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by Sneha Sneha

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GENOMIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN *PSEUDOMONAS AERUGINOSA*

A DISSERTATION

Submitted in partial fulfilment of the requirements for the degree of

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in

BIOTECHNOLOGY

Submitted by

SNEH PRIYA

2024/MSCBIO/19

Under the supervision of

Prof. JAI GOPAL SHARMA

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 110042

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Sneh Priya

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 110042

CANDIDATE'S DECLARATION

I, **Sneh Priya**, **Roll No. 24/MSCBIO/19** hereby certify that the work which is being presented in the thesis entitled **"Genomic Characterization of Antimicrobial Resistance in *Pseudomonas aeruginosa*"** is in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work carried out during the period from January 2026 to May 2026 under the supervision of Prof. Jai Gopal Sharma.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Place: Delhi

Date:

Sneh Priya

2k24/MSCBIO.19

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 110042

CERTIFICATE

Certified that Sneh Priya (2k24/MSCBIO/19) has carried out their search work presented in this thesis entitled "**Genomic Characterization of Antimicrobial Resistance in *Pseudomonas aeruginosa***" for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Prof. Yasha Hasija
Head of the Department
Department of Biotechnology
Delhi Technological University

Prof. Jai Gopal Sharma
Supervisor
Department of Biotechnology
Delhi Technological University

Date:

"Genomic Characterization of Antimicrobial Resistance in *Pseudomonas aeruginosa*"

Sneh Priya

Delhi Technological University, Delhi, India

Email: snehpriya2003@gmail.com

ABSTRACT

Antimicrobial resistance (AMR) is one of the most serious healthcare problems in the twenty-first century. According to data from the World Health Organization (WHO), AMR directly caused 1.27 million deaths and indirectly contributed to 4.95 million deaths in 2019. It is projected that without significant interventions, the number of deaths may rise to approximately 10 million annually by 2050. *Pseudomonas aeruginosa*, belonging to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), has been classified as a critical-priority pathogen by the WHO and U.S. Centers for Disease Control and Prevention. It is responsible for 10-16% of hospital-acquired infections and has an inherently complex resistance architecture due to the acquisition of new resistance determinants through horizontal gene transfer. While studies have used whole genome sequencing (WGS) for AMR surveillance, most remain limited to listing the resistance genes alone. It is therefore necessary to examine how these genes are organized and co-selected. A major factor contributing to the spread of resistance is the association of the resistance genes with mobile genetic elements (MGEs), which is yet to be fully understood.

This dissertation focuses on the development and application of a reproducible bioinformatics framework for the integrated characterization of the resistance genes and MGEs in clinical *P. aeruginosa* isolates. Whole genome sequences of ten clinical isolates representing pneumonia, bloodstream infections, and urinary tract infections from geographically diverse sources were retrieved from publicly available databases. Raw paired-end reads were processed through a quality-controlled assembly pipeline using FastQC, MultiQC, fastp, and SPAdes, followed by AMR gene detection using NCBI AMRFinderPlus. MGEs belonging to four categories, including insertion sequence (IS) elements, prophage regions, integrons, and plasmid replicons, were detected using ISEScan, PHASTER, IntegronFinder, and PlasmidFinder, respectively. All downstream analysis was implemented in Python, enabling transparent and reproducible execution.

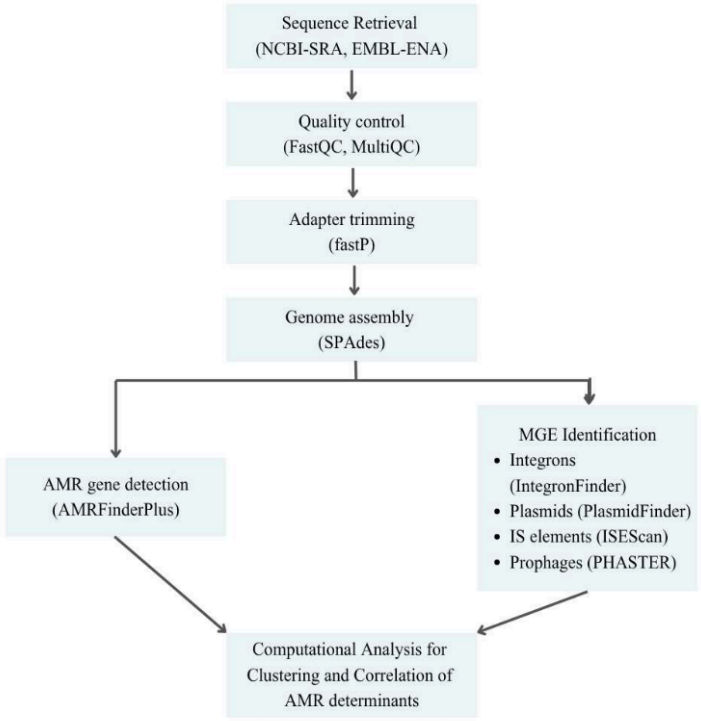
Across the ten isolates, 69 unique resistance determinants including both acquired resistance genes (85%) and chromosomal point mutations (15%) were identified. Three genes *aph(3')-IIb*, *catB7*, and *fosA*, were universally conserved. AMR gene burden varied significantly across isolates, with the highest burden isolate carrying 32 genes across multiple resistance classes. Co-occurrence network analysis was performed using a binary gene presence-absence matrix, revealing a structured resistome comprising 20 genes connected by 91 edges. The three core genes each showed maximum degree centrality. The *crpP* gene, encoding a ciprofloxacin resistance enzyme, exhibited disproportionately high connectivity relative to its

prevalence, indicating strong co-selection pressure. Hierarchical clustering using Jaccard distance identified high, moderate, and low burden groups, indicating non-random multi-class acquisition patterns. Shannon entropy indices ranged from 1.79 to 3.47 across isolates, with higher values in high-burden isolates. These findings suggest that resistance is distributed broadly across drug classes, and this has direct relevance to the treatment limitations faced in clinical settings.

Mobilome characterization revealed 297 IS elements, 15 intact prophage regions, integron-associated attC sites in six isolates, and plasmid replicons confined to the highest burden isolate. A composite MGE burden score integrating all four element types showed a statistically significant positive correlation with AMR gene count (Pearson's $r = 0.762$, $p = 0.010$, $R^2 = 0.580$). The values show a strong association between genomic plasticity and resistance accumulation within this population.

The findings of this study demonstrate that AMR in *P. aeruginosa* is not merely a collection of independent genetic events, but rather a structured, network-organized, and MGE-associated phenomenon. The framework utilized here generated quantitative outputs for each isolate, including network centrality scores, Shannon diversity indices, hierarchical cluster assignments, and MGE burden profiles, which are directly applicable to AMR surveillance. When applied to large sample sizes, these results may serve as a structured feature set for future machine learning-based resistance phenotype classification and outbreak risk prediction. This work contributes to the growing foundation of AI-driven infectious disease surveillance by bridging WGS-based genomic profiling with the computational framework required for large-scale clinical application.

GRAPHICAL ABSTRACT



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LIST OF PUBLICATIONS

1. The manuscript entitled "**Molecular Docking Analysis of Gedunin as a Potential Inhibitor of Penicillin-Binding Protein 2a (PBP2a) in Methicillin-Resistant *Staphylococcus aureus***" has been accepted in International Journal of Drug Delivery Technology (IJDDT)
2. A paper entitled "Traditional Antimicrobial Combinations In Modern Drug Development" was presented at the National Conference On History And Development Of Science In India: Bridging Ancient Wisdom To Contemporary Horizons held on April 15th-16th, 2026.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/Abbreviations	Meaning/Full form
AMR	Antimicrobial Resistance
WHO	World Health Organization
MDR	Multidrug-resistant
HAIs	Hospital-acquired infections
AST	Antimicrobial Susceptibility Testing
MGE	Mobile Genetic Element
WGS	Whole Genome Sequencing
IS	Insertion Sequence
HGT	Horizontal Gene Transfer
CARD	Comprehensive Antibiotic Resistance Database
NCBI	National Center for Biotechnology Information
HMM	Hidden Markov Model
CALIN	Clusters of Antibiotic Resistance Genes Linked to Integron
PHASTER	PHAge Search Tool Enhanced Release
ML	Machine Learning
ICE	Integrative and Conjugative Element
H′	Shannon Diversity Index
r	Pearson Correlation Coefficient
R^2	Coefficient of Determination
p	Statistical Significance Value

CHAPTER 1

INTRODUCTION

In modern era, one of the most persistent and challenging issues for healthcare is antimicrobial resistance (AMR). Due to the misuse of antibiotics, the resistance mechanisms in bacteria evolved and this has now led to a crisis that cannot be ignored. According to World Health Organization (WHO) data, AMR has caused about 1.27 million deaths, and contributed to additional 4.95 million deaths indirectly in 2019. It is projected that without significant intervention, the number of deaths might rise to about 10 million annually by 2050 (Murray et al., 2022). AMR has become one of the leading causes of mortality worldwide and without significant solutions to this problem, the future outcomes might become even worse as our therapeutic options to target resistant bacteria continue to become narrow.

The hospital environments contribute heavily to the AMR burden. Multidrug-resistant (MDR) pathogens are heavily concentrated in clinical settings, where antibiotic selection pressure is high. The patients are critically ill and immunocompromised becoming vulnerable to infections, Hospital-acquired infections (HAIs) caused by MDR pathogens lead to increased fatality rates, prolonged inpatient stays, and increased healthcare costs due to use of expensive last-resort therapeutic agents.

Pseudomonas aeruginosa is a member of the ESKAPE group of pathogens (which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp). This pathogen is responsible for 10-16% of HAIs, such as surgical site infections, bloodstream infections, urinary tract infections, and ventilator-associated pneumonia, and has a reported mortality rate ranging from 32-51% in clinical populations (Elfadadny et al., 2024).

Carbapenem-resistant *P. aeruginosa* has been designated as a critical-priority pathogen by both WHO and U.S. Centers for Disease Control and Prevention. This pathogen is considered so challenging due to its intrinsic resistance mechanisms. These include reduced outer membrane permeability, a network of *Mex* efflux pumps, and *AmpC* beta-lactamase production. In addition, the clinical isolates also acquire exogenous resistance determinants through horizontal gene transfer (HGT)(Fernández-Billón et al., 2023). The most clinically significant of these are the carbapenemases, particularly the metallo-beta-lactamases *bla*VIM and *bla*IMP, which have been detected in 30–50% of clinical *P. aeruginosa* isolates in some surveillance studies. This leads to carbapenems becoming ineffective, which are often a last-resort antibiotic. The combination of intrinsic and horizontally acquired resistance genes creates a resistance profile that makes the infections exceptionally challenging to fight (Elfadadny et al., 2024).

Despite the complexity of *P. aeruginosa* resistance, most healthcare settings still rely on phenotypic antimicrobial susceptibility testing (AST) for surveillance. While these methods provide the essential information needed to guide the treatment, they have several limitations, especially when MDR pathogens are involved. Phenotypic testing of sample requires 48-72 hours from collection to result. Also, this method can only report the bacterial isolate characteristics as performed under standard laboratory conditions, and cannot reveal the

underlying genomic architecture that provides more accurate information on resistance (Dolgusevs et al., 2024). The standard tests also ignore the information about mobile genetic elements (MGEs), whether the resistance genes are chromosomally encoded or plasmid-borne. The limitations create a gap between the genomic reality of resistance and the clinical information used for prescribing drugs to patients (Dolgusevs et al., 2024; Hassall et al., 2024).

Whole genome sequencing (WGS) is a method of AMR surveillance that captures the whole genetic blueprint of an isolate including resistance genes, chromosomal point mutations, mobile elements, and phylogenetic markers. For *P. aeruginosa* specifically, WGS can be used for outbreak investigation by reconstructing transmission chains and identifying specific mobile platforms carrying resistance determinants. However, most studies related to *P. aeruginosa* resistome remain confined to gene inventories without diving deep into their dissemination and mobility across clinical populations. The absence of reproducible, integrated computational frameworks makes it difficult to compare surveillance data across institutions (Sherry et al., 2025).

To understand the genomic architecture of resistance, the first step is characterizing the resistome. This explains which genes co-occur consistently and the distribution of resistance across drug classes. Network-based co-occurrence modelling and Shannon diversity quantification when applied to resistome provide a clear picture of the resistance complexity across samples. The mobilome refers to the full complement of mobile genetic elements in a genome including insertion sequence (IS) elements, prophages, integrons, plasmid elements. etc. These are responsible for spreading resistance across clinical environments. Most studies perform limited mobilome characterization, leaving the broader MGE landscape unstudied (Nodari et al., 2023). Covering this gap provides a direct measure of how genome plasticity translates into resistance and classifies the relationship between total MGE burden and resistance gene accumulation.

This study addresses three main objectives that define the scope of the work: first, to characterize the resistome of the selected clinical *P. aeruginosa* isolates including both the conserved resistance core and acquired determinants, and to describe its architecture through network and diversity analysis; second, to characterize the mobilome across four major MGE categories and compute a composite burden score per isolate; and third, to quantify the statistical relationship between MGE complexity and resistance gene burden, and to evaluate the structured numerical outputs of this framework as a foundation for future machine learning-based AMR prediction and surveillance applications.

CHAPTER 2
LITERATURE REVIEW

2.1. Antimicrobial Resistance: A Global Health Crisis

Antimicrobial resistance is one of the greatest challenges to global healthcare in the modern era. The misuse of antibiotics across human medicine, agriculture, and veterinary practice led to an imposed evolutionary pressure on the microbial populations, which accelerated the selection and spread of antimicrobial resistant strains worldwide (Aslam et al., 2018). A global systematic analysis reported that AMR led to 1.27 million deaths directly, and 4.95 million deaths indirectly in patients in 2019. The numbers might rise to annual 10 million deaths by 2050 if the problem of AMR continues to grow without significant solutions (Murray et al., 2022).

HAIs caused by MDR organisms represent a particularly severe clinical challenge, as they result in increased fatality and prolonged hospital stays, causing substantial economic burden to healthcare systems. A systematic review on clinical and financial burden of ESKAPE pathogen-associated HAIs confirmed that the infections vary significantly based on regional setting and type of pathogen (Woh & Zhang, 2025).

2.2 Pseudomonas aeruginosa as a Critical Priority Pathogen

2.2.1 Microbiological Characteristics and Virulence

Pseudomonas aeruginosa is a gram-negative, non-fermenting, aerobic bacillus with one of the largest bacterial genomes (~6.3 Mb), encoding a wide range of virulence factors, regulatory networks, and metabolic pathways (Elfadadny et al., 2024). The main virulence determinants involved in tissue and immune invasion include type III and type VI secretion systems, biofilm-forming exopolysaccharides (alginate, Pel, Psl), quorum-sensing circuits (*las*, *rhl*, *pqs*), proteases, pyocyanin, and rhamnolipids (Krūmiņa et al., 2026; Liao et al., 2022). These features make *P. aeruginosa* a challenging opportunistic pathogen causing bloodstream infections, urinary tract infections, ventilator-associated pneumonia, and surgical site infections, with reported mortality rates of 32–51% (Elfadadny et al., 2024). Recent WGS based studies of resistant strains of P. aeruginosa have confirmed co-occurrence of high virulence potential and MDR resistance profiles, highlighting the need of integrated genomic characterization (Pajaro-Castro et al., 2025).

2.2.2 Clinical burden of infection

P. aeruginosa is the fifth most frequent cause of HAIs globally, accounting for approximately 7.1% of all HAIs in tertiary care hospitals. The regional prevalence rates depend on local antibiotic stewardship and infection control, however multi-center metadata highlights that the prevalence of MDR *P. aeruginosa* ranges between 15% and 30% in general wards, and increases up to 48.7% within high-risk ICU environments (Salleh et al., 2025).

2.2.3 Genotypic-Phenotypic Mismatch

A major challenge in the diagnostics of MDR pathogens is the frequent mismatch between the genotypic predictions and phenotypic AST outcomes. A study documented these genotype-phenotype inconsistencies in ICU patients and linked them to gene expression, epistatic interactions, and regulatory mutations (Dolgusevs et al., 2024).

2.3 Mechanisms of AMR in P. aeruginosa

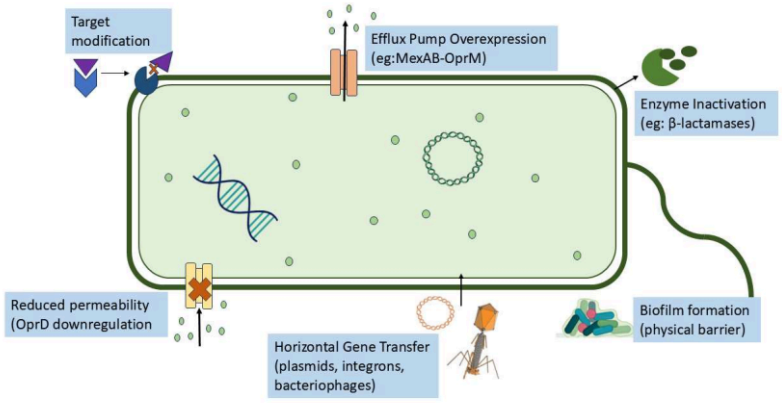

Fig 2.1: Major Mechanisms of AMR in *P. aeruginosa*

2.3.1 Intrinsic resistance mechanisms

In *P. aeruginosa*, the intrinsic resistance works at multiple levels. Reduced outer membrane permeability through downregulation of porin OprD restricts accumulation of carbapenems intracellularly. The constitutive expression of efflux systems (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM) pushes antimicrobial compounds including beta-lactams, fluoroquinolones, aminoglycosides, and tetracyclines out of the cell. The presence of *ampC* beta-lactamase provides resistance to aminopenicillins and extended-spectrum cephalosporins (Pang et al., 2019). Additionally, studies also show that biofilm formation can amplify intrinsic resistance by creating diffusion barriers and tolerant subpopulations (Liao et al., 2022).

2.3.2 Acquired Resistance Determinants

Beyond intrinsic mechanisms, *P. aeruginosa* acquires additional resistance genes by chromosomal mutations and horizontal gene transfer (HGT). Carbapenem resistance is frequently associated with acquired metallo-beta-lactamases such as blaVIM, blaIMP, and blaNDM, along with porin mutations and efflux overexpression (Oliver et al., 2024; Pang et al., 2019). Aminoglycoside-modifying enzymes, including phosphotransferase *aph(3')-IIb*, are found in many clinical *P. aeruginosa* strains. The chloramphenicol acetyltransferase *catB7* and fosfomycin resistance gene *fosA* are also acquired chromosomal determinants across species (Oliver et al., 2024). Studies report that ciprofloxacin-modifying enzyme *CrpP*, mediator of fluoroquinolone resistance has been spreading by highly mobilizable genomic islands in hospital settings (Hernández-García et al., 2021).

2.4 WGS for Resistome Profiling

Due to the reduction in cost of sequencing, WGS has become accessible for use in genomics research and surveillance operations. It allows simultaneous identification of acquired genes, chromosomal mutations, and MGEs within a single workflow, offering unparalleled resolution compared to conventional approaches. A review by Sherry et al. discusses how WGS can be adopted for AMR applications and identified reproducible bioinformatics pipelines (Sherry et al., 2025). A typical workflow comprises of raw read quality assessment using FastQC (S. Andrews, 2010) and MultiQC (Ewels et al., 2016), adapter trimming via fastP (Chen et al., 2018), *de novo* genome assembly using SPAdes (Bankevich et al., 2012), and target resistance gene annotation via AMRFinderPlus (Feldgarden et al., 2021). A comprehensive five-year longitudinal analysis of Spanish *Pseudomonas aeruginosa* clinical isolates successfully displayed the capacity of population-scale resistome characterization to monitor the shift in resistance trends and trace spread of high-risk bacterial clones (López-Causapé et al., 2017).

To use WGS as a tool in active surveillance studies, there is need of development of highly-curated, standardized reference databases and automated annotation pipelines. Bioinformatics tools are required to characterize the resistome from *de novo* genomic assemblies. They can distinguish between vertically inherited chromosomal mutations and horizontally acquired mobile resistance genes (Centner et al., 2026). Some public repositories include Comprehensive Antibiotic Resistance Database (CARD) which uses a specialized ontology-

driven system (Alcock et al., 2023), ResFinder that focuses on acquired determinants from clinical isolates (Florensa et al., 2022), and AMRFinderPlus (Feldgarden et al., 2021).

AMRFinderPlus is managed under National Center for Biotechnology Information (NCBI) Pathogen Detection Project, and uses the highly structured Bacterial Antimicrobial Resistance Reference Gene Database. It employs a hybrid multi-stage detection architecture that makes it better than the standard alignment-only search tools (like BLASTn alone). For alleles that are known and well-documented, the tool aligns their sequences against databases containing manually curated, position-specific BLAST cutoff rules. To detect highly divergent alleles, novel variants, or hidden orthologs within complex assemblies, it utilizes profile Hidden Markov Models (HMMs). In this manner, AMRFinderPlus can drastically reduce the rates of false-negatives when it profiles diverse clinical lineages. The "Plus" functional expansion of AMRFinderPlus refers to integration of specific curated databases tracking virulence factors, biocide resistance, and heavy metal tolerance, enabling more comprehensive, multi-dimensional genomic analysis of the target pathogen (Feldgarden et al., 2021, 2022).

2.5 MGEs: Drivers of Resistance

2.5.1 Integrons

Integrons are type of MGE that have a site-specific recombination system that allows capture, integration, and rearrangement of gene cassettes. Class I integrons are the most clinically prevalent and are major drivers of AMR dissemination in *P. aeruginosa*. IntegronFinder 2.0 (Néron et al., 2022) is a tool for identification of complete integrons that retain a functional integrase gene (*intI*) flanked by recombination sites (*attI*/*attC*), and chromosomally-fixed cassette arrays called CALINs (Clusters of Antibiotic Resistance Genes Linked to Integrons) which lack a functional integrase.

2.5.2 Plasmids

They serve as primary vehicles for inter- and intra-species transfer of AMR genes via conjugation. In *P. aeruginosa*, megaplasmids and broad-host-range incompatibility groups are often present in clinical lineages (Urbanowicz et al., 2021). These vectors serve as genetic scaffolds, utilizing transposons and integrons for the transfer of AMR determinants. PlasmidFinder (Carattoli et al., 2014) enables *in silico* detection and typing of plasmid replicons from assembled WGS data.

2.5.3 Insertion sequence elements

IS elements are the simplest and most abundant autonomous transposable genetic elements in prokaryotic genomes that drive genomic plasticity and resistome reorganization in *Pseudomonas aeruginosa.* They contain a transposase gene flanked by inverted repeats. ISEScan is used for automated identification and family classification of IS elements from

prokaryotic genome assemblies. The IS3 family is most prevalent in *P. aeruginosa* genomes and has been associated with expression of resistance genes (Xie & Tang, 2017).

2.5.4 Prophages

Prophages are bacterial viruses integrated stably into the host chromosome, and are a major part of the variable accessory genome in *P. aeruginosa*. PHAge Search Tool Enhanced Release (PHASTER) (Arndt et al., 2016) is used for automated classification of prophage regions into intact, questionable, and incomplete categories for systematic mobilome characterization.

Table 2.1: Overview of MGE categories and their Role in AMR Dissemination

Features	**Integrons**	**Plasmids**	**Insertion sequences**	**Prophages**
Size	0.5-3 kb (cassette arrays up to 10 kb)	1-400 kb	0.7-2.5 kb	10-200 kb
Type of element	Site-specific recombination system	Extrachromosomal circular DNA	Simplest transposable elements	Bacteriophage genomes integrated in host
Mechanism of transfer	Passive (depend on functional transposons or plasmids for HGT)	Active (conjugation, transformation)	Passive (require co-resident conjugative elements)	Passive (transduction)
Major AMR contribution	Multi-class resistance acquired via cassette capture	Inter-species AMR transfer (carbapenemases and ESBL)	OprD disruption, efflux pump upregulation, composite transposon formation	Regulatory gene disruption, lysogenic conversion, indirect resistance modulation
Prevalence in *P. aeruginosa*	High, especially class I integrons	Low to moderate	High, IS3 family is most prevalent	High
Key references	(Ali et al., 2024; Néron et al., 2022)	(Carattoli et al., 2014)	(Fernández-Billón et al., 2023; Xie & Tang, 2017)	(Arndt et al., 2016; Chang et al., 2025)

2.6 Integrated Resistome-Mobilome Analysis

2.6.1 Hierarchical Clustering and Distance Metrices

Hierarchical clustering of binary gene presence-absence matrices is a basic method to identify genomic relationships among bacterial isolates. In resistome data, clustering would reveal non-random resistance gene co-acquisition patterns which cannot be understood from individual gene counts. The Jaccard distance measures binary vector dissimilarity while ignoring joint absence, and is preferred for such analyses. It can be implemented within the SciPy scientific Python library (Virtanen et al., 2020). Visualization through Seaborn-generated clustered heatmaps (Waskom, 2021) shows isolate relationships and gene clustering patterns that helps to better understand the resistome architecture.

2.6.2 Diversity Quantification

Shannon entropy can be productively applied to resistome diversity quantification in genomic epidemiology. In the context of resistome, $H' = -\sum(\mathrm{p_i} \times \log_2 p_i)$, where p$i$ represents the proportion of AMR genes belonging to each resistance class (Shannon, 1948). Calculating Shannon entropy provides a normalized, scalar value measuring the absolute breadth, evenness, and distribution complexity of resistance classes across sequenced genomes, where high entropy scores would mean that complex, MDR genotypes are being heavily shaped by MGEs (Robertson et al., 2023).

2.6.3 Network-based Analysis and Gene Co-occurrence studies

Recent studies are going beyond simple gene inventories and focusing on characterization of the structural organization of the resistome using network-based approaches. Co-occurrence network analysis is one such approach where the nodes and edges represent the resistance genes and their co-presence across isolates, respectively (Choudhury & Andam, 2026). The NetworkX library (Hagberg et al., 2008) provides comprehensive tools for constructing and analyzing complex networks in Python.

The recently published Resistome Gene Association Inference Network (ReGAIN) pipeline combines AMRFinderPlus gene annotations with Bayesian network modelling, and evaluates conditional dependencies, quantifying the likelihood that a pathogen will acquire a specific resistance determinant given its existing genetic background. Network analysis show that resistance genes rarely occur in isolation. Instead, they cluster onto the mobile elements and drive multi-class resistance against drugs (Bring Horvath et al., 2024).

2.6.4 Resistome-Mobilome Correlation

The relationship between MGE burden and AMR gene count across multiple gram-negative surveillance studies consistently revealed strong positive correlations. Pearson correlation analysis can be used to obtain a quantitative measure of the association between genome plasticity and resistance burden. However, the correlation could have imperfect nature as highlighted by isolates with high IS element burden but low AMR gene count (Che et al., 2021). The mismatch means that non-mobilome factors such as chromosomal mutations, intrinsic resistance, and gene loss events also affect the resistance profiles independent of overall MGE content (Botelho et al., 2019).

A study by Noman et al. showed that WGS-derived genomic features, including gene presence–absence profiles can yield high classification accuracy (≥96%) in machine learning (ML) models predicting resistance to 12 antibiotic classes in *P. aeruginosa*. This highlights the importance of such structured quantitative genomic outputs from computational surveillance pipelines in ML applications to improve AMR surveillance (Noman et al., 2023).

CHAPTER 3

METHODOLOGY

3.1. Retrieval of WGS of Clinical Isolates

Genomes of ten clinical isolates of *P. aeruginosa* were obtained from publicly available databases NCBI Sequence Read Archive (Agarwala et al., 2018) and European Nucleotide Archive (David et al., 2026). These genomes represent bloodstream infections, pneumonia, and urinary tract infections from diverse geographic sources.

Table 3.1: Clinical *P. aeruginosa* isolates retrieved for resistome and MGE analysis

S. no.	Accession ID	Geographic origin	Collection Date	BioProject ID
1	ERR12310986	India	2020	PRJEB70413
2	SRR14087464	India	07-2018	PRJNA689041
3	SRR33445280	Italy	31-01-2024	PRJNA1259250
4	SRR35514394	Brazil	08-01-2018	PRJNA785542
5	SRR34755277	USA	25-06-2025	PRJNA288601
6	SRR35287142	USA	20-08-2025	PRJNA858823
7	SRR33818201	USA	2025	PRJNA288601
8	SRR35664086	USA	2025	PRJNA288601
9	SRR34900282	Australia	09-2015	PRJNA1220180
10	SRR35023004	Australia	2018	PRJNA1220180

3.2. Read Quality Control

The initial quality assessment of the raw paired-end reads was performed using FastQC (S. Andrews, 2010), producing quality reports for each sample that were then compiled through MultiQC (Ewels et al., 2016) for comparative analysis among the datasets. Adapter trimming and quality filtering followed using fastp (Chen et al., 2018) with qualified quality Phred ≥20, length ≥50 bp, sliding window 4:20, and automatic adapter detection. Post-trimming quality was re-assessed with FastQC and MultiQC to confirm improvement in per-base quality scores and adapter removal.

3.3. *De Novo* Genome Assembly

Filtered reads were assembled de novo using SPAdes (Bankevich et al., 2012) in careful mode with the k-mer sizes 21,33,55,77, and keeping contigs ≥500 bp in length.

3.4. AMR Gene Identification

The assembled contigs were screened with NCBI AMRFinderPlus (Feldgarden et al., 2021) keeping identity ≥90% and coverage ≥60% to detect acquired resistance genes and chromosomal point mutations.

3.5. MGE Detection

MGE belonging to four categories were characterized using the obtained SPAdes-assembled contigs of each Pseudomonas isolate.

3.5.1 IntergronFinder

Integrons were detected using Integronfinder (Néron et al., 2022) that analyzed the genomes for presence of complete integrons and CALINs. This allowed detection of both actively mobile integrons and chromosomally fixed resistance arrays.

3.5.2 PlasmidFinder

PlasmidFinder (Carattoli et al., 2014) was utilized in identification of the plasmid replicons present within the WGS data.

3.5.3 ISEScan

IS elements were searched using ISEScan (Xie & Tang, 2017) which recorded the total IS element count and number of distinct IS families for each isolate.

3.5.4 PHASTER

PHASTER (Arndt et al., 2016) detected and classified the prophage regions into three categories (intact, questionable, and complete). This provided an overall prophage landscape for MGE analysis, and only the intact prophage regions were used for the burden calculations due to high biological relevance and activity.

3.6. Computational Analysis of Resistance Genes and MGEs

The computational analysis was done using Python within a Google Colab environment to make a reproducible and accessible pipeline.

The AMRFinderPlus results were converted into a binary gene presence-absence matrix development. In the matrix, the rows represented isolates and the columns represented AMR genes identified. Then matrix multiplication of the transposed binary matrix produced a co-occurrence matrix. In this, each entry indicated the number of isolates in which two genes were simultaneously present. A co-occurrence network was made using NetworkX v3.x (Hagberg et al., 2008). Each node represented an AMR gene and edges were drawn between the genes that co-occur in multiple isolates. Node size was proportional to degree centrality and reflected the number of co-occurring relationships per gene. The different node colour displayed different resistance classes that highlighted their co-selection pattern.

Resistome diversity was quantified using the Shannon entropy index, calculated as follows:

$$H' = -\sum(\mathrm{p_i} \times \log_2 p_i)$$

where $\mathrm{p_i}$ describes the proportion of each resistance class among all AMR genes detected in that isolate (Shannon, 1948). This allowed comparison of resistome complexity across isolates.

Hierarchical clustering was performed using SciPy library (Virtanen et al., 2020) on the binary presence-absence matrix using Jaccard distance as the dissimilarity metric paired with the average linkage method. Jaccard distance is used because it is perfect for binary data and ignores joint absence. The dendrogram and clustered heatmap obtained were then viewed using the Seaborn clustermap function (Waskom, 2021), displaying both samples and genes simultaneously. This highlighted the co-clustering patterns and shared gene subsets among isolates.

For the integration of resistome and mobilome, complete MGE burden score per isolate was computed. This included calculating the arithmetic sum of the count of total IS elements, prophage regions, attC sites, and plasmid replicons. Pearson correlation coefficient calculation between MGE burden score and AMR gene count across all 10 isolates was done using the SciPy stats module, with statistical significance defined at $p < 0.05$. The resulting data is a measure of the relationship between resistance and genome plasticity burden in the selected clinical *P. aeruginosa* isolates.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 AMR gene burden

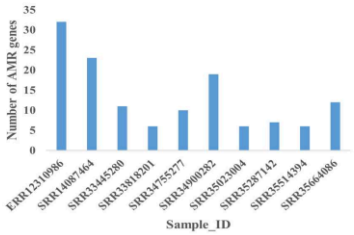

Fig. 4.I: Number of unique AMR genes detected per isolate

In this study, total 132 AMR genes were identified from WGS analysis of 10 clinical *P. aeruginosa* isolates. Out of all the genes, 69 were unique. The resistance determinants contained both acquired genes (85%) and chromosomal point mutations (15%).

The AMR gene burden was variable across isolates, with maximum 32 genes in ERR12310986 and minimum 6 genes in SRR33818201, SRR35023004, and SRR35514394 each.

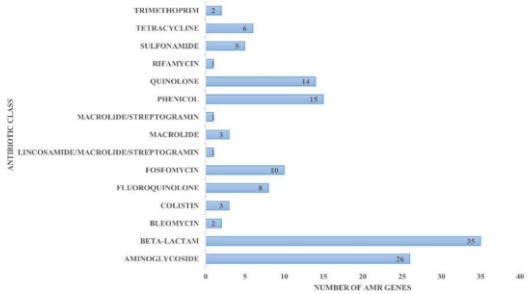

Fig. 4.2: Distribution of unique AMR genes by antibiotic class across isolates

Among all resistance classes, beta-lactam and aminoglycoside were the most prevalent classes, reflecting their well-documented dominance in clinical *P. aeruginosa* populations (Oliver et al., 2024).

4.2 Resistome architecture

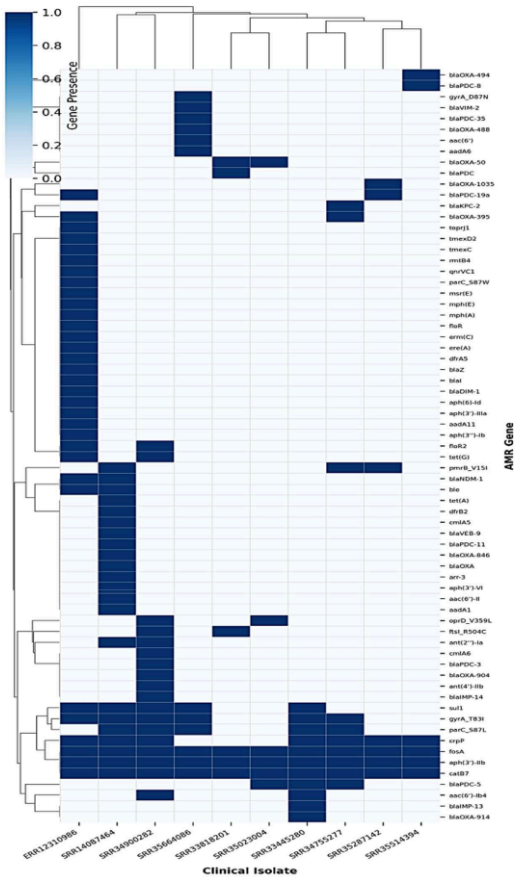

Fig 4.3: Hierarchical clustering heatmap of AMR gene distribution

Three genes *aph(3')-IIb*, *catB7*, and *fosA* were present in all ten samples, forming a conserved resistance core.

The high burden isolates ERR12310986, SRR14087464, and SRR34900282 shared broad overlapping resistance gene collection from multiple antibiotic classes. The moderate burden cluster included SRR33445280, SRR34755277, and SRR35664086, and showed partial gene sharing with the high burden group. The remaining isolates were low burden cluster and were mainly comprised of the conserved core genes.

4.3 Co-occurrence network and Resistance connectivity

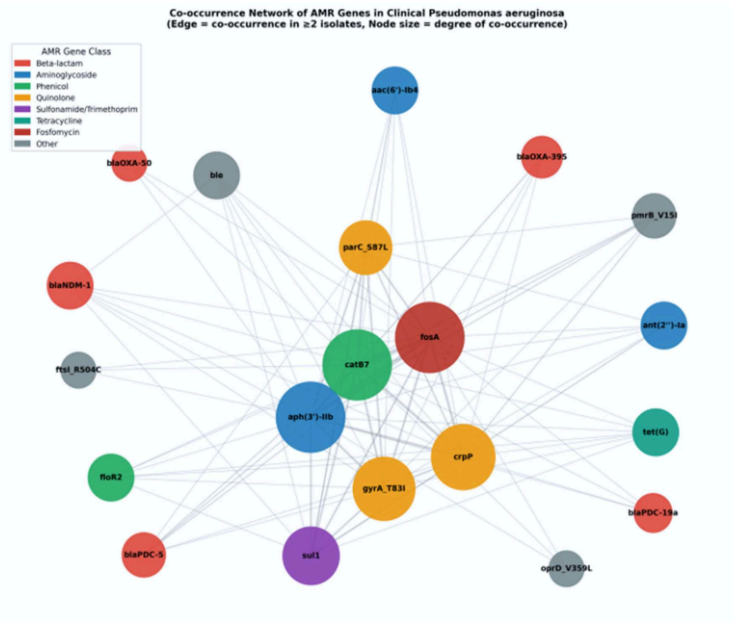

Fig 4.4: Co-occurrence network of AMR genes across isolates

Co-occurrence network analysis identified 20 genes and 91 edges. The three core genes *aph(3')-IIb*, *catB7*, and *fosA* each displayed 19 connections which is the maximum degree observed. This confirms their role as the structural backbone of the resistome network.

Interestingly, the *crpP* gene encoding for ciprofloxacin-modifying enzyme, showed 16 connections despite being present in only 7 isolates. This indicates its strong co-selection with other resistance genes (Chávez-Jacobo et al., 2018).

The presence of cross-class edges between beta-lactam, aminoglycoside, quinolone, and phenicol nodes shows that resistance is not class-specific. Co-selection of resistance determinants from multiple classes is a major cause for treatment failure (Choudhury & Andam, 2026; Schwartz et al., 2024).

4.4 Resistance Diversity

The Shannon diversity indices ranged from 1.79 in the low burden isolates to 3.47 in ERR12310986, with a mean of 2.40 across the 10 isolates. The consistency between Shannon index ranking and AMR burden cluster means that the isolates carrying more resistance genes had a more evenly distributed repertoire spanning a greater number of resistance classes. This is clinically relevant because an isolate with high diversity index is resistant across multiple drug classes simultaneously, and limits the treatment options (Choudhury & Andam, 2026; Schwartz et al., 2024).

4.5 MGE Landscape

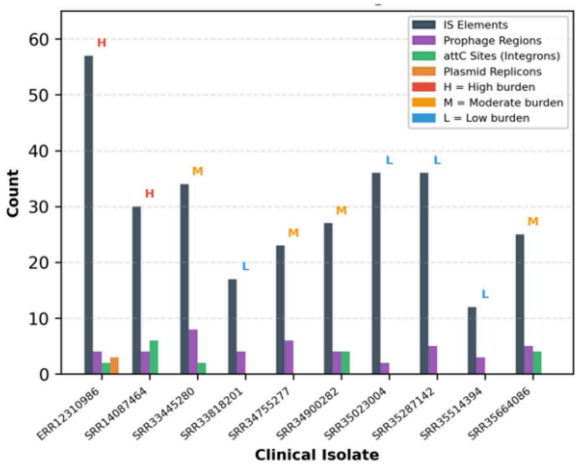

Fig 4.5: MGE landscape across all ten isolates

A total of 297 IS elements were detected, with a mean of 29.7 per isolate. IS3 was the dominant family, present in all isolates across varying copy numbers consistent with its known prevalence in Pseudomonas genomes (Fernández-Billón et al., 2023). ERR12310986 carried the highest IS burden at 57 elements across 17 distinct families.

45 prophage regions were identified in total across all categories, of which 15 were intact, present in eight of ten isolates. SRR33445280 harboured the highest intact prophage count at 4 regions, suggesting a history of repeated bacteriophage integration events.

Integrons were detected in total six isolates. SRR33445280 was the only isolate carrying a complete integron. CALIN structures were identified in four of them. Additionally, two isolates carried IntI fragment remnants alongside their CALINs, indicating partial integron decay.

Plasmid replicons were present in only one isolate (ERR12310986). Three replicons rep16/Inc18, rep5a/Rep3, and rep10/RepL were identified.

4.2 Correlation of AMR Genes and MGEs

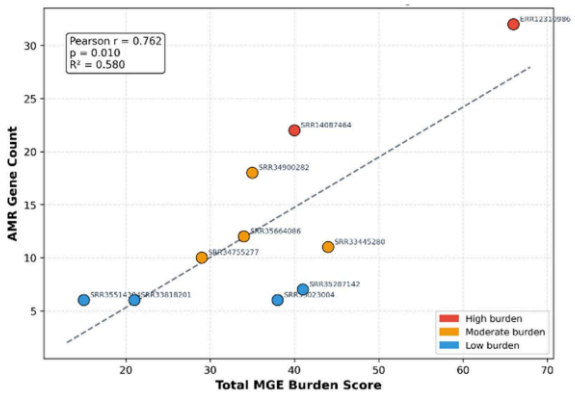

Fig 4.6: Pearson correlation between total MGE burden score and AMR gene count across isolates

Pearson correlation between the composite MGE burden score and AMR gene count resulted in r = 0.762, R^2 = 0.580, and p = 0.010. This means statistically significant positive association between resistance burden and mobilome complexity. Isolates with higher overall MGE scores also carried more AMR genes. The coefficient of determination value of 0.580 indicates that MGE burden accounts for approximately 58% of the variance observed in AMR gene count. The remaining 42% variance could have been caused by other factors such as chromosomal mutations, intrinsic resistance mechanisms, gene loss events, etc. Deviations from the regression line were seen in SRR35023004 and SRR35287142, which carried relatively high IS element counts of 36 each but low AMR gene counts of 6 and 7 respectively. This indicates that the accumulation of IS elements in these isolates occurred independently of resistance gene acquisition (Nodari et al., 2023).

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVES

Antimicrobial resistance in *Pseudomonas aeruginosa* is a structured, network-organised, and mobilome-associated genomic feature. This study aimed at demonstrating this, using an integrated bioinformatics pipeline to characterise the resistance genes and mobile elements of clinical *P. aeruginosa* isolates from geographically diverse sources. The result is a reproducible framework that goes beyond the single-tool gene inventory approaches and connects the overall AMR profile together.

The most consistent finding in the analysis was the universal conservation of the genes *aph(3')-IIb*, *catB7*, and *fosA* in every isolate regardless of geographic origin, infection type, or overall resistance burden. Each of these genes reached the maximum degree centrality of 19 connections in the co-occurrence network which is not coincidental. These genes are chromosomally fixed, near-intrinsic components of the *P. aeruginosa* genome, and have stable presence across diverse clinical populations. For surveillance purposes, they represent a reliable baseline. The clinical *P. aeruginosa* isolates carrying this core could be potentially high-risk isolates.

That accumulation is not random. Hierarchical clustering of the resistance gene matrix revealed low, moderate, and high burden tiers that represented an additive layer of multi-class resistance genes built upon the core. The high-burden isolates carried resistance spanning beta-lactam, aminoglycoside, quinolone, and phenicol classes simultaneously which was reflected in high value of Shannon diversity indices ($H' = 3.47$). This type of co-selection of multiple resistance classes is a consequence of MGEs, where resistance cassettes from different classes are mobilised together rather than being acquired overtime (Nodari et al., 2023). In the co-occurrence network results, *crpP* formed 16 connections despite being present in only 7 of 10 isolates. Such unbalanced connectivity occurs because *crpP* spreads mostly through integrative and conjugative elements (ICEs), and routinely co-localises with aminoglycoside resistance genes, mercury resistance operons, and virulence-associated fimbriae clusters (López et al., 2022).

The mobilome analysis confirmed that resistance complexity in this cohort scales is proportional to MGE complexity. The highest-burden isolate, ERR12310986, was the only one to carry all four MGE types simultaneously. The significant Pearson correlation between composite MGE burden and AMR gene count ($r = 0.762$, $p = 0.010$, $R^2 = 0.580$) provides direct quantitative evidence for this relationship across the cohort. The remaining 42% of unexplained variance is expected and occurs because of chromosomal mutations, gene loss events, etc. Two isolates (SRR35023004, SRR35287142) each carried 36 IS elements but only 6-7 resistance genes which means that IS element accumulation might not always be associated with the spread of resistance.

Overall, the workflow combined AMRFinderPlus screening, co-occurrence network modelling, Shannon diversity quantification, hierarchical clustering, and multi-MGE correlation in a reproducible computational environment. These generated structured numerical genomic profiles that are directly useful for clinical AMR monitoring. The framework addresses a gap that conventional WGS approaches leave open, which is that they identify what genes are present but do not quantify how those genes are organised, co-

selected, or connected to the mobile elements that disseminate them. It is shown by studies that systematic WGS-based tracking of high-risk clones can help to identify transmission routes and inform interventions that reduce nosocomial spread (Sherry et al., 2025; Stribling et al., 2025). The outputs obtained from our pipeline including the centrality scores, diversity indices, and per-isolate MGE profiles can be taken as standardised, quantitative features that would make the surveillance more scalable.

Looking forward, the most impactful next step is validation on a larger, phenotypically characterised cohort. While 10 isolates are enough to establish statistical significance and demonstrate a framework, they are not enough for generalization of outcomes for clinical decision. Incorporating phenotypic minimum inhibitory concentration data would also enable direct genotype-phenotype validation. Long-read sequencing methods could provide the complete structural context of resistance genes within different MGE types which is difficult with short-read assemblies.

The structured outputs of this kind of pipeline can be used as training features for machine learning-based AMR phenotype prediction. Random Forest models trained on *P. aeruginosa* WGS data have already achieved mean classification accuracies of ≥96% across 12 antibiotic families (Noman et al., 2023). Beyond the clinical laboratory, this type of analysis can be extended to environmental and veterinary isolates within a One Health framework that can help to track resistance across the ecological boundaries between hospital, community, and environmental reservoirs.

In conclusion, this study shows that characterising *P. aeruginosa* resistance genomics requires looking at both what genes are present and how they are organised and mobilised. The framework developed here makes that possible in a reproducible and scalable manner, and could be used in future for clinical AMR prediction and outbreak surveillance.

REFERENCES

Agarwala, R., Barrett, T., Beck, J., Benson, D. A., Bollin, C., Bolton, E., Bourexis, D., Brister, J. R., Bryant, S. H., Canese, K., Cavanaugh, M., Charowhas, C., Clark, K., Dondoshansky, I., Feolo, M., Fitzpatrick, L., Funk, K., Geer, L. Y., Gorelenkov, V., … Zbicz, K. (2018). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *46*(D1), D8–D13. https://doi.org/10.1093/nar/gkx1095

Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., Edalatmand, A., Petkau, A., Syed, S. A., Tsang, K. K., Baker, S. J. C., Dave, M., McCarthy, M. C., Mukiri, K. M., Nasir, J. A., Golbon, B., Imtiaz, H., Jiang, X., Kaur, K., … McArthur, A. G. (2023). CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Research*, *51*(D1), D690–D699. https://doi.org/10.1093/nar/gkac920

Ali, N., Ali, I., Din, A. U., Akhtar, K., He, B., & Wen, R. (2024). Integrons in the Age of Antibiotic Resistance: Evolution, Mechanisms, and Environmental Implications: A Review. *Microorganisms*, *12*(12), 2579. https://doi.org/10.3390/microorganisms12122579

Arndt, D., Grant, J. R., Marcu, A., Sajed, T., Pon, A., Liang, Y., & Wishart, D. S. (2016). PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Research*, *44*(W1), W16–W21. https://doi.org/10.1093/nar/gkw387

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, *Volume 11*, 1645–1658. https://doi.org/10.2147/IDR.S173867

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology*, *19*(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

Botelho, J., Grosso, F., & Peixe, L. (2019). Antibiotic resistance in Pseudomonas aeruginosa – Mechanisms, epidemiology and evolution. *Drug Resistance Updates*, *44*, 100640. https://doi.org/10.1016/j.drup.2019.07.002

Bring Horvath, E. R., Stein, M. G., Mulvey, M. A., Hernandez, E. J., & Winter, J. M. (2024). *Resistance Gene Association and Inference Network (ReGAIN): A Bioinformatics Pipeline for Assessing Probabilistic Co-Occurrence Between Resistance Genes in Bacterial Pathogens*. https://doi.org/10.1101/2024.02.26.582197

Carattoli, A., Zankari, E., García-Fernández, A., Voldby Larsen, M., Lund, O., Villa, L., Møller Aarestrup, F., & Hasman, H. (2014). *In Silico* Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. *Antimicrobial Agents and Chemotherapy*, *58*(7), 3895–3903. https://doi.org/10.1128/AAC.02412-14

Centner, C. M., Di Gregorio, S., Argimón, S., Brankin, A., Dean, A., Marcano Zamora, D., & Bertagnolio, S. (2026). Antimicrobial resistance databases: opportunities and challenges for public health. *Npj Antimicrobials and Resistance*, *4*(1), 1. https://doi.org/10.1038/s44259-025-00169-1

Chang, T. H., Pourtois, J. D., Haddock, N. L., Furukawa, D., Kelly, K. E., Amanatullah, D. F., Burgener, E., Milla, C., Banaei, N., & Bollyky, P. L. (2025). Prophages are infrequently associated with antibiotic resistance in *Pseudomonas aeruginosa* clinical isolates. *MSphere*, *10*(3). https://doi.org/10.1128/msphere.00904-24

Chávez-Jacobo, V. M., Hernández-Ramírez, K. C., Romo-Rodríguez, P., Pérez-Gallardo, R. V., Campos-García, J., Gutiérrez-Corona, J. F., García-Merinos, J. P., Meza-Carmen, V., Silva-Sánchez, J., & Ramírez-Díaz, M. I. (2018). CrpP Is a Novel Ciprofloxacin-Modifying Enzyme Encoded by the Pseudomonas aeruginosa pUM505 Plasmid. *Antimicrobial Agents and Chemotherapy*, *62*(6). https://doi.org/10.1128/AAC.02629-17

Che, Y., Yang, Y., Xu, X., Břinda, K., Polz, M. F., Hanage, W. P., & Zhang, T. (2021). Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. *Proceedings of the National Academy of Sciences*, *118*(6). https://doi.org/10.1073/pnas.2008731118

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Choudhury, S. T., & Andam, C. P. (2026). Genome-wide co-occurrence patterns link mobile genetic elements, antimicrobial resistance and defense systems in Pseudomonas aeruginosa. *BMC Genomics*, *27*(1), 226. https://doi.org/10.1186/s12864-026-12585-2

David, Y., Alisha, A., Awais, A., Rajkumar, D., Dipayan, G., Muhammad, H., Maira, I., Eugene, I., Vishnukumar, K., Amnon, K., Manish, K., Ankur, L., Isuru, L., Lili, M., Colman, O., Joana, P., Ruben, P., Stephane, P., Nadim, R., … Ugis, S. (2026). The European Nucleotide Archive in 2025. *Nucleic Acids Research*, *54*(D1), D120–D127. https://doi.org/10.1093/nar/gkaf1295

Dolgusevs, M., Jain, N., Savicka, O., Vangravs, R., Bodrenko, J., Bergmanis, E., Zemite, D., Selderina, S., Reinis, A., & Rozentale, B. (2024). Genomic and phenotypic inconsistencies in Pseudomonas aeruginosa resistome among intensive care patients. *Frontiers in Cellular and Infection Microbiology*, *14*. https://doi.org/10.3389/fcimb.2024.1335096

Elfadadny, A., Ragab, R. F., AlHarbi, M., Badshah, F., Ibáñez-Arancibia, E., Farag, A., Hendawy, A. O., De los Ríos-Escalante, P. R., Aboubakr, M., Zakai, S. A., & Nageeb, W. M. (2024). Antimicrobial resistance of Pseudomonas aeruginosa: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. *Frontiers in Microbiology*, *15*. https://doi.org/10.3389/fmicb.2024.1374466

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Feldgarden, M., Brover, V., Fedorov, B., Haft, D. H., Prasad, A. B., & Klimke, W. (2022). Curation of the AMRFinderPlus databases: applications, functionality and impact. *Microbial Genomics*, *8*(6). https://doi.org/10.1099/mgen.0.000832

Feldgarden, M., Brover, V., Gonzalez-Escalona, N., Frye, J. G., Haendiges, J., Haft, D. H., Hoffmann, M., Pettengill, J. B., Prasad, A. B., Tillman, G. E., Tyson, G. H., & Klimke, W. (2021). AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Scientific Reports*, *11*(1), 12728. https://doi.org/10.1038/s41598-021-91456-0

Fernández-Billón, M., Llambías-Cabot, A. E., Jordana-Lluch, E., Oliver, A., & Macià, M. D. (2023). Mechanisms of antibiotic resistance in Pseudomonas aeruginosa biofilms. *Biofilm*, *5*, 100129. https://doi.org/10.1016/j.bioflm.2023.100129

Florensa, A. F., Kaas, R. S., Clausen, P. T. L. C., Aytan-Aktug, D., & Aarestrup, F. M. (2022). ResFinder – an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microbial Genomics*, *8*(1). https://doi.org/10.1099/mgen.0.000748

Hagberg, A. A., Schult, D. A., & Swart, P. J. (2008). *Exploring Network Structure, Dynamics, and Function using NetworkX*. 11–15. https://doi.org/10.25080/TCWV9851

Hassall, J., Coxon, C., Patel, V. C., Goldenberg, S. D., & Sergaki, C. (2024). Limitations of current techniques in clinical antimicrobial resistance diagnosis: examples and future prospects. *Npj Antimicrobials and Resistance*, *2*(1), 16. https://doi.org/10.1038/s44259-024-00033-8

Hernández-García, M., García-Castillo, M., García-Fernández, S., López-Mendoza, D., Díaz-Regañón, J., Romano, J., Pássaro, L., Paixão, L., & Cantón, R. (2021). Presence of Chromosomal crpP-like Genes Is Not Always Associated with Ciprofloxacin Resistance in Pseudomonas aeruginosa Clinical Isolates Recovered in ICU Patients from Portugal and Spain. *Microorganisms*, *9*(2), 388. https://doi.org/10.3390/microorganisms9020388

Krūmiņa, A., Reinis, A., Jeske, A., Zeltiņa, I., & Vīksna, L. (2026). Molecular Mechanisms Underlying the Pathogenicity of Pseudomonas aeruginosa. *Medicina*, *62*(3), 462. https://doi.org/10.3390/medicina62030462

Liao, C., Huang, X., Wang, Q., Yao, D., & Lu, W. (2022). Virulence Factors of Pseudomonas Aeruginosa and Antivirulence Strategies to Combat Its Drug Resistance. In *Frontiers in Cellular and Infection Microbiology* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fcimb.2022.926758

López, M., Rojo-Bezares, B., Chichón, G., & Sáenz, Y. (2022). Resistance to Fluoroquinolones in Pseudomonas aeruginosa from Human, Animal, Food and Environmental Origin: The Role of CrpP and Mobilizable ICEs. *Antibiotics*, *11*(9), 1271. https://doi.org/10.3390/antibiotics11091271

López-Causapé, C., de Dios-Caballero, J., Cobo, M., Escribano, A., Asensio, Ó., Oliver, A., del Campo, R., Cantón, R., Solé, A., Cortell, I., Asensio, O., García, G., Martínez, M. T., Cols, M., Salcedo, A., Vázquez, C., Baranda, F., Girón, R., Quintana, E., … Cantón, R. (2017). Antibiotic resistance and population structure of cystic fibrosis Pseudomonas aeruginosa isolates from a Spanish multi-centre study. *International Journal of Antimicrobial Agents*, *50*(3), 334–341. https://doi.org/10.1016/j.ijantimicag.2017.03.034

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Néron, B., Littner, E., Haudiquet, M., Perrin, A., Cury, J., & Rocha, E. (2022). IntegronFinder 2.0: Identification and Analysis of Integrons across Bacteria, with a Focus on Antibiotic Resistance in Klebsiella. *Microorganisms*, *10*(4), 700. https://doi.org/10.3390/microorganisms10040700

Nodari, C. S., Opazo-Capurro, A., Castillo-Ramirez, S., & Mattioni Marchetti, V. (2023). Editorial: Mobile genetic elements as dissemination drivers of multidrug-resistant Gram-negative bacteria. *Frontiers in Cellular and Infection Microbiology*, *13*. https://doi.org/10.3389/fcimb.2023.1180510

Noman, S. M., Zeeshan, M., Arshad, J., Deressa Amentie, M., Shafiq, M., Yuan, Y., Zeng, M., Li, X., Xie, Q., & Jiao, X. (2023). Machine Learning Techniques for Antimicrobial Resistance Prediction of *Pseudomonas Aeruginosa* from Whole Genome Sequence Data. *Computational Intelligence and Neuroscience*, *2023*(1). https://doi.org/10.1155/2023/5236168

Oliver, A., Rojo-Molinero, E., Arca-Suarez, J., Beşli, Y., Bogaerts, P., Cantón, R., Cimen, C., Croughs, P. D., Denis, O., Giske, C. G., Graells, T., Daniel Huang, T.-D., Iorga, B. I., Karatuna, O., Kocsis, B., Kronenberg, A., López-Causapé, C., Malhotra-Kumar, S., Martínez, L. M., … Jeannot, K. (2024). Pseudomonas aeruginosa antimicrobial susceptibility profiles, resistance mechanisms and international clonal lineages: update from ESGARS-ESCMID/ISARPAE Group. *Clinical Microbiology and Infection*, *30*(4), 469–480. https://doi.org/10.1016/j.cmi.2023.12.026

Pajaro-Castro, N., Diaz-Morales, E., Hoyos, K., & Ibañez-Bersinger, C. (2025). Whole-Genome Sequencing of Resistance, Virulence and Regulation Genes in Extremely Resistant Strains of Pseudomonas aeruginosa. *Medical Sciences*, *13*(1), 6. https://doi.org/10.3390/medsci13010006

Pang, Z., Raudonis, R., Glick, B. R., Lin, T.-J., & Cheng, Z. (2019). Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnology Advances*, *37*(1), 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013

Robertson, J., Schonfeld, J., Bessonov, K., Bastedo, P., & Nash, J. H. E. (2023). A global survey of Salmonella plasmids and their associations with antimicrobial resistance. *Microbial Genomics*, *9*(5). https://doi.org/10.1099/mgen.0.001002

S. Andrews. (2010). *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Babraham Bioinformatics.

Salleh, M. Z., Zuraina, N. M. N. N., Deris, Z. Z., & Mohamed, Z. (2025). Current trends in the epidemiology of multidrug-resistant and beta-lactamase-producing Pseudomonas aeruginosa in Asia and Africa: a systematic review and meta-analysis. *PeerJ*, *13*(2). https://doi.org/10.7717/peerj.18986

Schwartz, B., Klamer, K., Zimmerman, J., Kale-Pradhan, P. B., & Bhargava, A. (2024). Multidrug Resistant Pseudomonas aeruginosa in Clinical Settings: A Review of Resistance Mechanisms and Treatment Strategies. *Pathogens*, *13*(11), 975. https://doi.org/10.3390/pathogens13110975

Shannon, C. E. (1948). A Mathematical Theory of Communication. *Bell System Technical Journal*, *27*(3), 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Sherry, N. L., Lee, J. Y. H., Giulieri, S. G., Connor, C. H., Horan, K., Lacey, J. A., Lane, C. R., Carter, G. P., Seemann, T., Egli, A., Stinear, T. P., & Howden, B. P. (2025). Genomics for antimicrobial resistance—progress and future directions. *Antimicrobial Agents and Chemotherapy*, *69*(5). https://doi.org/10.1128/aac.01082-24

Stribling, W., Hall, L. R., Powell, A., Harless, C., Martin, M. J., Corey, B. W., Snesrud, E., Ong, A., Maybank, R., Stam, J., Bartlett, K. V, Jones, B. T., Preston, L. N., Lane, K. F., Thompson, B., Young, L. M., Kwak, Y. I., Barsoumian, A. E., Markelz, A. E., … Lebreton, F. (2025). Detecting, mapping, and suppressing the spread of a decade-long Pseudomonas aeruginosa nosocomial outbreak with genomics. *ELife*, *13*. https://doi.org/10.7554/eLife.93181

Urbanowicz, P., Bitar, I., Izdebski, R., Baraniak, A., Literacka, E., Hrabák, J., & Gniadkowski, M. (2021). Epidemic Territorial Spread of IncP-2-Type VIM-2 Carbapenemase-Encoding Megaplasmids in Nosocomial Pseudomonas aeruginosa Populations. *Antimicrobial Agents and Chemotherapy*, *65*(4). https://doi.org/10.1128/AAC.02122-20

Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J.,

Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., … Vázquez-Baeza, Y. (2020). SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nature Methods*, *17*(3), 261–272. https://doi.org/10.1038/s41592-019-0686-2

Waskom, M. (2021). seaborn: statistical data visualization. *Journal of Open Source Software*, *6*(60), 3021. https://doi.org/10.21105/joss.03021

Woh, P. Y., & Zhang, X. (2025). The burden of ESKAPE pathogen-related hospital-acquired infections: clinical and financial perspective from a systematic review. *Journal of Hospital Infection*, *162*, 377–385. https://doi.org/10.1016/j.jhin.2025.06.006

Xie, Z., & Tang, H. (2017). ISEScan: automated identification of insertion sequence elements in prokaryotic genomes. *Bioinformatics*, *33*(21), 3340–3347. https://doi.org/10.1093/bioinformatics/btx433

LIST OF PUBLICATIONS WITH ACCEPTANCE LETTERS

Dear Author(s),

We are pleased to announce that your manuscript, "Molecular Docking Analysis of Gedunin as a Potential Inhibitor of Penicillin-Binding Protein 2a (PBP2a) in Methicillin-Resistant Staphylococcus aureus" has been accepted for publication in an upcoming issue of the *International Journal of Drug Delivery Technology (IJDDT)* (ISSN: 0975-4415).

This Scopus-indexed, peer-reviewed journal focuses on pharmaceutical sciences, drug delivery, clinical research, and biomedical fields. After thorough editorial review, peer evaluation, grammar checks, and similarity analysis, your paper stood out for its scientific excellence, originality, and relevance to clinical diagnostics and drug delivery innovations.

IJDDT offers a reliable venue for researchers, clinicians, and academics to disseminate cutting-edge work and promote collaboration in pharmaceuticals, medicine, and life sciences.

Congratulations on this milestone!

For any questions on the publication process, feel free to reach out.

Best regards,

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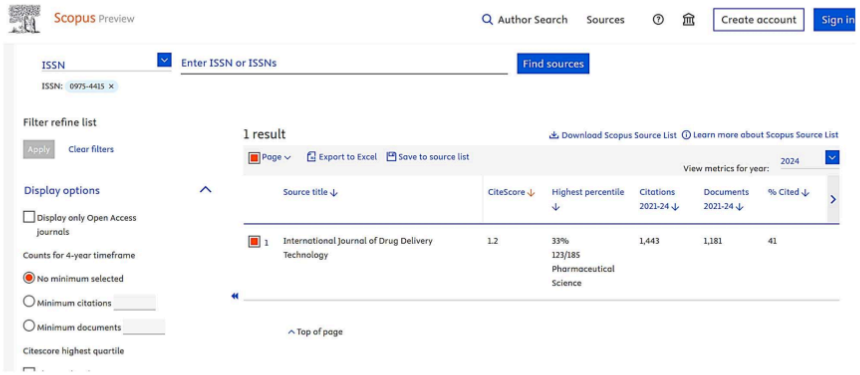

RAM LAL ANAND COLLEGE
UNIVERSITY OF DELHI
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DEPARTMENT OF MICROBIOLOGY
IN COLLABORATION WITH
DEPARTMENT OF HISTORY
HISTORY AND DEVELOPMENT OF SCIENCE IN INDIA:
BRIDGING ANCIENT WISDOM TO CONTEMPORARY HORIZONS

THIS IS TO CERTIFY THAT

SNEH PRIYA

HAS SUCCESSFULLY PRESENTED AT THE TWO-DAY NATIONAL CONFERENCE ORGANIZED BY THE DEPARTMENT OF MICROBIOLOGY IN COLLABORATION WITH THE DEPARTMENT OF HISTORY, RAM LAL ANAND COLLEGE, UNIVERSITY OF DELHI ON 15TH & 16TH APRIL 2026.

THE PRESENTATION TITLED:

Traditional Antimicrobial Combinations in Modern Drug Development

THE PARTICIPANT'S CONTRIBUTION IS SINCERELY APPRECIATED.
WE ACKNOWLEDGE THEIR VALUABLE EFFORT AND ACADEMIC ENGAGEMENT IN MAKING THE CONFERENCE A SUCCESS.

DR. SHALINI SWAMI
CONVENER&
ORGANISING
SECRETARY

DR. PRATIK KUMAR
CO-CONVENER

DR. VIKAS KUMAR
CO-CONVENER

PROF. RAKESH KUMAR GUPTA
PRINCIPAL

RAM LAL ANAND COLLEGE
UNIVERSITY OF DELHI
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HISTORY AND DEVELOPMENT OF SCIENCE IN INDIA:
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for **Best Oral Presentation** titled Traditional Antimicrobial Combinations In Modern Drug Development at the **Two-Day National Conference** organized by the Department of Microbiology in collaboration with the Department of History, Ram Lal Anand College, University of Delhi **dated 15th & 16th April, 2026.**

DR. SHALINI SWAMI
CONVENER&
ORGANISING
SECRETARY

DR. PRATIK KUMAR
CO-CONVENER

DR. VIKAS KUMAR
CO-CONVENER

PROF. RAKESH KUMAR GUPTA
PRINCIPAL

SNEH PRIYA

+91 8512097195 • snehpriya2003@gmail.com • New Delhi, India • www.linkedin.com/in/sneh-priya

EDUCATION

Master's in Biotechnology *2024 – Present*
Delhi Technological University, New Delhi
- CGPA: 9.00/10

Bachelor's (Hons.) in Microbiology *2021 –2024*
Ram Lal Anand College, University of Delhi, New Delhi
- CGPA: 8.811/10

Senior Secondary (XII), CBSE *2020-2021*
Vidya Bharati School, New Delhi
- Percentage: 95% (PCB)

Matriculation (X), CBSE *2018-2019*
Vidya Bharati School, New Delhi
- Percentage: 95%

RESEARCH EXPERIENCE

Department of Microbiology, University of Delhi
College Research Grant Projects

Evaluation of anti-ESKAPE potential of *Rauwolfia serpentina* and *Terminalia chebula* *Jan 2023 – Jun 2024*

Antimicrobial Activity of Selected Indian Plants against ESKAPE Pathogens *Aug 2022 -Dec 2023*

- Tested the antimicrobial activity of extracts from different Indian traditional plants.
- Techniques: Bacterial culturing, Microscopy, Antimicrobial Susceptibility Testing, Soxhlet extraction, Data analysis and Interpretation

INTERNSHIPS

Micro Crispr Private Limited – R&D Intern *July – Aug 2025*
R&D Department, New Delhi
- Contributed to R&D of anti-diabetic therapeutic peptides
- Techniques: Mammalian cell culturing, Mycoplasma testing, cAMP assay, Insulin ELISA
- Attended workshops on Bio-layer Interferometry and Nanopore sequencing

Bioinformatics Internship *June – Aug 2023*
Department of Microbiology, University of Delhi
- Predicted novel therapeutic protein targets in *Klebsiella pneumoniae* using AlphaFold
- Gained experience in deep learning, drug target identification, and structural analysis tools

AWARDS

Best Student Award, Department of Microbiology, Ram Lal Anand College (2022–23)

PUBLICATIONS

Accepted:

Raina, D., Priya, S., Sharma, J.G. "Molecular Docking Analysis of Gedunin as a Potential Inhibitor of Penicillin-Binding Protein 2a (PBP2a) in Methicillin-Resistant *Staphylococcus aureus*"
In the International Journal of Drug Delivery Technology (IJDDT)

Published:

Kapoor, S., Verma, S., Priya, S., Gupta, A., & Gupta, V. (2025). Algal Secondary Metabolites. In *Industrial Applications of Microbial Secondary Metabolites* (pp. 197–212). CRC Press. https://doi.org/10.1201/9781003408833-16

Patra, S., Verma, N., Priya, S., Gupta, A., & Gupta, V. (2024). Microbes as Biocontrol Agents for Sustainable Development. In *Microbial Approaches for Sustainable Green Technologies* (pp. 196–218). CRC Press. https://doi.org/10.1201/9781003407683-10

CERTIFICATIONS & WORKSHOPS

- Bioinformatics Summer Programme: Gene Editing, Molecular Cloning & CRISPR Applications – Bversity (June 2025)
- Hands-on Workshop on Bioinformatics and Molecular Docking – BioSoc, DTU (April 2025)
- National Workshop on PCR, RT-PCR & Their Applications – CIIDRET & DSSEED, University of Delhi (March 2023)
- Python Programming for Biological Problems – Udemy
- Basic Statistics for Biological Sciences (DBT Star College Scheme, 40 hrs)
- Biomedical Nanotechnology, NPTEL, IIT Roorkee, (Aug–Sep, 2022)

LANGUAGES

- English – Fluent
- Hindi – Native

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