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Response of *Escherichia coli* isolated from *Tympanotonos fuscatus* and *Pachymelania aurita* to conventional antibiotics, *Piper guineense* and seed extracts

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Abstract
Seafoods like periwinkles are reported to contain high bacterial load and serve as a route of ingesting pathogenic *E. coli* into human body as a result of poor sanitary conditions of the open markets and indiscriminate disposal of sewage into its water habitat. This study was carried out to investigate the Response of *Escherichia coli* Isolated from Periwinkles (*Tympanotonos fuscatus* and *Pachymelania aurita*) to Conventional Antibiotics and Uziza (*Piper guineense*) Leaf and Seed Extracts. Fifty-four (54) samples (raw and parboiled) were purchased from three different markets for three months using standard methods. Antibiotics disk diffusion method was carried out as well as extracts from Uziza leaf and seed was assayed using agar well and agar dilution methods. The susceptibility patterns of *E. coli* to antibiotics showed that all the isolates (100%) were susceptible to Levofloxacin and ofloxacin and a decreasing trend of resistance in the order; Ampiclox and Cefotaxime (89%) > Imipenem and Nalidixic acid (78%) > Amoxicillin-clavulanate (66.7%) > Cefexime (56%) > ceftriaxone-Sulbactam (33.3%) > Gentamycin (22%) > Cefuroxime (11%). The antimicrobial activity of (Uziza) leaf showed that at higher concentrations (300mg/ml) all the isolates were inhibited. While the minimum inhibitory concentration (MIC) observed for the leaf extract was 37.5mg/ml, the MIC of the seed was 9.375mg/ml. Molecular studies confirmed all the isolates screened had the blaTEM gene present in their genome. Out of the twenty-seven (27) *E. coli* isolates, (88.8%) had multidrug resistance index ≥ 0.2. The study therefore showed that the seed extract showed more potency than the leaf extract. Uziza leaf and seeds could therefore be used in the control of *E. coli* associated with sea food, while discouraging indiscriminate use of antibiotics.

Keywords: Conventional antibiotics, *Escherichia coli*, leaf and seed extracts, periwinkles, Uziza (*Piper guineense*)

1. Introduction
In Nigeria, especially Niger Delta region, periwinkle has been known for its versatility in preparing several delicacies such as in soup preparations like Afang soup, edikaikong soup and some native foods like Ekpang Nkukwo [1] (Lapsinkas, 2001). The method used to process periwinkles before consumption differs among the populace.
However, studies have shown that seafood harbor many pathogenic bacteria, like *E. coli* [2] and has been implicated in food-borne disease outbreaks leading to mortality and morbidity in the population [3]. Food is a major vehicle for transmission of pathogenic organism to the human guts [3].
As a result of the high rate of consumption, the microbiological quality of seafood has engendered great attention among consumers, regulatory agencies and food processors and is on the increase [4, 5]. Seafood (periwinkles) are highly perishable hence the need for it to be handled hygienically so as to prevent bacterial proliferation and preserve its natural characteristics [6].
Medicinal plants represent the most ancient form of medication used for thousands of years in traditional medicine in many countries [7]. West Africa is a region abundantly rich in therapeutic plants which are used to treat infectious disease arising from the consumption of contaminated food [8]. Traditionally, the crude extracts of different parts of plant such as seeds, leaves, root, stem, twigs, flower and fruits were used for the treatments of some human diseases [7]. Medicinal plant contain several phytochemicals such as flavonoids, alkaloids tannins and terpenoid which possess antimicrobial and antioxidant properties [9].
Antibiogram is an overall profile of antimicrobial susceptibility testing of specific microorganism to a group of conventional antibiotics [10].

It is a useful tool in the field of public health microbiology because it aids in detecting and monitoring trends in antimicrobial resistance organism to antimicrobial agents. Antimicrobial resistance in *E. coli* associated with this seafood is rapidly of public health concern [11]. Hence the importance to study the prevalence and response of *E. coli* isolated from *Tympanotonos fuscatus* and *Pachymelania aurita* to conventional antibiotics and Uziza extracts for the benefit of consumers.

Food poisoning due to pathogens is a major issue of public health concern worldwide with countries expending a lot of resources to overcome it.

Escherichia coli has been implicated in several disease conditions globally and known to cause severe illnesses in underaged children within five years of age, adults older than sixty-five years and people with weakened immune system.

The nutritional value and versatility of periwinkles in several delicacies, consumers tend to demand large quantities of periwinkles without considering the public health risk. Antibiotic resistance in *Escherichia coli* is a rapidly expanding problem due to the organism's ability to mutate, acquire and transmit plasmids and other mobile genetic elements encoding resistance genes [11]. The above has engendered interest in determining the susceptibility patterns of *E. coli* to both antibiotics and plant extracts, and to screen for the virulence factor and genes that confer resistance in order to protect consumer's health.

The study therefore aimed to investigate the Response of *Escherichia coli* Isolated from Periwinkles (*Tympanotonos fuscatus* and *Pachymelania aurita*) to Conventional Antibiotics and Uziza (*Piper guineense*) Leaf and Seed Extracts.

The research findings will therefore enlighten the public on the health risk associated with the consumption of contaminated seafoods. It will provide a guide on the effective drug of choice for the treatment of the microbe, as well recommend the medicinal plant used as an alternative to conventional antibiotics. This is very crucial especially with the current trends of the evolution of drug-resistant microorganisms primarily due to indiscriminate use of the antimicrobial agents and the ability of the organism to mutate and transmit plasmids and also advocate for standard safe practices in cause of handling and processing so as to reduce food intoxication in consumers.

2. Materials and Methods

2.1 Description of Study Area

The study was carried out in three different locations in Rivers State: Creek Road and Mile 3 markets in Port Harcourt City Local Government Area (4º75'8 N, 7º02'3 E and 4º80'42"N, 6º99'24"E) and as well as Oilmill market in ObioAkpor Local Government Area (4º85'85 N, 7º06; 8 E). These markets were selected based on several reasons such as at the Creek Road, the vendors receive high patronage of *T. fuscatus* and *P. aurita* on a daily basis due to the availability and easy accessibility to the market. The market is also in close proximity to the rivers where anthropogenic activities occur. At the oilmill market, the vendors receive high patronage of periwinkles; the poor sanitary condition of the market can contaminate the seafood leading to high buildup of bacterial load. The Mile 3 market is situated at a strategic place where it receives large populations of people on a daily basis [2].

2.2 Sample Collection

A total of 54 samples (parboiled and raw) comprising of *T. fuscatus* and *P. aurita* was purchased monthly for a duration of three months, from the different locations (Creek Road, Oilmill, and Mile 3 markets) in Rivers State, Port Harcourt, Nigeria. Twenty-seven (27) samples of *T. fuscatus* and twenty-seven (27) samples of *P. aurita* each were purchased. The samples were labeled properly, put in a sterile ice chest and transported to the Department of Microbiology Laboratory Rivers State University, Port Harcourt for bacteriological analysis.

2.3 Sample Preparation

Preparation of stock analytical unit was carried out by weighing 10g of edible part of *T. fuscatus* and *P. aurita* (raw and parboiled) samples and was homogenized using sterile blender in 90ml of normal saline (diluent).

2.4 Hemolysis Test

Each test organism was evenly streaked on the surface of distinct prepared blood agar plates and incubated at 35 to 37 °C for 24 hours. After incubation, the plates was removed from the incubator and observed lysing of red blood cells in the media. Many *E. coli* can be both beta hemolytic and gamma hemolytic. It was beta hemolytic when there was complete lysis of the red blood cells and the area around and under the streaked culture appear transparent, and also gamma hemolytic when there was no lysis of the red blood cell due to absence of hemolysin.

2.5 Storage of Pure Culture

Pure cultures of *E. coli* were stored in 10% (v/v) glycerol suspension in bijou bottles at 4 °C to 15^0C in a fridge.

2.6 Amplification of TEM Genes

TEM genes from the *E. coli* isolates were amplified using the forward; 5' CATTTCCGTGTCGCCCTTAT 3' and Reverse; 5' TCCATAGTTGCCTGACTCCC 3' primers on ABI 9700 Applied Biosystems thermal cycler at final volume of 40 microliters for 35cycles. The PCR mix includes 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward and reverse primer; 2µL of DNA template and then made up with 8.5µL Nuclease free water. The PCR conditions were as follows: initial denaturation, 95 °C for 5 minutes, denaturation 95 °C for 30secs, annealing, 56 °C for 30 seconds, extension, 72 °C for 45 seconds for 35 cycles and final extension, 72 °C for 5 minutes. The product was fixed in a 1% agarose gel at 150V for 30minutes and visualized on a UV transilluminator for a 793 bp amplicons.

2.7 Antimicrobial Susceptibility Testing

2.7.1 Preparation of Standard Bacterial Suspension

A 24 hours pure culture of the test organism was emulsified in a sterile nutrient broth tubes, and the turbidity adjusted to 0.5 Mcfarland turbidity standard using sterile normal saline. The standardized bacterial suspension was used for the susceptibility test. The McFarland standard was prepared as outlined in Clinical and Laboratory Standards Institute [10].

2.7.2 Susceptibility Testing

A sterile swab stick was dipped into the tube containing the standardized bacterial suspension and the swab was used to swab the entire surface of a prepared Mueller-Hinton agar.

The agar was allowed to dry for about 3-5 mins. With sterile forceps, impregnated antimicrobial disc was placed evenly on the surface of the inoculated plate. The head of the forceps was used to press down each disc slightly to make contact with the agar. After applying the disc, the plates were incubated in an inverted position at 35^0C for about 24hours. After incubation, the diameter of each zone of inhibition was measured in mm using a centimetre rule on the underside of the plate and recorded.

2.8 Determination of Multiple Antibiotic Resistance Index (MAR)

Multiple Antibiotic Resistance (MAR) index was carried out for each isolate using the formula, MAR index = a/b;
Where 'a' stands for the number of antibiotics to which the test isolate shows resistance
While 'b' stands for the total number of antibiotics to which the test isolate was evaluated for susceptibility [12].

2.9 Collection of Plant Samples

The samples of the leaves and seeds of *Piper guineense* (Uziza) were bought from Mile 3 market in Port Harcourt, Rivers State, Nigeria. The samples were taken to the Department of Plant Science and Biotechnology, Rivers State University and were identified by Dr. N. Chibuzor.

2.9.1 Preparation of samples and extraction procedure

The leaves of *P. guineense* was washed thoroughly with tap water to remove dirts and sand particles and rinsed with distilled water. The washed samples were dried at room temperature for 5 days. The seed was dried for 24hrs, after which the samples were finely ground. The ground samples were put into sterile screw capped containers. The ethanol extracts of the samples were prepared as described by Robinson *et al*., (2020) [13]. In this method 50g of each blended samples (powdered form) was transferred into 500ml beakers each containing 500ml ethanol. This was soaked for 48hrs before they were filtered using Whatman No 1 filter paper. The supernatants were evaporated to dryness in the oven at a temperature of 40 °C. A dark green and gold coloured mass of *Piper guineense* were obtained and stored in airtight bottles at 4 °C in a refrigerator for further use.

2.9.2 Preparation of Extract Concentrations

The stock solution of the extract was prepared as described by [13]. About 3g of the residue was dissolved in 10ml of Dimethyl sulfoxide (DMSO) which gave rise to 300mg/ml stock of the ethanol extract. Further two-fold dilution was carried out by diluting 5ml of the stock solution into 5ml of Dimethyl sulfoxide (DMSO) to achieve the concentrations of 150mg/ml, 75mg/ml 37.5mg/ml, 18.75mg/ml. 9.375 mg/ml and 4.687mg/ml The juices were diluted to produce similar concentrations.

2.10 Susceptibility testing of *E. coli* isolates to plant extracts

2.10.1 Using the well-in-agar method

Sterile swab sticks were dipped into the standard bacterial culture of each isolate in separate tubes and drained by pressing the swab stick above the fluid level. The swab stick were used to streak the surface of already prepared agar medium evenly. The media plates were allowed to dry for about five minutes. Holes were bored in the medium using a sterile 6 mm cork borer. The wells were crammed with equal concentrations of the different extract transferred into the holes using Pasteur pipette and plates were incubated at 37 °C for 24hours in an upright position. Well known antibiotics and sterile water were used as positive and negative control. The procedure was carried out in duplicates. This procedure was repeated for all *Escherichia coli* isolates. After incubation, plates were observed and diameters of zone of inhibition that developed were measured in millimeters. This indicated the degree of susceptibility or resistance of the test organisms to the extracts.

2.10.2 Using Agar Dilution Method

Varying concentrations of the extracts were prepared (300, 150, 75, 37.5, 18.75, 9.38, and 4.69) mg/ml [14], and 0.1ml of the standardized organisms and the various concentrations of the extracts were put into the sterile tubes of nutrient broth. These tubes were incubated at 37 °C for 18 to 24hours. Thereafter, the tubes were examined for visible growth or turbidity in the tubes and recorded. The MIC recorded as the concentration at which no visible growth was observed when compared with the controls. Tubes containing nutrient broth and organisms without extracts served as negative control while the tube containing only the nutrient broth and extracts without organism served as positive control.

3. Results

3.1 Genomic Characteristics and Detection of Antibiotics Resistance (TEM Genes) of the Isolates

The Agarose gel electrophoresis showing the amplified TEM gene of the 6 most resistant *E. coli* isolates to antibiotics showed that lane 1-6 revealed the TEM gene band at 793bp while lane M represented the 50bp DNA ladder. This showed that the six (6) *E. coli* isolates screened for TEM gene had the gene present in their genome as shown on Plate 1.

3.2 Susceptibility Pattern of *Escherichia coli* Isolated from *T. fuscatus* and *P. aurita*

The results of the susceptibility pattern of *E. coli* is as indicated in Table 1. The results showed all the isolates of *E. coli* were susceptible to levofloxacin (100%), Ofloxacin (100%), followed by Cefuroxime (89%), Gentamycin (78%), Nitrofurantoin (66.7%) and Ceftriaxone-sulbactam (66.7%). A decreasing pattern of *E. coli* resistance was noted as follows; Ampiclox (89%), Cefotaxime (89%), Imipenem (78%), Nalidixic acid (78%), Amoxicilin clavulanate (66.7%), Cefexime (56%), Ceftriaxone-sulbactam (33.3%), Gentamycin (22%) Cefuroxime (11%)

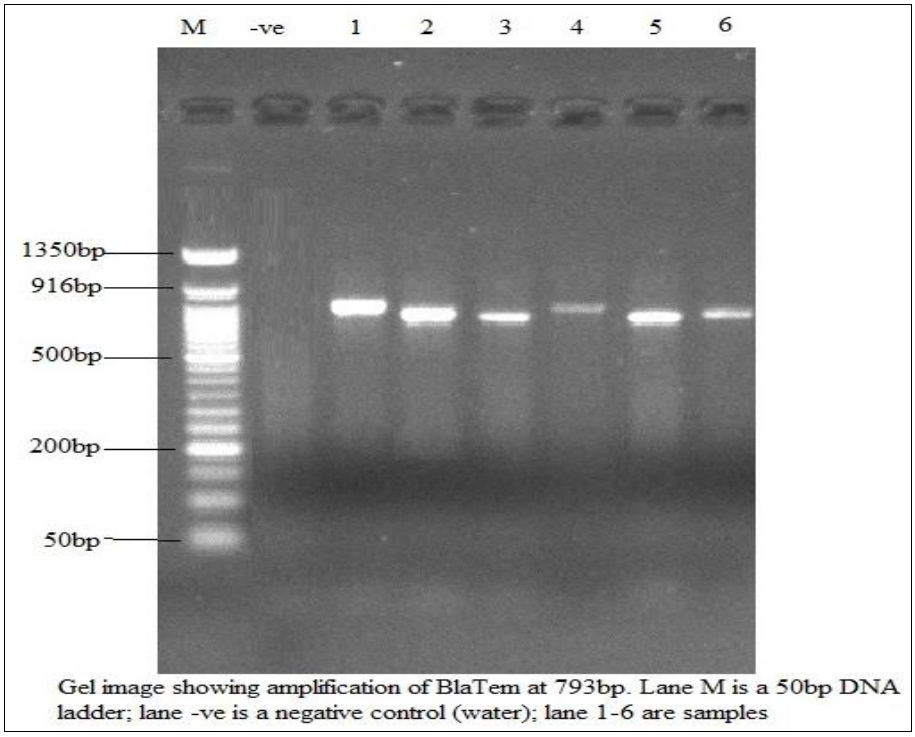

Plate 1: Amplified BlaTem gene bands of the six (6) *E coli* isolates on Agarose Gel after Electrophoresis

Table 1: Susceptibility Pattern of *Escherichia coli* Isolated from Seafood (*P. aurita* and *T. fuscatus*) in Port Harcourt, Rivers State

Antibiotics	WGT. (MG)	Susceptible n(%)	Resistant n(%)	Intermediate n(%)
LBC	5	27 (100)	0	0
CXM	30	24 (89)	3 (11)	0
ACX	10	3 (11)	24 (89)	0
CTX	25	0	24 (89)	3 (11)
IMP	10	6 (22)	21(78)	0
OFX	5	27 (100)	0	0
GN	10	21 (78)	6 (22)	0
NA	30	0	21 (78)	6 (22)
ZEM	5	6 (22)	15 (56)	6 (22)
AUG	30	9 (33.3)	18 (66.7)	0
CRO	45	18 (66.7)	9 (33.3)	0
NF	300	18 (66.7)	3 (11)	6 (22)

Key: LBC(Levofloxacin), CXM (Cefuroxime), ACX(Ampiclox), CTX(Cefotaxime), IMP(Imipenem), OFX (Ofloxacin), GN(Gentamycin), NA(Nalidixic acid), ZEM(Cefexime), AUG(AmoxicilinClavulanate), CRO(CeftriaxoneSulbactam), NF(Nitrofuanton)

3.3 Multiple Antibiotics Resistant indices of *E. coli*

The Multiple Antibiotic Resistance Index of *E. coli* isolated from *T. fuscatus* and *P. aurita* is shown in Table 2. Data showed that of the 27 *E. coli* isolates, 24(88.8%) had multidrug resistance index greater than 0.2.

Table 2: MAR Indices of *Escherichia coli* (N=27)

Mar index	Number (%)
0.1	3(11.1)
0.3	3(11.1)
0.4	3(11.1)
0.5	9(33.3)
0.7	3(11.1)
0.8	3(11.1)
10	3(11.1)
Total (≥ 0.02)	24(88.8%)

Key: Multiple Antibiotic Resistance (MAR)

3.4 Antibacterial activity of plant extracts of Uziza leaf and seed

The antibacterial activity of ethanolic extract of seed and leaf of *Piper.guineense* (Uziza) was determined on *Escherichia coli* isolates using agar well diffusion method for Minimum inhibitory concentration (MIC). Result of the antibacterial activity of the ethanolic extract of Uziza leaf is presented on Table 3. The obtained data showed that at higher concentrations (300mg/ml) inhibited all the test isolates with zone of inhibition ranging from 10-13. Also, at lower concentration the test isolates exhibited varying resistance to the extract. Data also reported that the least effective concentration observed for the leaf extract was 37.5mg/ml (Table 3).

Result of the antibacterial activity of the ethanolic extract of Uziza seed is presented in Table 4. The obtained data showed that at a concentration of 300mg/ml it inhibited all the test isolates. The diameter zone of inhibition for the ethanolic extract at 300mg/ml of the seed ranged between 10 mm -16 mm. The least effective concentration of seed extract was observed to be 9.375mg/ml. The ethanolic extract of the seed showed more activity than the leaf extract against the isolates tested.

Table 3: Antibacterial activity of ethanol extract of Uziza Leaf (*P. guineense*)

Isolate code	300mg/ml	150mg/ml	75mg/ml	37.5mg/ml	18.75mg/ml	9.375mg/ml	4.6875mg/ml
C/PAPWNS	13	10	9	8	0	0	0
C/PARAW	13	10	8	8	0	0	0
C/TFRAW	13	10	9	0	0	0	0
C/PAPWS	10	8	0	0	0	0	0
O/PAPWS	10	9	8	0	0	0	0
O/PARAW	10	8	0	0	0	0	0
M/TFPWNS	12	10	8	8	0	0	0
M/PAPWS	12	10	10	8	0	0	0
M/TFPWS	12	10	0	0	0	0	0
C/PAPWNS	13	10	9	8	0	0	0
C/PARAW2	13	10	8	8	0	0	0
C/TFRAW2	13	10	9	0	0	0	0
C/PAPWS2	10	8	0	0	0	0	0
O/PAPWS2	10	9	8	0	0	0	0
O/PARAW2	10	8	0	0	0	0	0
M/TFPWNS2	12	10	8	8	0	0	0
M/PAPWS2	12	10	10	8	0	0	0
M/TFPWS2	12	10	0	0	0	0	0
C/PAPWNS3	13	10	9	8	0	0	0
C/PARAW3	13	10	8	8	0	0	0
C/TFRAW3	13	10	9	0	0	0	0
C/PAPWS3	10	8	0	0	0	0	0
O/PAPWS3	10	9	8	0	0	0	0
O/PARAW3	10	8	0	0	0	0	0
M/TFPWNS3	12	10	8	8	0	0	0
M/PAPWS3	12	10	10	8	0	0	0
M/TFPWS3	12	10	0	0	0	0	0
Range of Inhibition	10-13	8-10	0-10	0-8	0	0	0
MIC Range 37.5mg/ml-150mg/ml							

Table 4: Antibacterial activity of ethanol extract of Uziza Seed (*P. guineense*)

Isolate code	300mg/ml	150mg/ml	75mg/ml	37.5mg/ml	18.75mg/ml	9.375mg/ml	4.6875mg/ml
C/PAPWNS	10	8	0	0	0	0	0
C/PARAW	14	12	10	8	8	6	0
C/TFRAW	10	8	8	0	0	0	0
C/PAPWS	12	10	8	6	0	0	0
O/PAPWS	12	10	8	0	0	0	0
O/PARAW	14	11	10	10	8	0	0
M/TFPWNS	15	12	11	8	0	0	0
M/PAPWS	16	11	8	0	0	0	0
M/TFPWS	12	10	8	0	0	0	0
C/PAPWNS	10	8	0	0	0	0	0
C/PARAW2	14	12	10	8	8	6	0
C/TFRAW2	10	8	8	0	0	0	0
C/PAPWS2	12	10	8	6	0	0	0
O/PAPWS2	12	10	8	0	0	0	0
O/PARAW2	14	11	10	10	8	0	0
M/TFPWNS2	15	12	11	8	0	0	0
M/PAPWS2	16	11	8	0	0	0	0
M/TFPWS2	12	10	8	0	0	0	0
C/PAPWNS3	10	8	0	0	0	0	0
C/PARAW3	14	12	10	8	8	6	0
C/TFRAW3	10	8	8	0	0	0	0
C/PAPWS3	12	10	8	6	0	0	0
O/PAPWS3	12	10	8	0	0	0	0
O/PARAW3	14	11	10	10	8	0	0
M/TFPWNS3	15	12	11	8	0	0	0
M/PAPWS3	16	11	8	0	0	0	0
M/TFPWS3	12	10	8	0	0	0	0
Range of Inhibition	10-16	8-12	0-10	0-10	0-8	0-6	0
MIC Range 9.375mg/ml-150mg/ml							

3.5 Hemolytic Pattern of *Escherichia coli* Isolates

The results obtained for the hemolytic pattern of the isolates are presented in Table 5. The data obtained showed that out of the twenty-seven (27) isolates, eighteen (18) isolates (66.7%) exhibited no hemolysis (Gamma hemolysis) while nine (9) isolates (33.3%) exhibited complete hemolysis (Beta hemolysis).

Table 5: Hemolytic patterns of *Escherichia coli* isolates

		Hemolytic Pattern (%)	
S/N	**Isolate code**	**Beta (β)**	**Gamma (γ)**
1	C/PAPWNS1	+	-
2	C/PARAW	+	-
3	C/TFPWS	-	+
4	C/TF RAW	-	+
5	O/PAPWS	+	-
6	O/PARAW	-	+
7	M/PAPWS	-	+
8	M/TF PWS	-	+
9	M/TFPWNS	-	+
10	C/PAPWNS2	+	-
11	C/PARAW2	+	-
12	C/TF PWS2	-	+
13	C/TF RAW2	-	+
14	O/PAPWS2	+	-
15	O/PARAW2	-	+
16	M/PA PWS2	-	+
17	M/TF PWS2	-	+
18	M/TFPWNS2	-	+
19	C/PAPWNS3	+	-
20	C/PARAW3	+	-
21	C/TFPWS3	-	+
22	C/TF RAW3	-	+
23	O/PAPWS3	+	-
24	O/PARAW3	-	+
25	M/PA PWS3	-	+
26	M/TF PWS3	-	+
27	M/TFPWNS3	-	+
	Total	9(33.3)	18(66.7)

4. Discussion

Antibiotic resistance in *E. coli* is rapidly an expanding problem due to the organism's ability to mutate, acquire and transmit plasmids and other genetic elements encoding resistance gene. The above has brought about its impacts in epidemiological studies. Results of the antibiotic sensitivity showed that a high percentage of *E. coli* were susceptible to levofloxacin and ofloxacin (100%). These are the most effective drug against *E. coli*. This result is in agreement with the work of [15] and Adekunle *et al*., 2022 [16]. The drug directly inhibits bacterial DNA synthesis; it interferes with DNA breaking the DNA strands by inhibiting DNA-gyrase in susceptible organisms which inhibit the relaxation of supercoiled DNA [17]. This susceptibility was followed by Cefuroxime (89%), Gentamycin (78%) and Nitrofurantoin and Ceftriaxonesulbactam (66.7%). Susceptibility of *E. coli* to cefuroxime in this study is as a result of its effectiveness against most Gram negative bacteria including *E. coli*. This drug acts by inhibiting the bacteria cell wall synthesis [18]. Gentamycin acts by inhibiting bacteria protein synthesis by binding to 30S ribosomes [19]. Nitrofurantoin binds to the bacterial ribosomes and inhibits bacterial enzymes involved in the synthesis of DNA, RNA, cellwall and protein synthesis and other metabolic enzyme [20]. Similar findings were reported by Saera *et al*., 2017 [21] where *E. coli* demonstrated susceptibility to cefuroxime, gentamycin, Nitofurantoin, ceftriaxone sulbactam, ofloxacin and levofloxacin.

A high percentage resistance of *E. coli* were observed with respect to the antibiotics tested. This resistance could be attributable to the modification of the drug target site, inactivation of the drug and active efflux of the drug. These mechanisms may be located on the bacterial chromosome (intrinsic) or acquired via a plasmid (TEM, SHV, CTX-M). The high resistance to the beta-lactam drugs ampiclox and amoxicillin could be as a result of the extensive and misuse as well as their affordability and acquisition of the blaTem, BLASHV and BLACTX [22, 23].

The high resistance to flouroquinolone Nalidixic acid observed in this study could be attributed to the acquisition via plasmids carrying quinolone resistance (qnr) and gyrA genes. The resistance to carbapenem (imipenem) can arise from high-level expression of plasmid-mediated class C β-lactamase combined with reduced outer membrane permeability or loss of an outer membrane protein deficiency [24]. Also, it may be due to efflux changes co-mediated by chromosomal AmpC β-lactamase overproduction and outer membrane protein loss [25]. The resistance of *E. coli* to β-lactam drugs, carbapenem, fluoroquinolone and cephalosporin is in agreement with the work of [26].

Resistance of *E. coli* to antibiotics is of great public health concern. The presence of multidrug resistant strains is an indication of different mechanisms developed by *E. coli* to evade and resist antibiotics therapy thereby making the treatment options expensive. The results of the Multiple Antibiotic Resistance (MAR) of *E. coli* isolated in this study showed that the percentage of isolates with MAR index values greater than 0.2 was (88.8%). A MAR index value greater than 0.2 is an indication of a high risk source of contamination where antibiotics are frequently used [27, 28]. This could also be due to the misuse of antibiotics arising from the infection of *E. coli* acquired from this source [23].

The study indicated the presence of BlaTem gene which is the most prevalent gene in *E. coli* that confer antibiotics resistance. This extended spectrum β-lactamase gene (BlaTEM) has been popularly known to be responsible for the resistance of *E. coli* to cephalosporin and penicillin antibiotics used in this study and its overproduction can increase the ability of the isolates to resist these antibiotics totally [23]. BlaTEM gene codes for the production of Bla TEM βeta-lactamase enzyme which destroys the Beta-lactam ring of the antibiotics thereby inhibiting the activity of the antibiotics [23, 29].

The antibacterial activity of the plant extract Uziza (leaf and seed) was concentration dependent. This means that higher concentrations of the extract had better antibacterial activity than lower concentrations. This study showed that the seed had better activity than the leaf on the isolates tested. This report is not in consonance with the previous study by Anyawu and Nwosu 2013 [30], which reported a higher activity in the leaf extract of *Piper guineense*. The leaf showed that at higher concentration (300mg/ml) it inhibited all the test isolates with zone of inhibition ranging from 10mm -13mm. Also, at lower concentration the test isolates exhibited varying resistance to the extract. The minimum inhibitory concentration (MIC) observed for the leaf extract was 37.5mg/ml. Studies on the seed extract showed that at a concentration of 300mg/ml, it inhibited all the test isolates

with the zone of inhibition for the seed extract ranging between 10mm and 16mm. The least effective concentration of the seed extract was observed to be 9.375mg/ml. This study therefore showed that *piper guineense* extracts contain antimicrobial compounds that could be useful for management of *E. coli* infections. Its effectiveness could be due to the phytonutrients such as saponins, glycosides, flavonoids, alkaloids (bioactive compounds) inherent in it. The presence of these phytonutrients supports the use of this herb as an antimicrobial agent.

The hemolytic pattern of *Escherichia coli* was observed to be 66.7% and 33.3% for Gamma hemolysis and Beta hemolysis, respectively. Gamma hemolysis indicates the absence of lysis of red blood cells (no hemolysis) while Beta hemolysis indicates a complete lysis of the red blood cells [31]. This indicated that 66.7% of *E. coli* lacked the ability to break down the red blood cells while 33.3% of the isolates showed the capability of complete breakdown of red blood cell and such breakdown could be due to the production of toxin (hemolysin) which is an important virulent factor in the pathogenesis of infections such as hemolytic uremic syndrome (HUS) which causes the destruction of red blood cells and can lead to renal failure. Hemolytic uremic occurs as a complication of a diarrheal infection usually by strains O157:H7 *E. coli*.

5. Conclusion

The antibiotics susceptibility pattern indicated that levofloxacin and ofloxacin could be used as the drug of choice for the treatment of foodborne infections of caused by *E. coli* However, the presence of blaTEM gene in the isolates tested was noted in all the isolates of *E. coli* in this study, indicating a possible plasmid mediated mode of drug resistance of the isolates recovered from the seafood.

More so, the antibacterial activity of the ethanolic extract of (Uziza) *piper guineense* leaf and seed were very effective and their effectiveness though concentration dependent, proved to be potent in the control of *E. coli* infection. This could be used to control *E. coli* foodborne illness especially since they are food spices. Furthermore, the uziza seed extract was more active that the leaf extract, since it showed a higher antibacterial activity at a minimum inhibitory concentration of 9.375mg/ml.

The study recovered *Beta* hemolytic as well as O157:H7 strains of *Escherichia coli* in the periwinkles. This therefore poses a serious public concern as exposure to these bacterial agents could lead to clinical conditions such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome which could lead to kidney failure and other grave health challenges. The use of herbs like Uziza seed and leaf as food spices is highly recommended.

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Author's Contribution

Not available

6. References

1. Deeyah FE, Williams JO, Sampson T. Prevalence and Molecular Characterization of *Escherichia coli* Isolated from Periwinkles (*Tympanotonos fuscatus* and *Pachymelania aurita*). Int J Microbiol Appl Sci. 2024;3(1):248-57.
2. Lapsinkas P. Omega-6 fatty acids, what, why, where and how? Leatherhead Food Research Association. Nutr Biochem Metab Clin Appl. 2001;2nd ed. Elsevier.
3. Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G. Antimicrobial activity of some of the South-Indian spices against Serotypes of *Escherichia coli*, Salmonella, Listeria monocytogenes and Aeromonas hydrophila. Braz J Microbiol. 2006;37:153-8.
4. World Health Organization, Food and Agriculture Organization of the United Nations. Prevention and reduction of food and feed contamination. Geneva: World Health Organization; Food and Agriculture Organization of the United Nations; c2012.
5. Food and Agriculture Organization of the United Nations. Good Hygienic Practices in the preparation and sale of street foods in Africa. Tools for training. Rome: Food and Agriculture Organization of the United Nations; c2009.
6. Fahim AES, Edris AAI, Fatin SH, Azza HE, Nairoz MA. Microbiological Evaluation of Some Heat-Treated Fish Products in Egyptian Markets. J Eur Comm Nutr. 2017;12(3):123-32.
7. Khan H. Medicinal Plants in Light of History: Recognized Therapeutic Modality. J Evid Based Complement Alternat Med. 2014;19(3):216-9.
8. Oguntibeju OO. Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. J Inflamm Res. 2018;11:307.
9. Talib WH, Mahasneh AM. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. Molecules. 2010;15(3):1811-24.
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, Twenty-first informational supplement. CLSI document M100-S21. Wayne, PA: Clinical and laboratory standards institute; c2017. p. 68-70.
11. Chroma M, Kolar M. Genetics methods for detection of antibiotic resistance: focus on extended-spectrum β-lactamases. Dept Microbiol, Fac Med Dent, Palacky Univ Olomouc, Czech Rep. 2010;154(4):289-96.
12. Sampson T, Moro K, Peekate LP. Conceptual Profile of Vibrio Species and the Associated Physicochemical Dynamics of Some Aquatic Reservoirs in Andoni Local Government Area of Rivers State, Nigeria. Acad J Life Sci. 2022;8(1):1-7.
13. Robinson VK, Nnamdi AU, Korobe BP. Antifungal activity of Local Gin (kai kai) extract of Andrographis paniculata on fungal isolates. Int J Res Innov Appl Sci. 2020;5(6):17-20.
14. Agwa OK, Uzoigwe CI, Mbaegu AO. Antimicrobial activity of Camwood (Baphia nitida) dyes on common human pathogens. Afr J Biotechnol. 2012;11(26):6884-90.
15. Williams JO, Douglas SI, Onyedibia GC. Antibiogram and Bacteriological Analysis of Biofilm Producing Isolates in Bore hole water from hospitals facilities in Port Harcourt, Rivers State. Int J Curr Microbiol Appl Sci; c2021.
16. Adekunle OC, Idris OJ, Wahab AA, Adekanle AM. Antibiotic susceptibility and detection of resistance genes of *E. coli* among healthy pregnant women in Designated Hospitals Osogbo, Osun State. J Appl Sci

Environ Manag. 2022;26(6):1151-5.
17. Nazia MS. National Library of Medicine, National Centre for Biotechnology information; c2022.
18. Vardanyan RS, Amby VJ. Synthesis of Essential Drugs. Antibiotics; c2006. p. 425-98.
19. Bruno JC, Prasanna T. Gentamicin. In: StatPearls. StatPearls Publishing; c2023.
20. Pereckaite L, Agnes G, Evelina BG, Marius V, Leva C, Vacis T. CTX-M-Producing *Escherichia coli* strains: resistance to temocillin, fosfomycin, nitrofurantoin and biofilm formation. Future Microbiol. 2022;17:10.
21. Saera SK, Ayesha N, Samina G, Farhat B, Jamal A. Antibiotic susceptibility in commonly isolated pathogens from urinary tract infection in a cohort of subjects from low socioeconomic strata. Pak J Med Sci. 2017;33(2):254-9.
22. Gourmelon M, Montet MP, Lozach SL, Mennec C, Pommepuy M, Beutin L, *et al*. First isolation of shiga toxin producing *Escherichia coli* variant strains in Shellfish from coastal areas in France. J Appl Microbiol. 2006;100:85-97.
23. Sampson T, Barika PN, Peekate L, Akani NP. Prevalence and antibiogram of *Escherichia coli* isolated from edible cockles (*Senilia senilis*) in Rivers state, Nigeria. Int Res J Public Environ Health. 2020;7(5):149-56.
24. Agnes AW, Van BR, Arenas J, Goessens WH, Tommassen J. Acquisition of carbapenem resistance by plasmid-encoded-AmpC-Expressing *Escherichia coli*. Antimicrob Agents Chemother. 2016;61(1):1413-6.
25. Suh B, Bae IK, Kim J, Jeong SH, Yong D, Lee K. Outbreak of Meropenem-resistant Serratia marcescens comediated by chromosomal AMPC beta-lactamase overproduction and outermembrane protein loss. Antimicrob Agents Chemother. 2010;54:5057-61.
26. Zhong H, Xwu G, Wang J. Temporal association between Antibiotic use and Resistance Gram-negative bacteria. Braz J Biol. 2023;83.
27. Rotchell D, Paul D. Multiple antibiotic resistance index. fitness and virulence potential in respiratory Pseudomonas aeruginosa from Jamaica. J Med Microbiol. 2016;65:251-71.
28. Ayandele A, Oladipo EK, Oyebisi O, Kaka MO. Prevalence of Multi-Antibiotic Resistant *Escherichia coli* and Klebsiella species obtained from Tertiary Medical Institution in Oyo State, Nigeria. Natl Libr Med. 2020;1:9.
29. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev. 2008;3(2):234-58.
30. Anyawu CU, Nwosu GC. Assessment of the antimicrobial activity of aqueous and ethanolic extracts of Piper guineense leaves. J Med Plant Res. 2014;8(10):436-40.
31. Kato GJ, Steinberg MH, Gladwin MT. Intravascular hemolysis and the pathophysiology of sickle cell disease. J Clin Invest. 2017;127(3):750-60.

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