Innovations

Plasmid Profiling of Pseudomonas Aeruginosa from Clinical Isolates – An Observation and Review

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Abstract

Problem: *The plasmid mediated quinolone resistance genes have been identified in many bacteria within the Enterobactericeae family and they have not been frequently documented in Pseudomonas aeruginosa isolates. The main objective of this study is to determine the plasmid mediated quinolone resistance in clinical isolates of Pseudomonas aeruginosa.* ***Methodology:*** *In this study, the identification was based on phenotypic characterization of P. aeruginosa, antibiotic resistance pattern and plasmid DNA profile. Processing the isolates and testing of antibiotic susceptibility was performed in Vitek2 Compact (Biomeriux, France) automated systems. DNA was isolated using the QIAamp DNA mini kit and quantified using Qubit. Genome data were analyzed and compared using the Plasmidfinder and pathogen.watch Database.* ***Findings:*** *Out of 157 Pseudomonas aeruginosa isolates, 10 isolates were determined as multidrug resistant strains according to MDR indexing. All were screening for the presence of MDR plasmids where three P. aeruginosa MDR strains from pus samples, were detected with four plasmids. Plasmids were identified by restriction mapping and this map of restriction is recognizing the sites within a particular plasmid.* ***Conclusions:*** *Plasmid mediated quinolone resistance genes have not been reported in the study region from P. aeruginosa isolates which indicates the need of analysis of antibiotic resistance phenotypes combined with plasmid profiling for curtailing the spread of resistance in these bacteria.*

Keywords: *Pseudomonas aeruginosa, quinolones,*

Introduction

Pseudomonas bacteremia is closely associated with significant morbidity and mortality up to 62% (Vidal *et al.,* 1996) and is of increasing trend in health care associated infections with multidrug resistant spectrum (Ng *et al.,* 2023). Sepsis is a potentially life threatening complications followed by multiorgan dysfunction (MOD) to multiorgan (MOF) failure and shock. The common manifestations of *Pseudomonas*

infections are pneumonia, urinary tract infections, surgical site infections and bacteremia with the prevalence of 7% among all healthcare associated infections (Magill *et al.,* 2014; Weiner *et al.,* 2016) and even higher in intensive care units (ICUs) with predilection of immune compromised patients (Bodey *et al.,* 1985).

P. aeruginosa can infect the susceptible hosts through various routes like contact and environmental contamination (Bedard *et al.,* 2016; Jefferies *et al.,* 2012; Pachori *et al.,* 2019). To understand the adaptability and high resistance characters, it can survive on extreme environment upto 6 months. The spreading of such healthcare associated infections can be avoided by decontaminating the fomites to reduce colonization and infection (Spagnolo *et al.,* 2021).

Among the possible reservoirs, *P. aeruginosa* have been spread through hospital water, a major source of healthcare-associated infections mainly through direct contact (bathing, washing, splashing of water), medical equipments cleaned with contaminated water and indirect contact through the hands of supportive health care workers and contaminated surfaces (Spagnolo *et al.,* 2016). It is believed that the antiseptics used for infection control is having its own antimicrobial properties, but upsurge of *Pseudomonas* growth still persisted (Cristina *et al.,* 2013).

P. aeruginosa exhibited high level of antibiotic resistance from both intrinsic and extrinsic environment and the frequencies depend on conferring genes and its transfer. The increase of MDR is due to the close association with mutations and other related transposans (Partridge *et al.,* 2018; Khaledi *et al.,* 2020). Further, treatment modalities with antibiotics narrowed due to the dearth of newer molecules (Bush and Page, 2017).

At the diagnostic level, testing the isolate for antibiotic sensitivity and resistance spectrum is questionable due to non-availability of newer generation antibiotics. Eventhough developed, the utilization may take long period of time, thus, testing and patient care is delayed. Practice of untested drugs in emergencies, inadequate and misuse of currently available antibiotics, course incompletion due to patient compliances and cross usage leads to variations in the gene expression profiles, sequence variations with the genes and mutation emergence. Identification of such molecular characters is crucial in the improving the modern healthcare practice. Even if the patient is symptomatically improved, the completion of antibiotic regimen is mandatory to prevent the resurgence of MDR *Pseudomonas* because when comparing with conventional methods of culture and sensitivity required 48 hours and above. The introduction of molecular diagnosis suggests the possibilities of point of care testing and reporting for quality health care (Lopez *et al.,* 2018).

As Pseudomonas strains have larger genomes (5 to 7 Mbp), the proportion of having regulatory genes and its related networks provide suitable survival to diverse and modified environment (Fimmersdorf *et al.,* 2010). There is a necessacity to evaluate the spread and contamination of *Pseudomonas* in hospital surfaces including clinical, laboratory and environmental wards, where the dissemination of resistant genes is higher (Fazeli *et al.,* 2012).

In natural environment, the survival and colonization of *Pseudomonas* is higher in burns and its related wards compared with other areas (Hurst and Sutter, 1966). While comparing with non hospital tap water, the hospital tap water provide suitable environment for survival, that leads to contamination of clinical equipments, cleaning clothes and surfaces (Hutchins *et al.,* 2017).

The emergence toward antibiotics arrived due to its multiple antibiotic resistance and tolerance phenotypes is high. Understanding the sudden modification in the bacterial phenotypes, it is important to avoid the recurrent and recalcitrant infections where a proper policy and algorithm required (Devin and Paul, 2021). Target site modification, enzymatic degradation, hyper expression of efflux pump genes can be accomplished higher in *Pseudomonas* conferring heteroresistance and adaptive mechanism (Jalal *et al.,* 2000).

The chemical composition of the antibiotics is disturbed due to multiple modifying enzymes coded with suitable mutational elements and plasmids harboring genes. Comparing with other bacterial pathogens, *Pseudomonas* has molecular existence by adopting with pH changes, antimicrobial exposure, anaerobic environments and starvation. Upregulation of efflux pumps, swarming motility and biofilm development provide adoption to the strains (Alford *et al.,* 2021).

Pseudomonas has one chromosomal DNA and one plasmid encoded with more than seven antimicrobial genes including carbapenem and fluoroquinolones resistant genes (Lin *et al.,* 2021). Finescaling the genomes to identify the genetic loci play a backbone for selecting antimicrobials for patient management and surface decontamination (Murray *et al.,* 2015). Changes in proteome will exploit the future antibiotics, and cytoplasmic modifications remain acquiring the task of antibiotic resistance (Pachori *et al.,* 2019).

Most of the times, the initiation of treatment regimen with broad spectrum antibiotics before analyzing sensitivity pattern lead to recovery but recurrent infection is more likely. Likewise, patient's non response to antibiotics renders the challenges in antibiotic selection. Thus, choosing 3rd generation antibiotics after getting sensitivity pattern of the isolates become mandatory. Emphasizing sterile techniques during invasive procedures also minimize the biofilm formation of superbugs and saving the effective antibiotics for future treatments (Sneha and Hemamalini, 2019).

The increasing incidence of antibiotic resistant genes has resulted due to increased usage of quinolones in parallel to increase in quinolone resistance. The first antibiotic resistance case was observed in Japan when it was identified as multidrug resistant during a single epidemic of diarrhea (Ramirez *et al.,* 2014). The new variations of plasmid molecules are accumulated the genetic determinants for visceral virulent factors and resistance.

The bacterial fitness for the environmental survival depends on the presence of plasmids, disseminating these genes among the most worrisome clinical pathogens. Among the plasmids, conjugative types are referred as the important drivers to develop antibiotic resistance strain groups in nosocomial pathogens which have pervasively happened. The microbiome with conjugated plasmids is largely present in the gut environment of the hospitalized patients leads to the unanswered resistance and colonization of resistome and plasmidome (Dib *et al.,* 2015).

Currently, the whole genome sequencing (WGS) databases are largely available that may help us to elucidate antibiotic resistance transmission routes. Bacterial cell have multiple plasmids; and a single plasmid has multiple replicons leading to increase in MDR (Johnson *et al.,* 2007). Various methods have been adopted like PCR, pulse field gel electrophoresis and DNA sequencing to detect the MDR genes. The RefSeq database of National Center of Biotechnology Information (NCBI) contains more than 8,000 complete plasmid sequences, and might be very much useful for the identification and comparison. With such a large and constantly growing database, it is conceivable to carry out quick monitoring for known (reference) plasmids rather than full-scale de novo assembly of plasmids in the sample (Roosaare *et al.,* 2018).

There are three types of information to identify plasmids

1. information about scaffold links and coverage in the WGS assembly
2. comparison to reference plasmid sequences
3. plasmid-diagnostic sequence features such as replication initiator proteins

While combining all three types of data for plasmid sequencing is important, focusing is only on using WGS assembly for plasmid reconstruction in a fully automatic fashion. Such plasmid sequencing efforts are important since many questions about plasmid function and evolution remain unanswered. To understand the contribution of *P. aeruginosa* in disseminating antibiotic resistance in clinical specimens, we have conducted a cross sectional study to screen and characterize MDR *P. aeruginosa* based on plasmid profiling.

Materials and Methods

Sample and isolation of *Pseudomonas aeruginosa*

This is a cross sectional study conducted in a tertiary care teaching hospital, Tiruchirapalli, India. A total of 157 *Pseudomonas aeruginosa* isolates were included in this study from January 2021 to December 2022. The study was started after getting approval from the institutional ethical committee (Ref: 1136/TSRMMCH&RC/ME-1/2023-IEC No: 112 dated 26.10.2023) and the samples were included as anonymous thus informed consent not required.

Bacteriological culturing, morphological (shape and gram reaction) and biochemical (H_2S generation, reduction of nitrates and fermentation of various carbohydrates) characteristics were determined for confirmation. All these identification methods were performed in Vitek2 Compact (Biomeriux, France) automated systems. In accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI), the antimicrobial susceptibility patterns were studied (CLSI, 2023).

Multiple drug resistance (MDR) indexing

P. aeruginosa were regarded as multiple drug-resistant (MDR) if any of them showed resistance to two or more antimicrobial drugs (Pitondo et al., 2014). MDR index for a single isolate was calculated as the following formula: "Number of antibiotics to which isolate is resistant (a)/Total number of antibiotics against which isolate was tested (b)" (Osundiya et al., 2013).

Whole-genome sequencing, assembly and annotation

DNA was isolated using the QIAamp DNA mini kit and quantified using Qubit as instructed by the manufacturer. Genome libraries with 450-bp insert size were prepared and sequenced on the Illumina platform with paired-end reads of 150-bp length. The data were assembled using the Spades assembler v.3.14 to generate contigs and annotated with Prokkav.1.5. Quality control of sequence data was performed using the GHRU quality control (QC) pipeline based on (i) the basic statistics of raw reads; (ii) the assembly statistics; (iii) contamination due to SNV and sequences from different species; (iv) species prediction using Bactinspector; and (v) overall QC as pass, warning, or fail of each isolate based on these different parameters as described in the pipeline.

Antimicrobial resistance determinants and virulence genes

Genome data were analyzed for the presence of virulence genes using the Virulence Factor Database. Resfinder was used to identify acquired AMR genes in the genomes using the GHRU Resfinder pipeline and mutations using the GHRU AMR pipeline with the PointFinder database.

Genotyping

The other genotypic characters were obtained by uploading the assembled genomes into the pathogen.watch and performing species-specific analysis. The MLST results, cgMLST, and Plasmid mapping and typing results were downloaded from the pathogen.watch by creating a collection of the study isolates.

Results

A total of 157 *P. aeruginosa* isolates were possible during the study period, among them 10 isolates (1 from sputum and 9 from pus) were determined as multidrug resistant strains according to MDR indexing (Osundiya et al., 2013). No plasmids identified from the sputum sample where as four plasmids identified from 3 pus samples. The breakdown determination of *P. aeruginosa* plasmid in this study was framed and presented in the figure 1.

All *P. aeruginosa* strains isolated were phenotypically confirmed and *in vitro* antimicrobial susceptibility screening was done in the automated machine there by 10 strains were grouped under the criteria of MDR. The detailed antibiotic susceptibility pattern of the MDR *P. aeruginosa* isolates were depicted in table 1.

Among the three *Pseudomonas aeruginosa* MDR strains from pus samples, there was possible to identify four plasmids. Among the plasmid containing *P. aeuruginosa* strains, the antibiotic susceptibility pattern showed maximum resistance towards various antibiotics, where all the three isolates were resistant to tigecycline. The detailed susceptibility pattern with number of isolates was interpreted in table 2.

A plasmid mapping is referred as a graphical representation, which representing various locations of major elements or landmarks of the plasmid. The comparative spots of elements within the plasmid are identified by restriction mapping and this map of restriction is recognizing the sites within a particular plasmid. The restriction maps of plasmids derived from MDR *P. aeruginosa* are shown in figures 2, 3, 4 and 5.

Discussion

Pseudomonas aeruginosa is a most frequent and severe causes of hospital-acquired infections, particularly affecting immunocompromised (especially neutropenic) and intensive care unit (ICU) patients (Spagnolo *et al*., 2021). Open wound infection is a serious problem especially with extended-spectrum beta lactamase (ESBL) producing Gram negative bacteria such as *P. aeruginosa* (Uyanga and Ibanga, 2019).

P. aeruginosa can be transmitted by a number of routes, including patient-to-patient and environmental contamination (Jefferies *et al*., 2012; Bedard *et al*., 2016). Due to its adaptable nature and high surviving ability, it can survive on dry inanimate surfaces in a hospital environment from 6 h to 6 months (Pachori *et al*., 2019). A further means of spreading infections that cannot be ignored is the hands of operators which can be contaminated following contact with a colonized or infected patient, or after using contaminated soap, cream or water (Spagnolo *et al.,* 2021).

The pressure of antibiotics has led to the rapid development of bacterial resistance. Among these antibiotics, Fluoroquinolones are some of the most commonly prescribed effective antimicrobials against *P. aeruginosa* infections where the drug resistance and drug failure observed (Yang *et al.,* 2015) and the same reflected in this study also. Therefore, infection control committee of the healthcare setting monitors the antimicrobial susceptibility which is very crucial to select effective antimicrobial agents in the treatment and reduce the antibiotic resistance (Jones *et al.,* 2001; Linder *et al.,* 2005).

The genetic repertoire of *P. aeruginosa* reflects the adaptive character of this bacterial species. The metabolic versatility is provided by genes encoding enzymes that participate in the metabolic pathways, transcriptional regulators, and regulatory systems (Sommer *et al.,* 2020). In addition, it is estimated that 150 of the genes identified in *P. aeruginosa* PAO1 encode outer membrane proteins related to adhesion, movement, antibiotics, and virulence factor output which represents a much higher number when compared to other genomes (Chevalier *et al*., 2017). In this study also, we identified the genomes that encoded with antibiotic resistant genes and enzymes.

P. aeruginosa is having mosaic genome that is composed of many core genes interspersed by strain-specific blocks of genes. While comparing the genomics within the species, *P. aeruginosa* divided into three groups that are depending on its characteristics including core, accessory and pan-genome. The core genome is present in some strains of *P. aeruginosa* that contain 90% of the whole genome, but absent in other strains of the same species. The accessory genome of *P. aeruginosa* consists of non-conserved, variable-length stretches of DNA, generally located in extrachromosomal elements, and blocks of inserted DNA in certain loci. The pan-genome can be represented as a circular chromosome with polymorphic strain-specific segments,

flanked by conserved genes referred to as anchors (Valot *et al.,* 2015; Chevalier *et al*., 2017; Freschi *et al.,* 2018; Freschi *et al*., 2019; Sommer *et al.,* 2020;)

Many studies demonstrated the presence of Extended Spectrum Beta Lactamase genes (bla CTX-M, bla SHV and bla TEM) and Carbapenemase genes (bla KPC and blaNDM) were from clinical isolates of *P. aeruginosa* with special observation against β-lactams, aminoglycosides, fluoroquinolones, sulfonamide, tetracycline, phenicol and fosfomycin (Akinloye *et al*., 2021; Ahmed, 2022). Additionally, the coexistence with occasional or new integrons with other gene cascades is conferring resistance towards fluoroquinolones, rifampicin, trimethoprim and β-lactam (Ana *et al*., 2021).

A restriction map was constructed for the plasmid for identifying the apparent differences and the two plasmid hybridization. Genotypic detection of resistance determinants revealed that all isolates were predicted to encode numerous AMR genes associated with resistance to aminoglycosides, fluoroquinolones, fosfomycin, sulfonamides and tetracyclines.

In *P. aeruginosa*, the natural expression of efflux pumps plays vital role in the increasing resistance to antibiotics further serious therapeutic challenges and threat associated with the chromosomally-encoded resistance mechanisms. When resistance emerges during therapy, clinical failure can occur and the therapeutic options for second-line therapy can become severely limited. To improve the patient outcome and survival, the Clinicians must be mindful of this threat when choosing an appropriate therapy by combinational drugs.

Since the standard combination of fluroquinolones and beta-lactam has been shown to be effective in preventing the emergence of some resistance problems, the search for more effective combinations must be a priority, but currently more resitance towards the combination occurs. In addition, genomic sequencing deep-rooted the presence of mutational imprints in antimicrobial resistance-associated genes are closely associated to *P. aeruginosa*. As common, some mutations observed in quinolone resistance determining regions detected which have been related to fluoroquinolone resistance and also efflux pump systems' overexpression (Subedi *et al.*, 2018; Do Nascimento *et al*., 2020).

Conclusion

This study confirmed the fact that the acquisition of antibiotic resistant genes (ARGs) depends on the resistance of *Pseudomonas* to antibiotics including fluoroquinolones, ie, the least resistant strain to antibiotics had the lowest acquisition of ARGs, while the most resistant strain to antibiotics had the highest acquisition of ARGs. In this study, MDR-*P. aeruginosa* showed evidence that ARGs are being transferred through plasmids.

Limitations

Plasmid map is made by programs available in gene banks; thus annotate everything, everywhere and ever overlapping data. Confirmation of the mapped plasmids may be done by comparing the sequences with commercially available reference plasmids for determining the similarities, variations and novelty.

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References

1. *Ahmed, O.B. (2022). Detection of antibiotic resistance genes in Pseudomonas aeruginosa by whole genome sequencing. Infection and Drug Resistance 15: 6703-6709. www.ncbi.nlm.nih.gov*

2. *Akinloye, A.O., Adefioye, J.O., Adekunle, C.O., Anomneze, B.U., Makanjuola, O.B., Onaolapo, O.J., Onaolapo, A.Y. and Olowe, O.A. (2021). Multidrug-Resistance Genes in Pseudomonas aeruginosa from Wound Infections in a Tertiary Health Institution in Osogbo, Nigeria. Infectious Disorders Drug Targets 21: 90-98. (www. pubmed.ncbi.nlm.nih.gov).*

3. *Alford, M.A., Baquir, B., An, A., Choi, K.G. and Hancock, REW. (2021). NtrBC selectively regulates host-pathogen interactions, virulence, and ciprofloxacin susceptibility of Pseudomonas aeruginosa. Frontiers in Cellular and Infection Microbiology 11: 694789. (www. pubmed.ncbi.nlm.nih.gov)*

4. *Ana, M.R., Elsa, D.L.C., Carlos, A.A., Christian, P., Eliana, R., Adriana, C., Maria, V.V. and Cesar, C. (2021). Genetic diversity of multidrug resistant Pseudomonas aeruginosa isolates carrying bla_{VIM-2} and bla_{KPC-2} genes that spread on different genetic environment in Colombia. Frontiers in Microbiology 12: Article 663020. (www.pubmed.ncbi.nlm.nih.gov)*

5. *Bedard, E., Prevost, M. and Deziel, E. (2016). Pseudomonas aeruginosa in premise plumbing of large buildings. Microbiology Open 5: 937-956. (www. pubmed.ncbi.nlm.nih.gov)*
6. *Bodey, GP, Jadeja, L, Elting, L. (1985). Pseudomonas bacteremia - retrospective analysis of 410 episodes. Archives in Internal Medicine 145: 1621-1629. (www. pubmed.ncbi.nlm.nih.gov)*

7. *Bush, K. and Page, MGP. (2017). What we may expect from novel antibacterial agents in the pipeline with respect to resistance and pharmacodynamic principles. Journal of Pharmacokinetics and Pharmacodynamics 44: 113-132. (www. pubmed.ncbi.nlm.nih.gov)*

8. *Chevalier, S., Bouffartigues, E., Bodilis, J., Maillot, O., Lesouhaitier, O., Feuilloley, M.G., Orange, N., Dufour, A. and Cornelis, P. (2017). Structure, function and regulation of Pseudomonas aeruginosa porins. FEMS Microbiology Reviews 41: 698-722. (www.pubmed.ncbi.nlm.nih.gov)*

9. *Clinical and Laboratory Standards Institute. Performance standards for Antimicrobial susceptibility testing (CLSI M100Ed33); 33rd ed. 2023; 402. (www.clsi.org)*

10. *Cristina, M.L., Spagnolo, A.M., Orlando, P. and Perdelli, F. (2013). The role of the environment in the spread of emerging pathogens in at-risk hospital wards. Reviews in Medical Microbiology 24: 104-112. (www. journals.lww.com)*

11. *Devin, S. and Paul, S. (2021). The many antibiotic resistance and tolerance strategies of Pseudomonas aeruginosa. Biofilm 2: 100056. (www.sciencedirect.com)*

12. *Dib, J.R., Martin, W., Maria, E.F. and Friedhelm, M. (2015). Strategies and approaches in plasmidome studies – uncovering plasmid diversity disregarding of linear elements? Frontiers in Microbiology 6: 463-474. (www.pubmed.ncbi.nlm.nih.gov)*

13. *Fazeli, H., Akbari, R., Moghim, S., Narimani, T., Arabestani, M.R. and Ghoddousi, A.R. (2012). Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. Journal of Research in Medical Sciences 2012; 17: 332-337. (www. pubmed.ncbi.nlm.nih.gov)*

14. *Freschi L., Bertelli C., Jeukens J., Moore M.P., Kukavica I.I., Emond R.J.G., Hamel J., Fothergill J.L., Tucker N.P. and McClean S. (2018). Genomic characterisation of an international Pseudomonas aeruginosa reference panel indicates that the two major groups draw upon distinct mobile gene pools. FEMS Microbiology Letter 365: 120-128. (www. pubmed.ncbi.nlm.nih.gov).*

15. *Freschi, L., Vincent, A.T., Jeukens, J., Emond, R.J.G., Kukavica, II., Dupont, M.J., Charette, S.J., Boyle, B. and Levesque, R.C. (2019). The Pseudomonas aeruginosa Pan-Genome Provides New Insights on Its Population Structure, Horizontal Gene Transfer, and Pathogenicity. Genome Biology and Evolution 11: 109-120. (www. pubmed.ncbi.nlm.nih.gov).*

16. *Frimmersdorf, E., Horatzek, S., Pelnikevich, A., Wiehlmann, L. and Schomburg, D. (2010). How Pseudomonas aeruginosa adapts to various environments: a metabolomic approach. Environmental Microbiology 12: 1734-1747. (www. pubmed.ncbi.nlm.nih.gov).*

17. *Hurst, V. and Sutter, V.L. (1966). Survival of Pseudomonas aeruginosa in the hospital environment. Journal of Infectious Diseases 116: 151-154. (www.jstor.org).*

18. *Hutchins, C.F., Moore, G., Thompson, K.A., Webb, J. and Walker, J.T. (2017). Contamination of hospital tap water: the survival and persistence of Pseudomonas aeruginosa on conventional and antimicrobial outlet fittings. Journal of Hospital Infection 2017; 97: 156-161. (www.sciencedirect.com).*

19. *Jalal, S., Ciofu, O., Hoiby, N., Gotoh, N. and Wretlind, B. (2000). Molecular mechanisms of fluoroquinolone resistance in Pseudomonas aeruginosa isolates from cystic fibrosis patients. Antimicrobial Agents and Chemotherapy 44: 710-712. (www.ncbi.nlm.nih.gov)*

20. *Jefferies, J.M.C., Cooper, T., Yam, T. and Clarke, S.C. (2012). Pseudomonas aeruginosa outbreaks in the neonatal intensive care unit – a systematic review of risk factors and environmental sources. Journal of Medical Microbiology 61: 1052-1061. (www. pubmed.ncbi.nlm.nih.gov)*

21. *Johnson, T.J., Wannemuehler, Y.M., Johnson, S.J., Logue, C.M., White, D.G. and Doetkott, C. (2007). Plasmid replicon typing of commensal and pathogenic Escherichia coli isolates. Applied Environmental Microbiology 73: 1976-1983. (www.pubmed.ncbi.nlm.nih.gov)*

22. *Jones, R.N., Beach, M.L. and Pfaller, M.A. (2001). Spectrum and activity of three contemporary fluoroquinolones tested against Pseudomonas aeruginosa isolates from urinary tract infections in the SENTRY Antimicrobial Surveillance Program (Europe and the Americas; 2000): More alike than different! Diagnostic Microbiology and Infectious Diseases 41:161-163. (www. pubmed.ncbi.nlm.nih.gov).*

23. *Khaledi, A., Weimann, A., Schniederjans, M., Asgari, E., Kuo, T.H., Oliver, A., Cabot, G., Kola, A., Gastmeier, P., Hogardt, M., Jonas, D., Mofrad, M.R., Bremges, A., McHardy, A.C. and Haussler, S. (2020). Predicting antimicrobial resistance in Pseudomonas aeruginosa with machine learning-enabled molecular diagnostics. EMBO Molecular Medicine 12: e10264.*

24. *Lin, J.W., Zhong, C.E., Ling, Y., Dong, H.F., Zhenbo, X. and Ding, Q.C. (2021). Emergence of clinical Pseudomonas aeruginosa isolate Guangzhou-PaeC79 carrying crpP, bla_{GES-5}, and bla_{KPC-2} in Guangzhou of China. Microbial Drug Resistance 27: 965-970. (www. pubmed.ncbi.nlm.nih.gov).*

25. *Linder, J.A., Huang, E.S., Steinman, M.A., Gonzales, R. and Stafford, R.S. (2005). Fluoroquinolone prescribing in the United States: 1995 to 2002. American Journal of Medicine 118: 259-268. (www.pubmed.ncbi.nlm.nih.gov).*

26. *Lppez, C.C., Sommer, L.M., Cabot, G., Rubio, R., Ocampo, S.A.A., Johansen, H.K., Figuerola, J., Canton, R., Kidd, T.J. and Molin, S. (2017). Evolution of the Pseudomonas aeruginosa mutational resistome in an international Cystic Fibrosis clone. Science Reports 7: 5555. (www.ncbi.nlm.nih.gov).*

27. *Magill, S.S., Edwards, J.R., Bamberg, W., Beldavs, Z.G., Dumyati, G., Kainer, M.A., Lynfield, R., Maloney, M., McAllister, H.L. and Nadle, J. (2014). Emerging infections program healthcare-associated infections and antimicrobial use prevalence survey team - multistate point-prevalence survey of health care-associated infections. New England Journal of Medicine 370: 1198-1208. (www.pubmed.ncbi.nlm.nih.gov).*

28. *Murray, J.L., Kwon, T., Marcotte, E.M. and Whiteley, M. (2015). Intrinsic antimicrobial resistance determinants in the superbug Pseudomonas aeruginosa. mBio 6: e01603-15.*
29. *Nascimento, A.P.B., Medeiros, F.F., Pauer, H., Antunes, L., Sousa, H., Senger, H., Albano, R.M., Santos, M.T.D., Ana P.C.A. and Fabricio ABS. (2020). Characterization of a SPM-1 metallo-betalactamase-producing Pseudomonas aeruginosa by comparative genomics and phenotypic analysis. Scientific Reports 10:13192. (www. pubmed.ncbi.nlm.nih.gov).*

30. *Ng, Q.X., Ong, N.Y., Lee, D.Y.X., Yau, C.E., Lim, Y.L., Kwa, A.L.H. and Tan, B.H. (2023). Trends in Pseudomonas aeruginosa (P. aeruginosa) Bacteremia during the COVID-19 Pandemic: a systematic review. Antibiotics (Basel) 12: 409. (www.ncbi.nlm.nih.gov)*

31. *Osundiya, O.O., Oladele, R.O. and Oduyebo, O.O. (2013). Multiple antibiotic resistance (MAR) indices of Pseudomonas and Klebsiella species isolates in Lagos University Teaching Hospital. African Journal of Clinical and Experimental Microbiology 14: 164-168. (www.ajol.info).*

32. *Pachori, P., Gothalwal, R. and Gandhi, P. (2019). Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes and Diseases 2019; 6: 109-119. (www. pubmed.ncbi.nlm.nih.gov)*

33. *Partridge, S.R., Kwong, S.M., Firth, N. and Jensen, S.O. (2018). Mobile genetic elements associated with antimicrobial resistance. Clinical Microbiology Reviews 31: e00088-17. (www.ncbi.nlm.nih.gov)*

34. *Pitondo, S.A., Martins, V.V., Fernandes, A.F. and Stehling, E.G. (2014). High level of resistance to Aztreonam and Ticarcillin in Pseudomonas aeruginosa isolated from soil of different crops in Brazil. The Science of the Total Environment 473: 155-158. (www. pubmed.ncbi.nlm.nih.gov).*

35. *Ramirez, M.S., Traglia, G.M., Lin, D.L., Tran, T. and Tolmasky, M.E. (2014). Plasmid-mediated antibiotic resistance and virulence in Gram-negatives: the Klebsiella pneumoniae Paradigm. Microbiology Spectrum 2: 1-15. (www. pubmed.ncbi.nlm.nih.gov).*

36. *Roosaare, M., Puustusmaa, M., Mols, M., Vaher, M. and Remm, M. (2018). PlasmidSeeker: identification of known plasmids from bacterial whole genome sequencing reads. Peer J 2018; 6: e4588. (www. pubmed.ncbi.nlm.nih.gov)*

37. *Sneha, S.S. and Hemamalini, G. (2019). Study of Antibiotic Sensitivity and Resistance Pattern of Bacterial Isolates in Intensive Care Unit Setup of a Tertiary Care Hospital. Indian Journal of Critical Care Medicine 23: 547-555. (www.pubmed.ncbi.nlm.nih.gov)*

38. *Sommer, L.M., Johansen, H.K. and Molin, S. (2020). Antibiotic resistance in Pseudomonas aeruginosa and adaptation to complex dynamic environments. Microbial Genomics 6: e000370.*

39. *Spagnolo, A.M., Orlando, P., Perdelli, F. and Cristina, M.L. (2016). Hospital water and prevention of waterborne infections. Reviews in Medical Microbiology 27: 25-32. (www. journals.lww.com)*

40. *Spagnolo, A.M., Sartini, M. and Cristina, M.L. (2021). Pseudomonas aeruginosa in the healthcare facility setting. Reviews in Medical Microbiology 32: 169-175. (journals.lww.com).*

41. *Subedi, D., Vijay, A.K., Kohli, G.S., Rice, S.A., and Willcox, M. (2018). Association between possession of ExoU and antibiotic resistance in Pseudomonas aeruginosa. PloS ONE 13: e0204936. (www. journals.plos.org)*

42. *Uyanga, F.Z. and Ibanga, I.A. (2019). Plasmid profile analysis of Pseudomonas aeruginosa isolated from wound infections in a general hospital in Southern Nigeria. Journal of Advances in Microbiology 18: 1-7. (www. journaljamb.com).*

43. *Valot, B., Guyeux, C., Rolland, J.Y., Mazouzi, K., Bertrand, X. and Hocquet, D. (2015). What It Takes to Be a Pseudomonas aeruginosa? The Core Genome of the Opportunistic Pathogen Updated. PLoS ONE 10: e0126468. (www.ncbi.nlm.nih.gov)*

44. *Vidal, F., Mensa, J., Almela, M., Martinez, J.A., Marco, F., Casals, C., Gatell, J.M., Soriano, E. and Jimenez de Anta, M.T. (1996). Epidemiology and outcome of Pseudomonas aeruginosa bacteremia, with special emphasis on the influence of antibiotic treatment - analysis of 189 episodes. Archives in Internal Medicine 156: 2121-2126. (www. pubmed.ncbi.nlm.nih.gov)*

45. *Weiner, L.M., Webb, A.K., Limbago, B., Dudeck, M.A., Patel, J., Kallen, A.J., Edwards, J.R. and Sievert, D.M. (2016). Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the national healthcare safety network at the centers for disease control and*

prevention, 2011–2014. Infection Control Hospital Epidemiology 37: 1288-1301. (www.pubmed.ncbi.nlm.nih.gov)

46. *Yang, X., Xing, B., Liang, C., Ye, Z. and Zhang, Y. (2015). Prevalence and fluoroquinolone resistance of Pseudomonas aeruginosa in a hospital of South China. International Journal of Clinical Experimental Medicine 8: 1386-1390. (www.ncbi.nlm.nih.gov).*

Table 1: Antibiotic susceptible pattern of MDR *Pseudomonas aeruginosa*

Antibiotics	**Susceptible pattern (n=10 isolates)**		
	Sensitive	**Resistant**	**Intermediate- sensitivity**
Amikacin (AMK)	3	7	0
Ceftazidime (CAZ)	2	8	0
Ciprofloxacin (CIP)	2	8	0
Colistin (COL)	10	0	0
Cefoperazone – sulbactum (CSL)	3	4	3
Doripenem (DRPM)	3	7	0
Cefipime (FEP)	3	7	0
Gentamycin (GEN)	2	7	1
Imipenem (IPM)	5	5	0
Levofloxacin (LVX)	6	4	0
Meropenem (MPN)	5	5	0
Tigecycline (TGC)	0	10	0

Table 2: Antibiotic susceptible pattern of MDR *Pseudomonas aeruginosa* with plasmids

Antibiotics	**Susceptible pattern (n= 3 isolates)**		
	Sensitive	**Resistant**	**Intermediate- sensitivity**
Amikacin (AMK)	1	2	0
Ceftazidime (CAZ)	2	1	0
Ciprofloxacin (CIP)	1	2	0
Colistin (COL)	3	0	0
Cefoperazone – sulbactum (CSL)	0	2	1
Doripenem (DRPM)	1	2	0
Cefipime (FEP)	1	2	0
Gentamycin (GEN)	0	2	1
Imipenem (IPM)	1	2	0
Levofloxacin (LVX)	1	2	0
Meropenem (MPN)	0	1	2
Tigecycline (TGC)	0	3	0

Figure 1: Breakdown of *Pseudomonas aeruginosa* plasmid determination

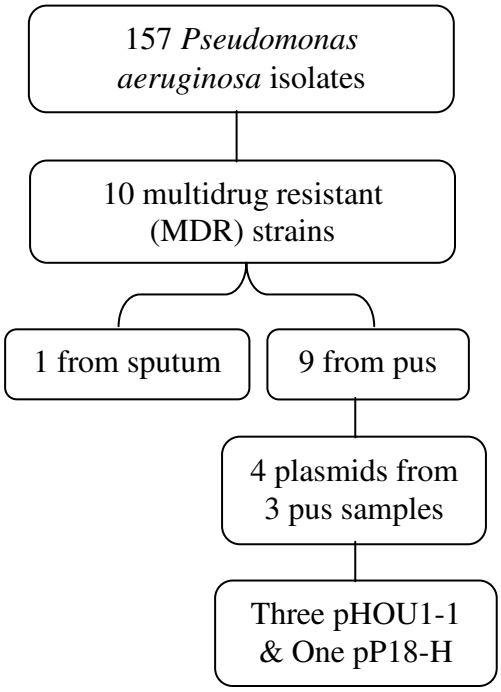

Figure 2: Plasmid map of pHOU1-1 indicated *P. aeruginosa* CpxR with ciprofloxacin resistance

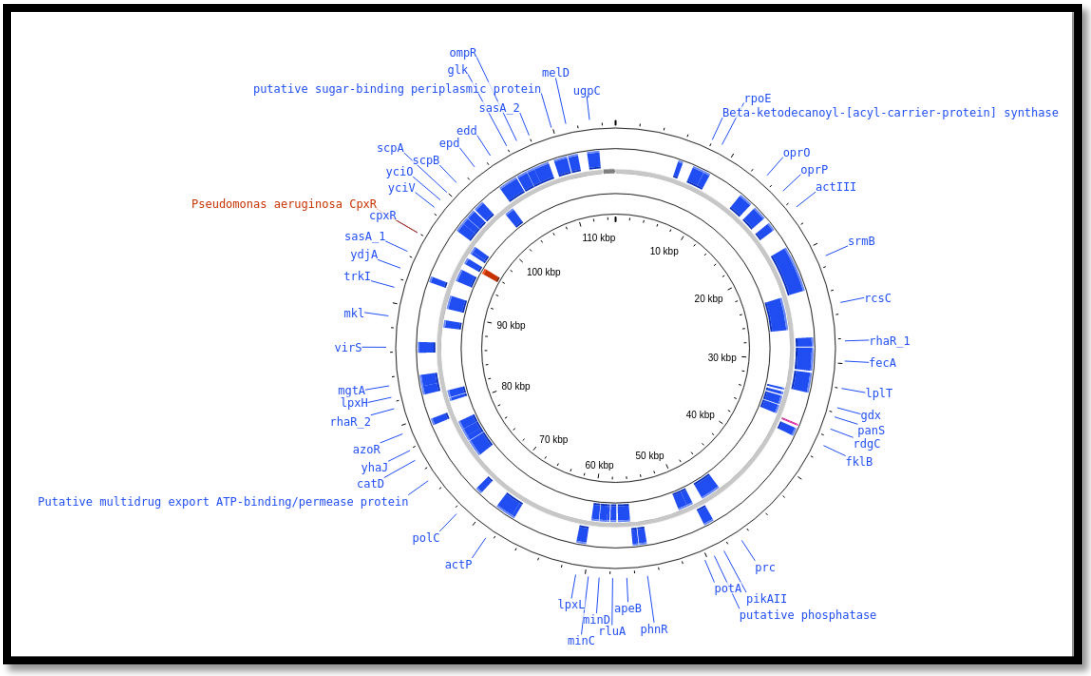

Figure 3: Plasmid map of pP18-H indicated *P. aeruginosa* with tetracycline resistance

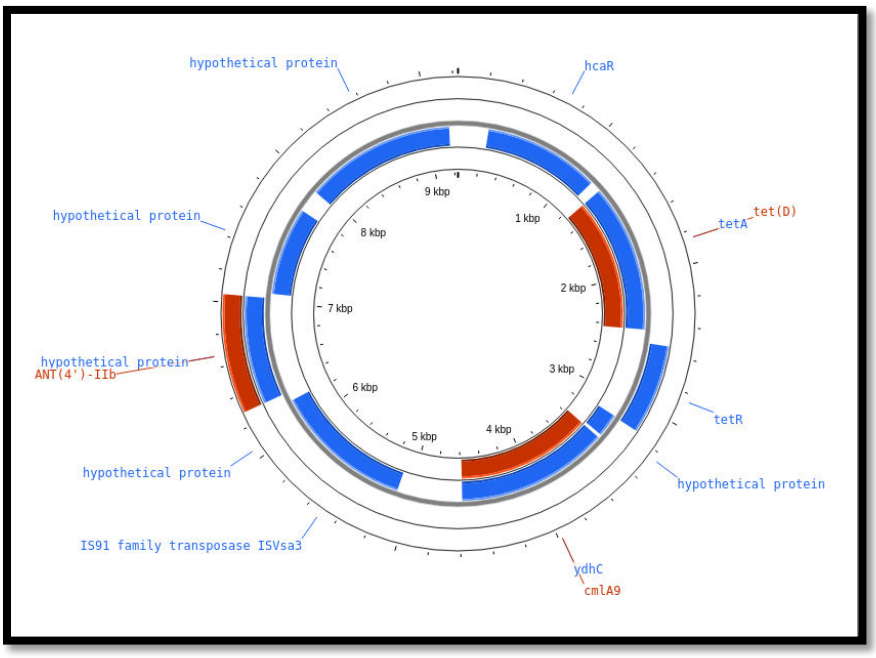

Figure 4: Plasmid map of pHOU1-1 indicated *P. aeruginosa* gyrA with fluoroquinolones resistance

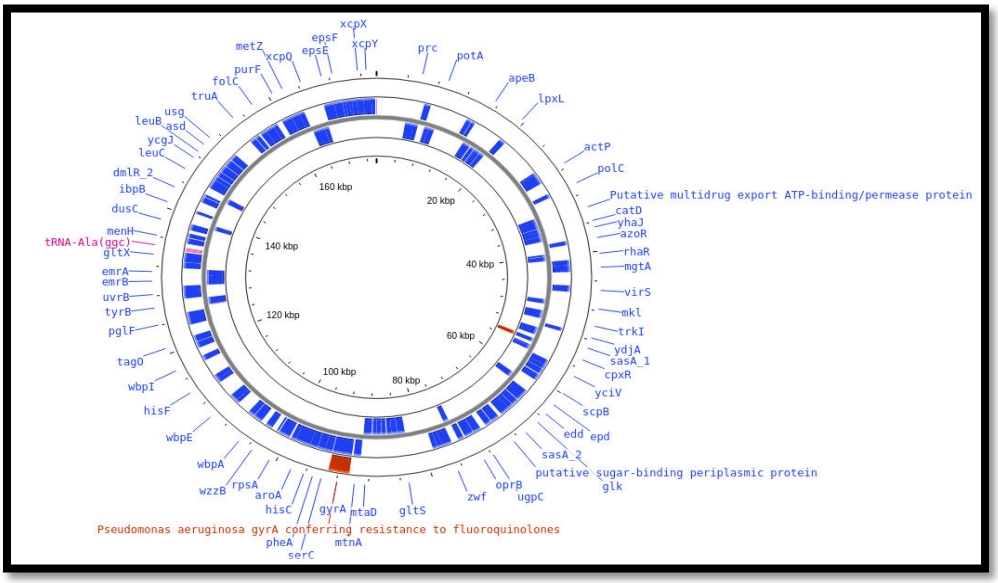

Figure 5: Plasmid map of pHOU1-1 indicated *P. aeruginosa* gyrA with fluoroquinoles resistance