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IN SILICO ANALYSIS AND NETWORK PHARMACOLOGY OF THE IMPACT OF GENES ASSOCIATED WITH MULTIDRUG-RESISTANT TUBERCULOSIS (MDR-TB)

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ABSTRACT

Drug-resistant tuberculosis (DR-TB) continues to be a public health crisis worldwide during 2018. It estimates approximately 5,58,000 cases (range, 4,83,000–6,39,000) developed TB resistant to rifampicin (RR-TB). The most effective first line drug, and of these, 82% had multidrug-resistant TB (MDR-TB). Three countries accounted for almost half of the world's cases of MDR/RR-TB: India (24%), China (13%) and the Russian Federation (10%). Ample of studies were performed in India, based on the previously informed mutations, in addition to which several novel mutations were also observed in the genes such as rpoB (rifampicin), katG, the ribosomal binding site of inhA (isoniazid), gyrA and gyrB (ofloxacin), rpsL and rrs (streptomycin). The current investigation was carried out to explore the gene-gene interaction which are supposed to be the master regulators in MDR-TB. A total of 12 genes were mined from 618 publications in MalaCard which are responsible for MDR-TB. STRING network database reported the genes namely IL10, SLC11A1, TNF, DEFA3, DEFA1 at the core region of the network which are supposed to play a key role in TB. These genes may be also responsible for differentially expressed in MDR-TB disease. The Drug association analysis of Web Gestalt has reported 15 drugs interacted with 12 genes. In the current investigation we would like to suggest for further in vivo and in silico analysis of the reported genes for therapeutics of MDR-TB.

KEYWORDS: MDR-TB; Isoniazid; Rifampicin; Mycobacterium Tuberculosis; STRING.

INTRODUCTION

Tuberculosis (TB) is an infectious bacterial disease caused by Mycobacterium tuberculosis which commonly affects the lungs and respiratory system. The TB morbidity was reported decreasing, but its remains alarming due to increase in incidence and prevalence of Multi Drug Resistance-TB (MDR-TB).[1] Tuberculosis can also affect other parts of your body, including your kidneys, spine or brain. When TB occurs outside your lungs, signs and symptoms vary according to the organs involved.[2]

Drug-Resistant Tuberculosis (DR-TB) is a form of TB where the bacteria and thus the patient do not respond to the first line of medication.

There are 2 types of TB:

- DS-TB or Drug Sensitive-TB
- DR-TB or Drug Resistance-TB There are 3 types of DR-TB:
- MDR – Multi-Drug-Resistant TB: resistant to at least isoniazid and rifampicin
- XDR – Extensively-Drug-Resistant TB: further resistant to fluoroquinolone (injectable)
- XDR (Also sometimes referred to as TDR) – Extremely or Totally Drug Resistant: resistant to more than one injectable

MDR-TB is a form of TB infection caused by bacteria that are resistant to treatment with at least two of the most powerful first-line anti-TB medications (drugs), isoniazid and rifampin.[3] TB can usually be treated with a course of four standard, or first-line, anti-TB drugs (i.e., isoniazid, rifampin and any fluoroquinolone).[4] Drugs are chosen with a stepwise selection process through five groups on the basis of efficacy, safety, and cost. Among the first group (the oral first-line drugs) high-dose isoniazid, pyrazinamide, and ethambutol are thought of as an adjunct for the treatment of MDR and Extensively-Drug-Resistant (XDR) tuberculosis. The second group is the fluoroquinolones, of which the first choice is high-dose levofloxacin. The third group are the injectable drugs, which should be used in the following order: capreomycin, kanamycin, and then amikacin. The fourth group are called the second-line drugs and should be used in the following order: thioamides, cycloserine, and then amino salicylic acid. The fifth group includes drugs that are not very effective or for which there are sparse clinical data. Drugs in group five should be used in the following order: clofazimine, amoxicillin with clavulanate, linezolid, carbapenems, thioacetazone, and then clarithromycin.[5]

Globally in 2015 there were about 4,80,000 people estimated to have become ill with MDR-TB. In addition there were an estimated additional 100,000 people who had rifampicin TB (RR-TB). So the total number of people estimated to have had MDR-TB or RR-TB was 580,000 in 2015. MDR-TB accounts for about 3.3% of new TB cases. Also, about 3.9% of new, and about 21% of previously treated TB cases were estimated to have either rifampicin or multi drug resistant TB in 2015. In 2015 MDR-TB and RR-TB caused approximately 250,000 deaths. A total of 125,000 patients were enrolled on MDR-TB treatment in 2015 (up from 111,000 cases in 2014).[6] In India, prevalence of primary MDR-TB in newly diagnosed cases has been observed to be 3.4 per cent or less. In a study conducted at a referral tuberculosis hospital in Amargadh, Gujarat, multidrug resistance in previously treated cases was observed to increase from 25.2 per cent in 1983 (n=305) to 33.8 per cent in 1986 (n=260). In the North Arcot district, between 1988-89, six per cent of the 3357 patients initiated on anti-tuberculosis treatment were found to have MDR-TB. In a study from Gujarat, the patterns of drug resistance were studied among previously treated tuberculosis patients who remained symptomatic or smear-positive despite receiving anti- tuberculosis drugs under the DOTS programme for a minimum period of five months. Of the 1472 patients studied, 804 (54.6%) were treatment failure cases and 668 (45.4%) were relapse cases; 822 patients (373 failures and 449 relapse) were culture positive. Of these 822 patients, 482 (58.6%, 261 failure and 221 relapse) were resistant to one or more drugs. Resistance to rifampicin and isoniazid with or without resistance to other drugs was seen in 289 of the 822 patients (35.2%).[7]

The genetic background of Mycobacterium tuberculosis related to INH resistance is complex. However, mutations in several genes, including katG (catalase peroxidase coding genes), ahpC, inhA, kasA, and ndh, have all been associated with INH resistance. Between 50% and 95% of INH-resistant strains contain mutations in codon 315 of the katG gene. Furthermore, 20% and 35% of INH-resistant strains contain mutations in the inhA regulatory region. The most common inhA mutation occurs in its promoter region (C15T) and this is frequently associated with mono resistance. Strains bearing mutations in the coding region of inhA show low-level resistance. Mutations in the RNA polymerase b subunit (rpoB) gene have been found in about 96% of RMP-resistant M. tuberculosis isolates. Mutations in codons 531 and 526 are the most frequently reported mutations. Information on the current prevalence of MDR-TB, hetero-resistance, and drug resistance mutations has not been documen-ted in Amhara National Regional State (ANRS), Ethiopia.[8]

MATERIALS AND METHODS

Mining of genes associated with MDR-TB from MalaCard

MalaCards is an integrated database of human maladies and their annotations, modeled on the architecture and richness of the popular GeneCards database of human genes. The MalaCards disease and disorders database is organized into "disease cards", each integrating prioritized information, and listing numerous known aliases for each disease, along with a variety of annotations, as well as inter-disease connections, empowered by the Gene Cards relational database, searches, and Gene Analytics set-analyses. Annotations include: symptoms, drugs, articles, genes, clinical trials, related diseases/disorders and more.[9]

Generation of gene network and its interactions using STRING database

Gene networks present a graphical view at the level of gene activities and genetic functions and help us to understand complex interactions in a meaningful manner. The STRING database (http://string-db.org/) aims to provide such a global perspective for as many organisms as feasible. Known and predicted associations are scored and integrated, resulting in comprehensive protein networks covering >1100 organisms.[10, 11]

Gene MANIA

Gene MANIA works best if most of the input genes are functionally related. If they are not, a disconnected network will result and the network weighting will not be optimal. If your query list consists of 6 or more genes, Gene MANIA will calculate gene list-specific weights. If your query list has less than 6 genes, Gene MANIA will make gene function predictions based on GO annotations patterns. Gene MANIA will be slower with an input gene list of more than 50 genes; if you have such large gene lists, we recommend using a gene list of no more than 100 genes. The Gene MANIA Cytoscape plugin is capable of handling larger gene lists.[12]

Gene-disease association study through Web Gestalt

Web Gestalt (WEB-based Gene SeT AnaLysis Toolkit), one of the first software applications that integrate functional enrichment analysis and information visualization for the management, information retrieval, organization, visualization and statistical analysis of large sets of genes. In addition to significant data expansion, Web Gestalt has also improved user friendliness and added new visualization features that help users better understand the enrichment results.[13, 14]

Uni Prot-(http://www.uniprot.org/)

The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and annotation data. The corresponding protein sequences encoded by these genes were retrieved from UniProtKB database.[15, 16]

Retrieval of Drugs and proteins

The Structure Data Format (SDF) 3D structure of the reported drugs were retrieved from the NCBI PubChem database[17,18] (http://www.ncbi.nlm.nih.gov/ pccompound/) along with its PubChem ID, Molecular weight and Molecular formula. The compounds were converted into pdb format structure using the PyMol[19] (academic version) tool, Discovery Studio v4.1 visualize tools[20] and online SMILES translator web server (https://cactus.nci.nih.gov/translate/) as per requirement. The structures of the corresponding proteins of reported genes were retrieved from PDB Protein Data Bank (PDB).[21]

RESULTS AND DISCUSSION

The MalaCard studies reported 618 publications of Multidrug-Resistant Tuberculosis with a total of 12 unique genes mapped to discrete genomic locations of human genome. The list of 12 unique genes is represented at **Table.1.**

From literature survey we got the genes related with MDR-TB. Different study shows different results in their survey. From the publications we got many drug compounds are used to secure from this disease. From this study we mined 22 drug compounds and their corresponding 52 targeted genes. Explored the drug compounds related with MDR-TB and the genes that are affected mostly by these drug compounds **Table.2.**[22,23]

A total of 12 genes obtained from significantly enriched biological processes are termed as key genes and were used for network construction of MDR-TB were analyzed through STRING database. The result of the string is represented in **Fig.1**. The MDR-TB network of STRING database reported the genes namely IL1A, IL10, SLC11A1, TNF, DEFA3, DEFA1 at the core region of the network. These genes may be said to play a key in MDR-TB as well as can be differentially expressed in MDR-TB disease.

A total of 12 genes obtained from MalaCard database significantly enriched biological processes are termed as key genes and the genes collected from literature survey were used for network construction for Gene-Gene interaction of MDR-TB were analyzed through Gene

Mania database. The result of the string is represented in **Fig.2**. The MDR-TB network of Gene Mania database reported the genes namely FSHB, CGA, RPL18, ITGA6, INHA, RPS2, RPS19, RPL27A, RPS5, RPS16, RPL21, RPS21, RPSA, MRPS2, KARS, GLUL, ACVR2A, PDIA3, FST, INHBB, SCN9A, NUDT2, VARS are the interacting genes from the above target genes. But from the network analysis 7 genes are at the core region of the network, they are CGA, FSHB, INHA, ITGA6, RPSA, RPL21, and RPS21. These genes may be said to play a key in MDR-TB as well as can be differentially expressed in MDR-TB disease.

Cytoscape**,** an open source bioinformatics software platform was used for visualizing molecular interaction networks and integrating with gene expression profiles and other state data. Additional features are available as plugins. Plugins are available for network and molecular profiling analyses, new layouts, additional file format support and connection with databases and searching in large networks. Plugins could be developed using the Cytoscape open Java software architecture by anyone as it is encouraged by the developer. The drug compounds and the targeted genes was uploaded in this server and could able to get a network of gene-gene interaction. The 22 drug compounds, 52 targeted genes and it's generate interaction network is shown in **Fig.3**

The Drug association analysis of Web Gestalt has reported 15 drugs interacted with 12 genes or its corresponding proteins. The results of Web Gestalt pertaining drugs against MDR-TB and its corresponding genes/proteins were cross checked by literature survey, substantially presented in **Table.3.** The genes related with the MDR-TB are derived from Web Gestalt database from Disease association analysis. From this analysis we got 4 genes are mostly related with MDR- TB, which is shown in below **Table.3.**

Likewise by Drug association analysis from Web Gestalt database we got 15 drugs, which are strongly associated with the MDR-TB disease. From this study we got the name of the genes and which genes are initiated by which drug was predicted. The result is given in below **Table.4.**

The Structure Data Format (SDF) 3D structure of the reported drugs were retrieved from the NCBI PubChem database (http://www.ncbi.nlm.nih.gov/ pccompound/) along with its PubChem ID, Molecular weight and Molecular formula. The compounds were converted into pdb format structure using the PyMol (academic version) tool, Discovery Studio v4.1 visulizer tools and online SMILES translator web server (https://cactus.nci.nih.gov/translate/)

as per requirement. The detail about the drugs, DRUG Name, PubChem CID, Molecular Formula, Molecular Weight and its corresponding Target is reported in **Table.5**.

The structure of the drug compounds are visualized by PyMol visualization tool. These structures shows the presence of different types of bonds and the presence of hydrogen at the end of each bond. These structures are retrieved from PubChem database, which contains the details of Drug compounds. These structures are derived from this database in SDF format. Then the structures are changed to PDB format by PyMol tool, which is shown in **figure.4**.

The structures of the targeted genes are retrieved from the UniProt database. This database contains the data of all the proteins / genes in all organisms. We took only the genes associated with Homo sapience (Human) and the structures of the genes are retrieved from the RCSB-PDB database in PDB format, which is shown in below **figure.5.**

Table 1: The MalaCards studies of MDR-TB with a total of 12 unique.

INHA	IL10	SLC11A1	TNF
DEFA3	IL1A	ABCB1	VDR
DEFA1	MT-RNR1	CD4	SLC17A5

Table 2: The literature studies of MDR-TB and prediction of Drug compounds and the targeted Genes.

S.L NO	NAME OF DRUGs	NAME OF TARGETs	REFERENCES
1	isoniazid	katG	[23]
		inhA	[23]
		ahpG	[23]
2	rifampicin	ahpC	[23]
	pyraxinamide	kasA	[23]
	enthambutol	Ndh	[23]
	streptomycin	rpoB	[23]
	fluoroquinolones	embB	[23]
		pncA	[23]
		rpsL	[23]
		rrs	[23]
3	rifampicin	rpoB	[22]
		rpoA	[22]
		rpoC	[22]
4	isoniazid	katG	[22]
		inhA	[22]
		ahpC	[22]
		kasA	[22]
		NDH	[22]
5	enthambutol	embCAB	[22]

		embB	[22]
		embB306	[22]
		embC	[22]
		Rv3806c	[22]
		Rv3792	[22]
6	pyraxinamide	pncA	[22]
		RpsA	[22]
7	streptomycin	rpsL	[22]
		rrs	[22]
		gidB	[22]
8	Fluoroquinolones	GyrA/ gyrB	[22]
		parC	[22]
		parE	[22]
9	Kanamycin	eis	[22]
	Capreomycin	TlyA	[22]
	Amikacin	rrs	[22]
	Viomycin		[22]
10	Ethionamide	ethA	[22]
		ethR	[22]
		inhA	[22]
11	Para-Amino Salicylic Acid	folC	[22]
		PAS	[22]
12	Cycloserine	alrA	[22]
		cycA	[22]
13	Thioacetazone		[22]
14	Macrolides	emr37	[22]
15	Clofazimine	H37Rv	[22]
		Rv0678	[22]
		MmpL5	[22]
16	Linezolid	linezolid	[22]
		rplC	[22]
		oxazolidinone	[22]
17	Bedaquiline	atpE	[22]
18	Delamanid	Rv3547	[22]
19	PA-824	nitroimidazo-oxazine	[22]
20	SQ-109	bedaquiline	[22]
		oxazolidinone	[22]
		mmpL3	[22]
21	Benzothiazinones	rv3790	[22]
		rv3791	[22]

Table 3: The result of Disease association from WebGetalt database.

S.L NO	*DISEASE*	*TOTAL NO OF GENE*	*Entrez Gene*	*GENE SYMBOL*
1	Tuberculosis, Multidrug-Resistant	4	1667	DEFA1
			1668	DEFA3
			6556	SLC11A1
			3623	INHA

Table 4: The result of Drug association from WebGetalt database.

S.L NO	DRUG	TOTAL NO OF GENE	Entrez Gene	GENE SYMBOL
1	prednisone	3	7124	TNF
			3586	IL10
			5243	ABCB1
2	anakinra	3	7124	TNF
			3586	IL10
			3552	IL1A
3	cyclosporine	3	7124	TNF
			3586	IL10
			5243	ABCB1
4	calcitriol	3	7421	VDR
			6556	SLC11A1
			3552	IL1A
5	saquinavir	2	920	CD4
			5243	ABCB1
6	amoxicillin	2	3552	IL1A
			5243	ABCB1
7	stavudine	2	7124	TNF
			5243	ABCB1
8	zalcitabine	2	7124	TNF
			5243	ABCB1
9	thalidomide	2	7124	TNF
			5243	ABCB1
10	mycophenolate mofetil	2	3586	IL10
			5243	ABCB1
11	prednisolone	2	3586	IL10
			5243	ABCB1
12	zidovudine	2	920	CD4
			5243	ABCB1
13	methotrexate	2	7124	TNF
			5243	ABCB1
14	dinoprostone	2	7124	TNF
			3586	IL10
15	immune globulin	3	7124	TNF
			920	CD4
			3586	IL10

Table 5: MDR-TB Drugs and their corresponding target genes/proteins from WebGestalt at significance level .05, Significance test Hypergeometric, MTC: BH and their details from PubChem.

S.L NO	*DRUG Name*	*PubChem CID*	*Molecular Formula*	*Molecular Weight*	*TARGET*
1	prednisone	5865	$C_{21}H_{26}O_5$	358.434 g/mol	7124
					3586
					5243
2	anakinra	No	No	No	7124
					3586
					3552
3	cyclosporine	6435893	$C_{62}H_{111}N_{11}O_{12}$	1202.635 g/mol	7124
					3586
					5243
4	calcitriol	5280453	$C_{27}H_{44}O_3$	416.646 g/mol	7421
					6556
					3552
5	saquinavir	441243	$C_{38}H_{50}N_6O_5$	670.855 g/mol	920
					5243
6	amoxicillin	33613	$C_{16}H_{19}N_3O_5S$	365.404 g/mol	3552
					5243
7	stavudine	18283	$C_{10}H_{12}N_2O_4$	224.216 g/mol	7124
					5243
8	zalcitabine	24066	$C_9H_{13}N_3O_3$	211.221 g/mol	7124
					5243
9	thalidomide	5426	$C_{13}H_{10}N_2O_4$	258.233 g/mol	7124
					5243
10	mycophenolate mofetil	5281078	$C_{23}H_{31}NO_7$	433.501 g/mol	3586
					5243
11	prednisolone	5755	$C_{21}H_{28}O_5$	360.45 g/mol	3586
					5243
12	zidovudine	35370	$C_{10}H_{13}N_5O_4$	267.245 g/mol	920
					5243
13	methotrexate	126941	$C_{20}H_{22}N_8O_5$	454.447 g/mol	7124
					5243
14	dinoprostone	5280360	$C_{20}H_{32}O_5$	352.471 g/mol	7124
					3586
15	immune globulin	119	$C_4H_9NO_2$	103.121 g/mol	7124
					920
					3586

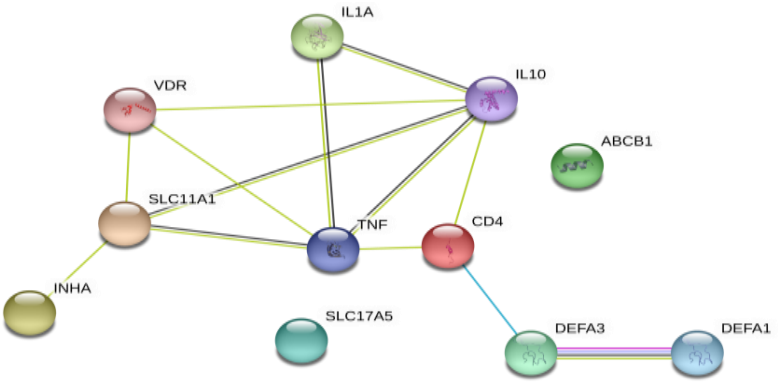


Fig. 1: Network construction of MDR-TB analyzed through STRING database.

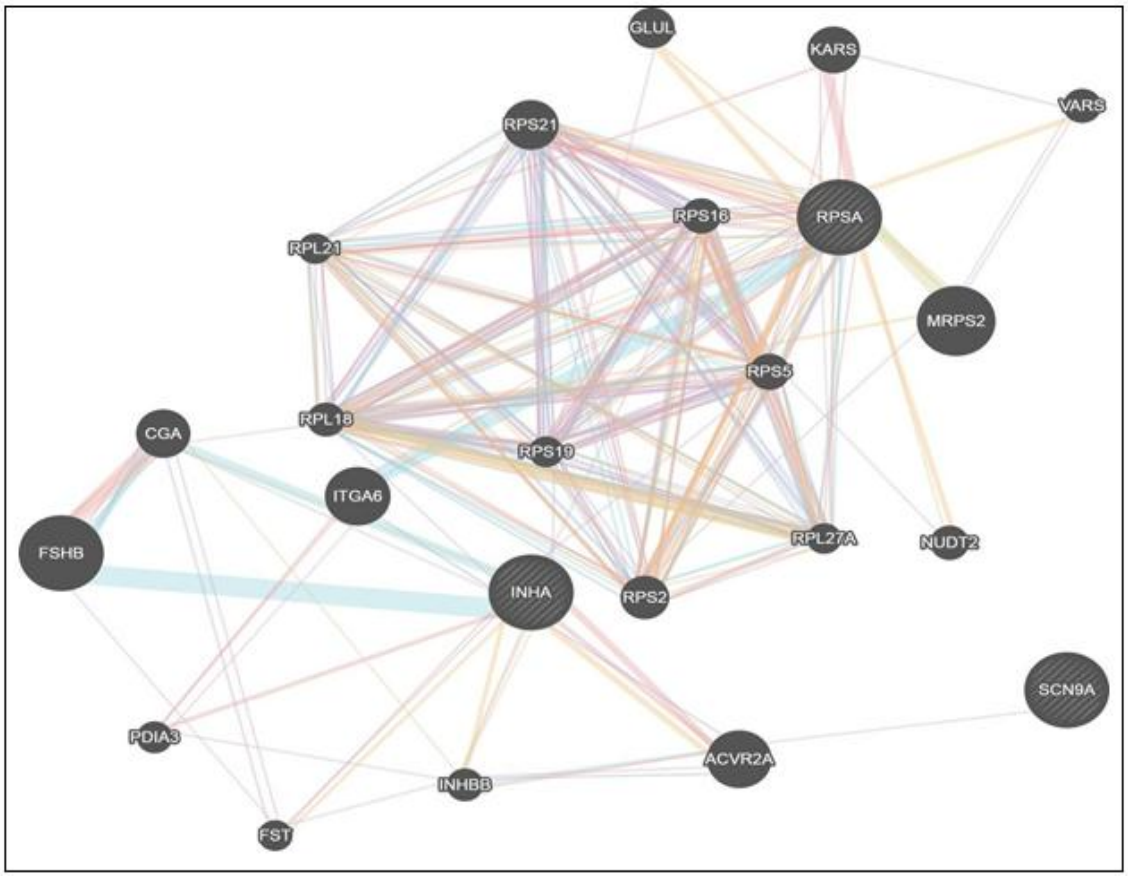


Fig. 2: Gene-Gene Network construction of MDR-TB analyzed through GeneMania database.

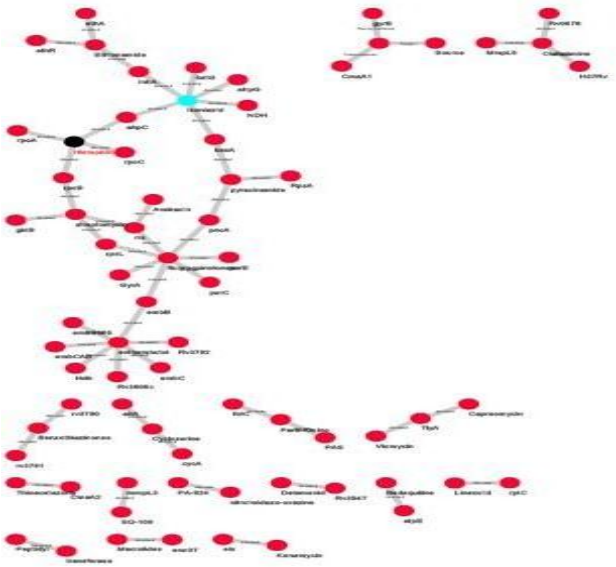
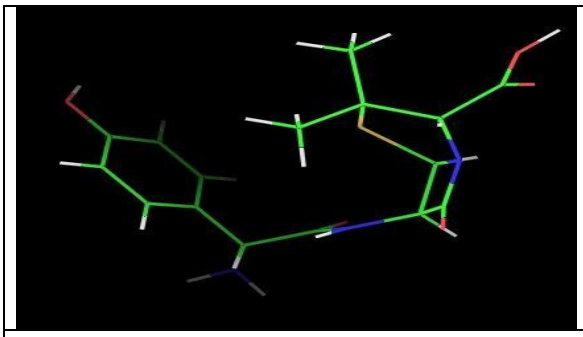
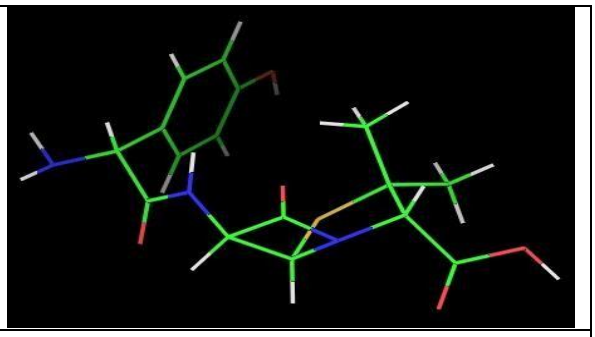
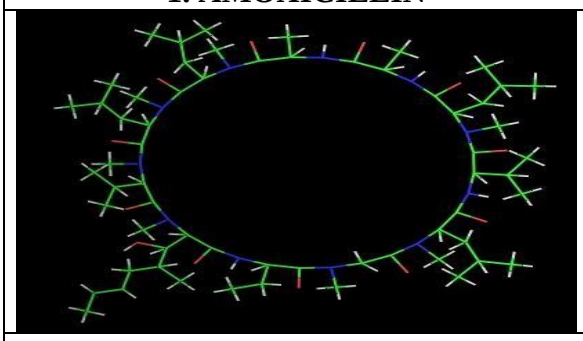
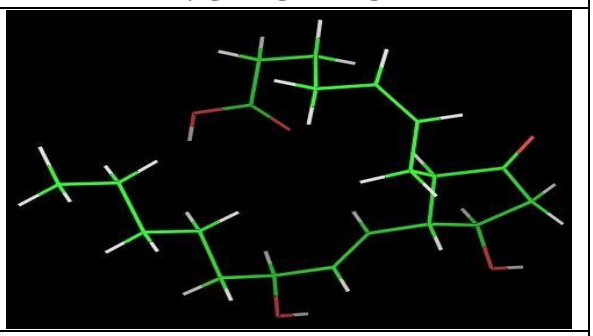
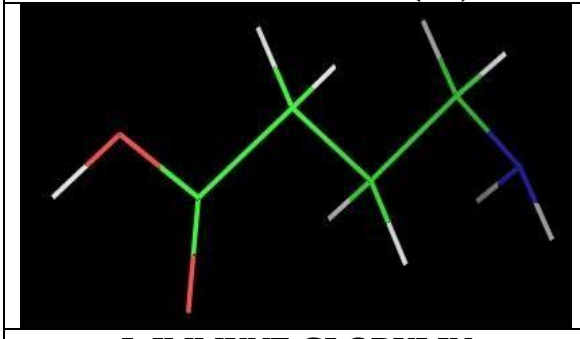
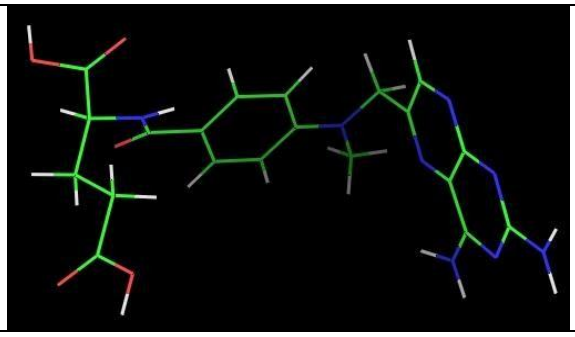


Fig. 3: Drug-Targeted Gene Network construction of MDR-TB analyzed through Cytoscape database.

	
1. AMOXICILLIN	**2. CALCITRIOL**
	
3. CYCLOSPORINE(2D)	**4. DINOPROSTONE**
	
5. IMMUNE GLOBULIN	**6. METHOTREXATE**

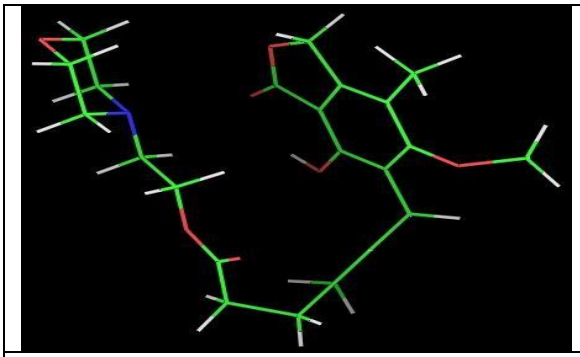
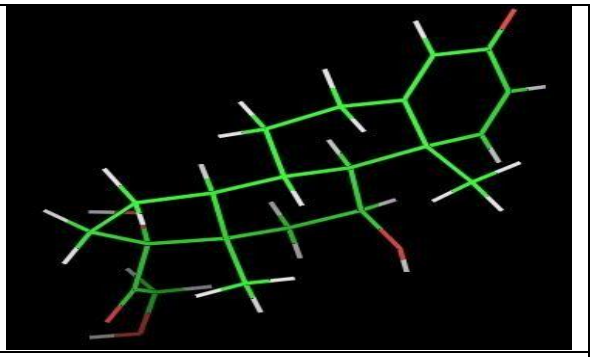
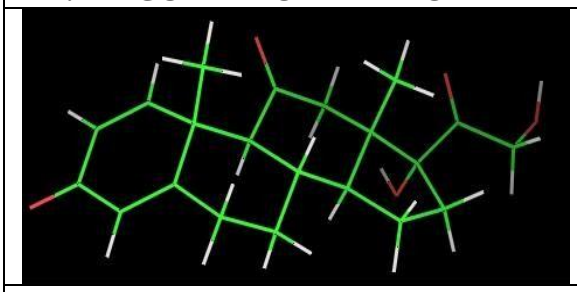
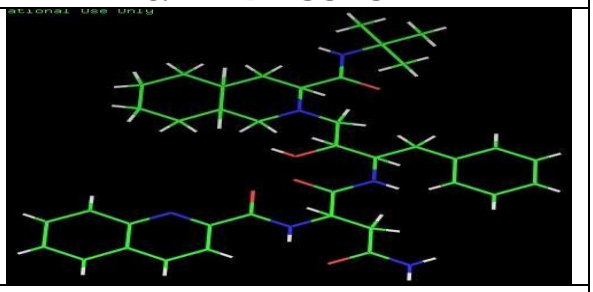
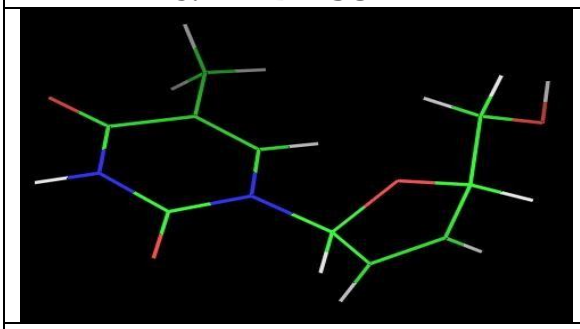
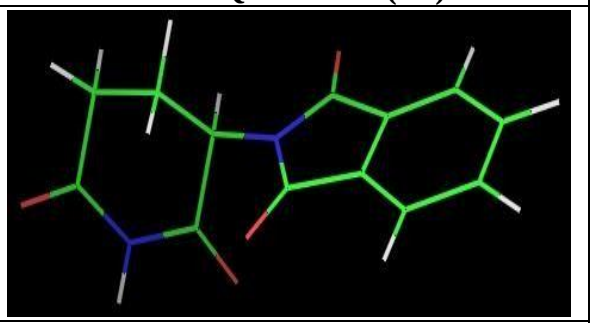
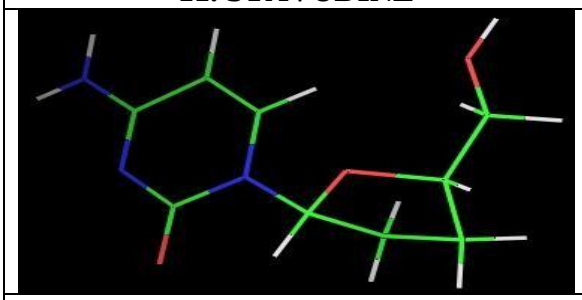
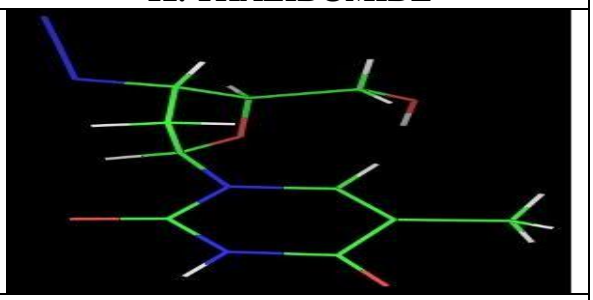
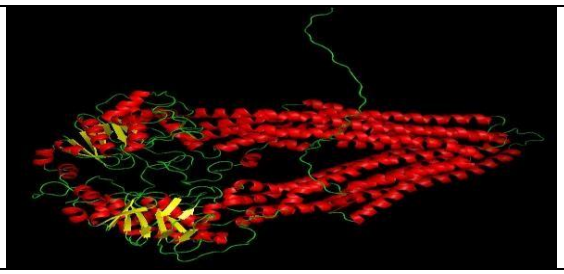
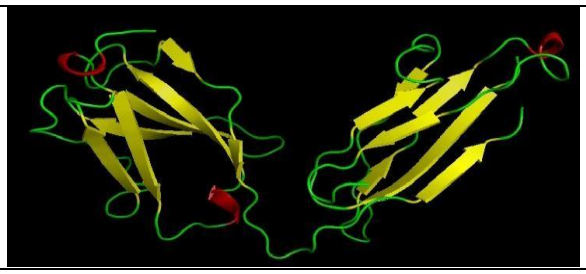
	
7. MYCOPHENOLATE MOFETIL	**8. PREDNISOLONE**
	
9. PREDNISONE	**10. SAQUINAVIR(2D)**
	
11. STAVUDINE	**12. THALIDOMIDE**
	
13. ZALCITABINE	**14. ZIDOVUDINE**

Fig. 4: The structure of the drug compounds associated with Multi Drug Resistant Tuberculosis.

	
ABCB1	**CD4**

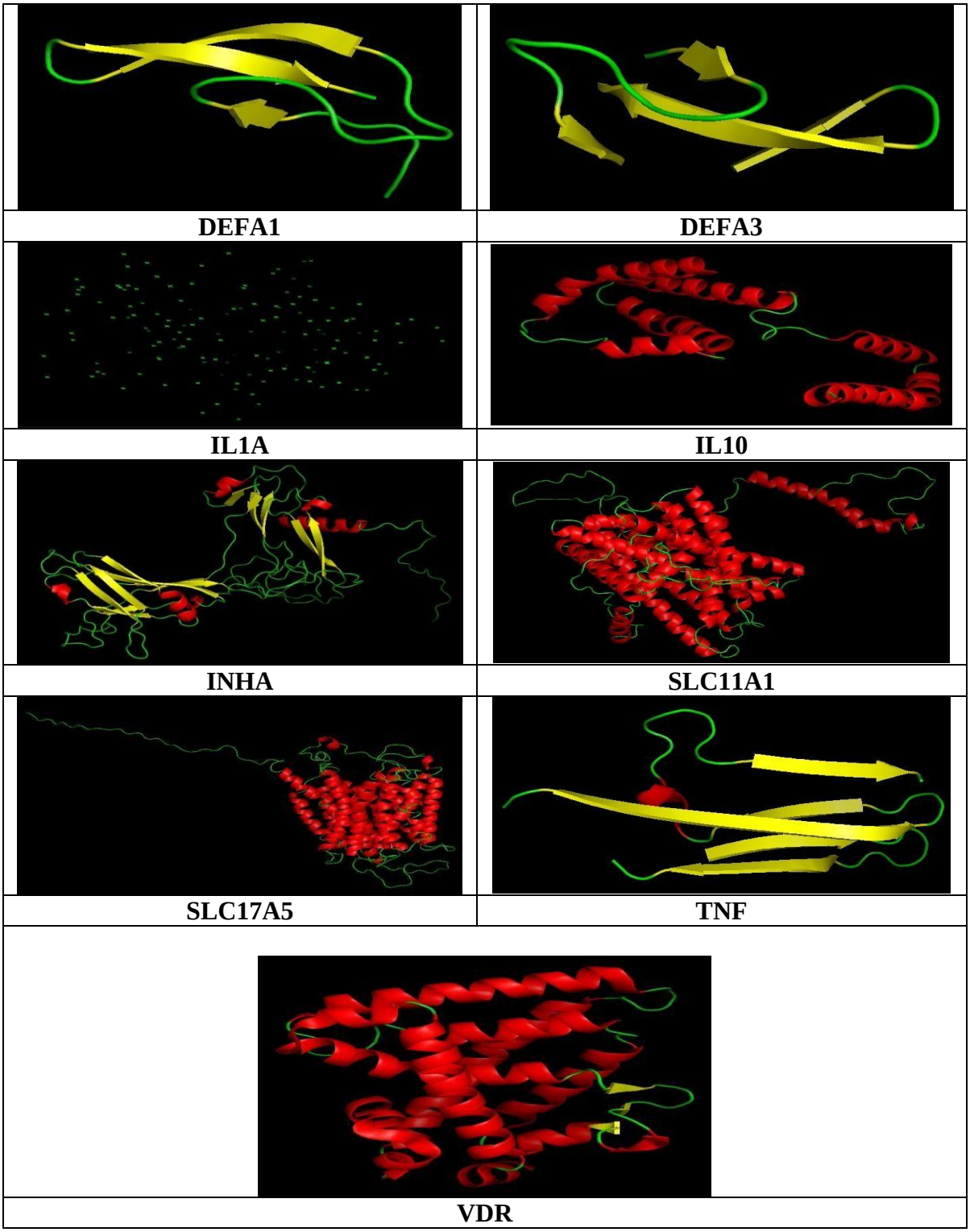


Fig. 5: The structure of the targeted Genes associated with Multi Drug Resistant Tuberculosis.

CONCLUSION

In the present investigation a total 12 unique genes were mined for MDR-TB from 618 publications in MalaCard. The MDR-TB network of STRING database reported the genes

namely IL10, SLC11A1, TNF, DEFA3, DEFA1 at the core region of the network. From the network analysis of Gene-Gene interaction of MDR-TB were analysed through Gene Mania database, 7 genes are at the core region of the network, they are CGA, FSHB, INHA, ITGA6, RPSA, RPL21, RPS21. These genes may be said to play a key in MDR-TB as well as can be differentially expressed in MDR-TB disease. The Drug association analysis of WebGestalt has reported 15 drugs interacted with 12 genes. In this study it is clear that episode of TB differs from person to person based on their genes, genetic interactions and expression levels that could recommend the clinicians to go for personalized medicine rather that generalized medicine for the patients with MDR-TB. Seeking the importance of genetic background of TB patients further studies can be done by mining of non- synonymous SNPs associated with genes for causing MDR-TB.

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