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THE USE OF CARD FOR SCREENING OF MULTI DRUG RESISTANCE VARIANT GENES (MDR-VG) IN *MYCOBACTERIUM TUBERCULOSIS*

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Abstract

Thirty complete sequences of Mycobacterium tuberculosis in FASTA formats were retrieved from the curated database of the NCBI; before being inputted into the Comprehensive Antibiotic Resistance Database (CARD). All the retrieved complete genome sequences of M. tuberculosis were randomly selected based on their location and accession numbers. Into Perfect and Strict Resistomes. The highest number of Mycobacterium tuberculosis complete genome sequences were retrieved from the America followed by Africa and Antarctica with 38%, 29%, 23% respectively. The continents with the least number of sequences were Asia and Europe with 6% and 4% respectively. The prevalence of genes categorized under the strict category from CARD database with

KEYWORDS:
Resistomes; CARD-RGI; Curated; Strain-relatedness; Antarctica; WGS

APH(6)-Id, APH(3'')-Ib, sul2, tet(B), ANT(3'')-Iic, adeJ, adeL, AmvA, adeN, adeR, AbaF, KpnH, gyrB, bacA, vanI, farB, mecR1, tet(45) ,cat86, rpsL, pncA, soxR, patB, oqxA, cmH-1, FosA2, ramA, parC, KpnG, OmpK37, FosA6, smeR, iri(100%) each. mdfA, AbaQ, gyrA, thyA, kasA, AAC(6')-Iy, emrB, Bla2, Bla1, mphL, arlR, blaZ, RbpA, mepR, marR (50%) occurrence each. The highest number of resistant mutants was found in the American continent. Some of the strict mutants in America were folC, msbA, baeR, tet(B), adeL, KpnH, gyrA, embB, GlpT, farB, blaZ and patB followed by Asia with tet(B), baeR, msbA and folC.

INTRODUCTION

Tuberculosis 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 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 MDR-TB incidence and 27% of global TB incidence among HIV-negative individuals (Global Tuberculosis Who Report [GWTR]x, 2018). There is an urgent need for improved diagnosis of TB, such as identification of markers to monitor transmission and effective treatment to deal with this deadly disease. Whole genome sequencing (WGS) studies from across the globe have revealed genetic diversity of *Mycobacterium tuberculosis* and have provided significant insights into its evolution and transmission (Casali *et al*., 2014. Undiagnosed drug resistance leads to further transmission, poor patient outcomes and potential for amplification of drug resistance, impeding the World Health Organization's (WHO) strategy to end tuberculosis by 2035. Several studies have shown association of the genetic variations with pathogenesis and drug resistance (Laurenzo and Mousa, 2011, Xu et al., 1996, Palomino, 2006). 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 (Gagneux and Small, 2007). There were an estimated 490,000 new cases of multidrug-resistant (MDR) TB, which is defined by phenotypic resistance to both isoniazid and rifampicin (WHO, 2016). Approximately 10% of MDR-TB cases globally can be

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classified as extensively drug-resistant (XDR), indicating that there is concomitant resistance to quinolones (such as the fluoroquinolones, levofloxacin, and moxifloxacin) and to a second-line injectable agent (amikacin, kanamycin, or Capreomycin) (WHO, 2016, Zaunbrecher *et al.,* 2009, Zhang & Yew, 2009).

As expected, drug-resistance patterns predicted treatment outcome; in 2015, TB treatment success overall was 83%, whereas the success rate was 54% for MDR-TB or rifampicin-resistant TB (RR-TB) and only 30% for XDR-TB (WHO, 2O18). Culture-based techniques remain the current reference standard for both diagnosis and drug-susceptibility testing of TB, but these processes are time-consuming and require specialized laboratory capacity. More recently, the use of rapid molecular tests for the diagnosis of TB has increased globally, particularly the use of Xpert MTB/RIF (Cepheid, Sunnyvale, CA), a PCR-based assay that simultaneously detects the presence of *M. tuberculosis* and resistance to rifampicin (Diacon et al., 2012, Engström et al., 2011.

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 (Rigouts *et al*., 2013). 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 (Rufai *et al*., 2014). Since there is considerable genetic heterogeneity among *M. tuberculosis* isolates from different geographic regions, large-scale sequencing efforts are required to map genetic variations and identify the genotypes associated with drug resistance. Genomics and bioinformatics are increasingly contributing to our understanding of infectious diseases caused by bacterial pathogens such as *Mycobacterium tuberculosis.* Bioinformatics and analysis is a key part of the WGS process and for optimal clinical Genomics and bioinformatics have contributed immensely to our understanding of infectious diseases: from disease pathogenesis, mechanisms and the spread of antimicrobial

resistance, to host immune responses. The direct benefit of whole-genome sequencing (WGS) is the ability to provide organism identification, strain relatedness and drug resistance of profile for characterized resistance conferring mutation. Owing to advances in technology and reductions in cost, whole-genome sequencing (WGS) has been transformed from a centralized service used by a select few to interrogate single genomes into a relatively decentralized lab technique used by many to detect and track infectious pathogens(Long *et al*.,2014). The strategy will contribute to a global agreement on WGS analytical approaches, epidemiological interpretation criteria and genomic nomenclature by surveillance objective, while retaining flexibility in order to explore improved methods. It will also help with multi-country evaluation of the public health effectiveness of WGS-based typing by measuring outcomes in terms of disease prevention and it will focus training efforts on developing a new, integrative 'genomic epidemiology' discipline, thereby building a common understanding which translates into public health risk assessment. The dawning of the new age of genome sequencing began to revolutionize our approach to human diseases, including TB. In 1998, (Cole *et al*.,1998) reported the complete genome sequence of the *M. tuberculosis* reference strain H37Rv, which was approximately 4.41 million base pairs in length and encoded approximately 4000 genes. The first sequencing of a clinical reference strain, CDC1551, quickly followed (Fleishman *et al*., 2012). An accompanying editorial optimistically stated. After several decades in the slow lane of classical microbiology, *M. tuberculosis* is once again at the cutting edge of science. However, even at the time of these breakthroughs, there was recognition that translating these genomic data into clinical benefit would prove challenging (Young *et al*., 2018). Despite these challenges, it is clear, more than 20 years later, that *M. tuberculosis* genomic data have been remarkably useful in improving our understanding of how drug-resistant TB evolves and spreads and in helping to inform diagnostics and therapies (Cabibbe *et al*., 2018). Comprehensive Antibiotic Resistance Database is a biological database that collects and organizes reference information

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on antimicrobial resistance genes, proteins and phenotypes (Jai *et al*., 2017). The database covers all types of drug classes and resistance mechanisms and structures its data based on an ontology. The CARD database was one of the first resources that covered antimicrobial resistance genes (Arthur *et al*., 2013).

Materials and Methods

Retrieval of complete genome sequence of *Mycobacterium tuberculosis*

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

Detection of antibiotic Resistant genes in *Mycobacterium tuberculosis*

The complete genome sequences of *Mycobacterium 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 AMR genes were categorized as perfect and strict.

Results

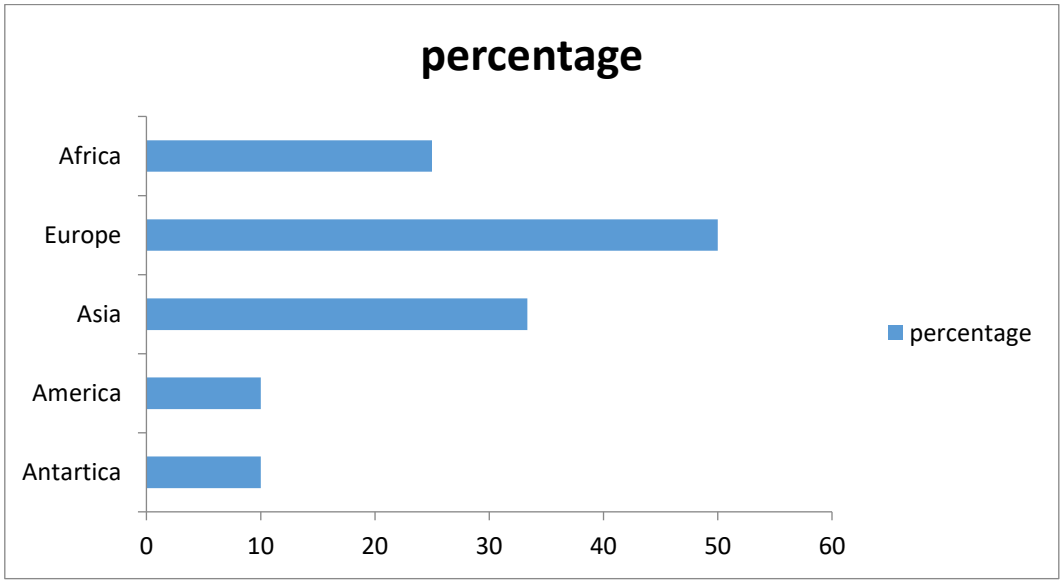

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Figure 1: Prevalence of MDR mutant genes by continent

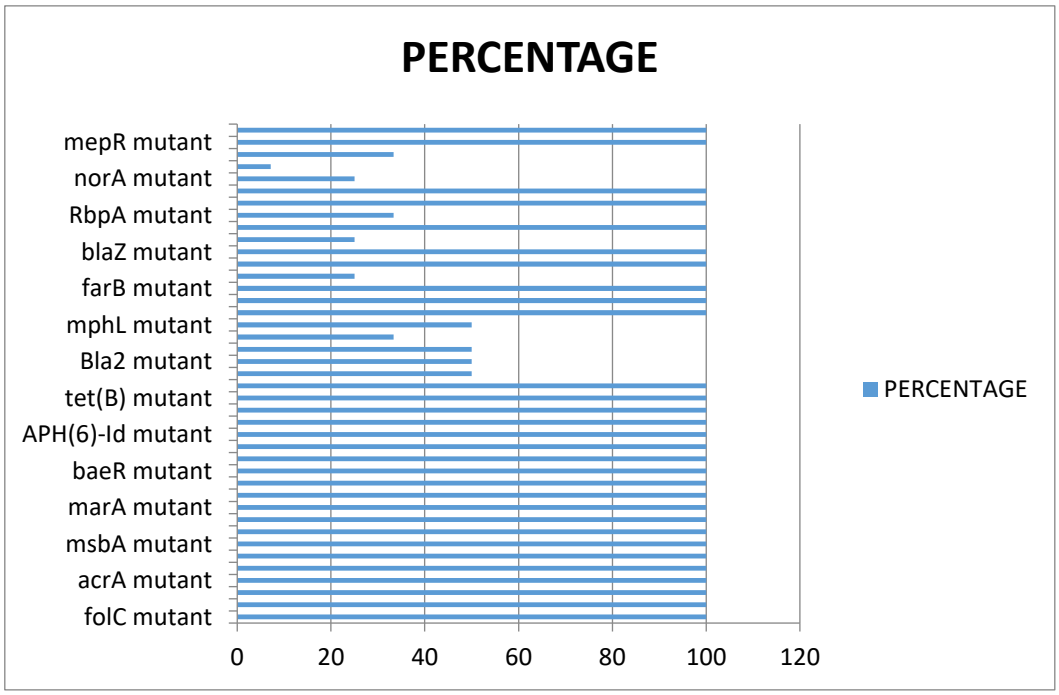

Figure 2: Prevalence of 'Strict' MDR mutant genes in the American Continent

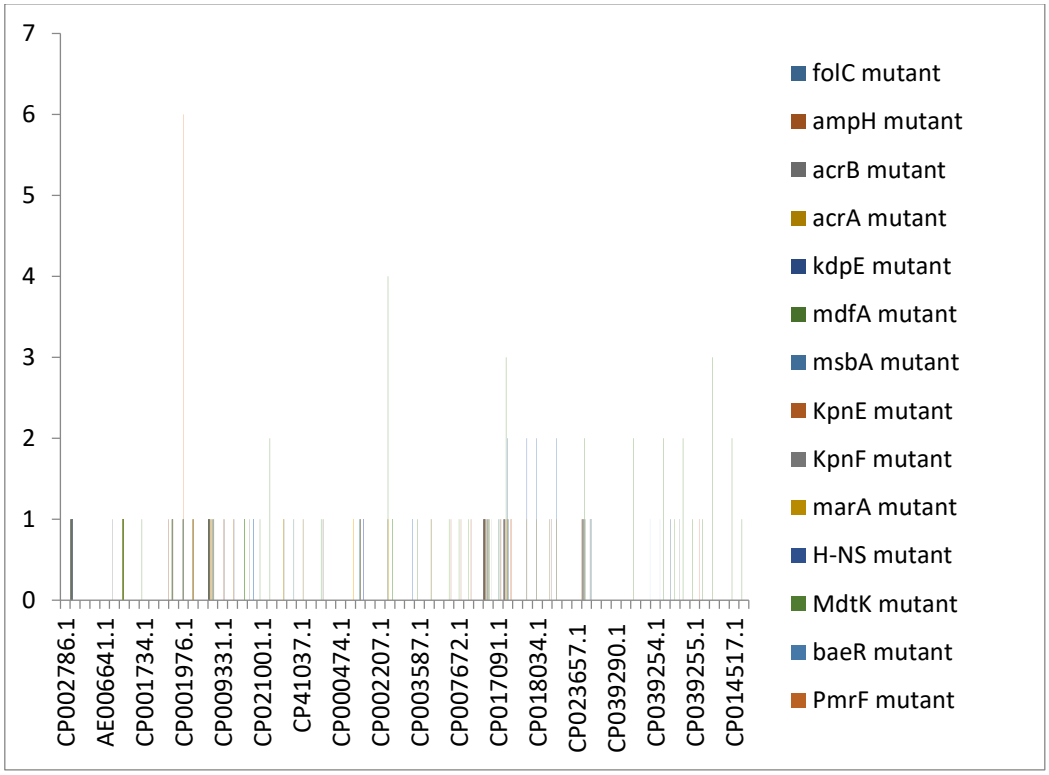

Figure 3: Distribution of 'Strict' in Antarctica

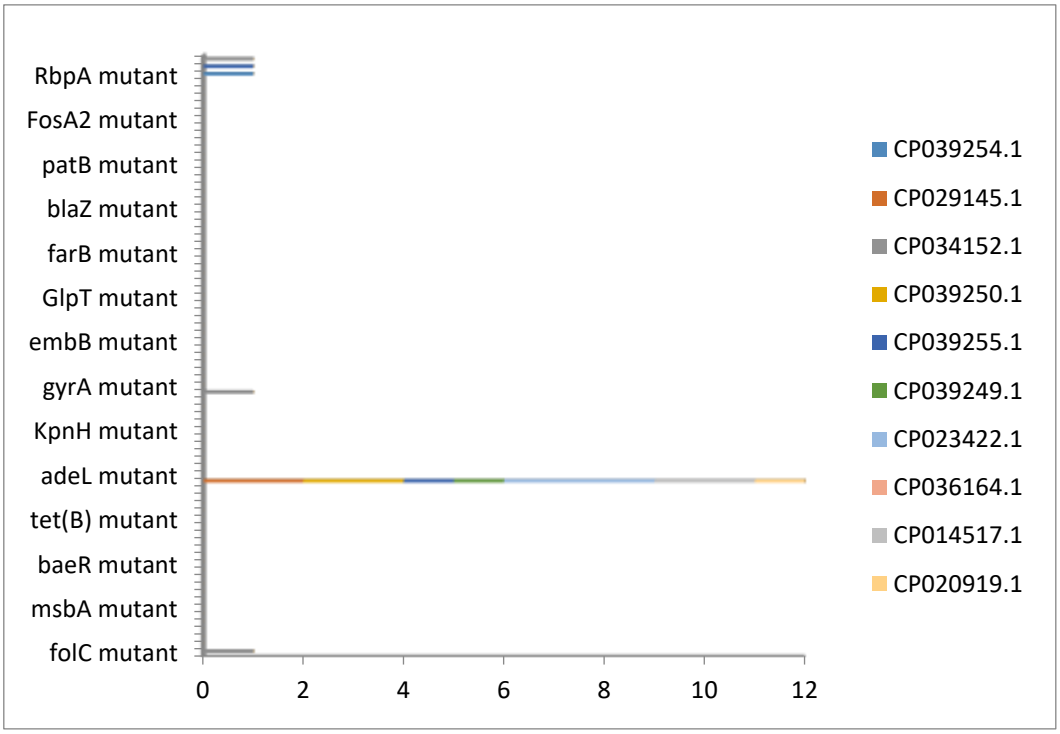

Figure 4: Prevalence of 'strict' Mutant genes in Africa

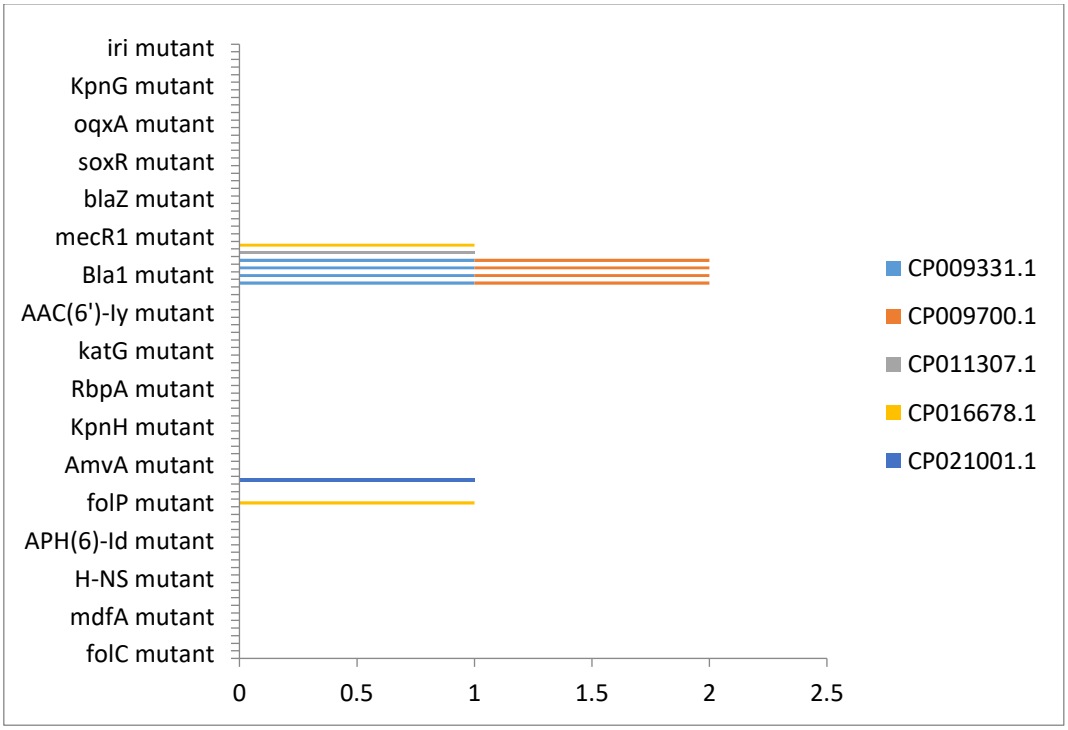

Figure 5: 'Strict' Mutant genes in Europe

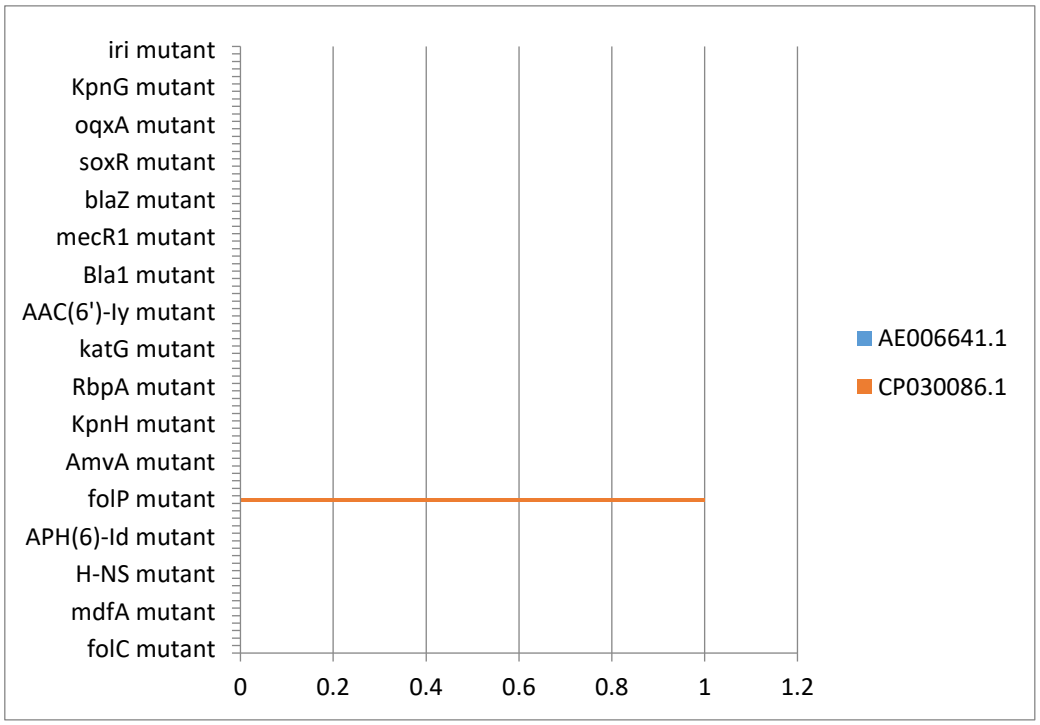

Figure 6: Mutant genes in Asia

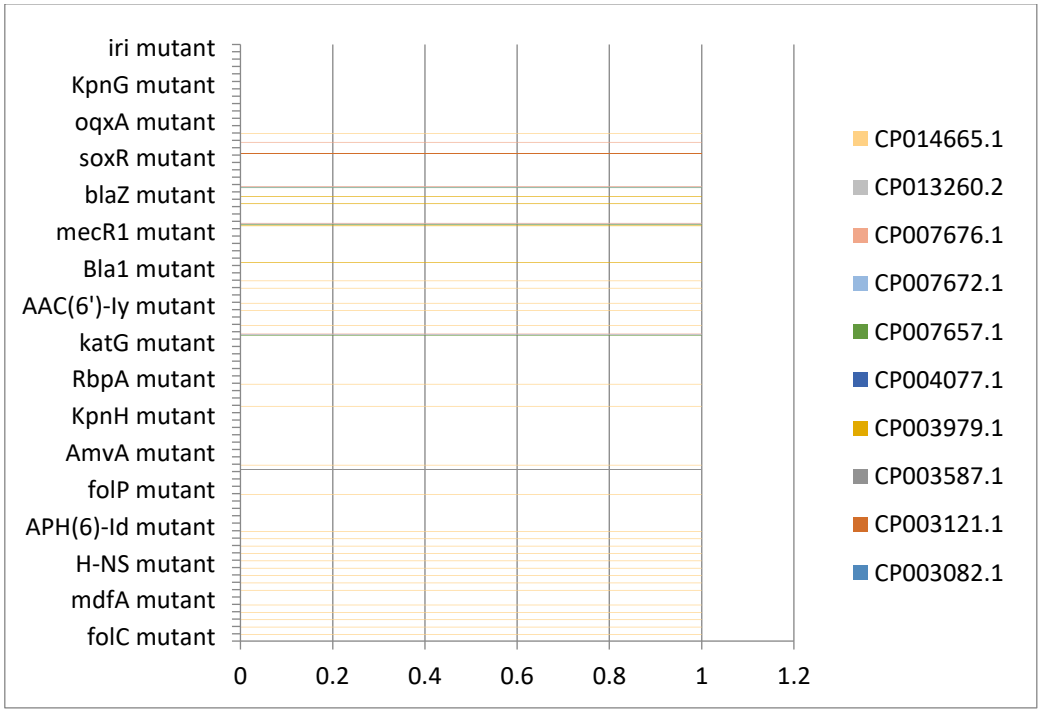

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Figure 7: Prevalence of 'perfect' MDR-VG in Antarctica

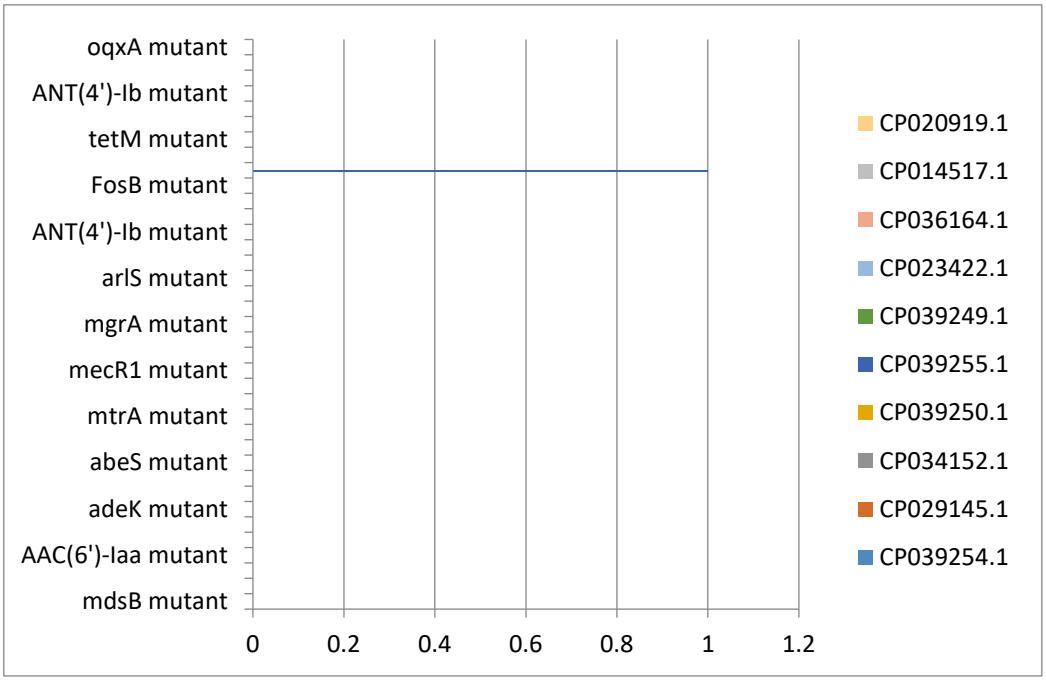

Figure 8: Prevalence of 'perfect' MDR-VG in America perfect

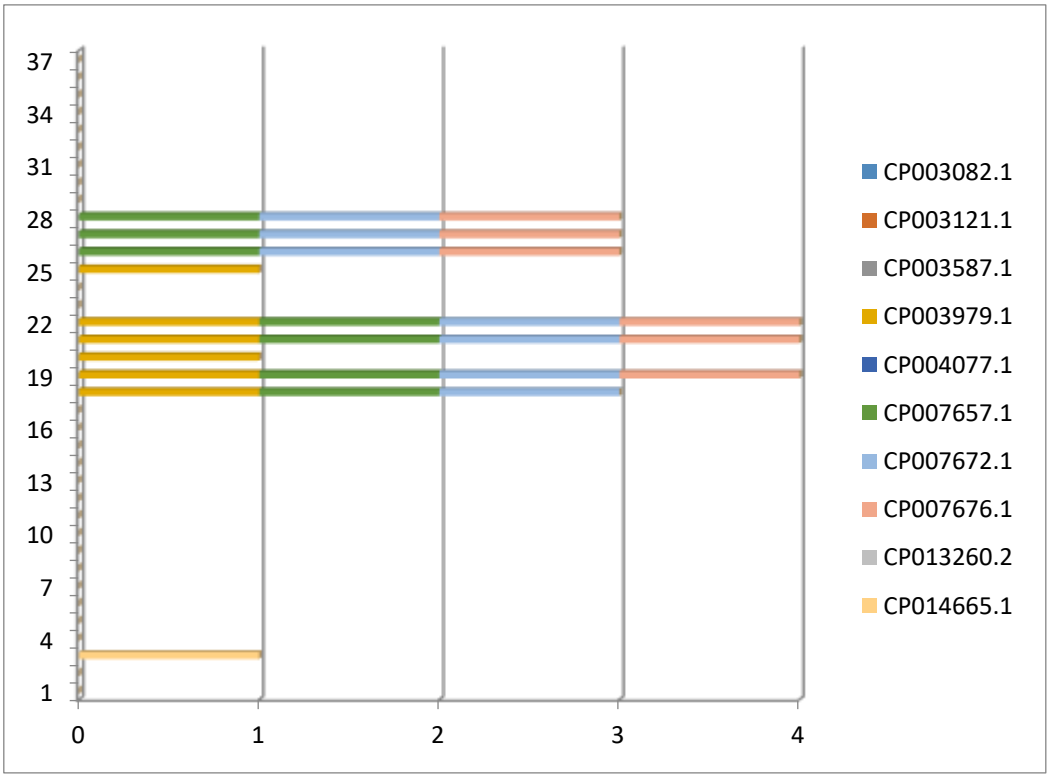

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Figure 9: Prevalence of 'perfect' MDR-VG in Africa

Discussion

Prevalence of 'strict' resistant genes present in the complete genome of *Mycobacterium tuberculosis* sequences and their clinical implication in five (5) continents:

In Asia "strict" Antibiotic efflux (acrB mutant, acrA mutant, kdpE mutant, mdfA mutant, msbA mutant, KpnE mutant, H-NS mutant, matK mutant, baeR mutant, tet(B) mutant, emrR mutant), Antibiotic inactivation (ampH mutant, ampC1 mutant, APH(6)-Id mutant, APH(3'')-Ib mutant), Antibiotic target alteration (folC mutant, pmrF mutant, sul2 mutant), Antibiotic efflux, reduced permeability to antibiotic (MarA mutant). In Europe "strict "Antibiotic efflux (adeJ mutant, adeF mutant, adeL mutant, AmvA mutant, adeN mutant, AbaQ mutant, adeR mutant, AbaF mutant), Antibiotic inactivation **(**ANT (3'')-Iic mutant), Antibiotic target alteration (folP mutant, parC mutant). In Africa "strict" Antibiotic efflux (acrB mutant, acrA mutant, KdpE mutant, mdfA mutant, msbA mutant, KpnF mutant, H-NS mutant, baeR mutant, tet(45) mutant, emrR mutant, adeF mutant, emrB mutant, sdiA mutant, FarB mutant, CRP mutant, KnpH mutant, arIR mutant, Lmrs mutant, norA mutant), Antibiotic inactivation (ampH mutant, ampC1 mutant, Bla2 mutant, Bla1 mutant, FosB mutant, mphL mutant, PC1 mutant, AAC(6')-Iy mutant), Antibiotic target alteration (gyrB mutant, PBP3 mutation, EF-Tu mutation, gyrA mutation, thyA mutation, KasA mutation, Kat mutation, murA mutation, embB mutation, rRNA mutation, KatG mutation, pmrF mutation, bacA mutation, GlpT mutation, VanI mutation, FolP mutation, rpsL mutation, pncA mutation), Antibiotic target alteration, antibiotic target replacement (rpoB mutation), Antibiotic target protection (RbpA mutation, adeF mutation), Antibiotic efflux, reduced permeability to antibiotic (marA mutation), Antibiotic target replacement (mecR1 mutation, mecA mutation).

In America "strict "Antibiotic efflux (norA mutation, acrB mutant, acrA mutant, KdpE mutant, mdfA mutant, msbA mutant, KpnF mutant, H-NS mutant, baeR mutant, tet(45) mutant, emrR mutant, adeF mutant, emrB

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mutant, sdiA mutant, FarB mutant, CRP mutant, KpnE mutant, arIR mutant, Lmrs mutant, AbaQ mutation, patB mutation, mepR mutation, mdtK mutation, mdfA mutation, kdpDE mutation, oqxA mutation, KpnG mutation, KpnH mutation), Antibiotic target alteration (GlpT mutation, murA mutation, gyr mutation, KatG mutation, pncA mutation, embB mutation, rRNA mutation, rspL mutation, FoIP mutation, norA mutation, baeA mutation, PmrF mutation, UhpT mutation, EF-Tu mutation, PBP3 mutation, parC mutation), Antibiotic inactivation (Cat86 mutation, blaZ mutation, FosB mutation, ampC1 mutation, ampH mutation, CMH-1 mutation, FosA2 mutation, FosA6 mutation), Antibiotic target protection (RbpA mutation), Antibiotic target alteration, antibiotic target replacement (rpoB mutation), Antibiotic target replacement (mecA mutation), Antibiotic efflux, reduced permeability to antibiotic (MarA mutation, ramA mutation, ompK37 mutation). In Antarctica "strict" Antibiotic efflux (adeF mutation, smeR mutation), Antibiotic target protection (RbpA mutation), Antibiotic inactivation (iri mutation), Antibiotic target alteration (folC mutation). A mutation at nucleotide 1401 or 1484 is associated with resistance to all these agents, whereas a mutation at nucleotide 1402 is associated with CAP resistance and low-level KAN resistance (Engström *et al*, .2011). Overexpression of eis (encoding the aminoglycoside acetyltransferase Eis), caused by mutations in the promoter region, confers low-level resistance to KAN (Zaunbrecher *et al*,.2009) Fluoroquinolones (FQs), e.g., Ofloxacin (OFL), which bind to DNA gyrase, inhibit proper regulation of supercoiling and cause chromosomal double-strand breaks (Xu et al,.1996). Spontaneous chromosomal mutations are the genetic basis for drug resistance in M. tuberculosis (Zhang and Yew, 2009), and a limited number of mutations account for the majority of phenotypic resistance to anti-TB drugs. RIF interacts with the ß-subunit (encoded by rpoB) of the RNA polymerase and inhibits the early steps of transcription (Campbell *et al.*, 2001).

Since sequences are highly conserved, certain mutations correlate well with phenotypic resistance, and a limited number of mutations account for the majority of phenotypic resistance to the important anti-tuberculosis

medications, various methods of genotypic testing have successfully been used for the rapid detection of *M. tuberculosis* resistance (Palomino, 2006)

CONCLUSION

Earlier studies revealed that as much as drug-resistance are induced and acquired; mixed infections are common, and often represent important intermediates in the evolution of drug resistance. Furthermore, its practical use in clinical settings holds great potential to improve public health through real-time molecular epidemiology tracking, to identify global hotspots of drug-resistance emergence, and to facilitate the development of improved approaches for the diagnosis and treatment of drug-resistant *Mycobacterium tuberculosis*.

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