Journal of Advances in Microbiology

21(4): 48-62, 2021; Article no.JAMB.67883
ISSN: 2456-7116

Title Antibiogram of Key Microorganisms of Health Importance, Found on Different Species of Banana (*Musa* spp.)

C. J. Ugboma[1], C. A. Nzurumike[1] and R. R. Nrior[1*]

[1]Department of Microbiology, Rivers State University, Nkpolu-Oroworukwo, P.M.B. 5080, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author RRN designed the study, author CAN performed the statistical analysis. Author RRN wrote the protocol, author CAN wrote the first draft of the manuscript, managed the analyses of the study and literature searches under the strict supervision of authors RRN and CJU. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2021/v21i430341
Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Fernanda dos Santos Nascimento, Universidade Estadual de Feira de Santana, Brazil.
(2) Ksenija Durgo, University of Zagreb, Croatia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67883

Original Research Article

Received 13 February 2021
Accepted 23 April 2021
Published 28 April 2021

ABSTRACT

Aim: The aim of this study was to isolate, enumerate, identify and ascertain the antibiotic profile of the bacterial isolates associated with key body parts (Cut stalk, Tip, Endocarp, Vascular Tissue and Epicarp) of Banana fruits (Cavendish, Dwarf Cavendish, Red, Lady Finger and Grand Nain Banana) collected from Port-Harcourt, Rivers State.
Study Design: The study employs statistical analysis of the data and interpretation.
Place and Duration of Study: Five local markets which includes Oil Mill, Fruit Garden, Creek Road, Mile One and Mile Three Markets, all located in the city of Port-Harcourt, Rivers State were used for this study. Sample collection lasted for a week and the analysis was carried out every day and it lasted for six months.
Methodology: A total of seventy-five (75) banana (Cavendish, Dwarf Cavendish, Red, Lady Finger and Grand Nain Banana) fruit samples were collected randomly, for a period of three months from five different markets (Oil Mill, Fruit Garden, Creek Road, Mile One and Mile Three Markets) in Rivers State. The collected samples were grouped into three groups (Unripe, Healthy-Looking and

**Corresponding author: E-mail: renner.nrior1@ust.edu.ng;*

Spoiled) and were subjected to standard microbiological procedures which includes standard plate counts, identification, sensitivity testing using Kirby-Bauer disk diffusion method and molecular identification of the isolates.

Results: A total of 83 bacteria were isolated from the different sampled parts (Cut stalk, Tip, Endocarp, Vascular Tissue and Epicarp) of the banana fruit samples. 18.88%. *Escherichia coli* showed high level of resistance to Cefotaxime (100%) > Augmentin (94.7%) > Gentamycin (78.9%) > Levofloxacin (10.5%). There was complete resistance to Cefotaxime, Levofloxacin, Imipenem/Cilastatin, Ofloxacin and Azithromycin in all isolates of *Staphylococcus aureus* and susceptibility at 70.8%, 79.2% and 20.8% to Augmentin, Ceftriaxone sulbactarm and Erythromycin, respectively. *Pseudomonas aeruginosa* were susceptible to Augmentin (25.0%), Imipenem/Cilastatin (66.7%), Gentamycin (83.3%) and Ceftriaxone Sulbactarm at 8.3%. While complete resistance was seen in Cefotaxime, Nalidixic Acid, Nitrofurantoin and Cefexime. The antimicrobial susceptibility pattern of all isolates of *Bacillus flexus* which shows complete resistance to Cefotaxime, Ceftriaxone Sulbactarm, Cefexime, Imipenem/Cilastatin and Azithromycin. While susceptibility was seen in Levofloxacin at 100% and 22.2%, 33.3% and 11.1% seen in Ciprofloxacin, Gentamycin and Augmentin, respectively. The isolates of *Proteus mirabilis* showed complete susceptibility in Imipenem/Cilastatin and Cefrtiaxone Sulbactarm and complete resistance in Augmentin, Cefotaxime, Gentamycin, Nalidixic Acid, Nitrofurantoin, Cefuroxime, Ampiclox, Cefexime and Levofloxacin. *Klebsiella pneumoniae* to Levofloxacin and 45.5% susceptibility to Augmentin, Ofloxacin, Ceftriaxone and Ampiclox at 54.5%, 27.3% and 18.2%, respectively. While complete resistance was seen in Cefotaxime, Nalidixic Acid, Nitrofurantoin, Cefuroxime and Cefexime.

Conclusion: Conclusively, this study revealed the Antibiotic susceptibility pattern of the isolated bacteria. Treatment guidelines for use of antibiotics should be formulated based on the hospital formulary and the sensitivity patterns. This should be reviewed occasionally to ensure rational use of antibiotics

Keywords: Antibiogram; cavendish; dwarf cavendish; red; lady finger and grand nain banana; Staphylococcus aureus; Bacillus flexus.

1. INTRODUCTION

Banana (*Musa* spp.) is one of the most widely cultivated tropical fruits in the world, grown in over 130 countries, along the tropics and subtropics of Capricorn. It is the second largest produced fruit after citrus, contributing about 16% of the world's total fruit production and the fourth most important staple food crops in the world after wheat, maize and rice [1]. According to FAOSTAT [2], the major banana-producing countries that contribute about 75% of total banana production are situated in developing countries. In Ethiopia, it is one of the most important fruit in terms of both production and consumption [3]. Also in Nigeria, it's one of the easily assessed fruit in all nook and crannies of each state. Most times it's taken as an appetizer, and in some cases, it's consumed as a main food. Also, it can be incorporated into confectionaries like the common banana cake.

Banana fruits are highly nutritious and easily digestible than many other fruits [4]. Its wide consumption is due to its sensory characteristics, particularly its attractive texture and flavor make banana popular by the consumers [5]. Moreover, it has high caloric contribution leading to high demands mainly by developed countries which account for nearly 70% of world's consumption [6,7]. It also contains low-fat, excellent source of dietary fiber, Vitamin C, Vitamin B_6, and Manganese [7]. The presence of Potassium and fiber in large amounts in bananas may help combat atherosclerosis, which can lead to heart attack and stroke [1]. Almost all types of bananas produced in Nigeria are consumed fresh and play an important role in feeding the low-income families as well as providing a source of income to them. The fact that it is an annual fruit that produces its fruit throughout the year adds to its importance as a cash crop in the growing region [8,9]. Although banana fruits are highly demanded as nutritious and economically important fruits, they face some limitations in post-harvest [10].

One of the limiting factors that influence the fruits' economic value is its relatively short shelf-life caused by postharvest pathogens attack. It is estimated that about 20-25% of the harvested bananas are decayed by pathogens

during post-harvest handling even in developed countries. Metabolic activities of microbes alter the condition of food, resulting in its spoilage [11]. In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contaminations [12,13]. Another factor that affects the fruits is the post-harvest handling, transportation (from the farm to the market or from a farmer (wholesaler) to the retailers), storage and marketing. Bananas are known to be very soft and perishable, and if not handled properly, it may result in decay and production of microorganisms, which become activated because of the change in the physiological state of the fruits [14].

Bananas contain high levels of sugars and nutrients element, and their low pH values make them particularly desirable to fungal decay [15]. It has been known that fruits constitute commercially and nutritionally important indispensable food commodity [16].

Microorganisms especially bacteria have been identified as major organisms causing deterioration of bananas by the secretion of extracellular cell wall degrading enzymes [17]. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the Enterobacteriaceae [18].

The microorganisms normally present on the surface of raw fruits may consist of chance contaminant from the soil or dust. These include bacteria that have grown and colonized by utilizing nutrient exuded from plant tissue. Among the group of bacteria commonly found include faecal coli forms such as *Klebsiella* and *Enterobacter* [19].

This study is aimed at revealing the prevalence of bacterial strains and their antibiotics susceptibility pattern.

2. MATERIALS AND METHODS

2.1 Study Area

The study areas for this research were five major markets (Oil-Mill Market, Fruit Garden Market, Mile Three Market, Mile One Market, and Creek Road Market) in Port-Harcourt, Rivers State, Nigeria.

2.2 Sample Collection for Analysis

A total of 75 samples of hybrid banana fruits (Cavendish Banana, Dwarf Cavendish Banana, Red Banana, Lady Finger Banana, and Grand Nain Banana) were purchased from the five different markets (Oil Mill Market, Fruit Garden Market, Mile One Market, Mile Three Market and Creek Road Market) in Port-Harcourt, Rivers State (Figs. 1-5). These hybrid Banana fruits were randomly selected because they (the banana fruits) are the readily available and most consumed Banana fruits in Port-Harcourt, Rivers State.

The fruits were collected in a polythene bag and transferred to the laboratory. The laboratory evaluation was conducted at the Microbiology laboratory of Rivers State University, Port-Harcourt, Nigeria.

The samples were categorized into three groups (Unripe, almost decaying (spoiled), Fresh and ripe (apparently healthy looking)).

Fig. 1. Banana sample analyzed - cavendish banana

Fig. 2. Banana samples analyzed –grand nain banana

Fig. 3. Banana samples analyzed –red banana

Fig. 4. Banana samples analyzed –dwarf cavendish banana

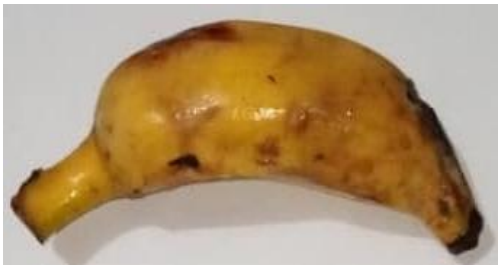

Fig. 5. Banana samples analyzed – lady finger banana

Fig. 6. Comparative morphology of Banana samples analyzed -dwarf cavendish, cavendish, gran nain and lady finger banana

2.3 Media Preparation

The under listed media were used for bacterial enumeration and isolation.

2.3.1 Nutrient agar

Nutrient Agar (NA) was used as a general-purpose medium because it supports the growth of a wide range of non-fastidious microorganisms. Nutrient agar of Becton Dickson and Company, USA was used for the isolation of Total Heterotrophic Bacteria (THB) by preparing, weighing out (with a normal calibration) 28grams of the Nutrient Agar into 1000ml of distilled water and then sterilized/autoclaved at 121^{o}C for 15 minutes according to the manufacturer's specification.

2.3.2 Mannitol salt agar

Mannitol Salt Agar was used for the isolation of *Staphylococcus aureus.* Media was prepared by following the manufacturer's instruction. 111 grams of the media was transferred into 1000ml of distilled water and then sterilized/autoclaved at 121^{o}C for 15 minutes according to the manufacturer's specification.

2.3.3 Mac-Conkey agar

Mac-Conkey Agar was used for the isolation of *Escherichia coli.* Media was prepared by following the manufacturer's instruction. 49.53 grams of the media was transferred into 1000ml of distilled water in a conical flask and then sterilized/autoclaved at 121^{o}C for 15 minutes according to the manufacturer's specification.

2.3.4 Salmonella-Shigella agar

Salmonella-Shigella Agar was used for the isolation of *Samonella* spp. and *Shigella* spp*.* Media was prepared by following the manufacturer's instruction. 60 grams of the media was suspended in 1000ml of distilled water in a conical flask and then sterilized/autoclaved at 121^{o}C for 15 minutes according to the manufacturer's specification.

2.3.5 Mueller-Hinton agar

Mueller-Hinton Agar was used to carry out the Antibiotic profile of the isolated bacteria. Media was prepared by following the manufacturer's instruction. 38 grams of the media was suspended in 1000ml of distilled water in a

conical flask and then sterilized/autoclaved at 121^{o}C for 15 minutes according to the manufacturer's specification.

2.4 Microbiological Analysis

2.4.1 Microbial estimation

The total heterotrophic bacterial count (THB), total coliform count (TCC), total *Staphylococcal* count (TSC), total *Bacillus* and *Proteus* count were determined using the spread plate count method on nutrient, Mac-Conkey and Mannitol Salt agar according to Cheesbrough [20].

2.4.2 Serial dilution

One gram of the different sampling parts of each banana sample were weighed out using an electric weighing balance and aseptically transferred into a sterile tube containing 9.0ml of normal saline. 10-fold serial dilution were carried out on each sample.

2.4.3 Inoculation and incubation

One-hundred microliter of 10^{-2} and 10^{-3} dilutions were spread plated onto sterile solidified MSA (Mannitol Salt Agar), NA (Nutrient Agar), Mac-Conkey Agar and Salmonella-Shigella Agar in triplicates and incubated for 24hrs.

2.4.4 Enumeration and isolation of pure culture

Colonies and spores that grew on the media after the incubation period were enumerated. Similarly, colonies were picked for subculture to get pure cultures using streak plate method. Pure culture of the bacterial isolates were stored in 10% glycerol, all in Bijou bottles.

The colonies counted were expressed as Colony forming unit (CFU) per gram of Banana samples using the formula:

$$T = N/V \times DF$$

Where,

T = Total number of colonies in CFU/g of banana.
N = Number of colonies counted on the pate.
V = Volume of inoculum plated. i.e. 0.1ml.
DF = Dilution factor used for plating (10^2 or 10^3).

2.4.5 Identification of microbial isolates

The microscopic and biochemical characteristics of the bacteria isolates are presented in Table 1. The results were classified based on comparison with [20]. The identities of the bacterial isolates were *Staphylococcus aureus, Escherichia coli, Bacillus* spp.*, Pseudomonas aeruginosa, Proteus* spp. and *Klebsiella* spp. These bacterial isolates were further subjected to molecular identification.

2.4.6 Molecular identification

2.4.6.1 DNA extraction (boiling method)

Four milimeters of an overnight grown culture broth in a Luria Bertani (LB) was spinned at 1400 rpm for 3 minutes. The bacterial cells were further suspended in 500 µl of normal saline and was heated at 95^{o}C for 20 minutes in a heating block. The heated bacterial cells were then cooled in an ice pack and then spinned for another 3 minutes at 1400rpm. After this process, the supernatant which contained the DNA of the bacteria or fungi was transferred to a 1.5ml microcentrifuge tube and was stored in the refridgerator at a temperature of 20^{o}C for other downstream reactions.

2.4.6.2 DNA quantification

The earlier extracted DNA genome was quantified using the Nanodrop spectrophotometer. The software for the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2µl of sterile distilled water and was blanked with the aid of normal saline. After the equipment has been blanked, 2µl of the extracted bacterial or fungal DNA was then loaded on the lower pedestal. The DNA concemtration of the genome was measured by clicking on the "measure" icon.

2.4.6.3 16S rRNA amplification

The 16S rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40µl for 35 cycles. The PCR mix contained: the X2 Dream taq, Mastermix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows:

i. Initial denaturation at 95°C for 5 minutes.
ii. Denaturation at 95°C for 30 seconds.
iii. Annealing at 52°C for 30 seconds.
iv. Extension at 72°C for 30 seconds for 35 cycles.
v. Final extension at 72°C for 5 minutes.

The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light illuminator.

2.4.6.4. Sequencing

Sequencing was carried out using the BigDye Terminator Kit on a 3510 ABt sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was carried out at a final volume of 10 µl, the components included a 0.25µl BigDye® terminator v1.1/v3.1, 2.25 µl of 5 x BigDye sequencing buffer, 10Mm primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10seconds, 55°C for 5 seconds and 60°C for 4 minutes.

2.4.6.5 Phylogenetic analysis

The obtained sequences were edited using the bioinformatics algorithm, trace edit, similar sequences were all downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTIN. These sequences were aligned with the aid of MAFFT. The evolutionary history of the sequence was inferred with the aid of the Neighbor-Joining method in MEGA 6.0 [21]. The bootstrap concensus tree was inferred from 500 replicates [22] was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [23].

2.5 Statistical Analysis

The data that were obtained from the bioremediation set up were subjected to statistical analysis using computer-based program, SPSS version 22 for analysis of variance (ANOVA) and multiple range tests to find the difference in the means at 5% (0.05) significant level.

2.6 Antibiotics Susceptibility Testing and Analysis

The susceptibility tests were performed using Kirby-Bauer Disc diffusion method as recommended by Cheesbrough [20] using Mueller Hinton Agar. The bacterial strains were tested against the following: AUG: Amoxicillin Clavulanate - 30µg**,** CTX: Cefotaxime - 25µg**,** CRO: Ceftriaxone Sulbactam - 45µg**,** ZEM: Cefexime -5µg**,** LBC: Levofloxacin - 5µg**,** CIP: Ciprofloxacin - 5µg**,** IMP: Imipenem/Cilastatin – 10/10µg**,** CXM: Cefruroxime - 30µg**,** OFX: Ofloxacin - 5µg**,** ERY: Erythromycin - 15µg**,** GN: Gentamycin - 10µg**,** AZN: Azithromycin - 15µg**,** NF: Nitrofurantoin - 30µg, ACX: Ampiclox -10µg and NA: Nalidixic Acid - 30µg.

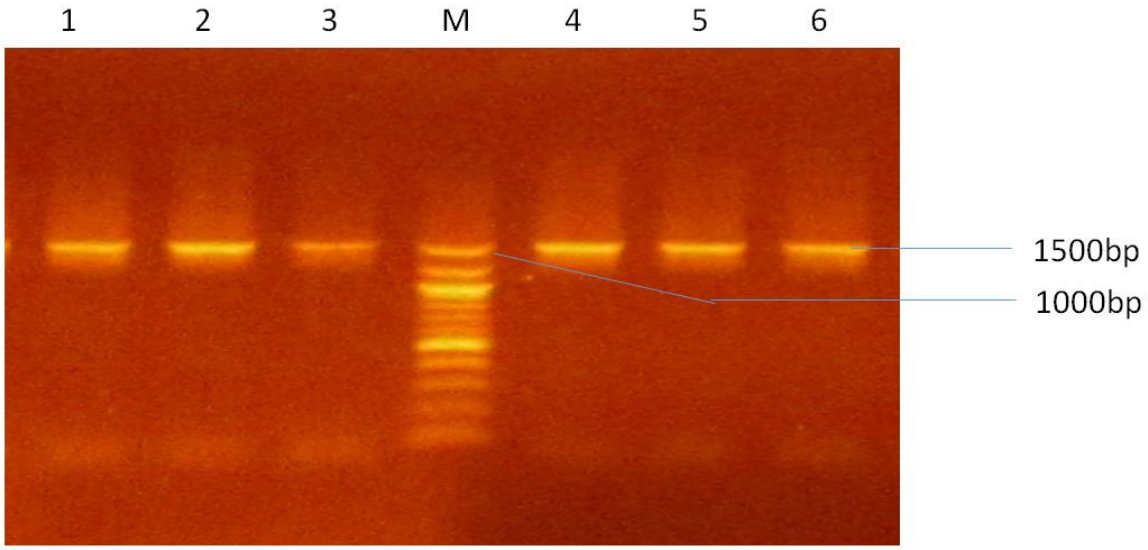

Fig. 7. Agarose gel electrophoresis showing 16SrRNA gene of some bacterial isolates, Lane 1 – 6 represents the 16SrRNA gene (1500BP), Lane M represents the 100 BP molecular ladder

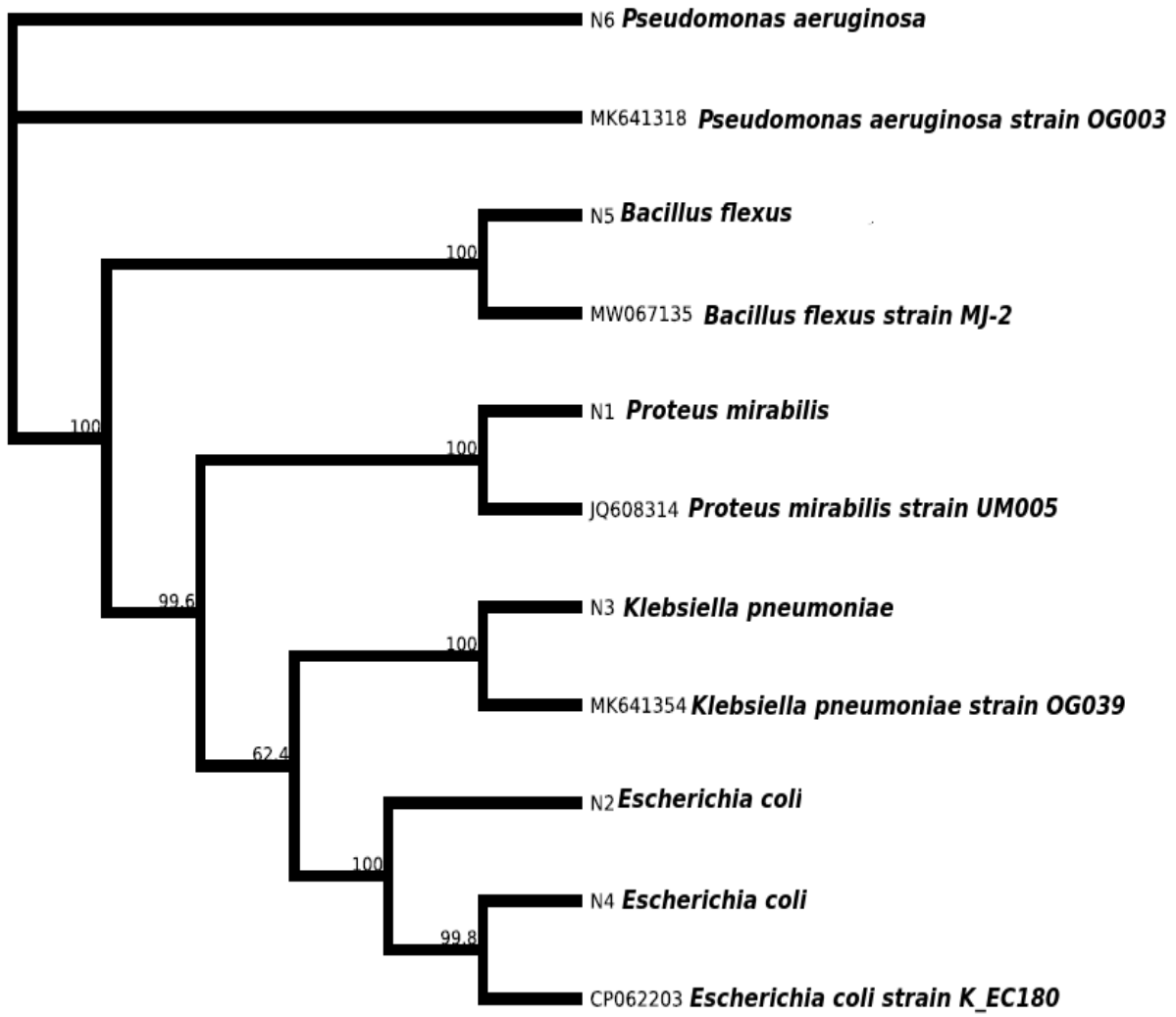

Fig. 8. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

3. RESULTS AND DISCUSSION

3.1 Potential Bacteria Isolated and Prevalence

Out of the 75 Banana (*Musa* spp.) fruit samples collected, 83 bacteria were isolated. Table 1 shows the cultural and biochemical characteristics of the bacterial isolates while the result of the molecular identification is seen in Fig. 4 and Fig. 5. The frequency of the isolates and their percentage prevalence are shown in Table 2. The isolates are potential bacteria associated with food-borne disease.

The findings of this study showed that *Staphyococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Kebsiella pneumoniae*, *Proteus mirabilis* and *Bacillus flexus* were found in Banana (*Musa* spp.) fruits sold in some markets in Port-Harcourt, Rivers State. In Table 1, the cultural and biochemical identities of these bacteria is seen with their percentage occurrence of these isolates from the different sampled parts seen in Table 2 and 3. The results obtained are in conformity with a study on Isolation and Identification of Microorganisms involved in the spoilage of Banana fruit (*Musa* spp.) sold in some selected markets in Eastern Nigeria [24].

From the above results (Tables 2 and 3), the Epicarp of the sampled Banana (*Musa* spp.) fruits had the most microbial load at 31.33% followed by the Tip at 27.71% and the Endocarp having the least count at 8.43%. The Grand Nain Banana had the most microbial load at 33.6% followed by the Dwarf Cavendish Banana at 22.8% and the least microbial load seen in the Red Banana at 9.6%.

Table 1. Cultural and biochemical characteristics of the bacterial isolates

Colonial morphology	Tests										Sugar fermentation					Probable organism
	GR	Co	Ca	Ox	Mo	Mr	Vp	In	Ci	Gl	Su	Ma	La	Fr	Ga	
Dry, donut shaped, dark pink in color and flat.	- Rod	-	+	-	-	+	-	+	-	+	-	+	+	+	-	***Escherichia coli*.**
Round, yellowish-white or golden yellow in color and elevated.	+ Cocci (cluster)	+	+	-	-	+	+	-	+	+	+	+	+	+	+	***Staphylococcus aureus.***
Greenish-blue in color, moist surface and elevated.	- Rod	-	+	+	+	-	-	-	+	-	-	+	-	-	-	***Pseudomonas aeruginosa.***
Opaque, round, creamy and elevated.	+ Rod	-	+	-	-	+	-	-	+	+	+	-	+	-	-	***Bacillus flexus.***
Colorless, round, smooth and elevated.	- Rod	-	+	-	+	+	-	-	+	+	-	-	-	-	-	***Proteus mirabilis.***
Large, shiny and dark pink in color and elevated.	- Rod	-	+	-	-	-	-	-	+	+	+	+	+	-	-	***Klebsiella pneumoniae.***

KEY: GR- Gram Reaction, Co- Coagulase, Ox- Oxidase, Mo- Motility, Mr- Methyl-Red, Vp- Voges Proskauer, In- Indole, Ci- Citrate, Gl- Glucose, Su- Sucrose, Ma- Mannitol, La- Lactose, Fr- Fructose, Ga- Galactose

Table 2. Percentage occurrence of isolates from the sampled parts of Banana (*Musa* Spp.) fruit

S/N	Banana (*Musa* spp.) Fruit samples	Sampling parts	No. of isolates (%)
1	Cavendish Banana	Cut stalk	3 (3.6)
		Tip	6 (7.2)
		Endocarp	1 (1.2)
		Vascular tissue	1 (1.2)
		Epicarp	4 (4.8)
2	Dwarf Cavendish Banana	Cut stalk	5 (6.0)
		Tip	7 (8.4)
		Endocarp	1 (1.2)
		Vascular tissue	1 (1.2)
		Epicarp	5 (6.0)
3	Red Banana	Cut stalk	2 (2.4)
		Tip	1 (1.2)
		Endocarp	1 (1.2)
		Vascular tissue	1 (1.2)
		Epicarp	3 (3.6)
4	Lady Finger Banana	Cut stalk	2 (2.4)
		Tip	4 (4.8)
		Endocarp	1 (1.2)
		Vascular tissue	2 (2.4)
		Epicarp	4 (4.8)
5	Grand Nain Banana	Cut stalk	6 (7.2)
		Tip	5 (6.0)
		Endocarp	3 (3.6)
		Vascular tissue	4 (4.8)
		Epicarp	10 (12.0)
Total			83 (100)

Table 3. Percentage occurrence of isolates from the sampled parts of Banana (*Musa* Spp.) fruit

S/N	Sampled parts of banana (M*usa* spp.) fruit	No. of isolates (%)
1	Cut stalk	18 (21.69)
2	Tip	23 (27.71)
3	Endocarp	7 (8.43)
4	Vascular tissue	9 (10.84)
5	Epicarp	26 (31.33)
Total		83 (100)

Table 4. Frequency and percentage of the bacteria isolates

Isolates	Frequency	Percentage prevalence (%)
Escherichia coli	19	22.89
Staphylococcus aureus	24	28.92
Pseudomonas aeruginosa	12	14.46
Bacillus flexus	9	10.84
Proteus mirabilis	8	9.64
Klebsiella pneumoniae	11	13.25
Total	83	100

Staphylococcus aureus was the most frequent occurring organism at 28.92% followed by *Escherichia coli* at 22.89% with the least seen in *Proteus mirabilis* at 9.64% (Table 4). The percentage occurrence of the isolates is also compared in the different study areas (Table 5) which indicates that Oil Mill market had the most microbial load at 32.53% followed by Mile One market at 19.28% and the least microbial load seen in Mile Three market at 14.46%.

Table 5. Percentage Occurrence of Isolates from the different study areas

Isolates	Study area (%)					Total (%)
	Oil mill (%)	Fruit garden (%)	Mile three (%)	Mile one (%)	Creek road (%)	
Escherichia coli	5 (4.03)	2 (1.61)	3 (2.42)	5 (4.03)	4 (3.23)	19 (15.32)
Staphylococcus aureus	7 (5.65)	3 (2.42)	4 (3.23)	4 (3.23)	6 (4.84)	24 (19.37)
Pseudomonas aeruginosa	4 (3.23)	2 (1.61)	2 (1.61)	2 (1.61)	2 (1.61)	12 (9.67)
Bacillus flexus	3 (2.42)	2 (1.61)	1 (0.81)	2 (1.61)	1 (0.81)	9 (7.26)
Proteus mirabilis	3 (2.42)	2 (1.61)	1 (0.81)	1 (0.81)	1 (0.81)	8 (6.46)
Klebsiella pneumoniae	5 (4.03)	2 (1.61)	1 (0.81)	2 (1.61)	1 (0.81)	11 (8.87)
Total	27 (32.53)	13 (15.66)	12 (14.46)	16 (19.28)	15 18.07)	83 (100)

3.2 Antibiogram Assay of the Isolates

The result of the Antimicrobial susceptibility pattern of the individual bacterial isolates (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus flexus, Proteus mirabilis* and *Klebsiella pneumoniae*) are represented in Tables 6-11. The susceptibility profile of the isolates was graded as susceptible, intermediate and resistant.

Escherichia coli were completely susceptible to Ofloxacin and some susceptible to Levofloxacin at 89.5%, with complete resistance to Cefotaxime, Imipenem/Cilastatin, Cefuroxime, Cefexime and Nitrofurantoin (Table 6). Similar observation occurred *Staphylococcus aureus*, there were complete resistance to Cefotaxime, Levofloxacin, Imipenem/Cilastatin, Ofloxacin and Azithromycin in all isolates of *Staphylococcus aureus* and susceptibility at 70.8%, 79.2% and 20.8% to Augmentin, Ceftriaxone sulbactarm and Erythromycin, respectively. Similar isolation of high susceptibility of *Staphylococcus aureus* to Ceftriaxone Sulbactarm has been previousy reported [25].

In Table 8, *Pseudomonas aeruginosa* were susceptible to Augmentin (25.0%), Imipenem/Cilastatin (66.7%), Gentamycin (83.3%) and Ceftriaxone Sulbactarm at 8.3%. While complete resistance was seen in Cefotaxime, Nalidixic Acid, Nitrofurantoin and Cefexime. This can be compared to a study on Antibiotic susceptibility pattern and analysis of plasmid profiles of *Pseudomonas aeruginosa* from human, animal and plant sources [26], which showed high susceptibility of *Pseudomonas aeruginosa* to Imipenem/Cilastatin.

Table 6. Antibiotic sensitivity pattern of *Escherichia coli* and its zone of inhibition (mm), N=19

Antibiotics	Susceptibility (%)	Intermediate (%)	Resistance (%)
AUG	1 (5.3)	0 (0.0)	18 (94.7)
CTX	0 (0.0)	0 (0.0)	19 (100)
IMP	0 (0.0)	0 (0.0)	19 (100)
OFX	19 (100)	0 (0.0)	0 (0.0)
GN	1 (5.3)	3 (15.8)	15 (78.9)
NA	0 (0.0)	5 (26.3)	14 (73.7)
NF	0 (0.0)	0 (0.0)	19 (100)
CXM	0 (0.0)	0 (0.0)	19 (100)
CRO	0 (0.0)	1 (5.3)	18 (94.7)
ACX	0 (0.0)	1 (5.3)	18 (94.7)
ZEM	0 (0.0)	0 (0.0)	19 (100)
LBC	17 (89.5)	0 (0.0)	2 (10.5)

KEY: AUG- Augmentin (30µg), CTX- Cefotaxime (25µg), IMP- Imipenem/Cilastatin (10µg), OFX- Ofloxacin (5µg), GN- Gentamycin (10µg), NA- Nalidixic Acid (30µg), NF- Nitrofurantoin (300 µg), CXM- Cefuroxime (30µg), CRO- Ceftriaxone Sulbactarm (45µg), ACX- Ampiclox (10µg), ZEM- Cefexime (5µg), LBC- Levofloxacin (5µg).

Table 7. Antibiotic sensitivity pattern of *Staphylococcus aureus* and its zone of inhibition (mm), N=24

Antibiotics	Susceptibility (%)	Intermediate (%)	Resistance (%)
AUG	17 (70.8)	7 (29.2)	0 (0.0)
CTX	0 (0.0)	0 (0.0)	24 (100)
CRO	19 (79.2)	0 (0.0)	5 (20.8)
ZEM	0 (0.0)	5 (20.8)	19 (79.2)
LBC	0 (0.0)	0 (0.0)	24 (100)
CIP	0 (0.0)	4 (16.7)	20 (83.3)
IMP	0 (0.0)	0 (0.0)	24 (100)
CXM	0 (0.0)	6 (25.0)	18 (75.0)
OFX	0 (0.0)	0 (0.0)	24 (100)
ERY	5 (20.8)	2 (8.3)	17 (70.8)
GN	0 (0.0)	4 (16.7)	20 (83.3)
AZN	0 (0.0)	0 (0.0)	24 (100)

KEY: AUG- Augmentin (30µg), CTX- Cefotaxime (25µg), CRO- Ceftriaxone Sulbactarm (45µg), ZEM- Cefexime (5µg), LBC- Levofloxacin (5µg), CIP- Ciprofloxacin (5µg), IMP- Imipenem/Cilastatin (10µg), CXM- Cefuroxime (30µg), OFX- Ofloxacin (5µg), ERY- Erythromycin (15µg), GN- Gentamycin (10µg), AZN- Azithromycin (15µg)

Table 8. Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* and its zone of inhibition (mm), N=12

Antibiotics	Susceptibility (%)	Intermediate (%)	Resistance (%)
AUG	3 (25.0)	0 (0.0)	9 (75.0)
CTX	0 (0.0)	0 (0.0)	12 (100)
IMP	8 (66.7)	0 (0.0)	4 (33.3)
OFX	0 (0.0)	2 (16.7)	10 (83.3)
GN	10 (83.3)	0 (0.0)	2 (16.7)
NA	0 (0.0)	0 (0.0)	12 (100)
NF	0 (0.0)	0 (0.0)	12 (100)
CXM	0 (0.0)	2 (16.7)	10 (83.3)
CRO	1 (8.3)	0 (0.0)	11 (91.7)
ACX	0 (0.0)	2 (16.7)	10 (83.3)
ZEM	0 (0.0)	0 (0.0)	12 (100)
LBC	0 (0.0)	3 (25.0)	9 (75.0)

KEY: AUG- Augmentin (30µg), CTX- Cefotaxime (25µg), IMP- Imipenem/Cilastatin (10µg), OFX- Ofloxacin (5µg), GN- Gentamycin (10µg), NA- Nalidixic Acid (30µg), NF- Nitrofurantoin (300 µg), CXM- Cefuroxime (30µg), CRO- Ceftriaxone Sulbactarm (45µg), ACX- Ampiclox (10µg), ZEM- Cefexime (5µg), LBC- Levofloxacin (5µg)

Table 9. Antibiotic sensitivity pattern of *Bacillus flexus* and its zone of inhibition (mm), N=9

Antibiotics	Susceptibility (%)	Intermediate (%)	Resistance (%)
AUG	1 (11.1)	3 (33.3)	5 (55.6)
CTX	0 (0.0)	0 (0.0)	9 (100)
CRO	0 (0.0)	0 (0.0)	9 (100)
ZEM	0 (0.0)	0 (0.0)	9 (100)
LBC	9 (100)	0 (0.0)	0 (0.0)
CIP	2 (22.2)	5 (55.6)	2 (22.2)
IMP	0 (0.0)	0 (0.0)	9 (100)
CXM	0 (0.0)	3 (33.3)	6 (66.7)
OFX	0 (0.0)	2 (22.2)	7 (77.8)
ERY	0 (0.0)	2 (22.2)	7 (77.8)
GN	3 (33.3)	0 (0.0)	6 (66.7)
AZN	0 (0.0)	0 (0.0)	9 (100)

KEY: AUG- Augmentin (30µg), CTX- Cefotaxime (25µg), CRO- Ceftriaxone Sulbactarm (45µg), ZEM- Cefexime (5µg), LBC- Levofloxacin (5µg), CIP- Ciprofloxacin (5µg), IMP- Imipenem/Cilastatin (10µg), CXM- Cefuroxime (30µg), OFX- Ofloxacin (5µg), ERY- Erythromycin (15µg), GN- Gentamycin (10µg), AZN- Azithromycin (15µg)

Table 10. Antibiotic sensitivity pattern of *Proteus mirabilis* and its zone of inhibition (mm), N=8

Antibiotics	Susceptibility (%)	Intermediate (%)	Resistance (%)
AUG	0 (0.0)	0 (0.0)	8 (100)
CTX	0 (0.0)	0 (0.0)	8 (100)
IMP	8 (100)	0 (0.0)	0 (0.0)
OFX	0 (0.0)	8 (100)	0 (0.0)
GN	0 (0.0)	0 (0.0)	8 (100)
NA	0 (0.0)	0 (0.0)	8 (100)
NF	0 (0.0)	0 (0.0)	8 (100)
CXM	0 (0.0)	0 (0.0)	8 (100)
CRO	8 (100)	0 (0.0)	0 (0.0)
ACX	0 (0.0)	0 (0.0)	8 (100)
ZEM	0 (0.0)	0 (0.0)	8 (100)
LBC	0 (0.0)	0 (0.0)	8 (100)

KEY: AUG- Augmentin (30µg), CTX- Cefotaxime (25µg), IMP- Imipenem/Cilastatin (10µg), OFX- Ofloxacin (5µg),GN- Gentamycin (10µg), NA- Nalidixic Acid (30µg), NF- Nitrofurantoin (300 µg), CXM- Cefuroxime (30µg), CRO- Ceftriaxone Sulbactarm (45µg), ACX- Ampiclox (10µg), ZEM- Cefexime (5µg), LBC- Levofloxacin (5µg)

Table 11. Antibiotic sensitivity pattern of *Klebsiella pneumoniae* and its zone of inhibition (mm), N=11

Antibiotics	Susceptibility (%)	Intermediate (%)	Resistance (%)
AUG	5 (45.5)	0 (0.0)	6 (54.5)
CTX	0 (0.0)	0 (0.0)	11 (100)
IMP	0 (0.0)	7 (63.6)	4 (36.4)
OFX	6 (54.5)	0 (0.0)	5 (45.5)
GN	0 (0.0)	3 (27.3)	8 (72.7)
NA	0 (0.0)	0 (0.0)	11 (100)
NF	0 (0.0)	0 (0.0)	11 (100)
CXM	0 (0.0)	0 (0.0)	11 (100)
CRO	3 (27.3)	5 (45.5)	3 (27.3)
ACX	2 (18.2)	0 (0.0)	9 (81.8)
ZEM	0 (0.0)	0 (0.0)	11 (100)
LBC	11 (100)	0 (0.0)	0 (0.0)

KEY: AUG- Augmentin (30µg), CTX- Cefotaxime (25µg), IMP- Imipenem/Cilastatin (10µg), OFX- Ofloxacin (5µg), GN- Gentamycin (10µg), NA- Nalidixic Acid (30µg), NF- Nitrofurantoin (300 µg), CXM- Cefuroxime (30µg), CRO- Ceftriaxone Sulbactarm (45µg), ACX- Ampiclox (10µg), ZEM- Cefexime (5µg), LBC- Levofloxacin (5µg)

Antimicrobial susceptibility pattern of all isolates of *Bacillus flexus* which shows complete resistance to Cefotaxime, Ceftriaxone Sulbactarm, Cefexime, Imipenem/Cilastatin and Azithromycin. While susceptibility was seen in Levofloxacin at 100% and 22.2%, 33.3% and 11.1% seen in Ciprofloxacin, Gentamycin and Augmentin, respectively (Table 9).

Alternate trend was observed with *Proteus mirabilis* which showed complete susceptibility in Imipenem/Cilastatin and Cefrtiaxone Sulbactarm and complete resistance in Augmentin, Cefotaxime, Gentamycin, Nalidixic Acid, Nitrofurantoin, Cefuroxime, Ampiclox, Cefexime and Levofloxacin (Table 10).

In the same vein, *Klebsiella pneumoniae* exhibited slight variation by showing complete susceptibility in all isolates of *Klebsiella pneumoniae* to Levofloxacin and 45.5% susceptibility to Augmentin, Ofloxacin, Ceftriaxone and Ampiclox at 54.5%, 27.3% and 18.2%, respectively. While complete resistance was seen in Cefotaxime, Nalidixic Acid, Nitrofurantoin, Cefuroxime and Cefexime (Table 11)

Similar types of bacterial contaminants had been identified in previous study in Bacteria associated with food in Awka-South LGA, Anambra State [27].

4. CONCLUSION

Conclusively, the present study reports the presence of bacterial contamination in Banana fruit (*Musa* spp.). Several pathogenic bacteria isolated from this study includes: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus flexus* and *Proteus mirabilis. Staphylococcus aureus* happened to be the most frequent occurring bacteria at 28.92% followed by *Escherichia coli* at 22.89%, with *Proteus mirabilis* being the least occurring bacteria at 9.64%.

The microorganisms normally present on the surface of raw fruits such as Banana may have high chances of contamination from the soil or dust. These include bacteria that have grown and colonized by utilizing nutrient exuded from plant tissue. Presence of these bacteria on bananas most especially Coliforms pose a serious threat to health of consumers as the organism could produce toxins, which are lethal when consumed.

Most times, contaminants in Banana fruits could be as a result of human contamination (This could be contamination from the microflora residing in the nostrils, mouth or skin of the vendor who's selling the fruit or from that of the consumer (buyer).

It is therefore necessary and important that both the farmers and sellers are to take necessary and appropriate precautions in preventing contamination and eating of contaminated fruits. This will however reduce the risk of toxins associated with bacterial contamination which are dangerous to human health.

Considering the high rate of resistance to antibiotics exhibited by the isolated bacterial strains, it can be concluded that there is wide spread of antibiotic resistance among microorganisms from different sources. This study emphasizes on the need for surveillance to prevent food-borne diseases associated with the consumption of infected banana fruits and also to detect emerging antimicrobial resistant bacteria especially in developing countries like Nigeria.

5. RECOMMENDATION

We therefore recommended that both the farmers and sellers are to take necessary and appropriate precautions in preventing contamination and eating of contaminated fruits. This will however reduce the risk of toxins associated with bacterial contamination which are dangerous to human health. An example is the wearing of gloves while harvesting or selling of Banana fruits if there are presence of wounds or infections on the wrist or hands, also not allowing their buyers touch their wares (Banana fruit) when trying to make a purchase.

Washing of hands before touching the fruits (Banana fruits) and washing of Banana fruits before consumption should be encouraged.

Treatment guidelines for use of antibiotics should be formulated based on the hospital formulary and the sensitivity patterns. This should be reviewed occasionally to ensure rational use of antibiotics.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67883