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Molecular Profiling of Multidrug-Resistant *Pseudomonas aeruginosa* Isolated from Health Care Settings for Targeted Antibiotic Development

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Abstract:

Multidrug-resistant *Pseudomonas aeruginosa* poses a significant public health challenge, particularly in healthcare settings, owing to its ability to evade multiple antibiotics. This study focused on the molecular profile of *P. aeruginosa* isolates to identify their resistance mechanisms and explore potential therapeutic targets. To characterize *P. aeruginosa,* its molecular diversity, multidrug resistance, genetic factors, and drug targets for new therapies. In total of 20 clinical samples were obtained, including *P. aeruginosa* (n=14), from three healthcare centers in Lahore, Pakistan. Samples underwent MDR assessment via Kirby-Bauer disc diffusion. gyrA/parC genes were detected by PCR, sequenced. Natural compounds docked to study binding. Following 14 identifies *P. aeruginosa* isolates, 28 % (n=4) isolates (NF12, NF22, NF24, and NF84) showed MDR characteristics to fluoroquinolones, carbapenems, and colistin, confirmation by 16S rRNA gene sequencing. PCR and sequencing identified gyrA and parC genes conferring fluoroquinolone resistance. Phylogenetic analysis revealed close clustering with *P. aeruginosa*. Berberine Hydroxide strongly bound GyrA and ParC (–6.6, –6.8 kcal/mol), while Curcumin had moderate binding, inhibiting resistant strains. This study highlights multidrug-resistant *P. aeruginosa* and shows strong binding potential with berberine hydroxide. Future research should develop effective therapies to address clinical antibiotic resistance.

Keywords: *Pseudomonas aeruginosa*, multidrug resistant, molecular profiling, berberine hydroxide, curcumin.

1.0 Introduction:

Pseudomonas aeruginosa belonging to the family Pseudomonadaceae while gram-negative, rod-shaped, non-spore-forming characteristics (Urganci et al., 2022). This bacterium is a common opportunistic pathogen that causes potentially harmful infections (De Sousa et al.,

2021). This microorganism is extraordinarily difficult to eradicate, appearing in hospital settings and natural habitats, and even in disinfectants, due to its unique physiological and biochemical characteristics (Crone et al., 2020).

The bacterium is motile, exhibiting polar flagella, and therefore, capable of chemotaxis (Xu et al., 2024). *P. aeruginosa* has the distinctive ability to produce well-known pigments such as pyocyanin (blue-green) and pyoverdine (yellow-green) (Abdelaziz et al., 2023). These pigments enable microbes to defend themselves and have significant effects on the degree of capability of *P. aeruginosa*. Pyocyanin is known to induce oxidative stress and immune responses in the host (Andrajevic et al ., 2023).

Pseudomonas is known to colonize and infect humans with necrotizing skin infections (Spernovasilis, Psichogiou, & Poulakou, 2021). Immunocompromised individuals are prone to this infection. These infections stimulate various immune signaling molecules that try to rid the body of these microbes above normal human body temperatures; these infections tend to colonize at 37- 42 ℃ (Best et al., 2020). Being an obligate aerobe capable of using nitrate as an alternative electron acceptor, *P. aeruginosa* grows on many media types, including nutrient agar, MacConkey, and cetrimide agars; the latter is selective for its isolation (Inkster et al., 2024).

Resource-limited environments pose challenges for sustaining life, but *P. aeruginosa* metabolic flexibility is one of the reasons why bacteria are capable of persisting in such conditions. Reported on the ability of *P. aeruginosa* to produce racially diverse organic compounds (Szklarczyk et al., 2021). Moreover, the bacterium's extracellular arsenal is extensive, comprising numerous extracellular enzymes, such as proteases, lipases, and elastases, which facilitate tissue destruction, nutrient harvesting, and immune response evasion (Heywood and lamton, 2020).

Pseudomonas aeruginosa has a broad range of virulence factors the organism is capable of causing infections (Seder et al., 2023). Exotoxin A is one of those who cause infections and it inhibits protein synthesis in host cells by the process of ADP-ribosylation. *P. aeruginosa* biofilms are composed of dense bacterial communities enclosed in an extracellular polymeric substance. (Yin et al., 2022) They provide protection against antibiotics and host defense. Biofilms are central to chronic infections, particularly in individuals with weakened immune systems.

Surgical site infections, ventilator pneumonia, bloodstream infections and Urinary tract infections are the most frequent hospital acquired infections that owing to *P. aeruginosa* (Abdalla et al., 2023). The pathogen is especially dangerous to some of the most vulnerable patients located in intensive care units, cancer wards, or those undergoing organ transplants. The risk of infection is also increased by mechanical ventilation, indwelling catheters including broad-spectrum antibiotics.

Many infections have also progressed to multidrug-resistant (MDR) phenotypes and even more serious extensively drug-resistant (XDR) phenotypes of *P. aeruginosa* (Almutairy, 2024). Resistance is often mediated through mobile genetic elements like integrons, transposons, and plasmids that allow horizontal gene transfer and broad-based circulation of resistance capabilities (del Barrio-Tofiño, López-Causapé and Oliver, 2020), (Gharakhyli *et al.*, 2023). Furthermore, the clonal expansion of high-risk strains ST235 and ST111 deepened the infection control Novick's problem.

These strains are resistant to multiple common antibiotics, even extending to last resort carbapenems (Santon et al., 2022). Additionally, self-protective mechanisms such as reduced outer membrane permeability, overactive efflux pumps, and β-lactamase production serve as standard treatments. The resistance of MDR *P. aeruginosa* to systematically treatable infections posed severe clinical hurdles (Elfadadny et al., 2024). Combination therapy commonly includes a combined antibiotic approach, but resistance to all categories, including colistin and tigecycline, has been documented (Santon et al., 2022). Strengthening antibiotic resistance requires immediate action through the development of new strategies including precision antibiotics and molecular profiling for tailored interventions.

Pseudomonas aeruginosa is an exceptional challenge posed as a pathogen due to the mechanisms it employs for virulence and resistance alongside incompatible adaptability to diverse environments (Hussain et al., 2023). Multifaceted intervention approaches incorporating advanced infection control procedures, heightened monitoring, and proactive innovative treatment research are essential to mitigate this problem.

1.1 Knowledge Gaps

The rise of multidrug-resistant *P. aeruginosa* infections in hospitals constrains a new approach. This project proposes using molecular profiling to understand the resistance

mechanisms of these bacteria, paving the way for targeted antibiotic development.

1.2 Questions:

How does the molecular profiling of multidrug-resistant Pseudomonas aeruginosa isolates contribute to identifying novel therapeutic targets and guiding effective antibiotic strategies in healthcare-associated infections?

1.3 Hypothesis:

Null Hypothesis (H_0)

There is no association between the molecular profile of *Pseudomonas aeruginosa* isolates from healthcare settings and their resistance to antibiotics.

There are no novel genetic determinants associated with multidrug resistance in Pseudomonas aeruginosa.

Alternate Hypothesis (H_1):

The molecular profile of Pseudomonas aeruginosa isolates from healthcare settings is associated with their resistance to antibiotics.

Molecular profiling will identify novel genetic determinants associated with multidrug resistance in Pseudomonas aeruginosa.

1.4 Objectives of study

The following were the study's objectives:

- To characterize the molecular diversity and antibiotic resistance profiles of ***P. aeruginosa*** isolates from health-care settings.
- To identify novel genetic determinants associated with multidrug resistance in ***P. aeruginosa*.**
- To explore potential drug targets for the development of precision therapies against multidrug-resistant ***P. aeruginosa*** strains.

2.0 Methodology:

This study had a cross-sectional design and was conducted from May 2024 to January 2025. This study was conducted at the Microbiology Department of the University of Central Punjab, Lahore. An aseptic technique was used for the sample collection. After collection of samples, inoculation was proceeded on MacConkey and blood agar. The plates were stored in 37°C incubator for 24 hours after labelling and inoculation. After incubation, the plates were examined for microbial growth.

Preliminary identifications were made using colony morphology, Gram staining, and biochemical tests. *P. aeruginosa* produced irregular colonies as large as 1 cm on Blood agar, which underwent beta-hemolysis. It appeared colorless and circular on MacConkey agar, indicating that it did not ferment lactose. *P. aeruginosa* gave nutrient agar a greenish tint with a fruity smell because of the substances pyocyanin and pyoverdin. Bacteria were differentiated by Gram staining.

For the rapid biochemical identification of *P. aeruginosa*, API (Analytical Profile Index)10S by bioMérieux for identification of gram-negative bacteria were used. API strip was incubated at 37°C for 18-24 hours after addition of bacterial growth and initial buffers. Subsequently color changes were submitted to the API web database for confirmation. Using this method, the identification of the isolate was accurate and was attained in a matter of hours.

Genomic DNA from MDR isolates NF12, NF22, NF24, and NF84 was purified using German Thermo-Fisher DNA extraction kit. The 16S rRNA, gyrA, and parC genes were PCR amplified using species-specific primers. During the PCR cycles, denaturation is performed for 1 min at 94 °C, while annealing is carried out at temperature of 55 °C for 1 min, extension step is performed at 72 °C for 2 min time, and amplification then occurs for 35 cycles, and finally, an elongation of 72 °C for 10 min is performed. The 2,000 base pairs amplicons were analyzed in 1.5 percentage agarose gels that had been stained with Ethidium Bromide. Sequencing is done by Macrogen (Seoul, Korea) kit pack while analyzed with the BLASTn online program and submitted to GenBank.

Phylogenetic study of Pseudomonas species was performed through multiple sequence alignment of the 16S rRNA, gyrA, and parC genes by ClustalW. A phylogenetic tree was constructed through MEGA 12 (ver. 05) program through neighbor-joining method technique. The homology of the sequences among the species was evaluated by bootstrap analysis with 1000 replicates. The data were analyzed through an automated programing integrated with the instruments. Additional analyses were performed using various bioinformatics tools to ensure comprehensive data interpretation.

The ProtParam tool (web.expasy.org) was used to analysed the physicochemical properties of GyrA and ParC proteins. Protein–protein interaction of GyrA and ParC was identified through STRING database (string-db.org), which predicts the associations of proteins from different organisms (Szklarczyk *et al.*, 2021). While the functional pathway was analyzed using KEGG

(genome.jp/kegg/pathway), which is a comprehensive resource for integrating information pertaining to genes, pathways, diseases, drugs, and metabolism (Szklarczyk *et al.*, 2021). The Psipred tool (bioinf.cs.ucl.ac.uk) is used for analyzing GyrA and ParC protein secondary structure prediction. structural characteristics based on 'position specific scoring matrices' derived from psi-blast. The protein sequences were uploaded for analysis in the FASTA format.

GyrA and ParC proteins' 3D structures were predicted from multiple sequence alignment using TrRosetta. Ramachandran plot, ERRAT, VERIFY 3D models were used to validate the 3D models of the proteins. ERRAT (servicesn. mbi. ucla) evaluated atomic interactions as claims and gave a score in the form of statistical evaluation. The site detailing The Ramachandran plot (saves. mbi. ucla) checked torsion angles (φ and ψ) of the amino acid residues and marked certain angles as allowed or disallowed, thus confirming the structure's integrity
Molecular docking of Berberine Hydroxide, Curcumin, Coumarins, and Caffeic acid was performed using AutoDock Vina on the PyRx platform. Docking ligands were tested against the GyrA and ParC proteins for possible interactions. Discovery through virtual screenings was done with the help of PyRx, while interaction details were captured using PyMOL and Discovery Studio, allowing a thorough view of binding on all sides in three dimensions.

3.0 Results:

Twenty clinical samples were collected from three different healthcare settings and immediately transported for microbiological analyses. The samples were inoculated onto Blood and MacConkey Agar with incubation time of 24 to 48 hours at 37°C. After completion of incubation time, 14 of 20 (70%) samples showed positive growth of *P. aeruginosa*, characterized by non-lactose fermenting colonies on MacConkey Agar and β-hemolytic activity on Blood Agar (Table 1). The remaining 6 samples (30%) did not exhibit the characteristic growth of *P. aeruginosa* under the given conditions.

A single colony from each positive sample was streaked onto MacConkey Agar plates to obtain pure colonies. The colonies appeared circular, small, and pigmented after 24 hours. The 14 identified isolates of *P. aeruginosa* were gram stained and appeared gram-negative red to pink. Rod shaped (bacilli) under a microscope were found either as single units or in pairs. No Spore formation was observed. *P. aeruginosa* identification was biochemically confirmed using an API 10S. The test isolates were oxidase, catalase, nitrate reduction, and arginine dihydrolase positive; however, they exhibited negative glucose fermentation, indole production, and urease

activity.

Four from the 14 isolates (NF12, NF22, NF24, and NF84) were multidrug-resistant, showing resistance to more than three antibiotics in the gram-negative drug panel, as shown in Figure 1. The sensitivity to these antibiotics was measured using Mueller-Hinton agar, as shown in Table 2. The MDR isolates showed varying resistance patterns as follows:

- Four MDR isolates were resistant to levofloxacin.
- Resistance to Ciprofloxacin observed in the NF22, NF24, and NF84 cells.
- Meropenem was ineffective against NF12, NF22, and NF84 strains.
- Colistin resistance was confirmed in the NF12, NF24, and NF84 cells.

Resistance to ceftazidime-avibactam, Imipenem and Tazobactam also differed among MDR isolates. Figure 1 shows a heat map of the antibiotic resistance patterns of *P. aeruginosa* strains.

Genomic DNA extraction performed from all four *P. aeruginosa* isolates; NF12, NF22, NF24, and NF84. The quality was confirmed by 1% agarose gel electrophoresis, which revealed clear and intact bands, indicating DNA suitability for PCR (Figure 2a). PCR amplification of the 16S rRNA gene was conducted to further affirm the molecular identification of *P. aeruginosa.* The MDR strains confirmed, NF12, NF22, NF24, and NF84, were used and run with specific primers targeting the 16S rRNA gene. Optimization was done to evaluate the specificity and efficiency of the PCR conditions while thermal cycling. The PCR products produced after amplification were analyzed by agarose gel electrophoresis. The bands from amplification were observed on UV transilluminator, indicated bands appearing at 1500 bp as shown in Figure 2b representing the 16S rRNA gene.

The PCR products of the GyrA and ParC genes (from NF12, NF22, NF24, and NF84) were loaded onto a 1.5% agarose gel to visualize the desired amplification, as indicated in Figure 2c & 2d. After gel electrophoresis, a distinct band corresponding to the expected amplicon size of the gyrA and parC genes was visible, which was a clear indication of successful amplification. The appearance of distinct bands indicated that the genes were subjected to successful amplification, which in turn confirmed the presence of the respective genes in the *P. aeruginosa* isolates.

In addition to gyrA and parC, isolates were screened for key resistance determinants. PCR screening and further gel electrophoresis confirmed the presence of blaVIM and mexAB-oprM efflux pump genes in NF22, while NF12 NF24 and NF84 carried mutations in gyrA and parC.

The absence of oprD was noted in NF84, potentially explaining its carbapenem resistance. Sequencing data for gyrA and parC genes have been deposited in GenBank under accession numbers PV46074.

A phylogenetic tree was constructed and evolutionary relationships determined among the isolated bacterial strains. The maximum likelihood phylogenetic tree is shown in figure 3 and is based on the 16S rRNA gene nucleotide sequence. The analysis revealed that the isolated strain clustered closely with *P. aeruginosa* reference sequences retrieved from GenBank. Comparative protein modeling revealed that modification in GyrA and ParC genes were correlated with fluoroquinolone resistance, as shown in Figure 4a & 4b. Phylogenetic trees suggest clonal dissemination of resistant strains within healthcare settings.

Molecular docking was conducted to assess the binding affinities of the selected ligands with GyrA and ParC, resistance-associated proteins of *P. aeruginosa*. The results showed Berberine Hydroxide displayed the highest binding (-6.6 kcal/mol for GyrA and -6.8 kcal/mol for ParC) and Curcumin followed closely behind it. Other coumarins and caffeic acid showed lower binding affinities, indicating a lower strength of interaction with the target proteins. This strongly confirms that Berberine Hydroxide and Curcumin are candidates for further optimization against fluoroquinolone-resistant bacterial strains, as shown in Table 3.

Berberine forms strong hydrogen bonds and hydrophobic interactions with key gyrA residues including Ser-2, Met-41, and Val-22. Similar to GyrA, Curcumin also interacted with Ser-2, Glu-3, and Val-22, indicating that curcumin binds to GyrA and inhibits DNA replication (Figure 5a & 5b). Berberine has been found to have stabilizing hydrogen bonds with ARG48 and GLY54 in ParC. Curcumin also had multiple strong hydrogen bonds and hydrophobic interactions with the ParC residues GLN43, ASP53, and SER78, confirming the inhibitory potential of curcumin (Figure 5c & 5d).

4.0 Discussion:

The development and spread of MDR *P. aeruginosa* pose a persistent challenge to healthcare systems because they have very limited treatment options and quickly evolve new drug resistance mechanisms that also reported in a study conducted by (Elahi *et al*,. 2024) The current study, which targeted the isolation and characterization of *P. aeruginosa* strains, included clinical samples obtained from health facilities from different geographical locations.

Microbiological, biochemical, and molecular methods were used to identify strains. Approximately 70% of the total clinical isolates were identified as *P. aeruginosa*, as they formed typical non-lactose fermenting colonies on MacConkey Agar and had β-hemolytic activity on Blood Agar as mentioned in (Jarych *et al.,* 2023) study. Morphological characterization accompanied by Gram staining further confirmed that these isolates were gram-negative bacilli with no spores. Biochemical profiling using the API 10S biochemical device identified *P. aeruginosa* based on the positive oxidase, catalase, nitrate reduction, and arginine dihydrolase reactions.

(de Siqueira e Oliveira *et al.*, 2021) stated about MDR strains which were able to confirm species with similar biochemical profiles. From 20, the 14 identified *P. aeruginosa* isolates, four were multidrug- resistant, showing resistance to fluoroquinolones (ciprofloxacin and levofloxacin),

carbapenems (meropenem and imipenem), and colistin. These alarming results should remind us that even though such organisms are currently under control, highly resistant *P. aeruginosa* is becoming common in healthcare settings as also mentioned in (Rosado et al., 2025) work. The molecular profiling of these isolates provides further knowledge about the genetic basis of resistance. PCR amplification and Genomic DNA extraction of the 16S rRNA gene performed to confirmed the molecular identity of *P. aeruginosa*. All four MDR strains, in which the bacteria were identified as Pseudomonas based on nucleotide sequence analysis through 16S rRNA gene sequencing, yielded an amplification of the PCR product of the predicted size of ~1500 bp.

Furthermore, amplification of both GyrA and ParC genes showed the role of mutations in the QRDR of GyrA and ParC in establishing fluoroquinolone resistance. This was corroborated by the report of (Gómez-Zorrilla *et al.*, 2024) regarding the role of variation in gyrA and parC in fluoroquinolone resistance in *P. aeruginosa*. On the basis of phylogenetic analysis using the 16 S rRNA of isolates similar to these reference *P. aeruginosa* strains, it was confirmed that there were genetic proximity and clonal spread of isolates within the healthcare setting. The identification of gyrA and parC mutations accounts for the fluoroquinolone resistance, as previously reported. The identification of blaVIM and mexAB-oprM further supports the multifactorial resistance profile. These results strongly suggest that new methods of action should evaluate efflux pump inhibitors and β-lactamase targeted therapies. Our docking results suggest that berberine hydroxide and curcumin may also serve as promising adjuvants to

restore fluoroquinolone activity by stabilizing GyrA and ParC protein-protein interactions. From a translational standpoint, the combination of molecular profiling followed by rational drug design can have significant implications in the development of precision therapies targeting MDR *P. aeruginosa*. The phylogenetic pattern observed was similar to that observed in other studies investigating nosocomial outbreaks of MDR *P. aeruginosa* (de Siqueira e Oliveira *et al.*, 2021), (Gómez-Zorrilla *et al.*, 2024)

Co-analysis of GyrA and ParC protein sequences is crucial for understanding the molecular basis of resistance. Our results pointed to shared mutations in these two proteins, thus establishing a possible link between these mutations and fluoroquinolone resistance, which correlates with the results of a previous study by (Kunz Coyne *et al.*, 2022). Numerous molecular docking studies have been conducted to target GyrA and ParC of *P. aeruginosa*. Berberine hydroxide was studied for binding with GyrA and ParC and obtained the highest score for receptor-ligand interaction, followed closely by curcumin. The favorable interactions of berberine with the key QRDR residues of GyrA-almost Ser-2, His-27, and Thr-37- are carbapenems (meropenem and imipenem), and colistin. These alarming results should remind us that even though such organisms are currently under control, highly resistant *P. aeruginosa* is becoming common in healthcare settings as also mentioned (Rosado et al., 2025) work. The molecular profiling of these isolates provides further knowledge about the genetic basis of resistance. PCR amplification and Genomic DNA extraction of the 16S rRNA gene performed to confirm the molecular identity of *P. aeruginosa*. All four MDR strains, in which the bacteria were identified as Pseudomonas based on nucleotide sequence analysis through 16S rRNA gene sequencing, yielded an amplification of the PCR product of the predicted size of

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A docking study of berberine with ParC was also credible because of the different stabilizing interactions formed by hydrogen bonds with ARG48 and Pi-alkyl interactions with PRO50. These interactions all favor further investigation of these compounds for the development of pharmacological agents. The hydrogen bonding and van der Waals interactions were reported and compared with those of (LaPlante *et al.*, 2022), who correctly established the ability of berberine to disrupt topoisomerase activity in gram-negative strains.

Based on the results of this study, there is potential for the use of berberine and curcumin for drug development against fluoroquinolone-resistant strains, since they show strong binding affinities to GyrA and ParC. Future studies should focus on in vivo validation and search for synergistic combinations with existing antibiotics to overcome resistance mechanisms. This study highlights the physicochemical characterization and molecular analysis of MDR *P. aeruginosa* isolates. Therefore, berberine and curcumin have already been used as natural compounds to develop CNS against MDR bacterial strains. Such work is important, as it further strengthens molecular docking and genomics as additional tools for the identification of new therapeutic agents targeting MDR pathogen

5.0 Conclusion

Molecular profiling of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolated from healthcare systems demonstrated significant genetic factors responsible for resistance and highlighted the need for developing effective therapy options. Investigators uncovered prominent genetic factors associated with multidrug-caused survival in a clinical setting. A genome-wide analysis provided mechanisms of resistance and facilitated the identification of compounds for new drug development. In addition, molecular docking studies further expanded upon compound identification with four natural compounds: Berberine Hydroxide, Curcumin, Coumarins, and Caffeic Acid designated inhibitors of GyrA and ParC. This represents a crucial step toward personalized antimicrobial strategies that combine molecular profiling with in silico drug discovery.

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Conflict of Interest Statement

We declare no conflict of interest. All data, research, and results presented in this manuscript are solely the work of the authors.

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Tables and Figures:

Table 1: Visualization of ligands protein interaction

Sr No	Ligands	Binding affinity with Gyr	Binding affinity with Par
1	Berberine Hydroxide	-6.6	-6.8
2	Curcumin	-6.5	-6.7
3	Coumarins	-5.3	-5.1
4	Caffeic acid	-5.6	-5.2

Table 1: Binding affinities (kcal/mol) of selected ligands with Gyr and Par proteins, indicating their interaction strengths.

Fig 1: Multidrug resistant Antibiotic Heatmap pattern

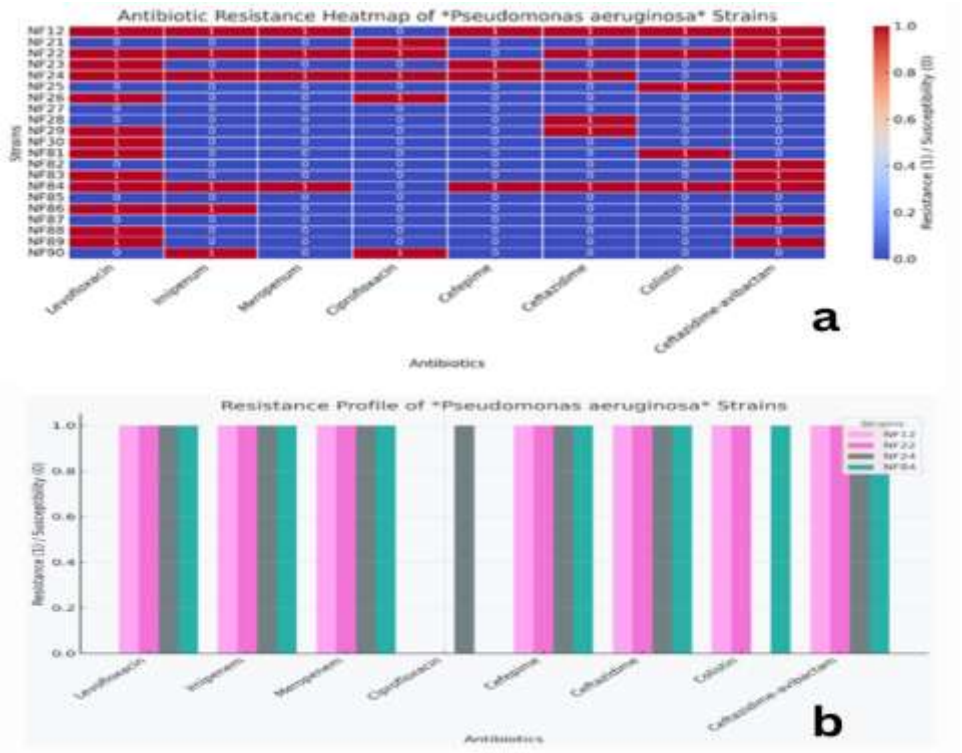

Figure1 (a) Heatmap showing antibiotic resistance patterns of P. aeruginosa strains.

(b) Resistance profile of four P. aeruginosa strains (NF12, NF22, NF24, and NF84) against eight antibiotics.

Fig 2: DNA Bands showing presence of Bacterial DNA, Amplified product of 16S rRNA gene, Gyr A and Par C gene through PCR.

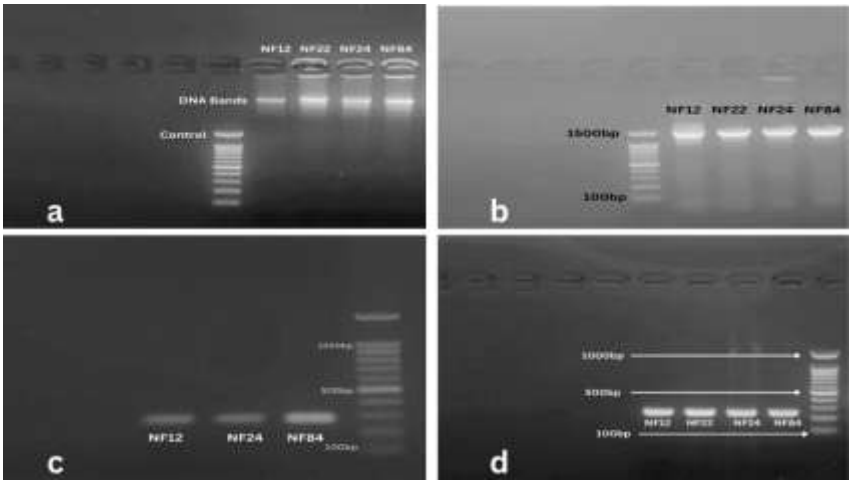

Figure 2 (a) DNA bands on 1.5% agarose gel confirming the presence of bacterial DNA.
(b) PCR results showing amplification of the 16S rRNA gene.
(c) Amplified product of Gyr A gene of P. aeruginosa on gel electrophoresis.
(d) Visualization of ParC gene amplification in Pseudomonas aeruginosa by gel electrophoresis

Fig 3: Neighbor-Joining Phylogenetic Analysis Showing Evolutionary Distances of Nine Nucleotide Sequences

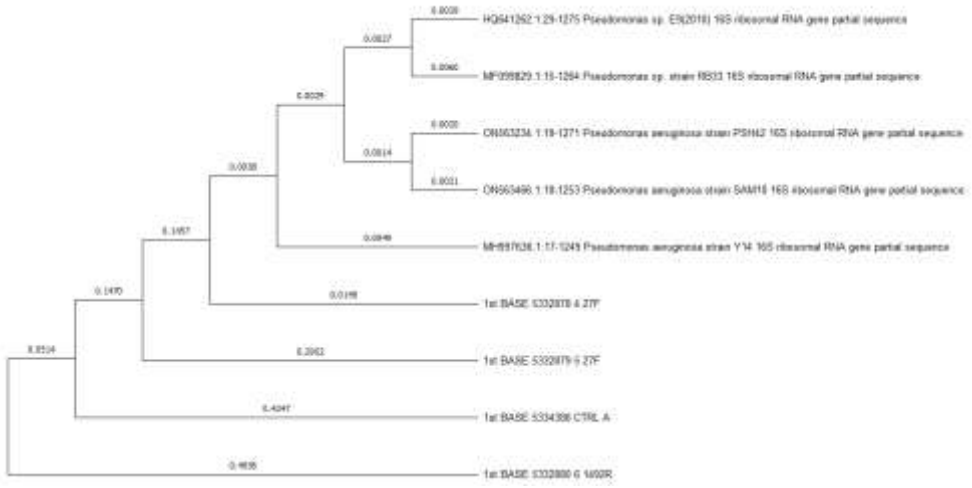

Figure 3 MEGA 11 analysis representing Phylogenetic tree inferred using the Neighbor-Joining method based on 9 nucleotide sequences.

Fig 4: 3D Structures of GyrA and ParC Proteins

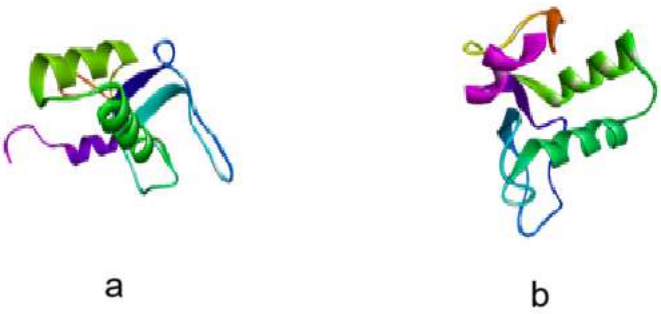

Figure 4 (a) 3D structure of the GyrA protein highlighting α-helices and β-sheets. (b) 3D model of ParC protein showing alpha helices and other secondary structures.

Fig 5: Docking analysis of Berberine and Curcumin with GyrA protein and Par C

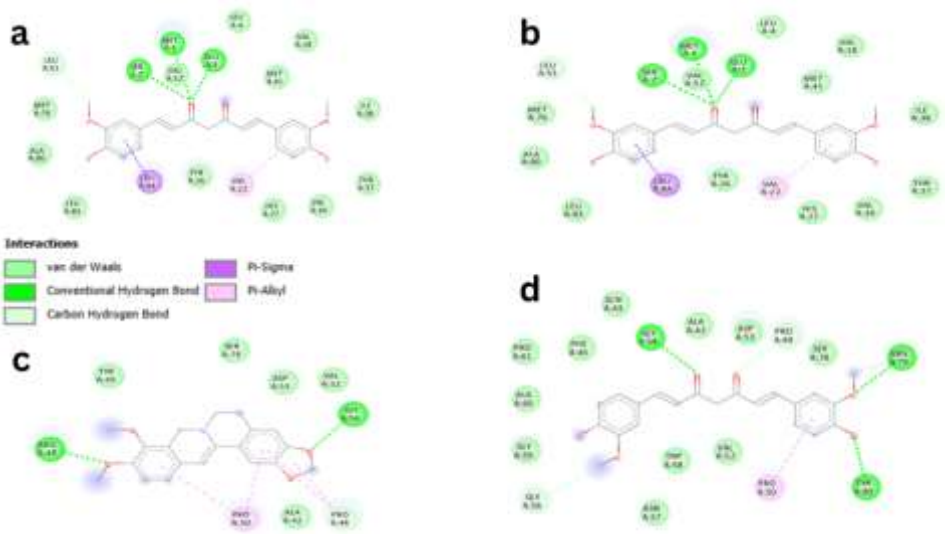

Figure 5 (a) Molecular docking of Berberine with GyrA of P. aeruginosa.

(b) Molecular docking of Curcumin with GyrA of P. aeruginosa.

(c) Visualization of berberine's 2D interactions with ParC.

(d) Curcumin–ParC molecular interactions showing multiple bonds and forces that support strong ligand–p