Molecular exploration of host-pathogen interactions in severe *Pseudomonas aeruginosa* infection through a multi-level data integration approach

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Abstract

Understanding host-pathogen interactions is crucial for explaining the variability in sepsis outcomes, with *Pseudomonas aeruginosa* (*PA*) remaining a significant public health concern. In this work, we explored *PA*-human host interaction mechanisms through a data integration workflow, focusing on protein-protein and metabolite-protein interactions, along with pathway modulation in affected organs during severe infections. A scoping literature review enabled us to construct a domain-based infection network encompassing pathogenesis concepts, molecular interactions, and host response signatures, providing a wide view of the relevant mechanisms involved in severe bacterial infections. Our analysis yielded a literature-based comprehensive description of PA infection mechanisms and an annotated dataset of 189 *PA*-human interactions involving 152 proteins/molecules (109 human proteins, 3 human molecules, 34 *PA* proteins, and 5 *PA* molecules). This dataset was complemented with gene expression analysis from *in vivo PA*-infected lung samples. The results indicated a notable overexpression of proinflammatory pathways and *PA*-mediated modulation of host lung responses. Our comprehensive molecular network of *PA* infection represents a valuable tool for the understanding of severe bacterial infections and offers potential applications in predicting clinical phenotypes. Through this approach combining omics data, clinical information, and pathogen characteristics, we have provided a foundation for future research in host-pathogen interactions and the mechanistic grounds to build dynamic computational models for clinical phenotype predictions.

Keywords: *P. aeruginosa;* host-pathogen interaction; bacterial infection; disease map; sepsis.

Introduction

Sepsis caused by multi-drug resistant pathogens remains a leading cause of mortality in intensive care units (ICU) and represents a significant public health concern (Kumar *et al.*, 2024; Rello *et al.*, 2017). While it is established that microbial infection outcomes depend heavily on host conditions and spatial interactions between microbes, hosts, and other microorganisms (Saarenpaa *et al.*, 2023, Mu *et al.*, 2023), many molecular details of these complex relationships remain unexplored. *Pseudomonas aeruginosa* (*PA*) is one of the most common pathogens for nosocomial infections, and, along with *Acinetobacter baumannii* and *Enterobacterales* resistant to carbapenem, it was listed among critical priority pathogens for WHO (WHO, 2022; 2024). The European Centre for Disease Prevention and Control (ECDC) has included *PA* in its antimicrobial resistance surveillance program (ECDC, 2022). As an opportunistic human pathogen particularly affecting Cystic Fibrosis (CF) patients, *PA*'s clinical significance stems from multiple drug resistance mechanisms, numerous virulence factors, and biofilm production capabilities, enhancing its infection and host colonization potential (Dahal *et al.*, 2023). Recently, computational approaches have aided in unravelling mechanistic insights of *PA* infections. A network-assisted silico experiment allowed the identification of novel genes for virulence and antibiotic resistance, confirmed through experimental validation, showing cross-resistance against multiple drugs due to the same genes (Hwang *et al.*, 2016). In another effort, a real-time deep-learning model was applied to sepsis patients aiming to estimate prognostic outcomes from early infection phases (Boussina *et al.*, 2024). The model addressed baseline acuity, comorbidities, seasonal effects, and secular trends over time, unravelling the strategic significance of computational modelling to improve the clinical outcomes in sepsis patients.

Mechanistic computational modelling, omics data analysis, and clinical research have emerged as crucial tools for bridging the gap between conceptual models and clinical practice in infectious diseases (Singh *et al.*, 2023, Montaldo *et al.*, 2021). By structuring key pathophysiological mechanisms and identifying conceptual domains, molecular diagrams provide novel insights into biomedical knowledge (Singh *et al.*, 2023, Hemedan *et al.*, 2022, Ostaszewski *et al.*, 2020). The value of network-based exploratory approaches was particularly evident during the COVID-19 pandemic, where rapid identification of molecular interactions between SARS-CoV-2 and human hosts became crucial (Steiner *et al.*, 2024). These virus-host interactome studies revealed infection mechanisms, explained clinical manifestations (Messina *et al.*, 2020, Messina *et al.*, 2021, Schmidt *et al.*, 2021, Lee *et al.*, 2021) and enabled a timely drug repurposing process for COVID-19 treatment (Gordon *et al.*, 2020, Zhou *et al.*, 2023). In that context, the resulting molecular maps of disease mechanisms

(e.g. a Disease Map - https://disease-maps.io/) provided biological meaning to apparently unrelated interactions, serving as a knowledge repository, representing a solid starting point for dynamic computational modelling and data analysis, capable of facilitating mechanistic understanding of complex disease processes (Ostaszewski *et al.*, 2021, Niarakis *et al.*, 2023).

Building upon these advances, our study presents a data integration workflow to build a molecular map of interaction between *PA* and human hosts in severe infection. Through extensive literature review, data curation, and gene expression meta-analysis, we have documented *PA* infection pathogenic mechanisms, direct protein-protein interactions (PPI), metabolite-protein interactions (MPI), and pathway activations in affected organs, organizing these findings into three conceptual domains.

Materials and methods

Scoping review

We conducted independent literature reviews compliant with international reference guidelines for scoping reviews (Peters *et al.*, 2015). For each domain, the scoping review outcomes were processed to identify features of *PA* interactions with the host and the direct or indirect effects that they cause within the host itself.

Using a structured search string in PubMed (Supplementary Text 1), we identified 532 articles after excluding duplicates and non-English publications. We supplemented this with 27 additional articles focusing on host response to *PA* infection in both mouse models and human patients through omics data analysis. The final selection comprised 150 articles which were categorized into three interaction levels: (1) “cell interaction level”; (2) “tissue interaction level”; and (3) “organ interaction level”. Full-text articles were evaluated by the curators to define the best possible conceptual domains (Peterson, 1996). Each article was assigned a unique reference ID (SR) and documented in Table-S1.

Conceptual domains

First, we identified the conceptual domains that organize the information obtained from the literature, providing a hierarchical model of host-pathogen interaction, following a previous experience on

mapping host-pathogen interactions in the COVID-19 Disease Map project (Singh *et al.*, 2023, Montaldo *et al.*, 2021). Three interaction levels within the host's system were identified: cell, tissue, and organ. For each level, we further identified conceptual domains, describing the interactions with the pathogen (Fig. 1). A comprehensive description of these interactions can be found on Supplementary Text 1, while a summary can be found below in the results section.

Molecular interaction dataset and human host - PA interactome

We documented PPI and MPI between *PA* and humans. All interaction details, including type, Uniprot ID, literature reference, and subdomains of the model, were compiled in the curated dataset (Table-S2). We constructed a network-based interaction model by exploring *PA*-host data gathered from the scoping review, following methodology established for SARS-CoV-2-human host interactions (Messina *et al.*, 2020, Messina *et al.*, 2021). Human PPI data was retrieved using R packages PSICQUIC and biomaRt (Aranda *et al.*, 2011, Smedley *et al.*, 2009), resulting in a comprehensive large network of 13,334 nodes and 73,584 interactions that included *PA*-human host interactions. The mechanisms of infection were estimated using the Random Walk with Restart (RWR) algorithm (Valdeolivas *et al.*, 2019), using each *PA* protein as a seed and limiting the output to the 200 closest host proteins per *PA* protein. Network visualizations were generated using GEPHI 0.9.2 (Bastian M *et al.*, 2009). Gene set enrichment analysis was performed using the R package enrichR was used to carry out gene set enrichment analysis(Kuleshov *et al.*, 2016), testing against Reactome 2022, KEGG 2021 and WikiPathways 2023 human pathways databases (Milacic *et al.*, 2024, Slenter *et al.*, 2018, Kanehisa *et al.*, 2017).

Meta-analysis of the whole transcriptome from animal model of PA-induced sepsis

We performed a meta-analysis of gene expression in mouse lung samples comparing *PA*-infected tissues with healthy controls using data from two projects. The first dataset comprised 12 bulk RNAseq samples from *PA*-infected lung tissues (PRJNA975462; GEO: GSE233206, SRA Study SRP439193) (Yang *et al.*, 2023), while the second included 6 bulk gene expression samples from acute and chronic *PA* pulmonary infection (PRJNA793679; GEO: GSE192890, SRA Study SRP353174) (Hu *et al.*, 2022). SRA data was processed using Prefetch and converted to FASTQ files

using the fastq-dump tool from the SRA Toolkit software v2.11.0 (Leinonen *et al.*, 2011, Han *et al.*, 2019). Reads were aligned to the mm10 mouse reference genome using HISAT2 (Kim *et al.*, 2015). Differentially expressed (DE) genes were identified using DESeq2 v.1.42.1 in R version 3.4.3 (Love *et al.*, 2014), with thresholds set at Log2FC >|1| and Benjamin-Hochberg False Discovery Rate <5% (BH-FDR). To account for batch effects between laboratories, we conducted a meta-analysis using metaRNASeq R packages, combining p-values from the two independent RNA-seq experiments using Fisher methods (Rau *et al.*, 2014). The analysis focused on 21,010 genes shared between datasets, generating combined BH-adjusted p-values and average Log2FC values. Genes meeting the thresholds of Log2FC >|1| and BH FDR <5% were classified as differentially expressed genes (DEGs).

Gene enrichment on DEGs in PA infection and healthy conditions

To deliver biological meaning from the data, we performed a gene enrichment analysis using Reactome, KEEG, and WikiPathways (Slenter *et al.*, 2018, Milacic *et al.*, 2024, Kanehisa *et al.*, 2017). The enrichR R package was used to conduct gene set enrichment analysis, with significance assessed through Fisher exact test (p-value) and false discovery rate (q-value: adjusted p-value for FDR) (Kuleshov *et al.*, 2016).

Results

Domain-based analysis of PA-human host interactions reveals detailed pathogenic mechanisms

At the cellular level, four key aspects characterize *PA*-host interaction: (i) bacterial adhesion/colonization (*PA*-Ad); (ii) bacterial invasion and innate immune response of the host (*PA*-In); (iii) *PA* exotoxins activity in infection (*PA*-Ex); (iv) Bacterial metabolic mechanisms (*PA*-Met). *PA* initiates infection through flagellum and type IV pili adherence, interacting with MUC1 ectodomains via NEU1 modulation (Hyun *et al.*, 2016, Hyun *et al.*, 2021) . The bacterium employs multiple adhesion strategies, including biofilm formation, psl adhesins (Kaya *et al.*, 2020, Byrd *et al.*, 2010), and various receptors binding to extracellular matrix components (Paulsson *et al.*, 2019, Beaufort *et al.*, 2013). During invasion, *PA* modifies host cell membranes through PI3K/PIP3/Akt pathway activation and uses specialized proteins like pilY1 for binding (Engel & Eran, 2011). The bacterium's survival in macrophages relies on mgtC and oprF (Garai *et al.*, 2019) (SR22), while its exotoxins (exoS, exoT, exoU, exoY, exoA) facilitate pathogenesis through various mechanisms,

including protein ribosylation, cytoskeleton modification, and membrane disruption (Henriksson *et al.*, 2000, Ghatak *et al.*, 2023, Jia *et al.*, 2003, Krall *et al.*, 2000) .

At the tissue level, *PA* affects three primary domains: (i) endothelial tissue (Endothelial Tissue - EnT); (ii) lower airway and alveolar epithelial tissue in the lung, including cystic fibrosis (CF) conditions (Airway Epithelial Tissue - AET); and (iii) other epithelial tissues such as desquamated bronchial and urinary epithelia (Other Epithelial Tissues- ETs). In endothelial tissue, particularly during severe infection, APOE exhibits antibacterial activity (Puthia *et al.*, 2022), while T3SS affects actin cytoskeleton dynamics (Huber *et al.*, 2014) . The bacterium adapts to blood survival by regulating metabolic pathways and virulence factors (Turner *et al.*, 2014, Elmassry *et al.*, 2019). In airway epithelial tissue, particularly relevant in CF conditions, *PA* flagella binds to asialoGM1 and MUC1, triggering inflammatory responses (Adamo *et al.*, 2004, Hyun *et al.*, 2021, Blohmke *et al.*, 2010). CFTR plays a crucial role in *PA* uptake and inflammation (Haenisch *et al.*, 2010, Schroeder *et al.*, 2002) . In other epithelial tissues, *PA* binds through HSPGs and N-glycans (Bucior *et al.*, 2012), with quorum sensing molecules affecting barrier integrity (Vikstrom *et al.*, 2009).

Finally, at the organ level, *PA* infection primarily impacts the lung and bloodstream. In lung infections, particularly in CF, *PA* causes intense inflammation with neutrophil infiltration and cytokine production (Blohmke *et al.*, 2010, Vega-Carrascal *et al.*, 2014). The infection involves various immune mechanisms, including TRPV4 (Scheraga *et al.*, 2020), TIM3/Gal-9 signalling (Vega-Carrascal *et al.*, 2014), and NET formation (Sung *et al.*, 2022). Single-cell analysis has revealed significant changes in immune cell composition during infection (Hu *et al.*, 2022). In bloodstream infections, *PA* affects the vascular endothelium through multiple mechanisms (Soong *et al.*, 2008), with TREM-1 playing a role in septic shock (Gibot *et al.*, 2019). The Hxu system contributes significantly to bloodstream infection capability (Yang *et al.*, 2022a), while single-cell profiling demonstrates differential immune cell responses (Oelen *et al.*, 2022). These multi-level interactions highlight the complexity of *PA* pathogenesis and its adaptive capabilities in different host environments.

Table 1: The table summarizes the main pathogenic mechanisms in *PA* infection for each domain, with comprehensive conceptual analysis provided in the Supplementary Text: A) Cell interaction level; B) Tissue interaction level; C) Organ interaction level. This structured approach enabled us to characterize specific mechanisms and experimental models of *PA* infections.

A)

		Key Molecules	**Biological Outcome**	**References**
Cell Interaction Level	*PA* Adhesion /Colonization	Flagellum, pilA	Adherence in upper respiratory tract, interaction with IRF-1	(Hyun *et al.*, 2021, Hyun *et al.*, 2016, Lory & Ichikawa, 2002)
		Flagellum, NEU1	Modulation of binding between flagellum and MUC1	(Hyun *et al.*, 2021, Lory & Ichikawa, 2002)
		Pilus	Interaction with asialo-GM1, asialo-GM2, glycosphingolipids; MMP7 expression induction	(Tynan *et al.*, 2017, Hsieh *et al.*, 2005, Wong *et al.*, 1995, Adamo *et al.*, 2004)
		psl	Biofilm formation, cell adhesion, flagellin-mediated NF-κB activation	(Byrd *et al.*, 2010)
		estA, oprD, oprG, oprQ, PA3923, Paf	Binding to LAMA1 (α4, α5) and FN1	(Paulsson *et al.*, 2019, Beaufort *et al.*, 2013)
		lecA	Binding to Gb3 and GPI-anchored CD59	(Brandel *et al.*, 2021)
		CD18, N-glycans	*PA* uptake facilitation via integrin-mediated uptake	(Demirdjian *et al.*, 2020)
	PA Invasion	mgtC, oprF	Macrophage survival	(Garai *et al.*, 2019)
		Flagellin	EGFR/TGF-α release, MUC1 phosphorylation, TLR5 association	(Kato *et al.*, 2016)
		IMPa	Leukocyte rolling adhesion via CD43, CD44, CD55, PSGL-1	(Bardoel *et al.*, 2012)
		pumA	NF-κB inhibition, interaction with TIRAP, MyD88, UBAP1	(Imbert *et al.*, 2017)
		LPS	SP-A interaction, TNF-α release limitation	(Hickling *et al.*, 1998)
		LL-37	IL-8 production inhibition, mucA mutagenesis	(Limoli *et al.*, 2014, Scott *et al.*, 2011)
		lasB	Protein degradation (elastin, collagen, laminin, IgG, C3, α1-AT, IFN-γ, IL-2)	(Barbey-Morel & Perlmutter, 1991)
		LPS	MUC5AC overproduction	(Song *et al.*, 2009)
		CD95/CD95 ligand	Apoptosis triggering, NF-κB/JNKs/GADD153/PLA 2 stimulation	(Grassme *et al.*, 2000)
		PTEN-CFTR complex	*PA* intracellular killing promotion	(Riquelme *et al.*, 2017)

	Exotoxins	Azurin	Cell proliferation inhibition via aldolase A secretion	(Choi *et al.*, 2023)
		3OC12-HSL	T-lymphocyte proliferation inhibition, MAPK-p38 activation	(Kahle *et al.*, 2013)
		PAI-1	Cyclooxygenase 2 activation in fibroblasts and ECs	(Williams *et al.*, 2004)
		PNC	Neutrophil death, mitochondrial dysfunction, IL-8 downregulation	(Ras *et al.*, 1990, Ghatak *et al.*, 2023, Manago *et al.*, 2015)
		pvrA	PC and fatty acid catabolism regulation	(Pan *et al.*, 2020)

B)

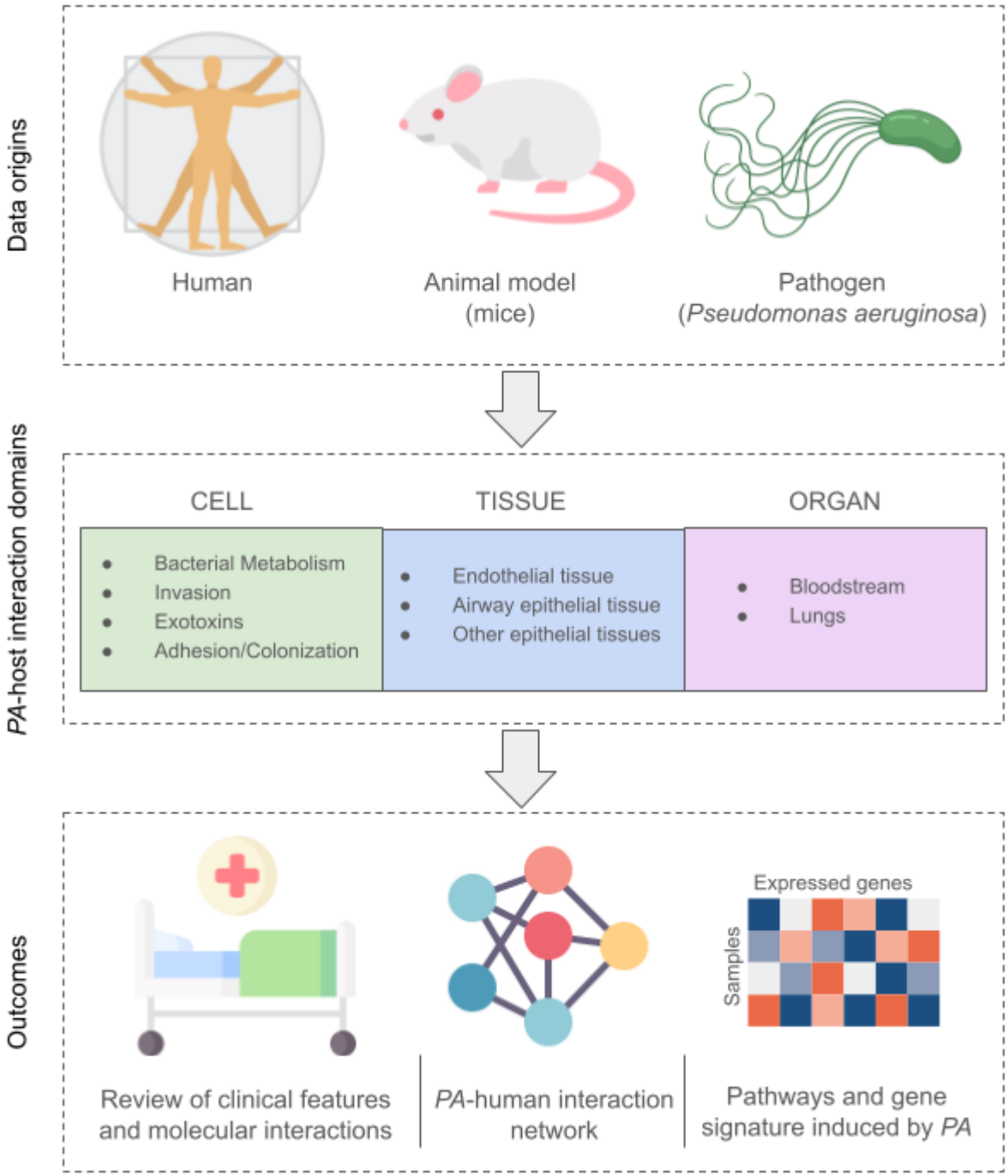
		Key Molecules	**Biological Outcome**	**References**
Tissue Interaction Level	Endothelial Tissue	APOE3	NF-κB reduction in monocytes, antibacterial activity	(Puthia *et al.*, 2022)
		exoS, exoT	Lim kinase-cofilin pathway modulation, GTPase inactivation	(Huber *et al.*, 2014)
		lasB/pseudolysin	Endothelial adherence disruption, cytotoxicity	(Soong *et al.*, 2008)
	Airway Epithelial Tissue	pilY1	PI3K/PIP3/Akt pathway activation, membrane remodeling	(Engel & Eran, 2011)
		Flagella	TLR5 activation, neutrophil respiratory burst	(Blohmke *et al.*, 2010, Koller *et al.*, 2008)
		pilA	Tight junction disruption, IRF-1 activation	(Roy *et al.*, 2014, Soong *et al.*, 2008, Barnes *et al.*, 2008, Yang *et al.*, 2022b)
		exoA	ADAM10 interaction, leukocyte migration alteration	(Aljohmani *et al.*, 2022)
		PNC	Ciliary dysfunction, mucus velocity alteration	(Forbes *et al.*, 2014)
		PA-IL, PA-IIL	Cilia binding, airway infection facilitation	(Gustke *et al.*, 2012)
		lasB	EC detachment via FN1/vWf degradation	(Beaufort *et al.*, 2013)
		Vav3	b1 integrin/FN1 complex formation in CF	(Badaoui *et al.*, 2020)
		CFTR	*PA* uptake regulation, NF-κB activation	(Schroeder *et al.*, 2002), (Haenisch *et al.*, 2010)
		Various	IL-6/CXCL8/TACE expression induction	(Gomez *et al.*, 2005)
	Other Epithelial Tissues	HSPGs	Enhanced apical surface binding	(Bucior *et al.*, 2012)
		pilA, Flagella	N-glycan and HSPG-mediated binding	(Bucior *et al.*, 2012)
		T3SS, LPS	Barrier function disruption	(Vikstrom *et al.*, 2009)
		exoS	Na/K-ATPase inhibition via FXYD3	SR40

		T3SS components	Keratitis development, tight junction disruption	(Yang *et al.*, 2022b)
		Fur regulator	Iron acquisition pathway regulation	(Lassek *et al.*, 2015)

C)

		Key Molecules	**Biological Outcome**	**References**
Organ Interaction Level	Lungs	LPS, CFTR	*PA* uptake, NF-κB activation	(Faudry *et al.*, 2007, Blohmke *et al.*, 2010, Vega-Carrascal *et al.*, 2014)
		CFTR/TLR4/TLR5	Phagocytosis regulation, inflammatory response	(Blohmke *et al.*, 2010, Haenisch *et al.*, 2010)
		TRPV4	Immune defence enhancement	(Scheraga *et al.*, 2020)
		Elastase	IgG cleavage, phagocytosis inhibition	(Bainbridge & Fick, 1989)
		LL-37, CLEC5A	NET formation, cytokine release	(Sung *et al.*, 2022)
		MIF	Lung inflammation reduction	(Tynan *et al.*, 2017)
		Various	Altered immune cell composition, pathway regulation	(Yang *et al.*, 2023, Hu *et al.*, 2022),
		--	Gut microbiota metabolism disruption	(Wen *et al.*, 2022)
	Bloodstream	TREM-1	Inflammatory response modulation	(Gibot *et al.*, 2019)
		QS genes, pqsH	Systemic infection adaptation	(Kruczek *et al.*, 2016)
		Hxu system	BSI pathogenesis regulation	(Yang *et al.*, 2022a)
		--	Blood metabolome alteration	(Elmassry *et al.*, 2020)
		--	Differential immune cell response	(Oelen *et al.*, 2022)

Figure 1. Structure of the data collection and analysis workflow. For each level, we identified further conceptual domains, describing the interactions with *PA* molecules.



PA-host proteins interaction network reveals key mechanisms modulated in humans by PA severe infection

We manually curated the molecular interactions between *PA* and human proteins during different infection stages. Analysis of 149 articles revealed multiple direct protein-protein interactions (PPI) and molecule-protein interactions (MPI), detailed in Table-S2 and annotated with Uniprot IDs, references, and model subdomains.

We identified 152 molecules (109 human proteins, 3 human molecules, 34 *PA* proteins, and 5 *PA* molecules), yielding 189 *PA*-human interactions and 7 human-human interactions. These interactions were categorized into four cellular domains: Adhesion process (*PA*-Ad), invasion and injury of tissue (*PA*-Inv), exotoxin production (*PA*-Ex) and bacterial metabolism (*PA*-meta).

Gene enrichment analysis revealed significant pathway associations across Reactome, WikiPathways and KEGG (Table-S3). Notable enrichments included the "Pathogenic *Escherichia coli* Infection WP2272" pathway (WikiPathway) and "Pertussis" (KEGG) with FDR<0.0001%. Reactome analysis highlighted three significant pathways (FDR<0.0001%), including Programmed Cell Death R-HSA-5357801, Toll-like Receptor Cascades R-HSA-168898, and Signalling by Interleukins R-HSA-449147. Key *PA* proteins, particularly exoS and exoT, demonstrated significant modulation of these pathways, through inhibition of interleukin proteins or degradation of occludin (OCLN), a cell death regulator (Barbieri, 2000). Notably, within the Signalling by Interleukins R-HSA-449147, we identified fibronectin (FN1), as an important player in cell adhesion, blood coagulation, and innate immunity. A full network of interactions between *PA* and human host proteins was built (Fig. 2). The network shows the overall cell response to infection, including new possible pathogenic mechanisms. The Complement Cascade Pathway (Reactome R-HSA-166658; 18/55; FDR<0.0001%) was modulated by outer membrane proteins oprH, oprQ, and the elastase lasB, contrasting cell damage by binding complement proteins like C3. These proteins also showed significant interactions with blood clotting factors (VWF, SERPINF2, PLAUR, PLAT, and PLG). GSEA analysis on the WikiPathways data highlighted the interaction with coagulation cascades (Complement and Coagulation Cascade WP558; 20/58; FDR<0.0001%), suggesting a potential involvement in thrombosis. Our network-based model also revealed a coordinated mechanism whereby exoS, exoY, and exoT trigger cell toxicity through interactions with cytoplasmic 14-3-3 proteins (e.g., YWHAB), further supporting exoS's central role in *PA* infection.

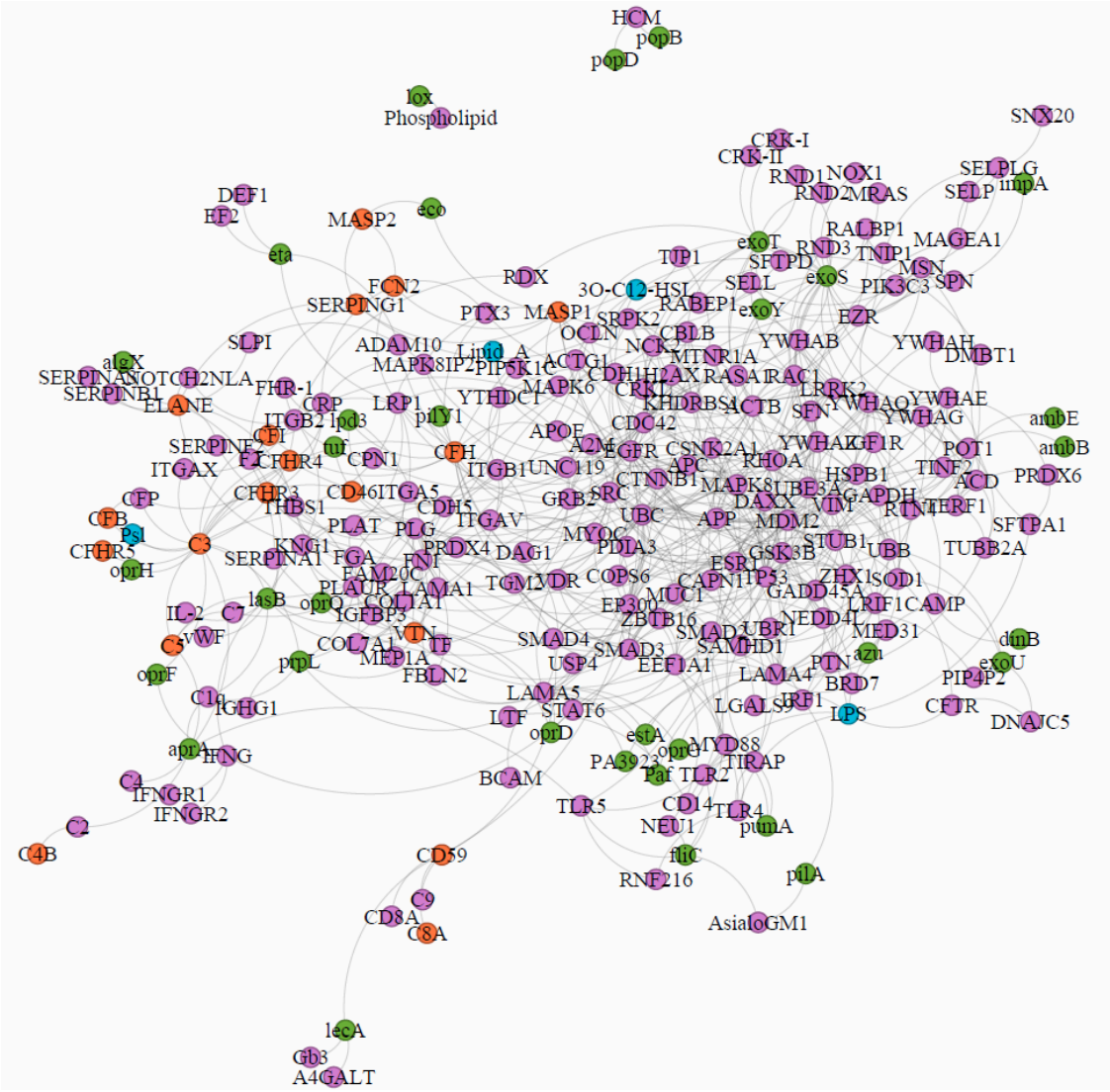


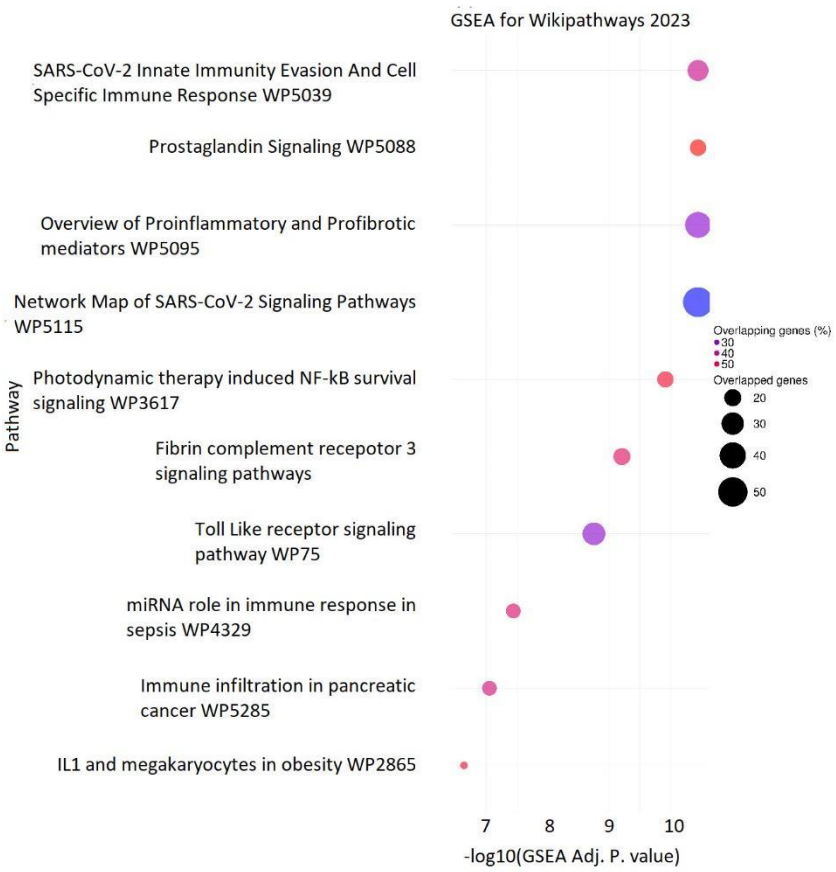
Figure 2. Network of *PA*-human host molecular interactions, with the top 200 nearest proteins found by the Random Walk with Restart (RWR) algorithm. Nodes have different colours to show different kinds of molecules: purple = human proteins, green = *PA* proteins, light blue = *PA* molecules, orange = human proteins belonging to the complement pathway.

Meta-analysis of whole transcriptome of PA-infected lung tissues from mice reveals selective modulation of pro-inflammatory pathways

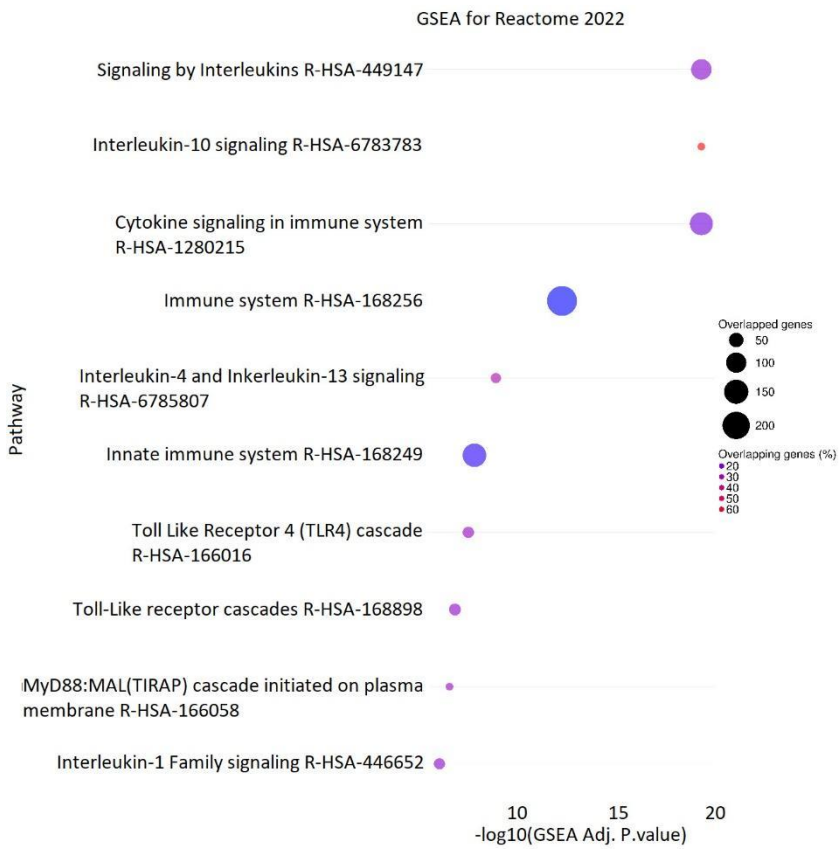
Our meta-analysis of gene expression of two bulk RNAseq datasets (GSE233206 and GSE192890) comparing *PA*-infected mice lung samples with healthy controls identified 1,560 upregulated and 383 downregulated genes (Log2FC > 1; FDR BH<0.05, Table S4).

Pathway analysis of upregulated genes using WikiPathways revealed significant enrichment in inflammation-related pathways, notably “Overview of Proinflammatory and Profibrotic Mediators

WP5095” (39/129, FDR<0.0001%). Reactome analysis aligned with our scoping review findings, highlighting significant enrichment (FDR<0.000001%) in key pathways: Signalling by Interleukins R-HSA-449147 (103/453), Interleukin-10 Signalling R-HSA-6783783 (31/45), Cytokine Signalling in Immune System R−HSA−1280215 (145/702) (Fig. 3a/b). Proinflammatory pathways were found nested into Interleukins R-HSA-449147 Reactome’s entry (Interleukin-2 family signaling R-HSA-451927; Interleukin-3, Interleukin-5 and GM-CSF signaling R-HSA-512988; Interferon alpha/beta signaling (Homo sapiens) R-HSA-909733; Interferon gamma signaling (Homo sapiens) R-HSA-877300; ISG15 antiviral mechanism (Homo sapiens) R-HSA-1169408; PKR-mediated signaling (Homo sapiens) R-HSA-9833482; TNFR2 non-canonical NF-kB pathway (Homo sapiens) R-HSA-5668541; Signaling by CSF1 (M-CSF) in myeloid cells (Homo sapiens); R-HSA-9680350), all presenting gene targets for *PA* exoU, exoS, azu, lasB, aprA, oprF, pilA, and LPS. These results suggest that these pathways are directly involved in initiating the innate response to *PA* infection, but also highlight the potential role of *PA* molecules in modulating and limiting this response, particularly for interleukin signalling.



a)



b)

Figure 3. GSEA with Reactome (A) and WikiPathways (B), based on upregulated DEGs in *PA* -infected samples, obtained from meta-analysis of two infection experiments in mouse lung tissues.

Discussion

In this work, we present our development of a comprehensive data integration model to understand *PA* infection through a detailed exploration of the literature and metanalysis of transcriptomics datasets. Our network-based explorative approach captures the complexity of severe bacterial infection by identifying specific molecular targets for each *PA* molecule, pathogenic mechanisms and host responses. *PA* serves as a complete model organism for studying severe systemic infections, given its multi-drug resistance capabilities, ability to cause acute and chronic infections in pulmonary disease patients, and its capacity to form biofilm in hypoxic conditions which makes it extremely difficult to treat [41, 42].

The computational analysis and data curation process established an annotated dataset of protein-protein interactions and molecule-protein interactions (PPI/MPI) between *PA* and human hosts, revealing several key mechanisms. Firstly, the central role of exoS during infection was confirmed, while an enhanced activity among exo family proteins, including exoY and exoT was widely

highlighted (Chadha *et al.*, 2022). ExoS functions by inhibiting several proteins of interleukin pathways and inducing the degradation of Occludin (OCLN), an integral membrane protein involved in cytokine-induced regulation of the tight junction permeability barrier ultimately inducing cell death (Soong *et al.*, 2008). Through its ADP RT activity, exoS modulates host cell apoptosis, inducing *PA*-infected cell death by targeting various Ras proteins (Jia *et al.*, 2006). The Complement Cascade Pathway undergoes modulation by *PA*'s outer membrane proteins oprH, oprQ, and elastase lasB, which trigger cytotoxic effects, and adhesion through complement protein binding, particularly C3 (Arhin & Boucher, 2010). This result mirrors the mechanism of activation of the complement system, in which C3 is the main actor against bacteria, through a link with oprF, a porin involved in ion transport (Na^+ and Cl^-) and anaerobic biofilm production (Sugawara *et al.*, 2012, Mishra *et al.*, 2015). A significant finding was the interaction between oprH, oprQ, and lasB with coagulation proteins, suggesting their activity in thrombotic processes. *PA* lasB's cleavage of a C-terminal- peptide FYT21 derived from thrombin inhibits activation of the transcription factors NFκ-B and activator protein 1 (AP-1). *PA* demonstrates sophisticated modulation of host immune responses through multiple pathways. The bacterial proteins aprA, lasB, and exoS exhibit inhibitory effects on interleukin pathways (Chadha *et al.*, 2022, Matsumoto, 2004, Phuong *et al.*, 2021), indicating an adaptive modulation that enhances *PA* survival within the host. Such an effect was confirmed in *PA* infection, where *PA*-derived DnaK negatively regulates IL-1β production by cross-talk between JNK and PI3K/PDK1/FoxO1 pathways (Lee *et al.*, 2020). Notably, decreased *PA* levels in CF patients correlate with reduced proinflammatory cytokines (Colombo *et al.*, 2005).

Our findings provide a deep view on molecular perturbations in *PA* systemic infection and serve as a foundation for developing specific disease maps for severe *PA* infection, supporting the integration of omics data from clinical cases into predictive computational models. Future developments may incorporate text mining and AI-assisted analysis for drug target identification (Niarakis *et al.*, 2023) and digital modelling of the human immune system under infection conditions (Niarakis *et al.*, 2024) to better predict real patient outcomes and test potential therapeutic strategies in a personalized fashion.

Several limitations warrant consideration. While we have documented numerous significant *PA*-human interactions, our model may not encompass all possible interactions. The PPI/MPI dataset requires iterative updates to incorporate new experimental findings from both *in vitro*, *in vivo* and clinical studies. Furthermore, since our interaction data derives primarily from *in vitro* experiments, the described pathogenic mechanisms require validation in the context of severe systemic infections. Finally, our differential expression meta-analysis, conducted in mouse models with limited sample

size, provides an overview of host gene-expression signatures in *PA* infection but requires confirmation through clinical data.

Conclusion

Our study presents a comprehensive collection and analysis of molecular mechanisms of *P. aeruginosa* infection through a systematic data integration approach. By combining literature-based evidence, protein-protein interaction analysis, and transcriptomic data from *in vivo* studies, we have constructed a detailed dataset of *PA*-host interactions across cellular, tissue, and organ levels. Our findings highlight the complex interplay between *PA* virulence factors and host responses, particularly the role of exoS in modulating interleukin pathways and the involvement of outer membrane proteins in complement cascade regulation. The identification of 189 *PA*-human interactions involving 152 molecules provides a detailed understanding of molecular pathophysiology in invasive bacterial infections and direct molecular interactions between *PA* and host proteins, as well as involvement of molecular pathways linked to clinical phenotypes of sepsis.

The integration of differential expression analysis from mouse models further strengthens our understanding of host response patterns, particularly in proinflammatory and immune signaling pathways. As antimicrobial resistance continues to pose significant challenges in healthcare, such comprehensive molecular understanding may prove invaluable for applying precision medicine approaches to severe bacterial infections and improving patient-tailored treatments in severe systemic infections.

Contributors

CF, CR and FM designed the study, developed the search strategy with feedback from LL and BS. FM, CR, VD and MP screened and selected studies. CR, VD, SC, MP and BR extracted the data and prepared the data for analysis. FM, CR, LL, BS, LG, MGB, GC and CF verified the study data. FM, LL and SC analysed the data. FM, CR, VD and MP wrote the first draft of the manuscript. FM, LL, MGB, GC and CF, writing—review and editing. MGB and CF, supervision. CF and FM, project administration; FM, LG, and CF, funding acquisition. All authors critically revised the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit it for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The datasets generated and analysed in this meta-analysis are published in the supplementary text and in supplementary tables.

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