AMR Surveillance Open Data Re-use Challenge

Proposal Title: Novel approach to antibiogram analysis: looking at the composite resistance phenotype

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Add more rows if needed

Datasets included in the analysis (Tick all those that apply):

	GSK – SOAR 201818
	Johnson & Johnson – Bedaquiline Drug Resistance Assessment in MDR-TB (DREAM)
	Paratek - KEYSTONE
✔	Pfizer – ATLAS_Antibiotics
	Pfizer – ATLAS_Antifungals
	Shionogi – SIDERO-WT
✔	Venatorx – GEARS
	Other data (please provide details): _____

Objectives

The escalating challenge of antimicrobial resistance (AMR) poses a significant global concern for public healthcare systems[1,2]. Current AMR surveillance and molecular mechanism studies traditionally focus on specific drug-bug combinations, like carbapenem-resistant *Klebsiella pneumoniae*, designated as a priority pathogen by the WHO[3]. While wastewater and environmental surveillance aim to detect *Klebsiella* species and genes conferring carbapenem resistance[4], it is known that resistance genes for one antibiotic often coexist with genes for resistance to others[5]. To address these complexities, we propose a novel analysis approach using *Klebsiella pneumoniae* as a model. We consider the resistance profile of an isolate for a set of common antibiotics across two datasets, ATLAS and GEARS. We term this composite phenotype, encompassing resistance/sensitivity to a group of antibiotics, a 'subtype' of the pathogen. Our primary objective is to track and study the prevalence of different subtypes across time and space, enabling a more comprehensive understanding of AMR dynamics.

We then explore the impact of climatic parameters on the prevalence of different *Klebsiella pneumoniae* subtypes, aiming to uncover additional insights into antibiotic resistance patterns. Rising temperatures and climate change have been associated with recent antibiotic resistance developments, as bacterial growth and genetic material dissemination are closely tied to temperature conditions[6]. Heavy rainfall has been linked to bacterial mutagenesis and antibiotic resistance gene expression[7]. Rising local temperatures in the United States and Europe have shown correlations with increased antibiotic resistance at the population level in various pathogens[8,9].

Methods

1) Generating distinctive subtypes and determining their occurrences across various countries

As an example, we selected *Klebsiella pneumoniae*, a priority pathogenic species, to demonstrate our proposed analysis approach. We utilized Pfizer-Atlas and Venatorx datasets, encompassing diverse specimens such as blood, urine, and pus, with data on *K. pneumoniae*. The MIC values from the ATLAS and GEARS datasets were interpreted using the CLSI 2020 and 2021 breakpoint criteria, respectively. For consistency, our subtype study encompassed data from 2018 to 2021, allowing for a comprehensive comparison between the two datasets.

Subsequently, we identified five common antibiotics for *K. pneumoniae* in both datasets: cefepime, ceftazidime, gentamicin, levofloxacin, and piperacillin-tazobactam. Each isolate's antibiogram was then represented as a unique string of five 'R' (for resistant) or 'S' (for sensitive) characters, signifying its resistance status to the corresponding antibiotic. We termed each distinct string of 'R' and 'S' as a subtype, facilitating a more comprehensive analysis of the resistance patterns within the *K. pneumoniae* population.

2) Testing the impact of climatic parameters on the subtype prevalence

We chose five different climatic parameters, two were the proxies of precipitation (daily average rainfall in mm and percent relative humidity) and other three were the proxies of temperature (maximum, minimum and mean temperature). Average daily measurements of rainfall (mm), maximum, minimum, and mean temperature (°C), as well as relative humidity (%), were collected from January 1, 2018, to December 31, 2021, at a 2-meter height from ground level. Yearly average values were calculated based on the data obtained from the National Aeronautics and Space Administration (NASA) Langley Research Center Prediction of Worldwide Energy Resource Project (NASA. POWER LARC Data Access Viewer, Available at https://power.larc.nasa.gov/data-access-viewer/ accessed on July 29, 2023). The data originates from the Modern-Era Retrospective analysis for Research and Applications version 2 (MERRA-2) dataset merged with Goddard Earth Observing System Model (GEOS) version 5.12.4. We utilized the Python request module with the provided API (https://power.larc.nasa.gov/docs/tutorials/service-data-request/api/) to extract the climate parameters, specifically relative humidity, specific humidity, and precipitation, for subsequent regression analysis. Prior to regression, we $\log_{10}$ transformed these climate parameters for optimal analysis.

3) Multivariate Regression analysis

We conducted regression analysis on the prevalence of thirty-two *Klebsiella pneumoniae* subtypes across 63 countries from 2018 to 2021, considering six climate variables (768 regression analyses in total). A *p*-value less than 0.05 was considered statistically significant for each regression model.

To perform the analysis, we utilized Python (version 3.1.12) and R (version 4.2.1) statistical programming languages. For reproducibility, we employed the Conda environment. In Python, we employed the statsmodels (version 0.14.0), pandas (version 1.5.3), matplotlib (version 3.7.1), seaborn (version 0.12.2), and geopandas (version 0.9.0) modules for data analysis and visualization. In R, data visualization was achieved using the ggplot2 (version 3.4.0), ggpubr (version 0.4.0), and RColorBrewer (version 1.1.3) packages.

Results

Antibiotic subtype shows significant association with climate variables

The subtype study carried out using the merged ATLAS and GEARS dataset and focusing on five antibiotics (cefepime, ceftazidime, gentamicin, levofloxacin, and piperacillin-tazobactam) identified 33 distinct subtypes of *Klebsiella pneumoniae* between 2018 and 2021 (Table 1). Notably, the analysis highlighted that the extreme subtypes, RRRRR (~13%) and SSSSS (~55%), accounted for approximately 70% of the isolates (Table 1).

We then explored the relationship between the regional patterns (averaged) of the most prevalent subtypes in the merged dataset (RRRRR and SSSSS) and the global climatic variables including precipitation, relative humidity (RH) and temperature (mean, minimum and maximum temperature) averaged over 2018-2021. Precipitation and relative humidity showed higher correlations with many of the subtypes than the temperature variables over the years (Figure 1). Owing to the consistent strength of correlation of precipitation and relative humidity with the most prevalent subtypes (RRRRR and SSSSS) across the

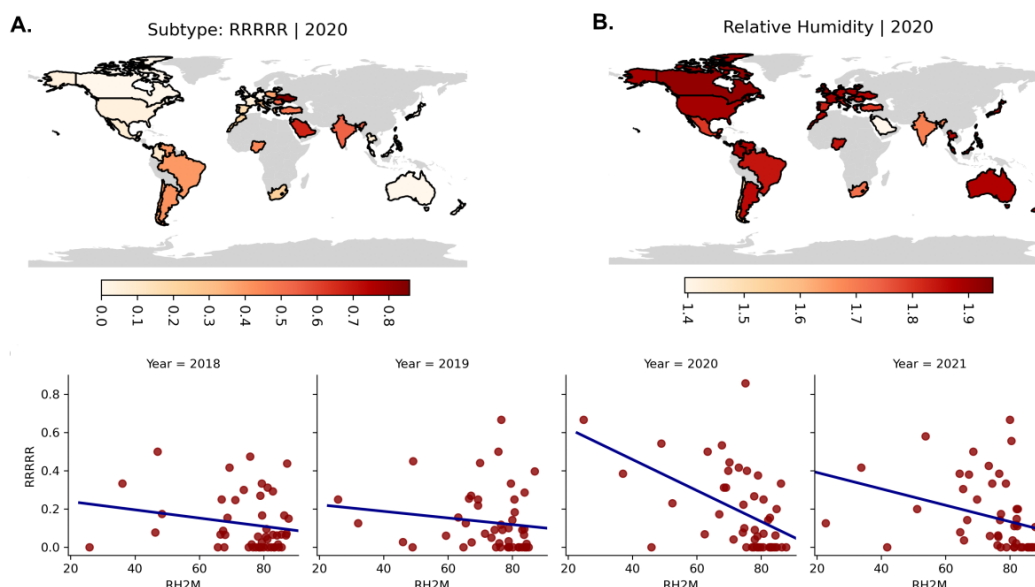years, we focused our analysis on the association between these parameters and the predominant subtypes.

Table 1: Prevalence of 13 subtypes of *K. pneumoniae* from 2018 to 2021.

Subtype	2018 (%)	2019 (%)	2020 (%)	2021 (%)
SSSSS	55.79	48.23	56.32	56.66
SSSSR	2.39	2.88	3.24	2.65
SRSSS	1.85	1.25	1.83	2.99
SRSRR	0.39	0.59	0.42	0.94
SRRSS	0.69	0.72	0.92	1.11
RSSRR	0	0	0.17	0.26
RSRRR	0.08	0.07	0.08	0.09
RRSSS	3.32	2.69	2	1.88
RRSSR	0.77	1.11	0.92	1.37
RRSRS	1.77	2.36	2	0.85
RRSRR	5.56	7.6	5.32	7.08
RRRSS	2.39	2.23	1.91	1.19
RRRRR	11.96	14.94	11.56	13.99
Total isolates (N)	2592	3052	2404	2344

Association of relative humidity with all-susceptible-subtype (SSSSS) and all-resistance-subtype (RRRRR)

Post the multivariable regression analysis, we found that there is a strong association between the all-susceptible subtype and the relative humidity (Figure 3) and this was consistent across all the regions. We see that there is a significant inverse relationship between RH and SSSSS subtype with time (2021). We saw a similar trend between RH and RRRRR subtype across all the regions. With 2020, showing a significant inverse relationship between the RRRRR subtype and RH.

Figure 1: Bubble plot representing significant association post regression analysis across different subtypes of *K. pneumonia* and global climatic parameters from 2018 to 2021 using ATLAS and GEARS data. The color intensity represents the R-squared value and size of the point represents the $-\log_{10}(p\text{-value})$, where bigger size implies statistical significance.

Figure 2: Regression analysis between all-susceptible-subtype prevalence (0-1) with relative humidity ($\log_{10}$ transformed). Heatmap showing (A) prevalence of all-susceptible-subtype (SSSSS) and (B) $\log_{10}$(relative humidity) for 2018 across 63 countries. (C) Scatterplot between SSSSS subtype and relative humidity (RH2M) with the regression line from 2018 to 2021 (for 2018 and 2021 corresponding p-values were less than 0.05).

Figure 3: Regression analysis between all-resistance-subtype prevalence (0-1) with relative humidity ($\log_{10}$ transformed). Heatmap showing (A) prevalence of all-resistance-subtype (RRRRR) and (B) $\log_{10}$(relative humidity) for 2018 across 63 countries. (C) Scatterplot between RRRRR subtype and relative humidity (RH2M) with the regression line from 2018 to 2021 (for 2020 corresponding p-value was less than 0.05).

Impact and limitations of the work:

Majority of the AMR studies consider a drug-bug combination as the standard unit of analysis. We propose extending this approach to include the resistance status against all the relevant antibiotics. This proposed 'subtype analysis' is more relevant for molecular mechanisms of resistance as well as resistance evolution as it considers the entire resistance phenotype of the isolate. One logistic limitation of this approach is that only those antibiotics may be considered which are common across the different datasets or specimens that one aims to study.

The analysis of spatiotemporal interaction between prevalent subtypes with climate variables is an example of how subtype analysis can be integrated into a one-health paradigm. It can potentially provide important insights into the intricate relationships that lead to the transmission and persistence of antibiotic resistance in the environment. This understanding can guide targeted therapies and strategies to address the multifaceted challenge of antibiotic resistance while also preserving antibiotic efficacy for future generations. Future work in this area can focus on defining the most relevant proxies for climatic parameters.

References

Burnham, J. P. (2021). Climate change and antibiotic resistance: a deadly combination. *Therapeutic Advances in Infectious Disease*, *8*. https://doi.org/10.1177/2049936121991374

Frost, I., Kapoor, G., Craig, J., Liu, D., & Laxminarayan, R. (2021). Hot Topic Status, challenges and gaps in antimicrobial resistance surveillance around the world. *Journal of Global Antimicrobial Resistance*, *25*, 222–226. https://doi.org/10.1016/j.jgar.2021.03.016

Icmr. (2021). *Annual Report 2021: Antimicrobial Resistance Research and Surveillance Network*.

MacFadden, D. R., McGough, S. F., Fisman, D., Santillana, M., Brownstein, J. S., & Clim Chang Author manuscript, N. (2018). *Antibiotic Resistance Increases with Local Temperature*. *8*(6), 510–514. https://doi.org/10.1038/s41558-018-0161-6

McGough, S. F., MacFadden, D. R., Hattab, M. W., Mølbak, K., Santillana, M., Sarah, M. F., Derek, M. R., Mohammad, H. W., Kåre, M., & Mauricio Rates, S. (n.d.). *Rates of increase of antibiotic resistance and ambient temperature in Europe: a cross-national analysis of 28 countries between 2000 and 2016*. *1*. https://doi.org/10.2807/1560

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

Pal, C., Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. G. J. (2015). Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics*, *16*(1), 1–14. https://doi.org/10.1186/S12864-015-2153-5/FIGURES/9

Ratkowsky, D. A., Olley, J., McMeekin, T. A., & Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, *149*(1), 1–5. https://doi.org/10.1128/JB.149.1.1-5.1982

WHO publishes list of bacteria for which new antibiotics are urgently needed. (n.d.). Retrieved July 31, 2023, fromhttps://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed