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Characterisation and antibiotic susceptibility pattern of bacterial isolates obtained from some shellfish sold in Lagos-West, Nigeria

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Abstract

Shellfish are rich in essential nutrients and are widely accepted globally. The bacterial contamination, antibiotic susceptibility and heavy metal concentrations on some shellfish muscles above the tolerance permissible limit by WHO/FAO require an in-depth study. An investigative study was carried out on tiger shrimp (*Penaeus monodon*), pink shrimp (*Penaeus notialis*) and Lagoon crabs (*Callinectes amnicola*) obtained from Makoko fish market/landing site on their bacterial quality, antibiotic susceptibility patterns and heavy metal accumulation of bacteria isolated were carried out using standard methods. The highest Total bacterial count ($2.48\pm0.02\times10^7$cfu/g) was observed in Lagoon crab (*C. amnicola*) while the lowest count ($1.20\pm0.02\times10^7$cfu/g) was observed in tiger shrimp (*P. monodon*). However, the highest Total Faecal count ($1.42\pm0.02\times10^4$cfu/g) was observed in pink shrimp (*P. notialis*) while the lowest count was observ ed in *P. monodon* ($1.13\pm0.03\times10^4$cfu/g). The bacterial isolates were molecularly identified as (*Morganella morganii and Proteus vulgaris)* isolated from tiger shrimp, Lagoon crabs had *(Proteus mirabilis)* while pink shrimp showed (*Alcaligenes faecalis)*. The isolates were 100% susceptible to ciprofloxacin, azithromycin and erythromycin and resistant to cefotaxime, cefuroxime, imipenem/clastatin, augmentin and nitrofurantoin. The mean heavy metals concentration was as follows zinc>iron>copper>nickel>chromium>manganese > Cadmium while Lead and cobalt were not detected in the samples. The study has shown a possible unhygienic environment indicative of the bacteria isolated, a possible environmental spread of antibiotic-resistant bacteria and a heavy metal-contaminated water body. There is a serious need for constant monitoring to lessen future health problems for humans in and around the environment.

Keywords: antibiotic-resistant; contamination; Makoko

1. Introduction

Bacterial contamination especially coliforms and faecal coliforms in shellfish demonstrates the level of pollution of their environment because these organisms are not the normal bacterial flora in fish (Mohamed *et al*., 2017). Shellfish contamination is mainly difficult to determine whether the contamination occurred in the water body, during their handling or marketing (Mohamed *et al*., 2017). Shellfish are known benthic feeders and they are chosen as biomarkers for studies in aquatic environments because they are good bio-indicators of pollutants in an aquatic environment (Farkas *et al*., 2002). The study of heavy metals contamination in food and environment is of special concern because of their toxicological effect on human beings, animals and other living organisms (Bruins *et al*., 2000). They persist in nature and consequently tend to accumulate in food chains. The World Health Organization reported that the world's foodborne diseases each year almost 1 in 10 people fall ill after consuming contaminated food (Udoekong *et al*., 2021).

Several organisms have been implicated in food-borne diseases from shellfish. Resistant bacterial infections associated with seafood are very cumbersome to treat because the organism can be resistant to many antibiotics due to the unhealthy environment of the water bodies (Mohamed *et al*., 2017). These antibiotics are used therapeutically and prophylactically in animal production and they still find their way into the environment (Perez *et al*., 2020). The environmental dissemination of antibiotics is the main problem because it promotes the spread of antibiotic-resistant bacteria (Hembach *et al*., 2017). Research has also proven that drug-resistant genes can be transferred from animals to humans co-existing in the same environment (Perez *et al*., 2020).

Heavy metals distribution varies among fish species and it depends on the age of the fish, development status and other physiological factors (Adams, 2002). Furthermore, the tolerance of these fish species to heavy metals has been proposed as an indicator of the potential toxicity of heavy metals in the environment where the organism was isolated (Lima *et al*., 2012). Therefore, there is a dramatic increase in the interest in studying the residues of heavy metals in shellfish.

The presence of bacteria in the muscles of shellfish with relatively high concentrations of antibiotic resistance and some heavy metals prompted a study of the bacteria contamination, antibiotic susceptibility and residue of heavy metals in some shellfish sold in the Lagos metropolis hence the objective of this study was to isolate and characterize the bacteria, their

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antibiotic resistance and determine the heavy metal residues of some shellfish sold in Lagos, Nigeria and to further highlight the implications of these contaminants in the food chain and environment in general.

2. Materials and Methods

2.1 Description of the study area

The study area, Better Life Fish market/landing site in Makoko, Lagos State, Nigeria, is located between longitudes 3° 23' and 3° 39" E and Latitudes 6° 23' and 6° 44" N.

2.2 Sample collection

Twenty-five fresh samples each of Tiger shrimp (*Penaeus monodon*), Pink shrimp (*Penaeus notialis*), and Blue Crab (*Callinecte s amnicola*) were randomly obtained from the Fish market/landing site in the Makoko area of Lagos State Nigeria. These samples were collected aseptically, picked at random using sterile gloves and immediately kept in an icebox and transferred to the Microbiology Laboratory of the Department of Fish Technology of the Nigerian Institute for Oceanography and Marine Research, Victoria Island for bacteriological and chemical analysis.

2.3 Characterisation and Identification of the Isolates

2.3.1. Bacteriological analysis

Ten (10) grams of randomly selected shellfish samples each were transferred to a sterile polythene bag and 90 ml of 0.1% sterile buffered peptone H_2O in a blender at 2000 rpm for 1-2 minutes to provide a homogenous solution of 1/10 dilution. One ml from the original dilution was transferred with a sterile pipette to another sterile test tube containing 9 ml of sterile buffered peptone water (0.1%) and mixed to make the next dilution, from which further decimal serial dilutions were prepared. The prepared dilutions were subjected to an enumeration of Total Bacteria Count as described by (USDA/FSIS (1998) and Total Coliform as described by AOAC (1980).

The purified bacterial colonies were characterized for identification using cultural, morphological and standard biochemical tests for identification of microorganisms namely: Gram reaction and standard biochemical tests such as; oxidase, urease, indole, Voges-Proskauer, hydrogen sulphide production, catalase, citrate utilization and sugar fermentation tests (Cheesbrough, 2002). Bacterial isolates were further confirmed by molecular identification using the universal 16S rRNA bacterial primers (Colan *et al*., 2012).

2.3.2 Molecular analysis (16S rRNA gene sequence, BLASTn and phylogenetic analysis)

This test was carried out according to the method of (Omoya and Ajayi, 2020). Identification of strain was done by sequencing the PCR products of 16S rRNA (bacteria) using a sequencer, determined sequences were compared with sequences available in GenBank, derived sequence aligned by Basic Local Alignment Search Tool (BLAST) algorithm, the highest value with identified species in the Sequence match search. Using the results received through BLASTn a phylogenetic tree is created using the MEGA X application (Kumar *et al*., 2018; Stecher *et al*., 2020).

2.3.3 Antimicrobial Susceptibility Test

The antimicrobial susceptibility of bacterial isolates was performed by using the Kirby Bauer Disk diffusion method on Mueller Hinton Agar following the clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). Concisely, the bacterial strain was grown on a TSA plate overnight at 37 ^{o}C. A loopful of the bacterial colony was transferred into 5 ml of sterile tryptic soy broth (TSB). The turbidity of the just-inoculated suspension was adjusted to that of a 0.5 McFarland standard (CLSI, 2018). After standardization, a sterile cotton swab was dipped into the culture suspension and streaked on the entire surface of a sterile Mueller Hinton agar plate. The inoculum was allowed to dry for 3 to 5 min, but no more than 15 min. The antibiotic disks were placed on the plate and, after incubation for 24 hrs at 37^{o}C, the diameter of the inhibition zone was measured with callipers. The antibiotic susceptibility pattern was examined by using commercial antibiotic discs including Amoxicillin-clavulanate (30 µg), Cefotaxime (25 µg), Ofloxacin (5 µg), Gentamycin (10 µg), Nalidixic acid (30 µg), Cefuroxime (30 µg), Ceftriaxone/sulbactam (45 µg), Ampiclox (10 µg), Cefixime (5 µg), Erythromycin (15 µg), Azithromycin (15 µg), Ciprofloxacin (5 µg), Imipenem/Clastatin (10/10 µg), Nitrofurantoin (300 µg), Levofloxacin (5 µg). The zones of inhibition were measured and recorded. The antibiotic sensitivity was termed susceptible when the recorded zones of inhibition (mm) were $\geq$ 15 and resistant when the zones of inhibition to the antibiotic were $\leq$13 (Falomir *et al*., 2010).

2.3.4 Determination of Heavy metals residues in the shellfish

Reagent/apparatus: All reagents used were of analytical grade. Distilled water was used for solution preparation and dilutions. All glasses were soaked in 10% HNO_3 for 24 hours and later rinsed with distilled water before use for heavy metal analysis. Analysis of heavy metals: The wet method of digestion was used to carry out the sample digestion. Exactly 5.0g of the homogenized samples were weighed and digested with 25ml 6N HNO_3. The digested sample was analysed for zinc (Zn), copper (Cu), cadmium (Cd), chromium (Cr), iron (Fe), nickel (Ni), manganese (Mn), lead (Pb) and cobalt (Co) using scientific flame atomic absorption spectrophotometer (Varian spectrophotometer AA 600 model).

2.4 Statistical analysis

The data obtained were analysed using the prism version 5.03 statistical software programs (Graph pad software, San Diego, CA. USA). Descriptive statistics including percentages were used to summarize the data.

3. Results and Discussion

3.1. Results

3.1.1. Microbial culture and isolation of bacteria

The Total Bacterial Count (TBC) in shellfish samples is presented in Table 1. Results showed that the highest TBC ($2.48\pm0.02\times10^7$cfu/g) was observed in blue crab (*C. amnicola*) while the lowest count ($1.20\pm0.02\times10^7$cfu/g) was observed in tiger shrimp (*P. monodon*). The mean Total Faecal count (TFC) for the samples is shown in Table 2. The result revealed that the highest TFC ($1.42\pm0.02\times10^4$cfu/g) was observed in pink shrimp (*P. notialis*) while the lowest count was observed in ($1.13\pm0.03\times10^4$cfu/g).

Table 1
Mean values of total bacterial count in sampled shellfish

Samples		Total Bacterial Count $\times10^7$ cfu/g
Tiger shrimp	*P. monodon*	$1.20\times10^7\pm0.02$
Pink Shrimp	*P. notialis*	$1.48\times10^7\pm0.01$
Blue Crab	*C. amnicola*	$2.28\times10^7\pm0.02$

*Data represent mean ± SD

Table 2
Mean values of total faecal count in sampled shellfish

Samples	Total Faecal Count $\times 10^4$cfu/g	
Tiger shrimp	*P. monodon*	$1.13\times10^4\pm0.03$
Pink Shrimp	*P. notialis*	$1.42\times10^4\pm0.02$
Blue Crab	*C. amnicola*	$1.02\times10^4\pm0.05$

*Data represent mean ± SD

Table 3
Phenotypic characterisation of bacterial isolates

	Isolate Code	Gram Reaction	Colony Morhology	Indole	Motility	Methyl Red	Vokes Proskuer	Urease	Oxidase	Catalase	Citrate	Glucose	Lactose	Maltose	Sucrose	Mannose	Gas Production	Hydrogen Sulphide (H_2S)	Probable Organism
1.	C1BM1	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
2.	C1BS1	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
3.	C2BVY	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
4.	C2BS1	-	Circular smooth	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
5.	C2BVB	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
6.	C1AS1	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	-	+	+	+	+	*Proteus* spp.
7.	C1AT1	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
8.	S1BS1	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
9.	S1BM2	-	Circular smooth	+	-	-	+	-	-	-	+	+	+	+	-	+	+	+	*Proteus* spp.
10.	S1BT1	-	Feathery Irregular Edges	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	*Alcaligenes* spp.
11.	S1BT2	-	Circular Smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
12.	S2BT1	-	Circular smooth	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
13.	S3BVY	-	Circular Smooth	+	-	-	+	-	-	-	+	+	+	+	+	-	+	+	*Morganella* spp.
14.	S3BT2	-	Circular Smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.
15.	S3BVB	-	Circular Smooth	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	*Proteus* spp.

3.1.2. *Phenotypic characterisation of the isolates*

The phenotypic characterisation inclusive of cultural, morphological and biochemical tests of the bacterial isolates is shown in Table 3. Fifteen (15) were isolated, out of which 13 were *Proteus* spp. The probable identity of the remaining two isolates were *Alcaligenes* spp. and *Morganella* spp.

3.1.3. *Molecular characterisation of the isolates*

From all the samples collected and processed, isolates were selected for molecular identification based on their cultural morphology and phenotypic characterisation. After all the 16S rRNA Sequences of the isolates, the 4 (four) isolates obtained were blasted in GenBank of NCBI. The bacterial isolates were identified and belonged to three different genera *Morganella*, *Proteus* and *Alcaligenes* as presented in Table 4.

The phylogenic tree made from sequenced 16SrRNA region of the four (4) bacterial isolates from the shellfish with evolutionary analyses done with MEGA X is shown in (Table 4). The phylogenic grouping showed that strains with similar sequences to the ones got from the gene bank were clustered into three groups marked in red dots as shown in Figure 1. The maximum likelihood phylogenic tree based on the 16SrRNA genes of the isolated strains and their closest related species Bootstrap values calculated for 500 replications are indicated in Figure 1 with the sum of branch length of 0.28112020. The scale length of the tree was 0.020, with branch lengths in the same units as those of the evolutionary distances used to inter the phylogenic tree. The analysis involved 8 nucleotides sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 963 positions in the final dataset.

Table 4
Identities of the bacterial isolates

Samples	Identified organisms	Strains	Accession number
Tiger shrimp (*P. monodon*)	*Morganella morganii*	S3BVY 16S ribosomal RNA gene, partial sequence	MT780936
	Proteus vulgaris	S3BVB 16S ribosomal RNA gene, partial sequence	MT785901
Blue Crab (*C. amnicola*)	*Proteus mirabilis*	C1ATI 16S ribosomal RNA gene, partial sequence	MT785771
Pink Shrimp (*P. notialis*)	*Alcaligenes faecalis*	S1BT1 16S ribosomal RNA gene, partial sequence	MT785895

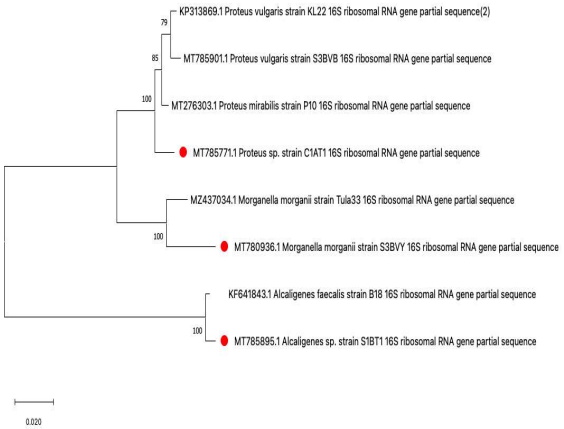

Figure 1. Phylogenic tree of the nucleotide sequence of 4 bacteria isolates from shellfish, i.e. MT780936, MT785901, MT785771 and MT785895. Four isolates from GenBank were MZ437034.1, MT276303.1, KP313869.1 and KF641843.1

3.1.4. Drug susceptibility and resistance of the isolates

The vernier calliper was used to measure the diameter of the zones of inhibition on the petri dish for susceptibility and resistance of isolated organisms to the fifteen tested antimicrobial agents depicted in Table 5.

Table 5
Zones of inhibition (mm) of antibiogram patterns of the Microorganisms

Antibiotics	*P. vulgaris*	*P. mirabilis*	*M. morganii*	*A. faecalis*
Cefixime	18	0 (R)	19	20
Ceftriaxone	7 (R)	15	21	22
Cefuroxime	6 (R)	0 (R)	23	23
Cefotaxime	11(R)	3 (R)	19	0(R)
Gentamicin	19	10 (R)	15	16
Imipenem/Clastatin	16	20	0 (R)	0(R)
Ampiclox	24	0 (R)	25	26
Augmentin	6 (R)	4 (R)	19	18
Nalidixic acid	19	20	19	0(R)
Ciprofloxacin	21	24	21	23
Ofloxacin	15	20	0 (R)	17
Levofloxacin	17	21	0(R)	17
Azithromycin	18	18	19	19
Erythromycin	23	23	24	24
Nitrofurantoin	17	21	0(R)	0(R)

The heavy metals residues in the studied shellfish are presented in Table 6 below.

Table 6
Heavy metals residues in the studied shellfish (mg/kg)

Species	Zn	Cu	Cd	Cr	Fe	Ni	Mn	Pb	Co
P. notialis	14.1±0.3	4.64±0.7	BDL	2.62±0.7	10.6±0.85	2.14±0.65	BDL	BDL	BDL
P. monodon	6.57±0.1	0.1± 0.14	BDL	0.1±0.5	4.51±0.46	2.19±0.3	BDL	BDL	BDL
C. amnicola	28.7±0.2	12.8±1.1	0.06±0.63	12.8±0.4	25.1±3.3	2.82±0.65	54.7±0.2	BDL	BDL
WHO/FAO (2012)	0.03	0.4	0.05	0.05	4.0	0.6	0.10	0.5	0.015

*BDL- below detectable limit
*Zinc (Zn), copper (Cu), cadmium (Cd), chromium (Cr), iron (Fe), nickel (Ni), manganese (Mn), lead (Pb) and cobalt (Co)

3.2. Discussion

The bacterial data obtained across the studied samples shows that the quality standard of the shellfish varies and this could be attributed to handling techniques as reported by Afolayan *et al*. (2020) that the microbiological quality of shellfish depends on various factors such as post-harvest handling. The total bacterial count in this study was a little similar to the report by Afolayan *et al*. (2020) which recorded a microbial count that ranged from $2.71\pm 0.03 \times 10^8 - 1.00\pm 0.4\times 10^8$ CFU g^{-1} in shellfish obtained from better life fish market Makoko Lagos. Total bacterial count often reveals the quality of good sanitation and freshness.

The presence of TFC is an indication of sewage contamination. The mean values of the total faecal count recorded in this study exceeded the acceptable limit of coliforms <100 CFU g^{-1} for fish and shellfish as specified by the International Commission on the Microbiological Specification for Foods (1986). The studied samples are benthic feeders and they stay more on the ocean floor seeking calcium to get a thicker shell (Wicksten *et al*., 2017). In doing so, they tend to bio-accumulate more faecal contaminants while feeding, this may account for the high coliform count observed in this study. The high coliform count can be attributed to the cleanliness of the water body where sea animals were caught, and handled by the fisher's folk as previously reported by Bojarczuk *et al*. (2018). Our data recapitulated the study on shrimps by Omoya and Ajayi, (2020) where a high coliform count from shellfish was reported and they attributed it to the aquatic environment.

The genus *Morganella* was named as *M. morganii* (MT780936). It had the closest similarity sequence of 97 % with *M. morganii* strain Tula 33 reported to have exhibited multiple drug resistance and was isolated from a water source in Adamawa State, Nigeria with accession number MZ437034.1 (Tula et al.,). Then two *Proteus* genera were named *P. vulgaris* and *P. mirabilis* (MT785901 and MT785771 respectively) *P. vulgaris* had a 98% similarity sequence with *P. vulgaris* strain KL 22 isolated from sea sediment in Thailand with accession number KP3138669.1 while, *P. mirabilis* had 97% similarity with *P. mirabilis* strain P 10 with accession number MT276303.1 isolated in faeces of Mink and *A. faecalis* (MT785895) had 96 % sequence similarity with *A. faecalis* isolated in the rumen of a camel from China (source GenBank of NCBI).

In this study, *M. morganii, P. vulgaris*, *P. mirabilis* and *A. faecalis* are widely distributed in nature, they are pathogenic bacteria inhabiting estuarine ecosystems and they can act as opportunistic pathogens (Liu et al., 2016). They are known to be a major cause of foodborne illness associated with the consumption of raw or undercooked contaminated shellfish (Udoekong *et al*., 2021). All the bacterial pathogens found in this present study have been previously linked with commonly consumed shellfish in southwestern Nigeria (Afolayan *et al*., 2020; Udoekong *et al*., 2021). The isolates from this study have been documented as clinically relevant species that could cause foodborne diseases associated with seafood and can also cause other health problems like urinary tract infections in humans (Tosun *et al*., 2016).

Antibiotic-resistant bacteria are those bacteria that were not inhibited or killed by the tested antibiotics. They survive and even increase in the presence of the tested antibiotics. Bacteria that are resistant to many antibiotics are known as multi-resistant organisms. In this study, the *Proteus* species showed 100% resistance to cefuroxime, cefotaxime and augmentin, more so, *P. vulgaris* resisted ceftriaxone, *P. mirabilis* showed resistance to cefixime and ampiclox apart from the initially listed antibiotics. *M morganii* showed 100% resistance to four (Imipenem/clastatin, ofloxacin, levofloxacin and nitrofurantoin) out of the fifteen tested antibiotics while *A. faecalis* showed 100% resistance to cefotaxime, imipenem/clastatin, nalidixic acid and nitrofurantoin. The resistance of the strains to antibiotics could be explained by the anthropogenic activities (disposal of sewage from homes and hospitals, non-biodegradable compounds etc) going on around the Lagos Lagoon, allowing the presence of residual antibiotics in shellfish products). This result compares well with previous studies of Adeyemi *et al*. (2008) that isolated *Vibrio* species from seafood in Lagos State Nigeria with most of them showing resistance to cefuroxime, cefotaxime, ofloxacin, nitrofurantoin augmentin and ampiclox. Also, in a study conducted by Udoekong *et al*. (2021), most of the organisms isolated from shellfish were resistant to one or more antibiotics and cefepime, chloramphenicol, ciprofloxacin and trimethoprim-sulfamethoxazole were the least effective drugs against the *Proteus* species and *M. morganii* while the imipenem is the most effective antibiotics.

All of the organisms isolated in this study have shown multidrug resistance patterns since they were resistant to more than three antibiotics (Ekwealor *et al*., 2016). The development of antibiotic resistance by the isolates may be due to mutation

or environmental pollution (Elbashir *et al*., 2018; Watt *et al*., 2017). The increase in the spreading of antibiotic-resistant organisms in seafood has continued to generate interest, especially where the potential of transferring antibiotic resistance genes could occur between opportunistic pathogens and normal flora in the gut. This has the potential of becoming virulent in immunocompromised individuals who consume seafood from the Lagos coastal water.

Cadmium which is among the most toxic heavy metals to humans and has high environmental and health risks occurred in *C. amnicola*. Manganese also occurred only in *C. amnicola*. Nickel was recorded in all the shellfish samples used with the highest value (2.82±0.65mg/kg) in *C. amnicola*. The highest value of iron (25.1 ± 3.3 mg/kg) was observed in *C. amnicola* while the lowest value (4.51±0.46mg/kg) was recorded in *P. monodon.* The highest value of Chromium (12.8±0.4 mg/kg) was recorded in *C. amnicola* while the lowest value (0.1±0.5 mg/kg) was recorded in *P. monodon*. The highest value of zinc (28.7±0.2 mg/kg) was recorded in *C. amnicola* while the lowest value (6.57±0.1 mg/kg) was recorded in *P. monodon*. The study has shown that the heavy metals concentration occurred in the following order: zinc>iron>copper>nickel>chromium>manganese>Cadmium while Lead and cobalt were not detected in the samples. This is similar to the heavy metal concentration reported by Jumbo *et al*. (2015) in some fin and shellfish from Ogoni land, southern Nigeria.

The bacteria isolated and characterised from the studied shellfish have shown a possible environmental spread of antibiotic-resistant bacteria that needs constant monitoring to abate future health problems while the heavy metal contamination of the water body should be in periodic monitoring with risk assessment to forestall future problems to humans and animals in and around the environment.

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Bibliography

Adams, S.M. 2002. Biological indicators of aquatic ecosystem stress: Introduction and overview. In Biological Indicators of Aquatic Ecosystem Stress. *American Fish Society* Bethesda Maryland 1-11.

Adeyemi, A., Enyinnia, V., Nwanze, R., Smith, S., and Omonigbehin, E. 2008. Antimicrobial susceptibility of potentially pathogenic halophilic Vibrio species isolated from seafoods in Lagos, Nigeria. *African Journal of Biotechnology*, 7: 3791-3794.

Afolayan, O.A., Moruf, R.O., and Lawal-Are, A.O. 2020. Bacterial Contamination and Heavy Metal Residues in Frozen Shellfish Retailed Within Lagos Metropolis, Nigeria. *Science World Journal*, 15: 1597-6343.

AOAC (Association of Official Analytical Chemists). 1980. Official methods of analysis. 534. W. Horwitz (Ed.). Arlington, V.A, Washington DC: AOAC.

Bojarczuk, A., Jelonkiewicz, L., and Lenart-Boron A. 2018. The effect of anthropogenic and natural factors on the prevalence of physicochemical parameters of water and bacterial water quality indicators along the river Bialka, Southern Poland. *Environmental Science and Pollution Research International*, 25: 10102-10114.

Bruins, M.R., Kapal, S., and Oehme, W.F. 2000. Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety*, 45: 198-207.

Cheesbrough, M. 2002. *District laboratory practices in tropical countries*, Part II. United Kingdom: Cambridge University Press, 182-187.

CLSI (Clinical and Laboratory Standards Institute). 2018. Performance Standards for Antimicrobial Susceptibility testing. 28th ed. CLSI supplement M100. Wayne, PA: CLSI; p15.

Conlan, S., Kong, H.H., and Segre, J.A. 2012. Species-Level Analysis of DNA Sequence Data from the NIH Human Microbiome Project, 7:10.

Ekwealor, P.A., Ugwu, M.C., Ezeobi, I., Amalukwe, G., Ugwu, B.C., Okezie, U., Stanley. C., and Esimone, C. 2016. Antimicrobial evaluation of bacterial isolates from urine specimens of patients with complaints of urinary tract infections in Awka, Nigeria. *International Journal Microbiology,* Article ID 9740273, 6.

Elbashir, S., Parveen, P., Schwarz, J., Rippen, T., Jahncke, M., and Depaola, A. 2018. Seafood pathogens and information on antimicrobial resistance: A review. *Food Microbiology*, 70: 85-93.

Falomir, M.P., Gozalbo, D., and Rico, H. 2010. Coliform bacteria in fresh vegetables: From cultivated lands to consumers. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 2: 1175–1181.

Farkas, A., Salanki, J., and Specziar, A. 2002. Relation between growth and the heavy metal concentration in organs of Bream *Abramis brama* L. populating Lake Balaton. *Archives Environmental Contamination Toxicology*, 43: 236-243.

Hembach, N., Schmid, F., Alexander, J., Hiller, C., Eike, T.R., and Schwart, J. 2017. Occurrence of the mcr-1 colistin resistance gene and other clinically relevant antibiotic resistance genes in microbial populations at different municipal wastewater treatment plants in Germany. *Frontiers in Microbiology*, 8: 1282.

ICMSF (International Commission on Microbiological Specifications for Foods). 1986. Sampling plans for fish, shellfish: Microorganisms in foods 2. Sampling for microbiological analysis: principles and specific Applications 2nd Edn. University of Toronto Press, Toronto, Canada. p 181-195.

Jumbo, A., Wegwu, M.O., Belonwu, D.C., and Okerenta, B.M. 2015. Assessment of heavy metal concentrations of selected fin and shell fish from Ogoniland. *Journal of Environment and Earth Science*, 5: 15-19.

Kumar, S., Stecher, G., Li. M., Knya, Z.C., and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis

across computing platforms. *Molecular Biology and Evolution*, 2018; 35: 1547-1549.

Lima de Silva, A.A., de Carvalho, M.A., de Souza, S.A. Dias, P.M., Filho, R.G., Saramago, C.S., Bento, C.A., and Hofer, E. 2012. Heavy metal tolerance (Cr, Ag AND Hg) in bacteria isolated from sewage. *Brazilian Journal of Microbiology*, 43: 1620-31.

Liu, H., Zhu, J., Hu, Q., and Rao, X. 2016. *Morganella morganii*, a non-negligent opportunistic pathogen (Review). *International Journal of Infectious Diseases*, 50: 10-17.

Mohamed, M.A., Al Shabeeb, S., Al Ramadhan, G.H., and Imran, P.M. 2017. Isolation of enterobacteriaceae from raw seafoods sold in fish markets in eastern province of Saudi Arabia. *International Journal of Advanced Research*, 5: 1711-1718.

Omoya, F.O., and Ajayi, A.T. 2020. Assessment of the microbial quality of seafood and effects of salt concentration and temperature on isolated microorganisms. *Journal of Microbiology and Antimicrobials*, 12: 17-31.

Pérez-Etayo, L., González, D., Leiva, J., and Vitas, A.I. 2020. Multidrug-Resistant Bacteria Isolated from different Aquatic Environments in the North of Spain and South of France. *Microorganisms,* 8: 1425.

Stecher, G., Tamura, K., and Kumar, S. 2020. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution*, 37: 1237–1239.

Tosun, S.Y., Ucok D., and Mol, A.S. 2016. Isolation of Salmonella spp and other members of Enterobacteriaceae from horse Mackerel (*Trachurus trachurus*), sold in public markets of Istanbul, Turkey. *Journal of Food and Health Science*, 2: 82-89.

Tula, M.Y., Enabulele, O.I., and Ophori, E.A. 2021. Prevalence of parasite and MDR Enteropathogenic bacteria from water sources in Adamawa- North senatorial zone, Adamawa State Nigeria. Unpublished From GenBank, NCBI.

Udoekong, N.S., Bassey, B.E., Asuquo, A.E., Akan, O.D., and Ifeanyi, C.I.C. 2021. Multi-Drug Resistance Genes associated with some Gram-Negative Bacteria Isolates from Shellfish in Iko and Douglas River Estuaries, in Nigeria. European Journal of Biology, 2: 19-27.

USDA/FSIS (United States Department of Agriculture/ Food Safety and Inspection Service). 1998. Microbiology Laboratory Guidebook, 3rd Ed., Washington, DC: USDA–FSIS.

Watts, J.E.M., Schreier, H.J., Lanska, L., and Hale, M.S. 2017. The Rising Tide of Antimicrobial Resistance in Aquaculture: Sources, Sinks and Solutions. *Marine Drugs*, 15:158.

Wicksten, M., De Grave, S., France, S., and Kelley, C. 2017. Presumed filter-feeding in a deep-sea benthic shrimp (Decapoda, Caridea, Stylodactylidae), with records of the deepest occurrence of carideans. *ZooKeys*, 646: 17-23.