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Original article

The intercontinental 'Omics' mining of antibiotic resistant genes in *Mycobacterium tuberculosis*

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ABSTRACT

Background: By the year 2030, it will be seven more times difficult and seven times more of 14,600 pills to treat one person with drug resistant tuberculosis (TB) if we fail in the use of 'Omics therapy'. A curated database of the National Centre for Biotechnology Institute (NCBI): Comprehensive Antibiotic Resistance Database (CARD) provides high-through-put referenced information on antibiotics resistance. **Methods:** This study employed a less expensive but high accuracy software-based approach; Comprehensive Antibiotics Resistance Database to identify the resistance genes within the genomes of *Mycobacterium tuberculosis* across the five continents. Thirty complete genomes of *Mycobacterium tuberculosis* were retrieved and their respective accession numbers and locations were categorized into Perfect and Strict genes. **Results:** The least number of *Mycobacterium tuberculosis* complete genome sequences were retrieved from the America followed by Antarctica and Africa with 10%, 10%, 25% prevalence respectively. The continents with the highest number of sequences were Asia and Europe which account for 25% and 33.3% respectively. The prevalence of genes categorized under the strict category from CARD database with mdsB, mdsA, AAC(6')-Iaa, sdiA, golS TEM-1, tetW , ANT(4')-Ib, had 100% prevalence each (present in all the 30 complete genome sequences retrieved), arlR, arlS, mepR, mgrA had the prevalence rates of 25%, each, mepA, Lmrs, FosB had the prevalence rates of 33.3% each.

Introduction

Tuberculosis (TB) is one of the leading causes of death worldwide. The emerging drug resistance is a serious global threat and poses significant challenge to public health. According to the recent world health organization (WHO) report, there were an estimated 10.0 million cases of TB and 1.3 million deaths during the year 2017. India alone accounted for 24% of global multi-drug resistant TB (MDR-TB) incidence and 27% of global TB incidence among HIV-negative individuals [1]. Whole genome sequencing (WGS) studies from across the globe have revealed genetic diversity of *Mycobacterium tuberculosis (M. tuberculosis)* and have provided significant insights into its evolution and transmission [2]. They have also revealed specific genotypes associated with drug resistance. However, these tests rely on a limited number of mutations. There have been several instances where phenotypic resistance could not be explained by known mutations associated with drug resistance [3-8]. Global frontline molecular diagnostics such as line probe assays and Xpert MTB/RIF used for diagnosis of drug resistant TB, have been developed based on these genetic markers [9]. However, these tests rely on a limited number of mutations. There have been several instances where phenotypic

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resistance could not be explained by known mutations associated with drug resistance [3]. A recent study comparing the efficacy of Xpert MTB/RIF with line probe assay for detection of rifampicin mono-resistant *M. tuberculosis* reported the utility of country specific probes, to increase the sensitivity of Xpert MTB/RIF in India [4, 9-15].

As earlier stated, the continued emergence of MDR, extensively drug resistant (XDR) and total drug resistant (TDR) strains have further hampered the effective disease control. For the whole world not to be launched into the complete era of TDR strains; a more high-through-put method of diagnosis is of great necessity. Conventional methods of diagnosis are greeted with flaws resulting from dearth in power especially in Africa where expertise requirement, political and economic instability and other technical fallibilities is deeply rooted. Hence, the opportunities offered by whole genomes (totality of genetic makeup) through Bioinformatics (Biomolecular information of an organism) approach. This study employed a less expensive but high accuracy software-based approach 'omics therapy'; (CARD) to identify the resistance genes within the genomes of *M. tuberculosis* across the five continents.

Materials and Methods

Retrieval of complete genome sequence of *Mycobacterium tuberculosis*

A total of 30 different complete genome sequences (FASTA format) of *M. tuberculosis* were retrieved from NCBI nucleotide database.

FASTA is a DNA and protein sequence alignment software package first described in 1985. Its legacy is the FASTA format which is now ubiquitous in bioinformatics. It stands for Fast Adaptive Shrinkage Threshold Algorithm.

Detection of antibiotic resistant genes in bacteria group

The complete genome sequences of *M.tuberculosis* were analyzed to detect the presence or absence of antibiotic resistant genes and mutants Analysis were carried out using the Comprehensive Antibiotic Resistance Database (CARD). The Resistant Gene Identifier (RGI) was employed for detection of the resistant genes and mutants present. The antimicrobial resistance (AMR) genes were categorized as perfect and strict.

Statistical analysis

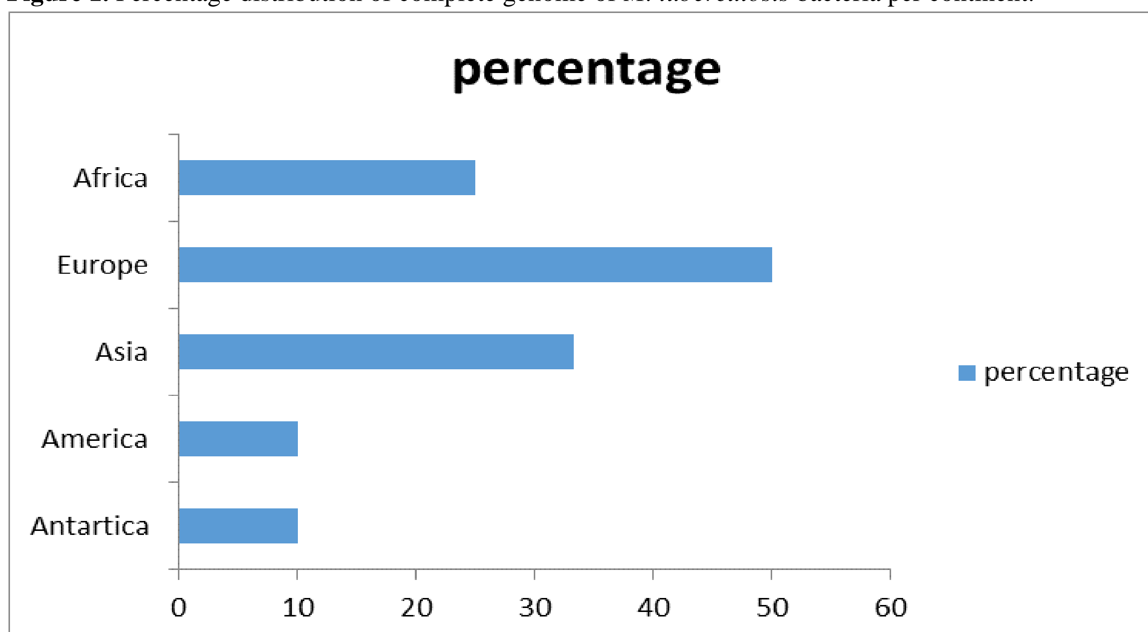
All the resultant data were exported from RGI pool for statistical analysis using Excel Microsoft word version 2013.

Results

Distribution of complete genome sequences of *M.tuberculosis* per location

The locations from which the whole genome of *M.tuberculosis* sequences retrieved for this study was shown in **figure (1)**. The highest number of *M. tuberculosis* were retrieved from the Antarctica and America then followed by Africa, with 10%, 10% and 25% respectively. The continents with the least number of sequences are Asia and Europe with 33.3%, and 50% respectively.

Figure 1. Percentage distribution of complete genome of *M. tuberculosis* bacteria per continent.



Prevalence of 'perfect' resistant genes present in the complete genome of *M.tuberculosis* sequences

Figure 2. Percentage distributions of mutants' genes categorized under 'Perfect'.

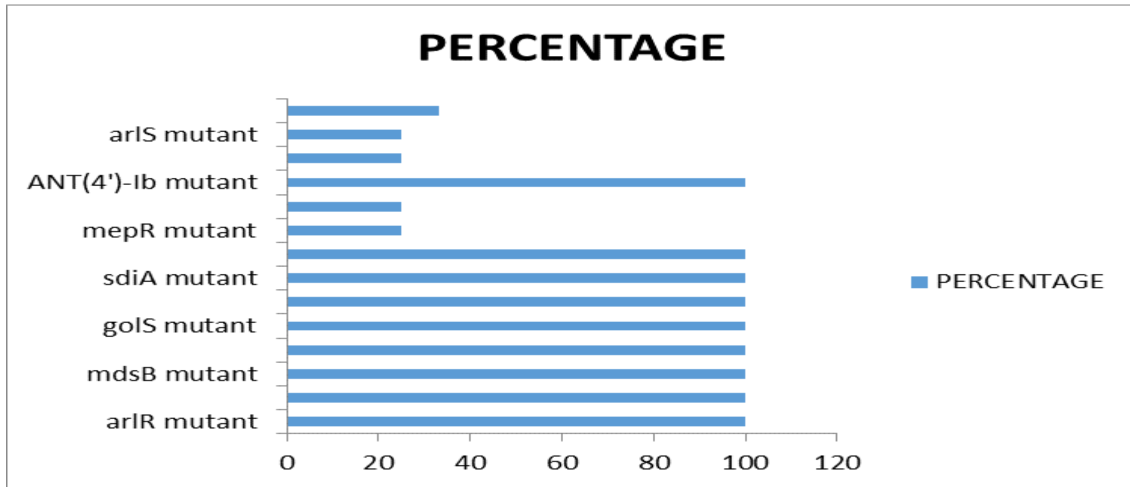
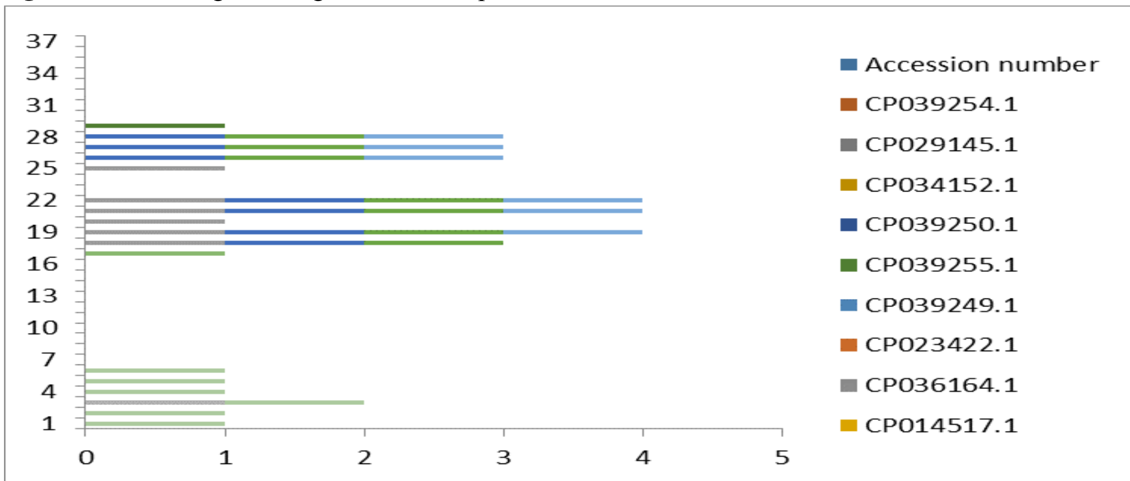


Figure 3. Resistant gene categorized under "perfect" and their accession numbers.



Percentage distribution of 'strict' resistant genes present in the complete genome of *M.tuberculosis* sequences

Figure 4. Percentage distribution of resistant genes categorized under 'strict'

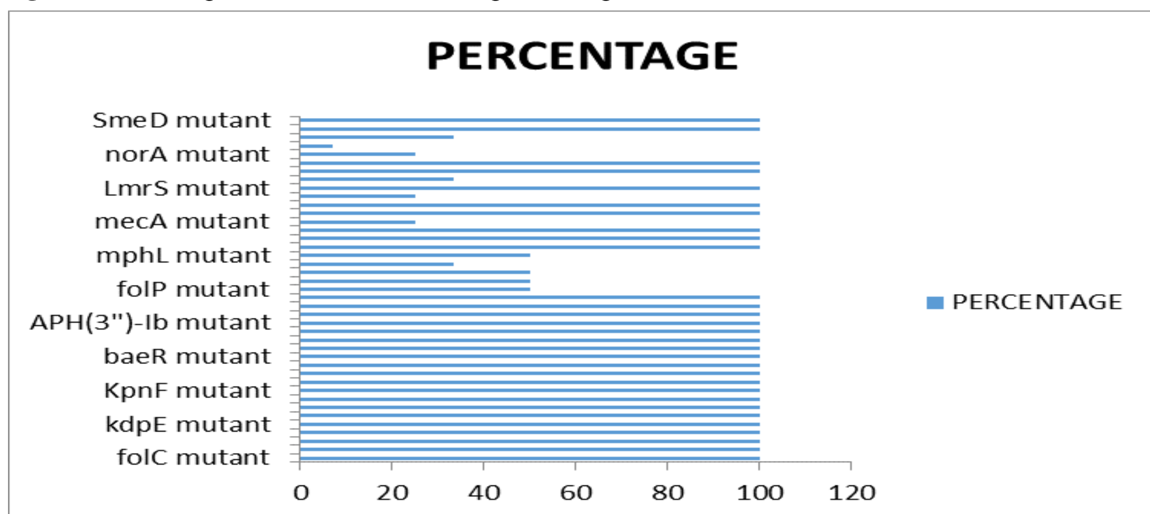
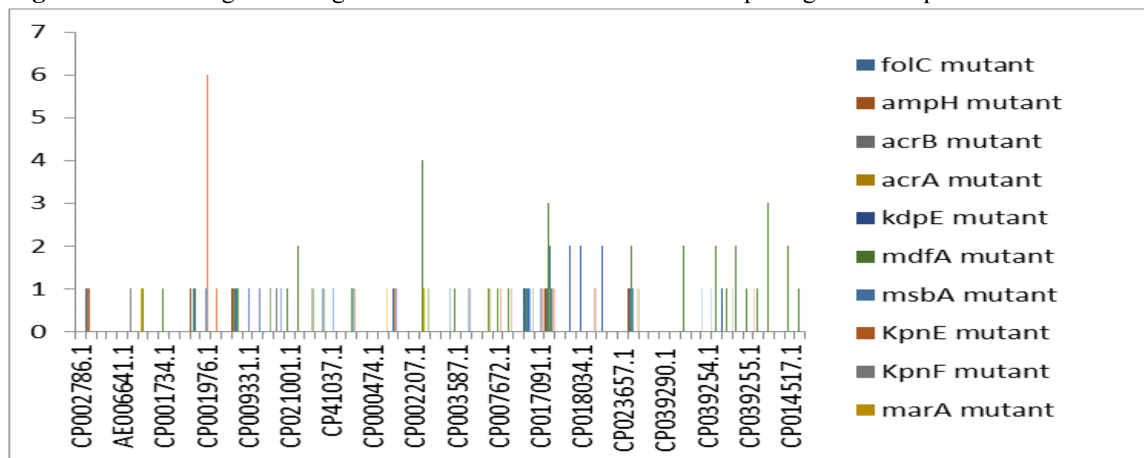


Figure 5. Resistant genes categorized under 'strict' in all the 30 complete genome sequences retrieved.



Discussion

All the prevailing resistance genes as indicated in **figure (2)** were similar to works in earlier studies. The presence of genes under the 'perfect' category from CARD-RGI analysis in each of the whole genome sequences represented by their accession numbers is shown in **figure (3)**. **Figure 4** shows the prevalence of genes categorized under the strict category from CARD database with APH(6)-Id, APH(3")-Ib, sul2, tet(B), KpnF, KpnE, gyrB, bacA, vanI, farB, mecR1, tetW, patB, smeD, smeR, iri, emrR, ampC1, baeR, marA, H-NS, mdtK, msbA, mdfA, KdpE, acrB, ampH, mepR, LmrS, blaZ, (100%) each. folC, FolP, Bla2, Bla1, mphL (50%)each. FosB, RbpA, murA, (33.33%) each. The mutant genes norA, mecA, (25%) each. adeF (7.14%). The presence of genes under the 'strict' category from CARD-RGI analysis in each of the whole genome sequences represented by their accession number is shown in **figure (5)**. Drug resistance in *M. tuberculosis* is the result of chromosomal mutations in existing genes that are passed along through vertical descent that is, passed from mother to daughter cells. Unlike many other bacterial pathogens, *M. tuberculosis* rarely recombines via lateral exchange of DNA [1] and also lacks plasmids. Many of the resistance determinants were discovered before the sequencing of the *M. tuberculosis* genome was completed. The resistance genes are shorted into two, which are perfect and strict. In all there are fourteen(14) perfects and forty-one(41) stricts mdsB, mdsA, AAC(6')-Iaa, sdiA, golS TEM-1, tetW , ANT(4')-Ib, arlR, arlS, mepR, mgrA, mepA, Lmrs, FosB, APH(6)-Id, APH(3")-Ib, sul2, tet(B), KpnF, KpnE, gyrB, bacA, vanI, farB, mecR1, tetW, patB, smeD, smeR, iri, emrR, ampC1, baeR, marA, H-NS, mdtK, msbA, mdfA, KdpE, acrB, ampH, mepR, LmrS, blaZ, folC, FolP, Bla2, Bla1, mphL, FosB, RbpA, murA, norA, mecA, adeF mutants have been successfully identified. The drug-resistant tuberculosis outbreaks in Tugela Ferry and other regions of South Africa highlight the need for early and accurate diagnosis of drug resistance [16,17]. Several studies have shown association of the genetic variations with pathogenesis and drug resistance [8]. Global frontline molecular diagnostics such as line probe assays and Xpert MTB/RIF used for diagnosis of drug resistant TB, have been developed based on these genetic markers [9, 18-20].

Conclusions

The current omics approach has yielded predictive theories about the prevailing drug resistance mutants across various regions and locations thereby laying solid foundation for its usage and applicability for wet laboratory analysis.

Conflict of interest: None.

Financial disclosure: None.

References

1- **World Health Organization.** Global Tuberculosis Report 2018. Geneva: WHO; 2018 . Available at: http://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf?ua=1. Accessed July , 2019.

2- **Casali N, Nikolayevsky V, Balabanova Y, Harris SR., Ignatyeva O, Kontsevaya I, et al.** Evolution and transmission of drug-resistant tuberculosis in a Russian population.Nat Genet 2014;46: 279–286.

3- **Rigouts L, Gumusboga M, De Rijk WB, Nduwamahoro E, Uwizeye C, DeJong B, et al.** Rifampin resistance missed in automated liquid culture system for *Mycobacterium tuberculosis* isolates with specific rpoB mutations. J Clin Microbiol 2013;51: 2641–2645.
4- **Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S.** Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-mono resistant *Mycobacterium tuberculosis* .J Clin Microbiol 2014; 52:1846–1852.
5- **World Health Organization.** WHO treatment guidelines for drug-resistant tuberculosis. 2016 update.Geneva:WHO;2016. Available at: https://apps.who.int/iris/bitstream/handle/10665/250125/9789241549
6- **Diacon AH, Donald PR, Pym A, Grobusch M, Patientia RF, Mahanyele R.** Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. Antimicrobial Agents Chemother 2012; 56:3271–6.
7- **Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, Sasaki H, et al.** OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med 2006; 3:2131–44.
8- **Laurenzo D, Mousa SA.** Mechanisms of drug resistance in *Mycobacterium tuberculosis* and current status of rapid molecular diagnostic testing .Act a Trop 2011;119: 5–10.
9- **Gagneux S, Small PM.** Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. Lancet Infect. Dis 2007;7: 328–337.
10- **Bloom BR, Atun R, Cohen T, Dye C, Fraser H, Gomez GB, et al.** Tuberculosis .In: Holmes KK, editors. Major infectious diseases .Washington DC: The International Bank for Reconstruction and Development/The World Bank. 2017. Available at: https://www.ncbi.nlm.nih.gov/books/NBK525174/. Accessed July 9, 2019.
11- **Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al.** Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 1998; 393:537–44.
12- **Fleischmann RD, Alland D, Eisen JA, Carpenter L, White O, Peterson J, et al.** Whole-genome comparison of *Mycobacterium tuberculosis* clinical and laboratory strains. J Bacteriol 2002; 184:5479–90.
13- **Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, et al.** "CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database". Nucleic Acids Research 2017; 45 (D1): D566–D573.
14- **Long S, Beres S, Olsen R, Musser J.** Absence of patient-to-patient intra hospital transmission of Staphylococcus *aureus as* determined by whole-genome sequencing. Bio5 2014; (5): e01692e1714.
15- **Young DB.** Blueprint for the white plague. Nature 1998; 393:515–6.
16- **Huson DH, Scornavacca C.** Dendro scope 3: an interactive tool for rooted phylogenetic trees and networks. Syst Biol 2012; 61:1061–1067.
17- **Cabibbe AM, Walker TM, Niemann S, Cirillo DM.** Whole genome sequencing of *Mycobacterium tuberculosis*. Eur Respir J 2018; 52:537–45.

18-**Casali N, Broda A, Harris SR, Parkhill J, Brown T, Drobniewski F.** Whole genome sequence analysis of a large isoniazid-resistant tuberculosis outbreak in London: a retrospective observational study. PLoS Med 2016; 13(10):e1002137.

19-**Akhter Y, Ehebauer MT, Mukhopadhyay S, Hasnain SE.** The PE/PPE multigene family codes for virulence factors and is a possible source of mycobacterial antigenic variation: Perhaps more? Biochimie 2012; 94:110–6.

Aderibigbe TS, Micheal AO. The intercontinental 'Omics' mining of antibiotic resistant genes in *Mycobacterium tuberculosis*. Microbes Infect Dis 2022; 3(2): 428-433.