COMPARATIVE GENOMICS ANALYSIS OF ANTIBIOTIC RESISTANCE GENES IN *ALCALIGENES FAECALIS*

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by

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CANDIDATE'S DECLARATION

I Palak hereby certify that the work which is being presented in the thesis entitled "**Comparative Genomic Analysis of Antibiotic Resistance Genes in *Alcaligenes faecalis*"** in partial fulfillment of the requirements for the award of Master of Science in Biotechnology, submitted in the Department of Biotechnology , Delhi Technological University is an authentic record of my own work carried out during the period from 2024 to 2026 under the supervision of Prof Yasha Hasija.

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.

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Certified that **Palak** has carried out their search work presented in this thesis entitled **"Comparative Genomics Analysis of Antibiotic Resistance Gene in *Alcaligenes faecalis*"** for the award of **Master of Science in Biotechnology** 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/herself 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.

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ABSTRACT

Alcaligenes faecalis, opportunistic gram-negative bacteria which has a rod shape structure. It is associated with raising concern of Antibiotic Resistance, which is responsible of causing infections among Humans. This study presents a in-depth comparative genomics analysis of 24 complete genome of *Alcaligenes faecalis* retrieved from NCBI. Genome annotation conducted through the RAST Server, leading to Phylogenetic analysis of 24 strains. Phylogenetic tree which reveals their evolutionary relationship among themselves. Resistome profile has built using database like CARD and Resfinder, shows Antibiotic resistance gene presence among those strains. Pan genome analysis has performed using Roary uncovered 1907 conserved core genes, 9612 acquired accessory genes, also resulted in quantitative ARGs frequency helped to classify gene into core and accessory. ARGs were mapped on Pan genome revealed presence on ARGs on core genes and Accessory genes. Phylogenetic comparison between core gene and accessory gene allowing discrimination between lineage-based divergence and traits acquired from Horizontal gene transfer. Strains were categorized based on their isolation source shows *A. faecalis* is widely distributed in Environment, also revealed host associated strains. This study investigated phylogenetic relationship and distribution of the genes responsible for Antibiotic resistance, involving in adaptation distribution and in pan genome analysis.

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List of Abbreviations

1. Abbreviation Full Form
2. A. faecalis -Alcaligenes faecalis
3. ABC ATP-Binding Cassette
4. AMR Antimicrobial Resistance
5. ARG Antimicrobial Resistance Gene
6. ATCC American Type Culture Collection
7. bp Base Pair
8. CARD Comprehensive Antibiotic Resistance Database
9. CDS Coding DNA Sequence
10. CLSI Clinical and Laboratory Standards Institute
11. DNA Deoxyribonucleic Acid
12. GI Genomic Island
13. HGT Horizontal Gene Transfer
14. iTOL Interactive Tree Of Life
15. MDR Multidrug Resistance
16. Mb Megabase
17. NCBI National Center for Biotechnology Information
18. ORF Open Reading Frame
19. PCR Polymerase Chain Reaction
20. RAST Rapid Annotation using Subsystem Technology
21. REALPHY Reference Sequence Alignment-Based Phylogeny Builder
22. RGI Resistance Gene Identifier
23. RNA Ribonucleic Acid
24. rRNA Ribosomal RNA
25. SNP Single Nucleotide Polymorphism
26. SSTI Skin and Soft Tissue Infection
27. tRNA Transfer RNA
28. UTI Urinary Tract Infection
29. XDR Extensively Drug Resistant

CHAPTER 1

INTRODUCTION

1.1 Background

Comparative genomics is the comparison between genomes of different species or different strains of the same species. It involves a comprehensive systematic approach using a variety of computational tools for comparison. It is argued, however, that a genome taken in isolation from a single organism doesn't reveals much by itself as it reveals if studied in comparison with another one that'swhy this comparative genome analysis have been done to identify the antibiotic resistance genes involved in that particular retrieved genomes. In the case of prokaryotes, which are single-celled organisms lacking a well developed nucleus, a comparative genomics provides valuable information about their genomic diversity, adaptation, and the molecular basis of their biological traits. This resistance creates complication in the treatment of infectious diseases that can also increases healthcare costs, and higher mortality rates which needs to get under controlled. There are mainly two major ways to bacteria become resistant to antibiotic: Intrinsic resistance and Acquired resistance. Certain bacteria naturally possess intrinsic resistance while acquired resistance occurs through mutations or acquisition of new genes from other organisms. Processes like transformation, transduction, and conjugation, horizontal gene transfer(HGT) plays significant role in spread of Antimicrobial resistance genes. Mobile gene element like plasmids, integrons, transposons also help spread and integrate these resistance genes among various bacteria. Comparative genomics being a powerful tool for understanding the genetic basis of antibiotic resistance. It also help to identification of both accessory and core genes within species. Comparative genomic analysis is especially useful for determining strain-specific resistance patterns of antibiotic resistance genes in *Alcaligenes faecalis*.

Therefore, a comprehensive comparative genomic approach is needed to examine the distribution, diversity, and evolution of antibiotic resistance genes in *Alcaligenes faecalis.* Such research advances our knowledge of phylogenetic relationship and their relativeness to antibiotic resistance gene pattern in different strains.

1.2 About *Alcaligenes faecalis*

Alcaligenes faecalis, from the Alcaligenaceae family is a rod-shaped, aerobic motile, gram-negative bacteria. It has extensive distribution in a range of habitats such as soil, water, sewage and plant-associated niches, which demonstrates its high ecological adaptability. *A. faecalis* is reported as opportunistic pathogen that associated with infections including infection like pneumonia, urinary tract infection, bacteremia[1] and extensive drug resistance (XDR)[2]. Previous studies revealed its biotechnological[3] and industrial potential which comprises pollutant biodegradation[4], toxic metal tolerance that involves metal resistance genes like *mer[5], [6],* found on genomic islands shared with antimicrobial resistance gene, plant growth promotion, also has biodegradation capacity[7]. It is also involved in induction of clinical infection associated with breach of physical barriers shows opportunistic behaviour in immunocompromised host and its antimicrobial resistance[8]. The inclusion of host associated clinical strains such as MUB14 and NY11312 highlights the role of these strains in emergence of multidrug resistant infections and hospital-associated diseases burden while also offering crucial insight into genomic characteristics of antimicrobial resistance in *Alcaligenes faecalis.* PGB1 environmental strain isolated from penicillin waste drugs shows resistance to penicillin and β- lactam antibiotic which is primarily mediated by β-lactamase production, Antibiotic efflux system, plasmid mediated aminoglycoside resistance gene revealed by genomic analysis of strain PGB1[9]. Prolonged exposure to antibiotic promotes strong selective pressure favouring survival and adaptation among resistant strains, as widely documented in antimicrobial resistance studies.

1.3 Research gap

Although antimicrobial resistance has been studied in major clinical pathogen but Alcaligenes faecalis is being underexplored in the level of Antibiotic resistance genes. The following research gaps which i have identify is listed:

- Comparative genomic analysis of multiple strains of Alcaligenes faecalis
- Distribution and diversity of Antibiotic resistance genes among strains.
- Pan genome analysis to identify genes contributing to resistance
- Correlation of evolutionary pattern and resistance profile.
- Host associated strains and their clinical relevance study.

1.4 Objectives

This study aims to investigate the antibiotic resistance profile of Alcaligenes faecalis by comparative genomics approach. The objectives have listed below:

- To retrieve and analyse complete genome of 24 strains of Alcaligenes faecalis
- Genome annotation for identification of coding sequence and functional genes.
- To identify and compare Antibiotic resistance genes with 24 strains using database.
- To perform pan genome analysis to identify genetic makeup in core and accessory genes.
- To study evolutionary relationship among strain through core genome phylogenetic tree construction
- To correlate genomics features with habitat and with resistance profile.

1.5 LITERATURE REVIEW

1.5.1 Antimicrobial Resistance Mechanisms

There are various Antibiotic Resistance mechanism are involve that allow bacteria to survive in exposure of various antibiotic through a variety of complex biological processes. The primarily mechanism is Enzymatic Activation of antibiotics where enzyme degrades antibiotic agents make them ineffective. Intrinsic resistance to beta-lactam antibiotic is demonstrated by A. faecalis through generation of class beta-lactamase that hydrolyses carbenicillin (CARB-type enzyme). In PGB1 strain, penicillin resistance is mediated through beta-lactamase[9]. Acetyltransferases, adenylyltransferases, and phosphotransferases that neutralize aminoglycosides like streptomycin and neomycin are encoded by genes like aac, aad, and aph. There are active efflux pumps are involved that actively expels antibiotics from th cell. The RND family exhibits most significant efflux system in A. faecalis that exports antibiotics including fluoroquinlone, tetracycline and beta-lactams[10]. Transporters such as teta are involved in transporting tetracycline out of cell thus exhibiting antibiotic resistance. In clinical strains MUB14, NY11312, some mutation can be seen which alter molecular target of antibiotics. Mutations in DNA gyrase, tropoisomerase, RNA polymerase can confer resistance to antibiotics such as fluoroquinolone, rifampicin. ARGs are often associated with MGE facilitating their transfer between strains.

1.5.2 Previous Studies

Neonatal bacteremia was one of the earliest and most important epidemics in which *A. faecalis* was a causative agent[11]. Several bloodstream infections in newborns were previously reported in which the organism led to septicemia and resulted in high mortality rates, owing to the lack of therapeutic options and antimicrobial resistance. Neonates are at increased risk due to their immature immune system, long hospital stays, and exposure to contaminated hospital equipment[11]. *A. faecalis* has proved to be resilient in moist hospital environments, including respirators, intravenous fluids and hemodialysis systems, which enabled nosocomial transmission during these infections[12]. It is also correlated with bacteremia, meningitis, endocarditis, pneumonia, urinary tract infections (UTIs), peritonitis, endophthalmitis, otitis media, diabetic foot infections, and skin and soft tissue infections (SSTIs)[8]. The contamination of medical devices and aqueous environments in hospitals is a significant source of HAIs. The severity of the infection and the implications of multidrug resistance (MDR) have made *A. faecalis* infections a growing concern. The clinical study was a retrospective study with pneumonia being one of the main categories of infections, with some patients developing into severe pneumonia following previous antibiotics use and polymicrobial infection[2]. The necropsy revealed a large number of lesions in the lungs, septicemia and multiple organ involvement, highlighting the invasive pathogenic capabilities of the organism. Several cases of SSTI with vascular ulcers, chronic ischemia, postoperative wounds, and diabetic complications were reported[8]. The majority of patients had predisposing factors like any recent surgery, chronic vascular disease, or prolonged exposure to contaminated water or moist environment. The authors stressed that *A. faecalis* should not be considered as a contaminant as it may act as a true pathogen, able to cause persistent and hard to treat infections. They are also resistant to many of the antibiotics we are routinely using, which makes treatment more difficult, particularly in the case of chronic wound infections. Another significant symptom is a UTI (urinary tract infection). It is found that UTI was one of the most common infections reported *with A. faecalis*, especially among the elderly with neurological comorbidities, catheterization and obstructive uropathy[2].

Multidrug-resistant (MDR), extensively drug-resistant (XDR) *of A. faecalis* are a significant threat to infections with this organism[10]. Multiple studies have reported increasing resistance to *β-lactams, carbapenems, aminoglycosides, fluoroquinolones, and cephalosporins.* Some resistance mechanisms involve the production of β-lactamases, efflux pumps and acquisition of resistance genes via horizontal gene transfers[9]. Resistance to almost all the antibiotics tested has been shown by some clinical isolates, limiting treatment and leading to poor clinical outcomes. In general, literature reveals that *Alcaligenes faecalis* is no longer just an environmental commensal but an emerging opportunistic pathogen of clinical relevance.

CHAPTER 2

MATERIALS AND METHODS

2.1 Data Collection

A total 24 genome of *A. faecalis* has retrieved from National Centre for Biotechnology Information (NCBI)[13] genome database. Collected sequence with filter of complete or Chromosome level assembly and their associated data like GC content, isolation source, size and total genes were recorded. Based on their isolation, strains has categorized into Environmental or Clinical category. These sequences were then curated for downstream comparative analysis.

2.2 Genome Annotation

All recovered draft genome sequence in Fasta format were subjected for functional annotation using RAST server[14]. Coding sequence, tRNA count, protein sequence and subsystem based functional assignments were generated by annotation pipeline. The annotated .faa, .gbk files were used for subsequent analysis. Orthologs were identified with eggNOG-mapper v2[15] using annotated protein files as input.

2.3 AMR Gene Identification

To identify Antimicrobial resistance genes, genomes were screened against CARD- RGI[16] and ResFinder[17], allowing constructed of a curated resistome dataset. A non-redundant list of AMR genes for every strains was created by manually curating outputs from both the databases. A binary presence-absence matrix has prepared with distribution of genes among these strains, which was further used for comparative analysis and visualization by generating heatmaps.

2.4 Pan-genome Analysis

We conducted pan-genome analysis to analyse core and accessory gene using Roary[18], [19] with annotated GFF3 files using minimum percentage identify for blastp-95 at default parameter. Output files was further used for phylogenomic based on accessory and core genes.

2.5 Phylogenetic Analysis

In order to determine evolutionary relationship among 24 strains of A. faecalis phylogenetic tree is constructed using whole-genome based approach implemented in REALPHY. The tree was visualized and annotated using iTOL[20] from the obtained result. Resulted dataset from Roary was used to generate phylogenetic trees based on core and accessory gene to compare lineage-based evolution with gene content variation among strains.

2.6 Genomic island analysis

Genomic islands were predicted using IslandViewer 4[21] by multiple methods such as SIGI-HMM, IslandPick and Island-Path-DIMOB. Compared distribution of AMR among different strains. Circular map was created using proksee.

CHAPTER 3

RESULTS

3.1 Genome Statistics

A total 24 complete genome of *A. faecalis* were analyzed for comparative genome analysis. Their size vary from 3.9 to 4.6 Mb from which strain CAB20, J481 has smallest size and strain NY11312 has largest size among these strains. Their GC% lies in a range of 55% to 57%. Among all of strains, MUB14 has highest number of chromosome which is 3. The strains has categorized into environmental and clinical strains, based on their source of isolation. *A. faecalis* prominently contain environmental strains but few strains such as NY11312 and MUB14 are host associated clinical strains and *A. faecalis subsp faecalis* ATCC8750 isolated from Pure culture has referred as Reference strain. Total gene count ranged from 3700 to 4200 genes. These variations point to moderate level of genomic plasticity which is characterized of opportunistic bacteria that can adapt to various environments. Previous study reveals, strain PGB1 which is isolated from penicillin waste dregs exhibits metabolic adaptation to degrade Beta-lactam antibiotics[9]

3.2 AMR Gene Analysis

Comparative resistance analysis among total 24 strains reveals their AMR distribution. Genotypic Antimicrobial resistance profile of strains showed in Fig. 3. The *adeF* and *qacG* are present in almost strains. Strains including BDB4, Mc250, J481 has only one antimicrobial gene. There are almost all AMR genes that are present in clinical strain excepts genes *catB3*(chloramphenicol acetyltransferase B3) and *aac(6')-31*(Aminoglycoside 6'-N-acetyltransferase 31) which is only present in strain ZD02. Predominant enrichment of AMR gene in clinical isolates indicating their strong exposure to antibiotics(*Beta-lactams, Aminoglycosides, Sulfonamides, Phenicols, Tetracycline*). Environmental strains has comparatively reduced but distinct ARGs including *catB3, aac(6')-31, AAC6'-ib9, sul2, floR, tetA, aph(3'')-ib* suggesting their ecological adaptability to their polluted, aquatic, soil-surfaced environments include antibiotics(*Tetracycline, Chloramphenicol*). This presents environmental isolates retain functionally diverse adaptive resistome shaped by ecological pressure while clinical strains exhibits higher and clinical significant ARGs.

Table. 1 GENERAL CHARACTERISTIC OF GENOME AMONG STRAINS

S No.	Strain	Isolation	Category	Size (Mb)	GC %	tRNA	Total Gene
1	c16	Wastewater Sludge	Environmental	4.3	56.5	57	3959
2	DSM 30030	Soil Surface	Environmental	4.1	56.5	57	3782
3	DY-8	Cadmium-polluted paddy soil	Environmental	4.2	56.5	57	3845
4	NY11312	Host Homo Sapiens	Clinical	4.6	57	56	4252
5	D334	Mangrove	Environmental	4.2	56.5	57	3859
6	FDAARGOS_ 491	Environment	Environmental	4.1	56.5	57	3778
7	FDAARGOS_ 1024	Unknown Source	Unknown	4.1	56.5	57	3756
8	J481	Salt marsh sediment	Environmental	3.9	55.5	58	3561
9	JF101	Undersea Mud	Environmental	4.1	56.5	57	3815
10	JLAF9	Chicken Manure	Environmental	4	56.5	57	3,734
11	JQ135	Wastewater	Environmental	4.1	56	58	3723
12	MUB14	Host Associated	Clinical	4.5	57	58	4082
13	P156	Surface Soil	Environmental	4	56.5	57	3798
14	SCAU 6	Sludge	Environmental	4.1	56.5	58	3795
15	SCSIO B001	Marine	Environmental	4	57	56	3683
16	ATCC8750	Pure Culture	Reference Strain	4.1	56.5	57	3902
17	T17	Sediment	Environmental	4.3	56.5	58	3961
18	ZD02	Nametode	Environmental	4.2	57	56	3845
19	AU14	Wheat Roots	Environmental	4.2	56.5	50	3857
20	BDB4	Soil Surface	Environmental	4.2	57	54	3582
21	CAB12	Marine environment	Environmental	4.1	56	59	3629
22	Mc250	Rhizosphere	Environmental	4.2	56.5	57	3834
23	PGB1	Penicillin waste dregs	Environmental	4.4	56.5	57	4054
24	CAB20	Marine environment	Environmental	3.9	56	58	3559

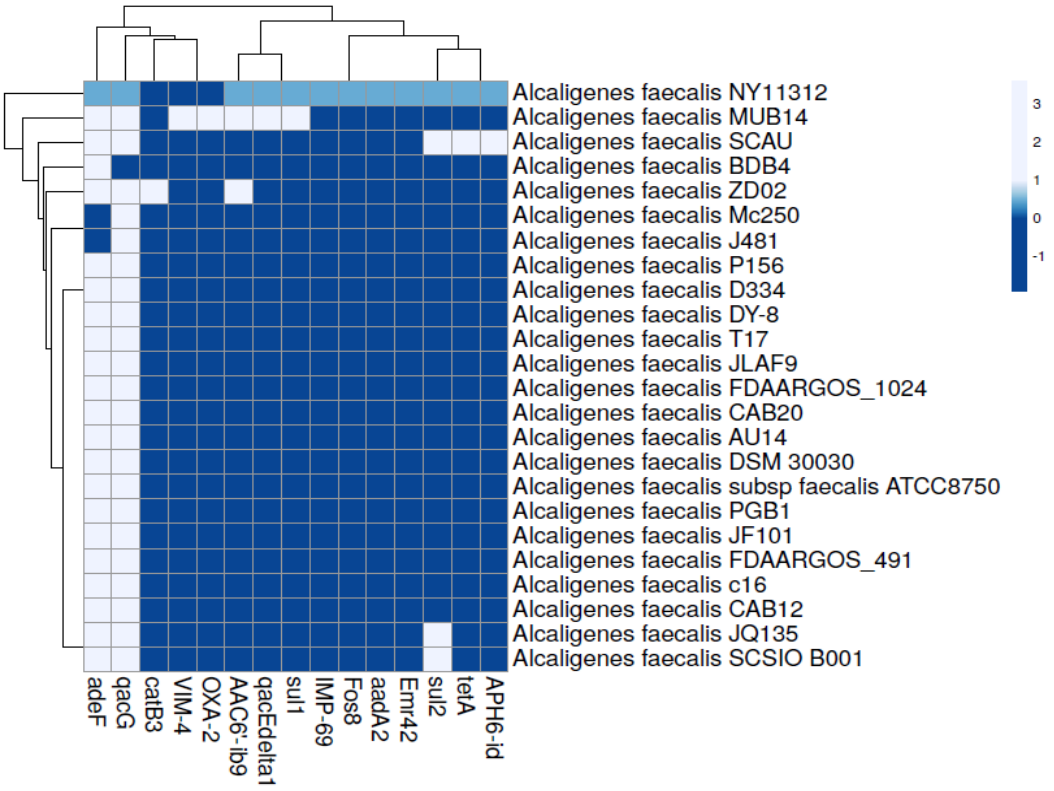

Fig. 1. Heatmap constructed by using absence-presence matrix from the data collected using CARD-RGI. The blue block shows absence of Antimicrobial Resistance Gene(ARGs) and white block represents presence of ARGs.

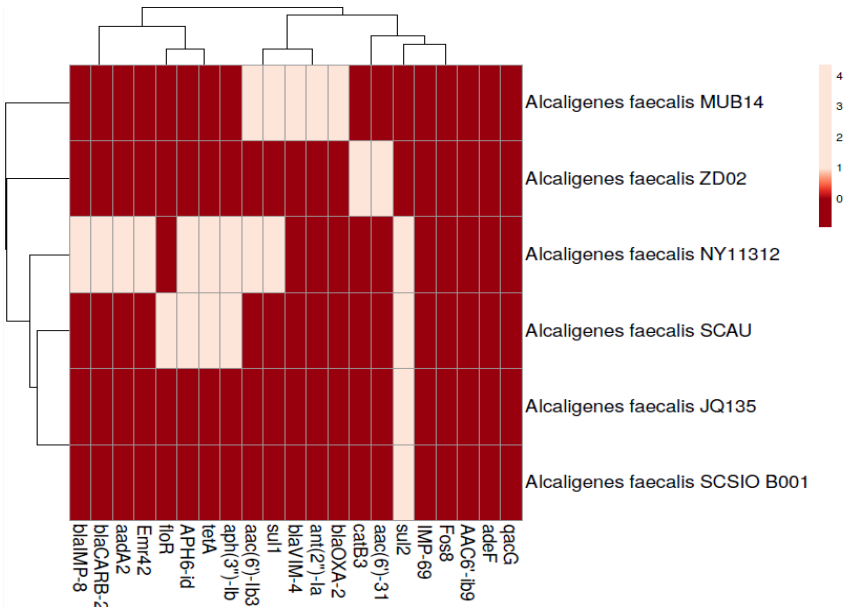

Fig. 2. ResFinder data based Heatmap constructed. The Red block shows absence of ARGs and orange one shows presence of ARGs.

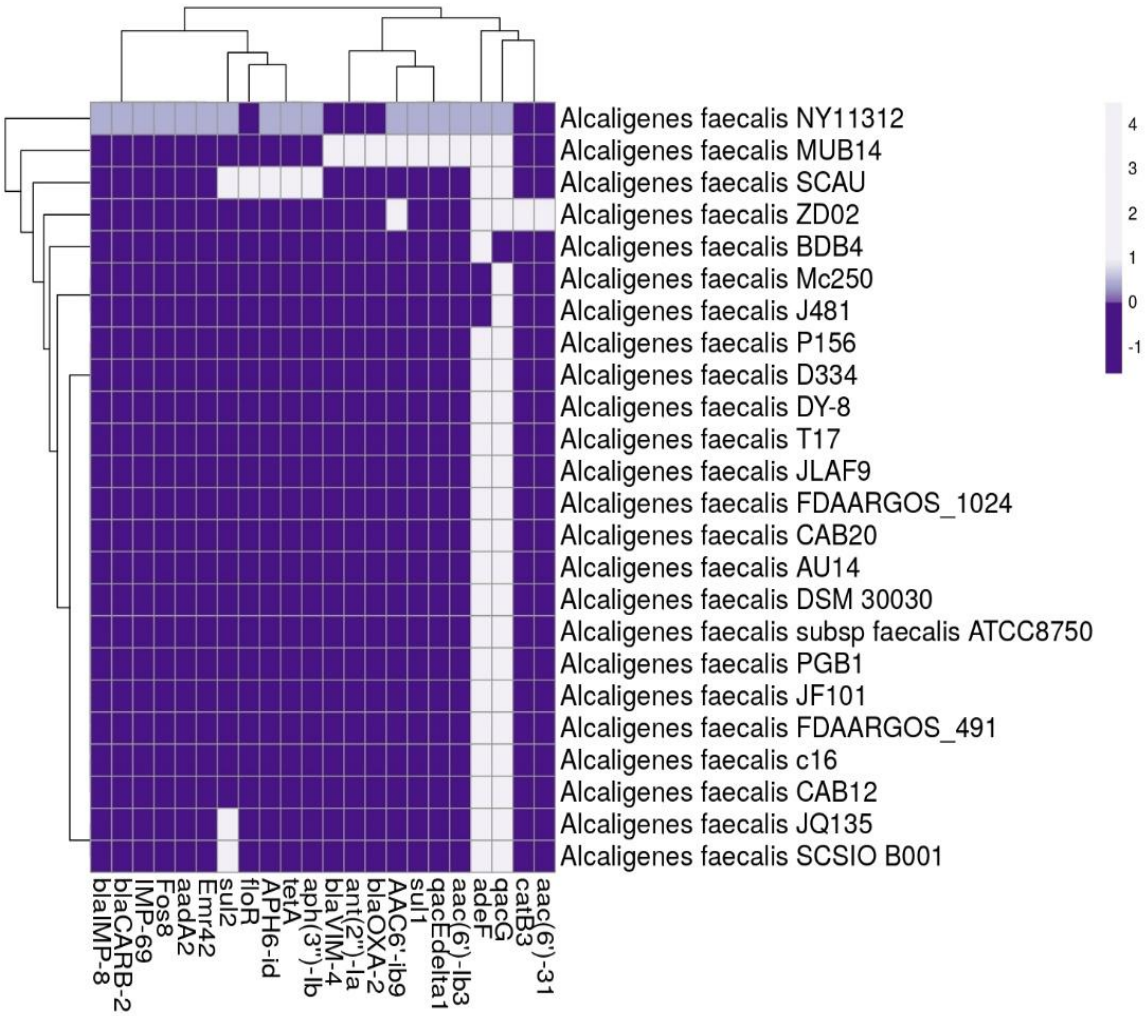

Fig. 3. Distribution of Antimicrobial Resistance Genes among all 24 strains. Grey block represents presence of ARGs and Violet block represents absence of ARGs.

3.3 Determination of Core and accessory gene variations

Pan-genome analysis was conducted to determine ARGs distribution and to study their genetic diversity, adaptive potential. Its expanding gene repertoire indicating open-genome architecture. It helped us to identify core and accessory genes. Core gene typically being present >=99% of strains, shared across all strains, generally involves in metabolism, transcriptional regulation and essential cellular processes. In contrast, accessory gene being unique gene found in only few strains, usually provides organism selective advantage to adapt in environment. Pan-genome genes distribution in *A. faecalis* has presented in Fig. 4. where core gene comprise 1907 gene count while accessory gene comprise 9612 gene count. ARGs has classified into core or accessory gene category on the basis of gene frequency in Table(1). It demonstrated that majority of ARGs encoding Beta-lactamase(*blaIMP, blaVIM, blaOXA variants*), aminoglycoside-modifying enzymes(*aad,aac,aph*) and sulfonamide resistance(*sul1, sul2*) were distributed among strains that indicates the acquisition through horizontal gene transfer. Core ARGs suggest intrinsic resistance whereas accessory ARGs suggest acquired resistance. From Table. 2 we can easily see large fraction of accessory ARGs and distribution of accessory gene from Fig. 3. which highlights evolutionary plasticity of *A. faecalis* and its ability to adapt into diverse environment.

TABLE 2 – CLASSIFICATION OF ANTIMICROBIAL RESISTANCE GENE BASED ON CALCULATED GENE FREQUENCY

ARGs	Isolates present	Total Isolates	Frequency	Classification
EmrA	24	24	1	Core
qacG	23	24	0.96	Soft-core
adeF	22	24	0.92	Soft-core
sul2	4	24	0.17	Accessory (shell)
AAC6'-ib9	3	24	0.13	Accessory (cloud)
tetA	2	24	0.08	Accessory (cloud)
sul1	2	24	0.08	Accessory (cloud)
qacEΔ1	2	24	0.08	Accessory (cloud)
aadA2	1	24	0.04	Accessory (cloud)
VIM-4	1	24	0.04	Accessory (cloud)
IMP-69	1	24	0.04	Accessory (cloud)
OXA-2	1	24	0.04	Accessory (cloud)
catB3	1	24	0.04	Accessory (cloud)
Fos8	1	24	0.04	Accessory (cloud)
Emr42	1	24	0.04	Accessory (cloud)

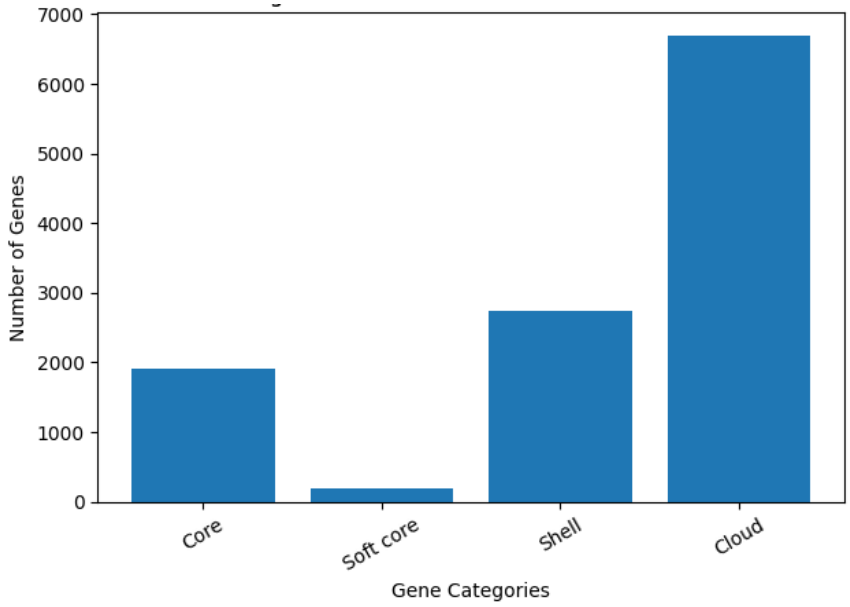

Fig. 4. Pan-genome gene distribution *of A. faecalis*

3.4 Phylogenetic Analysis

Phylogenetic tree has constructed using whole-genome data, core genome alignment and accessory genome alignment to study evolutionary relationship among strains. Whole genome based tree has formed and demonstrated in Fig. 5. It shows evolutionary relationship against whole genome including both conserved and variable gene content, resulting in cluster formation. Conserved isolates that remained clustered in all trees includes DSM30030, susp. faecalis ATCC 8750, FGAARGOS 491, FGAARGOS 1024 suggesting genomic stability and limited divergence. Core-genome based tree has constructed, shown in Fig. 6. It displays strong conservation among strains such as ZD02, PGB1, MUB13, SCAU that reflects vertical inheritance of conserved genes. The tree reflected the relationship of strain based on core-gene that being conserved into the lineage and being evolutionary backbone. It exhibits more stable topology and formed cluster based on shared conserved and essential genes. Accessory-genome based tree presented in Fig. 7. that shows gene acquired from environment. The tree showed adaptive pattern and greater separation between strains due to gene acquisition or loss. It captured adaptive divergence exhibited by ecological pressure. Accessory gene tree elucidates highest divergence among strains BDB4, JF101, D334, AU14, c16 and Mc250 that appears more dispersed.

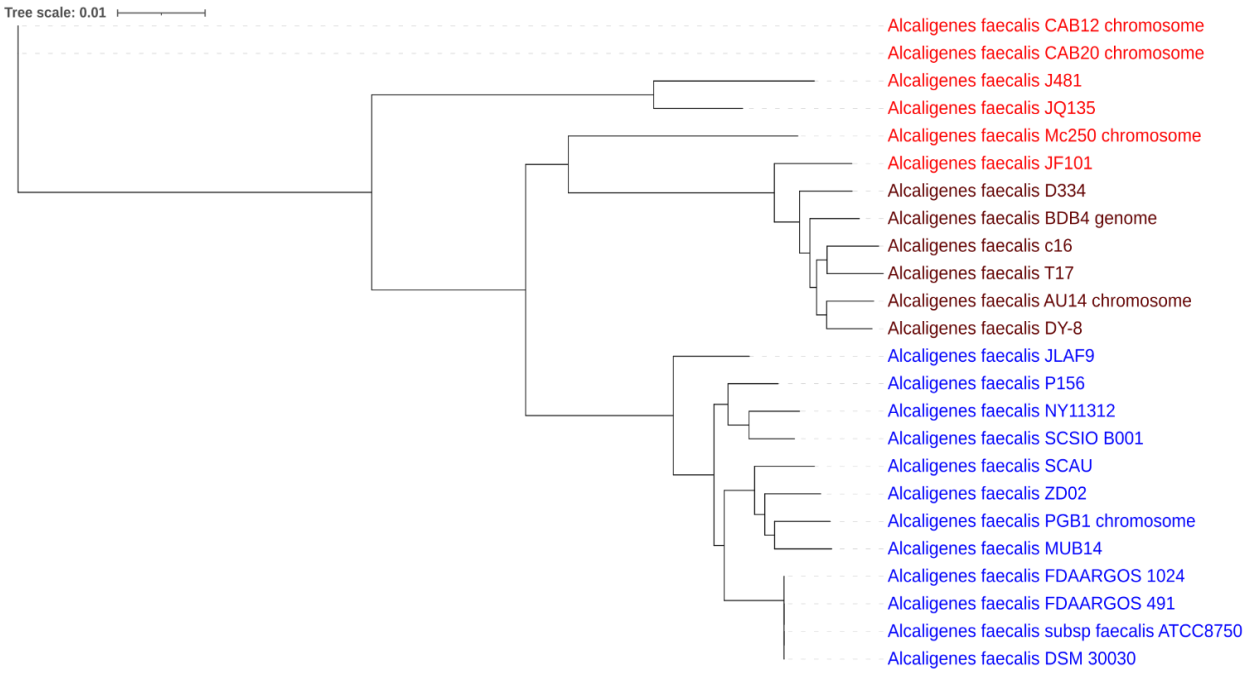

Fig. 5. Phylogenetic Tree constructed based on whole-genome data

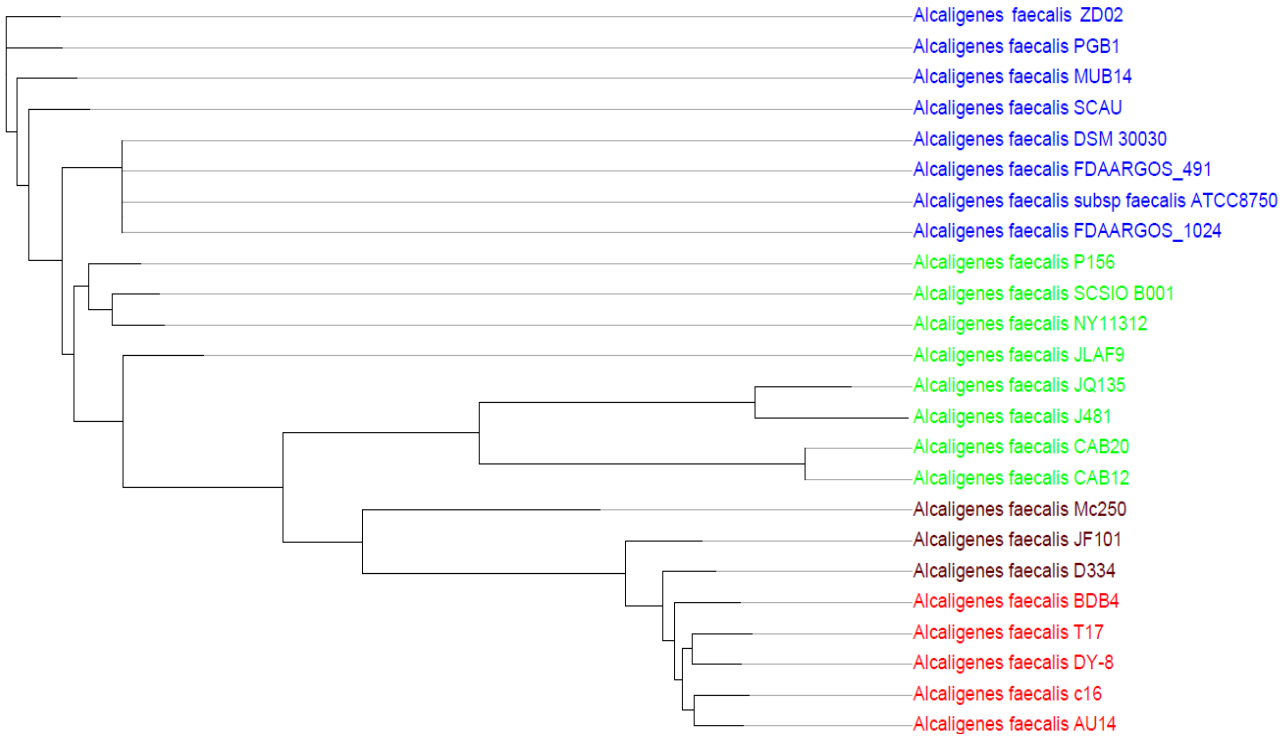

Fig. 6. Core-genome based Phylogenetic Tree

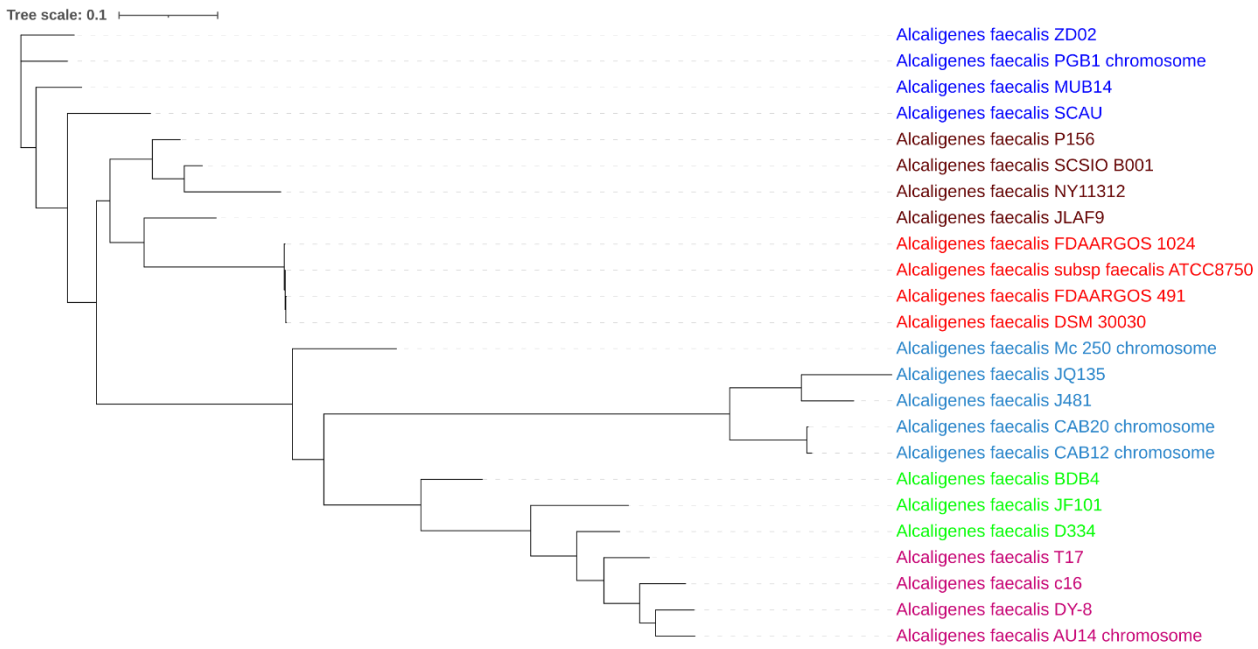

Fig. 7. Accessory genome alignment based Phylogenetic Tree.

3.5 Detection of Genes in Genomic island

Genomics islands of *A. faecalis* has predicted using Island viewer that identified most of ARGs in the genomic island. The majority ARGs were localized and there were significant presence of enzymes like Integrase, translocase, recombinase indicating that horizontal gene transfer shaped the resistome within these strains as shown in Table 3. There were a limited number of ARGs such, *adeF* (an RND efflux pump component) and *qacG* conferring quaternary ammonium compounds, found outside the genomic island. Presence of *adeF* and *qacG* outside the genomic island and their presence in almost all the strain suggesting its intrinsic resistance and represent a stable gene linked to long-term environment adaptation. Co-localization of resistance gene with functional component such as efflux system(*RND, Multidrug*), Multidrug resistance or transporter and stress related response gene. Indicating multifunctional adaptation. *A. faecalis* strains also have shown phage acquisition that may be crucial in protecting them from environment stress. Genomic island analysis has demonstrated ARGs localized within genomic island, that has aquired from the environment through horizontal gene transfer. The presence of ARGs outside genomic island suggests intrinsic resistance from prolonged exposure, also helps us distinguishes between intrinsic mechanism and adaptive resistance from environment, highlighting deeper insights of genomic and evolutionary mechanism among strains.

TABLE 3. GENOMIC ISLAND PREDICTION AND ITS DISTRIBUTION

S No.	Strain	Antimicrobial Resistance	Recombinase	Tranposase /integrase	Multidrug resistance	Multidrug Efflux system
1	c16		5	9	7	8
2	DSM 30030		4	7	7	8
3	DY-8		4	6	6	8
4	NY11312	tetA, aph(3")-ib, APH6-id, Sul2, fos8	4	23	10	12
5	D334		5	8	5	12
6	FDAARGOS_ 491		5	12	7	8
7	FDAARGOS_ 1024		4	10	8	8
8	J481		5	13	6	4
9	JF101		4	12	6	10
10	JLAF9		5	13	8	8
11	JQ135	sul2	4	15	8	4
12	MUB14	AAC6'-ib9, qacEdelta1, aac(6')-Ib3, sul1, ant(2")-Ia, blaOXA-2, blaVIM-4	5	20	8	10
13	P156		4	5	8	7
14	SCAU 6	tetA, aph(3")-ib, APH6-id, Sul2, floR	4	5	6	10
15	SCSIO B001	sul2	3	9	6	10
16	ATCC8750		5	9	8	8
17	T17		7	6	7	8
18	ZD02	AAC6'-ib9	3	10	8	10
19	AU14		1	4	6	9
20	BDB4		2	1	8	10
21	CAB12		3	3	7	5
22	Mc250		6	7	8	7
23	PGB1		4	4	7	10
24	CAB20		3	3	8	5

CHAPTER 4

DISCUSSION

4.1 Categorisation based on Isolation and Adaptative gene distribution

This analysis provides categorization of strain based on their isolation, that presents a view of how these strains adapts to their relative ecological niches through gene acquisition, focusing on Antimicrobial resistance gene. Fig() clearly shows that clinical strain contain high number of ARGs, particularly beta-lactamases(*blaIMP, blaVIN, blaOXA*)and aminoglycoside modifying enzymes. The enrichment of those genes indicates clinical strains have evolve to endure environments characterized by frequent antibiotic use, resulting in accumulation of specialized resistance determinants. Environment isolates exhibits unique genomic profile, showing gene linked to broad spectrum stress tolerance and metabolic flexibility. Genes like *tetA, adeF and Emr42* were more common among them which encodes for efflux systems, that can expels wide range of harmful substance, such as heavy metals, pollutants and antibiotics. Occurance of *floR* implies adaptation to environment where agriculture or aquaculture practices are common. Also, the distribution of qac variants that give resistance against disinfectants and biocides pointing to environmental strains survibility in contaminated and chemically complex habitat.

Intrestingly, there was some overlap in genes that was evident between two groups. This suggests that environmental strains may hold adaptive genes, including ARGs that can potentially based on clinical strains highlighting possible ecological link between them. Overall, this implies that clinical strains adopt distinct yet synergistic adaptive strategies with specializing in antibiotics resistance and environmental strains possesses versatile low specificity mechanism that maintain broader ecological fitness where bacteria encounters fluctuating stress rather than consistent antibiotic pressure.

4.2 Resistome profile

To study distribution pattern of ARGs among strains, heatmaps were generated using CARD-RGI, ResFinder and combined gene matrix. This resistome profile reveals diverse, distinct yet uneven distribution pattern of Antimicrobial resistance genes.

Gene distribution shown using RGI Fig. 1. which predicts resistomes from nucleotide or protein data according to homology and SBP models. It composed both conserved and variable resistance determinants indicating combination of intrinsic and acquired mechanism. Genes including*, adeF and Emr42* were prevalent across strains highlighting their function as part of intrinsic resistome that contribute to adaptability. The co-occurrence of *sul1 and qacE1* indicates integron-associatesd gene clusters while genes like catB3 and Fos8 implies recent acquisition. ResFinder finds acquired genes and chromosomal mutation that cause antibiotic bacterial resistance in total DNA sequence . ResFinder generated heatmap Fig. 2. reveals key resistance genes such as Beta-lactamases and aminoglycosides resistance genes were unevenly distributed and concentrated among strains including ZD02 and MUB12. Some strains such as SCSIO B001 and JQ135 exhibits comparative low ARGs count, suggesting lower exposure to antibiotics.

The combined gene matrix heatmap Fig. 3. integrating CARD and ResFinder resultants comprises both intrinsic and acquired resistance determinants. The clustering of ARGs in clinical strains indicates their rapid acquisition which is driven by HGT, facilitating adaptation in selective antimicrobial pressure. Stains including MUB12, NY11312 , and ZD02 exhibits higher density of ARGs tends to cluster together forming distinct resistome cluster, whereas some strains exhibits low resistance highlighting ongoing evolution of resistance.

4.3 Pan-genome analysis and Phylogenetic findings

Phylogenetic analysis reveals layered evolutionary structure and their relativeness shaped by both conserved and variable gene acquisition. The whole-genome phylogenetic tree which represents an overall pattern shaped by integrating both conserved and variable genome regions. Fig. 5. tree also presents an intermediate topology. Conserved cluster was identified such as ATCC8750, FDAARGOS and DSM30030 strain reflect their stable lineage. Core-genome Tree shows highly conserved pattern within genome reflecting relationship based on conserved house keeping gene. Closely grouped strains with minimal branch divergence include strain like ZD02, MUB14, SCAU and PGB1. These strains reflects their close ancestry and divergence through gene acquisition. Common divergence was observed from accessory and whole-genome tree that aligns with resistome distribution pattern, it indicates to acquisition of resistance gene contributes significantly to divergence. There was also found some consistent cluster includes strains like DSM30030, ATCC8750 that highlights stable lineage and forming conserved backbone.

4.4 Identification and prediction of Genomic island

To study Genomic island, this analysis conducted which gives us critical insight into adaptation of *A. faecalis.* The findings points that majority of ARGs are localized within genomic islands. Few genes such as *adeF and qacG* were found outside the GI suggesting that they are possibly part of core genome. These genes are also associates with efflux-mediated system indicating intrinsic role so they must be in long-term adaptive mechanism rather than recent acquisition. The GI predicted resistome pattern clearly align with accessory genome fraction hat suggests us resistance evolution and gene acquisition is driven mainly through mobile gene elements. Beyond ARGs, genomic island also exhibits genes that are also involve in stress response, efflux system, metal efflux and xenobiotic degradation reveals their involvement extends beyong ARGs. Phage related genes were also identified, highlighting their gene acquisition and involvement of prophage-mediated HGT that can contribute to genome plasticity.

Distribution of genomic islands presents on genomic circular map Fig. 8. It provides a comprehensive visualization of genome organization, presenting distribution of coding sequence, AMR determinants, RNA genes. Concentric rings demonstrate forward and reverse strand, coding sequence(CDS) with annotated features such as t-RN, r-RNA and origin of replication.

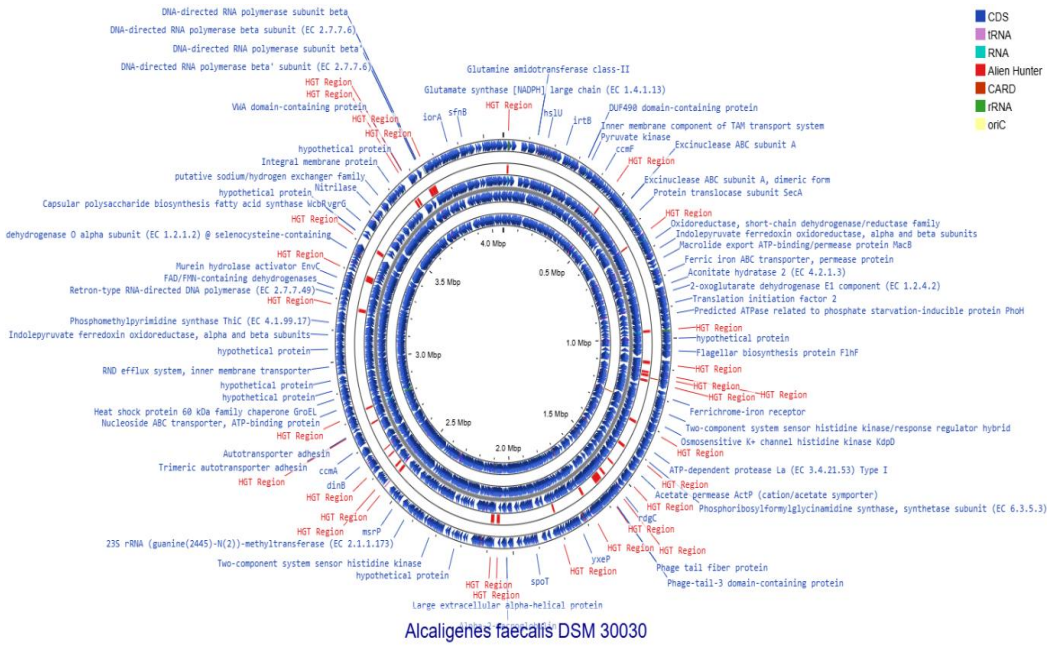

Fig. 8. Genomic circular map showing distribution of genomic island

CHAPTER 5

CONCLUSION

This study employs a comprehensive comparative genomic perspective on antimicrobial resistance in *A. faecalis*. AMR distribution pattern in strain reveals open pan-genome, highlighting its adaptability by acquiring new genes. Many ARGs have predicted to be acquired through HGT but small subsets of gene such as *adeF, qacG* were found outside genomic island indicating intrinsic resistance. Clinical isolates have higher density of clinical relevant antibiotic resistance indicates prolonged exposure of antibiotics. The clustering patterns of trees also suggested that strain with similar environment tends to converge in their resistome profile. Overall, this study elucidates ecological pressure have shaped evolving accessory genome of *A. faecalis*, resulting in antimicrobial resistance.

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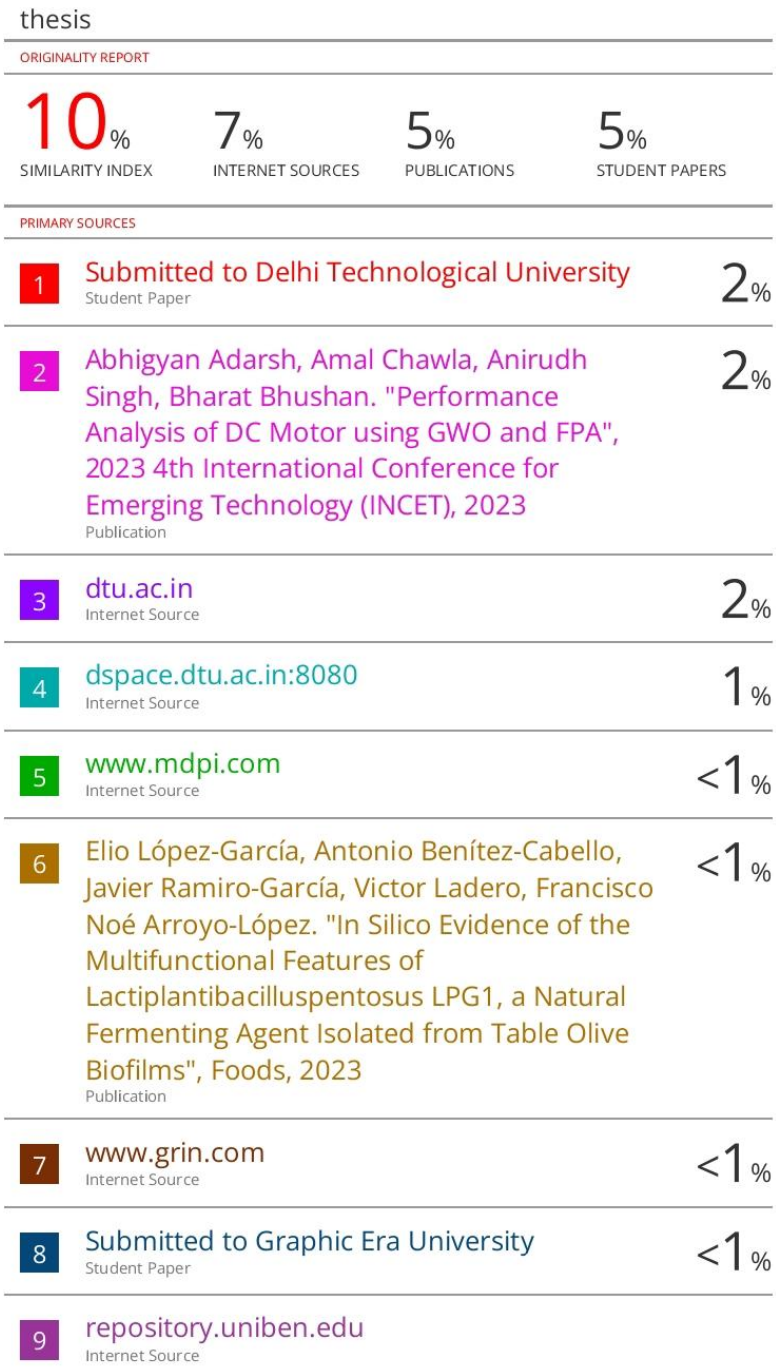

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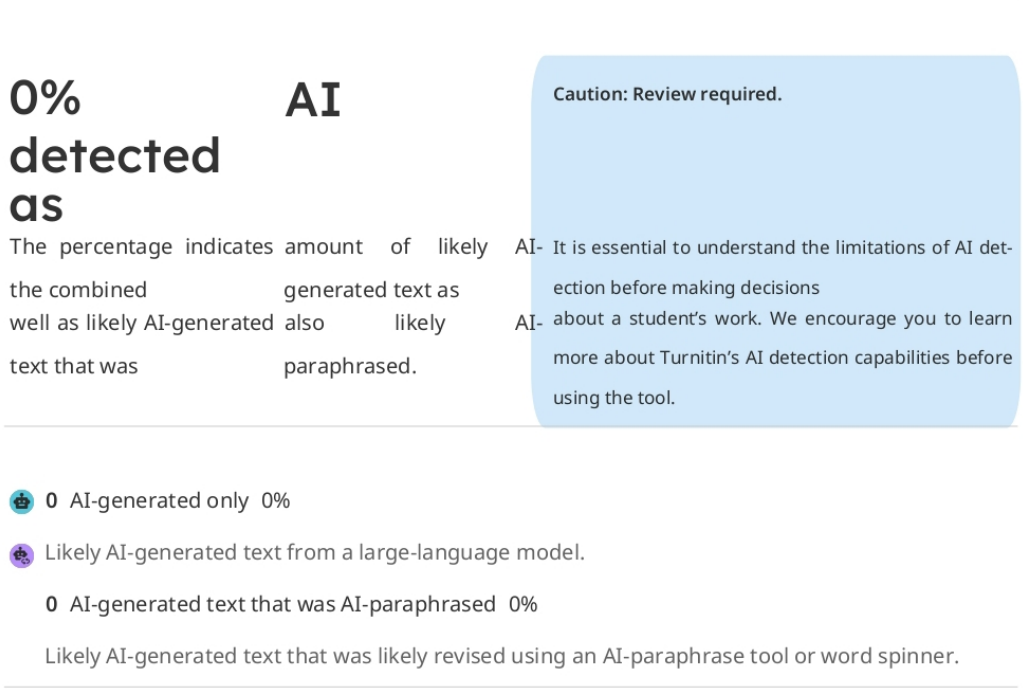
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