RESEARCH ARTICLE

Pangenomic analyses of tuberculosis strains to identify resistomes using computational approaches

Dur-e-Shahwar Tariq

Abstract

Objective: To locate resistomes in tuberculosis strains, to determine the severity of drug resistance, and to infer its implications with respect to high tuberculosis prevalence in a Third World setting.

Method: The pangenomic study was conducted from October 2022 to January 2023 in Sir Syed University of Engineering and Technology, Karachi, and comprised 2012-22 data on multiple sequence alignment to assess the genetic evolution of tuberculosis strains. Antibiotic resistance drug classes were identified using the Canadian Antibiotic Resistance Database, which entailed multidrug-resistant and extremely drug-resistant strains. Also, GenBank was used for tuberculosis genome FASTA (fast-all; nucleotide and protein sequence representation) files, prediction of resistome sequences on the basis of Canadian Antibiotic Resistance Database, and multiple sequence alignment was done in Mauve.

Results: Evolutionarily, the 6 strains identified were structurally similar with polymorphisms in their core chromosomal regions. Their resistome genes showed perfect hits for isoniazid, rifamycin, cephalosporin, fluoroquinolone, aminoglycosides, penem, penam and cephamycin.

Conclusion: Drugs discovered in antibiotic resistance genes are now less effective in treatment, and have the potential to develop into more dangerous bacteria, if not monitored. For treatment, staying long durations in hospitals for quality healthcare and supervision in third world countries is unaffordable.

Key Words: Tuberculosis, Resistomes, Isoniazid, Fluoroquinolone, Aminoglycosides, MDR-TB, XDR-TB, CARD Database, TB treatment, Multiple sequence alignment

(JPMA 74: S-74 (Supple-2); 2024) **DOI: https://doi.org/10.47391/JPMA-DUHS-S15**

Introduction

In developing countries, there is a severe public health issue of responsible treatment of infectious diseases and the general lack of awareness in this regard.[1] This makes preventable infectious diseases a cause of death that may otherwise have been curable through careful stewardship, antibiotics, and full-course treatment.[2] Tuberculosis (TB) is one such disease that is rampant in many parts of South Asia and is one that requires long-term careful treatment.

Mycobacterium (M.) TB, the pathogen that causes TB has characteristics that are usual of bacteria, including the ability to code for antibiotic resistance, evolve itself to adapt to different environmental conditions, and the ability to sustain itself through a host as a parasite.[3] However, these qualities paired with mycolic acid (waxy substance) coated cell walls enhances its tendency to cause a highly contagious and sometimes life-threatening disease in human beings. M. TB exists in multiple forms: active TB, multidrug resistant TB (MDR-TB) and extremely drug resistant TB (XDR-TB).[4] All are transmitted by means of airborne droplets suspended in the air or adsorbed on a surface by someone infected with TB.

TB, in retrospect, is milder in comparison to other pulmonary diseases in terms of symptoms. However, its pathogen has a tendency to blend with somatic cells and remain undetected by the body's immune system as it continues to harm the body. This, coupled with the pathogen's ability to evolve and become resistant to antibiotics, can make this preventable disease all the more dangerous, with potential to become incurable.[2] Currently, there are two lines of antibiotics used to treat TB: first line, which consists of rifampicin, isoniazid, pyrazinamide and ethambutol, and second line, which consists of aminoglycosides, capreomycin, ethionamide and fluoroquinolones.[5]

Patients are treated for TB by following 6 months of first-line drugs. Then, continuing treatment with second-line drugs if the patient is still not cured, and keeping them under supervision of their attending physician for these 6 months or even up to a year at times.[4] This is a regular regimen followed for all patients worldwide. However, for a patient affected by MDR-TB or XDR-TB, this treatment

3rd year student of Biomedical Sciences, Sir Syed University of Engineering and Technology, Karachi, Pakistan

Correspondence: Dur-e-Shahwar Tariq. Email: durree.s.t@gmail.com

becomes ineffective, and physicians may transition to second-line drugs directly without first-line drugs being used, or treat the patient with other medications altogether if they have XDR-TB.[5] Patients may also have longer hospitalisation periods during this time.

The current study aimed to observe the evolution of TB strain isolates and the severity of first-line and second-line antibiotic resistance in TB strains. It was hypothesised that the resulting antibiotics resistomes would consist mainly of first-line antibiotics.

Materials and Methods

The pangenomic study was conducted from October 2022 to January 2023 in Sir Syed University of Engineering and Technology in Karachi, and comprised 2012-22 data on multiple sequence alignment (MSA) to assess the genetic evolution of TB strains. Antibiotic resistance drug classes were identified using the Canadian Antibiotic Resistance Database (CARD)[6] to see how similar nucleotide sequences in the selected TB strains were to resistome sequence using basic local-alignment search tool (BLAST)-like algorithms[7].

The study design was based on qualitative secondary research principles whereby bioinformatic tools and databases were used to observe the evolution and antibiotic resistance gene clusters of TB strains from various years. TB isolates strains were extracted from GenBank[8]. Qualitative analyses, such as MSA pangenomic analysis, were done using Mauve plugged into CARD5 to determine resistome sequences linked to current drugs used in TB treatment.

The exclusion and inclusion criteria centred around a qualitative perspective where the genome's specific core and non-core regions were uniquely discussed with relevance to antibiotic resistance and its overall evolution. Hence, studying the total number of proteins in the TB genome was outside the scope of the study.

There were 6 sequences that were selected in FASTA file formats (shortened from Fast-All; represents protein and nucleotide sequences)[9] and extracted from GenBank. In the FASTA sequences below, the GCA counterpart indicates their source as NCBI Genbank, numbers up to the full stop are identifiers for the specific genome assembly of the sequence, and .1/.2 indicates the version of the FASTA sequence[8]. These were filtered by years ranging 2012-22. The files contained TB genomes, including MDR-TB and XDR-TB genomes as well.

The strains analysed included GCA_022870345.1, GCA_000023625.1,GCA_002116835.1, GCA_000655215.1, GCA_000648395.1, and GCA_000154605.2. These sequences were first plugged into Mauve to perform multiple sequence alignment to align them. Mauve recognises localised co-linear blocks (LCB) in genomes.[10] The colourful LCBs are core chromosomal regions that are conserved. As genomes evolve, their chromosomes promote re-arrangements through mechanisms, such as insertions and deletions, to help the bacteria adapt to its environment and survive.[11] Hence, even though core regions are maintained evolutionarily in TB strains, their arrangement can vary[12].

The FASTA sequences were then plugged into CARD where loose, perfect, and strict hits[5] were noted for potential matching resistant gene sequences in resistomes.

Loose hits are sequences not reliable to consider, but approximately match the resistome records present on CARD. They are the most in number and are labelled in red.

Strict hits are sequences present on CARD that approximately match sequences on the strain, but carry a level of uncertainty about the existence of that particular resistome being found in the TB strain. They are labelled in yellow.

Perfect hits are the most reliable to consider and are sequences that can definitively be considered to be found in the strains. They are labelled in green.

The results obtained from these steps were then used to derive conclusions based on the evolution of TB strains and resistomes. The significance of investing efforts into studying antibiotic resistance evolution of TB ties into how this socially impacts Third World countries, including Pakistan, where patients bear the consequences of resistant TB strains that continue to evolve throughout the duration of treatments[13,14].

Results

The pangenomic analyses provided evidence on the evolution of M. TB core regions (Figure 1). Evolutionarily, the 6 strains identified were structurally similar with polymorphisms in their core chromosomal regions.

The interconnecting lines and colours matching between the segments of deoxyribonucleic acid (DNA) in each strain represented consistent evolutionary descent. The matching LCBs showed that all strains shared the same core genetic sequences with some rearrangements in the TB strains' structure. This may have occurred to maintain competitiveness in the bacteria's adaptability to survive in changing environments.

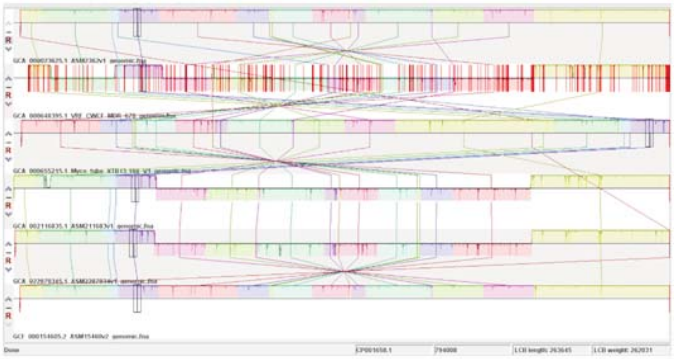

Figure-1: Multiple sequence alignment (MSA) pangenomic analysis of tuberculosis (TB) strains. This was carried out using the Progressive Mauve setting on the freely-accessible Mauve software. The coloured blocks represent localised co-linear blocks of core chromosomal regions connected to where that segment is found again in the next strain. Evolutionarily, all strains share the same less mutating core regions.[16]

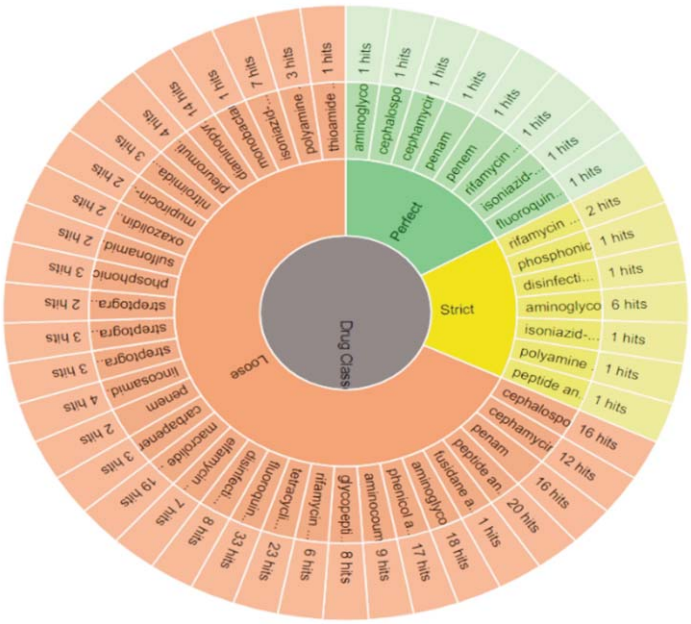

Figure-2: Perfect, Strict and Loose hits for resistomes using the Canadian Antibiotic Resistance Database (CARD). The result encapsulates the results discovered in the other 5 strains on antibiotic resistance that CARD discovered in their resistomes. Green perfect hits are most reliable to consider as evidence of antibiotic resistance, whereas yellow are potential hits[5]

Contrary to the study hypothesis, perfect matches were found for both first-line and second-line drugs, such as fluoroquinolone, isoniazid, rifampicin, aminoglycosides and others.

Their resistome genes showed perfect hits for isoniazid, rifamycin, cephalosporin, fluoroquinolone, aminoglycosides, penem, penam and cephamycin. (Figure 2).

All 6(100%) strains were found to contain 3-5 perfect hits. There were 5(83.3%) strains resistant to rifamycin and isoniazid, and 1(16.7%) strain displayed a strict hit. All 6(100%) strains were resistant to fluoroquinolones, aminoglycosides, cephalosporin, cephamycin, penam and penem.

Discussion

Based on the findings, the antibiotic resistomes detected in the M. tuberculosis strains analysed included sequences for both first-line and second-line drugs.

Evolutionarily, TB strains have not strewn too far from each other·[15,16] Still, they vary within their respective antibiotic resistance capacities based on the environments that the strains survived in. Possible factors might also include the approximate timeframe they developed in, where, prevalence and severity of resistant or extremely resistant TB cases in their particular regions varied.[17]

With second-line antibiotics found in the most recent strains of TB, the task now is how to come up with better, more updated treatment regimens to treat TB. Second-line drugs are originally used to facilitate treatment for patients for whom first-line drugs are not feasible,[16] but now several of these drugs are ineffective. This may suggest that the current available antibiotics are being overused and inappropriately dispensed.[16] It is also important to consider how the treatment regimen works for TB patients altogether when considering the costs of maintaining and supervising them as a part of responsible stewardship, which is an integral part of their treatment. This may be unaffordable for many, especially in Third World countries. Effective TB treatment in such countries entails elevated cost of staying at a hospital for a duration of 6 months or more and being under the supervision of doctors. This cost is often beyond the capacity of even middle to upper middle class patients.

TB is highly prevalent in South Asia, and the region contributes to the highest annual number of global cases of TB. Pakistan and India rank in the top 5, with Pakistan rolling in an average of over 500,000 TB cases and 15,000 drug-resistant TB cases annually.[17] With Pakistan in particular, where the current study took place, it comes as no surprise that it ranks highly in the TB leaderboard of annual infections.[16] Though antibiotics tend to be government-mandated and made to be more affordable to the people, any further care is often not as accommodated. Government-supported hospitals may

be able to help in these circumstances where they can provide free treatment to as many patients as possible and provide them with low-cost antibiotics.[18] However, this is still not sufficient to maintain the traffic that comes with many more patients afflicted with TB who cannot afford treatment.

Being a Third World country with a population of more than 220 million people, Pakistan has more than 25% of the population living below the international poverty line.[19] The international poverty line indicates people survive on just $2.15/day. At just PKR611.68, paying for living costs, daily meals, and having access to high-quality healthcare is an unrealistic standard for a satisfactory quality of life.[19] Healthcare systems in developing countries face an ever-growing risk of not being sufficient or helpful to those who need medical attention[20] due to a lack of funding, resources and medications.

However, to become better prepared for what TB antibiotic resistances can develop into, predictions through precision medicine can possibly be made using the strict and loose hits found on CARD.[21] These predictions can help doctors prescribe antibiotic regimens that patients can follow with diligence and stewardship to prevent more resistant genes from developing.[12] Antibiotic resistomes, despite being complex to study, should be invested into as they offer valuable information that can be used in other areas, such as drug development.[15] They may also help lead to predictions about what antibiotic resistance can be anticipated in future patients to tailor their treatment accordingly.

The current study has limitations as it was not a primary research that could monitor TB resistomes in patients from a particular region or a country and could have explored TB pathogen's evolution further. The former is the key to understanding the rate at which TB strains have evolved.[14] Also, other aspects such as personalised long-term permanent treatment and the efficacy of experimental procedures were not studied. Although, better treatments can be developed by studying the antibiotic resistomes in more depth, such as through combination therapy, and also exploring links between latent TB and active TB to see how they affect a regular patient. Though latent TB is mostly asymptomatic and dormant,[22] it has the potential to aggravate the matter, especially in case of immunocompromised patients.

Conclusion

The severity of first-line and second-line antibiotic resistance was observed in TB strains, especially MDR-TB and XDR-TB strains. Over the past decade, this has made drug development more difficult with treatment of continuously evolving TB strains. Core chromosomal regions remained the same in every strain, but evolutionarily jumped to different locations, as suggested by the pangenomic analysis. In Third World countries with struggling healthcare systems, long term care facilities and stewardship during treatment is still a determinant for M. TB antibiotic resistance. Combination therapy with new drugs may be promising, but experimental treatments must also be explored further as suitable alternatives.

Acknowledgement: We are grateful to Fareha Masood for facilitating the study.

Disclaimer: The text was presented as a Poster at the 1st International Conference on Biomedical Sciences, 2023, at the Dow University of Health Sciences, Ojha Campus, Karachi.

Conflict of Interest: None.

Source of Funding: None.

References

1. Rufai SB, Ozer EA, Singh S. Pan-genome analysis of Mycobacterium tuberculosis identifies accessory genome sequences deleted in modern Beijing lineage. bioRxiv 2020. doi: 10.1101/2020.12.01.407569
2. Moule MG, Cirillo JD. Mycobacterium tuberculosis Dissemination Plays a Critical Role in Pathogenesis. Front Cell Infect Microbiol 2020;10:e65. doi: 10.3389/fcimb.2020.00065.
3. Galagan Lab, Boston University. TB database home. [Online] 2013 [Cited 2023 May 11]. Available from URL: http://tbdb.bu.edu/
4. Miggiano R, Rizzi M, Ferraris DM. Mycobacterium tuberculosis Pathogenesis, Infection Prevention and Treatment. Pathogens 2020;9:385. doi: 10.3390/pathogens9050385.
5. Myneedu VP, Singhal R, Khayyam KU, Sharma PP, Bhalla M, Behera D, et al. First and second line drug resistance among treatment naïve pulmonary tuberculosis patients in a district under Revised National Tuberculosis Control Programme (RNTCP) in New Delhi. J Epidemiol Glob Health 2015;5:365-73. doi: 10.1016/j.jegh.2015.04.002.
6. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 2020;48:D517-25. doi: 10.1093/nar/gkz935.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10. doi: 10.1016/S0022-2836(05)80360-2.
8. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res 2016;44:D67-72. doi: 10.1093/nar/gkv1276.
9. Lipman DJ, Pearson WR. Rapid and sensitive protein similarity searches. Science 1985;227:1435-41. doi: 10.1126/science.2983426.
10. Darling Labs. Mauve: Multiple Genome Sequence Alignment. [Online] 2023 [Cited 2023 May 11]. Available from URL: https://darlinglab.org/mauve/mauve.html
11. Naz A, Aslam MA, Khan AUH, Rasul S, Manzoor H, Iqbal R, et al. Genetic polymorphism in association with susceptibility to tuberculosis: a study in a Pakistani population. Braz J Microbiol

2019;50:429-34. doi: 10.1007/s42770-019-00048-8.
12. Long Q, Smith H, Zhang T, Tang S, Garner P. Patient medical costs for tuberculosis treatment and impact on adherence in China: a systematic review. BMC Public Health 2011;11:393. doi: 10.1186/1471-2458-11-393.
13. Rajbhandary SS, Marks SM, Bock NN. Costs of patients hospitalized for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2004;8:1012-6.
14. Zakham F, Sironen T, Vapalahti O, Kant R. Pan and Core Genome Analysis of 183 Mycobacterium tuberculosis Strains Revealed a High Inter-Species Diversity among the Human Adapted Strains. Antibiotics (Basel) 2021;10:500. doi: 10.3390/antibiotics10050500.
15. Wright GD. The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 2007;5:175-86. doi: 10.1038/nrmicro1614.
16. Arshad H, Gillani AH, Akbar J, Abbas H, Bashir Ahmed A, Gillani SNH, et al. Knowledge on Multi-Drug Resistant Pathogens, Antibiotic Use and Self-Reported Adherence to Antibiotic Intake: A Population-Based Cross Sectional Survey From Pakistan. Front Pharmacol 2022;13:e903503. doi: 10.3389/fphar.2022.903503.
17. World Health Organization (WHO). Pakistan: Tuberculosis. [Online] 2021 [Cited 2023 May 11]. Available from URL: http://www.emro.who.int/pak/programmes/stop-tuberculosis.html
18. Vermund SH, Altaf A, Samo RN, Khanani R, Baloch N, Qadeer E, et al. Tuberculosis in Pakistan: Adecade of progress, a future of challenge. J Pak Med Assoc 2009;59(Suppl 1):s1-8.
19. The World Bank. From $1.90 to $2.15 a day: The Updated International Poverty Line. [Online] 2022 [Cited 2023 May 11]. Available from URL: https://ourworldindata.org/from-1-90-to-2-15-a-day-the-updated-international-poverty-line
20. Awan HA, Sahito AM, Sukaina M, Khatri G, Waheed S, Sohail F, et al. Tuberculosis amidst COVID-19 in Pakistan: a massive threat of overlapping crises for the fragile healthcare systems. Epidemiol Infect 2022;150:e41. doi: 10.1017/S0950268822000358.
21. Yang T, Zhong J, Zhang J, Li C, Yu X, Xiao J, et al. Pan-Genomic Study of Mycobacterium tuberculosis Reflecting the Primary/Secondary Genes, Generality/Individuality, and the Interconversion Through Copy Number Variations. Front Microbiol 2018;9:e1886. doi: 10.3389/fmicb.2018.01886.
22. Hasan R, Jabeen K, Ali A, Rafiq Y, Laiq R, Malik B, et al. Extensively drug-resistant tuberculosis, Pakistan. Emerg Infect Dis 2010;16:1473-5. doi: 10.3201/eid1609.100280.