WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 5, 1552-1559. Research Article ISSN 2277– 7105

VIRULENCE FACTORS AND ANTIBIOTICS RESISTANCE MECHANISMS IN *MYCOBACTERIUM TUBERCULOSIS*

Alaa S. Hamzah[1]* and Riad A. Dellol[2]

[1]Lecturer in Middle Technical University, Institute of Medical Technology/Baghdad, Department of Nursing, Iraq.

[2]Assist Prof. in Albayan University, Iraq.

Article Received on
20 Feb. 2019,
Revised on 10 Mar. 2019,
Accepted on 31 Mar. 2019
DOI: 10.20959/wjpr20195-14746

***Corresponding Author**
Alaa S. Hamzah
Lecturer in Middle Technical University, Institute of Medical Technology/Baghdad, Department of Nursing, Iraq.

ABSTRACT

Mycobacterium tuberculosis caused tuberculosis disease that become more distribution comparison with HIV infection in WHO statistics. This bacterium has different virulence factors like secreted proteins factors, cell surface components proteins that synthesis of cell wall, enzymes involved in metabolism, transcriptional regulators factor that regulate virulence gene expression. Ability of this bacterium to the resistance drugs that used in treatment due to found genetic mutations in genes that responsible of resistance. These genes of resistance are: *katG*, *inhA* for Isoniazid resistance, *rpoB* for Rifampicin, *pncA*, *rpsA* for Pyrazinamide, *embB* for Ethambutol, *rpsL*, *rrs*, *gidB* for Streptomycin, *gyrA*, *gyrB* for Quinolones, *tlyA* for Capreomycin, *ethA* for Ethionamide and *thyA*, *folC* for Para-aminosalicylic acid.

KEYWORDS: Virulence factors, Antibiotics, *Mycobacterium tuberculosis*, Mechanisms Resistance.

INTRODUCTION

Mycobacterium tuberculosis cause tuberculosis disease that exceed HIV infection which led to highest number of mortalities in about 1.8 million deaths and 10.4 million new tuberculosis infection worldwide in 2015 documented in world health organization in 2016.[1] This bacterium is a part of Mycobacterium tuberculosis complex and genetically included related species cause tuberculosis in human and some other organisms.[2] Mycobacterium tuberculosis complex divided into eight lineages are L1 the Philippines and Indian Ocean, L2 East Asia, L3 India and East Africa, L4 Europe and Americas, L5 West Africa 1, L6 West

Africa 2, L7 Ethiopia and L8 animal-adapted isolates. Group (L1, L2, L3, L4, and L7) comprise Mycobacterium tuberculosis. Also mycobacterium complex can divided into ancient group (L1, L5-8) and modern strains (L2-4) depend on found or absence of Mycobacterium tuberculosis specific deletion.[3,4]

Genetic analysis of mycobacterium tuberculosis complex sequence showed identical in percentage (99%) at the nucleotide level and rare genome rearrangements but it they exhibit diverse in pathogenic phenotypes.[5,6] Different infection with mycobacterium tuberculosis complex which L1 to L7 lineages mainly infect humans and rarely infect animals, L5 and L6 lineages infect human in West Africa but L7 lineage documented in Ethiopia, L1-4 geographically distributions.[7] That's different in infection led to different in virulence of strains (invade tissue of human) which consider modern strains (L2-L4) more virulent of human infection but ancient strains group (L1,L5-8) cause today tuberculosis cases. Beijing sub-lineage of the L2 lineage is more virulent than the other modern isolates because it developed mechanisms of drug resistance and adaptation in host tissues. Mycobacterium tuberculosis contain different virulence factors are cell surface proteins, lipid and fatty acid metabolism proteins.[8,9] Tuberculosis now consider very important clinical problem because distribution of infection and developed drug resistance against drug choice that use in treatment of this dangerous bacterium in all countries of the world. According to all mention above, this subject review aim to explain virulence factors of Mycobacterium tuberculosis and its containing mechanisms that used for resistance antibiotics.

Virulence factors in *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is bacilli in shape caused tuberculosis which reach the alveoli and infect the alveolar macrophages in the lungs and it is an intracellular bacterium. This bacterium may be in latency phase in about 10% of the infected cases. In latency phase occur granuloma formation and found giant cells. *Mycobacterium tuberculosis* can release when occur necrosis of granuloma and become extracellular bacteria and infect other patient in anaerobic condition, often it cause calcification, dissemination and meningitis stages. Cell envelop of this bacterium composed of arabinoglactan on top of the peptidoglycan, mycolic acid, Phenolic Glycolipid (PGL), Lipoarabinomannan (LAM) that's increase virulence of bacteria and resist immunity system defenses as shown in figure 1.[10,11]

Genome of *Mycobacterium tuberculosis* consist of one circular chromosome (4.4 Mb) that include about 4,000 genes without extra chromosomal DNA. These genes enable this

bacterium to live in different condition include aerobic, microaerophilic and anaerobic. Also it encoded to the complex potential regulatory contain thirteen sigma factors, eleven two-component systems, histidine kinase factors and eleven Serine Threonine Protein Kinases. Target site in new drugs that used in treatment of this bacterium is kinase. Genome of mycobacterium tuberculosis contain a large number of genes required in, metabolism of the cell wall lipids, as well as degradative enzymes (20 cytochrome P- 450), insertion elements (IS6110) that are consider basis of genotyping.[12,13] Also found about 160 members of the PE/PPE genes but not knew function exactly. In sequencing details of these bacteria showed no clearly pathogenicity island but found virulence genes as shown in table 1. This bacterium through transmission by air infect lungs mainly but it can also infect glands, bones, central nerves system and may be infect all body parts. These virulence gene encoded to virulence factors (table 1) that increase from pathogenocity of Mycobacterium tuberculosis.[14] Virulence factors included secreted protiens factors (HspX, Esat6/CFP-10 and 19-kD) that locate in outside of bacteria and use in vaccine and diagnosis, cell surface components proteins that synthesis of cell wall, enzymes involved in metabolism that employed in cellular metabolism like enzymes required in metabolism lipid and fatty acid enable bacteria to grow on fatty acids, transcriptional regulators like PhoP/PhoR response regulatory that consider important virulence factor in regulation virulence gene expression.[15,16]

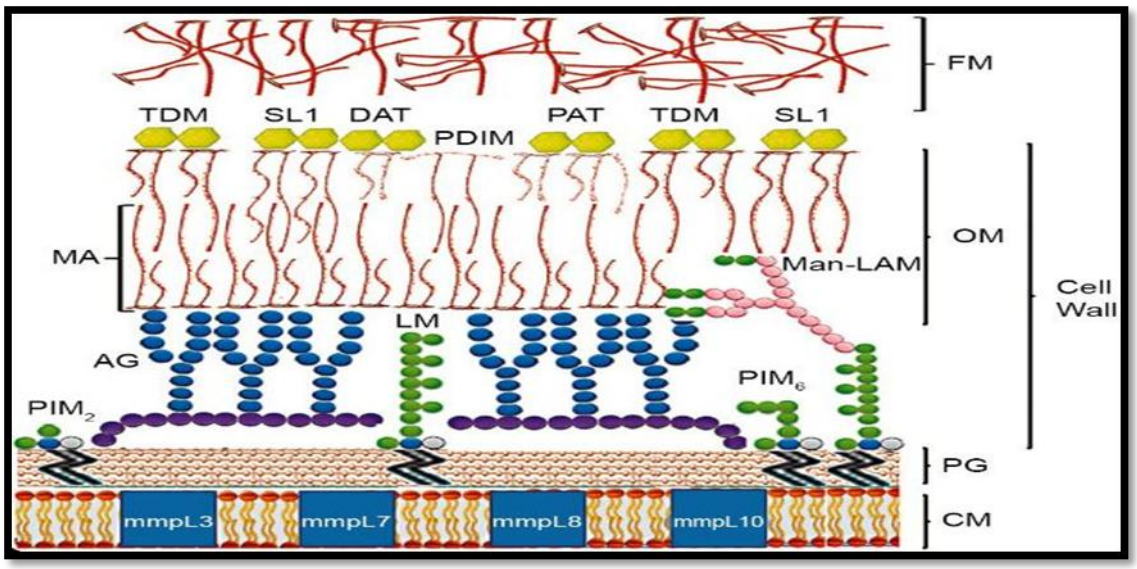

Figure 1: Component of cell envelop of *Mycobacterium tuberculosis* "CM, PG, and covalently attached macromolecules: AG, PIM2, PIM6, LM, and Man-LAM; an OM composed of MA covalently attached to AG, TDM, DAT, PAT, PDIM, and SL-1; and an outermost layer of FM; FM: free mycolic acid; TDM: trehalose dimycolate; SL-1:

sulphoglycolipid; DAT: diacyltrehalose; PDIM: phthiocerol dimycocerosate; PAT: poliacyltrehalose; MA: mycolic acid; Man-LAM: mannose-capped lipoarabinomannan; OM: outer membrane; AG: arabinogalactan; LM: lipomannan; PIM2: phospho-*myo*-inositoldimannoside; PIM6: phospho-*myo*-inositol-hexamannoside; PG: peptidoglycan; CM: cell membrane; mmpL: mycobacterial membrane protein large".[17]

Table 1: Virulence factors with genes that encoded them in *Mycobacterium tuberculosis.* [18,19,20, 21]

Virulence factor	Genes	Effect of virulence factor
Protein of the system of fatty acid synthesis	*fas, kasAkasB, mmaA1-14, pks1, fbpA*	Genes deletion caused KOE decrease starting from the first week of infection
Transcription factors	*WhiB1, WhiB2, WhiB3, WhiB4, WhiB5, whiB6, whiB7*	Reactivation chronic tuberculosis, cell division of bacteria, antibiotics resistance
Serine/threonine protein kinase	*pknD, pknG, pknE*	Gene mutation in gene that decrease number of bacteria, increase survival of bacteria in organs.
Proteins in ESX-1 type VII of secretion system	*espA, espC, espD, espR, espF, espG1, espH, espH, eccA1, eccB1, eccCa1*	Increase bacterial growth, increase bacterial loading on organ.

Resistance *Mycobacterium tuberculosis* of antibiotics

Ability of Mycobacterium tuberculosis to the resistance antibiotics were developed because occur mutation in genes that responsible for antibiotics resistance as shown in table 1, which transformed resistance bacteria to the multidrug resistance and extensively drug resistance mycobacterium tuberculosis.[22] There are two lines of drugs that used in treatment infection with mycobacterium tuberculosis include first line of treatment (*Rifampicin, Isoniazid,* Ethambutol, Pyrazinamide and Streptomycin), second line of treatment (Fluoroquinolones, Kanamycin, Capreomycin, Amikacin, Viomycin, Ethionamide, Para-Amino Salicylic Acid, Cycloserine, Thioacetazone, Macrolides, Clofazimine and Linezolid). Other drugs now under experimented by Authors and pharmaceutical drugs companies that reduce time of treatment include (*Bedaquiline, Delamanid, PA-824, SQ-109 and Benzothiazinones) which are tested in vitro and invivo and appered excellent results in reduce period of treatment. About 450000*

multidrug resistance in 2012 that caused in death 170,000 persons, which resist rifampicin and isoniazid.[23,24]

Table 1: Drugs that used in treatment of mycobacterium tuberculosis with genes that involved in activation and mechanisms.[25, 26, 24]

Name of Drug	Name of Gene	Mechanism of action
Isoniazid	*katG, inhA*	Catalase/peroxidase; enoyl reductase
Rifampicin	*rpoB*	RNA polymerase
Pyrazinamide	*pncA, rpsA*	Pyrazinamidase; ribosomal protein 1
Ethambutol	*embB*	Arabinosyl transferase
Streptomycin	*rpsL, rrs, gidB*	S12 ribosomal protein, 16A rRNA, 7-methylguanosine methyltransferase
Quinolones	*gyrA, gyrB*	DNA gyrase
Capreomycin	*rrs, tlyA*	16S rRNA, rRNA methyltransferase
Kanamycin/Amikacin	*Rrs*	16S rRNA
Ethionamide	*ethA*	Enoyl-ACP reductase
Para-aminosalicylic acid	*thyA, folC*	Thymidylate synthase A

Anti – tuberculosis antibiotic most effect is rifampicin and together with isoniazid were responsible for basis of the multidrug treatment *Mycobacterium tuberculosis* in period six months. This antibiotic was active against *Mycobacterium tuberculosis* that growing and non-growing (slow metabolizing), which it is link with β-subunit in RNA polymerase And led to inhibition process of protein synthesis through inhibition elongation of mRNA.[27] *Mycobacterium tuberculosis* development mechanisms to resist rifampicin by occur mutation in codons (516,526,531) in *rpoB* gene that codes for target site of this antibiotic (β-subunit of the RNA polymerase) result in low affinity to bind it with target site.[28, 29]

Streptomycin antibiotic produced by *Streptomyces griseus*. It is consider first drug used to treatment infection with mycobacterium tuberculosis which inhibition of protein synthesis through inhibition of translation in active growing bacteria which it affect on 30S of ribosome (S12 and 16S rRNA) that it encoded by *rpsL* and rrs genes. When occur mutation in these genes led to resist *Mycobacterium tuberculosis* against streptomycin.[30] Site of mutation in *rpsL* gene in codon 43 that led to transform amino acid lysine to arginine but the site of mutation in *rrs* gene occur around nucleotides 530 and 915 that led to increase resistance of this bacterium against streptomycin.[31]

CONCLUSION

Tuberculosis considers major disease before HIV infection in documents of WHO. Virulence factors included secreted proteins factors, cell surface components proteins that synthesis of cell wall, enzymes involved in metabolism, transcriptional regulators factor that regulate virulence gene expression. Distribution of TB infection resulted from multidrug resistance against drugs that used in treatment like streptomycin, Rifampicin, Isoniazid and Quinolones. Mechanisms of resistance is occur genetic mutation in some genes like *katG, inhA, rpoB, pncA, rpsA, embB, rpsL, rrs, gidB, gyrA, gyrB, tlyA, ethA, thyA* and *folC*.

REFERENCES

1. World Health organization (WHO). 2016. Global treatment guidelines for drug resistant tuberculosis, 1-64.
2. Galagan, J. E. 2014. Genomic insights into tuberculosis. Nat. Rev. Genet, 15: 307–320.
3. Brosch, R., Gordon, S. V., Marmiesse, M., Brodin, P., Buchrieser, C. and Eiglmeier, K. 2002. A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc. Natl. Acad. Sci. U.S.A, 99: 3684–3689.
4. Bentley, S. D., Comas, I., Bryant, J. M., Walker, D., Smith, N. H. and Harris, S. R. 2012. The Genome of Mycobacterium africanum west african 2 reveals a lineage-specific locus and genome erosion common to the M. tuberculosis complex. PLoS Negl. Trop. Dis, 6: e1552.
5. Comas, I., Coscolla, M., Luo, T., Borrell, S., Holt, K. E. and Kato-Maeda, M. 2013. Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. Nat. Genet, 45: 1176–1182.
6. Rodriguez-Campos, S., Smith, N. H., Boniotti, M. B. and Aranaz, A. 2014. Overview and phylogeny of Mycobacterium tuberculosis complex organisms: implications for diagnostics and legislation of bovine tuberculosis. Res. Vet. Sci, 97: S5–S19.
7. Winglee, K., Manson McGuire, A., Maiga, M., Abeel, T., Shea, T. and Desjardins, C. A.2016. Whole genome sequencing of Mycobacterium africanum strains from Mali provides insights into the mechanisms of geographic restriction. PLoS Negl. Trop. Dis, 10: e0004332.
8. Grace, P. S. and Ernst, J. D. 2016. Suboptimal antigen presentation contributes to virulence of Mycobacterium tuberculosis in vivo. J. Immunol, 196: 357–364.
9. Xinmiao, J., Li, Y., Mengxing, D., Suting, C., Lingna, L., Dandan, C., Jing, F.,Tingting, Y., Ju, Z., Xiangli, Z., Yuanyuan, S., Guirong, W., Yongjie, S., Hairong, H. and Fei, C.

2017. The Bioinformatics Analysis of Comparative Genomics of Mycobacterium tuberculosis Complex (MTBC) Provides Insight into Dissimilarities between Intra specific Groups Differing in Host Association, Virulence, and Epitope Diversity. Frontiers in Cellular and Infection Microbiology, 7: 1-4.

10. Ehrt, S. and Schnappinger, D. 2009. Mycobacterial survival strategies in the phagosome: defence against host stresses. Cell Microbiol, 11: 1170-8.
11. Meena, L.S. an Rajni,R. 2010. Survival mechanisms of pathogenic Mycobacterium tuberculosis H37Rv. FEBS J, 277: 2416-27.
12. Balabanova, Y., Nikolayevskyy, V. and Ignatyeva, O. 2011. Survival of civilian and prisoner drug_sensitive, multi_ and extensive drug_resistant tuberculosis cohorts prospectively followed in Russia, *PLoS One*, 6: 20-53.
13. Udwadia, Z.F. 2012. MDR, XDR, TDR tuberculosis: omi_nous progression, *Thorax*, 67: 286–288.
14. Marina, A., Forrellad, L., Klepp,.I, Andrea, G. and Julia, Y.2013. Virulence factors of the Mycobacterium tuberculosis complex. Virulence, 4: 1, 3–66.
15. Brudey, K. 2006. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology, *BMC Microbiol,* 6: 23-25.
16. Homolka, S., Projahn, M. and Feuerriegel, S.2012. High resolution discrimination of clinical *Mycobacterium tuberculosis* complex strains based on single nucleotide polymorphisms, *PLoS One*, 7: 39-55.
17. Adriano, Q. and Lee, W. R. 2017. Bacterial immunostat: Mycobacterium tuberculosis lipids and their role in the host immune response. Rev Soc Bras Med Trop, 50: 9-18.
18. Philips, J.A. and Ernst, J.D. 2011. Tuberculosis pathogenesis and immunity, *Annu. Rev. Pathol*, 7: 353–384.
19. Be, N.A., Bishai, W.R. and Jain, S.K. 2012. Role of *Myco_bacterium tuberculosis pknD* in the pathogenesis of central nervous system tuberculosis, *BMC Microbiol*, 12: 22-24.
20. Shur, K., Maslov, D. and Bekker, O. 2013. *WhiB7* gene polymorphism and its regulon genes in *Mycobacterium tuberculosis*, as a new mechanism of drug resistance, *FEBS J*, 280: 366-367.
21. Prozorov, A.A., Fedorova, I.A., Bekker, O.B. and Danilenko, V.N. 2014. The Virulence Factors of *Mycobacterium tuberculosis*: Genetic Control, New Conceptions. Russian Journal of Genetics, 50: 885-908.

22. Vibol, I., Silaphet, S., Yves, B., Nicolas, S., Franck, B., Monique, C., Phannasinh, S., Phouratsamy, N., Alain, R., Jean-Luc, B. and Phimpha, P. 2013. Resistance of Mycobacterium tuberculosis to antibiotics in Lao PDR: first multicentric study conducted in 3 hospitals. BMC Infectious Diseases, 13: 1-27.
23. Christian, L., Andrew, V. and Mario, C. R. 2010. New drugs and new regimens for the treatment of tuberculosis: review of the drug development pipeline and implications for national programmes. Current Opinion in Pulmonary Medicine, 16: 186–193.
24. Juan, C. P. and Anandi, M. 2014. Drug Resistance Mechanisms in *Mycobacterium tuberculosis*. Antibiotics, 3: 317-340.
25. Udwadia, Z.F., Amale, R.A., Ajbani, K.K. and Rodrigues, C. Totally drug-resistant tuberculosis in India. Clin. Infect. Dis, 54: 579–581.
26. Migliori, G.B., Centis, R., D'Ambrosio, L., Spanevello, A., Borroni, E., Cirillo, D.M. and Sotgiu, G. 2012. Totally drug-resistant and extremely drug-resistant tuberculosis: The same disease? Clin. Infect. Dis, 54: 1379–1380.
27. Siu, G.K., Zhang, Y., Lau, T.C., Lau, R.W., Ho, P.L., Yew, W.W., Tsui, S.K., Cheng, V.C., Yuen, K.Y. and Yam, W.C. 2011. Mutations outside the rifampicin resistance-determining region associated with rifampicin resistance in *Mycobacterium tuberculosis*. J. Antimicrob. Chemother, 66: 730–733.
28. Brandis, G. and Hughes, D. 2013. Genetic characterization of compensatory evolution in strains carrying *rpoB* Ser531Leu, the rifampicin resistance mutation most frequently found in clinical isolates. J. Antimicrob. Chemother, 68: 2493–2497.
29. De Vos, M., Muller, B., Borrell, S., Black, P.A., van Helden, P.D., Warren, R.M., Gagneux, S. and Victor, T.C. 2013. Putative compensatory mutations in the *rpoC* gene of rifampin-resistant *Mycobacterium tuberculosis* are associated with ongoing transmission. Antimicrob. Agents Chemother, 57: 827–832.
30. Palomino, J.C. and Martin, A. 2013. Tuberculosis clinical trial update and the current anti-tuberculosis drug portfolio. Curr. Med. Chem, 20: 3785–3796.
31. Zhao, F., Wang, X.D., Erber, L.N., Luo, M., Guo, A.Z., Yang, S.S., Gu, J., Turman, B.J., Gao, Y.R. and Li, D.F. 2014. Binding pocket alterations in dihydrofolate synthase confer resistance to para-aminosalicylic acid in clinical isolates of *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother, 58: 1479–1487.