Using Genomic Tools to Predict Antimicrobial Resistance and Markers in Clinical Bacterial Samples

Tony Hong-Wei Chang[1], Julie D Pourtois[1], Naomi Haddock[2], Daisuke Furkuawa[1], Thomas Hong[3], Derek Amanatullah[3], Elizabeth Burgener[4] and Paul Bollyky†

[1]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
[2] University of California San Francisco Medical School, San Francisco, CA, USA
[3]Department of Orthopaedic Surgery, Stanford Hospital and Clinics, Redwood City, CA, USA
[4]Division of Pediatric Pulmonology & Sleep Medicine | Children's Hospital Los Angeles, Keck School of Medicine at USC, Los Angeles, CA, USA

†Address correspondence to pbollyky@stanford.edu

Abstract

Antimicrobial resistance (AMR) poses a critical threat to hospital infections particularly in the context of hospital-acquired infections (HAIs). This study leverages genomic tools to predict AMR and identify resistance markers in clinical bacterial samples associated with HAIs. Using comprehensive genomic and phenotypic analyses, we evaluated the genetic profiles of Pseudomonas aeruginosa and Staphylococcus aureus to uncover resistance mechanisms. Our results demonstrate that genomic tools, such as CARD-RGI and the Solu platform, can accurately identify resistance genes and predict AMR phenotypes in nosocomial pathogens. These findings underscore the potential of integrating genomic approaches into clinical practice to enhance the management of resistant infections in hospital settings and inform the development of novel antimicrobial strategies.

Importance

This study investigates the impact of prophages on antibiotic resistance in two clinically significant bacteria, Pseudomonas aeruginosa and Staphylococcus aureus. Understanding how prophages influence resistance mechanisms in these pathogens is crucial, as Pseudomonas aeruginosa is known for its role in chronic infections in cystic fibrosis patients, while Staphylococcus aureus, including MRSA strains, is a leading cause of hospital-acquired infections. By exploring the relationship between prophage presence and resistance, this research provides insights that could inform the development of more effective treatment strategies and enhance our ability to combat antibiotic-resistant infections, ultimately improving patient outcomes and public health.

Introduction

Antimicrobial Resistance in Hospital-Acquired Infections

Hospital-acquired infections (HAIs), particularly those caused by ESKAPE pathogens—*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* [1]species—are a major public health concern due to their high levels of antimicrobial resistance[2]. These pathogens are significant contributors to HAIs, leading to severe complications and increased mortality[3,4,5].

Pseudomonas aeruginosa

Pseudomonas aeruginosa (Pa) is a Gram-negative rod-shaped opportunistic pathogen known for its high resistance to antibiotics and disinfectants[6,7]. It is particularly problematic in patients with compromised immune systems and is known to form biofilms that enhance its resistance. The large genome of *Pa*, typically ranging from 5.5 to 7 million base pairs, contributes to its adaptability and complex resistance mechanisms[8,9], including intrinsic resistance through its outer membrane, acquired resistance via mutations, and horizontal gene transfer[10,22,12,13,14]. *Staphylococcus aureus (Sa)* is a Gram-positive bacterium commonly found on the skin and in the nasal passages[15]. While usually harmless, it can cause a range of infections from minor skin conditions to severe diseases like pneumonia and sepsis, especially when the skin barrier is breached, or the immune system is compromised[16,17]. *Sa* has a smaller genome (2.7 to 3.0 million base pairs) but exhibits high virulence and resistance, notably in methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Sa also forms biofilms on medical devices, making it resistant to antibiotics[18,19].

Genomic Tools for Predicting AMR

Advances in genomic sequencing have revolutionized the study of antimicrobial resistance (AMR). Tools like the Comprehensive Antibiotic Resistance Database (CARD) and its Resistance Gene Identifier (RGI) facilitate the identification of resistance genes in bacterial genomes[20,21,22,23], aiding in the prediction of AMR phenotypes. Additionally, a platform like Solu.bio provide efficient DNA sequencing and analysis, enabling rapid identification of resistance patterns[24,25,26,27,28,29].

Temperate Phages and AMR

Temperate phages can integrate into bacterial genomes as prophages, contributing to genetic diversity and potentially carrying AMR genes [30,31,32,33,34,35,36,37]. These prophages can transfer resistance genes through lysogenic conversion and transduction, impacting bacterial resistance mechanisms[38,39]. The role of prophages in biofilm formation and bacterial virulence further complicates treatment strategies for infections[40,41].

Study Focus

This study evaluates the use of genomic tools to predict AMR and identify resistance markers in clinical samples of *Pseudomonas aeruginosa* (*Pa*) and *Staphylococcus aureus (Sa)*. By integrating genomic and phenotypic data, the research aims to enhance the understanding of resistance mechanisms and inform the development of new antimicrobial strategies.

Results

Correlation of ARO Genetic Terms with Antibiotic Resistance

Tobramycin Resistance

The correlation of ARO genetic terms of *Pa* with tobramycin resistance is illustrated in Figure 1. We mapped bacterial genes associated with antibiotic resistance using both bar plots and box-and-whisker plots. A Wilcoxon test was employed to determine the statistical significance of differences in resistance profiles among variants of the aadA gene(Figure 1A). The analysis revealed that the aadA gene was significantly associated with antibiotic resistance (Wilcoxon test, p-value = 0.018), indicating that its presence likely contributes meaningfully to the resistance phenotype (Figure 1B). Conversely, the aadA6 variant did not contribute significantly to antibiotic resistance (Wilcoxon test, p-value = 0.698), suggesting it does not play a substantial role in conferring resistance under the conditions studied (Supplemental Figure 3A). The differential significance of these aadA variants highlights the complex genetic underpinnings of antibiotic resistance and underscores the necessity of targeted genetic analysis in understanding and combating resistance[44].

Ciprofloxacin Resistance

We also explored the association of specific ARO terms—gyrA and adeF—with *Pa* phenotypic resistance to ciprofloxacin. These genes were selected based on bar plots that indicated their potential influence on AMR45(Figure 1C). The gyrA gene showed a nonsignificant relationship with resistance (Wilcoxon test, p-value = 0.0787), indicating that its presence alone may not be sufficient to confer significant resistance to ciprofloxacin (Figure 1D)[49]. Similarly, the adeF gene failed to establish a significant correlation with resistance (Wilcoxon test, p-value = 0.242) (Supplemental Figure 3B). This analysis sheds light on the intricate genetic interactions underlying antibiotic resistance, emphasizing the importance of identifying specific genetic markers that influence resistance phenotypes[40].

Genes associated with AMR in *Pa* clinical samples

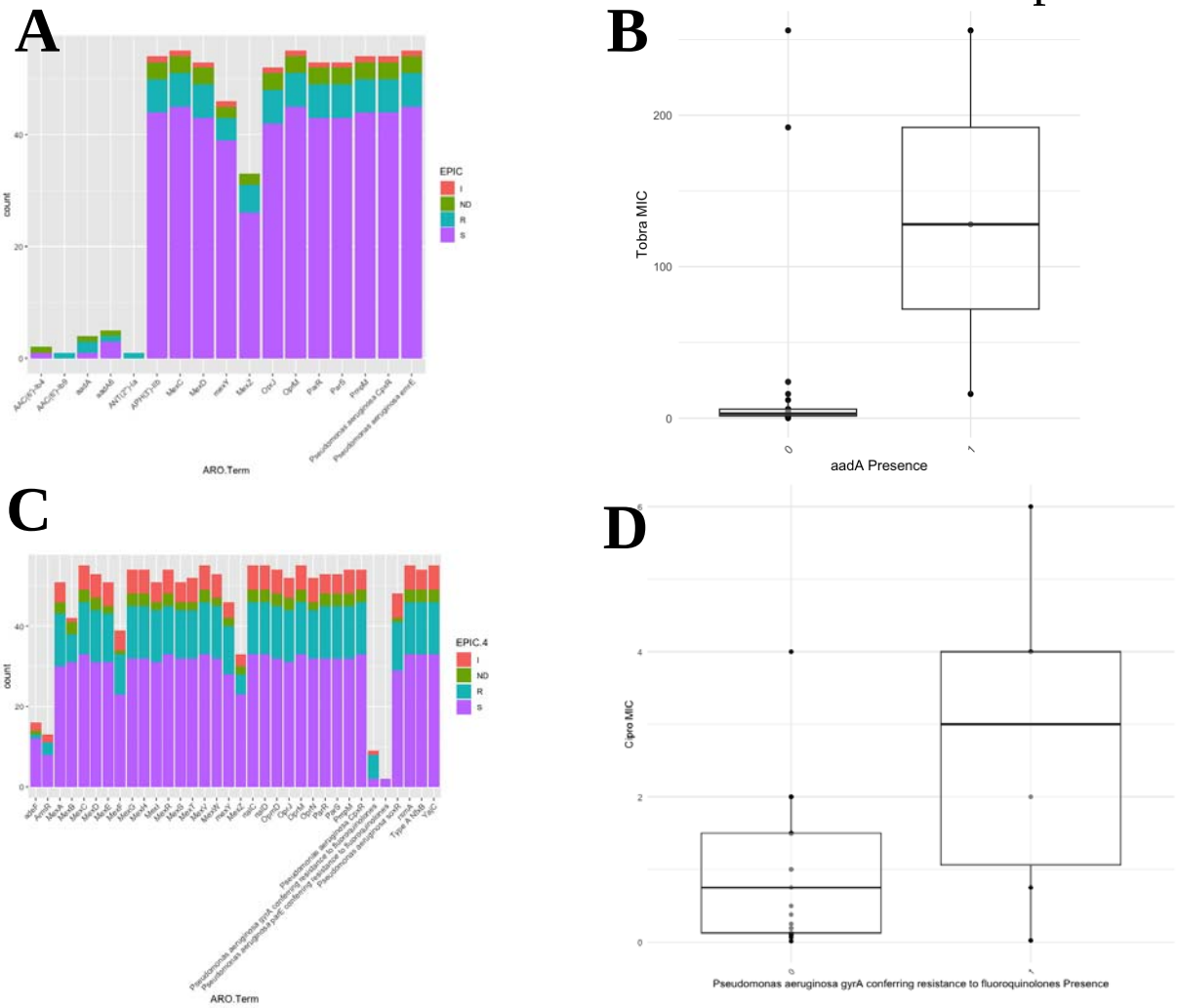


Figure 1. *Pa* clinical dataset has ARO terms that contribute to tobramycin resistance. ARO term aadA is significantly associated with antibiotic resistance **A.** Barplot of *Pa* ARO terms **B.** The box and whisker plot indicates that *Pa* clinical samples with the aadA ARO term display tobramycin resistance with minimum inhibitory concentrations (MICs) exceeding 100 µg/ml, contrasting with susceptible samples that have an MIC of 0 µg/ml. *Pa* clinical dataset has ARO terms that contribute to ciprofloxacin resistance specifically ARO term gyrA. EPIC.4 is Ciprofloxacin, I as indeterminate, ND, no data provided, R=Resistance, S= Susceptibility **C.** Manhattan plot on Aeruginosa ARO terms mapped below to see which ARO terms are more prominent in resistance samples **D.** Box and whisker plot showing that the ARO term gyrA in *Pa* clinical samples that display ciprofloxacin resistance MIC as over 5ug/ml vs susceptible was 1ug/ml

CARD RGI vs Solu

The presented heatmaps illustrate the analysis of *Pa* data on the top and *Sa* data on the bottom. Each row represents different samples, and each column corresponds to specific resistance genes. The left panels display the Solu Bio analysis results, while the right panels show the CARD-RGI analysis results. Despite utilizing different vocabularies, both analysis methods produce highly similar patterns, demonstrating the effectiveness of each platform in identifying resistance genes.

For *Pa* data, the Solu Bio analysis identifies the presence and abundance of resistance genes across the samples, highlighting a consistent pattern of gene distribution. The CARD-RGI analysis corroborates these findings, demonstrating comparable gene presence and abundance across the same set of samples. Specific genes such as mexA, mexB, oprJ, and oprM exhibit consistent patterns across both analyses, indicating their prominent role in resistance.

Similarly, for *Sa* data, the Solu Bio analysis identifies resistance genes with a consistent pattern of gene distribution across the samples. The CARD-RGI analysis supports these results, showing comparable gene presence and abundance. Genes such as *mecA*, *mecR1*, *blaZ,* and *femA* are consistently detected across all samples by both Solu Bio and CARD-RGI [47,48,49,50,51,52.]

These results underscore the success of both Solu Bio and CARD-RGI in accurately identifying resistance genes in clinical bacterial samples. The highly similar patterns observed between the two analysis methods highlight their reliability and robustness, making them effective tools for genomic analysis of antibiotic resistance.

Genomic Tools offer high degrees of predictability

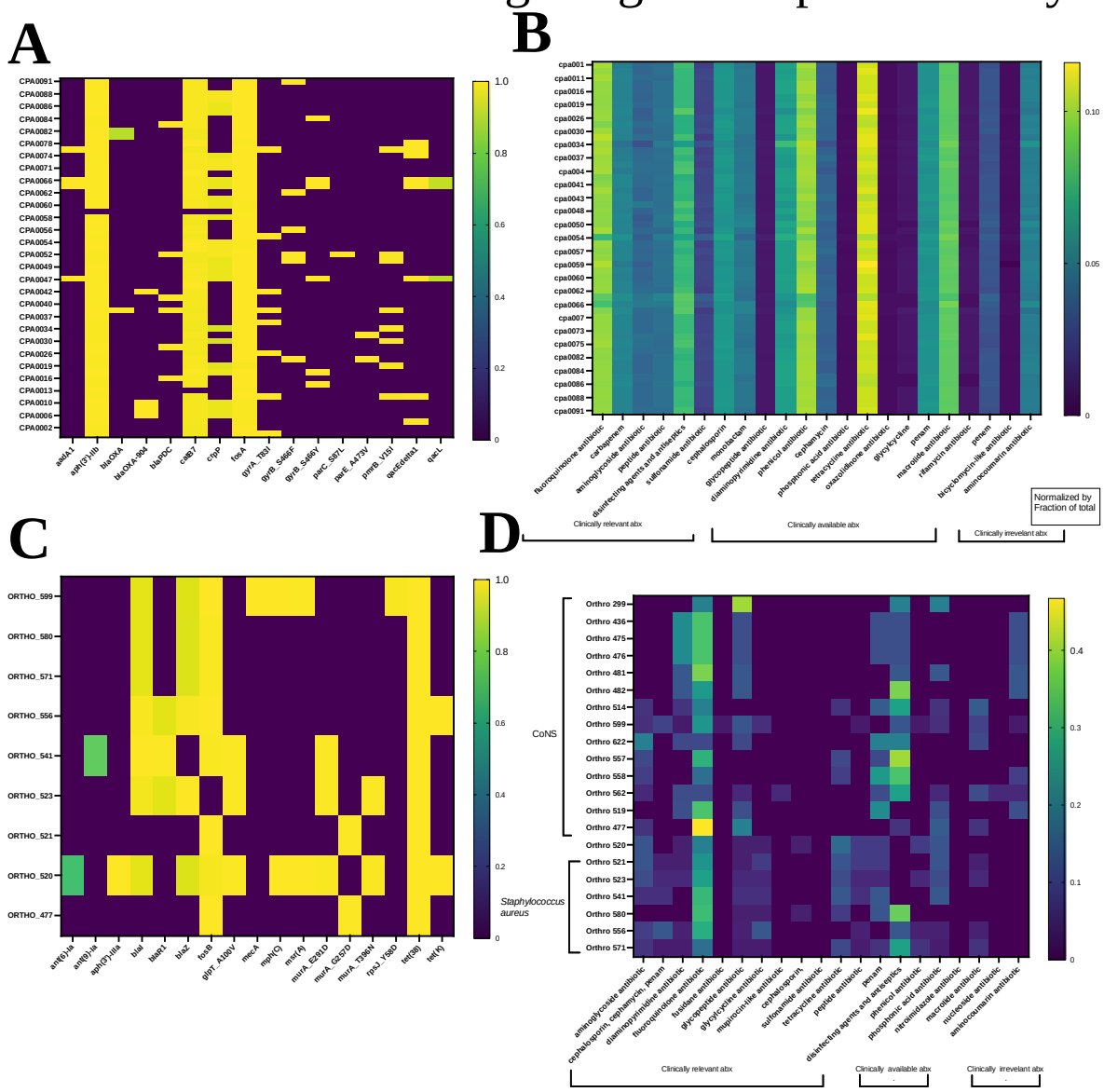


Figure 2: The presented heatmaps illustrate the analysis of *Pa* data on the top and *Sa* data on the bottom. Each row represents different samples, and each column corresponds to specific resistance genes.
A. the Solu Bio analysis results for *Pa*, while the **B.** CARD-RGI analysis results *Pa*. Despite utilizing different vocabularies, both analysis methods produce highly similar patterns. For *Pa* data, specific genes such as **mexA**, **mexB**, **oprJ**, and **oprM** exhibit consistent patterns across both analyses, indicating their prominent role in resistance. **C**. The Solu Bio analysis for *Sa* identifies the presence and abundance of resistance genes across the samples, highlighting a consistent pattern of gene distribution. **D.**The CARD-RGI analysis corroborates these findings, demonstrating comparable gene presence and abundance across the same set of samples. *Sa* data, genes such as **mecA**, **mecR1**, **blaZ**, and **femA** are consistently detected across all samples by both Solu Bio and CARD-RGI analyses.

Number of prophages has no impact on resistance profiles of both *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Impact of Prophage Numbers on Resistance in Pseudomonas aeruginosa

We hypothesized that the number of prophages in a bacterial sample might affect its resistance. To investigate this, we utilized both *Pa* and *Sa* datasets, uploading the complete bacterial genomes onto CARD-RGI. Resistance was quantified by the number of unique ARO terms per sample. Each sample, even from the same patient but with different bacteria, was treated separately. The number of ARO terms and prophages were then graphed and plotted with linear regression to evaluate correlation (Figure 3A).

For *Pa*, linear regression revealed that the number of prophages did not influence resistance (p-value = 0.3776) (Figure 3A). We then analyzed two factors: the range of antibiotic classes to which resistance is shown and the count of resistance mechanisms identified through genomic data. The number of prophages did not impact antibiotic drug class resistance (Linear regression, p-value = 0.6295) (Figure 3C) nor the number of resistance mechanisms (Linear regression, p-value = 0.4887) (Figure 3E).

To further enhance this analysis, we connected the prophage samples to clinical data from the clinical microbiology lab, investigating Pa resistance to ciprofloxacin and tobramycin. Ciprofloxacin disrupts bacterial DNA replication, and tobramycin interferes with protein synthesis, both being commonly prescribed antibiotics. We found that prophage count for resistance to ciprofloxacin (Shapiro-Wilk, p-value = 0.4320) and to tobramycin (Shapiro-Wilk, p-value = 0.4043) were not statistically significant (Supplemental Figure 2 A, B).

Impact of Prophage Numbers on Resistance in Staphylococcus aureus

For *Sa*, linear regression showed that the number of prophages did not correlate significantly with resistance (Linear regression, p-value = 0.0646) (Figure 3B). We further analyzed resistance to the number of antibiotic classes and the number of resistance mechanisms using genomic data. The number of prophages seemed to have an impact on antibiotic drug class resistance (Linear regression, p-value = 0.0121) (Figure 3D), but not on the number of resistance mechanisms (Linear regression, p-value = 0.1690) (Figure 3F).

Connecting the prophage samples to clinical data, we investigated Sa resistance to clindamycin and erythromycin. Clindamycin is active against many aerobic Gram-positive cocci, and erythromycin is active against many Gram-positive bacteria. We found that the prophage count for clindamycin (Shapiro-Wilk test, p-value = 0.6224) and erythromycin (Shapiro-Wilk test, p-value = 0.1566) resistance were not statistically significant when compared within susceptible and resistant categories (Supplemental Figure 3 A, B).

These results indicate that the number of prophages does not significantly influence phenotypic resistance in *Pa* and *Sa*, with specific impacts on *Sa's* ability to take the infections of prophages requiring further investigation.

Prophages have minimal impact on Antimicrobial resistance

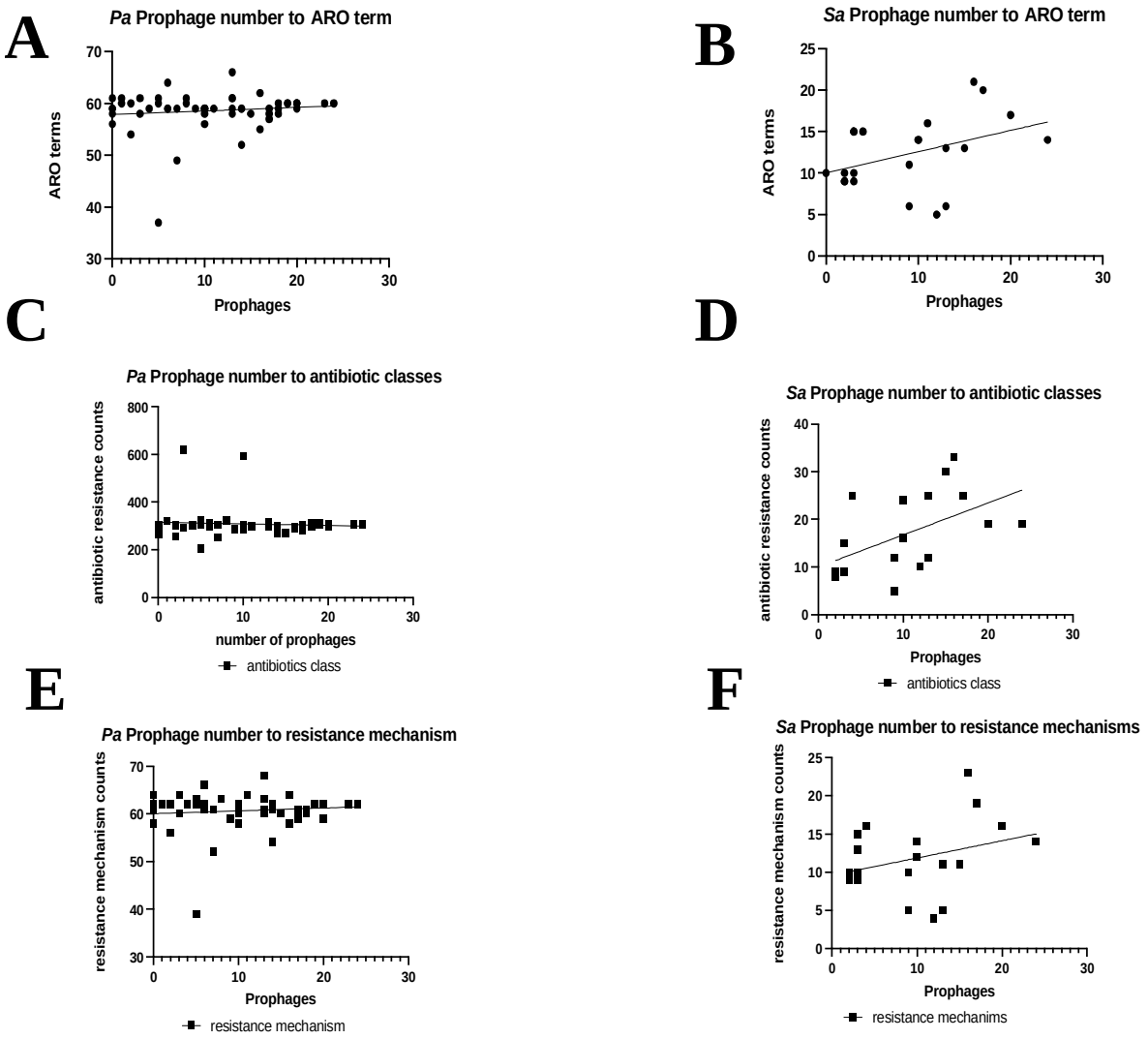


Figure 3:Effects of prophage presence on AMR. **A.** *Pa* prophage numbers has no effect on AMR **B.** *Sa* prophage numbers has no effect on AMR **C.** *Pa* prophage numbers has no effect on number of antibiotic resistance **D.** *Sa* prophage number has no effect on number of antibiotic resistance **E.** *Pa* prophage has no effect on number of resistance mechanisms **F.** *Sa* prophage has no effect on number of resistance mechanism

Impact of Prophage Presence on Antimicrobial Resistance in Pseudomonas aeruginosa and Staphylococcus aureus

The analysis of prophage presence in *Pa* and *Sa* was conducted to determine their influence on resistance to specific antibiotics. The results are summarized in the provided figure, which depicts the p-values for the correlation between prophage presence and antibiotic resistance in these bacterial species. For tobramycin (blue dots), the p-values indicate no significant correlation between the presence of various prophages and resistance in *Pa*. The resistance profiles remained consistent regardless of prophage presence, as shown by the high p-values for all prophages tested. Similarly, for ciprofloxacin (red dots), the p-values reveal no significant effect of prophage presence on resistance in *Pa*. The resistance levels to ciprofloxacin did not vary significantly with the presence of prophages, further supporting that prophages do not influence *Pa* resistance to these antibiotics. In contrast, for erythromycin (blue dots), the presence of prophages in *Sa* showed some effect on resistance. Lower p-values for certain prophages suggest a significant correlation, indicating that prophage presence may enhance resistance to erythromycin in *Sa*. This finding highlights a potential role for prophages in contributing to erythromycin resistance. Conversely, for clindamycin (red dots), the p-values did not show a significant correlation with prophage presence. The resistance profiles for clindamycin remained unaffected by the presence of prophages, indicating that prophage-related resistance mechanisms may be specific to certain antibiotics like erythromycin.

The study's findings highlight a stark contrast between the impact of prophages on antibiotic resistance in *Pa* and *Sa*. In *Pa*, prophages do not significantly affect resistance to tobramycin and ciprofloxacin, suggesting that other genetic or environmental factors are more critical in determining *Pa* resistance to these antibiotics. Conversely, in *Sa*, prophages appear to influence resistance to erythromycin, but not to clindamycin, indicating that prophage-related resistance mechanisms in *Sa* may be specific to certain antibiotics. These results underscore the importance of considering the role of prophages in antibiotic resistance studies and the potential for targeted genetic analysis to better understand and combat resistance in different bacterial species.

Prophages as Markers for Antimicrobial resistance

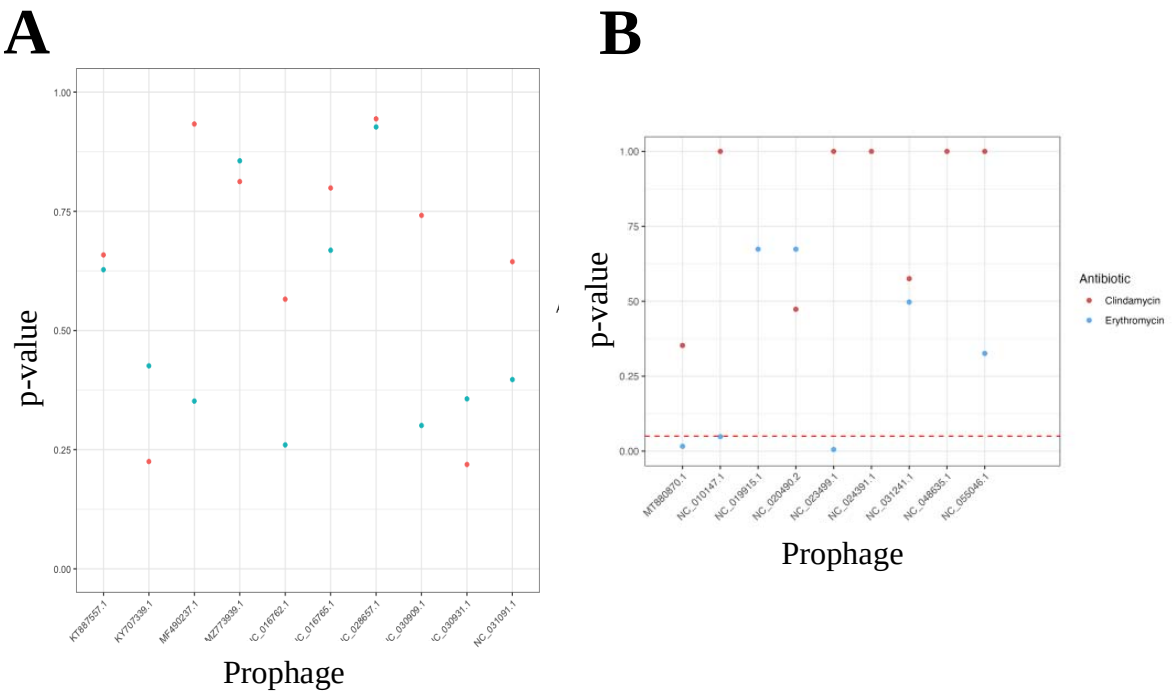


Figure 4: The analysis of prophage presence in *Pa* and *Sa* aimed to determine their influence on resistance to specific antibiotics, summarized in Figures 4A and 4B. In Figure A, the impact on *Pa* resistance to tobramycin (blue dots) and ciprofloxacin (red dots) is shown. The p-values indicate no significant correlation between prophage presence and resistance to tobramycin or ciprofloxacin in *P*a. Resistance profiles remained consistent across all prophages, suggesting that prophages do not influence *Pa* resistance to these antibiotics. In contrast, Figure B illustrates the impact on *Sa* resistance to erythromycin (blue dots) and clindamycin (red dots). The presence of prophages in Sa showed some effect on resistance to erythromycin, with lower p-values for certain prophages indicating a significant correlation. This suggests that prophage presence may enhance resistance to erythromycin in *Sa*. However, for clindamycin, no significant correlation was found between prophage presence and resistance, indicating that prophage-related resistance mechanisms in *Sa* may be specific to certain antibiotics like erythromycin.

Discussion

Genomic Tools for Predicting Antimicrobial Resistance

Throughout the research, the utilization of bacterial genomic data to investigate the genomic basis of antibacterial resistance. This was first demonstrated clinically through the development of tests to detect the mecA gene in *Staphylococcus aureus* (*Sa*), a marker identifying methicillin-resistant *Sa* (MRSA). Applying similar genomic approaches to *Pseudomonas aeruginosa* (*Pa*), I have identified trends and resistance mechanisms that are not readily apparent through phenotypic analysis alone. This work highlights the importance of genomic tools in uncovering resistance mechanisms and emphasizes the complementary nature of genotypic and phenotypic data.

Identification of Resistance Genes:

Pseudomonas aeruginosa (*Pa*): The analysis revealed significant resistance genes such as gyrA, aadA, aph(3')IIB, catB7, fosA, aadA1, blaOXA, and sul1. These genes encode enzymes that mediate resistance by various means, including antibiotic modification, degradation, or target site alteration. Particularly, the presence of genes like qacEdelta1, although not encoding an RND efflux pump, produces a protein that serves as an efflux mechanism for quaternary ammonium compounds, potentially reducing antibiotic susceptibility[73].

Staphylococcus aureus (*Sa*): Key genes identified include blaI, tet38, fosB, and erm(A), which are involved in beta-lactamase production, efflux pumps, fosfomycin resistance, and ribosomal modification, respectively. These findings align with the CARD data, which shows a trend of resistance to fluoroquinolones, glycopeptides, and phosphonic acid class antibiotics[84].

Comparison of Genomic Tools:

The CARD-RGI and Solu platforms demonstrated a high degree of consistency in predicting AMR profiles. Both tools effectively identified significant resistance genes in Pa and Sa, validating their use in clinical settings. The Solu software's user-friendly interface and integrated visualization tools, such as phylogenetic trees and heatmaps, enhance the interpretability of comparative genomic studies, making it accessible for users with limited computational expertise.

Phenotypic vs. Genotypic Data:

While the genotypic data provides comprehensive insights into the presence of resistance genes, it is important to note that not all identified genes correspond to phenotypic resistance sometimes the genomic data is more comprehensive than what is clinically noted. This discrepancy underscores the need for a combined approach, utilizing both phenotypic resistance experiments and genomic data to gain a complete understanding of bacterial resistance profiles.

Prophages as Markers of Resistance

Role of Prophages in AMR:

The analysis of prophages in bacterial genomes has revealed their potential role in resistance mechanisms. In *Sa*, the correlation between prophage numbers and resistance suggests that prophages may contribute to the bacteria's resistance profile, possibly through regulatory or indirect pathways. In contrast, *Pa* showed no clear correlation between prophage numbers and resistance capabilities, likely due to its larger genome masking these effects.

Clinical Implications:

Understanding the prophage-resistance nexus in *Sa* could lead to new strategies for countering bacterial resistance. Prophages may serve as surrogate markers for resistance testing, enhancing clinician response times to antibiotic resistance in hospitals and clinical infections. For *Pa*, the lack of a clear association between prophages and resistance highlights the need for further investigation into other genetic or environmental factors influencing resistance.

Future Directions:

Future studies should isolate prophages in vitro to dissect their resistance mechanisms and pathways of transmission. Techniques such as gene knockout or alterations in signaling pathways could provide deeper insights into how prophages impact bacterial resistance. Additionally, integrating genomic tools with clinical data will be crucial in validating the accuracy and applicability of these tools in predicting resistance phenotypes.

Conclusion

This research has broadened the application of genomic-based diagnostic tools, demonstrating their versatility across bacterial pathogens like *MRSA* and *Pseudomonas aeruginosa*. By identifying specific resistance genes, these tools enhance our understanding of antibiotic resistance mechanisms and support the development of targeted treatments. The Solu software, with its efficient processing of large-scale genomic data and user-friendly interface, represents a significant advancement in confronting antibiotic resistance. This work underscores the potential of genomic tools to complement phenotypic data, providing a rapid and precise detection system that informs more tailored treatment decisions and fosters the development of innovative therapeutic strategies.

Materials and Methods

Sample Collection

From June 2020 to June 2023, 55 *Pseudomonas aeruginosa (Pa)* isolates from respiratory cultures of cystic fibrosis patients and 21 *Staphylococcus aureus (Sa)* isolates from prosthetic joint infections were collected at Stanford Hospital under IRB approvals #11197 and #54462, respectively. Samples were biobanked with patient consent, de-identified using unique codes, and associated antibiotic histories were extracted from electronic medical records.

DNA Extraction and Sequencing

DNA was extracted from Pa and Sa isolates using the DNeasy Blood and Tissue Kit (Qiagen, 69504) and sequenced at the Stanford Illumina sequencing core. The extraction involved bacterial lysis, DNA purification, and elution, ensuring high-quality DNA suitable for sequencing. Sequencing reads were quality-checked using FASTQualityControl and trimmed with Trimmomatic 0.39. Quality reports were assembled using MultiQC55.

Genomic Analysis

Sequencing reads were mapped to reference genomes (Pa: GCF_000006765.1, Sa: NC_007795.1) using Bowtie2 v2.4.4, and mapping reads were quantified with samtools. Draft genomes were assembled using SPAdes, and FASTA files were generated for further analysis[55].

CARD-RGI Analysis

FASTA files were uploaded to the CARD-RGI platform (https://card.mcmaster.ca/analyze/rgi) to predict antibiotic resistance genes. The platform identified "perfect" and "strict" hits, categorizing resistance genes based on the Antibiotic Resistance Ontology (ARO). Results were downloaded, and resistance genes were analyzed using R 4.2.0 for statistical computing and data visualization[56].

Solu Platform Analysis

The Solu platform (https://platform.solu.bio/) facilitated bulk upload and analysis of genomic sequences, identifying bacteriophage annotations and resistance genes. Samples were processed using the VIBRANT tool for prophage detection, and results were compiled into an Excel workbook for further analysis[47].

Data Normalization

After categorization and quantification of the total ARO terms, the number for each class of samples was normalized by dividing by the total number samples and plotted on a heatmap

Statistical Analysis

Statistical analyses were performed using Prism 9.3.1 and R. Test used included linear regression, chi squared test. After categorization and quantification, the number of resistance genes for each clinical sample was normalized by dividing by the total number of samples and plotted on a heatmap. Prophage presence and resistance mechanisms were graphed, with no pre-determined sample sizes. Investigators were blinded during DNA extraction, library preparation, and phage genome annotation, but not during the final analysis. * $P \leq 0.05$

Acknowledgements

T.C. discloses support from the Cystic Fibrosis grant student traineeship and Stanford Bio-X foundation grant. P.L.B. discloses support from National Institutes of Health grant R01 HL148184-01, National Institutes of Health grant R01 AI12492093, National Institutes of Health grant R01 DC019965, Cystic Fibrosis Foundation grant, and Emerson Collective grant. The contents are those of the authors and do not necessarily represent the view of the funding agencies.

Competing interests

The authors declare no competing interests

References

1.De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, et al. Antimicrobial Resistance in ESKAPE Pathogens. Clinical Microbiology Reviews [Internet]. 2020 May 13;33(3). Available from: https://journals.asm.org/doi/10.1128/CMR.00181-19

2.Santajit S, Indrawattana N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. BioMed Research International [Internet]. 2016;2016:1–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4871955/

3.Denissen J, Reyneke B, Waso-Reyneke M, Havenga B, Barnard T, Khan S, et al. Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. International Journal of Hygiene and Environmental Health [Internet]. 2022 Jul 1;244:114006. Available from: https://www.sciencedirect.com/science/article/pii/S143846392200089X

4.Centers for Disease Control and Prevention. Current HAI progress report [Internet]. CDC.gov. 2022. Available from: https://www.cdc.gov/hai/data/portal/progress-report.html

5.Healthcare-Acquired Infections (HAIs) – PatientCareLink [Internet]. www.patientcarelink.org. Available from: https://www.patientcarelink.org/improving-patient-care/healthcare-acquired-infections-hais/#:~:text=In%20American%20hospitals%20alone%2C%20the

6.Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. Journal of the Royal Society of Medicine [Internet]. 2002;95 Suppl 41(Suppl 41):22–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1308633/

7.Dai P, Jiao F, Yang L, Ousman Bajinka, Abdelhalim KA, Wu G, et al. Multiple Mechanisms Synergistically Induce Pseudomonas Aeruginosa Multiple Drug Resistance. 2023 May 6;14(2):627–34.

8.Cabot G, López-Causapé C, Ocampo-Sosa AA, Sommer LM, Domínguez MÁ, Zamorano L, et al. Deciphering the Resistome of the Widespread Pseudomonas aeruginosa Sequence Type 175 International High-Risk Clone through Whole-Genome Sequencing. Antimicrobial Agents and Chemotherapy. 2016 Dec;60(12):7415–23.

9.Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, et al. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature. 2000;406(6799):959–64.

10.Jurado-Martín I, Sainz-Mejías M, McClean S. Pseudomonas aeruginosa: An Audacious Pathogen with an Adaptable Arsenal of Virulence Factors. International Journal of Molecular Sciences [Internet]. 2021 Mar 18;22(6). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8003266/

11.Gellatly SL, Hancock REW. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathogens and Disease. 2013 Mar 15;67(3):159–73.

12.González M, Blasco L, María López, Ortiz-Cartagena C, Inés Bleriot, Pacios O, et al. Prophage identification and molecular analysis in the genomes of *Pseudomonas aeruginosa* strains isolated from critical care patients. mSphere. 2023 Aug 24;8(4).

13.Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, et al. Pseudomonas aeruginosa: pathogenesis, Virulence factors, Antibiotic resistance, Interaction with host, Technology Advances and Emerging Therapeutics. Signal Transduction and Targeted Therapy. 2022 Jun 25;7(1).

14.Jurado A, Fernández L, Rodríguez A, García P. Understanding the Mechanisms That Drive Phage Resistance in Staphylococci to Prevent Phage Therapy Failure. Viruses [Internet]. 2022 May 1 [cited 2024 Jan 24];14(5):1061. Available from: https://www.mdpi.com/1999-4915/14/5/1061/htm

15.Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of Staphylococcus aureus. Virulence [Internet]. 2021 Jan 31;12(1):547–69. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7872022/

16.Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H. Expression of Virulence Factors by Staphylococcus aureus Grown in Serum. Applied and Environmental Microbiology [Internet]. 2011 Sep 16;77(22):8097–105. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3208999/

17.Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clinical Microbiology Reviews [Internet]. 2015 May 27;28(3):603–61. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4451395/

18.Rao S, Linke L, Magnuson R, Jauch L, Hyatt DR. Antimicrobial resistance and genetic diversity of Staphylococcus aureus collected from livestock, poultry and humans. One Health [Internet]. 2022 Dec 1;15:100407. Available from: https://www.sciencedirect.com/science/article/pii/S2352771422000398

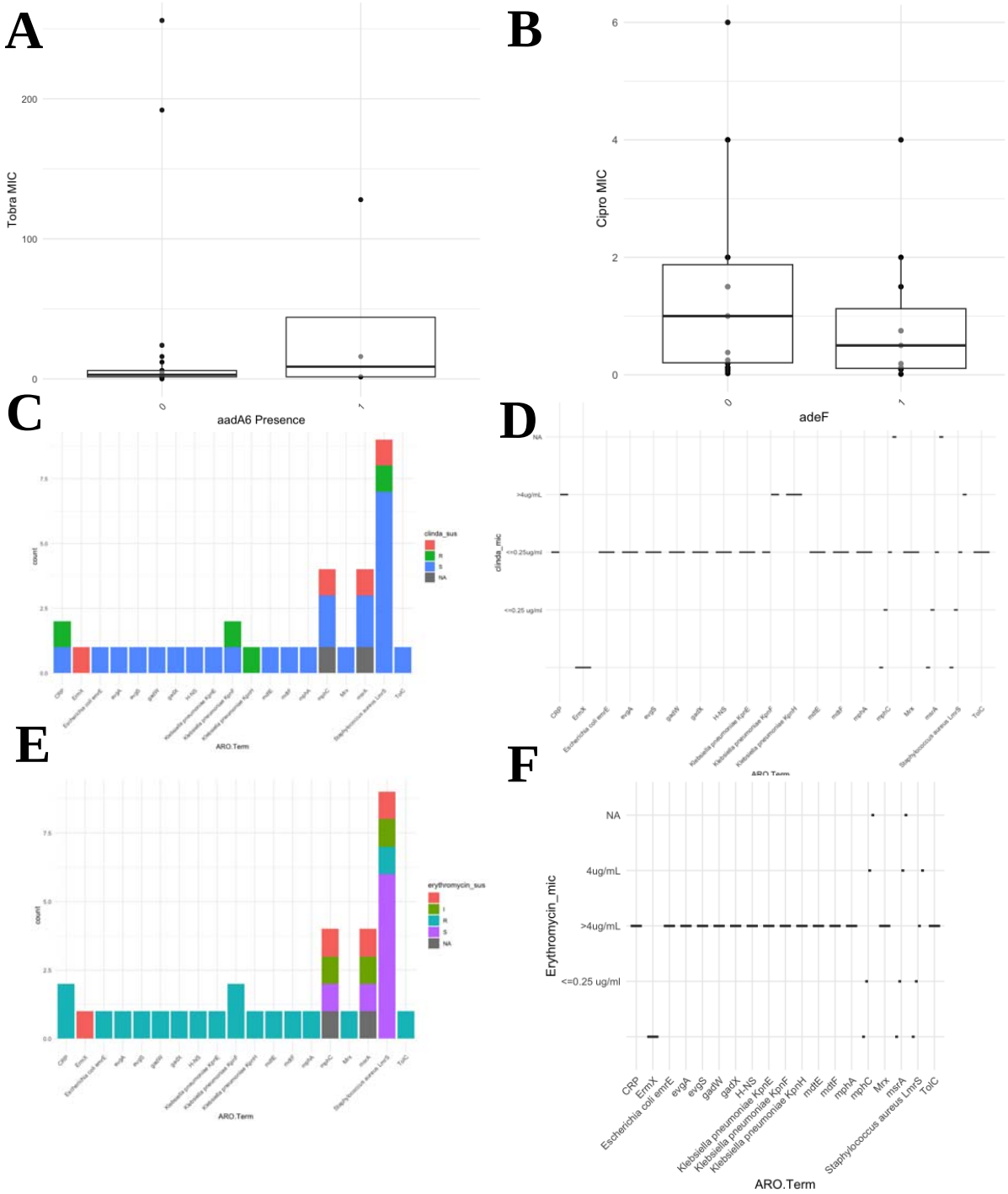
19.Moller AG, Lindsay JA, Read TD. Determinants of Phage Host Range in Staphylococcus Species. Johnson KN, editor. Applied and Environmental Microbiology. 2019 Mar 22;85(11).

20.Diallo K, Dublanchet A. Benefits of Combined Phage–Antibiotic Therapy for the Control of Antibiotic-Resistant Bacteria: A Literature Review. Antibiotics. 2022 Jun 22;11(7):839.

21.Djordjevic SP, Jarocki VM, Seemann T, Cummins ML, Watt AE, Drigo B, et al. Genomic surveillance for antimicrobial resistance — a One Health perspective. Nature Reviews Genetics [Internet]. 2023 Sep 25;1–16. Available from: https://www.nature.com/articles/s41576-023-00649-y
22.Hendriksen RS, Munk P, Njage P, van Bunnik B, McNally L, Lukjancenko O, et al. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. Nature Communications. 2019 Mar 8;10(1).
23.Köser CU, Ellington MJ, Peacock SJ. Whole-genome sequencing to control antimicrobial resistance. Trends in Genetics [Internet]. 2014 Sep;30(9):401–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4156311/
24.Domingues F, Rebelo JS, Dionísio F, Nogueira T. Multi-Drug Resistance in Bacterial Genomes—A Comprehensive Bioinformatic Analysis. International Journal of Molecular Sciences [Internet]. 2023 Jul 14 [cited 2024 Jan 27];24(14):11438–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10380340/
25.Van Nieuwenhuyse B, Van der Linden D, Chatzis O, Lood C, Wagemans J, Lavigne R, et al. Bacteriophage-antibiotic combination therapy against extensively drug-resistant Pseudomonas aeruginosa infection to allow liver transplantation in a toddler. Nature Communications [Internet]. 2022 Sep 29;13(1):5725. Available from: https://www.nature.com/articles/s41467-022-33294-w
26.Ren Y, Chakraborty T, Doijad S, Falgenhauer L, Falgenhauer J, Goesmann A, et al. Prediction of antimicrobial resistance based on whole-genome sequencing and machine learning. Birol I, editor. Bioinformatics. 2021 Oct 6;38(2):325–34.
27.Clokie MRJ, Millard AD, Letarov AV, Heaphy S. Phages in nature. Bacteriophage [Internet]. 2011 Jan [cited 2024 Jan 24];1(1):31–45. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3109452/
28.Fillol-Salom A, Alsaadi A, de Sousa JAM, Zhong L, Foster KR, Rocha EPC, et al. Bacteriophages benefit from generalized transduction. PLoS Pathogens [Internet]. 2019 Jul 5;15(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6636781/
29.Chiang YN, Penadés JR, Chen J. Genetic transduction by phages and chromosomal islands: The new and noncanonical. Kline KA, editor. PLOS Pathogens [Internet]. 2019 Aug 8;15(8):e1007878. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6687093/
30.Wendling CC, Refardt D, Hall AR. Fitness benefits to bacteria of carrying prophages and prophage□encoded antibiotic□resistance genes peak in different environments. Evolution. 2020 Dec 21;
31.Touchon M, Moura de Sousa JA, Rocha EP. Embracing the enemy: the diversification of microbial gene repertoires by phage-mediated horizontal gene transfer. Current Opinion in Microbiology. 2017 Aug;38:66–73.
32.Nanda AM, Thormann K, Frunzke J. Impact of Spontaneous Prophage Induction on the Fitness of Bacterial Populations and Host-Microbe Interactions. Journal of Bacteriology [Internet]. 2015 Feb 1;197(3):410–9. Available from: https://jb.asm.org/content/197/3/410
33.Bach MS, de Vries CR, Khosravi A, Sweere JM, Popescu MC, Chen Q, et al. Filamentous bacteriophage delays healing of Pseudomonas-infected wounds. Cell Reports Medicine [Internet]. 2022 Jun 21 [cited 2023 May 15];3(6):100656. Available from: https://www.sciencedirect.com/science/article/pii/S2666379122001884?via%3Dihub
34.Secor PR, Burgener EB, Kinnersley M, Jennings LK, Roman-Cruz V, Popescu M, et al. Pf Bacteriophage and Their Impact on Pseudomonas Virulence, Mammalian Immunity, and Chronic Infections. Frontiers in Immunology [Internet]. 2020 Feb 21;11. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7047154/
35.Sweere JM, Van Belleghem JD, Ishak H, Bach MS, Popescu M, Sunkari V, et al. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. Science (New York, NY) [Internet]. 2019 Mar 29;363(6434). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6656896/
36.Yee R, Dien Bard J, Simner PJ. The Genotype to Phenotype Dilemma: How Should Laboratories Approach Discordant Susceptibility Results? Journal of Clinical Microbiology. 2021 Jan 13;
37.Rose R, Nolan DJ, Ashcraft D, Feehan AK, Velez-Climent L, Huston CD, et al. Comparing antimicrobial resistant genes and phenotypes across multiple sequencing platforms and assays for Enterobacterales clinical isolates. BMC Microbiology. 2023 Aug 18;23(1).
38.Solu – Where bacterial DNA lives. [Internet]. www.solu.bio. [cited 2024 Jan 24]. Available from: https://www.solu.bio/
39.Vanstokstraeten R, Piérard D, Crombé F, De Geyter D, Wybo I, Muyldermans A, et al. Genotypic resistance determined by whole genome sequencing versus phenotypic resistance in 234 Escherichia coli isolates. Scientific Reports. 2023 Jan 9;13(1).
40.Suzuki S, Horinouchi T, Furusawa C. Prediction of antibiotic resistance by gene expression profiles. Nature Communications. 2014 Dec;5(1).

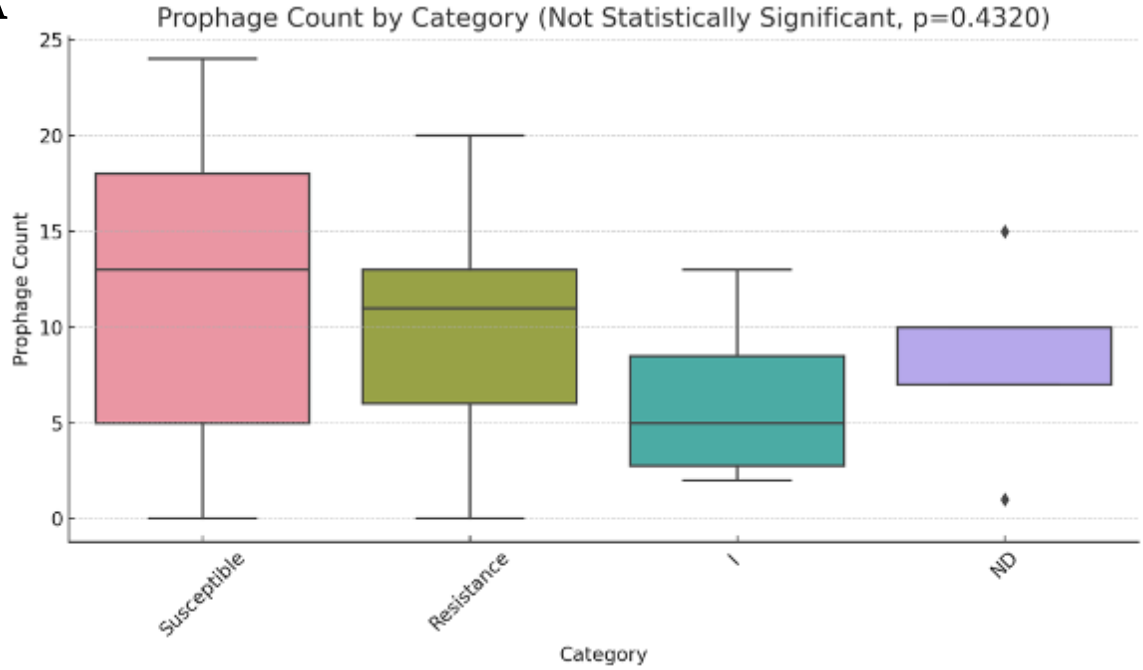
41.Haddock NL, Barkal LJ, Ram-Mohan N, Kaber G, Chiu CY, Bhatt AS, et al. Phage diversity in cell-free DNA identifies bacterial pathogens in human sepsis cases. Nature Microbiology [Internet]. 2023 Aug 1 [cited 2024 Jan 23];8(8):1495–507. Available from: https://www.nature.com/articles/s41564-023-01406-x
42.The Comprehensive Antibiotic Resistance Database [Internet]. card.mcmaster.ca. Available from: https://card.mcmaster.ca/ontology/37157
43.Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology. 2012 May;19(5):455–77.
44.Cameron A, Klima CL, Ha R, Gruninger RJ, Zaheer R, McAllister TA. A Novel aadA Aminoglycoside Resistance Gene in Bovine and Porcine Pathogens. Gales AC, editor. mSphere. 2018 Feb 28;3(1).
45.Shenagari M, Bakhtiari M, Mojtahedi A, Zahra Atrkar Roushan. High frequency of mutations in gyrA gene associated with quinolones resistance in uropathogenic Escherichiacoli isolates from the north of Iran. 2018 Dec 1;21(12):1226–31. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6312683/
46.Dashtbani-Roozbehani A, Brown MH. Efflux Pump Mediated Antimicrobial Resistance by Staphylococci in Health-Related Environments: Challenges and the Quest for Inhibition. Antibiotics. 2021 Dec 7;10(12):1502.
47.Hainrichson M, Yaniv O, Cherniavsky M, Nudelman I, Shallom-Shezifi D, Yaron S, et al. Overexpression and Initial Characterization of the Chromosomal Aminoglycoside 3′- O -Phosphotransferase APH(3′)-IIb from Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy [Internet]. 2007 Feb;51(2):774–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1797760/
48.Wang J, Liu JH. Mutations in the chloramphenicol acetyltransferase (S61G, Y105C) increase accumulated amounts and resistance in Pseudomonas aeruginosa. FEMS microbiology letters [Internet]. 2004 Jul 15 [cited 2022 Jul 21];236(2):197–204. Available from: https://pubmed.ncbi.nlm.nih.gov/15251197/#:~:text=A%20chloramphenicol%20acetyltransferase%20%28catB7%29%20gene%20containing%20two%20point
49.Heshmatipour Z, Arabameri N, Eftekhar Ardebili S, Jafari Bidhendi Z. The role of Gene Mutations (gyrA, parC) in Resistance to Ciprofloxacin in Clinical Isolates of Pseudomonas Aeruginosa. Iranian Journal of Pathology. 2021 Aug 1;16(4):426–32.
50.Zwama M, Nishino K. Ever-Adapting RND Efflux Pumps in Gram-Negative Multidrug-Resistant Pathogens: A Race against Time. Antibiotics. 2021 Jun 25;10(7):774.
51.Pourakbari B, Yaslianifard S, Yaslianifard S, Mahmoudi S, Keshavarz-Valian S, Mamishi S. Evaluation of efflux pumps gene expression in resistant Pseudomonas aeruginosa isolates in an Iranian referral hospital. Iranian Journal of Microbiology [Internet]. 2016 Aug 1 [cited 2024 Mar 2];8(4):249–56. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5296939/
52.Struble JM, Gill RT. Reverse Engineering Antibiotic Sensitivity in a Multidrug-Resistant Pseudomonas aeruginosa Isolate. Antimicrobial Agents and Chemotherapy. 2006 Jul;50(7):2506–15.
53.Glycopeptide Antibiotics [Internet]. PubMed. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012 [cited 2024 Mar 2]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547958/#:~:text=Introduction
54.Grossman TH. Tetracycline Antibiotics and Resistance. Cold Spring Harbor Perspectives in Medicine [Internet]. 2016 Mar 17;6(4):a025387. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4817740/
55.Wendling CC, Refardt D, Hall AR. Fitness benefits to bacteria of carrying prophages and prophage□encoded antibiotic□resistance genes peak in different environments. Evolution. 2020 Dec 21;
56.Card RM, Mafura M, Hunt T, Kirchner M, Weile J, Rashid MU, et al. Impact of Ciprofloxacin and Clindamycin Administration on Gram-Negative Bacteria Isolated from Healthy Volunteers and Characterization of the Resistance Genes They Harbor. Antimicrobial Agents and Chemotherapy. 2015 Aug;59(8):4410–6.

Supplemental Figures

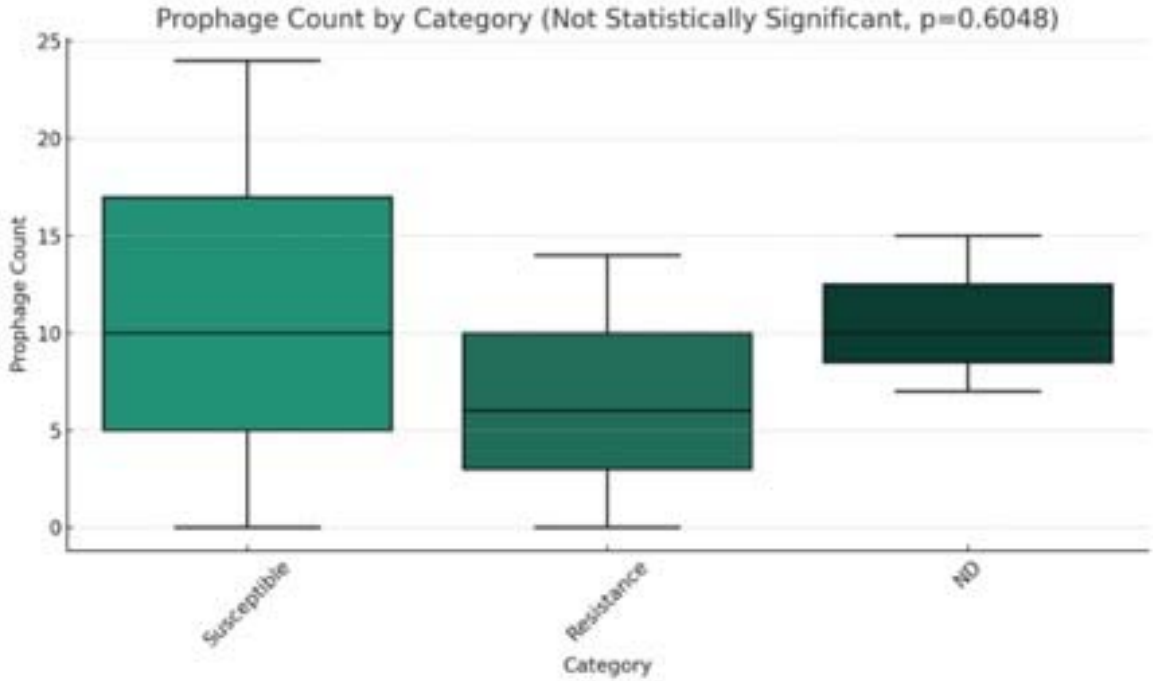


Supplemental Figure 3 A. The box and whisker plot indicates that *Pa* clinical samples containing the 'aadA6' ARO term demonstrate tobramycin resistance with MICs below 100 µg/ml, while susceptible samples maintain an MIC at approximately 0 µg/ml. **B.** Box and whisker plot showing that the ARO term adeF in *Pa* clinical samples that display ciprofloxacin resistance MIC as over 5ug/ml vs susceptible was 1ug/ml. **C**. Manhattan plot on *Sa* ARO terms mapped below to see which ARO terms are more prominent in clindamycin resistance samples. **D**. spread plot on the ARO terms for clindamycin resistance. **E**. Manhattan plot on *Sa* ARO terms mapped below to see which ARO terms are more prominent in erythromycin resistance samples **F.** spread plot on the ARO terms for erythromycin resistance.

A

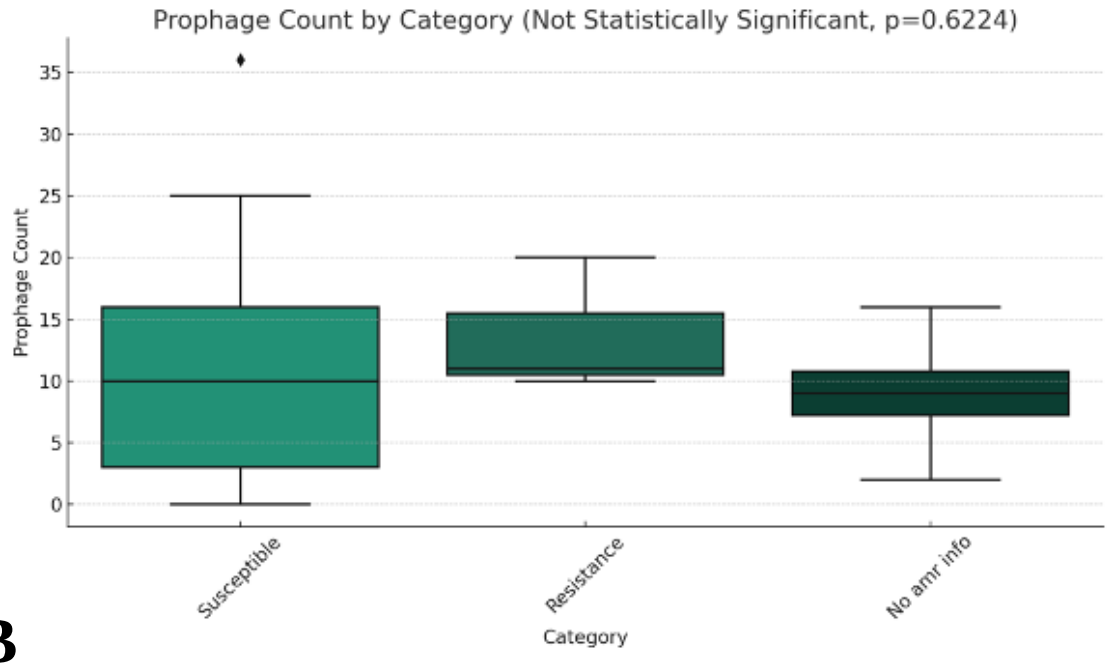


B

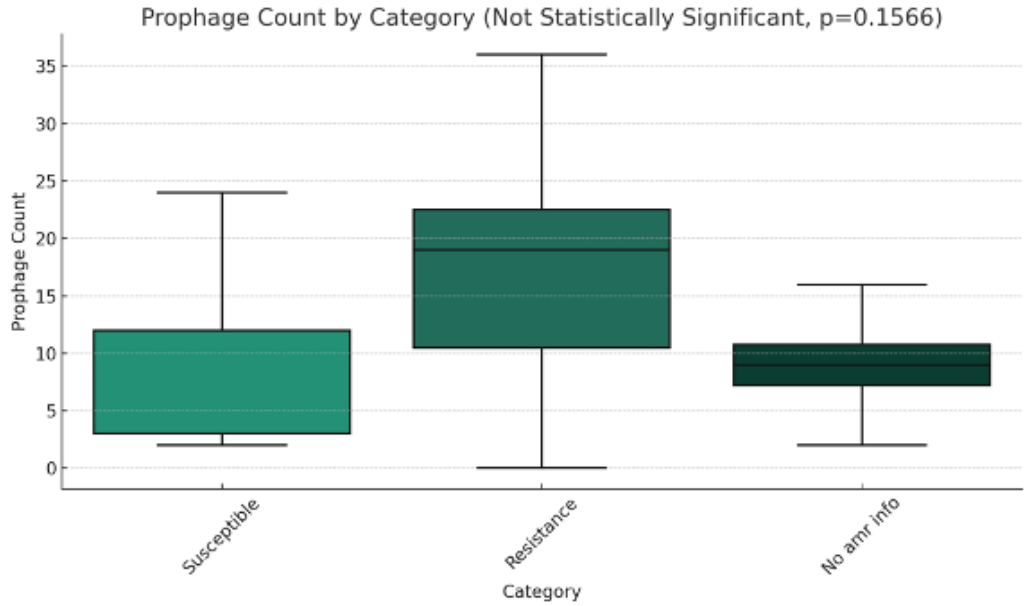


Supplemental Figure 2 Prophage numbers do not have an effect on bacterial antimicrobial resistance in *Pa*. **A.** A box and whisker plot illustrating the distribution of *Pa* prophage counts in samples that are either susceptible or resistant to ciprofloxacin (Shapiro-Wilk, p-value=0.4320). **B.** A box and whisker plot illustrating the distribution of *Pa* prophage counts in samples that are either susceptible or resistant to tobramycin (Shapiro-Wilk, p-value= 0.6048).

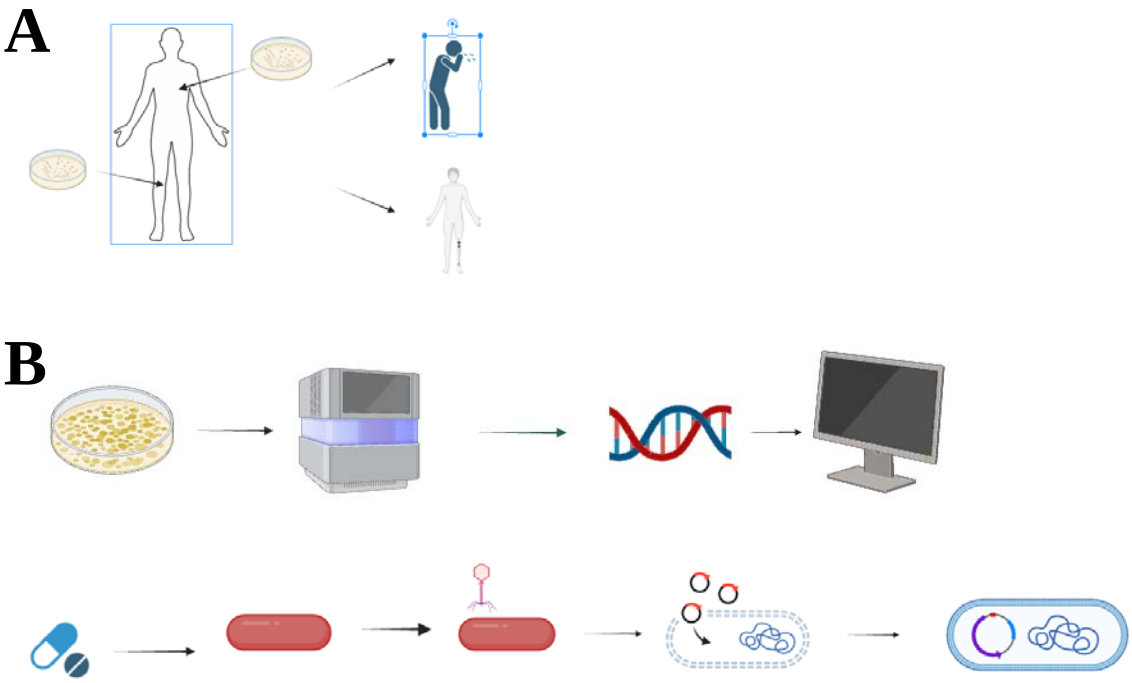
A



B



Supplemental Figure 3. Prophage numbers do not influence bacterial antimicrobial resistance in *Sa* bacteria. **A.** A box and whisker plot illustrating the distribution of *Sa* prophage counts in samples that are either susceptible or resistant to clindamycin (Shapiro-Wilk, p-value= 0.6224). **B.** A box and whisker plot illustrating the distribution of *Sa* prophage counts in samples that are either susceptible or resistant to erythromycin (Shapiro-Wilk, p-value =0.1566).



Scheme 1: The schematic represents the sampling method workflow that was established for acquiring prophage sequencing. Samples were acquired from the operating room or bacterial lung samples. The bacteria samples were then isolated and sequenced via illuminia sequencing then displayed digitally on a computer. The schematic represents the lysogenic cycle, where a bacteriophage (a virus that infects bacteria), shown at the top, injects its DNA into a bacterial cell. Instead of immediately replicating and destroying the host (lytic cycle), the viral DNA (prophage) integrates into the bacterial genome. This integrated DNA can include genes that provide antibiotic resistance (AMR genes). When the bacterium replicates, it also copies the prophage DNA, which may now include these resistance genes. Consequently, the bacterial progeny inherits the antibiotic resistance, enabling them to survive in the presence of antibiotics that would otherwise inhibit or kill them.