Journal of Advances in Microbiology

20(6): 37-45, 2020; Article no.JAMB.57932
ISSN: 2456-7116

Molecular Characterization and Detection of Antibiotic Resistance Genes in *Pseudomonas* Species Isolated from *Tympanotonus fuscatus*

T. Sampson[1*], N. P. Akani[1] and I. O. Hakam[1]

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

Authors' contributions

This work was carried out in collaboration among all authors. Author TS supervised the entire study, managed the analyses of the study and also provided extensive discussion for the study. Author NPA designed the study, performed the literature searches and performed the statistical analysis. Author IOH managed the laboratory proceedings and wrote the first draft of manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2020/v20i630252
Editor(s):
(1) Dr. C. Graciela Castro Escarpulli, Mexico.
Reviewers:
(1) Abhishek Mishra, Houston Methodist Research Institute, USA.
(2) Amal Alsulaiman, Damascus University, Syria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57932

Original Research Article

Received 20 April 2020
Accepted 25 June 2020
Published 04 July 2020

ABSTRACT

Aim: This was carried out to characterize *Pseudomonas* species isolated from the West African Mud Creeper (*Tympanotonus fuscatus*) molecularly and as well detect the possible presence of inducible AmpC gene that mediates resistance to cephalosporins and most penicillins.
Sample: *Tympanotonus fuscatus* (West African Mud Creeper), a gastropod mollusc found in brackish waters of West Africa was used for the study.
Place and Duration of Study: This study was carried out between February and August 2019 at the Department of Microbiology, Rivers State University, Port Harcourt, Nigeria.
Methodology: Thirty two (32) *Pseudomonas* species were isolated and identified culturally from *T. fuscatus*. *Pseudomonas* species isolates were subjected to a group of ten (10) antibiotics using the Kirby-Bauer disc diffusion method and resistant isolates were screened molecularly for the presence of resistance gene (AmpC). AmpC screening was carried out in a step wise process of DNA extraction, quantification, amplification of *ampC* gene using appropriate primer and Agarose

**Corresponding author: E-mail: tonye4good62@yahoo.com;*

gel electrophoresis to reveal which DNA extracts had *ampC* genes amplified. The two most resistant isolates had their *16S rRNA* sequenced, identified and were also profiled for plasmids by extracting plasmid DNA.

Results: Results revealed that 96.67% of isolates had MAR index greater than 0.2 indicating high a risk source of contamination where antibiotics are often used. Results also showed the presence of *ampC* gene in seven (7) out of the twelve (12) isolates screened for *ampC* gene. Molecular characterization via sequencing of the *16S rRNA* gene of the two (2) most resistant isolates confirmed that both isolates were strains of *Pseudomonas aeruginosa*. Profiling of plasmids also revealed the presence of plasmid DNA of about 10 kilo base pairs in both isolates profiled.

Conclusion: This study has revealed the resistance ability of *Pseudomonas* and some reasons behind this resistance. Appropriate investigation into antimicrobial resistance is recommended for the administration of drugs for the treatment of food-mediated *Pseudomonas* infections.

Keywords: Resistant genes; Pseudomonas species; AmpC; molecular characterization; Tympanotonus fuscatus.

1. INTRODUCTION

The treatment of bacterial infection is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents [1]. Bacterial resistance to these antimicrobial agents is basically as a result of one or more resistance determinants which reduce the antimicrobial activities of the drugs [2].

Pseudomonas species are Gram-negative bacteria belonging to the phylum Proteobacteria, class, Gammaproteobacteria, family *Pseudomonadaceae* and genus *Pseudomonas* in which members demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches ranging from water, soil, plant seeds, air [3].

Pseudomonas aeruginosa represents a classical phenomenon of antimicrobial resistance since virtually all known mechanisms of antimicrobial resistance can be seen in it. These mechanisms include but not limited to the de-repression of chromosomal AmpC cephalosporinase, production of plasmids or integron-mediated β-lactamases from different molecular classes (carbenicillinases and extended-spectrum β-lactamases belonging to class A, class D oxacillinases and class B carbapenem-hydrolysing enzymes), diminished outer membrane permeability (loss of OprD proteins), overexpression of active efflux systems with wide substrate profiles, synthesis of aminoglycoside modifying enzymes (phosphoryltransferases, acetyl transferases, adenylyltransferases); and structural alterations of topoisomerases II and IV determining quinolone resistance [4].

P. aeruginosa has naturally occurring chromosomal AmpC b-lactamase or cephalosporinase which relates to β-lactams such as Penicillin G; Aminopenicillins including those combined with β-lactamase inhibitors; first and second generation cephalosporins. AmpC β-lactamase is encoded by the *ampC* gene and it is the primary cause of the organism's resistance to β-lactams. Enzyme production is the major mechanism of acquired resistance of P. *aeruginosa* to β-lactam antibiotics. β-lactamases rupture the amide bond of the β-lactam ring leaving obtained products which lack antimicrobial activity [5]. *P. aeruginosa* is naturally susceptible to carboxypenicillins, ceftazidimes and aztreonams; however, it can acquire resistance to third generation cephalosporins. This happens readily through the constitutive excessive production of AmpC β-lactamase [5]. The AmpC β-lactamase enzyme belonging to molecular class C is naturally produced in low quantities by *P. aeruginosa* and determines resistance to aminopenicillins and some of the early cephalosporins [6]. However, chromosomal cephalosporinase production may increase from 100 to 1000 times in the presence of inducing β-lactams (including imipenem) [5]. *Pseudomonas* species is highly resistant to cephalexin, cephalosporin, ampicillin, amoxicillin/Clavulanic acid and nalidixic acid [7] and also to a variety of medicinal herbs such as bitter cola seed and turmeric [8].

This study was carried out to characterize and detect antibiotic resistant genes in some *Pseudomonas* species isolated from the West African Mud Creeper using molecular techniques.

2. MATERIALS AND METHODS

2.1 Study Period

This study was carried out between February and August 2019.

2.2 Sample Description, Size and Collection

A total of 42 edible samples of *Tympanotonos fuscatus* were collected between February and May 2019 from three different locations in Rivers State Nigeria; Mile 1 market in Port Harcourt City Local Government Area (4.7918° N, 6.9986° E), Rumueme Market in Obio/Akor Local Government Area (4.8273° N, 6.9820° E) and Mile 3 Market in Port Harcourt City Local Government Area (4.8042° N, 6.9924° E). Microbiological examination was carried out at the Microbiology laboratory, Rivers State University, Port Harcourt, Nigeria.

2.3 Isolation and Identification of Bacteria

Bacteria was isolated from sample using cultural means, following the process of serial dilution, spread plating, incubating over night and sub culturing to obtain pure cultures. Morphological and biochemical tests were carried out for identification of *Pseudomonas* species. Biochemical tests such as oxidase test, motility test, catalase test, starch hydrolysis test, indole test, methyl red test, Voges Proskaeur test, citrate utilization test were carried out on isolates [9]. Molecular characterization was employed to confirm the identities of some isolates using the method of Queipo-ortuno et al. [10]; Al-Awadhi et al. [11].

2.4 Antibiotic Sensitivity Testing

Standardization of isolates was carried out by adjusting the turbidity of isolates in test tubes to that of a 0.5 McFarland standard. The antimicrobial susceptibility profiles of the isolates to conventional antibiotics were determined using the Kirby Bauer disk diffusion method [12] on sterile Mueller-Hinton agar. The surface solid media plate was inoculated with bacterial suspension by streaking the swab over the agar plate surface; being sure that no zone of the surface is left free of inoculum. This procedure was repeated several times, rotating the agar plate 60° each time to ensure even distribution of the inoculum to the edge of the agar. The plates were left to dry for 3–5 min to allow absorption of any moisture prior to applying the antibiotic disks. Antibiotic disks of ten conventional antibiotics (Cephalexin (CEP) – 10 µg, Ofloxacin (OFX) -10 µg, Nalidixic acid (NA) – 30 µg, Pefloxacin (PEF) – 10 µg, Gentamycin (CN) – 10 µg, Amoxicillin/Clavulanic acid (AU) – 30 µg, Ciprofloxacin (CPX) – 10 µg, Trimethoprim (SXT) – 30 µg, Streptomycin (S) – 30 µg and Ampicillin (PN) – 30 µg) were aseptically placed on the surface of the inoculated agar plate with sterile forceps. Each disk was pressed down to ensure full contact with the surface of the agar. At least 24 mm was left between the centres of the disks, and not less than 15 mm from the border of the plate too. The plates were then inverted and placed in an incubator within 15 min of applying the disks. Finally, the plates were incubated for 24 hours at 33 to 35°C [12]. After incubation, the plates (control and test plates) were examined to ensure growth was confluent or near confluent. On the underside of the plate, the diameter of each zone of inhibition for those that had zones of inhibition were measured in millimetre (mm) using a meter rule. The measurement included the diameter of the disc. For interpretation MIC Analysis and Susceptibility Testing, the criteria provided by CLSI were followed.

2.5 Molecular Studies

Molecular screening was carried out on the most resistant strains of *Pseudomonas* isolated for identification, plasmid profiling and detection of antibiotic resistance gene (AmpC).

2.5.1 DNA extraction and quantification

DNA extraction was carried out using the Boiling method by Bell et al. [13]. 24 hour old pure cultures of *Pseudomonas* species were put in Luria-Bertani (LB) Broth and allowed to incubate at 37°C. After 24 hours, the cells were washed thrice in microcentrifuge tubes with normal saline by centrifuging for 3 minutes at 14,000 xg and decanting supernatant leaving the DNA at the base. The DNA was washed with 1 ml of normal saline and vortexed to mix and then centrifuged again. The cells were re-suspended in 500 ul of normal saline and heated at 95°C for 20 min in a heating block after which it was cooled on ice and then centrifuged for 3 minutes at 14000 xg. Using a 1.5 ml microcentrifuge tube, the supernatant containing the DNA was transferred and stored at 20°C [13]. The extracted genomic DNA was quantified by using the Nanodrop 1000 Spectrophotometer as described by Olsen and Morrow [14].

2.5.2 16S rRNA amplification

The method as described by Srinivasan et al. [15] was adopted. An ABI 9700 Applied Biosystems Thermal Cycler was used to amplify the *16S rRNA*. The *16S rRNA* region of the rRNA genes of *Pseudomonas* species isolates were amplified using the forward primer; 27F: 5' AGAGTTTGATCMTGGCTCAG-3 and Reverse Primer 1492R: 5'-CGGTTACCTTGTTACGACTT-3' at a final volume of 30 µl for 35cycles. The PCR cocktail was prepared using the primers at 0.6µM concentration, the Template (the extracted DNA), Buffer 1X, water, PCR Mix (15M) which consists of; dNTPs, MgCl and Taq Polymerase. The conditions for PCR were as follows; Initial denaturation, 95°C for 5 minutes; Denaturation, 95°C for 30 seconds; Annealing 52°C for 30 seconds; Extension, 72°C for 5 minutes. The product was fixed in a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator.

2.5.3 DNA sequencing

The amplified products were labelled using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems). The sequencing was done at a final volume of 10 µl; the components included 0.25 µl BigDye terminator v1.1/v3.1, 2.5 µl of 5x BigDye sequencing buffer, 10 µM Primer PCR primer and 2-10 ng PCR template per 100 bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10 seconds, 55°C for 5 seconds and 60°C for 4 minutes [15].

2.5.4 Phylogenetic analysis

Sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 [16]. The evolutionary distances were computed using the Jukes-Cantor method [17].

2.6 Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple Antibiotic Resistance Index was determined from Sensitivity testing Results using the formular, MAR index =a/b, where a, = the number of resistance to antibiotics displayed and b = the total number of antibiotics tested, as described by Osundiya et al. [18].

3. RESULTS

The result shown on Fig. 1 is the agarose gel electrophoresis of the amplified 16S rRNA gene of two selected and most resistant *Pseudomonas* isolates before sequencing.

The evolutionary distance between the *P. aeruginosa* isolates from this study and the accession numbers of their closest relatives on the phylogenetic tree is shown on Fig. 2.

The agarose gel electrophoresis showing the plasmid DNA bands of the most resistant *Pseudomonas* isolates is displayed on Fig. 3.

The agarose gel electrophoresis image showing the amplified *ampC* gene of the 12 most resistant *Pseudomonas* isolates to antibiotics is shown

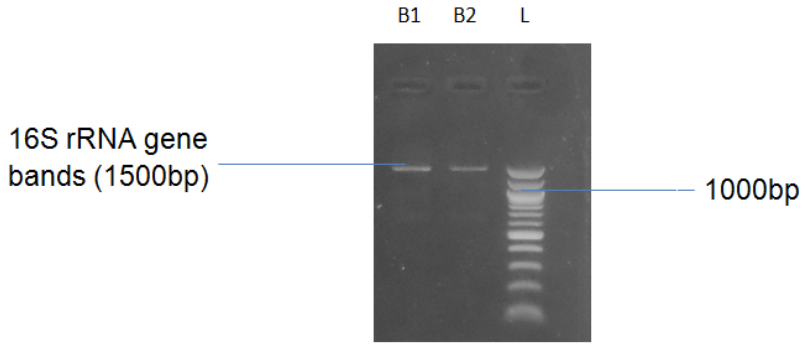

Fig. 1. Agarose gel electrophoresis of the 16S rRNA gene of selected most resistant bacterial isolates

Lanes B1 and B2 represent the 16SrRNA gene bands (1500bp) while lane L represents the 100bp molecular ladder

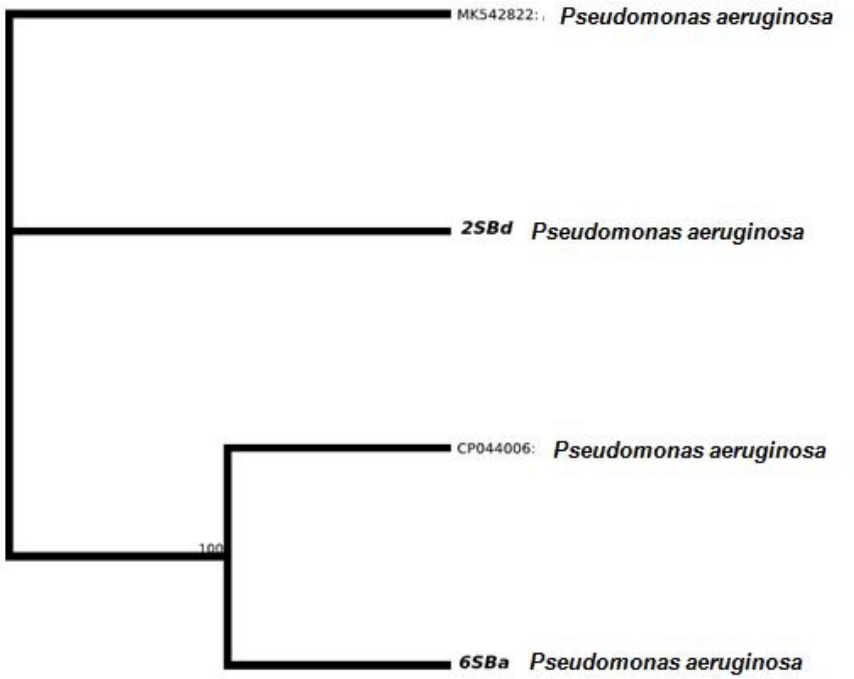

Fig. 2. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

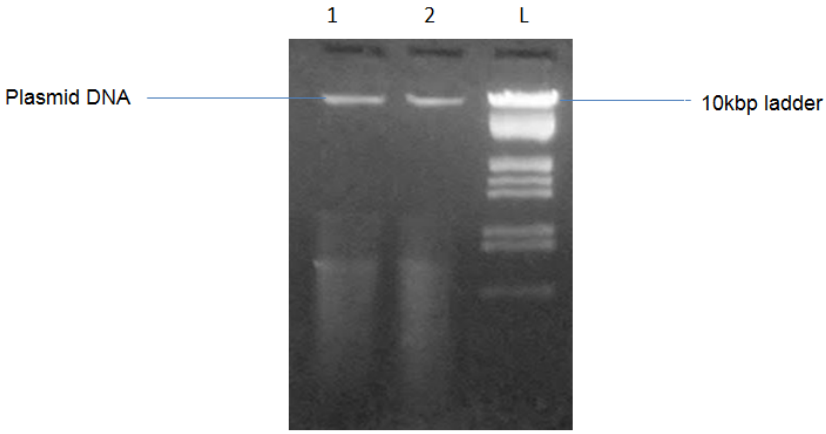

Fig. 3. Agarose gel electrophoresis showing the plasmid DNA bands of the *Pseudomonas aeruginosa* isolates

Lane 1 and 2 showing plasmid DNA bands at >10 kbp while lane L represents the 10 kbp molecular ladder. Plasmid DNA from both strains bands at regions greater than 10 kilobase pairs. This represents high molecular weight plasmids

on Fig. 4. Lane L represents the 100 bp molecular ladder, while Lane 1-3, 6-8 and 10 showing the *ampC* band at 500 bp. From the result it was observed that 7 out of the 12 isolates (58.3%) screened for *ampC* gene had the gene present in their genetic material.

Table 1 shows the Multiple Antibiotic Resistance (MAR) Index of *Pseudomonas* isolates after subjection to a group of 10 conventional antibiotics. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used.

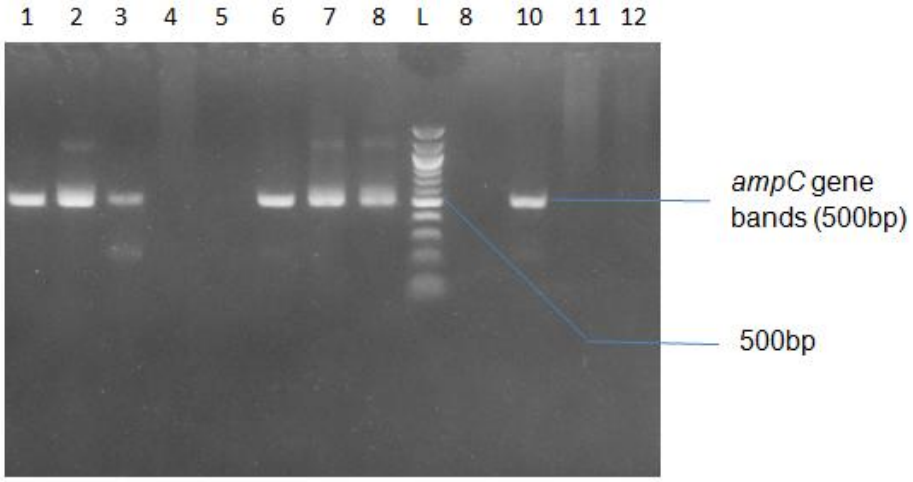

Fig. 4. Agarose gel electrophoresis showing the amplified ampC gene of the 12 most resistant *Pseudomonas* isolates to antibiotics

Table 1. Multiple Antibiotic Resistance (MAR) index of *Pseudomonas* species

MAR index	Number (%)
0.1	1 (3.33)
0.2	3 (10.0)
0.3	5 (16.67)
0.4	6 (20.0)
0.5	11 (36.67)
0.6	4 (13.33)

4. DISCUSSION

The contributions of bacterial species in the epidemiology of disease are influenced by the method used in the identification and characterization of the bacterial isolates, as researchers employ various microbiological techniques to achieve this aim. Molecular characterization has recently been employed as a high-throughput approach which enable a reliable identification of disease causing agents. PCR has the potential for identifying microbial species rapidly by amplification of sequences unique to a particular organism [19]. This research therefore explored the use of a culture-based molecular technique in accessing and probing of *Pseudomonas* species isolated from West African Mud Creeper (*Tympanotonus fuscatus)*, for the presence of genes that confer antibiotic resistance, such as *ampC* gene. Results from the molecular study showed that the obtained 16S rRNA sequence from the selected most resistant isolates produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. Also, the evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates 2SBd and 6SBa within the *Pseudomonas* sp. which revealed a close relatedness to *Pseudomonas aeruginosa* than other *Pseudomonas* sp.

Persistence of antibiotic resistant bacteria, including multidrug resistant (MDR) pseudomonads, is an important environmental health problem associated with food samples from environmental samples. Beyond the phenotypic determination of antibiotic resistance pattern earlier reported [7], the presence of antibiotic resistance gene (*ampC*) which could mediate the antibiotic resistance in the *Pseudomonas* sp. was screened through a molecular technique. It followed that these two isolates reported in this paper were the most resistant to almost all the antibiotics tested. The result further revealed the presence of plasmid DNA of over 10 kilo base pairs. Several resistant attributes are borne on the plasmid DNA of bacteria and the heavier or longer the plasmid, the more likely it is to carry several genes coding for extra bacterial attributes [20].

Resistant gene analysis for the detection of *amp*C gene in twelve (12) resistant *Pseudomonas* species isolates revealed that seven (7) out of twelve isolates (58.3%) screened had the *ampC* gene present in their genome. *amp*C is largely responsible for the resistance of *Pseudomonas* species isolates to

cephalosporin antibiotics used in the study and its overproduction can even further increase the ability of isolates to resist these antibiotics completely [21]. *amp*C gene codes for the production of *amp*C beta-lactamase enzyme which acts on the beta-lactam ring of antibiotics to inhibit their activity [22].

There is paucity of information relating to studies on the molecular characterization of antibiotic resistance genes among Nigeria environmental pseudomonads, therefore, making it difficult to compare with studies from Nigeria [23]. However, among few of the studies, Chikwendu et al. [24] reported the presence of bla_{SHV} and bla_{TEM} among environmental pseudomonads from Nigeria while Odumosu et al. [25] reported $bla_{oxa\text{-}10}$, *ampC* β-lactamase in 50 and 70% of *Pseudomonas aeruginosa*, respectively from Nigeria clinical source.

MAR index of *Pseudomonas* species isolated in this study revealed that the percentage of isolates with MAR index ≥ 0.2 was 96.67%. It is important to note that MAR index values > 0.2 indicate high risk source of contamination where antibiotics are often used [18,22]. This shows that 96.67% of the *Pseudomonas* species isolated in this study are likely to show multiple resistance to the antibiotics used in this study. This finding is very critical as it indicates that these antibacterial agents may not be potent in the management of infections caused by these species of *Pseudomonas.*

The presence of resistant strains of *P. aeruginosa* in *T. fuscatus* which may have risen from the market environment due to poor hygienic practices poses a problem for public health as it can cause a wide range of infections such as endocarditis, gastrointestinal infections, osteomyelitis, septicaemia and meningitis especially in immunocompromised individuals [26]. The study of bacterial genomics informs our understanding of resistance mechanisms and comparatively analysing these genes can provide relevant information on the evolution of resistant strains of organisms and on resistance genes [27].

5. CONCLUSION

This study has shown that the resistance of most *Pseudomonas* species to some antibiotics is largely due to the presence of the resistance *ampC* gene, presence of heavy plasmids amongst other factors not covered by the current study. This molecular identification of *Pseudomonas* species has shown that genomic studies are needed to confirm the exact taxonomic identity of *Pseudomonas* spp due to their public health importance. Further studies are however needed to decipher the factors influencing their high genetic plasticity. The risks of resistance of *P. aeruginosa* to antibiotics are also very high considering the MAR index values obtained from the study.

From this study it can also be inferred that West African Mud Creeper (*Tympanotonus fuscatus)* harbours bacterial populations that can lead to serious foodborne ailments with associated multiple antibiotic resistant traits. This observation is not however unconnected with microbiological quality of its marine habitat. This further buttresses the fact that the marine environment serves as a sink for antibiotic resistant microbial population. Proper waste disposal/management is therefore recommended to check and prevent the build-up or proliferation antibiotic resistant microbial community in the environment vis-à-vis the invaluable importance of the aquatic environment to man.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fred CT. Mechanisms of antimicrobial resistance in bacteria. American Journal of Infection Control. 2006;34(5):3–10.
2. Kumar S, Lekshmi M, Parvathi A, Nayak BB, Varela MF. Antibiotic resistance in seafood-borne pathogens. In: Food Borne Pathogens and Antibiotic Resistance, O. V. Singh (Ed.); 2017.
3. Madigan M, Martinko J. Brock biology of microorganisms (11th Ed.). Prentice Hall; 2005. ISBN: 0-13-144329-1.
4. Tanya S, Daniel Y. *Pseudomonas aeruginosa* – A phenomenon of bacterial resistance. Journal of Medical Microbiology. 2009;58:1133–1148.
5. Bagge N, Ciofu O, Hentzer M, Campbell JI, Givskov M, Hoiby N. Constitutive high expression of chromosomal b- lactamase in *Pseudomonas aeruginosa* caused by a new insertion sequence (IS1669) located in ampD. Antimicrobial Agents and Chemotherapy. 2002;46:3406–3411.

6. Langaee TY, Gagnon L, Huletsky A. Inactivation of the ampD gene in *Pseudomonas aeruginosa* leads to moderate-basal-level and hyperinducible AmpC β-lactamase expression. Antimicrobial Agents and Chemotherapy. 2000;44:583–589.
7. Akani NP, Hakam IO, Sampson T. Prevalence and antibiogram of *Pseudomonas aeruginosa* isolated from West African Mud Creeper (*Tympanotonus fuscatus*). South Asian Journal of Research in Microbiology. 2019;5(2):1-8.
8. Hakam IO, Akani NP, Sampson T. Antimicrobial efficacy of some herbs on resistant strains of *Pseudomonas* species isolated from West African Mud Creeper. Singapore Journal of Scientific Research. 2020;10:73-78.
9. Aditi FY, Rahman SS, Hosain MM. A study on the microbiological status of mineral drinking water. The Open Microbiology Journal. 2017;11:31–44.
10. Queipo-Ortuño MI, De Dios Colmenero J, Macias M, Bravo MJ, Morata P. Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. Clinical and Vaccine Immunology. 2008;15(2):293–296.
11. Al-Awadhi H, Dashti N, Khanafer M, Al-Mailem D, Ali N, Radwan S. Bias problems in culture-independent analysis of environmental bacterial communities: A representative study on hydro-carbonoclastic bacteria. Springer Plus. 2013;2:369.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, twenty-first informational supplement. CLSI Document M100-S21 (ISBN 1-56238-742-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA. 2017;30(1):68-70.
13. Bell JM, Paton JC, Turnidge J. Emergence of vancomycin resistant enterococci in Australia: Phenotypic and genotypic characteristics of isolates. Journal of Advances in Biology. 1998;36(8):2187–2190.
14. Olsen ND, Morrow JB. DNA extract characterization process for microbial detection methods development and validation. BMC Research Notes. 2012;5: 668.
15. Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S, Nadarajan L, Brodie EL, Lynch SV. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. Plos One. 2015;10(2):1–22.
16. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987;4(4):406–425.
17. Jukes TH, Cantor CR. Evolution of protein molecules. In Munro HN, Editor, Mammalian Protein Metabolism, Academic Press, New York. 1969;21–132.
18. Osundiya OO, Oladele RO, Oduyebo OO. Multiple antibiotic resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. African Journal of Clinical and Experimental Microbiology. 2013;14(3): 164–169.
19. Virupakshaiah DBM, Hemalata VB. Molecular identification of *Pseudomonas aeruginosa* from food borne isolates. Int J Curr Microbiol App Sci. 2016;5:1026-1032.
20. Banu H, Prasad KP. Role of plasmids in microbiology. Journal of Aquaculture Research and Development. 2017;8(1): 466–474.
21. Berrazeg M, Jeannot K, Enguene VYN, Broutin I, Loeffert S. Mutations in β-lactamase AmpC increase resistance of *Pseudomonas aeruginosa* isolates to antipseudomonal cephalosporins. Antimicrobial Agents and Chemotherapy. 2015;59(10):6248–6255.
22. Kong K, Schneper L, Mathee K. Beta-lactam antibiotics: From antibiosis to resistance and bacteriology. APMIS: Acta Pathologica, Microbiologica, et Immuno-logica Scandinavica. 2010;118(1): 1–36.
23. Adesoji AT, Ogunjobi AA, Olatoye IO. Molecular characterization of selected multidrug resistant *Pseudomonas* from water distribution systems in South-Western Nigeria. Annals of Clinical Microbiology and Antimicrobials. 2015;14(39). DOI:https://doi.org/10.1186/s12941-015-0102-4
24. Chikwendu CI, Ibe SN, Okpokwasili GC. Detection of bla_{SHV} and bla_{TEM} beta-lactamase genes in multi-resistant *Pseudomonas* isolates from environmental

sources. Afr J Microbiol Res. 2011;5:2067–2074.

25. Odumosu BT, Adeniyi BA, Chandra R. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant *Pseudomonas aeruginosa* from Southwest Nigeria. Ann Clin Microbiol Antimicrob. 2013;12:29–35.
26. Rossolini GM, Thaller MC. Coping with antibiotic resistance: Contributions from genomics. Genomic Medicine. 2010;2(2): 15–20.
27. Mena KD, Gerba CP. Risk assessment of *Pseudomonas aeruginosa* in water. Rev Environ Contam Toxicol. 2009;201:71–115.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57932