Antimicrobial Resistance of Bacterial Isolates from Locally and Industrially Processed NONI (*Morindacitrifolia*) Drinks Sold in Port Harcourt

ABSTRACT

Public health is seriously threatened by the emergence of antibiotic resistance, especially in food products. The purpose of this study was to evaluate the antimicrobial resistance patterns of bacterial isolates from noni (*Morindacitrifolia*) drinks that are sold in Port Harcourt, Nigeria, both locally and through industrial processing. Noni samples were bought from three distinct locations in Rivers State while the fruits were locally fermented into noni beverages. Conventional microbiological methods were employed to identify the bacterial isolates, and the disc diffusion method was employed to assess the antibiotics' susceptibility. Characterization of the bacterial isolates was further done using molecular techniques.The total heterotrophic bacteria of the beverages seen in the study ranged from 3.5±0.7 to $5.9\pm0.3\times10^4$cfu/ml. The total coliform counts ranged from 1.7±1.9 to $3.8\pm1.2\times10^4$ CFU/ml and faecal coliform count in all the samples was zero (0). Data showed that the locally produced noni sample had the highest bacterial counts (1.6×10^3) while the lowest count was observed in samples from location B (1.50×10^2). The presence of multiple bacterial species, including *Flavobacterium*sp (16.7%), *Enterobacter*sp (25%), *Serratia*sp (25%), *Micrococcus*sp (8.3%) and *Bacillus*sp (25%) was indicated by the results.Total (100%) resistance was particularly noticeable against commonly used antibiotic such ascefuroxime, cefotaxime, cefixime and meropenem gentamycin, vancomycin and 66.7% to ampiclox.The Data of the virulence of the bacterial isolates showed that*Bacillus*spwas positive for biofilm production, starch hydrolysis and haemolysis. Ampicillin resistance gene (AmpC gene) was detected in *Serratia marcescens* AB061685.1and *Bacillus subtilis* AB192294.2. These findings highlight the significance of strict quality control measures in the production and sale of Noni drinks to mitigate the risk of antimicrobial resistance transmission through food consumption.

Key words: Noni Drink, Antimicrobial Resistance, Locally, Industrially, Bacteria, Port Harcourt

1.0 INTRODUCTION

The demand of consumers for safer, fresh, higher-quality foods with long shelf life has increased [1]. This has led to the development of preservation technologies through minimal processing techniques such as applying natural additives (such as polyphenols, organic acids, essential oils, and plant extracts) and physical methods (ozone-based treatment, ultraviolet radiation, pulsed light, cold plasma, ultrasound, and novel packing practices) [1,2]. Consequently, this kind of processing has become a subject of special care due to the presence of certain pathogens such as *Escherichia coli* O157: H7, which is considered by the WHO [3] as one of the 14 pathogens causing severe gastrointestinal diseases. Nonetheless, there is little information about the bacterial isolates in local and commercial Noni juices. More so, antibiotic resistance of bacteria

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is a health problem at the global level, in 2013, 700,000 deaths were reported as generated by these resistant bacteria and it is expected that by 2050, this will be main cause of death with around 10 million cases, and it can become one of the first causes of death worldwide [4]. *M. citrifolia* contains phytochemicals that have antibacterial, antiviral, antifungal, antitumor, anthelminthic, analgesic, hypotensive, anti-inflammatory and immune-enhancing effects [5]. A variety of beverages, powders, cosmetic products, oil, and leaf powders from the various part of the plant have been introduced into the consumer market. Noni is sometimes called a "starvation fruit", implying that it was used by indigenous peoples as emergency food during times of famine. Despite its strong smell and bitter taste, the fruit was nevertheless eaten as a famine food. The seeds are edible when roasted. In Thai cuisine, the leaves (known as bai-yo) are used as a green vegetable and are the main ingredient of kaeng bai-yo, cooked with coconut milk. The fruit (luk-yo) is added as a salad ingredient to some versions of somtam[6]. The fruits are used in making the Noni juice in Nigeria and the consumption of the juice due to its benefit is increasing and little attention is known of its bacterial quality as well as the antibiotic resistant of possible bacteria. Thus, the present study seeks to investigate the bacteria associated with the Noni fruit juice and determines its antibiotic susceptibility.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

The study was carried out in the Microbiology Laboratory of the Department of Microbiology, Rivers State University. The samples were locally produced noni beverages and commercially produced noni beverages. The commercially fermented noni beverages used in this study were bought from three different locations: Rivers State University Shopping complex; Everyday Supermarket, New GRA; and Market Square Mall Choba, all in Rivers State.

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2.2 Preparation of Local Noni

Ripe noni fruits were harvested and collected into a sterile polyethene bag from Dilomat farm at Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, and immediately transported to the Microbiology Laboratory, Department of Microbiology, Rivers State University for analysis. In the lab, the fruits were sorted, washed with clean water, placed into a sterile wide-mouth container with a lid and stored at ambient temperature (25-30°C) for 6 days and noni juices were obtained (fermented drip extract of noni fruit). The flow chart of the production process is illustrated in Fig. 1.

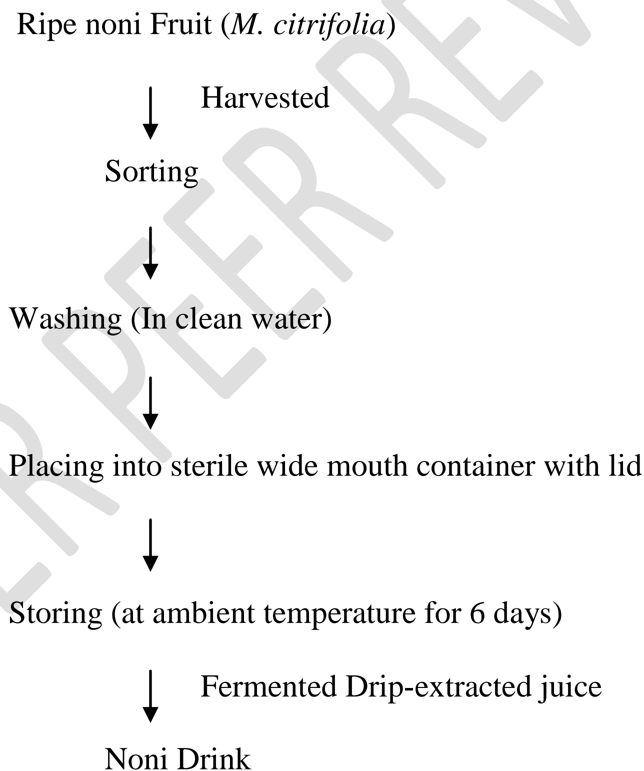

Figure 1: Flow chart for the preparation of noni drink(*M. citrifolia*)

2.3Isolation and Enumeration of Bacteria

Samples from commercially and locally produced noni juice were subjected to ten-fold serial dilution. In this method, one millilitre (1ml) of the sample waswithdrawn and transferred into a

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test tube containing sterile 9ml normal saline to give a dilution of 10^{-1}. After which, another 1mL was withdrawn from the previous dilution and transferred into another test tube containing sterile 9mL normal saline. To obtain a dilution of 10^{-2}. This was further diluted serially to obtain a final dilution of 10^{-6}. Aliquots of 10^{-2} and 10^{-3} dilutions were inoculated onto Eosin methylene blue agar for isolation of coliform, mannitol salt agar for isolation of *Staphylococcus* sp and Nutrient agar plates for the enumeration and isolation of total heterotrophic bacteria (THB). The inoculums were spread using sterile bent glass rod and the plates were incubated at 37 ℃ for 24-48 hours. After incubation, plates were observed for bacterial growth. Counts were made for the respective plates and distinct colonies were sub-cultured for further identification.Discrete colonies that grew on the respective agar plates were sub-cultured on freshly prepared dried nutrient agar plates using a sterile wire loop. The plates were incubated for 24 hours at 37°C. After incubation, the plates were observed for pure cultures and pure cultures (cultures void of contaminants) were preserved by inoculating them on freshly prepared nutrient agar slants and refrigerated until required for further analysis.

2.4. Characterization of Bacterial Isolates

Identification of the isolates were based on their colonial morphology, Gram reaction, biochemical (catalase, oxidase, Citrate utilization, Indole, and sugar fermentation ability) and molecular characterization. The colonial, gram reaction and biochemical responses of the isolates were compared with information in Bergey's Manual of Determinative Bacteriology [7]. The tests were carried out as described in Cheesbrough [8].

2.5 Molecular Identification

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All bacterial isolates obtained were molecularly identified following various processes. DNA extraction was carried out using boiling methods as described by Abdelhai*et al.,*[9]DNA was then quantified using the Nanodrop 1000 Spectrophotometer. 16S rRNA amplification was done using polymerase chain reaction (PCR) technique. Sequencing was carried out using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa following manufacturers prescription.Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN and ampCgene from the isolates were amplified on an ABI 9700 Applied Biosystems thermal cycler.

2.6 Antibiotic Susceptibility Testing

A 0.5 McFarland Standards was prepared by dissolving 0.5ml of 0.048 $BaCl_2$ (1.175% $BaCl_2$-$2H_2O$) in 99.4ml of 0.18*M* H_2SO_4 (1%v/v) as specified in CLSI [10]. This was used to standardize the turbidity of the test organism.The pure isolates were grown in nutrient broth for 18-24 hours, after which its turbidity is reduced by adding sterile normal saline until it matches McFarland's. Sensitivity of isolates to several antibiotics Streptomycin (30µg), Norfloxacin (10µg), Chloramphenicol (30µg), Ciprofloxacin (10µg), Erythromycin (10µg), Levofloxacin (5µg), Gentamycin (10µg), Ampiclox (30µg), Rifampicin (30µg), Amoxicillin (30µg),tetracycline (20µg) clotrimazole (30 µg), gentamycin (10µg), cefuroxime (30µg), cefotaxime (30µg), ceftriaxone (30µg), ciprofloxacin (30 µg), amoxicillin (30µg), vancomycin (10µg), cefixime (30µg) and meropenem (30 µg)was determined on Mueller Hinton Agar using the Kirby Bauer disk diffusion method. Onto a sterile solid Muller Hinton agar, each of the test organisms from the already prepared McFarland standard was aseptically inoculated using a sterile swab stick. The test isolates were seeded horizontally and vertically [11] and allowed to

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dryfor 5minutes before antibiotic disc was aseptically placed on the solid media using sterile forceps. The plates were incubated at 37°C for 24hours after which the diameter of the zones of inhibition was measured to the nearest millimeter and the readings recorded as resistance, susceptible or intermediatedas described by the CLSI [10].

3.0 Result

3.1 Bacterial Load in the Noni Samples

The bacterial load of the noni beverages from the different locations is presented in Table 1. In the Table, it can be seen that the total heterotrophic bacteria of the beverages ranged from 3.5 ± 0.7 to $5.9 \pm 0.3 \times 10^4$ CFU/ml. The total coliform counts ranged from 1.7 ± 1.9 to $3.8 \pm 1.2 \times 10^4$ CFU/ml and the faecal coliform counts in all the samples was zero (0). Thus, they were void of faecal contamination. More so, there was no significant differences ($P>0.05$) in the total heterotrophic bacteria and coliform counts of the beverages in all the samples.The locally produced noni sample had the highest total heterotrophic counts 1.6×10^3 cfu/ml while the least count was in noni from everyday supermarket 1.50×10^2cfu/ml. For coliform count, the locally produced noni hada greater load of 3.50×10^2 cfu/ml when compared to samples from Everyday supermarket GRA which had the least 5.0×10^1 cfu/ml.

3.2 Prevalence of Bacterial species

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As shown in Fig 2. *Flavobacterium*sp, *Enterobacter*sp, *Serratia*sp, *Micrococcus*sp and *Bacillus*sp had a prevalence rate of 16.7, 25, 25, 8.3, and 25%, respectively. Thus, *Bacillus* sp, *Serratia*sp, and *Enterobacter*sp, was the most prevalent while *micrococcus* sp, was the least encountered isolate. All the bacterial isolates were isolated from the local noni samples while only *Bacillus* sp, *Enterobacter* sp and *Micrococcus* sp was isolated from the industrial noni samples (Table 2).

3.3 Identity of the Bacterial Isolates

Twelve bacterial isolates belonging to the genera*Flavobacterium*, *Enterobacter*, *Serratia*, *Micrococcus* and *Bacillus*were identified.The 16S rRNA sequence of the bacterial isolates provided an exact match with sequences of bacteria stored on the NCBI non-redundant nucleotide (nr/nt) database as shown in fig 3.In accordance with the phylogenetic placement of the isolates' 16S rRNA within the bacterial isolates (N4, N11, N12, N2 and N1), the evolutionary distances computed using the Jukes-Cantor technique revealed a close relatedness to *Micrococcus aloeverae*NZSMVK01000044.1, *Bacillus subtilis*AB192294.2,*Serratia marcescens*AB061685.1,*Enterobacter aerogenes* DQ836222.1and *Chryseobacteriumvrystaatense*FQVE01000001.1 respectively. The amplified 16S rRNA gene bands of the isolates on agarose gel after electrophoresis is presented in Plate 1. In the plate, lane Mrepresent the 16S rRNA of the molecular markerwhile lanes 1-5 represent *Micrococcus aloeverae, Bacillus subtilis,Serratia marcescens, Enterobacter aerogenes* and *Chryseobacteriumvrystaatense*, respectively.

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3.4Virulent Attributes of the Bacterial Isolates

Table 3 displayed the virulence of the bacterial isolates. All the isolates were negative for haemolysis expect*Bacillus* spwhich was positive for biofilm production, starch hydrolysis and haemolysis.

3.5Antibiotic Susceptibility of the Identified Bacteria

The antibiotic susceptibility of the identified Gram negativeisolates can be seen in Tables 4a and b. All the *Flavobacterium* sp were completely (100%) resistant to cefuroxime, cefotaxime, cefixime and meropeme. *Enterobacter* sp were susceptible to ceftriaxone but highly (100%) resistant to all other tested antibiotics while *Serratia* isolates showed 100% resistant to gentamycin, cefuroxime, vancomycin and cefixime.

The Gram positive isolates,*Bacillus* sp were completely susceptible to rifampicin and streptomycin but showed 66.7% resistance to ampiclox while *Micrococcus* sp, was completely susceptible to chloramphenicol, gentamycin, streptomycin, erythromycin and levofloxacin and showed no resistance to all the antibiotics tested.

3.6Multiple Antibiotics Resistant Index (MARI) of the Isolates

The Multiple Antibiotic Resistance (MAR) index showed that the MAR index of the microorganisms ranged from 0.2 to 0.8. *Serratia, Bacillus* and *Enterobacter* isolates had a MAR index greater than 0.2 (Table 5).

3.7 Detection of AmpC resistant Genes in the Isolates

The amplified resistant gene bands on agarose gel electrophoresis (Plate 2) shows that *ampC*antibiotics resistant gene (*ampC*gene) was present in *Serratia* sp and *Bacillus* sp while

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Enterobacter sp, *Micrococcus* sp and *Flavobacterium* sp were negative for the *ampC*gene (Table 6).

Table 1. Microbial Load of Commercially Produced Noni Beverages

Sample	**THB** ($\times10^4$)	**TCC** ($\times10^4$)	**FC** ($\times10^4$)
Noni from RSU	4.2±1.6 [a]	2.0±2.8 [a]	0.0±0.0
Noni, Every day Supermarket	3.6±0.7 [a]	1.7±1.9 [a]	0.0±0.0
Noni, Market Square	3.5±0.7 [a]	1.9±1.4 [a]	0.0±0.0
Locally fermented	5.9±0.3 [a]	3.8±1.2 [a]	0.0±0.0
P-value	0.180	0.331	-

Keys: THB =Total heterotrophic bacteria, TCC =Total coliform count, FC = Faecal Coliform

Table 2. Distribution of Isolates across the Samples

Isolate	**Local Noni**	**A**	**B**	**C**
Enterobacter sp	+	+	+	+
Micrococcus sp	+	+	+	+

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Bacillus sp	+	+	+	+
Serratia sp	+	-	-	-
Flavobacterium sp	+	-	-	-

Keys: + = isolated; - = not isolated

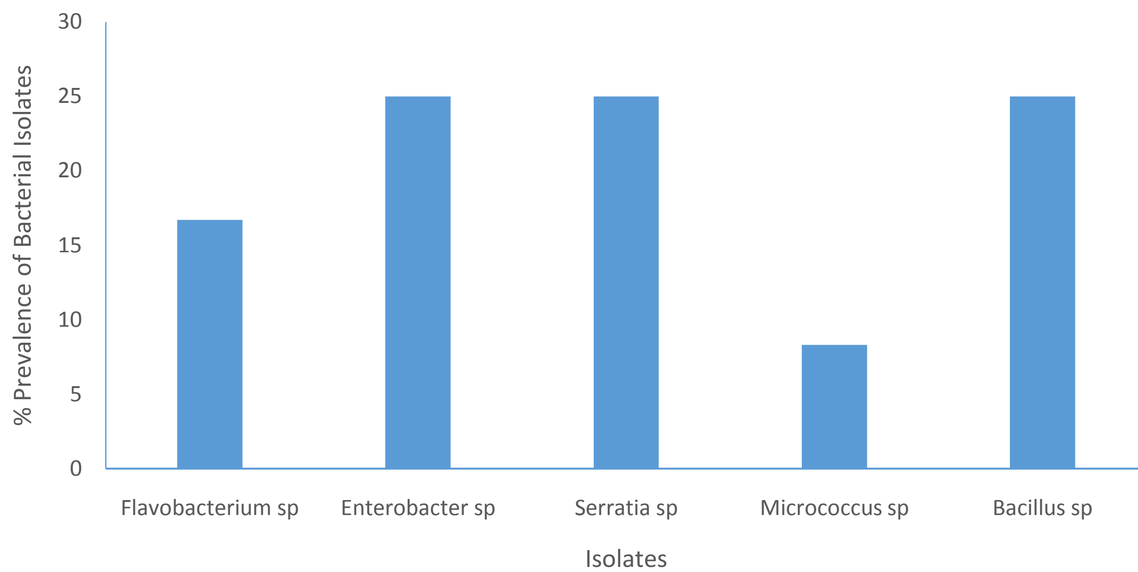

Fig. 2: Percentage occurrence of bacterialIsolates in the beverages

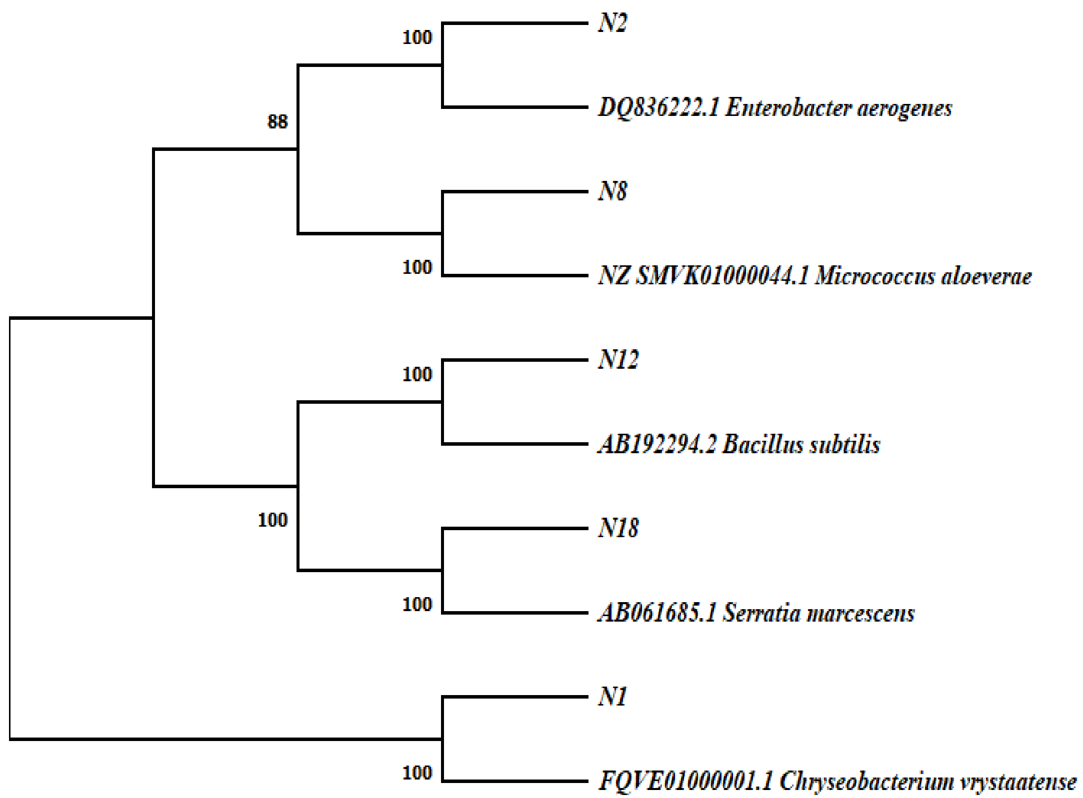

Fig. 3: Phylogenetic Tree displaying the Evolutionary Distance between the Bacterial Isolates

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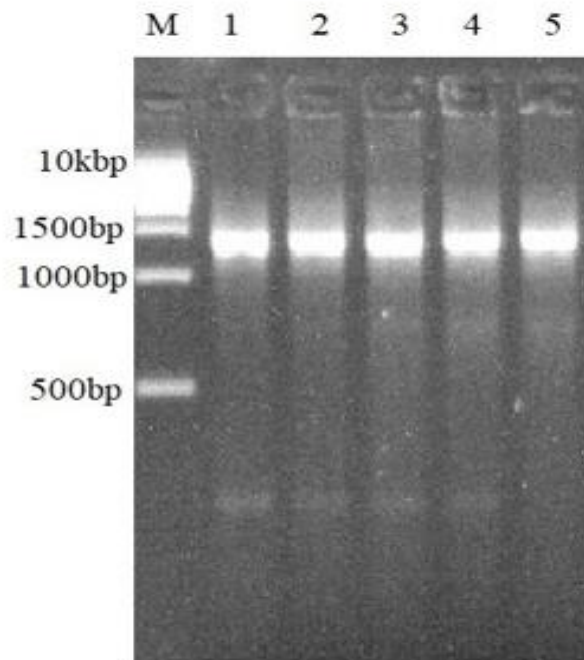

Plate 1: Amplified 16SrRNA Gene bands at 1500bp on Agarose Gel after Electrophoresis

Table 3. Virulent Attributes of the Bacterial Isolates

Virulence	***Flavobacterium* sp**	***Enterobacter* sp**	***Serratia* sp**	***Micrococcus* sp**	***Bacillus* sp**
Motility	+	+	+	-	+
Haemolysis	-	-	-	-	-
Biofilm	-	-	-	-	+

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Starch hydrolysis	**-**	**-**	**-**	**-**	+

Keys: +: positive for the test; -: negative for the test

Table 4a: Antibiotic Susceptibility of Identified Gram-negative Bacterial Isolates

Antibiotics	*Flavobacterium* (n = 2)			*Enterobacter*sp (n = 3)			*Serratia*sp (n = 3)		
	R	I	S	R	I	S	R	I	S
TET	0	0	2 (100)	3 (100)	0	0	1 (25)	0	2 (75)
COT	0	0	2 (100)	3 (100)	0	0	0	0	3 (100)
GEN	0	0	2 (100)	3 (100)	0	0	3 (100)	0	0
CRX	2 (100)	0	0	3 (100)	0	0	3 (100)	0	0
CHL	0	0	2 (100)	3 (100)	0	0	0	0	3 (100)
CTX	2 (100)	0	0	0	3 (100)	0	0	0	3 (100)
CTR	0	2 (100)	0	0	0	3 (100)	0	0	3 (100)
CIP	0	0	2 (100)	3 (100)	0	0	0	0	3 (100)
AMK	0	0	2 (100)	3 (100)	0	0	0	0	3 (100)
VAN	0	0	2 (100)	3 (100)	0	0	3 (100)	0	0
CPZ	2 (100)	0	0	3 (100)	0	0	3 (100)	0	0
MEM	2 (100)	0	0	3 (100)	0	0	0	0	3 (100)

Keys: TET = tetracycline, COT = cotrimazole, GEN = gentamycin, CRX =cefuroxime, CHL = chloramphenicol, CTX = cefotaxime, CTR = ceftriaxone, CIP = ciprofloxacin, AMK = amoxacillin, VAN = vancomycin, CPZ = cefixime, MEM = meropenem

N or n= Number of isolates, R= Resistance, I= Intermediate Resistance, S= Susceptible

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Table 4b: Antibiotics Susceptibility of Identified Gram-positive Isolates

Antibiotics	*Bacillus*sp (n = 3)			*Micrococcus*sp (n = 1)		
	R [N (%)]	I [N (%)]	S [N (%)]	R [N (%)]	I [N (%)]	S [N (%)]
CH	1 (20)	0	2 (80)	0	0	1 (100)
CPX	0	2 (40)	1 (60)	0	1 (100)	0 (0)
E	1 (33.3)	0 (0)	2 (66.7)	0 (0)	0	1 (100)
LEV	0	1 (33.3)	2 (66.7)	0	0 (0)	1 (100)
CN	1 (33.3)	0	2 (66.7)	0 (0)	0	1 (100)
APX	2 (66.7)	0 (0)	1 (33.3)	0 (0)	1 (100)	0 (0)
RD	0	0	3 (100)	0 (0)	1 (100)	0 (0)
AMX	0	1 (33.3)	2 (66.7)	0	1 (100)	0 (0)
S	0	0	3 (100)	0	0	1 (100)
NB	2 (33.3)	0 (0)	1 (66.7)	0 (0)	1 (100)	0 (0)

Keys: S: Streptomycin, NB: Norfloxacin, CH: Chloramphenicol, CPX: Ciprofloxacin, E: Erythromycin, LEV: Levofloxacin, CN: Gentamycin, APX Ampiclox, RD: Rifampicin, AMX: Amoxilicin

Table 5: Multiple Antibiotics Resistant Index (MARI) of the Isolates

MARI	*Bacillus* sp	*Micrococcus* sp	*Flavobacterium* sp	*Enterobacter* sp	*Serratia* sp
0.1	0	0	0	0	0

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0.2	2 (66.7)	1 (100)	2 (100)		
0.3	0	0	0	0	3 (100)
0.4	1 (30.3)	0	0	0	0
0.5	0	0	0	0	0
0.6	0	0	0	0	0
0.7	0	0	0	0	0
0.8	0	0	0	3 (100)	0
0.9	0	0	0	0	0

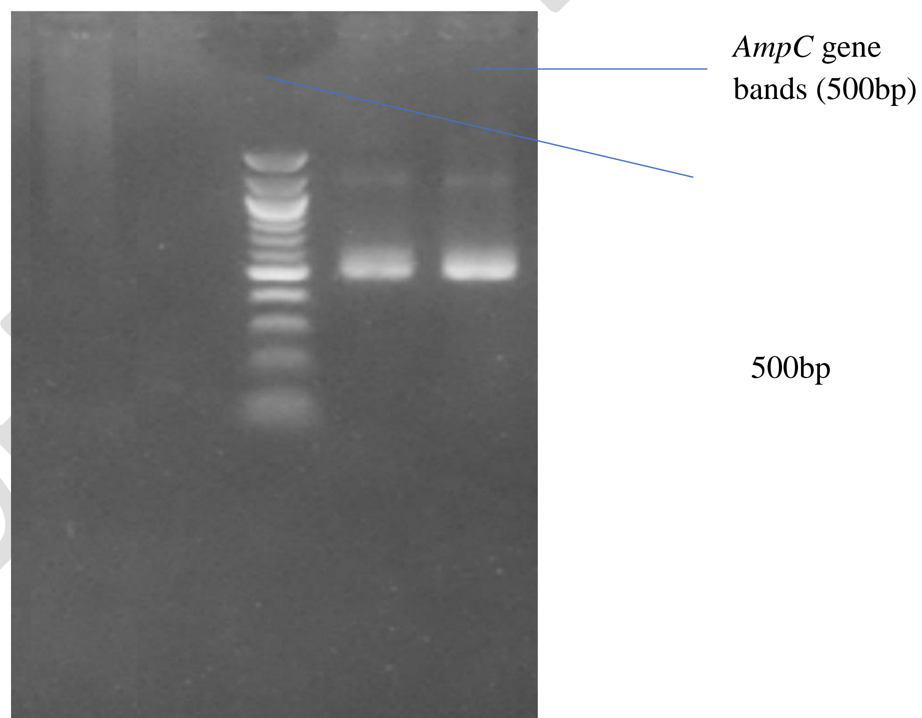

Plate 2: Amplified *AmpC* Gene band at 500bp on agarose gel after electrophoresis.
Key: 1= *Enterobacter aerogenes,* 2= *Micrococcus aloeverae*, 3=*Chryseobacteriumvrystaatense*

, L= Molecular marker, 4= *Serratia marcescens*, 5= *Bacillus subtilis*

Table 6. Phenotypic, Genomic and Resistant Gene of the Isolates

Isolates	Phenotypic Identity	Genomic Identity	Accession number	Resistant gene (ampC gene)
N2	*Enterobacter* sp	*Enterobacter aerogenes*	DQ836222.1	-
N4	*Micrococcus* sp	*Micrococcus aloeverae*	NZSMVK01000044.1	-
N11	*Bacillus* sp	*Bacillus subtilis*	AB192294.2	+
N12	*Serratia* sp	*Serratia marcescens*	AB061685.1	+
N1	*Flavobacterium* sp	*Chryseobacteriumvrystaatense*		-

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4.0 DISCUSSION

4.1 Population of bacteria across all Samples

Microorganisms are responsible for the spoilage of food and drinks. Their presence could cause infectious diseases such as cholera, diarrhoea, and typhoid [12] or could be associated with the breakdown of food components (fermentation).The total heterotrophic bacterial load and coliform load in the noni samples were very high especially when compared with WHO permissible limits of 100 cfu/ml for total viable bacteria and 0.0cfu/ml for faecal coliform [13]. A similar study has also reported counts in noni beverage which corroborate present study.

Amadi *et al.* [14] in their study reported that the total heterotrophic bacterial load ranged from $1.5 \pm 0.6 \times 10^4$ to $9.2 \pm 0.8 \times 10^4$CFU/ml.

The presence of these bacteria in the noni samplescould be attributed to contamination from the environment, processing and production processes [15]. Another possible cause of high coliform from this same location could be poor storage conditions.This is similar to previous findings byAgwa*et al*., [16] who reported highcoliforms inready-to-eat foods sold in some markets in Port Harcourt, Rivers State.

Similar cases of microbial contamination of noni fruits have also been reported in Indonesia by Sogandi and Nilasari, [17]. The noni beverages in the present study were void of *Salmonella-Shigella* and this is contradictory to Galvin *et al.,*[18] who reported the presence of *Salmonella* and *Shigella* in their study. The most common water source used for thenoni processing and preparation leading to the localisation and dispersal of microorganisms in the noni samples within the study area, as wellasstorage conditions and personnel activities predominantly could all contribute to the detection ofhigh bacterial load[19].

4.2 Prevalence of Bacterial Isolates in all Samples

The bacteria isolated and identified in this study from noni samples include *Enterobacter sp*, *Serratiasp*,*Flavobacterium sp*, *Bacillus sp*, and *Micrococcus sp.* Even though most of the microorganisms obtained in this study may not be extremely pathogenic,their presence in food and drink samples indicates contamination and could cause opportunistic infections [20]. *Enterobacter*spis a Gram-negative bacteriumproducing protease, amylase, and cellulase enzymeswhich are used as probiotics that provide benefits to humans [17]. *Serratia* sp is a rod-shaped Gram-negative bacterium in the family of Yersiniaceae. It is a facultative anaerobe and

opportunistic pathogen in humans. Due to its abundance in the environment, it is commonly found in places such as dirt and subgingival biofilm of teeth causing staining of teeth [21]. *Flavobacterium*spis a genus of Gram-negative non spore forming and motile, rod-shaped bacteria that are found in soil and fresh water in a variety of environments. Several species are known to cause disease in freshwater fish. These microbes are also mostly found in aquatic ecosystems and wet areas with freshwater or seawater [22]. *Bacillus*sp are spore-formingorganisms and can act as opportunistic pathogens causing illnesses such as cerebrospinal fluid shunt infections, endocarditis, endophthalmitis, meningitis, as well as bacteremia [23]. *Micrococcus*sp. is generally thought to be a saprotrophic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, causing some infections such as recurrent bacteremia, septic shock, arthritis, endocarditis, meningitis[23]. The prevalence of microorganisms in this study revealed *Bacillus* spp, *Enterobacter* and *Serratia* spphad the highest prevalence of 25% across all samples, and*Micrococcus* sp. was only 8.3%. This finding is comparable to that of Bernardet*et al*. [22], who also found a high prevalence of *Bacillus* and *Flavobacterium spp*at 74% and 43% respectively.The low frequency of these bacterial isolates in this study could be due to the extreme measures adopted to reduce contamination during the production of the local noni and the analysis of both the local and commercial noni beverages. A previous study has reported that unhygienic settings, cross-contamination, areas with high population density, anthropogenic activities, and the storageconditions of the food and drinks could increase microbial load and frequency of microorganisms [24].

4.3 Susceptibility of Bacterial Isolates to Antibiotics

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Antibiotic resistance in bacterialinked to food and drinks has been a concern worldwide, and its susceptibility patterns revealed significant variance in response to antibiotics. Streptomycin and rifampicin were the most potent antibiotics against isolates of *Bacillus* sp as complete inhibition of the isolates was recorded. Isolates of *Micrococcus* exhibited resistance against gentamycin and ampiclox but were susceptible to streptomycin and chloramphenicol. This is contradictory to a previous study [25] who reported 100% susceptibility of *Micrococcus* sp to Gentamycin and Ampiclox. Isolates of *Flavobacterium* showed resistance to cefixime, meropenem, cefotaxime and cefuroxime antibiotics. The resistance of some of the isolates to the different antibiotics especially to cefuroxime, cefotaxime and cephalosporin antibiotics could be attributed to the presence of the *ampC*gene. *AmpC* genes are one of the Beta-lactamases that confer bacteria the ability to resist most antibiotics such as penicillin and first-generation cephalosporins [26]. The most potent antibiotics were Streptomycin, rifampicin and chloramphenicol since they possessed greater antibiotics properties. Streptomycin and Gentamycin belong to the aminoglycoside antibiotics that function by binding to the 30S ribosomal subunit thereby disrupting the proof-reading function which leads to the synthesis of toxic proteins caused by wrong interpretation of the mRNA [27]. Ciprofloxacin functions by adhering to DNA gyrase, thereby preventing bacteria DNA replication, transcription, and recombination [28]. Thus, the ability to completely inhibit the bacterial isolates.

The intermediate response to antibiotics known to be the last resort for infections caused by these Gram-negative bacterial isolates in this current study is of public health importance as they are tending towards resistance. This could be due to the overuse of antibiotics and the indiscriminate prescription of antibiotics which may finally lead to multidrug resistance. Obire*et al.,* [29] reported that continued exposure to antibiotics as a result of self-medication could lead to the

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upsurge of multi-drug resistant bacterial isolates. Other methods that could increase resistance to antibiotics include the production of inactivating enzymes such as lactamases that hydrolyse the active component of the antibiotic [27].

5.0 CONCLUSION

Overall, the bacterial counts in the commercial and locally fermented noni beverages were high and could be a major public health threat.

Bacteria such as *Micrococcus aloeverae*NZSMVK01000044.1, *Bacillus subtilis*AB192294.2,*Serratia marcescens*AB061685.1,*Enterobacter aerogenes* DQ836222.1and *Chryseobacteriumvrystaatense*FQVE01000001.1 were isolated from the noni samples. These bacteria could be virulent and may pose a threat to consumers especially those with weak immune systems.

The antibiotics resistance gene,*ampC*was detected in two of the isolates and this could be part of the reasons for the resistance to antibiotics recorded. Although some of the isolates do not possess the gene, other mechanisms could account for the observed antibiotics resistancereported in the study. The study therefore accentuated the bacteriological quality as well as antibiotics resistance profile of bacterial associated with NONI (*Morindacitrifolia*) Drinks Sold in Port Harcourt.

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