the repetitive groups. T7_907-R(H08.23.abI isolates has similar sequence with *Enterococcuscasseliflavus* strain CMGS-1 with accession number KT602856.

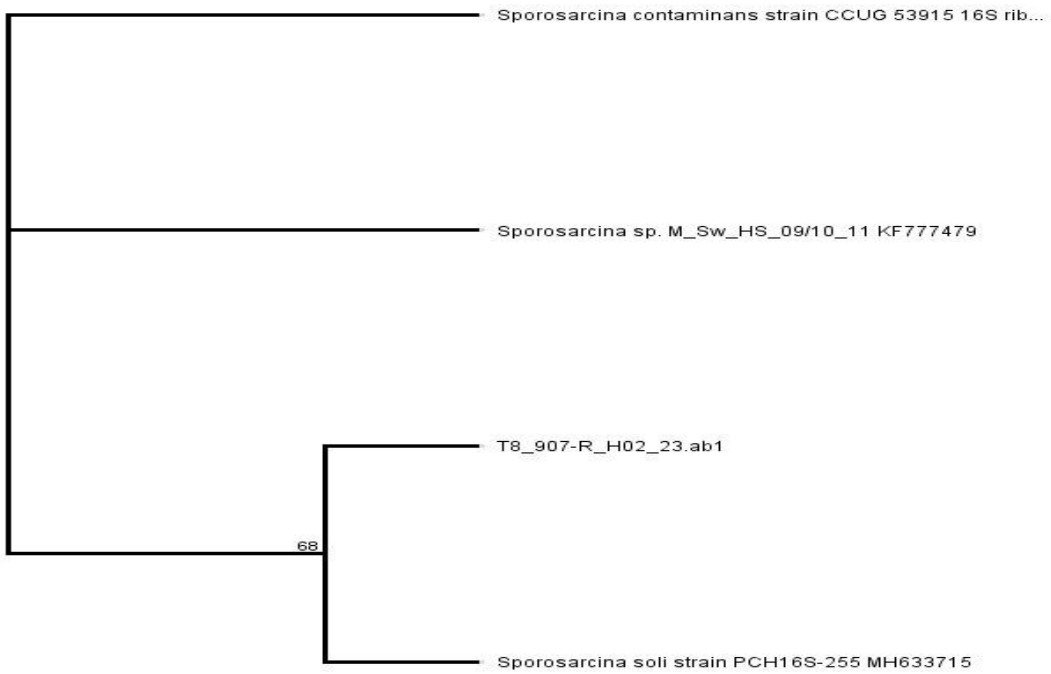


Figure 7: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers of occurrences of the repetitive groups. T8_907-R(H02.23.abI isolates has similar sequence with *Sporosarcina sp.m-sw-Hs-09/10-* 11 with accession number KF777479.

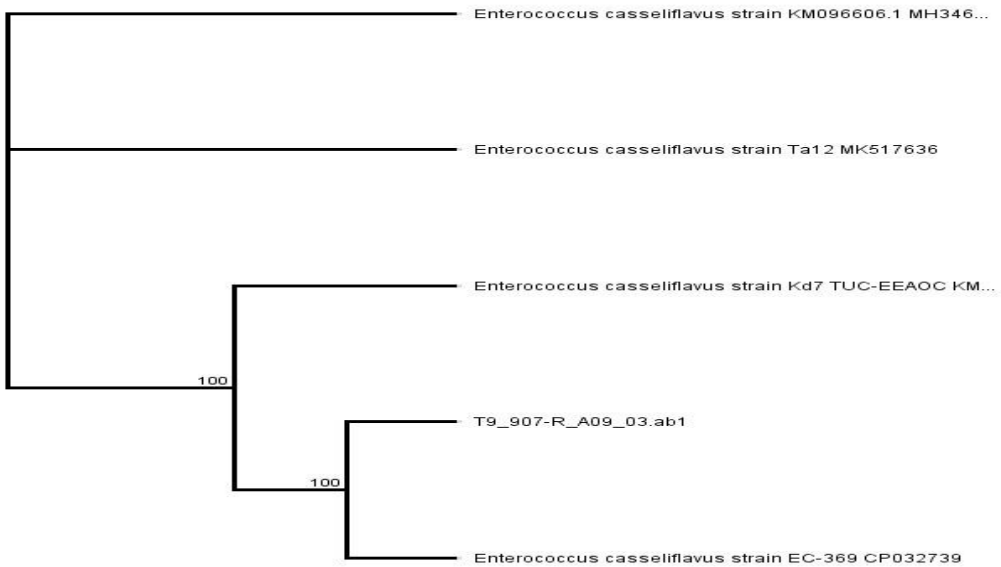


Figure 8: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers of occurrences of the repetitive groups. T9_907-R(A09.03.abI isolates has similar sequence with

Enterococcus casseliflavus strain EC 291with accession number Kd7TUC—EEAOC KM.

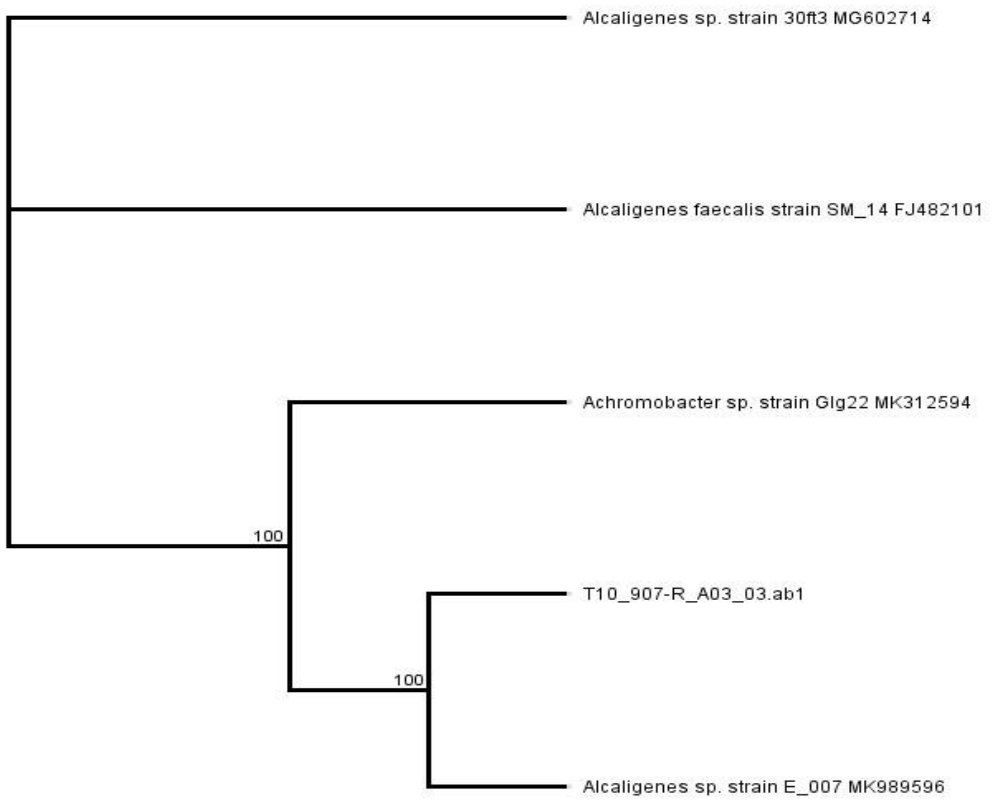


Figure 9: Phylogenetic analysis of a bacterium based on nucleotide sequence of part of the 16srRNA nucleotide sequence of the isolate.

The phylogenetic tree was constructed by Neighborjoining method program in the Geneious package (version 9.0.5).The numbers of the forks shows the numbers ofoccurrences of the repetitive groups. T10_907-R(AO3.abI isolates has similar sequence with *Alcaligenes faecalis* strain SM-1`4 with accession number FJ 482101.

DISCUSSION

Vegetables are extraordinary dietary source of nutrients, micronutrients, vitamins and fibers for human and essential basic material for food industry. Vegetables become contaminated while still on the plant in fields, orchards, during harvesting, transport, processing, distribution and marketing or even at home (Mahari and Kia, 2006). Vegetabes are regarded as harmful when the bacteria load whether pathogenic or not is high even if bacteria are known to be harmful.

This study showed high level of bacterial and parasitic contamination of edible vegetables sold at Nnewi markets.

Ten (10) bacterial species were isolated with *Pseudomonas specie* been predominate. Apart from *sporosarcina contaminans, Ochrobactrum anthropi, Enterococcus gallinarum, Enterococcus casseliflavus, Alcaligenes feacalis.* All isolates are enteric organism indicating faecal contamination from either irrigation source or sewage fertilizers.Contamination may have resulted from contaminated manure, manure compost, sewage sludge, irrigation water, runoff water from livestock operations or directly from wild and domestic animals.

Bacteria isolates recovered from Nkwo Edo market recorded the highest prevalence of 1.6% (*Escherichia coli* and *pseudomonas species)* respectively. While in Orie Agbor market, *pseudomonas species, Klebsiella pneumonia and Enterococcus gallinarum* (1.1%) recorded the highest prevalence. This could be because Nkwo- Edo is larger market, where vendors source their vegetables.

Vegetables may not only be contaminated from the farm, but also from unclean environmental sources as well as vegetable vendors due to poor sanitary status.Oranusi and Braid (2012) also noted the role of vendors as intermediary between the farmer and the final consumer, playing a role in the distribution of fruits and vegetable produce and distribution of contaminants.

Pseudomonas Species was predominately present in the two markets (Orie Agbor and Nkwo-Edo Nnewi). The contamination of vegetables by *Pseudomonas species*

may occur during handling, processing, transit and harvesting. *Pseudomonas Species* is recacitrant survivals, has the ability to form biofilms and has intrinsic antibiotic resistant ability to various classes of antibiotic making them tenatious survivals in the environment despite the presence of antibiotic mold resistance in the soil.It has the ability to thrive in harsh condition as a result of hardy cell wall that contains porins as noted by Ryan and Ray, (2004).*Pseudomonas species* favours its existence in nature. Cornelius,(2008) also noted the hypermutative nature of *Pseudomonas species* .Vegetables may also come in contact with soil, insects and contaminated water. The presence of *Pseudomonas* in animal feces (which is used as fertilizer) is suggested a possible source of contamination.

The most contaminated vegetables in Orie Agbor market was carrot and lettuce in Nkwo Edo market. Carrot is a root crop that grows inside the soil and may have been contaminated by animal wastes used as fertilizers, water used for washing and from handlers. Contamination of this vegetable could be as a result of poor handling and storage.

The most contaminated vegetables in Nkwo Edo was Lettuce. Lettuce leaves have a large surface area (whorls) suitable for air contact and fecal dropping from the birds making it more susceptible to contamination than other vegetables. Its crevices retain dirt containing organism which may not be easily be removed by slight washing, unlike Utazi Leaf which is a simple leaf and has high Amino acis content. Another reason could be the differences in their phytochemical constituents as most phytochemical elements have antimicrobial properties (Burt, 2004).

Highest unacceptable bacteria count observed in leafy vegetables (Spinach, Lettuce, Pumpkin) as well as tuber Vegetable (Irish Potato),could be attributed to their morphology of plant parts as well as other cultivation practices. Uzeh *et al*. (2009) also recorded a bacteria count on the vegetables.Vegetables are regarded as harmful when the bacteria load is high even if the bacteria are not known to be harmful. There is possibility that these sellers may be washing their vegetables with contaminated water collected from river or stream.

CONCLUSION

Conclusively, there is high level of parasitic and bacteria contamination of vegetables from the study site from animal contamination through water and sewage manure. DNA sequencing of isolates proved that isolated bacteria are possible human pathogens and parasites isolated also cause human infestation.

REFERENCES

1. Agbo C.U, Baiyeri K.P, Obi I.U Indigenous knowledge and utilization of *Gongronema latifolium* Benth: a case study of women in university of Nigeria Nsukka. *Biological Research journal*, 2005; 3(2): 66-69.
2. Ahmed H Factors that influence the environmental sanitation on people's health in Nigeria. *Journal of Environmental Science, Toxicology and Technology*, 2018; 12(2): 73-76.
3. Aminu F and Ali M. Isolation and identification of microorganisms associated with spoilage of cabbage (*Brassica oleracea*) in Sabon Gari market Kano, Nigeria. *International Journalof Advanced Academic Research, Sciences, Technology and Engineering*, 2017; 3: 1-8.
4. Bekele F, Tefera T, Biresaw G, Yohannes T Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. *Infectious Diseases of Poverty*, 2017; 6: 19.
5. Berg G, Erlacher A, Smalla K and Krause R Is there a connection among opportunistic infections, human health and our 'gut feeling'? Microbial Biotechnology, 2014; 7: 487-495.
6. Burt S. Essential oils: Theirs antibacterial properties and potential applications in foods. *International journal of food microbiology*, 2004; 94(3): 223-253.
7. Cheesbrough M. District Laboratory practice in tropical countries part I. Cambridge University Press, 2006.
8. Cornelius P. *Pseudomonas*:Geneious and Molecular Biology. First Edition,Caister Academic Press, 2008; 19-26.
9. Ejike B.U, Ejike E.N Ogbonna C.R, Onu E.O Geohelminth contamination of vegetables sold in some markets in Aba North, South East, Nigeria, *Nigerian Journal of Parasitology*, 2018; 39(2): 194-198.
10. Ikpeze O.O and Chima S.C. Soil transmitted healminth Parasites contaminating edible raw vegetables and fruits sold at Nkwo Edo Markets Nnewi, Nigeria. The Bioscientist, 2017; 5(I): 165-72.
11. Jay M.James. Modern food Microbiology, Wayne State University.CBS Publishers and distributors.4th edition, 2005; 79-114.
12. Mahvi A. H and Kia E.B. Helminth Eggs in raw and treated waste water in the Islamic Republic of Iran. *East Mediterranean health journal*, 2006; 12(1-2): 137-143.
13. Oranusi U.S and Braide W. A study of microbial safety of ready-to –eat foods vended on highways:Onitsha Owerri ,Southeast Nigeria. *International Research Journal of Microbiology*, 2012; 3(2): 66-71.
14. Prescott L.M, Harley J .P, Klein D.A. Microbiology, 5th Edition., McGraw-Hill, New York, 2002.
15. Ray K.J, Ray C.G. Sherris Medical Microbiology.Fourth edition.McGraw Hill, 2004; 85-89.
16. Atsika S, Karamanoli K, Karayanm H., Genitsaris S. Metagenomic Characterization of Bacterial Communities on Ready-to-Eat vegetables and

effects of household washing on their diversity and composition. *Pathogen*, 2019; 8: 1-18.
17. Uzeh R.E, Alade F.A, Bankole M The microbial quality of prepacked mixed vegetable salad in some retail outlets in Lagos, Nigeria. *African Journal of food science*, 2009; 3(9): 270-272.