South Asian Journal of Research in Microbiology

7(3): 35-46, 2020; Article no.SAJRM.60111
ISSN: 2582-1989

Bacterial Isolates from Bivalve Clams (*Galatea paradoxa*, Born 1778): Occurence, Multi-drug Resistance, Location of Antibiotic Resistance Marker and Plasmid Profiles

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MUO and CUI designed the study and wrote the protocol. Author OJA wrote the first draft of the manuscript, managed the literature searches and statistical analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2020/v7i330174
Editor(s):
(1) Dr. Ana Claudia Coelho, University of Tras-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Vanessa Acosta de Matheus, Universidad Técnica de Manabí, Ecuador.
(2) Dhouha Boussoufa, University Tunis El Mannar, Tunisia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60111

Original Research Article

Received 08 June 2020
Accepted 15 August 2020
Published 21 August 2020

ABSTRACT

The occurrence of bacterial isolates in *Galatea paradoxa* (Born 1778) was determined using standard bacteriological method. The multi-drug resistance, location of antibiotic markers, plasmid DNA extraction and electrophoresis was determined by disc diffusion, acridine orange, TENS alkaline lysis and 0.8% agarose gel electrophoresis, respectively. Of the 63 bacterial isolates from *G. paradoxa*, *Staphylococcus aureus* and *Streptococcus pyogenes* had the highest and lowest percentage of occurrence with 40.0% and 5.0%, respectively. *Escherichia coli* was 25.0%, *Pseudomonas aeruginosa* (17.5%), *Enterococcus* spp and *Salmonella* spp (15.0%) each, *Bacillus subtilis* (12.5%), *Klebsiella pneumoniae* and *Enterococcus faecalis* (10.0%) each while *Vibrio cholerae* was (7.5%). The results showed Streptomycin and Ciprofloxacin as the most effective antibiotics against bacterial isolates from *G. paradoxa*. *Bacillus subtilis* and *P. aeruginosa* displayed 100% sensitivity to Streptomycin; *Salmonella* spp and *E. faecalis* were 100% sensitive to Augmentin. *V. cholerae* and *S. pyogenes* showed 100% resistance to Penicillin and Rifampicin,

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respectively. Of the 63 bacterial isolates, 43 (68.3%) were multidrug resistant (MDR) isolates, of which *S. aureus* and *E. coli* had the widest multiple antibiotic resistance (MAR) indices ranging from 0.3 to 0.8, while S. *pyogenes* had the least MAR ≤ 0.5. Of the 43 MDR bacterial isolates, 16.3%, 23.3% and 60.5% had their entire antibiotic resistance encoded on plasmid, chromosome and both plasmid and chromosome, respectively. The agarose gel electrophoresis showed that MDR bacterial isolates from *G. paradoxa* had plasmid DNA with molecular weights ranging from 23.1 to 31.5kb. This study has showed that *G. paradoxa* harboured bacteria which could pose serious health risks and *G. paradoxa* should be adequately cooked before consumption.

Keywords: Clams; plasmids; multi-drug resistance; antibiotics; bacteria.

1. INTRODUCTION

Galatea paradoxa (Born 1778) previously *Egeria radiata* (Lamark 1804), freshwater clam, is a bivalve, filter feeding mollusc, belonging to order 'Veneroidea'; superfamily 'Tellinoidea' and family 'Donacidae' [1]. This invertebrate aquatic animal, endemic to the West African sub-region such as the Volta (Ghana), Cross and Nun (Nigeria) and Sanaga (Cameroun) has two hinged calcareous shells that when closed tightly aid in its protection [2,3].

Galatea paradoxa is a suitable bioindicator of environmental pollution [4] and can occasionally accumulate toxins in their soft tissues via feeding on toxic phytoplankton and when ingested by humans may frequently cause food-related diseases [5]. *Galatea paradoxa* has high nutritional value [6,7] and constitutes an important source of food for human due to its protein, vitamins (A, B_2, B_6, B_{12}) and other essential minerals such as iron, phosphorus, potassium, zinc, copper, manganese, lipids and selenium [2,6]. The soft tissue of *G. paradoxa* is consumed after frying, smoking, roasting, steaming or cooking [7,3] and *G. paradoxa* also serves as a means of livelihood to young men and women in some parts of Southern Nigeria.

Antibiotic resistant bacteria from human and animals, released into wastewater, may find their way into the soil and water environments [8]. The multiple antibiotic resistances of micro-organisms especially *E. coli* from clams and other aquatic organisms have been reported [9,10,11]. Antimicrobial resistance exhibited in micro-organisms could be linked to the presence of plasmid and its heterogenous nature [12]. Plasmids are circular extrachromosomal deoxyribonucleic acid (DNA) capable of autonomous replication [13]. Plasmids, either conjugative or non-conjugative [14], allow the movement of genetic materials, such as virulence factors and antibiotic resistance genes (ARGs) between bacterial species and genera [15,16] through a horizontal genetic transfer [13]. Plasmid profiles determination is the earliest DNA-based method used as serotype-specific reference patterns for detecting certain strain with possible variation in plasmid content which is very important in epidemiological studies [15]. This study determined the occurrence, antibiotic susceptibility profiles, location of antibiotic resistant marker and plasmid molecular weights of bacterial isolates obtained from *Galatea paradoxa*.

2. MATERIALS AND METHODS

2.1 Collection and Identification of *Galatea paradoxa*

Forty freshly harvested *Galatea paradoxa* (Clams) were obtained from Itam and Akpan Andem markets in Uyo, (Akwa Ibom State, Nigeria) using sterile wide-mouth plastic containers and were immediately transported to the Department of Microbiology Laboratory, University of Uyo. The *G. paradoxa* were identified and confirmed by a Fish Taxonomist in Fisheries and Aquaculture Department, University of Uyo. The *G. paradoxa* were extensively washed with sterile distilled water, rinsed with normal saline to remove all extraneous materials before shucking. The edible part (meat) was aseptically removed as described by APHA [17] and was transferred into sterile containers for bacteriological analysis.

2.2 Bacteriological Analyses of Samples

Ten (10) grams of fleshy blended parts of *G. paradoxa* was aseptically suspended into 90 mL of sterile distilled water and vigorously shaken to dislodge adhered bacteria. Ten-fold serial dilutions of the homogenates were made and 1 mL of aliquot was pour-plated in triplicates onto

each plate of MacConkey Agar (MCA), Nutrient Agar (NA), Eosine Methylene Blue (EMB) Agar and aerobically incubated at 37°C for 24 hr. After incubation, the colonies on the positive plates were counted to obtain the Total Heterotrophic Counts (THBC), Total Coliform Counts (TCC) and Total Faecal Coliform Counts (TFCC), respectively. Thereafter, the discrete colonies were sub-cultured onto plates of freshly prepared nutrient agar and aerobically incubated at 37°C for 24 hr. The pure cultures of isolates were streaked onto nutrient agar slants, incubated at 37°C for 24 hr and stored in the refrigerator at 4°C. All isolates were Gram stained and subjected to convectional biochemical tests [18].

2.3 Antibiotic Susceptibility Profile of the Pathogenic Bacterial Isolates from *G. paradoxa*

In-vitro antibiotic susceptibility of bacterial isolates was determined using Kirby-Bauer disc diffusion technique [19]. Briefly, 10 µL of each bacterial isolate, prepared directly from a 16-hr-old agar plate and adjusted to 0.5 McFarland Standard, was inoculated on each plate of Mueller Hilton Agar (MHA). The antibiotic discs tested on Gram positive bacterial isolates were: Ciprofloxacin (CPX, 10 µg), Norfloxacin (NB, 10µg), Gentamycin (CN, 10µg), Amoxicillin (AML, 20 µg), Streptomycin (S, 30 µg), Erythromycin (E, 30 µg), Ampicloxacillin (APX, 20 µg), Chloramphenicol (CH, 30 µg), Levofloxacin (LEV, 10 µg) and Rifampin (RD, 20 µg), while Ciprofloxacin (CPX, 10 µg), Pefloxacin (PEF, 10 µg), Augmentin (AUG, 30 µg), Cephalothin (CEP, 10 µg), Streptomycin (S, 30 µg), Nalidixic Acid (NA, 30 µg), Ofloxacin (OFX, 10 µg), Trimethoprim-Sulphamethoxazole (SXT, 30 µg) and Ampicillin (PN, 30 µg) were used for Gram negative bacterial isolates. The antibiotic discs were aseptically placed on the surfaces of the culture plates with sterile forceps, and the plates were incubated at 37°C for 18 hr. Thereafter, inhibitory zones were observed and measured in millimeters (mm). The interpretation of the measurement as sensitive and resistant was determined based on the criteria of CLSI [19].

2.4 Determination of Multiple Antibiotic Resistance Index

Multiple antibiotic resistance index (MAR) was determined using the formula: MAR=x/y, where 'x' was the number of antibiotics to which the test isolate displayed resistance and 'y' was the total number of antibiotics to which the test isolate has been evaluated for sensitivity Akinjogunla and Enabulele [16]. Isolates that were resistance to three or more classes of antibiotics were taken to be multiple antibiotic resistant [20].

Fig. 1. *G. paradoxa*

2.5 Location of Antibiotic Resistance Markers of Bacterial Isolates from *G. paradoxa*

The presumptive location of antibiotic resistance markers of bacterial isolates was ascertained using acridine orange [21]. Each bacterial isolate was grown for 24 hr at 37^{o}C in a test-tube containing 9 ml of nutrient broth (pH: 7.6) and 1 ml of acridine orange (1 g/1000 ml). After 24 hrs, the broth culture was agitated, a loopful was sub-cultured onto MHA plates and antibiotic sensitivity testing was carried out. Cured antibiotic resistance markers were determined by comparing the pre-curing and post-curing antibiograms of the bacterial isolates. Absence of zone of inhibition on MHA indicated plasmid-mediated resistance, while presence of zone of inhibition on MHA indicated chromosome-mediated.

2.6 Plasmid DNA Extraction/Plasmid Profile and Gel Electrophoresis of Bacterial Isolates

The method of Xiuhua et al. [22] was used for Plasmid DNA extraction. Each 24 hr old broth culture was centrifuged for 1 min at 10,000 rpm, supernatant was discarded leaving about 100 µL together with the cell pellet, vortexed to homogenize, 300 µL of TENS solution was added, mixed by inverting tubes and 150 µL of sodium acetate was added and vortexed. This solution was centrifuged at 10,000 rpm for 10 mins to pellet cell debris and chromosomal DNA, the supernatant was transferred into fresh tube,

mixed with ice-cold absolute ethanol (900 µL) and centrifuged for 15 mins at 10,000 rpm. Thereafter, the supernatant was discarded, pellet washed in 1 mL ethanol (70 %), air-dried and re-suspended in 40 µL of Tris-EDTA buffer. The agarose gel was prepared, boiled and allowed to cool, 40 µL ethidium bromide was added and poured into casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30 mins, the comb was removed and 12 µL of the plasmid DNA sample was loaded into the wells after mixing with 4 µL of bromophenol blue. A DNA molecular weight marker, Hind III digest of DNA, was also loaded into one of the wells, the gel was thereafter electrophoresed in a horizontal tank (Gel XL Model 01951) at a constant voltage of 100 V for 1 hr 30 min and plasmid DNA bands were viewed under a short wave ultraviolet light (UV) transilluminator and the photographs were taken using a photo documentation system. The DNA bands were matched with those for Lambda DNA Hind III digest molecular weight marker in the range 0.1 – 23.1kb. The approximate molecular weight of each plasmid was consequently obtained by extrapolation on graphical plots of molecular weight of marker against the distance traveled by the respective band.

2.7 Statistical Analysis

The Statistical Package for Social Sciences (IBM SPSS Version 22.0) was used for data analysis.

3. RESULTS

A total of sixty-three (63) bacterial isolates, belonging to 10 genera, comprising 29 (46.1%) Gram positive bacteria (GPB) and 34 (53.9%) Gram negative bacteria (GNB) were isolated from *G. paradoxa*. Of the 63 bacterial isolates from *G. paradoxa*, *S. aureus* had the highest percentage of occurrence (40.0%), followed by *E. coli* (25.0 %), *P. aeruginosa* (17.5%), *Enterococcus* spp and *Salmonella* spp (15.0 %) each, *B. subtilis* (12.5%), *K. pneumoniae* and *E. faecalis* (10.0 %) each, *V. cholerae* (7.5%), while *S. pyogenes* (5.0 %) had the least percentage of occurrence (Fig. 2).

The varied antibiotics susceptibility profiles of GPB and GNB from *G. paradoxa* are presented in Tables 1 and 2. The GPB were highly sensitive to CN, S, CPX and LEV with percentage sensitivities ranging from 65.5% to 82.8%, while between 55.2% and 58.6% GPB were resistant to NB and RD. All (100%) *B. subtilis* were S and LEV sensitive; 60% *B. subtilis* displayed resistance to NB, AMX and RD; *S. pyogenes* exhibited 100% resistance to NB and RD; 75% *S. aureus* were sensitive to CPX and LEV, while 50% *S. aureus* showed resistance to AMX (Table 1). The susceptibility profiles of GNB to ten (10) antibiotics are shown in Table 2. The GNB showed between 70.5% and 82.4% sensitivities to S, AUG, OFX and CPX. The *P. aeruginosa* showed a high level of sensitivity to CPX (n=7/7; 100%) and S (n=7/7; 100%); above 70% *E. coli* were sensitive to OFX, CPX, AUG and PN; *Salmonella* spp and *E. faecalis* showed 100% sensitivity to AUG, while *V. cholerae* showed 100% sensitive to CN (Table 2).

The MAR indices of GPB and GNB from *G. paradoxa* are presented in Table 3. Of the 63 isolates obtained, 11.1% isolates were sensitive to all the antibiotics tested; 20.6% were non-MDR isolates, while 68.3% isolates exhibited multiple antibiotic resistance. *S. aureus* and *E. coli* had the widest MAR indices ranging from 0.3 to 0.8, while *S. pyogenes* had the least MAR indices ranging from 0.3 to 0.5. The MAR indices of *Enterococcus* spp, *K. pneumoniae*, *P. aeruginosa* and *V. cholerae* ranged between 0.3 to 0.7 (Table 3).

The pre-curing and post-curing antibiograms of multi-drug resistant (MDR)-GPB and GNB from *G. paradoxa* are shown in Tables 4 and 5. Of the 19 MDR-GPB from *G. paradoxa,* 3 (15.8%) isolates comprising *S. aureus* G-SA12, G-SA16 and *S. pyogenes* G-SP28 had their entire antibiotic resistant markers encoded on the plasmids; 3 (15.8%) isolates comprising *Enterococcus* spp G-ES09, G-ES23 and *B. substilis* G-BS05 had their entire antibiotic resistant markers located on the chromosomes, while 13 (68.4%) isolates had their antibiotic resistant markers located on both the plasmids and chromosomes (Table 4). Of the 24 MDR-GNB obtained , 4 (16.7%) isolates had their entire antibiotic resistant markers encoded on the plasmids; 7 (29.2%) isolates had their entire antibiotic resistant markers located on the chromosomes, while 13 (54.2%) isolates had their antibiotic resistant markers located on both the plasmids and chromosomes (Table 5).

Table 6 shows the molecular weights of plasmids in some MDR-bacterial isolates obtained from *G. paradoxa*. The results of agarose gel electrophoresis showed that MDR-bacterial isolates had varied resistance plasmids with molecular weights ranging between 23.1 and 31.5 kb (Fig. 3).

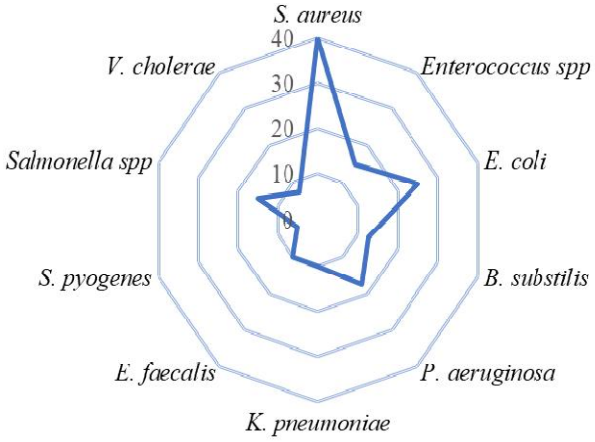

Fig. 2. Percentage occurrence of bacterial isolates in *G. paradoxa*

Table 1. Antibiotics susceptibility profiles of gram-positive bacterial isolates from *G. paradoxa*

Antibiotics		Gram Positive Bacteria / No (%) Susceptibility				
		***S. aureus* (n=16)**	***Enterococcus* spp (n=6)**	***B. subtilis* (n=5)**	***S. pyogenes* (n=2)**	**Total No (%)**
CPX	S	12(75.0)	4(66.7)	4(80.0)	2(100)	22(75.7)
	R	4(25.0)	2(33.3)	1(20.0)	0(0.0)	7(24.3)
NB	S	9(56.3)	2(33.3)	2(40.0)	0(0.0)	13(44.8)
	R	7(43.7)	4(66.7)	3(60.0)	2(100)	16(55.2)
CN	S	10(62.5)	5(83.3)	3(60.0)	1(50.0)	19(65.5)
	R	6(37.5)	1(16.7)	2(40.0)	1(50.0)	10(34.5)
AMX	S	8(50.0)	3(50.0)	2(40.0)	2(100)	15(51.7)
	R	8(50.0)	3(50.0)	3(60.0)	0(0.0)	14(48.3)
S	S	11(68.7)	4(66.7)	5(100)	1(50.0)	21(72.4)
	R	5(31.3)	2(33.3)	0(0.0)	1(50.0)	8(27.6)
RD	S	7(43.7)	3(50.0)	2(40.0)	0(0.0)	12(41.4)
	R	956.3)	3(50.0)	3(60.0)	2(100)	17(58.6)
E	S	8(50.0)	4(66.7)	3(60.0)	1(50.0)	16(55.2)
	R	8(50.0)	2(33.3)	2(40.0)	1(50.0)	13(44.8)
CH	S	10(62.5)	3(50.0)	4(80.0)	2(100)	19(65.5)
	R	637.5)	3(50.0)	1(20.0)	0(0.0)	10(34.5)
LEV	S	12(75.0)	5(83.3)	5(100)	2(100)	24(82.8)
	R	4(25.0)	1(16.7)	0(0.0)	0(0.0)	5(17.2)
APX	S	9(56.3)	3(50.0)	3(60.0)	1(50.0)	16(55.2)
	R	7(43.7)	3(50.0)	2(40.0)	1(50.0)	13(44.8)

Keys: S: Sensitive; R: Resistant; CPX: Ciprofloxacin; E: Erythromycin; CH: Chloramphenicol; APX: Ampicloxacillin, AMX: Amoxicillin; S: Streptomycin; RD: Rifampicin; NB: Norfloxacin; LEV: Levofloxacin; CN: Gentamycin

4. DISCUSSION

The *G. paradoxa* is a good source of protein, however, it has a great tendency to harbour pathogenic microorganisms, especially those that are harmful to human health due to the unsanitary conditions of the water bodies where aquatic animals are cultivated [23]. The isolation of bacterial isolates from *G. paradoxa* agrees with the findings of Adebayo-Tayo et al. [23] who reported the occurrence of bacteria in aquatic organism from two different creeks in Nigeria. In this study, *S. aureus, E. coli*, *P. aeruginosa*, *Enterococcus* spp, *K. pneumoniae, E. faecalis* and *S. pyogenes* were isolated from *G. paradoxa. Staphyloccocus aureus*, a predominant bacterial isolate, has been reported to cause food poisoning in human.

Table 2. Antibiotics susceptibility profiles of gram-negative bacterial isolates from *G. paradoxa*

Antibiotics		Gram Negative Bacteria / No (%) Susceptibility						Total No (%)
		K. pneumoniae (n=4)	*Salmonella* spp (n=6)	*E. faecalis* (n=4)	*V. cholerae* (n=3)	*E coli* (n=10)	*P. aeruginosa* (n=7)	
OFX	S	3(75.0)	5(83.3)	4(100)	2(66.7)	7(70.0)	6(85.7)	27(79.4)
	R	1(25.0)	1(16.7)	0(0.0)	1(33.3)	3(30.0)	1(14.3)	7(20.6)
PEF	S	2(50.0)	4(66.7)	1(25.0)	2(66.7)	5(50.0)	4(57.1)	18(52.9)
	R	2(50.0)	2(33.3)	3(75.0)	1(33.3)	5(50.0)	3(42.9)	16(47.1)
CPX	S	4(100)	4(66.7)	3(75.0)	2(66.7)	8(80.0)	7(100)	28(82.4)
	R	0(0.0)	2(33.3)	1(25.0)	1(33.3)	2(20.0)	0(0.0)	6(17.6)
AUG	S	3(75.0)	6(100)	4(100)	1(33.3)	8(80.0)	3(42.9)	25(73.5)
	R	1(25.0)	0(0.0)	0(0.0)	2(66.7)	2(20.0)	4(57.1)	9(26.5)
CN	S	2(50.0)	3(50.0)	3(75.0)	3(100)	7(70.0)	5(71.4)	23(67.6)
	R	2(50.0)	3(50.0)	1(25.0)	0(0.0)	3(30.0)	2(28.6)	11(32.4)
S	S	3(75.0)	6(100)	2(50.0)	0(0.0)	6(60.0)	7(100)	24(70.6)
	R	1(25.0)	0(0.0)	2(50.0)	3(100)	4(40.0)	0(0.0)	10(29.4)
CEP	S	2(50.0)	2(33.3)	3(75.0)	2(66.7)	6(60.0)	5(71.4)	20(58.8)
	R	2(50.0)	4(66.7)	1(25.0)	1(33.3)	4(40.0)	2(28.6)	14(41.2)
NA	S	1(25.0)	4(66.7)	1(25.0)	1(33.3)	6(60.0)	4(57.1)	18(52.9)
	R	3(75.0)	2(66.7)	3(75.0)	2(66.7)	4(40.0)	3(42.9)	16(47.1)
SXT	S	2(50.0)	3(50.0)	2(50.0)	1(33.3)	8(80.0)	6(85.7)	22(64.7)
	R	2(50.0)	3(50.0)	2(50.0)	2(66.7)	2(20.0)	1(14.3)	12(35.3)
PN	S	0(0.0)	4(66.7)	3(75.0)	0(0.0)	7(70.0)	5(71.4)	19(55.9)
	R	4(100)	2(33.3)	1(25.0)	3(100)	3(30.0)	2(28.6)	15(44.1)

Keys: S: Sensitive; R: Resistant; OFX: Ofloxacin; PEF: Pefloxacin; CPX: Ciprofloxacin; AUG: Augmentin; CN: Gentamycin; S: Streptomycin; CEP: Cephalothin; NA: Nalidixic Acid; SXT: Trimethoprim-Sulphamethoxazole; PN: Ampicillin

Table 3. Multiple antibiotic resistance index of bacterial isolates from *G. paradoxa*

MAR index	No (%) of Gram Positive Bacteria				No (%) of Gram Negative Bacteria						Total No (%)
	S. aureus	*S. pyogenes*	*B. substilis*	*Enterococcus* spp	*K. pneumoniae*	*Salmonella* spp	*E. faecalis*	*P. aeruginosa*	*V. cholerae*	*E. coli*	
0.0	1(6.3)	0(0.0)	1(20.0)	0(0.0)	0(0.0)	1(16.7)	0(0.0)	2(28.6)	0(0.0)	2(20.0)	7(11.1)
0.1	2(12.5)	0(0.0)	1(20.0)	1(16.7)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)	1(10.0)	6(9.5)
0.2	3(18.8)	0(0.0))	0(0.0)	1(16.7)	0(0.0)	1(16.7)	0(0.0)	2(28.6)	0(0.0)	0(0.0)	7(11.1)
0.3	2(12.5)	1(50.0)	0(0.0)	0(0.0)	1(25.0)	1(16.7)	0(0.0)	0(0.0)	0(0.0)	3(30.0)	8(12.7)
0.4	1(6.3)	0(0.0))	1(20.0)	2(33.3)	1(25.0)	2(33.3)	2(50.0)	2(28.6)	1(33.3)	2(20.0)	14(22.2)
0.5	0(0.0)	1(50.0)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	1(25.0)	0(0.0)	1(33.3)	0(0.0)	4(6.3)
0.6	5(31.3)	0(0.0)	2(40.0)	1(16.7)	0(0.0)	1(16.7)	0(0.0)	0(0.0)	0(0.0)	1(10.0)	10(16.7)
0.7	0(0.0)	0(0.0)	0(0.0)	1(16.7)	1(25.0)	0(0.0)	0(0.0)	1(14.3)	1(33.3)	0(0.0)	4(6.3)
0.8	2(12.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(10.0)	3(4.8)

Keys: MAR: Multiple Antibiotic Resistance; Values in parenthesis represent percentages

Table 4. Pre-curing and post-curing antibiogram of multi drug resistant gram positive bacterial isolates from *G. paradoxa*

Bacterial isolates	Codes	Pre-curing Antibiogram	Post-curing Antibiogram
S. aureus	G-SA01	CPX-NB-AMX-S-RD-E-CH-APX	CPX-NB-E-CH-APX
	G-SA03	NB-CN-AMX-RD-E-CH	NB-CN-CH
	G-SA04	CPX-NB-AMX-S-RD-E-CH-APX	CPX-NB-E-CH-APX
	G-SA07	CPX-AMX-RD-E-LEV-APX	AMX-E-LEV
	G-SA10	NB-CN-AMX-RD-E-CH	NB-AMX-CH
	G-SA11	CPX-CN-AMX-RD- LEV-APX	CPX-CN- LEV-APX
	G-SA12	CN-E-CH	-------
	G-SA13	NB-CN-AMX-RD-E-CH	NB-RD-E-CH
	G-SA15	NB-S-E-APX	NB-S-APX
	G-SA16	AMX-S-RD	----
Enterococcus spp	G-ES02	CPX-NB-AMX-RD-E-CH	CPX-NB-AMX
	G-ES09	CPX-NB-AMX-RD-E-CH-LEV	CPX-NB-AMX-RD-E-CH-LEV
	G-ES15	CN-AMX-CH-APX	AMX-CH
	G-ES23	NB-S-RD-APX	NB-S-RD-APX
B. substilis	G-BS31	AMX-RD-E-APX	AMX-E-APX
	G-BS14	CPX-NB-CN-AMX-RD-E	CPX-CN
	G-BS05	NB-CN-AMX-RD-CH-APX	NB-CN-AMX-RD-CH-APX
S. pyogenes	G-SP28	NB-CN-RD	-----
	G-SP39	NB-S-RD-E-APX	NB-S-E-APX

Keys: Ciprofloxacin; E: Erythromycin; CH: Chloramphenicol; APX: Ampicloxacillin; AMX: Amoxicillin; S: Streptomycin; RD: Rifampicin; NB: Norfloxacin; LEV: Levofloxacin; CN: Gentamycin.

Table 5. Pre-curing and post-curing antibiogram of multi drug resistant gram negative bacterial isolates from *G. paradoxa*

Bacteria isolates	Codes	Pre-curing Antibiogram	Post-curing antibiogram
K. pneumoniae	G-KP05	S-NA-SXT-PN	---
	G-KP14	AUG-CN-PN	AUG-CN-PN
	G-KP22	PEF-CEP-NA-SXT-PN	CEP-NA-PN
	G-KP40	OFX-PEF-CN-S-CEP-NA-PN	OFX-CN-S-NA-PN
Salmonella spp	G-SS17	OFX-CPX-CN-CEP-SXT-PN	CPX-CN-PN
	G-SS22	PEF-CN-CEP-SXT	PEF-CN-CEP
	G-SS30	PEF-CN-CEP-SXT	PEF-CN-CEP-SXT
	G-SS32	CPX-CEP-NA	CPX-CEP-NA
E. faecalis	*G*-EF06	PEF-CPX-CN-SXT	PEF-CN-SXT
	G-EF10	PEF-S-NA-SXT	PEF-S-NA-SXT
	G-EF25	PEF-S-CEP-NA-PN	PEF-NA-PN
V. cholerae	G-VC18	AUG-S-NA-PN	AUG-S-NA-PN
	G-VC29	OFX-CPX-AUG-S-CEP-SXT-PN	OFX-CPX-AUG
	G-VC38	PEF-S-NA-SXT-PN	PEF-S-NA-SXT-PN
P. aeruginosa	G-PA08	OFX-AUG-CN-PN	OFX-AUG-PN
	G-PA19	PEF-AUG-CN-NA	AUG-NA
	G-PA40	PEF-AUG-CEP-NA-SXT-PN	PEF-AUG-NA-PN
E. coli	G-EC01	S-CEP-NA	----
	G-EC04	CPX-CN-SXT-PN	CN-SXT-PN
	G-EC35	PEF-CN-NA	----
	G-EC38	OFX-PEF-AUG-CN	OFX-PEF-AUG-CN
	G-EC34	OFX-PEF-CPX-AUG-CEP-NA-SXT-PN	OFX-PEF-CPX-AUG
	G-EC27	OFX-PEF-S-CEP-NA-PN	OFX-PEF-S-CEP-PN
	G-EC19	PEF-S-CEP	----

Keys: OFX: Ofloxacin; PEF: Pefloxacin; CPX: Ciprofloxacin; AUG: Augmentin; CN: Gentamycin; NA: Nalidixic Acid; S: Streptomycin; CEP: Cephalothin; SXT: Trimethoprim-Sulphamethoxazole; PN: Ampicillin

Table 6. Plasmid Molecular Weight of Bacterial Isolates from *G. paradoxa*

Bacterial isolates	Code of isolates	Molecular weight of plasmids (Kb)
S. aureus	G-SA01	29.2
S. aureus	G-SA04	23.1
Enterococcus spp	G-ES09	23.1
B. subtilis	G-BS05	28.8
S. pyogenes	G-SP39	25.2
K. pneumoniae	G-KP40	31.5
Salmonella spp	G-SS17	23.1
E. faecalis	G-EF25	25.2
P. aeruginosa	G-PA40	25.2
E. coli	G-EC27	28.8
E. coli	G-EC34	27.5

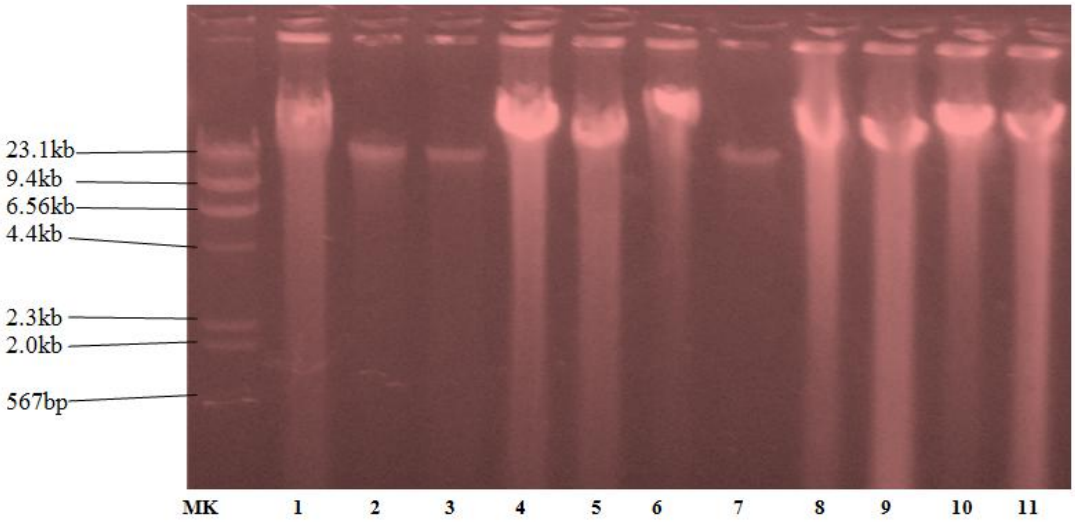

Fig. 3. Agarose electrophoresis showing plasmid profiles (molecular weight) of bacterial isolates from *G. paradoxa*

Line 1: G-SA01, 29.2kb); (Line 2: G-SA04, 23.1kb); (Line 3: G-ES09, 23.1kb); (Line 4: G-BS05, 28.8kb); (Line 5: G- SP39, 25.2kb); (Line 6: G-KP40, 31.5kb); (Line 7: G-SS17, 23.1kb); (Line 8: G-EF25, 25.2kb); (Line 9: G-PA40, 25.2kb); (Line 10: G-EC27, 28.8kb); (Line 11: G-EC34, 27.5kb); MK: Molecular weight marker (Hind 111 digest)

The high incidence of *S. aureus* and *E. coli* in our study corroborates the studies carried out by Ekanem and Adegoke [6]; Oranusi et al. [24] and Ekanem [25], who observed high levels of pathogens in aquatic organisms. However, our results differ from those obtained by Udoh et al. [26] who obtained fewer pathogenic bacteria in *G. paradoxa* samples from Cross River, Nigeria. The presence of *Salmonella* spp and *E. coli* indicates the possible contamination of *G. paradoxa* with human or animal faeces that can cause foodborne infections when eaten without proper cooking processes (Udoh [27]. The occurrence of pathogenic bacteria, especially *E. coli* in aquatic animals, can influence human health by inducing disease or infection and causing abdominal pain, acute gastroenteritis, bloody / mucoid diarrhea, nausea, vomiting, and fever [28].

The antibiotic susceptibility testing of bacterial isolates from *G. paradoxa* ascertained the extents of their susceptibility to frequently used antibiotics. This study revealed the varied levels of multi-drug resistance among bacterial isolates from *G. paradoxa* and confirms the findings of Adedeji et al. [29] who reported antibiotic resistance among bacterial isolates from different aquatic environments in Ibadan, South-West, Nigeria. The GNB were highly sensitive to S, AUG, OFL and CPX, while the GPB were highly sensitive to CN, S, CPX and LEV in this study.

The high sensitivity of GNB to AUG and OFL are in conformity with findings of Adebayo-Tayo et al. [30] who reported in Uyo, Nigeria that most GNB from sea-foods were highly sensitive to AUG and OFL.

In this study, 43(68.3%) bacterial isolates from *G. paradoxa* exhibited multiple antibiotic resistance with MAR indices ranging between 0.3 and 0.8. The MAR index higher than 0.2 could be a marker of contamination from high-risk sources, hence, indicating a potential human health risk [31]. Globally, multiple drug resistance by organisms has been reported to be concomitant with outbreak of major epidemics [32,33]. The isolation of the 68.3% MDR bacterial isolates from *G. paradoxa* in our study reflects a high alarm index, since these values suggest serious public health problems, with the consequences of managing infections caused by the bacteria.

Some of bacterial isolates from *G. paradoxa* had their antibiotic resistance markers encoded on the plasmids; chromosome or both and these results are consistent with the work of Yah et al. [34]. The loss of antibiotic resistance markers in MDR bacterial isolates from *G. paradoxa* in this study using acridine orange is in agreement with the reports of Akortha and Filgona [13]. The molecular weights of some bacterial isolates in this study indicated strain-specific than species-specific and thus agrees with result of Akinjogunla and Enabulele [16].

5. CONCLUSION

This study shows that G. *paradoxa* harbours different types of pathogenic MDR bacteria which produce serious public health risks by generating outbreaks of diseases transmitted by contaminated food. Therefore, it is suggested to carry out educational campaigns, raising awareness of the risks of consuming mollusks raw bivalves, especially *G. paradoxa.*

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Moses SB. Growth, biomass, mortality, production and potential yield of the West African clam, *Egeria radiata* (Lamarck) (Lamellibranchia, Donacidae) in the Cross River system, Nigeria. Hydrobiologia. 1990;196:1–15.
2. Etim L, Brey T. Growth, productivity and significance for fishery of the bivalve *Egaria radiata* (*Donacidae)* of live bivalve molluscs. 1994;1-19.
3. Villalobos LB, Elguezabal LA. Microbiological quality of the bivalves *Pinctada imbricate* commercialized in Cumana, Venezuela. Food Technology. Acta Cientifica Venezolona. 2001;52:55–61.
4. Chiesa LM, Nobile M, Malandra R, Pessina D, Panseri S. Food safety traits of mussels and clams: Distribution of PCBs, PBDEs, OCPs, PAHs and PFASs in sample from different areas using HRMS-Orbitrap and modified QuEChERS extraction followed by GC-MS/MS. Food Additives and Contaminants. 2018;35:5.
5. Hatha AAM, Christi KC, Singh R, Kumar S. Bacteriology of the fresh water bivalve clam *Batissa violacea* (Kai) sold in the suva market. The South Pacific Journal of National Science. 2005; 23:48–50.
6. Ekanem EO, Adegoke GO. Bacteriological study of West African clam (*Egeria radiata Lamarch*) and their overlying waters. Food Microbiology. 1995; 12:381-385.
7. King RP. Population structure, growth performance and mortality rates of the freshwater clam *G*. *paradoxa* Born 1778, in Nun River, Nigeria. Archive of Fisheries and Marine Research. 2000;48(1):21-30.
8. Citterio B, Andreoni F, Simoni S, Carloni E, Magnani M, Mangiaterra G, Cedraro N, Biavasco F, Vignaroli C. Plasmid replicon typing of antibiotic- resistant *Escherichia coli* from clams and marine sediment. Frontier Microbiology. 2020;11: 1101.
9. Akinjogunla OJ, Inyang CU, Akinjogunla VF. Bacterial species associated with anatomical parts of fresh and smoked Bonga fish (*Ethmalosa fimbriata*): prevalence and susceptibility to cephalosporins. Research Journal of Microbiology. 2010;6(1):87-97.
10. Vignaroli C, Distante L, Leoni F, Chierichetti S, Ottaviani D, Citterio B, et al. Multidrug resistant and epidemic clones of

Escherichia coli from natural beds of Venus clam. Food Microbiology. 2016;59: 1-6.

11. Pormohammad A, Nasiri MJ, Azimi T. Prevalence of antibiotic resistance *Escherichia coli* strains simultaneously isolated from human, animals, food and the environment: A systemic review and meta-analysis. Infect. Drug Resist. 2019; 12:1181–1197.
12. Olukoya DK, Olasupo NA. Drug resistance and plasmid profiles of diarrheagenic bacterial isolates in Nigeria (1988-1996). Nigerian Quarterly Journal of Hospital Medicine. 1997; 7:26-32.
13. Akortha EE, Filgona J. Tansfer of gentamicin resistance genes among Enterobacteriaceae isolated from the outpatient with UTIS attending 3 hospital in Mubi, Adamawa State. Scientific Research and Eassy. 2009;4(8):745–752.
14. Prescott, LM, Harley JP, Klein DA. Microbiology. New York: McGraw Hill. 2008; 7:966.
15. Miranda S, David MG, Peter JC. Evolution of multi- resistant plasmids in Austria clinical isolates of *Escherichia coli.* Microbiology. 2004; 150:1539–1549.
16. Akinjogunla OJ, Enabulele IO. Virulence factors, plasmid profiling and curing analysis of multi-drug resistant *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. isolated from patients with acute otitis media. Journal of American Science. 2010;6(11): 1022–1033.
17. APHA (American Public Health Association). Standard methods for the examination of water and wastewater. 16th Edition, Washington D.C; 1998.
18. Holt JG, Krieg NR, Sneath PHA, Stately JT, Williams ST. St. Bergey's manual of determinative bacteriology (9^{th} Edition). Baltimore, Williams and Wilkins; 1994.
19. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility testing (26^{th} edn), Wayne, Pennsylvania, U.S.A; 2016.
20. Jan MB, John DT, Sentry A. High prevalence of oxacillin resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-pacific and South Africa: results from sentry antimicrobial surveillance program, (1998-1999), Antimicrobial Agents and Chemotherapy. 2002;46(3):879-881.
21. Akinjogunla OJ, Adenugba IT, Inyang UO. Extracellular hydrolytic enzymes and location of multidrug resistance markers in urinary bacterial isolates. World Journal Biomedical Research. 2017;4(1):50-60.
22. Xiuhua C, Xiaodong D, Wen-Yuan S. Isolation of plasmid DNA from single colonies of *Agrobacterium tumefaciens* by means of rolling circle amplification. Plant Mol Bio Rep. 2003;21:411–415.
23. Adebayo-Tayo BC, Onilude AA, Ogunjobi AA, Adeoye DO. Bacteriological and proximate analysis of periwinkles from two different creeks in Nigeria. World Applied Science Journal. 2006;1(2):87-91.
24. Oranusi IS, Effiong ED, Duru NU. Comparative study of microbial, proximate and heavy metal compositions of some gastropods, bivalve and crustacean seafood. African Journal of Clinical and Experimental Microbiology. 2018;19(4): 291–302.
25. Ekanem EO, Adegoke GO, Ossai GEA. Bacteriological and organoleptic characteristics West African Dam (*Egeria radiate* Lamarch) meat. Nigeria Food Journal. 1994; 12:66-73.
26. Udoh DI, Udo IU, Udoh EI. Microbiological analysis of the freshwater clam (*Galatea paradoxa,* Born 1778) caught from Cross River, Nigeria. Nigeria Journal of Agriculture, Food and Environment. 2017; 13(3):59–64.
27. Udoh SM. Pollution associated with shrimps harvest from Cross River Estuary in Nigeria. Journal of Educational Laboratory Sciences. 1994;7(2):34–44.
28. Akinjogunla OJ, Eghafona NO, Ekoi OH. Diarrheagenic *Escherichia coli* (DEC): Prevalence among in and ambulatory patients and susceptibility to antimicrobial chemotherapeutic agent. Journal of Bacteriology Research. 2009; 1(3):034-038.
29. Adedeji OB, Tiamiyu AM, Emikpe BO. The antibiotic resistant patterns of bacterial flora of fish from different aquatic environments from Ibadan, South-west Nigeria. Advances in Environmental Biology. 2011;5(8):2039–2047.
30. Adebayo-Tayo, AC, Odu NN, Michael MU, Okonko, IO. Multi-drug resistant (MDR) organisms isolated from sea-foods in Uyo, South-Southern Nigeria. Nature and Science. 2012;10(3):61-70.
31. Gwendelynne BT, Son R, Nishibuchi M, Raha, AR, Suhaimi N, Lesley M,

et al. Characterization of *Vibrio parahaemolyticus* from coastal seawater in Peninsular Malaysia. Southeast Asian Journal of Tropical Medicine. 2005;36: 940–945.

32. Canton R, Coque TM, Baquero F. Multi-resistant gram-negative *Bacilli*: From epidemics to endemics. nosocomial and hospital related infections. Current Opinion in Infectious Diseases. 2003;16:315-325.
33. Levy SB. Antibiotic resistance: Consequences of inaction. Clinical Infectious Diseases. 2001;33:124-129.
34. Yah SC, Eghafona NO, Oranusi S, Abouo AM. Widespread plasmid resistance genes among *Proteus* species in diabetic wounds of patients in the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. African Journal of Biotechnology. 2007;6(15): 1757-1762.

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