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Original Research Article

Bioinformatics Lens for Deciphering the Antimicrobial Resistance Genes (AMR): Mycobacterium tuberculosis at the epicenter of using Comprehensive Antibiotics Resistance Database (CARD)

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Abstract

The extensive use of antibiotics including anti-tuberculosis drugs through the development of mutations, the emergence and the spread of multidrug-resistant mechanisms is recognized as one of the most dangerous threats to global control and eradication. The prevalence of genes under the 'strict' category from CARD-RGI analysis in each of the whole genome sequences represented by their accession number. Seventy complete genomes of bacteria which spanned across 24 families were polled in FASTA format from the NCBI database using their respective accession numbers. The individual genomes were concurrently allowed to run at the Comprehensive Antibiotics Resistance database. The prevalence of perfect AMR genes under the strict category with 100% are: 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. Those on 50% prevalence are: mdfA, AbaQ, gyrA, thyA, kasA, AAC(6')-Iy, emrB, Bla2,Bla1, mphL, arlR, blaZ, RbpA, mepR, marR (50%)each. folC, acrB, acrA, kdpE, KpnF, MdtK, folP, adeF. EF-Tu, RbpA, rpoB, katG, sdiA, GlpT, uhpT. Strict mutant AMR genes of 25% prevalence are: PmrF, ampC1, CRP, embB, FosB (25%). In all of the seventy complete genomes, there were thirty-seven (37) perfects and Eighty-one (81) stricts APH (6)-Id mutant.Conversely, antibiotic resistance is a product of genetic change; early detection through surveillance offers high promising specificity and sensitivity in mutant detection.

Keywords: AMR genes, Resistomes, Mutants, FASTA, Intergenic regions, SNP

INTRODUCTION

Emergence of antibiotic resistant strains and rapid spread due to globalization poses a serious challenge to the global health. Genomics has served as an important milestone in bacterial drug discovery. It is now possible to understand causes of emergence of antibiotic-resistant strains and to identify potential drug targets through combinatorial approaches involving comparative genomics, metabolomics, phylogenomics, evolutionary and structural biology/bioinformatics [1]. Tuberculosis (TB) is one of most dangerous chronic infectious diseases and it is caused by *Mycobacterium tuberculosis* (M.tb) infection. *Mycobacterium tuberculosis* is typically transmitted by aerosols and reaches the lungs. The extensive treatment courses result in poor compliance and drug resistance, and the emergence of multidrug-resistant strains has become a serious public health threat and represents a new challenge in TB control [2]. The advent of antimicrobial resistance has added significantly to the impact of infectious diseases, in number of infections, as well as added healthcare costs. Even though we have a very large number of antimicrobial agents from which to choose for potential infection therapy, there is documented antimicrobial resistance to all of these, and this resistance occurs shortly after a new drug is okayed for use. These concerns prompted the WHO to launch a Global Action Plan on antimicrobial resistance in 2015 [2]. TB control is hampered by anti-mycobacterial resistance, multidrug resistance (MDR) and,

recently, extensively drug resistant (XDR) mycobacterial strains [3]. Genomics analysis has immensely contributed to the identification of drug resistance-conferring mutations and surveillance [4].

Whole genome analyses have demonstrated that mycobacterial drug resistance is largely attributed to single nucleotide polymorphisms (SNPs); for example, rifampicin (RIF) resistance arises from mutations in the rpoB gene and mutations in the katG and inhA lead to isoniazid resistance [5]. Newly characterized genetic mutations in bacteria genomes have also been shown to play key roles in the emergence of anti-mycobacterial drug resistance [6].

Analyses of 161 drug resistant *M. tuberculosis* genomes identified 72 genes, 28 intergenic regions and 21 SNPs with strong and consistent associations with drug resistance [7]. Genomic analysis has also identified lineage mutation rate differences and predicted the emergence of anti-mycobacterial resistance [8]. A retrospective analysis of thousands of *M. tuberculosis* genomes collected from African and European patients identified 120 resistance-determining mutations for first and second line anti-mycobacterial drugs, which could be valuable in developing new assays for drug susceptibility testing [9]. It is possible for AMR to develop in bacteria, but it can also originate in non-bacteria such as fungi, parasites, and viruses.

Furthermore, genomics through the use of GWAS has been used to identify novel mutations associated with resistance to cycloserine, ethionamide, and paraaminosalicylic acid, suggesting the involvement of efflux pump in the emergence of resistance [10]. A number of genomics-based tools have been developed to detect drug resistance including Mykrobe Predictor, PhyResSE, and TB-Profiler, which are easy to use by researchers with no bioinformatics expertise and can predict drug resistance within minutes after obtaining sequences. Mykrobe Predictor has a sensitivity and specificity of 82.6 and 98.5%, respectively [11].

TB-Profiler was developed using a mutation library consisting of 1,325 mutations in different genes associated with drug resistance in 15 anti-tuberculosis drugs and had more than 75% sensitivity as well as more than 90% specificity for all drugs tested [10]. A recent study evaluating the performance of these tools showed that their sensitivity ranges from 74 to 80% along with a specificity of more than 95% [12]. Antibiotics work in different ways. For example, penicillin works by indirectly causing the bacterium's cell wall to weaken and burst, so it dies. Tetracycline, on the other hand, do not kill bacteria but inhibit their growth by stopping the bacteria from making proteins. Some antibiotics can be used to treat a broad range of infections, while others are used to treat infections caused by specific types of bacteria. Most antibiotics can cause some side effects (e.g., stomach upset, diarrhea), though some have a higher risk of causing serious side effects (e.g., hearing damage, kidney damage).

However, there is still a need for optimization of analysis pipelines to make them applicable in field settings where the disease burden is usually the highest.
Genomics analysis has also been used to determine the evolutionary history and spread of mycobacterial strains such as the Beijing strain, demonstrating its spread from the Far East [13]. An investigation of *M. tuberculosis* transmission dynamics is important in monitoring outbreak; [14] demonstrated that whole genome analysis can be used to monitor infections to decipher transmission dynamics. Furthermore, genomics has also been applied to decipher transmission dynamics of *M. tuberculosis* in Vietnam, suggesting that SNPs in ESX-5 type VII secreted protein EsxW could potentially contribute to enhancing transmission [15].

Furthermore, genomics has been applied to investigate TB outbreaks, genotyping of the outbreak associated lineages, and their evolution during the outbreak [16].Indeed, analysis tools have been developed for the prediction of *M. tuberculosis* spoligotypes from raw sequence reads, and in combination with other analysis tools also determine antibiotic resistance as well as transmission dynamics [10].

Some genomics methods can also be employed to identify mixed infections as well as infections with a single strain and have recently been applied to clinical isolates from Malawi [18].

MATERIALS AND METHODS

Retrieved Accession Numbers from NCBI:
CP002786.1, CP019649.1, KC131129.1, NC_023568.1, AE006641.1, CP030086.1, CP038262.1, CP003385.1, CP001734.1, CP002545.1,CP002466.1, CP001875.2, CP001976.1, CP001662.1, CP007507.1, CP009331.1, CP009700.1, CP011307.1, CP016678.1,CP021001.1,CP028127.1, CP033505.1, CP033506.1, CP041037.1, CR767821.1, AJ938182.1, AM286280.1, CP000474.1, CP000813.4, NZ_CP012506.2, CP001098.1, CP002207.1, CP003041.1, CP003082.1, CP003121.1, CP003587.1, CP003979.1, CP013701.1, CP004077.1, CP007657.1, CP007672.1,CP007676.1, CP013260.2, CP014665.1, CP017091.1, CP017475.1, CP017685.1, CP018032.1, CP018034.1, CP018768.1, CP019035.1, CP021644.1, CP023477.1, CP023657.1, CP024458.1, CP014513.1, CP040018.1, CP039290.1, CP01729.1, CP014771.1, CP039253.1, CP039254.1, CP029145.1, CP034152.1, CP039250.1, CP039255.1, CP0234622.1, CP036164.1, CP014517.1, CP020919.1.

Retrieval of complete genome sequence of Bacteria group

A total of 70 different complete genome sequences in FASTA format of Bacteria which fall into the major group taxonomic groups were retrieved from NCBI nucleotide database. All the seventy bacteria fall into 24 taxonomic families, namely: Staphylococcaceae (1), Enterobacteriaceae, Corynebacteriaceae, Streptococcaceae, Acetobacteriaceae, Micrococcacceae, Dermabacteriacecceae, Norcadardiaceae, Sphingomonadaceae, Xanthomonadaceae, Oxalobacteriaceae, Intrasporangiaceae, Comanadaceae, Thermogaceaae, Anaplasmoataceae, Neiseseriaceae, Bacillaceae, Mycobacteriaceae, Erwiniaceae, Pseudomonadales, Burkholderiaceae, Sulfolobaceae, Vibrionaceae and Gloeobacteriaceae

Procedure of retrieving 70 strains of Bacteria from National Center for Biotechnology information (NCBI)

Go to NCBI

↓

Click on National Center for Biotechnology information

↓

Click on Nucleotide

↓

Type any organism (e.g. Mycobacterium tuberculosis or bacteria name)

↓

Click on your desire stain

↓

Click on summary

↓

Click on FASTA text

↓

Align all and copy to Note pad

↓

Then save.

Detection of antibiotic Resistant genes in Bacteria group

The complete genome sequences of Bacteria group 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.

Flow chart for Analysis of Antimicrobial Resistance from CARD database

Open new tab

↓

Type Compressive Antimicrobial Resistance Data base (CARD)

Click on Analyze

Click on RGI

Enter a GenBank accession(s)

Then submit

Click on AMR Gene

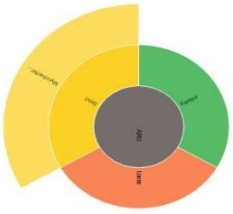
Click on AMR Gene Family

Click on Drug Class

Click on Resistance Mechanism

How to sort out into continents

RESULTS



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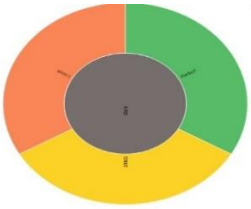

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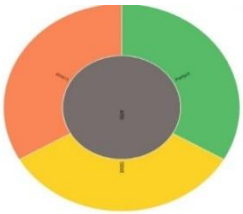

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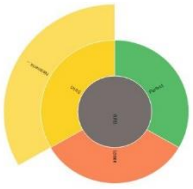

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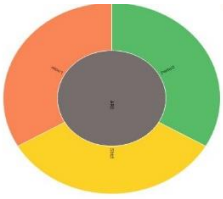

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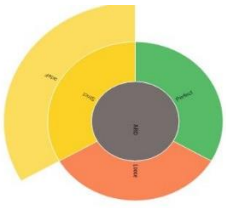

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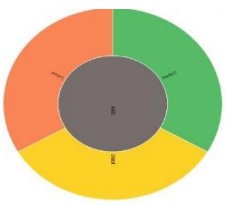

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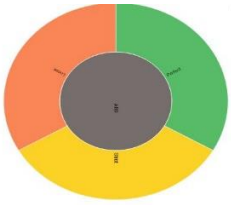

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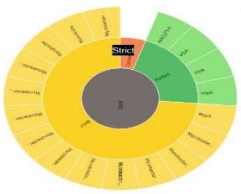

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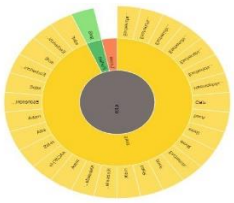

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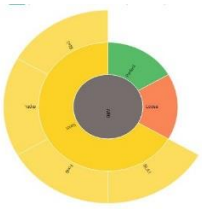

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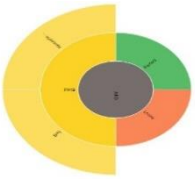

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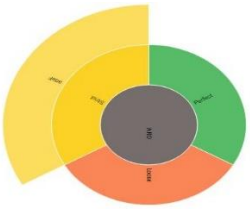

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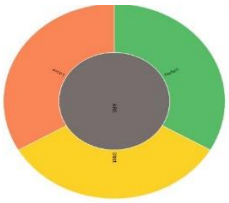

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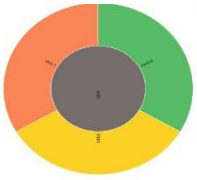

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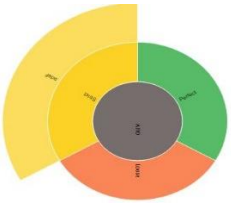

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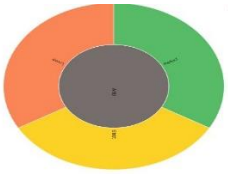

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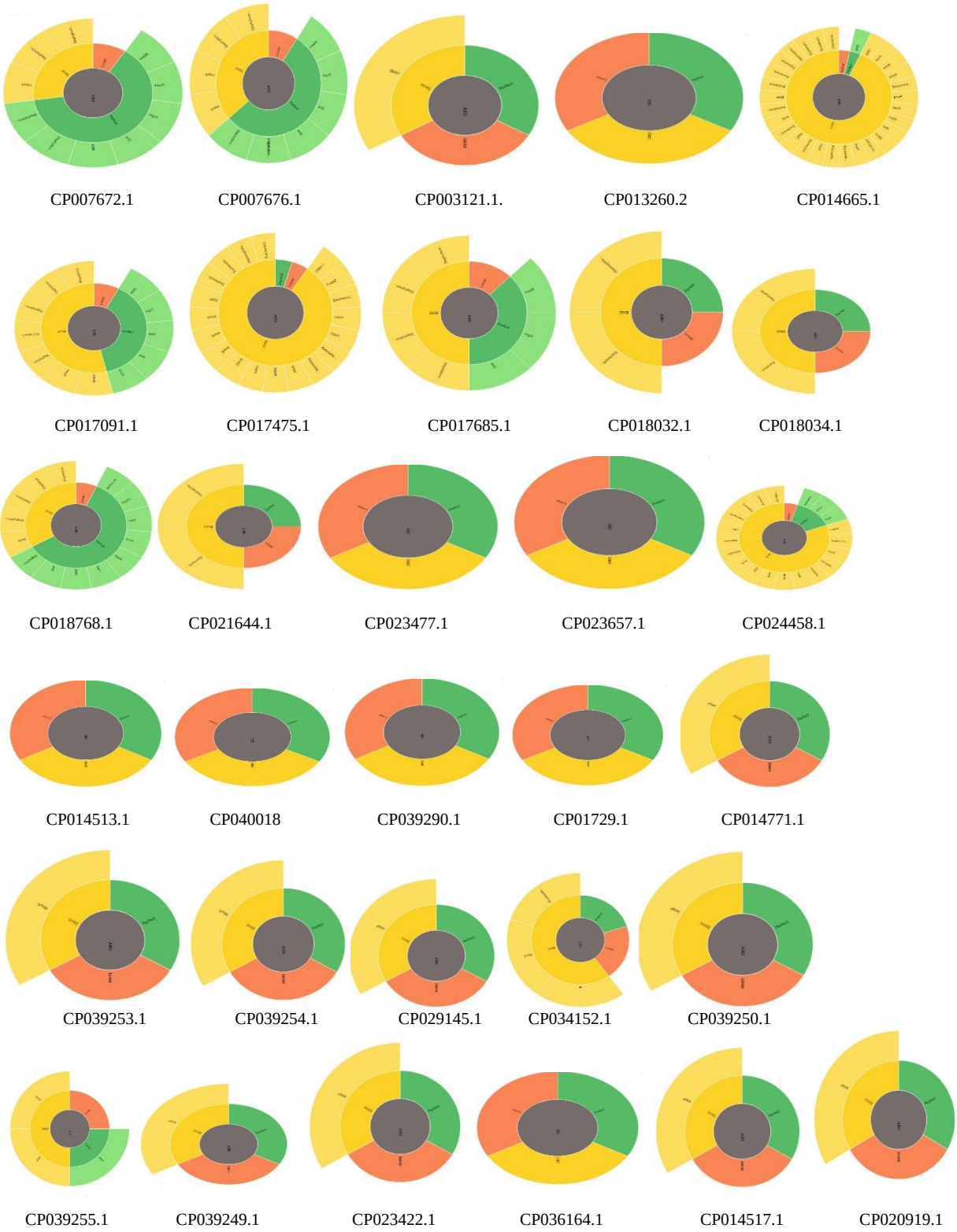

Table-1: Summarized Prevalence of AMR genes in the resistomes.

Range/Categories of AMR genes	0-25	26-50	51-75	76-100	Total
Perfect	45 (64.4)	16 (22.9)	9 (12.9)	0 (0.0)	70
Strict	22 (31.4)	22 (31.4)	25 (35.7)	1 (1.4)	70
Loose	68 (97.1)	02 (2.9)	0(0.0)	0 (0.0)	70

DISCUSSION

Comprehensive Antibiotic Resistance Database resistant genes identifier (CARD-RGI) have been successfully used in this study to understand antibiotic resistance genes in about 70 complete genome sequences and accessions of Bactria group retrieved from the NCBI. In this study, the NCBI database was used to retrieve 70 complete genome sequences of Bactria Group and the CARD RGI was used to understand what antibiotic resistance genes are present in theses genome. The lowest number of complete genome sequences retrieved from Asia and Europe, (6% and 4% respectively) due to the limited number of the complete genome sequences of bacteria Group deposited in the NCBI GenBank from these locations. Drug resistance in 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 [19] and also lacks plasmids. Many of the resistance determinants were discovered before the sequencing of the *M. tuberculosis* genome was completed.

The resistance genes were shorted into: perfect, strict and loose genes. In all, there were thirty-seven(37) perfects and Eighty-one(81) stricts resistant mutants, some of which included: APH(6)-Id mutant, APH(3")-Ib mutant, sul2 mutant, tet(B) mutant, ANT(3")-Iic mutant, adeJ mutant, adeL mutant, AmvA mutant, adeN mutant, adeR mutant, AbaF mutant, KpnH mutant, gyrB mutant, bacA mutant, vanI mutant, farB mutant, mecR1 mutant, tet(45) mutant ,cat86 mutant, rpsL mutant, pncA mutant, soxRv mutant, patB mutant, oqxA mutant, cmH-1 mutant, FosA2 mutant, ramA mutant, parC mutant, KpnG mutant, OmpK37 mutant, FosA6 mutant, smeR mutant, iri mutant, mdfA mutant, AbaQ mutant, gyrA mutant, thyA mutant, kasA mutant, AAC(6')-Iy mutant, emrB mutant, Bla2 mutant, Bla1 mutant, mphL mutant, arlR mutant, blaZ mutant, RbpA mutant, marR mutant, folC mutant, acrB mutant, acrA mutant, kdpE mutant, KpnF mutant, MdtK mutant, folP mutant, adeF mutant, EF-Tu mutant, RbpA mutant, rpoB mutant, katG mutant, sdiA mutant, GlpT mutant, uhpT mutant, PmrF mutant, ampC1 mutant, CRP mutant embB mutant, FosB mutant, msbA mutant, emrR mutant, ampH mutant, KpnE mutant, marA mutant, H-NS mutant, baeR mutant, mecA mutant, norA mutant, murA mutant, rRNA mutant, LmrS mutant, AAC(6')-Iaa mutant, mtrA mutant, AAC(2')-Ic mutant, SHV-1 mutant, efpA mutant, tetM mutant, tet(K) mutant, FTU-1 mutant, BPU-1 mutant, ANT(4')-Ib mutant, smeD mutant, mecI mutant, ADC-6 mutant, abeS mutant OXA-67 mutant, mdsB mutant, mdsA mutant, TEM-1 mutant, adeK mutant, adeI mutant, tetW mutant , , golS mutant, mecR1 mutant, ErmA mutant, mfpA mutant, 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 [19]. Several studies have shown association of the genetic variations with pathogenesis and drug resistance [20]. 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 [21].

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 [22]. 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 [23]. 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.

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