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Research Article

Structural and Functional Annotation of Ribosomal Protein S4 (*rpsD*) Gene in *Pseudomonas aeruginosa*

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ABSTRACT *Pseudomonas aeruginosa* is one of the opportunistic microorganisms that can cause severe infections mainly in the immunocompromised individuals, cystic fibrosis and patients with burn wounds. It has developed mechanisms like quorum sensing and antibiotic resistance that makes the antimicrobials ineffective on it. Therefore, it is necessary to develop new drug candidates with altered therapeutic targets that can combat the antimicrobial resistance. The ribosomal protein S4 (*rpsD*) gene is involved in the synthesis of structural proteins of the ribosomes (S4 protein). Ribosomes are crucial in protein synthesis that are of key significance for cellular processes in the cell. If there is no protein synthesis, this will ultimately lead to the death of the cell. That's why this gene have therapeutic potential when we think of a novel drug candidate against *Pseudomonas aeruginosa* infections. In this study, our focus is to structurally and functionally annotate the *rpsD* gene, analyzing it with different bioinformatics tools to get to know of its therapeutic potential. GEO2R is used for the analysis of gene expression profiles of treated and normal samples. There is the use of 2 GEO datasets; GSE27674 (Protoanemonin-treated vs. untreated) and GSE39044 (PA2449 regulation, treated vs. nontreated) for the identification of differently expressed genes involved in the *Pseudomonas aeruginosa* infection and to make it resistant. STRING and Cytoscape tools were used to check the protein-to-protein interactions. To elucidate the biological roles of differentially expressed genes (DEGs) pathway enrichment analysis (Gene Ontology and Kyoto Encyclopedia of Genes and Genomes) was done. The miRNA/transcription factor analysis and molecular docking had helped with analysis of therapeutic potential of the compound that showed complementarity with the target. GEO2R has shown that in infected sample there are 877 DEGs from GSE27674 dataset, 438 gene were upregulated out of the 877 DEGs, on the other hand GSE39044 data set has revealed 1607 DEGs out of which 803 were upregulates. Protein to protein interaction has revealed that there are 10 hub genes *RPSD*, *RMPE*, *RMPI*, *RPSU*, among others, important in the infection caused by *Pseudomonas aeruginosa.* The pathway enrichment analysis has let us identified that quorum sensing and ribosomal function are associated with prognosis of disease caused by *Pseudomonas aeruginosa*. The molecular docking has confirmed that PA4222 is a best target for a compound that shows complementarity to it.

KEYWORDS Pseudomonas, rpsD, annotation, docking, ribosomal protein

Introduction

Pseudomonas aeruginosa belong to gram negative group of microorganisms. It is characterized as opportunistic pathogen because it specifically infects immunocompromised individuals. It causes a lot of severe nosocomial infections, which includes bloodstream infections, UTIs (Urinary tract infections), lung infections in cyclic fibrosis patients and ventilator associated pneumonia (Lyczak *et al*, 2000; Driscoll *et al*, 2007). It is also associated significantly with burn wound infections, leading cause of disease in people undergoing chemotherapy or have Acquired Immune Deficiency Syndrome (Church *et al*, 2006). The global prevalence of *Pseudomonas aeruginosa* infections is alarming with around 10-15% hospitals acquired infections (Gales *et al*, 2001). *Pseudomonas aeruginosa* colonize the lungs of Cystic Fibrosis patients about 80% by adulthood, which leads to chronic lung damage and could be life threatening (Folkesson *et al*, 2012). It becomes challenging to treat with commercially available antibiotics as it has the ability to develop antibiotic resistance, engage in quorum sensing and form biofilms, which makes it global health concern (Davies *et al*, 1998; Bjarnsholt *et al*, 2009).

Pseudomonas aeruginosa have a diverse and large genome that ranges from 5.5 to 7 million bp, the proportion of regulatory genes in its genome is about 8-10 % that make it to survive into harsh and diverse environmental conditions (Stover *et al*, 2000). PAO1 strain is one of the reference strains and which is most studied and it has 6265 genes and these are involved in metabolic flexibility, antibiotic resistance and virulence factors (Winsor *et al*, 2016). In the genome of PA01, there is a rpsd gene and it is at locus PA0004. It is responsible for encoding of 30S ribosomal protein (S4) and it is of key significance for the mRNA binding and initiation of translation(Grundy and Henkin, 1991). If there are any mutations or downregulation of this gene, it can lead to the death of the cell that's why it can be a good therapeutic target (Lister *et al*, 2009). *Pseudomonas aeruginosa* is pervasive and developing resistant to multiple drugs, and resistance rate is 30-50% in some regions of the world (Li *et al*, 2005; Lister *et al*, 2009). The resistance mechanisms that evolved in bacteria are efflux pump, biofilm formation and the production of beta lactamase (Poole, 2011). The traditional method of treatment relies on the antibiotic such as tobramycin, colistin to counter the infection and to overcome the resistance they are administered in combinations(Elborn *et al*, 2016). Bioinformatics is a promising field that can help us to understand the gene expression, regulatory network and drug interactions to look for novel drug targets(Walsh, 2000). In this study, different bioinformatic approaches were utilized to understand the therapeutic potential of our gene of interest. Datasets were obtained from GEO (Gene Expression Omnibus) database which is a significant resource of High throughput expression data from the lab experiments of the researchers. This approach ranged from comparison of datasets to miRNA analysis and protein-to-protein interactions. This approach was followed by molecular docking to better understand the therapeutic potential.

Materials and Methods

Data extraction

For this study, data were retrieved from the GEO (www.ncbi.nlm.nih.gov/geo) database hosted by NCBI (National Center of Biotechnology Information). Two data sets (i.e. GSE27674 and GSE39044), from *Pseudomonas aeruginosa* infections treated and non-treated individuals, were selected and retrieved. Both datasets were originally studied from microarray analysis. The dataset GSE27674 had 18 samples (9 treated and 9 untreated) which studied effects of a natural quorum sensing inhibitor called Protoanemonin. The second dataset GSE39044 was produced from studies related to the impact of PA2449 transcriptional factors regulator. It had 12 samples such as 6 treated and 6 untreated. Thus, a total of 30 samples were included for downstream analysis.

Differentially expressed genes analysis

GEO2R platform was used for the investigation of gene expression profiles and to look for differentially expressed genes (DEGs) in datasets obtained earlier (Bayat, 2002). GEO2R interface comes under Gene Expression Omnibus web-based tool managed by NCBI. The lima algorithm, obtained via the Bioconductor project, was used to get the significant DEGs in the GSE39044 and GSE27674 datasets with an adjusted P-value < 0.05 and a log2 fold change (FC) ≥ 2. This log2 fold change (FC) ≥ 2 threshold level was deliberately set to get meaningful results. GEO2R platform illustrated the results via volcano plots and Venn diagrams.

Protein-protein interaction

Following the identification of DEGs, next step is to check their interactions. The main focus was to assess the physical interaction in the proteins expressed by genes. The STRING database is good to examine the physical associations and interaction between the proteins. To get the reliable and robust results confidence score of 0.9 was used which is a good fit to get significant results.

Gene Ontology (GO) and Pathway Enrichment Analysis

The gene profiler was used for the Gene Ontology and pathway enrichment analysis for differentially expressed genes. G: profiler gives pathway information and functional annotations derived from different biological databases such as Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO) etc. It provides accurate understanding of different biological processes such as molecular functions, and cellular processes etc.

MicroRNAs and Transcription Factors

The mirDIP (Micro RNA Data Integration Portal) was used to determine the interactions between DEGs and the miRNAs with the help of its relationship prediction module. This tool was further involved in identification of Transcription Factors (TFs). This tool is best suited for the analysis of post transcriptional regulations associated with DEGs. It derives data from 26 miRNA databases to derive comprehensive results.

Identification of Small Molecule Drugs

DGIdb (https://www.dgidb.org/) was used to investigate the small molecules that could be possible potential drug of choice for the DEGs. This tool retrieve data from >30 databases of drug gene interactions. It is best suited platform to screen out potential drugs can interact with DEGs from the datasets GSE27674 and GSE39044 datasets.

Molecular docking

Molecular docking is a significant step in the drug development. Following the identification of potential small molecule, the next step is to check the complementarity of potential proteins and small molecule. Patch Dock was used to screen the drug candidates which shows high binding affinity for the protein of interest. Molecular modeling give insight into the biochemical processes and behavior of the small drug candidate into the binding regions of the rpsD protein.

Results

Comparisons of experimental groups

GEO2R helped in identifying 1607 DEGs in GSE39044 and 877 in GSE27674 while P-value was adjusted to P-value (P_adj) < 0.05 and |log2FC| ≥ 1 (Fig 1). The DEGs with top P-values were selected for further analysis of the rpsD gene, ribosomal protein S4, can be a potential therapeutic target for the drugs. The volcano plot for GSE27674 has exhibited that there are 439 upregulated genes indicated by red dots and 438 downregulated gene out of 877 differentially expressed genes in response to treatment with protoanemonin. On the other hand, volcano plot for GSE39044 has suggested that there are 402 downregulated genes and 1205 upregulated having log values log2FC extending to +8 exhibiting that PA2449 regulation is associated with resistance mechanism. The nonsignificant genes in the volcano plot are represented by grey dots (P_adj ≥ 0.05 or |log2FC| < 1) around the x-axis. The Ven Diagram help to understand the Differentially expressed genes count. In case of GSE27674, 5023 genes were analyzed and out of which 877 were DEGs with no overlap seen in the comparison of treated and non-treated samples. For GSE39044, there was the analysis of 4293 genes out of which 1607 exhibited to be DEGs, without any overlap in expression, pointing out PA2449-regulated response.

Protein-Protein Interaction Network

Protein to Protein Interactions of the upregulated DEGs in GSE27674 and GSE39044 have given insight into the physical interactions and structural characteristics. STRING was used to get the PPI networks and visualized in Cytoscape to identify shared upregulated DEGs which includes rpsG, rpsJ, and rplL rpsd, and rpsE among others. The PPI network of GSE27674 upregulated genes have shown a highly linked structures with various hubs, exhibiting how bacteria adapt itself to antibiotics to develop resistance. In case of GSE39044 PPI network of 1205 upregulated genes have less dense and interlined connectivity pattern. So GSE27674 PPI network is intricate hub structure for the analysis of novel drug candidates. Maximal Clique Centrality method helped this study to identify the top 10 hub genes (Fig. 2). These genes are rpsd, rpsE, rpsG, rpsJ, rpsR, rpmI, rpmE, rpsU, rpmH, and rplL. In cytoscape a color gradient was applied to exhibit the Cytoscape hubs. Red color corresponds to high MMC score while yellow color reflects decrease in MMC score. MMC method is effective for the identification of highly connected genes in the large clique, underscoring their importance in biological processes. Gene rpsD have shown the highest score of 4354704, and 24 Node degree, exhibiting key role in the PPI network with greatest number of direct interactions. Other genes such as rpsE, rpSG, rpSJ, rpsR have slightly low score and node degree showing slightly less connections but still have significant connectivity. All these hub genes have central role in ribosomal protein synthesis and survival under stress conditions like antibiotics.

DEGs' gene ontology and pathway enrichment analysis

The CC method analysis pointed out that enrichment in HDL particle, showing involvement of acute phase proteins such as HDL particle and SAA1, a phenomenon driven by *Pseudomonas aeruginosa* induced inflammation, which is sustained by the production of bacterial virulence factors due to the involvement of the *rpsD* gene (Table 1). The KEGG pathway enrichment analysis further highlighted the involvement of interleukin 17 signaling pathway, interaction between the different cytokines via receptors, cell signaling in epithelial cells, chemokine signaling pathway, all of these pathways have the involvement of the DEGs. This analysis collectively illustrated that *rpsD* gene have crucial role in sustaining bacterial virulence and if we target this gene, we can combat the antibiotic resistance and look for the drug candidate which have more therapeutic potential in relevance to this gene.

Micro RNA, transcription factor, and DEG interaction prediction

Relationship among the DEGs and miRNA, transcription factors was determined using miRNet tools. To recognize interaction of miRNA and DEGs database was taken from mirDIP, however TRRUST was utilized to recognize the relationship between TFs and DEGs. Analysis thorough miRNet the interlinkage between these is visually representation, shows us the controlled adjustment effected by *P. aeruginosa* in A549 lungs cells, in GAE27674 dataset (Fig. 3). The hub genes such as *rpaD*, *rmpI*, and *rmpE*, indicated prominent significance appears as central nodes just by its immense interchanging activity. The gene rpsD possessed the top rank of miRNA interlinkage after that rmpI and rmpE with addition of DEGs such as rpsU and rpmH also indicated the miRNA interconnection. The has-miR-155-5p showed link with *rpsD*, *rpmE*, *rpsU*, and *rpmH* denoted the largest controlling role in P aeruginosa meditate inflammation. Also, *rmpE* which was regulated by hsa-miR-155-5p and 2 TFs one of which was NF-KB. Important step of post translational controlling was indicated by involvement of these miRNA nodes it helps in reduction of inflammation produce by pathogenic factors of *P. aeruginosa*.

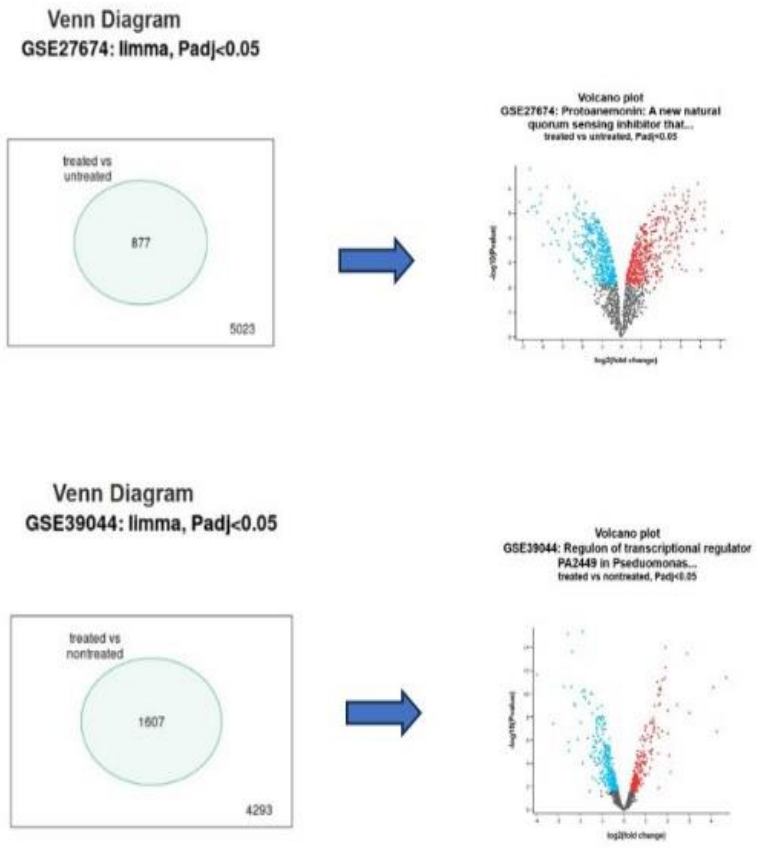

Fig. 1: The Venn diagrams and Volcano plots for datasets GSE27674 and GSE39044 with an adjusted P value < 0.05. The genes are represented by colored dots: grey genes are not significant, blue genes are upregulated, and red genes are downregulated

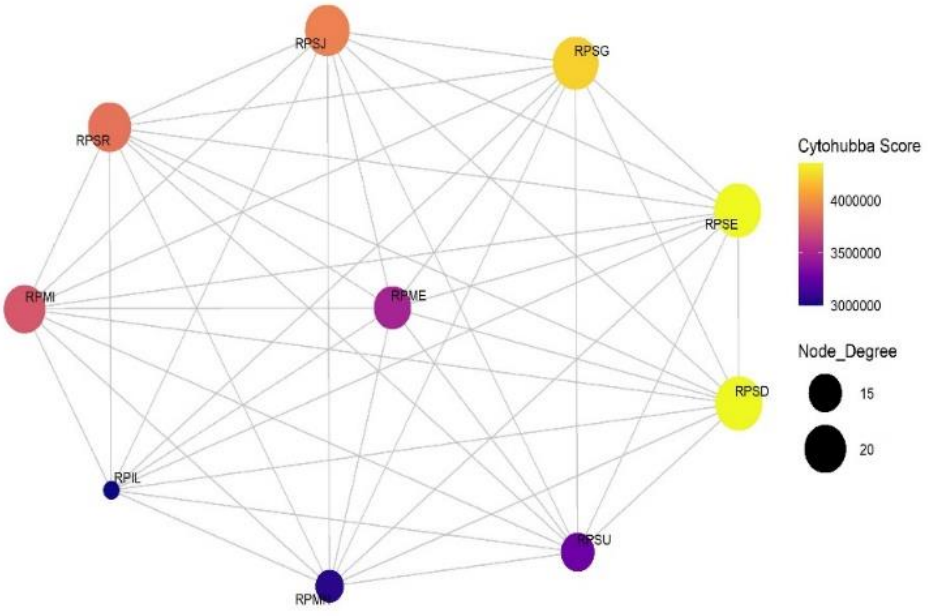

Fig. 2: The MCC method rank in the top ten in STRING network

Table 1: Summary of Top Enriched GO Terms and KEGG Pathways.

Category	Term name	GO/KEGG ID	Adjusted p value	Biological Relevance
GO-MF	signal receptor binding	GO:0005102	3.155×10^{-10}	Important for immune inflammatory response
	Molecular function regulator	GO:0098772	1.149×10^{-5}	Reduce host regulatory activity
GO-BP	cytokine-mediated signaling pathway	GO:0019221	1.203×10^{-10}	Amplify inflammation
	antimicrobial humoral response	GO:0019730	2.103×10^{-8}	Production of antimicrobial peptides and acute phase protein
	cellular response to stimulus	GO:0051716	1.779×10^{-3}	Involve in rpsD dependent protein synthesis
GO-CC	high-density lipoprotein particle	GO:0034364	3.940×10^{-2}	Reduce bacterial virulence by lowering acute phase protein
KEGG pathway	IL-17 signaling pathway	KEGG:04657	1.390×10^{-19}	Perform tissue damage
	Cytokine-cytokine receptor interaction	KEGG:04060	1.512×10^{-11}	Produce exotoxins, produced under rpsD-dependent translation
	Chemokine signaling pathway	KEGG:04062	3.789×10^{-8}	Recruit cells to infection site and stop it
	Epithelial cell signaling in *Pseudomonas aeruginosa*	KEGG:04062	4.509×10^{-4}	Reflects that factors produced through rpsD-dependent synthesis overlaps

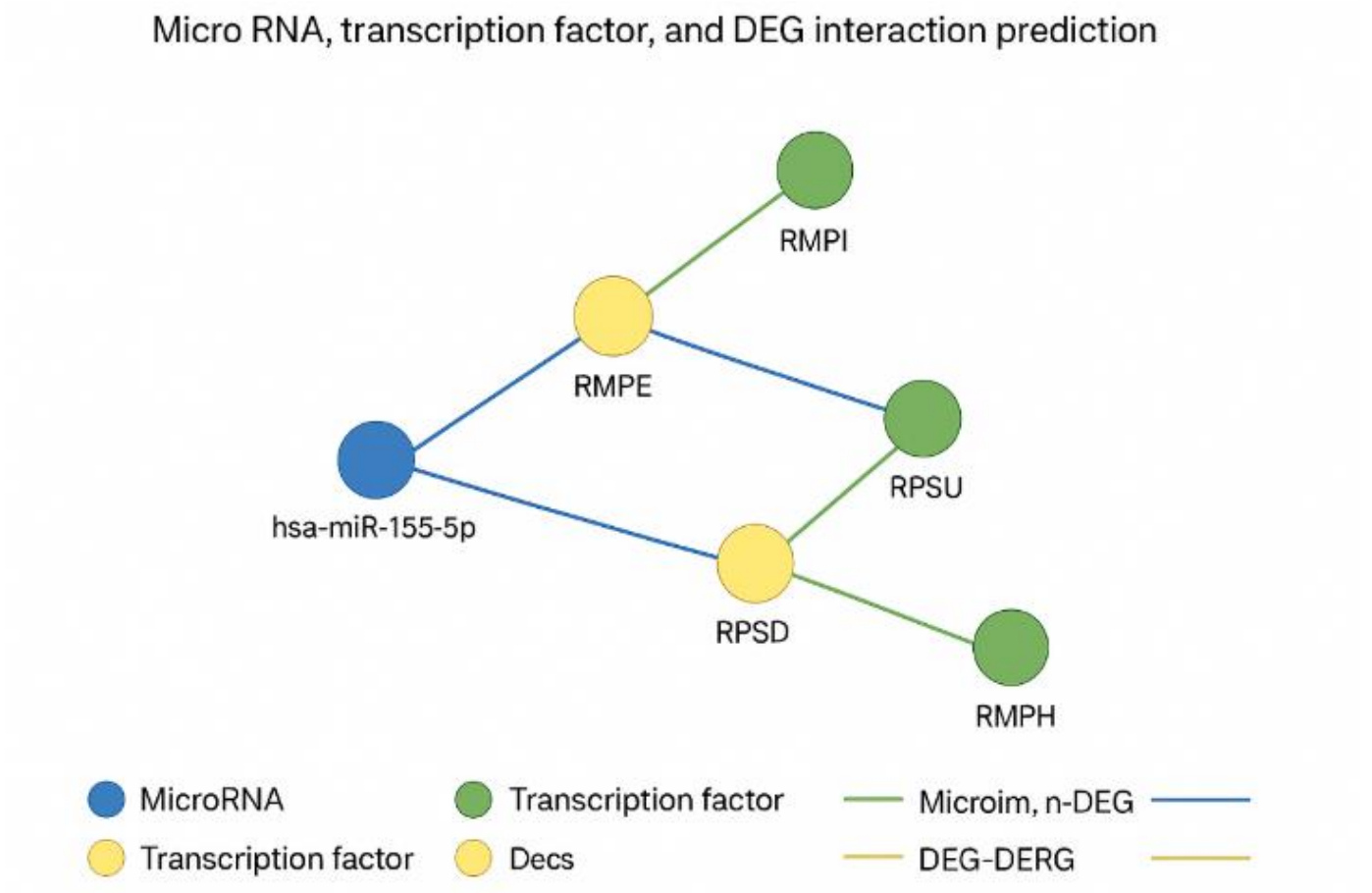

Fig. 3: miRNet tools generated a network of these miRNAs, TFs, and DEGs using different color and relationship. Relationship between DEGs and miRNA was shown by blue color while green represent the transcription factors and yellow represent the DEGs.

Protein-Drugs analysis

The identification of the rpsD gene as therapeutic target lead the way for the selecting the potential therapeutic agents (Fig. 4). The DGIdb database was used for the identification of the that can interact with proteins rpsD, rpmI, and rpmE from *P. aeruginosa*. These genes encode for the ribosomal proteins S4, L35, and L31 respectively. These entire proteins showed significant role in the synthesis of bacterial proteins that can sustains virulence factors. The DGIdb has helped with the prediction of crucial interactions between DEGs and drugs to inhibit the bacterial protein synthesis which paly role in their antibiotic resistance. There is a list of Drugs (gentamicin, thalidomide, golimumab, adalimumab, infliximab, etanercept, certolizumab pegol, lenalidomide, meropenem anhydrous, mycophenolate mofetil, cyclosporine, atorvastatin calcium trihydrate, rifampin, and cefotaxime sodium) that could target the rpsD gene. In case of rpmi major drugs that are identified includes (celecoxib, naproxen sodium, ibuprofen sodium salt, meloxicam, and etoricoxib), on the other hand rpme has shown association with (aspirin, ketoprofen, diclofenac sodium, indomethacin, flurbiprofen, and sulindac). The selection of these drugs is based on the interaction scores and their FDA approval. Out of this gentamicin is of key importance with an interaction score of 0.04881140585216039. It is an approved drug that can bind to rpsd S4 ribosomal protein to inhibit its function of protein synthesis. Other approve drug have antibacterial and anti-inflammatory responses when interact with proteins of the pathway like IL-17 signaling pathway. As for RPMI, meloxicam and etoricoxib, have role in reduction of prostaglandin mediated inflammation.

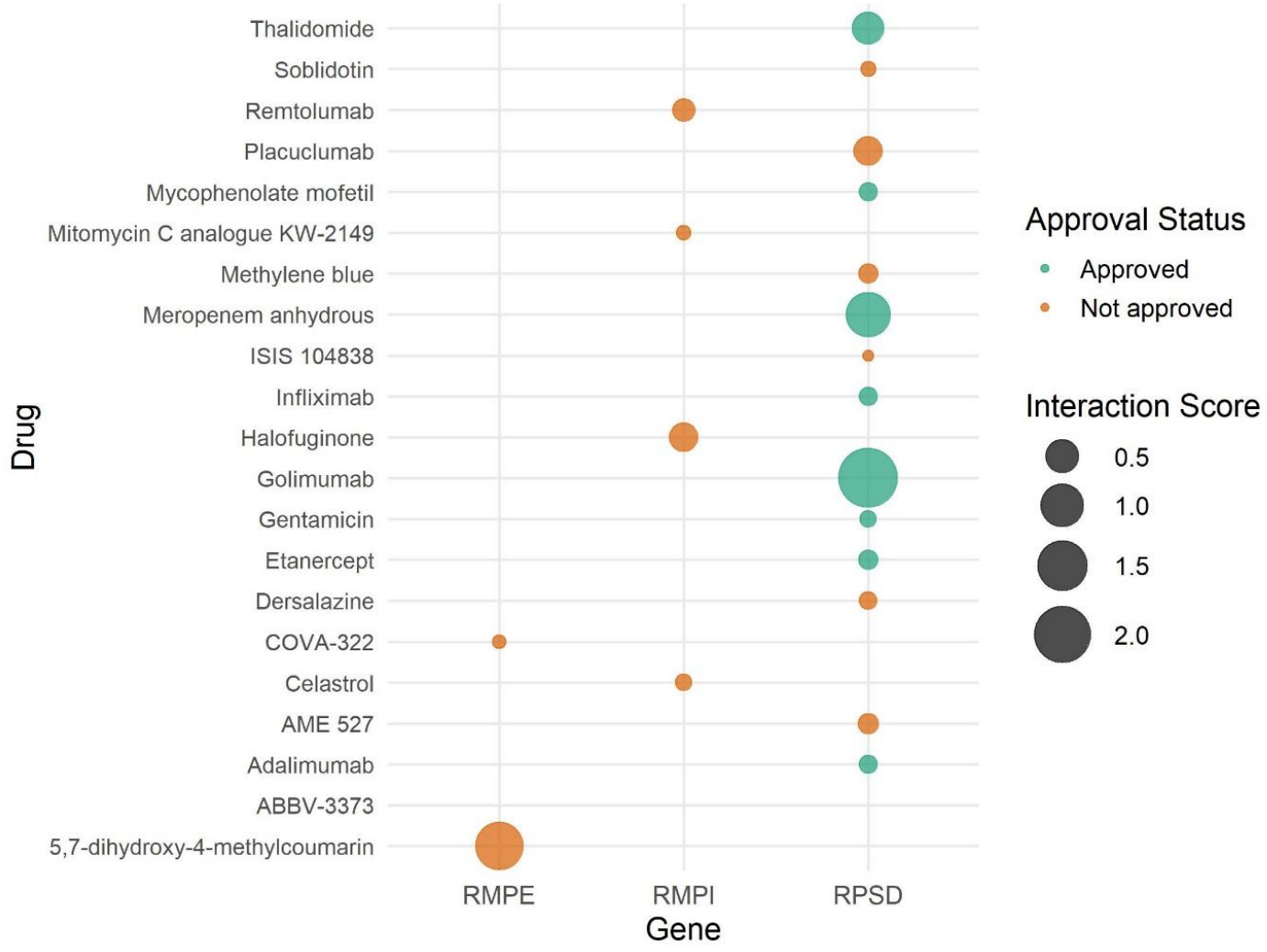

Fig. 4: Correlation analysis between affected body parts and affected activities. Correlation is significant at the 0.05 level (2-tailed). The color and size of circles help visualize both the direction and strength of associations, with blue indicating positive and red indicating negative correlations.

Molecular docking

Molecular docking analysis were performed on the genes rpsD, rpsI from the *Pseudomonas aeruginosa,* indicated by PDB ID-8CDI and 6spe (Fig. 5). These analyses were done to screen the small drug molecules that can inhibit the protein synthesis when interact with S4 protein, causing the *Pseudomonas aeruginosa* to lose its virulence. The binding pocket of 30S ribosomal subunit was studied in detail for the generation of precise docking grid (Table 2). Then for rpsD, a grid with measurement as 20 × 20 × 20 Å³ was constructed, centered at coordinates (15.0, 10.0, 25.0) Å, making sure significant coverage of the S4 active site and adjacent 16S rRNA regions have shown interaction with antibiotics like gentamicin. All the drug candidates that were screened out through DGIdb database were docked one by one with 30S ribosomal subunit, focusing their interaction with S4 protein of the subunit as it is the Position that encoded by the rpsD gene. Gentamicin and Meropenem anhydrous interaction with S4 protein has shown biding score -8.1 kcal/mol and -7.8 kcal/mol, respectively, suggesting highest affinity and potential inhibitory on the synthesis of protein. The binding energies below -6 kcal/mol are considered strong. On the other hand, the molecules which has shown moderate binding like Golimumab (-6.5 kcal/mol) and Infliximab (-6.4 kcal/mol) also plays roles in mitigating the inflammatory responses in the host due to the virulence factors of the rpsD gene. The drugs which showed the moderate affinity need further optimization to make them best suited therapeutic candidate against *Pseudomonas aeruginosa* infections.

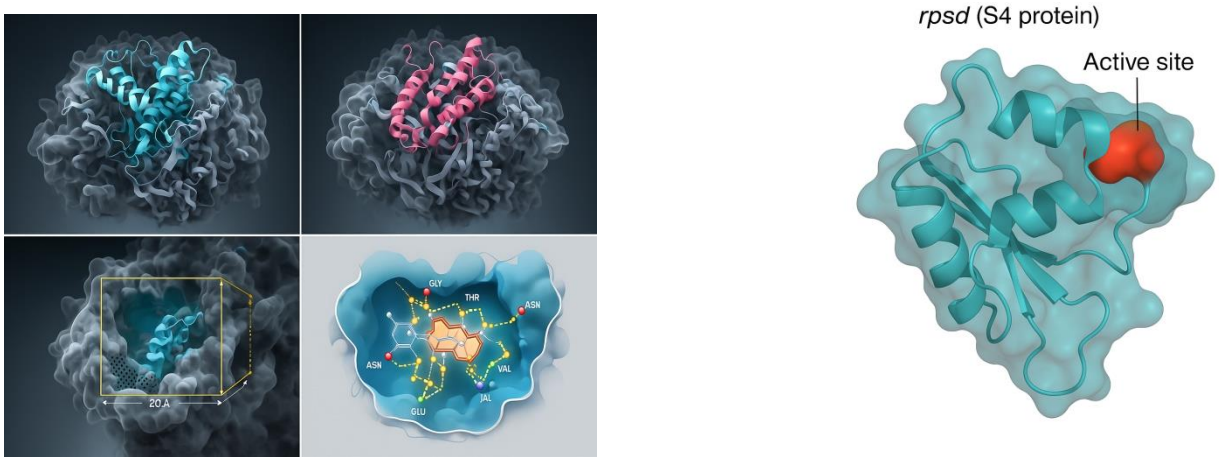

Fig. 5. Molecular docking visualization and schematic illustration of ribosomal proteins rpsD and rpsI. (a): 3D structure of S4 protein (rpsD, cyan) with semi-transparent surface highlighting the overall protein topology. **(b):** 3D structure of S5 protein (rpsI, pink) showing molecular surface. **(c):** Binding pocket used for docking on rpsD, depicted as a yellow box (20×20×20 Å³) to indicate the docking grid covering the active site. **(d):** Schematic ligand-binding diagram illustrating a small molecule interacting with key residues. Hydrogen bonds and other interactions are shown with dotted lines. This panel emphasizes the conceptual binding mode of screened drugs targeting the S4 protein. **(e).** 3-D structure of the ribosomal S4 protein (rpsD) of *Pseudomonas aeruginosa*. The protein is represented in a ribbon (cyan), overlaid with a semi-transparent molecular surface (light blue) to highlight the overall secondary structure elements. The active site, corresponding to the drug-binding pocket targeted for molecular docking, is highlighted in red-orange. This visualization provides structural context for potential drug interactions.

Table 2: Molecular Docking results revealing the binding score, gene, and drugs.

Gene	Drug	Binding score (kcal mol)	Gene	Drug	Binding score (kcal mol)
RPSD	Meropenem anhydrous	-8.1	RMPI	Celastrol	-6
RPSD	Gentamicin	-7.8	RMPI	Halofuginone	-5.7
RPSD	Golimumab	-6.5	RMPI	Mitomycin C analogue KW-2149	-5.5
RPSD	Infliximab	-6.4	RMPI	AME-527	-5.3
RPSD	Adalimumab	-6.3	RMPI	ABBV-3373	-5.2
RPSD	Etanercept	-6.2	RMPI	Remtolumab	-5.1
RPSD	Mycophenolate mofetil	-5.6	RMPE	5,7-dihydroxy-4-methylcoumarin	
RPSD	Thalidomide	-5.2	RMPE	COVA-322	
RPSD	Placulumab	-5.1			
RPSD	Soblidotin	-5			
RPSD	ISIS 104838	-4.9			
RPSD	Methylene blue	-4.8			
RPSD	Dersalazine	-4.7			

Discussion

This study has highlighted the importance of *rpsD*, *rpmI* as important genes after analyzing their DEGs profile which vary in the treated and nontreated sample of the microarray dataset GSE27674 and GSE39044. The analysis of the dataset identified a significant number of upregulated genes, that can form the diverse PPI network with multiple interaction hubs (Finan *et al*, 2017; Wang *et al*, 2021). The top hub genes that were identified are RPSD, RPMI, RPME which have shown crucial role in the regulatory network that involves differentially expressed gene miRNAs, and transcription factors (TFs), particularly with the miRNA hsa-miR-155-5p and the TF NF-κB.

The DGIdb tool has helped with the protein-drug interaction prediction, highlighted some drugs that can target the rpsD, rpmI, rpmE (Wagner *et al*, 2016). These identified drugs can inhibit the function of S4 protein ultimately there will be Pseudomonas aeruginosa cell death. Molecular docking has further optimized the results highlighted the top drug candidate with high binding affinity (Agu *et al*, 2023). Meropenem anhydrous (-8.1 kcal/mol) and Gentamicin (-7.8 kcal/mol) have shown highest affinity with rpsD. On the other hand, Golimumab (-6.5 kcal/mol) and Infliximab (-6.4 kcal/mol) have shown moderate binding affinity with rpsD.

The rpsD gene encodes the ribosomal protein S4 that is of central importance for the bacterial protein synthesis, it facilitates the synthesis of virulence factors that are important in quorum sensing mechanism and biofilm formation (Markowska *et al*, 2024). These also upregulates the host DEGs IL17, 8 signaling pathways and CXCL1. In the same way rpmI and rpmE have role in 50s ribosomal subunit protein synthesis helping with persistence of bacterial infection. This study has indicated that rpsd, rpmI and rpmE could be potential target for the novel drugs.

The use of GEO2R allowed reliable identification of differentially expressed genes between treated and untreated samples. The strict cut-off values improved the quality of the selected genes (Grundy and Henkin, 1991). The datasets revealed a large number of altered genes. This confirmed strong transcriptional changes in infected conditions. The volcano plots clearly separated significant and non-significant genes while the Venn diagrams showed distinct expression patterns between groups. These findings support the reliability of the computational pipeline. The method reduced background noise in the data. The selection of two independent datasets strengthened the study design.

Protein–protein interaction analysis provided insights into biological connectivity. The use of STRING with a high confidence score improved interaction accuracy. Cytoscape visualization helped interpret complex networks. The MCC method efficiently identified hub genes. rpsD showed the highest node degree and interaction score. This confirmed its central biological role in survival of this pathogen (Grundy and Henkin, 1991; Walsh, 2000; Wagner *et al*, 2016). Other ribosomal genes also showed strong connectivity. These results matched known ribosomal functional clusters while the dense interaction pattern suggested coordinated regulation. The method helped prioritize genes for downstream analysis. The PPI approach strengthened target gene validation.

Gene ontology and KEGG enrichment analysis clarified functional roles of the DEGs. The enrichment in ribosomal and quorum sensing pathways aligned with bacterial survival strategies. The IL-17 signaling pathway showed strong association with inflammatory responses. These findings suggested a link between bacterial virulence and host immune activation as agreed in the previous studies (Winsor *et al*, 2016; Wang *et al*, 2021). The GO terms supported involvement in protein synthesis and stress response. The KEGG pathways highlighted relevant infection-related mechanisms. The results were consistent with known Pseudomonas pathogenesis while the enrichment results supported the therapeutic value of rpsD (Grundy and Henkin, 1991; Markowska *et al*, 2024). This strengthened the biological relevance of the study.

miRNA and transcription factor analysis revealed post-transcriptional regulation. The use of miRNet and mirDIP improved interaction prediction. TRRUST helped identify key transcription factors. *rpsD* showed strong miRNA connectivity. hsa-miR-155-5p appeared as a major regulator. NF-κB involvement linked bacterial infection with inflammation control. These findings indicated complex regulatory control of ribosomal genes and the results highlighted coordinated gene regulation under stress (Bjarnsholt *et al*, 2009; Elborn *et al*, 2016). The network structure showed functional interdependency of genes. These regulatory results strengthened the proposed role of rpsD.

Drug–gene interaction analysis provided clinically relevant insights. DGIdb enabled rapid screening of FDA-approved compounds. Several antibiotics showed predicted interactions with rpsD. Gentamicin showed strong relevance among selected drugs. Anti-inflammatory drugs also appeared as potential modulators. These findings suggested dual antimicrobial and anti-inflammatory potential. The method supported drug repurposing opportunities. The result connected gene targets with therapeutic candidates. This approach reduced time for lead identification. The predicted interactions were biologically plausible as previously shown in the studies (Grundy and Henkin, 1991; Stover *et al*, 2000; Wagner *et al*, 2016). The method added translational value to the study. It strengthened the therapeutic relevance of ribosomal targets.

Molecular docking validated the computational drug screening results as per previous studies (Chao *et al*, 2024). PatchDock showed strong binding affinity for selected compounds (Grundy and Henkin, 1991; Agu *et al*, 2023). Gentamicin and meropenem showed the highest binding scores. These results supported experimental targeting of the S4 protein. The docking grid design ensured accurate active site coverage. The binding energies indicated strong molecular interactions. Moderate binding drugs suggested opportunities for optimization. The visualization improved understanding of structural compatibility. Docking confirmed inhibition potential at the ribosomal level. The results aligned with protein synthesis inhibition mechanisms (Grundy and Henkin, 1991; Agu *et al*, 2023). These findings demonstrated the strength of combined bioinformatics and structural analysis. The study supports *rpsD* as a strong candidate for novel drug development.

Declaration of Competing Interest

The authors declare that they have no competing or conflict of interests.

Author Contributions

MZ: Conceptualization, Methodology, formal analysis, Writing—original draft preparation, Writing—review and editing. This author has read and agreed to the published version of the manuscript.

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