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# Molecular Characterization and Plasmid Profile of Antibiotics Resistant Genes in *Staphylococcus aureus* and *Pseudomonas aeruginosa* from Clinical Specimens

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**A B S T R A C T**

One of the global health challenges is the antibiotic-resistant pattern exhibited by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study aimed to characterize *S. aureus* and *P. aeruginosa* from clinical specimens using molecular methods and to carry out plasmid profiles of antibiotic-resistant genes. Twenty isolates comprised of 10 *S. aureus* and 10 *P. aeruginosa* isolates from clinical specimens which exhibited multi-drug resistance were collected from the Department of Microbiology, Rivers State University. Polymerase chain reaction and DNA sequencing were employed to identify the isolates. Additionally, plasmid profiling was conducted to examine the presence of mecA, TEM, CTX-M and NDM-1 genes in the plasmid and the eno gene. Results showed that the 16S rRNA of the isolates showed a 99-100% similarity to the isolates of *S. aureus* with accession numbers (AF065394.1, NC007795.1, KV829593.1, NZJXHU01000160.1, KV841461.1, CP031670.1, CP031664.1, BX571856.1, AP017922.1 and CP003045.1) and *P. aeruginosa* (CP104590.1, NR026078.1, MN911415.1, CP007224.1, NZCP041945.1, NSPN01000008.1, NZNIZN01000087.1, KY086497.1, KJ482599.1, and KJ482590.1), respectively. Results of the plasmid profiling showed that 90% of the isolates possessed the *blaTem* gene, 40% of *P. aeruginosa* possessed New Delhi metallo-β-lactamase (NDM-1), 80% of both *S. aureus* and *P. aeruginosa* possessed the *CTX-M* gene while 40% of the *S. aureus* isolates possessed the *mecA* gene. Results also showed that 55% of all the isolates possessed the eno gene. The identification of plasmid-borne resistant genes emphasizes the need for continued surveillance and control measures to mitigate the spread of antibiotic resistance in healthcare settings.

## Introduction

Antibiotics have proven to be an effective treatment for a wide range of microbiological infections. However, misuse or overuse of antibiotics has resulted in the emergence of a new breed of antibiotic-resistant bacteria (Al-Zahrani *et al.*, 2017). The European Centre for Disease Prevention and Control (ECDC) and the Centers

for Disease Control and Prevention (CDC) have developed terminology to define the various levels of the acquired antibiotic resistance profiles in microorganisms as follows: multidrug-resistant (MDR) was defined as "acquired non-susceptibility to at least one agent in three or more antibiotics", extremely drug-resistant (XDR) was defined as "non-susceptibility to at least one agent in all but two or fewer antimicrobial categories", and pan drug-resistant (PDR) was defined as "non-susceptibility to all agents in all antibiotics" (Al-Zahrani *et al.,* 2017).

Many bacteria strains including *P. aeruginosa*, *Klebsiella pneumoniae*, *S. aureus* and *Enterococcus spp*., *E. coli*, and *Acinetobacter* spp have been identified as MDR, XDR or PDR (Magiorakos *et al.,* 2012; Theuretzbache, 2013; Giamarellou *et al.,* 2013; Pontikis *et al.,* 2014). Also, infections caused by bacterial multidrug-resistant (MDR) pathogens for which conventional antibiotic therapies are ineffective are becoming a rising problem in hospitals and communities and one of the most serious risks to human health is antimicrobial resistance (Franci *et al.,* 2015). *Pseudomonas aeruginosa* is one of the most common Gram-negative rods, capable of infecting a wide range of tissues and causing significant morbidity and mortality in hospital patients. It's the second most common cause of nosocomial pneumonia, the third most common cause of urinary tract infection, the fourth most common cause of surgical site infection, the fifth most frequently isolated pathogen from all sites, and the seventh most frequently isolated pathogen from the bloodstream (Olivares *et al.,* 2020). It is an opportunistic human pathogen, found in almost every habitat and could cause a variety of life-threatening acute and chronic infections, especially in people who have weakened immune systems. It is particularly important since it is the largest cause of morbidity and mortality in cystic fibrosis (CF) patients, as well as one of the most common nosocomial bacteria infecting hospitalized patients, and it is intrinsically resistant to a wide range of antibiotics (Moradali *et al.,* 2017). *Staphylococcus* species are generally considered commensal bacteria (Park *et al.,* 2019; Parlet *et al.,* 2019). They can, however, cause skin and soft tissue infections, acute or chronic infections, osteomyelitis, pneumonia, endocarditis, implantable device-associated infections, and other disorders when immunosuppressed or under other situations (Williams *et al.,* 2019). *S. aureus* is one of the most common bacteria associated with hospital and community-acquired illnesses (Miller *et al.,* 2020). Although *S. aureus* can infect any tissue in the body, a skin break is the most common source of infection (Ansari *et al.,* 2019) and the majority of bacterial cutaneous infections, such as surgical site infections, purulent cellulitis, cutaneous abscesses, and many others, are caused by *S. aureus* (Parlet *et al.,* 2019; Heilmann *et al.,* 2019). More recently, it has been discovered that community-acquired methicillin-resistant *S. aureus* (MRSA) infections exceed hospital-acquired infections (Heilmann *et al.,* 2019), increasing the importance of the development of effective therapeutic alternatives (Dos Santos *et al.,* 2018). Studies have shown that *Pseudomonas aeruginosa* and *Staphylococcus aureus* has a high resistance to conventional antibiotics making empirical antibiotic therapy ineffective against these bacteria as they are known to use inherent and acquired resistance mechanisms to most conventional antibiotics (Yayan *et al.,* 2015; Pang *et al.,* 2019). Furthermore, adaptive antibiotic resistance by *Pseudomonas aeruginosa* and *Staphylococcus aureus* is a newly identified mechanism that combines biofilm-mediated resistance and the generation of multidrug-tolerant persister cells. This is responsible for recurrence and antibiotic resistance has been a major concern in recent decades, resulting in difficult-to-treat infections and a huge burden on the global healthcare system (Al-Zahrani *et al.,* 2014; Moradali *et al.,* 2017; Pang *et al.,* 2019). For effective control of resistant isolates, it would be important to carry out a plasmid profile. Thus, the justification of the present study.

## Materials and Methods

### Collection of Isolates

A total of twenty (20) isolates comprised of ten (10) *Staphylococcus aureus* and (10) *Pseudomonas aeruginosa* isolates from clinical specimens (wound, urine, stool and ear) which exhibited multi-drug resistance were collected from the Department of Microbiology, Rivers State University. These isolates were confirmed phenotypically using cultural, morphological and biochemical techniques (Prescott *et al.,* 2011). The isolates were further used for further studies after confirmation.

### DNA Extraction

The extraction of bacteria DNA was done using the boiling technique adopted in a previous study (Robinson and Wemedo, 2019). Twenty-four hours old cultures were put in Luria Bertani (LB) medium and incubated for twenty-four (24) hours. After incubation, the bacterial

isolate was spun at 14000 rpm for three minutes. The cells were heated for 20 minutes at 95°C after being re-suspended in 500 µl of ordinary saline.

The heated bacterial suspension was spun for three minutes at 14000 rpm after cooling on ice. The DNA-containing supernatant was put into a 1.5 ml microcentrifuge tube and kept at -20 °C (Felsenstein, 1985). The extracted DNA was quantified using the Nanodrop 1000 spectrophotometer

## Amplification of the 16S rRNA

Saitou and Nei's techniques (1987) were adopted. The 16s rRNA of the rRNA genes of isolates were amplified using the 27F and 1492R primers on the ABI9700 Applied Biosystems thermal cycler for 35 cycles. The X2 Dream taq Master mix from Inqaba, South Africa (including taq polymerase, DNTPs, and MgCl), the forward and reverse primers at a concentration of 0.4M, and the extracted DNA serving as the template make up the PCR mix. Initial denaturation was adjusted to 95℃ for 5 minutes, followed by denaturation for 30 seconds at 95 ℃, annealing at 52 ℃, extension at 72 ℃ for 30 seconds for 35 cycles, and final extension at 72 ℃ for 5 minutes.

## Sequencing and Phylogenetic Analysis

Sequencing was performed by Inqaba Biotechnological, Pretoria, South Africa, using a 3510 ABI sequencer and the BigDye Terminator kit. After downloading related sequences from the National Center for Biotechnology Information (NCBI) database using BLASTN, phylogenic analysis was performed on the resultant sequences using the bioinformatics technique Trace edit tool. MEGA 11.0 used ClustalX to align downloaded sequences, and the Neighbor-Joining technique to estimate evolutionary history (Saitou and Nei, 1987). The evolutionary history of the taxa under study was assumed to be represented by the bootstrap consensus tree generated from 500 replicates (Felsenstein, 1985). The Jukes-Cantor technique was used to compute the evolutionary distances (Jukes and Cantor, 1969).

## Amplification of *mecA*, SHV, TEM, CTX-M and NDM-1 Genes

Applied Biosystems ABI 9700 thermal cycler was used to amplify the *mecA* genes from the isolates using the primers *mecA* Forward: 5' AAAATCGATG-GTAAAGGTTGGC-3' and mecA Reverse: 5'-AGTTCTGCAGTACCGGATTTTGC-3' for 35 cycles. The SHV genes were amplified using the primers SHV F: 5' CGCCTGTGTATTATCTCCCT3' and SHVR: 5'CGAGTAGTCCACCAG ATCCT-3' for 35 cycles. The TEM genes was amplified using the primers TEMF: 5'-ATGAGTATTCAA CATTTCCGTG3' and TEMR: 5'TTACCAATGC TTAATCAGTGAG-3' for 35 cycles, the The CTX-M genes from the isolates were amplified using the primers CTX-MF: 5'CGCTTTG CGATGTGCAG-3' and CTX-MR: 5'-ACCGCGATATCGT TGGT-3' for 35 cycles while the NDM genes from the isolates were amplified using the primers NDMF: 5′- GGTTTGGCGATC TGGTTTTC-3′ and NDMR: 5′-CGGAATGGCT CATCACGATC- 3′ for 35 cycles.

The PCR mixture for each gene contained: the Taq polymerase, dNTPs, and MgCl in the X2 Dream Taq Master mix provided by Inqaba, South Africa, the primers at a concentration of 0.4M, and 50ng of the extracted DNA as template. The following were the PCR conditions: Initial denaturation was carried out at 95°C for 5 minutes, followed by subsequent denaturations at 95°C for 30 seconds, 55°C for 40 seconds, annealing, 72°C for 50 seconds for 35 cycles, and final extension for 5 minutes at 72°C. The product was visualised using a blue light trans-illuminator for a 500bp product size after being resolved on a 1% agarose gel prepared with EZ vision dye at 120V for 25 minutes (Peterson, 2010).

## Biofilm (ENO) Amplification

On an ABI 9700, the eno gene was amplified using the primers enof: 5-ACGTGCAGCAGCTGACT-3 and enoR: 5CAACAGCATCTTCAGCCTTC-3. 35 cycles in an Applied Biosystems thermal cycler with a final volume of 40 microliters. The X2 Dream tag Master mix from Inguba South Africa, primers at a concentration of 0.4M, and 50mg of the extracted DNA as template are all included in the PCR mixture. The following temperatures were used for the PCR conditions: initial denaturation at 95 °C for 40 seconds; annealing at 50 °C for 45 seconds; extension at 72 °C for 40 seconds for 35 cycles; and final extension at 72 °C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.The result was visualised on an UV transilluminator for a 281bp product size after being resolved on a 1% agarose gel at 120V for 25 minutes. (Peterson 2010).

## Results and Discussion

Results of the agarose gel electrophoresis of representative bacterial isolates are presented in Plate 1. Results showed that isolates in Lane 1 – 20 were within the 1500bp of the 16SrRNA bands.

Results of the phylogenic tree showing the evolutionary distance and relationship of the bacterial isolates is presented in Fig.1. Results also showed that isolates U3, S2, E2, E1, W3, W1, U2, U1, S1 and W2 which were previously characterized phenotypically as *S. aureus* have very high relatedness with *S. aureus* while isolates EB1, WB1, SB2, SU1, SB1, WB6, UP2, SU2, EB2 and UP1 which has been phenotypically characterized as *P. aeruginosa* showed very high relatedness as illustrated in the phylogenetic tree.

Results of the antibiotics resistant genes is presented in Plates 2-5. Results of the *blaTEM* gene of the bacterial isolates is presented in Plate 2. Results showed that out of the twenty isolates screened for the *blaTEM* resistant genes, 90% of the isolates possessed the genes while 10% were negative for the gene. Lanes 1-10 represents the *S. aureus* isolates while lanes 11-20 represents the *P. aeruginosa* isolates. Thus, isolates 5 and 20 do not possess the *blaTEM* gene.

Results of the New Delhi metallo-β-lactamase (NDM-1) of the *P. aeruginosa* isolates is presented in Plate 3. Results showed that out of the ten *P. aeruginosa* isolates screened for the gene, only four (40%) isolates possessed the genes.

The agarose gel electrophoresis of the *CTX-M* gene of the twenty bacterial isolates is presented in Plate 4. Results showed that out of the twenty isolates, 80% possessed the genes while 20% of the isolates do not possess the *CTX-M* gene.

Results of the *mecA* gene showed that out of the ten *S. aureus* isolates screened for the genes, only 40% of the isolates possessed the *mecA* gene while 60% of the isolates were negative for the gene (Plate 5).

Results of the slime (biofilm) forming gene (eno gene) of the isolates showed that 55% of the isolates possessed the eno genes while the other 45% lacked the slime (biofilm) gene. Moreover, results showed that 70% of the *S. aureus* isolates were biofilm formers (presence of *Eno* genes) while 30% do not possess the genes, thus referred to as non-biofilm formers. Similarly, 40% of *P. aeruginosa* isolates showed presence of the eno genes, 60% do not possess the gene (Plate 6).

In line with the phenotypic characteristics of the isolates which clearly showed they belonged to the *S. aureus* and *P. aeruginosa*, the molecular identities were not far from the phenotypic characterization as it showed very high similarity index of the isolates against those stored in the NCBI data base.

The agarose gel electrophoretic ladder confirmed they were within the 1500 base pair of the 16srRNA molecular ladder. The advantages of molecular characterization of microorganisms over phenotypic characterization is in the accurateness and precision of the results.

The molecular method have been regarded as the most precise method of classifying microorganisms since it relies on the genetic make-up (genome) of the microorganisms, it could describe the phylogenic relationship of the microorganisms and changes that have taken place over a long period of time (Ali and Latif, 2016; Fernández-espinar *et al.*, 2011). Unlike the phenotypic characterization which mostly relied on the metabolic characteristics that could be controlled by a particular gene thereby leading to variations in physiological response (Fernández-espinar *et al.*, 2011).

Antibiotic resistant genes are genes known to confer resistance to microorganisms that possess them. These genes are mostly carried in the plasmids and could be transferred to none carriers of the gene via horizontal gene transfer (Prescott *et al.*, 2011).

In the present study, resistant genes such as $bla_{TEM}$, New metallo-β-lactamase ($bla_{NDM}$) also abbreviated as NDM-1, *CXT-M*, *mecA* and *Eno* gene were investigated. The presence of these genes in the bacterial isolates (*S. aureus* and *P. aeruginosa*) could be the major factors responsible for the high resistance to antibiotics observed in the present study.

For instance, the NDM-1 ($bla_{NDM}$) gene is one of the genes responsible for carbapenem and other clinically significant resistance in bacterial isolates especially gram-negative bacteria (Ettu *et al.*, 2021). The most common mechanism of bacterial resistance to β-lactamase antibiotics such as carbapenem is the production of β-lactamase enzymes (Palzkill, 2012).

**Plate.1** Agarosegel electrophoresis of some selected bacterial isolates. Lane 1–20 represents 16SrRNA traitbands (1500 bp). Lane M represents the 100 bp

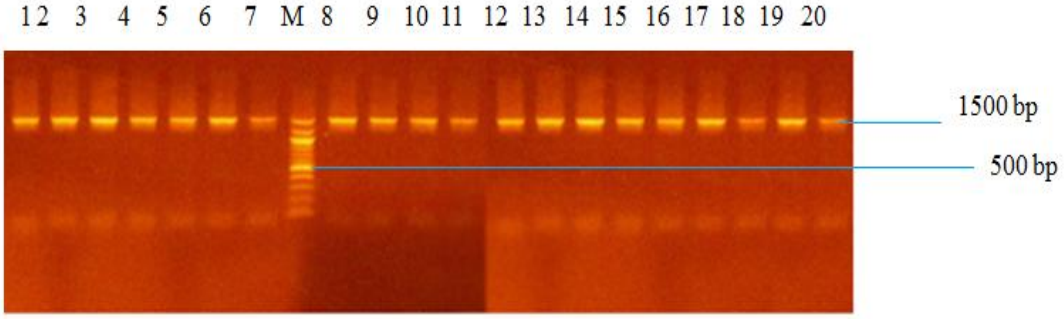



**Plate.2** Agarose gel electrophoresis of *BlaTem* gene of some selected isolates. Lane 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and 19 represents the *blaTem* gene bands (400bp). Lane M represents the 100bp Molecular ladder

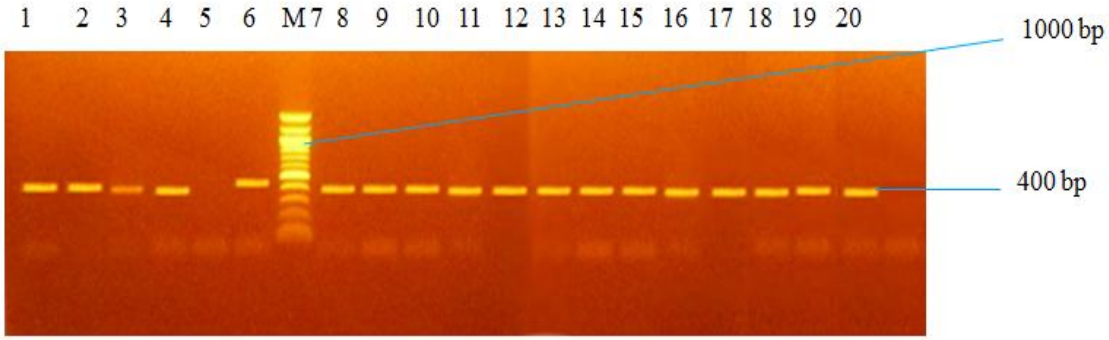



**Plate.3** Agarose gel electrophoresis of NDM gene of bacterial isolates. Lane 2, 3, 9 and 10 represent the NDM-1 gene band (621bp). Lane M represents the 100bp Molecular ladder of 1500bp.

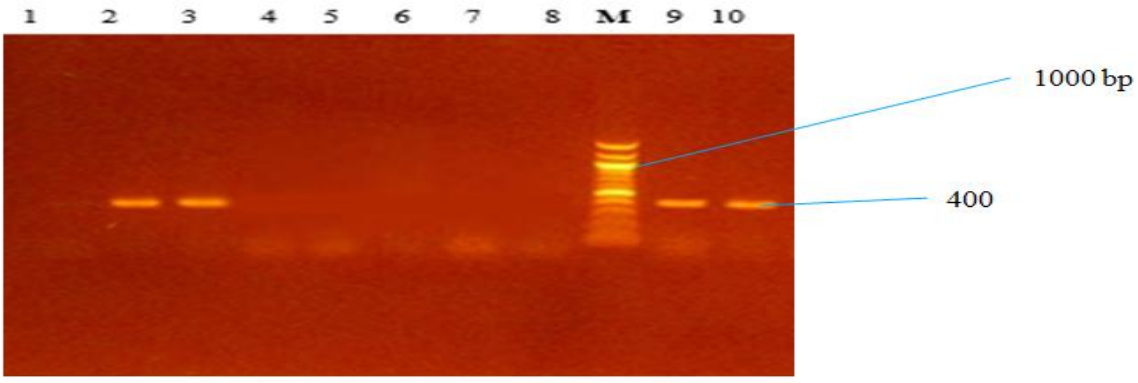


**Plate.4** Agarose gel electrophoresis of CTX-M gene of the bacterial isolates. Lane 1-7, 9, 11-15 and 18-20 represents the CTX-M gene bands (550 bp). Lane M represents the 100 bp Molecular ladder.

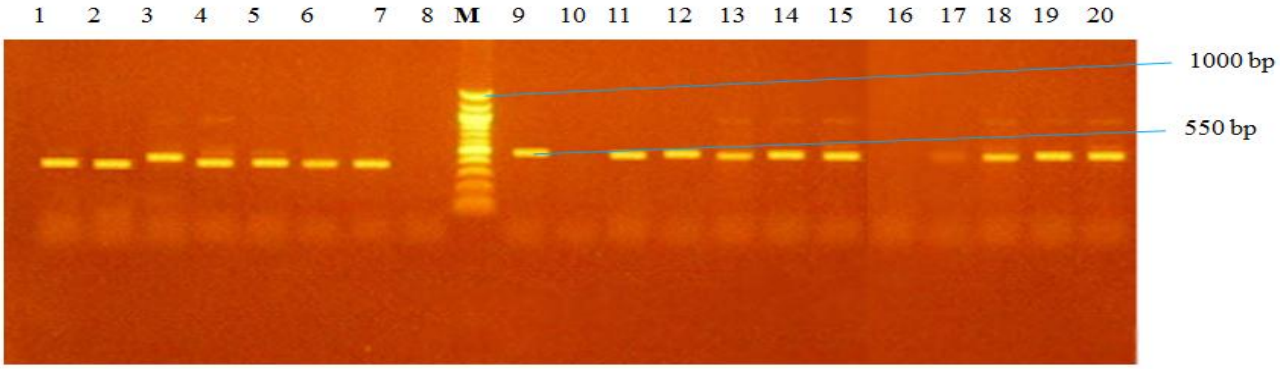



**Plate.5** Agarose Gel Electrophoresis Showing the Amplified *mecA* Gene Bands at 500bp. Lanes 1, 3-5 and 10 represent the *mecA* gene bands (500 bp). Lane M represents the 500 bp Molecular ladder.

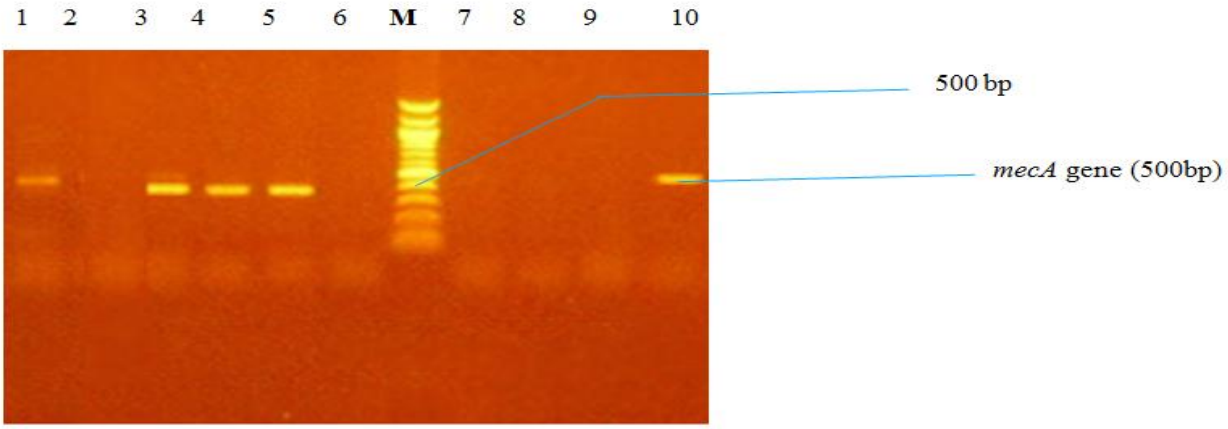



**Plate.6** Agarose gel electrophoresis showing the amplified Eno gene from the bacterial isolates. Lanes 2, 4-9, 13-14 and 19-20 showed the Eno gene bands at 300 bp while lane L represents the 50 bp molecular ladder

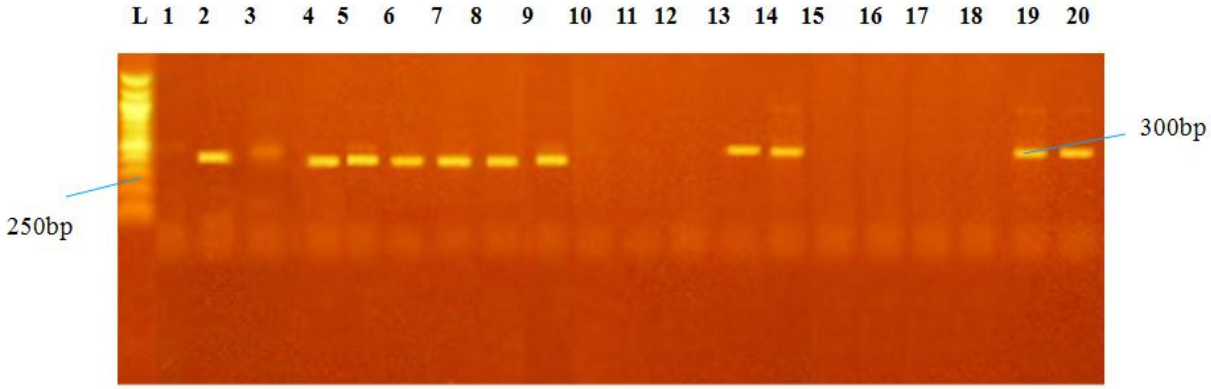
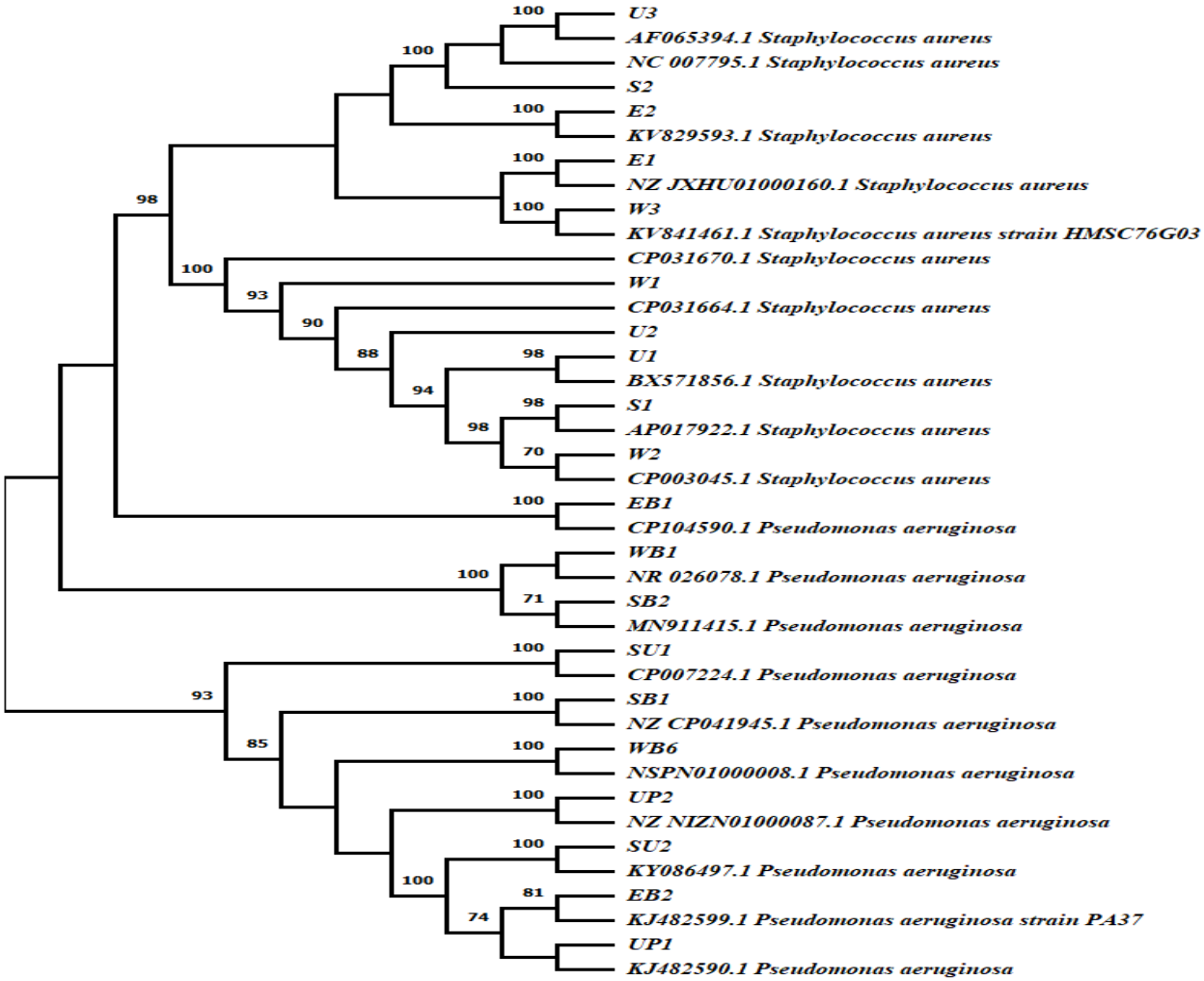


**Figure.1** Phylogenic Tree of Isolates showing the evolutionary distance between Isolates




The New Delhi metallo-lactamase (NDM-1) is a novel metallo-lactamase that is encoded by the blaNDM-1 gene, and this gene is found on large plasmids that are easily transferred, and it is said to be resistant to the majority of antibiotics, making spread to relevant bacteria quick and easy (Fernando *et al.*, 2021). Most blaNDM-1 strains are antibiotic resistant and carry several additional resistance genes to sulfonamides, aminoglycosides, fluoroquinolones, and macrolides (Shanthi *et al.*, 2014). Thus, this gene could be said to have influenced the resistance of *P. aeruginosa* isolates to aminoglycosides, fluroquinolones and penicillin in the present study since it was detected. Except for aztreonam, all β-lactamase can be hydrolyzed by the New Delhi metallo β-lactamase (Mayers *et al.*, 2017). This could be the resistance observed in ceftazidme in the present study. Additionally, isolates that produce metallo β-lactamase frequently exhibit aminoglycoside resistance (Alby and Miller, 2018). It is believed that MBL genes and aminoglycoside resistant genes are related, as bacteria carrying MBL genes frequently display co-resistance to aminoglycosides, making it difficult to administer effective therapeutic regimens (Walsh, 2005). Thus, the resistance to gentamycin an aminoglycoside in the present study could be attributed to the presence of NDM-1 gene in these isolates. A previous study of carbapenem resistant clinical specimens in Yola, Nigeria reported out of the 119 bacterial isolates, 25.2% had the $bla_{NDM}$ genes which is also found in 40% of our isolates in the present study. The prevalence of $bla_{NDM}$ is not unexpected given that Nigeria was one of the nations with the highest percentage of isolates containing $bla_{NDM}$ according to a previous international study (Kazmierczak *et al.*, 2016).

The *mecA* gene is a gene known to confer resistance to *S. aureus* isolates against methicillin antibiotics. The *mecA* gene is a penicillin binding protein 2a that is carried on the staphylococcal cassette chromosome mec (SCCmec) of which there are at least six different types recognized, and this results in resistance to all beta-lactam antibiotics (Humphreys, 2012). Furthermore, blaTEM and CTX-M genes are known to confer resistance against beta-lactam antibiotics.

The TEM gene ($bla_{TEM}$) genes is a Beta-lactamase gene that confers bacteria the ability to resist most antibiotics such as penicillin and first-generation cephalosporins including cephaloridine (Amirkamali *et al.,* 2017). In a previous study, it was reported that TEM genes in *Pseudomonas* isolates from clinical specimens contributed to 97.5% and 95% resistance to tetracycline and amoxicillin.

The CTX-M type enzymes are a subset of class A extended spectrum beta-lactamases (ESBLs) that, in general, have much higher levels of activity against cefotaxime and ceftriaxone than ceftazidime. Furthermore, the presence of CTX-M type ESBLs is frequently linked to co-resistance phenotypes, including resistance to tetracycline, fluoroquinolones, and aminoglycosides (Zeynudin *et al.,* 2018). The presence of this genes could be part of the reasons of the high level of resistance recorded in both isolates.

This study showed that the isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* had a wide variety of antibiotic resistance genes. It has also improved our understanding of some of the genes responsible for the resistance of antibiotics by these isolates. Although, plasmid profiling revealed the connection between a few resistance genes and mobile genetic elements, emphasising the role that horizontal gene transfer could play in the spread of resistant genes. To slow the spread of antibiotic resistance in healthcare settings, the discovery of plasmid-borne resistance genes highlights the necessity of ongoing surveillance and control measures.

## Author Contribution

V. K. Robinson: Investigation, formal analysis, writing—original draft. M. Okpokiri: Validation, methodology, writing—reviewing. F. O. Osakaude:—Formal analysis, writing—review and editing. S. A. Wemedo: Investigation, writing—reviewing.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Research Funding:** Not applicable

**Ethical Approval:** Not applicable.

**Consent to Participate:** Not applicable.

**Consent to Publish:** Not applicable.

**Conflict of Interest:** The authors declare no competing interests.

## References

Alby, K., and Miller, M. B. (2018). Mechanisms and Detection of Antimicrobial Resistance. Principles and Practice of Pediatric. *Infectious Diseases*, 1467-1478.e4. https://doi.org/10.1016/B978-0-323-40181-4.00290-5

Al-Zahrani, A., Al-Haj, N., Omer, H. and Al-Judaibi, A. (2014). Impact of Extractsof Marine Macroalgae on Multidrug-Resistant Bacteria. *Journal of Microbiology Research*, 4, 18-24.

Ali, M. S., and Latif, Z. (2016). Molecular characterization of yeast strains isolated from different sources by restriction fragment length polymorphism. *Pakistan Journal of Botany*, 48(1), 363–370.

Al-Zahrani, A., Al-Judaibi, E., Omar, H. and Al-Judaibi, A. (2017). Effects of Biochemical and Molecular Inhibitors of Plant Extracts on Pathogenic Bacteria. *Journal of Biosciences and Medicines*, 5, 44-55. https://doi.org/10.4236/jbm.2017.55005

Amirkamali, S., Naserpour-Farivar, T., Azarhoosh, K., & Peymani, A. (2017). Distribution of the bla OXA, bla VEB-1, and bla GES-1 genes and resistance patterns of ESBL-producing *Pseudomonas aeruginosa* isolated from hospitals in Tehran and Qazvin, *Iran. Revista da Sociedade Brasileira de Medicina Tropical,* 50(3), 315–320 https://doi.org/10.1590/0037-8682-0478-2016

Ansari, S., Jha, R. K., Mishra, S. K., Tiwari, B. R. and Asaad, A. M. (2019). Recent advances in *Staphylococcus aureus* infection: focus on vaccine development. *Infection and Drug Resistance,* 12, 1243–1255. https://doi.org/10.2147/IDR.S175014

Dos Santos, L. D. R, Dos Santos, A. E. S., Cera´volo, I. P., Figueiredo, F. J. B. and Dias-Souza, M. V. (2018). Antibiofilm activity of black tea leaf extract, its cytotoxicity and interference on the activity of antimicrobial drugs. *Biointerface Research in Applied Chemistry*, 8, 3565–3569

Ettu, A. O., Oladapo, B. A., and Oduyebo, O. O. (2021). Prevalence of carbapenemase production in *Pseudomonas aeruginosa* isolates causing clinical infections in Lagos University Teaching Hospital, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 22(4), 498–503. https://dx.doi.org/10.4314/ajcem.v22i4.10

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39:783-791. https://doi.org/10.2307/2408678

Fernández-espinar, M. T., Llopis, S., Querol, A., and Ferna, M. T. (2011). Molecular Identification and Characterization of Wine Yeasts. In Molecular Wine Microbiology; Carrascosa, A.V., Munoz, R., Gonzalez, R., Eds.; Elsevier Academic Press Inc.: San Diego, CA, USA. 111–141.

Fernando, B. J. U., Antonio, M. O. B., De Guzman, K. M. A., Gatbonton, J. C. Y., Vendivil, S. T., Tiongco, R. E. G., and Tesalona, S. D. (2021). The Prevalence of blaNDM-1 in Clinical Isolates of Carbapenem-resistant *Pseudomonas aeruginosa*: A Systematic Review. *SciMedicine Journal*, 3(4), 387–398. https://doi.org/10.28991/SciMedJ-2021-0304-9

Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G. and Galdiero, M. (2015) Silver Nano-particles as Potential Antibacterial Agents. *Molecules*, 20 (5), 8856-8874. https://doi.org/10.3390/molecules20058856

Giamarellou, H., Galani, L., Baziaka, F. and Karaiskos, I. (2013) Effectiveness of a Double-Carbapenem Regimen for Infections in Humans Due to Carbapenemase-Producing Pandrug-Resistant *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy*, 5, 2388-2390. https://doi.org/10.1128/AAC.02399-12

Heilmann, C., Ziebuhr, W. and Becker, K. (2019). Are coagulase-negative staphylococci virulent? *Clinical Microbiology and Infection*, 25, 1071–1080. https://doi.org/10.1016/j.cmi.2018.11.012

Humphreys, H. (2012). *Staphylococcus*: Skin infections; osteomyelitis; bloodstream infection; food poisoning; foreign body infections; MRSA, Editor(s): David Greenwood, Mike Barer, Richard Slack, Will Irving, Medical Microbiology (Eighteenth Edition), Churchill Livingstone,176-182.

Jukes, T. H. and Cantor, C. R. (1969). Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism, (pp. 21-132) Academic Press, New York. http://dx.doi.org/10.1016/B978-1-4832-3211-9.50009-7

Kazmierczak, K. M, Rabine, S., Hackel, M., McLaughlin, R. E., Biedenbach, D. J. and Bouchillon S. K. (2016). Multiyear, multinational survey of the incidence and global distribution of metallo-β-lactamase-producing Enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrobial Agents Chemotherapy*, 60 (2), 1067–1078. https://doi.org/10.1128/AAC.02379-15

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T. and Monnet, D. L. (2012) Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Clinical Microbiology and Infection*, 18, 268-281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Mayers, D. L., Sobel, J. D., Ouellette, M. and Kaye, K. S. (2017). Antimicrobial Drug Resistance. Mechanism of Drug Resistance. *Springer International Publishing*, 1101 https://doi.org/10.1007/978-3-319-46718-4

Miller, L. S., Fowler, V. G., Shukla, S. K., Rose, W. E. and Proctor, R. A. (2020). Development of a vaccine against *Staphylococcus aureus* invasive infections: Evidence based on human immunity, genetics and bacterial evasion mechanisms. *FEMS Microbiology Reviews*, 44, 123–153. https://doi.org/10.1093/femsre/fuz030

Moradali, M. F., Ghods, S. and Rehm, B. H. A. (2017). *Pseudomonas aeruginosa* Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. *Frontiers in Cellular and Infection Microbiology*, 7, 39. https://doi.org/10.3389/fcimb.2017.00039

Olivares, E., Badel-Berchoux, S., Provot, C., Prévost, G., Bernardi, T. and Jehl, F. (2020). Clinical impact of antibiotics for the treatment of Pseudomonas aeruginosa biofilm infections. *Frontiers in Microbiology*, 10, 2894.

Palzkill, T. (2012). Metallo-β-lactamase structure and function. Annals of the New York *Academy of Sciences*, 1277 (1), 91– 104. https://doi.org/10.1111/j.1749-6632.2012.06796.x

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J. and Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnology Advancement*, 37 (1), 177–192.

https://doi.org/10.1016/j.biotechadv.2018.11.013

Park, Y. J., Kim, C. W. and Lee, H. K. (2019). Interactions between Host Immunity and Skin-Colonizing Staphylococci: No Two Siblings Are Alike. *International Journal of Molecular Sciences*, 20 (3), 718; https://doi.org/10.3390/ijms20030718

Parlet, C. P., Brown, M. M., Horswill, A. R. (2019). Commensal Staphylococci Influence *Staphylococcus aureus* Skin Colonization and Disease. *Trends Microbiology*, 27, 497–507. https://doi.org/10.1016/j.tim.2019.01.008

Peterson, G. (2010). Development of microarray and multiplex polymerase chain reaction assays for identification of serovars and virulence genes in *Salmonella enterica* of human or animal origin. Journal of Vetinary Diagnostic Investiture, 22(6), 559-569. https://doi.org/10.1177/104063871002200410

Pontikis, K., Karaiskos, I., Bastani, S., Dimopoulos, G., Kalogirou, M., Katsiari, M., Oikonomou, A., Poulakou, G., Roilides, E. and Giamarellou, H. (2014) Outcomes of Critically Ill Intensive Care Unit Patients Treated with Fosfomycin for Infections Due to Pandrug-Resistant and Extensively Drug-Resistant Carbapenemase-Producing Gram-Negative Bacteria. *Journal of Antimicrobial Agents*, 43, 52-59. https://doi.org/10.1016/j.ijantimicag.2013.09.010

Prescott, L. M., Willey, J. M., Sherwood, L. and Woolverton, C. J. (2011). Microbiology New Nature, 47-49.

Robinson, V. K. and Wemedo, S. A. (2019). Molecular Characterization of Indoor air Microorganisms of a Model Primary Molecular Characterization of Indoor air Microorganisms of a Model Primary Health Care. *Asian Journal of Biotechnology and Genetic Engineering*, 2 (1), 1-9.

Saitou, N. and Nei, M. (1987) The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. Molecular Biology and Evolution, 4, 406-425.

Shanthi, M., Sekar, U., Kamalanathan, A., and Sekar, B. (2014). Detection of New Delhi metallo beta lactamase-1 (NDM-1) carbapenemase in *Pseudomonas aeruginosa* in a single centre in southern India. *The Indian journal of medical research*, 140(4), 546–550.

Theuretzbache, U. (2013) Global Antibacterial Resistance: The Never-Ending Story. *Journal of Global Antimicrobial Resistance*, 1, 63-69. https://doi.org/10.1016/j.jgar.2013.03.010

Walsh, T. R. (2005). The emergence and implications of metallo-β-lactamases in Gram-negative bacteria. *Clinical Microbiology and Infection*, 11, 2–9. https://doi.org/10.1111/j.1469-0691.2005.01264.x

Williams, M. R., Costa, S. K., Zaramela, L. S., Khalil, S., Todd, D. A. and Winter, H. L. (2019). Quorum sensing between bacterial species on the skin protects against epidermal injury in atopic dermatitis. *Science Translational Medicine*, 11 (490):eaat8329. https://doi.org/10.1126/scitranslmed.aat8329

Yayan, J., Ghebremedhin, B. and Rasche, K. (2015). Antibiotic Resistance of *Pseudomonas aeruginosa* in pneumonia at a Single University Hospital Center in Germany over a 10-year period. WebberMA, ed. *PLoS One*, 10 (10), e0139836. https://doi.org/10.1371/journal.pone.0139836

Zeynudin, A., Pritsch, M., Schubert, S., Messerer, M., Liegl, G., Hoelscher, M., Belachew, T., and Wieser, A. (2018). Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β - lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. *BMC Infectious Diseases*, 18, 524

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