Comparative Structural Analysis of Beta Lactamases From *Klebsiella pneumoniae* and *Mycobacterium tuberculosis*: An *in silico* approach

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Abstract

Development of antimicrobial resistance by infectious pathogens is a major challenge to the medical practitioners to treat those diseases. Production of beta lactamase is one of such mechanism that develops resistance against beta lactam antibiotics. The beta lactamases of the resistant pathogens cleave the beta lactam ring of the antibiotic and renders it inactive. Thus methods to inactivate this enzyme are an essential area of research to combat the resistant strains. This study focuses on the sequence and structural characterization of β lactamases of Klebsiella pneumoniae and Mycobacterium tuberculosis, two priority pathogens as per WHO guidelines using established computational tools.

Keywords: Beta lactamases, Priority Pathogens, Ligands, Structure, Computational Tools.

Introduction

Antibiotics are the small organic secondary metabolites produced by a group of actinomycetes and fungi that kills or inhibits the growth of the non related bacteria. Depending upon the chemical structure of their active component, antibiotics may be classified into large number of groups like beta lactam antibiotics, aminoglycoside antibiotics etc. Among them beta lactam antibiotics may be considered as most anciently and widely used antibiotic for restricting microbial growth. Beta lactam antibiotics contain beta lactam ring at its active center. The beta lactam ring is a cyclic amide in which a nitrogen atom is attached to the beta carbon atom relative to the carbonyl group (Figure1). Beta lactam antibiotics inhibit the biosynthesis of the peptidoglycan layer, of the bacterial cell wall.

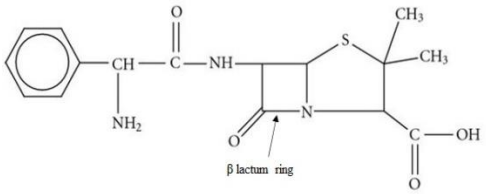


Figure 1: Beta lactam ringcontaining Ampicillin antibiotic

Antibiotic resistance, now a days has become a global concern. Most widely accepted and well documented strategies include degradation of antimicrobial skeletal structure, effluxing of antibiotic from the cell, alteration of the target site. Bacteria follow Darwinian principle of selection to develop some stringent strategies to evade the lethal effects of different antibiotics (1). Due to continuous and inefficient usage of these antibiotics certain bacterial species which were susceptible

to these antibiotics have now become resistant. This resistance is caused by the enzyme, Beta Lactamase. The Beta lactamase was first reported in *Escherichia coli* by Edward Abraham in 1940. Bacterial beta lactamases are enzymes that deactivate the effect of the beta lactam containing antibiotics such as Penicillin by attacking their beta lactam ring. Four primary mechanisms for development of resistance against beta lactamases have been reported so far. These include production of inhibitory enzymes against beta lactamase, modification of active site in PBP, decreased expression of outer membrane protein and introduction of efflux pumps (2) Beta lactamases are being classified based on amino acid sequence and/or on functionality. On the basis of Structure, beta lactamases were divided into four classes, Class A-D by Ambler (3). Among them, classes A,C,D areserine ester hydrolase and class B beta lactamases aremetallo-beta lactamases require a zinc ion as cofactor. Depending on the functionality beta lactamases can be devided into three major groups: Group 1 Cephalosporinases (Class C), Group 2 Serine beta lactamases(Class A and Class D) and Group 3 Metallo-beta lactamases(Class B)(4)

Klebsiella pneumoniae is a WHO enlisted priority pathogen that are responsible against global cause of Pneumonia. *Klebsiella pneumonia*e belongs to the *Enterobacteriaceae* family where the resistance determinants are encoded on the chromosome, plasmids, integrons and transposon (5). *Klebsiella pneumoniae* shows resistance against many beta lactam antibiotics particularly through the expression of beta lactamase gene. Cephalosporinases such as Extended Spectrum Beta lactamases (ESBLs) and Carbapenemases are found as the most important of them (6).

Mycobacterium tuberculosis is another WHO priority pathogen, responsible for tuberculosis which affects almost one third of the global population. *Mycobacterium tuberculosis* is resistantto beta lactam antibiotics as it contains beta lactamase enzyme which is encoded by *blaC* gene (7). The beta lactamase enzyme belongs to the Class A of Ambler classification system. Clavulanic Acid, an inhibitor is very less susceptible to the enzyme (8) in comparison to that of the enzyme from *Klebsiella pneumoniae.*

Methodology

Two pathogens *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* was selected for WHO priority pathogen list between whom the beta lactamase from the first one is plasmid borne and the second one is chromosome mediated metallo beta lactamase.

Amino acid sequences of these two enzymes were obtained from UNiProt server using NCBI database. Secondary structures were predicted using RaptorX tool (9) and probability of stability of each amino acid in their corresponding position in the secondary structure were calculated by deriving Ramachandran Plot using Molprobity tool (10). Post translational modifications of each enzyme were predicted using NetPhos and NetAcet software. The active sites of both the enzymes were marked using CastP (11). Finally common structural pockets for both the enzymes have been pointed out using TM Align (12) and DoGSite Scorer server (13). Finally, the evolutionary tree

analysis was done by Mega X software. The trees were generated by Maximum Parsimonious method with respect to an out group microorganism (14).

Results

AMINOACID SEQUENCES

Amino acid sequences for beta lactamase enzymes from *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* obtained from NCBI database are as follows

The Amino Acid Sequence of Beta lactamase enzyme from *Klebsiella pneumoniae* is

>MELPNIMHPVAKLSTALAAALMLSGCMPGEIRPTIGQQMETGDQRFGDLVFRQLAPNVWQ
HTSYLDMPGFGAVASNGLIVRDGGRVLVVDTAWTDDQTAQILNWIKQEINLPVALAVVTHA
HQDKMGGMDALHAAGIATYANALSNQLAPQEGMVAAQHSLTFAANGWVEPATAPNFGPL
KVFYPGPGHTSDNITVGIDGTDIAFGGCLIKDSKAKSLGNLGDADTEHYAASARAFGAAFPK
ASMIVMSHSAPDSRAAITHTARMADKLR

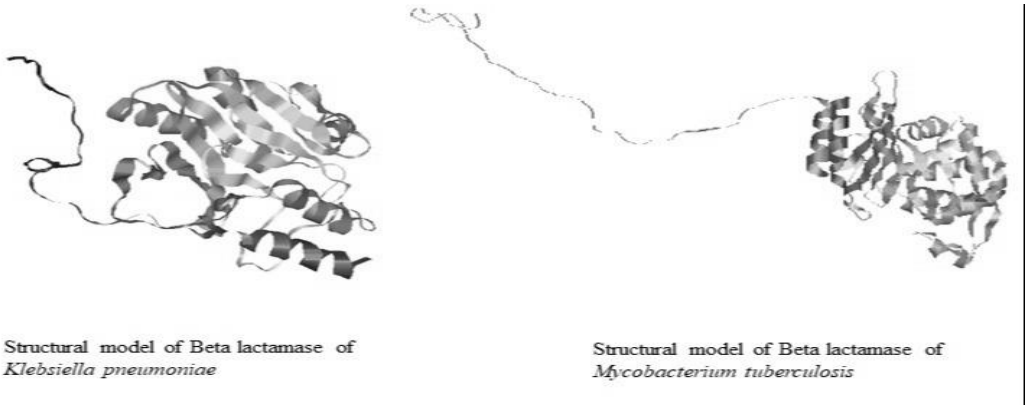
The Amino Acid Sequence of Beta lactamase enzyme from *Mycobacterium tuberculosis* is

>MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAELERRYDARL
GVYVPATGTTAAIEYRADERFAFCSTFKAPLVAAVLHQNPLTHLDKLITYTSDDIRSISPVAQ
QHVQTGMTIGQLCDAAIRYSDGTAANLLLADLGGPGGGTAAFTGYLRSLGDTVSRLDAEEP
ELNRDPPGDERDTTTPHAIALVLQQLVLGNALPPDKRALLTDWMARNTTGAKRIRAGFPAD
WKVIDKTGTGDYGRANDIAVVWSPTGVPYVVAVMSDRAGGGYDAEPREALLAEAATCVA
GVLA

SECONDARY STRUCTURE OF BETA LACTAMASE

Klebsiella pneumoniae:

Beta lactamase enzyme from *Klebsiella pneumoniae* is a periplasmic enzyme which is encoded by the gene *blaNDM-1*located in the plasmid.The secondary and tertiary structure was determined by Raptor X server. The beta lactamase enzyme from *Klebsiella pneumoniae* is made up of 270 amino acids in which amino acids 1-28 is the signal peptide and amino acids 29-270 is the original peptide. The beta lactamase enzyme from *Klebsiella pneumoniae* is composed of 7 α helical Structures, 19 β strands, 29 Turns. UsingRaptorX tool it was identified that the enzyme is composed of2000 Atoms and is made up of 2045 bonds in which 162 are hydrogen bonds (Figure 2).

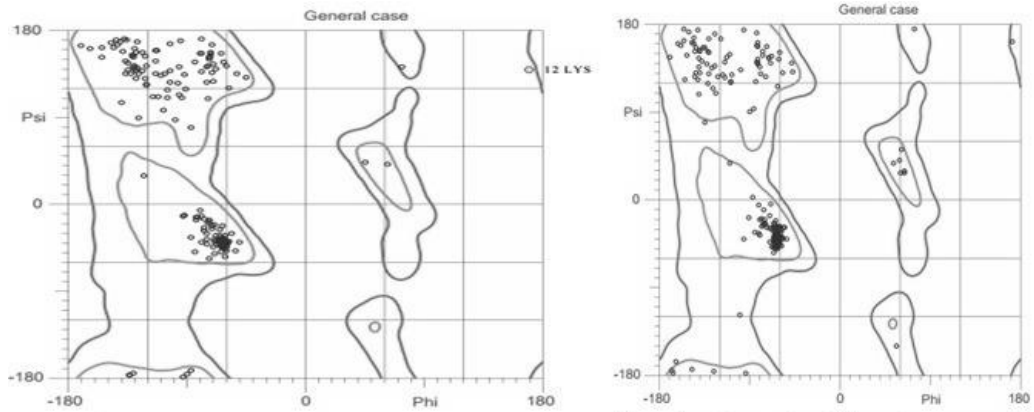


Structural model of Beta lactamase of *Klebsiella pneumoniae*

Structural model of Beta lactamase of *Mycobacterium tuberculosis*

Figure 2: Ribbon structure of Beta lactamase enzyme from *Klebsiella pneumoniae*and and *Mycobacterium tuberculosis*

The Ramachandran Plot of the secondary structure depicts 98.4% (264 out of 268) residues were in the favourable region and 98.9% (265/268) were in the allowable region(Figure3). Amino Acids in only three positions, Lysine-12, Glycine-83, Glycine-188 are in the forbidden region.



Ramachandran plot of Beta lactamase of *Klebsiella pneumoniae*

Ramachandran plot of Beta lactamase of *Mycobacterium tuberculosis*

Figure3: Ramachandran Plot for beta lactamase from *Klebsiella pneumoniae*and and *Mycobacterium tuberculosis.*

Mycobacterium tuberculosis:

The beta lactamase from *Mycobacterium tuberculosis* wasa membrane bound enzyme located at the inner cell membrane and periplasm. It was encoded by chromosomal gene *blaC* and found to be made up of 307 amino acids in which amino acids 1-34 is the signal peptide and amino acids 35-307 is the original peptide. The secondarystructure of the enzyme has 16 α helical Structures, 10 strands, 27 turns. Total number of atom present in the enzyme was 2289 that are joined by 2332 bonds in which 187 were hydrogen bonds.

The Ramachandran Plot of the secondary structure depicts 97.7% (298/307) residues in the favourable region and 100% (307/307) residues in the allowable region. No amino acids are in the forbidden regions (Figure3).

With respect to solvent accessibility both polypeptide chains of the beta lactamases were similarly exposed to external environment with 40% exposed, around 25% medium and 35% buried regions. An overall comparison of these two enzymes were given in Table1.

Table1: Table for overall structural comparison between beta lactamase enzymes from *Klebsiella pneumoniae* and *Mycobacterium tuberculosis.*

Beta lactamase enzyme from *Klebsiella pneumoniae*	**Beta lactamase enzyme from *Mycobacterium tuberculosis***
There are 270 amino acids in the Beta Lactamase enzyme.	There are 307 amino acids in the Beta Lactamase enzyme.
The Beta Lactamase enzyme has a signal peptide at its amino terminal end.	The Beta Lactamase enzyme has a signal peptide at its amino terminal end.

48(17%) amino acids positions are predicted as disordered.	46(14%) amino acids positions are predicted as disordered.
Secondary structure predictions for the Beta Lactamase enzyme: 25% helix, 21% sheet , 52% random coil.	Secondary structure predictions for the Beta Lactamase enzyme: 38% helix, 14% sheet, 47% random coil.
Solvent accessibility for the Beta Lactamase Enzyme: 40% exposed, 24% medium, 35% buried.	Solvent accessibility for the Beta Lactamase enzyme: 40% exposed, 28% medium, 31% buried.
As per Ramachandran plot, 98.4% (264/268) residues are in the favourable region and 98.9 % (265/268) are in the allowable region.	As per Ramachandran Plot, 97.7%(298/307) residues are in the favourable region and 100% (307/307) residues are in the allowable region.
Amino Acids Lys-12, Gly-83, Gly-188 are in the forbidden region.	No amino acids are in the forbidden region.

POST-TRANSLATIONAL MODIFICATIONS

Post translational modification in terms of net phosphorylation and net acetylation of beta lactamase enzymes from *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* was studied using NetPhos and NetAcet.

Beta lactamase from *Klebsiella pneumoniae* shows thatserine was the major residue for phosphorylation while tyrosine and threonine share very little percentage. The highest phosphorylation score was exhibited by serine 251 (0.994) followed by serine 217 (0.975) and serine 232 (0.911) (Table2).

Table2: Table for Probable Phosphorylation Sites of beta lactamase enzyme from *Klebsiella pneumoniae*

NAME OF AMINO ACID	POSITION	SIMILARITY	SCORE	PROBABILITY
Serine	14	PKA	0.577	Low
Serine	14	PKG	0.575	Low
Serine	14	cdc2	0.501	Low
Serine	24	cdc2	0.533	Low
Tyrosine	64	Unspecified	0.933	High
Tyrosine	94	Unspecified	0.582	Low
Threonine	162	PKC	0.820	High
Threonine	201	PKC	0.725	High
Serine	213	PKC	0.533	Low
Serine	213	PKA	0.588	Low
Serine	217	Unspecified	0.975	High
Serine	217	PKA	0.519	Low
Tyrosine	229	Unspecified	0.723	High
Serine	232	PKC	0.911	High
Serine	249	Unspecified	0.857	High
Serine	251	Unspecified	0.994	High
Tyrosine	260	PKC	0.663	Low

For beta lactamase enzyme from *Mycobacterium tuberculosis* all the three amino acids share the equal probability of phosphorylation. The highest phosphorylation score was exhibited by Serine 118 (0.988) followed by Threonine 196 (0.937) and Serine 35 (0.904)(Table3).

In both the cases most of the phosphorylations have similarity with the signal transduction proteins and cell cycle regulatory protein.

Table3: Table for Probable Phosphorylation Sites of beta lactamase enzyme from *Mycobacterium tuberculosis*

Name of amino acid	Position	Similarity	Score	Probability
Threonine	22	PKC	0.526	Low
Serine	29	PKA	0.680	Low
Serine	35	Unspecified	0.904	High
Serine	35	PKA	0.683	Low
Threonine	36	Unspecified	0.771	High
Threonine	36	PKC	0.643	Low
Threonine	68	PKC	0.768	High
Threonine	69	PKG	0.583	Low
Serine	84	PKC	0.771	High
Threonine	85	PKC	0.862	High
Serine	111	CKII	0.508	Low
Serine	111	cdc2	0.508	Low
Serine	118	Unspecified	0.988	High
Serine	142	PKA	0.682	Low
Serine	142	Unspecified	0.586	Low
Serine	142	cdc2	0.558	Low
Threonine	161	cdc2	0.508	Low
Serine	170	Unspecified	0.950	High
Serine	170	PKA	0.549	Low
Threonine	174	cdc2	0.515	Low
Serine	176	Unspecified	0.789	High
Serine	176	cdc2	0.524	Low
Serine	176	CKII	0.510	Low
Threonine	196	Unspecified	0.937	High
Threonine	197	Unspecified	0.848	High
Threonine	198	Unspecified	0.559	Low
Threonine	198	p38MAPK	0.534	Low
Threonine	224	PKC	0.578	Low
Threonine	231	Unspecified	0.800	High
Threonine	231	PKC	0.800	High
Tyrosine	256	Unspecified	0.590	Low
Tyrosine	267	cdk5	0.562	Low
Tyrosine	267	p38MAPK	0.551	Low
Tyrosine	267	CKI	0.543	Low
Tyrosine	267	GSK3	0.509	Low
Serine	279	PKC	0.512	Low

The beta lactamase enzyme does not possess any acetylation sites as amino acids Serine, Threonine, Glycine or Alanine were not present in positions 1-3.

ACTIVE SITE AND SUBSTRATE BINDING SITE INFORMATION

From the active and substrate Binding Site information it was found that the beta lactamase from *Klebsiella pneumoniae* is a metal binding or metallo-enzyme. The enzyme is a zinc ion (Zn^{2+}) binding enzyme that has two zinc ions in the active site. The first zinc ion is bound to the enzyme by Histidine 120,122 and 189 while the second ion is bound by Aspartic acid 124, Cysteine 208 and Histidine 250 residues.Substrate enzyme interaction is mediated by basic amino acids Lysine 211 and Asparagine 220 from enzymatic part (Table4)(Figure4).

Table4: Table for Active Site and Binding Site information of beta lactamase enzyme from *Klebsiella pneumoniae*

Feature of the binding site	Name of the amino acid	Position of the amino acid	Description
Metal ion binding Site	Histidine	120	Zinc 1; via tele Nitrogen
Metal ion binding Site	Histidine	122	Zinc 1; via pros Nitrogen
Metal ion binding Site	Aspartic Acid	124	Zinc 2
Metal ion binding Site	Histidine	189	Zinc 1; via tele Nitrogen
Metal ion binding Site	Cysteine	208	Zinc 2
Metal ion binding Site	Histidine	250	Zinc 2; via tele Nitrogen
Substrate Binding Site	Lysine	211	Substrate
Substrate Binding Site	Asparagine	220	Substrate; via Amide Nitrogen

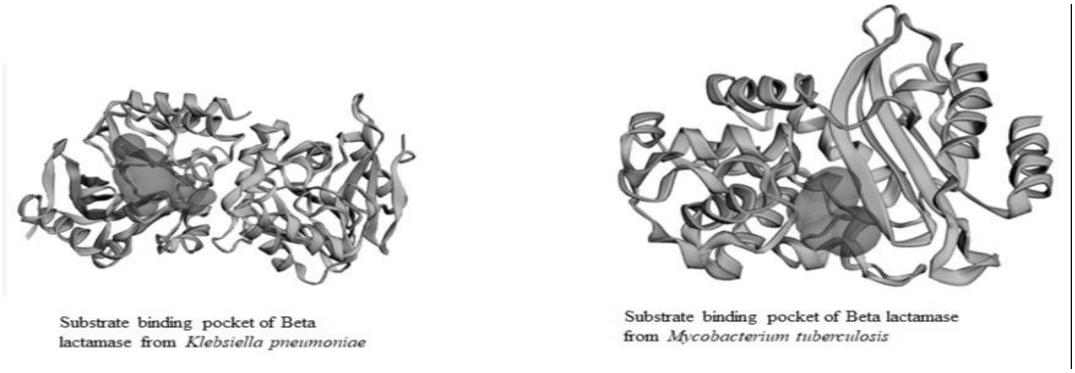


Figure 4: Figure depicting the active site or substrate binding site (grey sack) for beta lactamase from*Klebsiella pneumoniae* and *Mycobacterium tuberculosis*

The β lactamase from *Mycobacterium tuberculosis* is Serine β lactamase enzyme as its active site or substrate binding site is composed of Serine amino acid. Serine 130 is involved in substrate binding while Serine 70 is involved in the formation of the acyl-ester intermediate complex for catalysis. Isoleucine 105 plays an important role in this enzyme-substrate interaction by regulating the entry of the substrate to the active site and Lysine 73 strengthen the interaction by increasing the nucleophilicity of the Serine in active site.

Table-5: Table for Active Site and Binding Site information of beta lactamase enzyme from *Mycobacterium tuberculosis*

Feature of the binding site	Name of the amino acid	Position of the amino acid	Description
Active Site	Serine	70	Acyl-ester intermediate
Active Site	Glutamic Acid	166	Proton acceptor
Substrate Binding Site	Serine	130	Substrate
Site	Lysine	73	Increases the nucleophilicity of the Serine of Substrate binding Site
Site	Isoleucine	105	Controlsthe accessibility of the substrate to the enzyme active sites

DETERMINATION OF COMMON DRUGGABLE SITE

Beta Lactamase enzymes from *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* have certain sequences which are aligned due to their similar domain fold structure or motif. The Beta lactamase

enzymes from both different bacterial strains are said to be Orthologous i.e, the enzymes are doing same catalytic reaction, having same structural homology but their mode of action is different and is found in different bacterial strains, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis.*

The sequences which are aligned are composed of amino acids of same property. The Aligned Chains are made up of the α carbon backbone (Figure 5). The following information was obtained from the TM Align algorithm,

a) Length of Chain:2- 270 residues (For beta lactamase from *Klebsiella pneumoniae*)1- 307 residues (For beta lactamase from *Mycobacterium tuberculosis*)
b) Aligned Length-124 residues
c) RMSD- 5.60
d) TM Score-; 0.29482 (if normalized by length of *Klebsiella pneumoniae*Chain), 0.26760 (if normalized by length of *Mycobacterium tuberculosis*Chain)

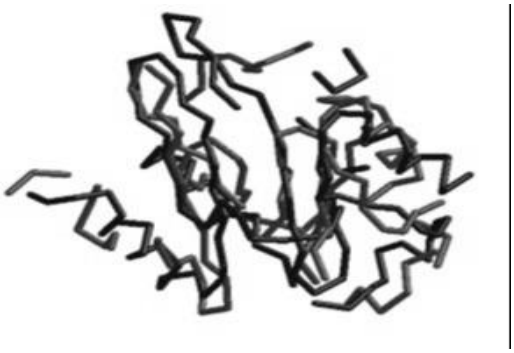


Figure 5: Depicting aligned alpha carbon backbones of beta lactamase from *Klebsiella pneumoniae* (light grey) and from *Mycobacterium tuberculosis* (dark grey).

Discussion

The Beta lactamase enzyme from *Klebsiella pneumoniae*is encoded by the gene *bla*NDM-1. The enzyme is made up of 270 amino acids in which amino acids 1-28 is the signal peptide and 29-270 amino acids is the original peptide. The enzyme is located in the Periplasm and Periplasmic Space. The Secondary Strurcture of the enzyme is composed of 7 Helical structures, 19 β strands, 29 turns. The enzyme is composed 2000 Atoms and is made up of 2045 bonds in which 162 are Hydrogen bonds. From the Ramachandran Plot analysis, the favourable, allowable regions and the forbidden regions of the enzyme can be determined. 98.4% (264/268) residues are in the favourable region and 98.9%(265/268) are in the allowable region. Amino Acids Lys-12, Gly-83, Gly-188 are in the forbidden region. The beta lactamase enzyme has phosphorylation sites and Serine amino acids are the most probable sites for phosphorylation. The beta lactamase does not have any probable acetylation sites as amino acids Serine, Threonine, Glycine and Alanine are not present at positions 1-3. The beta lactamase enzyme is a Zinc ion binding enzyme. Amino acids Histidine, Aspartic Acid, Cysteine are involved in Metal (Zinc ion) binding. Amino acids Lysine and Asparagine are involved

in Substrate binding. So, the beta lactamase from *Klebsiella pneumoniae* is a metal binding or a metallo-enzyme.

The beta lactamase enzyme from *Mycobacterium tuberculosis* (ATCC 25618/H37Rv) is encoded by the gene *bla*C. The enzyme is made up of 307 amino acids in which amino acids 1-34 is the signal peptide and 35-307 amino acids is the original peptide. The enzyme is located in the Inner Cell Membrane and Periplasm. The secondary structure of the enzyme is composed of 16 helical structures, 10 β strands, 27 turns. The enzyme is composed 2289 Atoms and is made up of 2332 bonds in which 187 are Hydrogen bonds. From the Ramachandran Plot analysis, the favourable, allowable regions and the forbidden regions of the enzyme can be determined. 97.7%(298/307) residues are in the favourable region and 100% (307/307) residues are in the allowable region. No amino acids are in the forbidden regions. The beta lactamase enzyme has phosphorylation sites, Serine and Threonine amino acids are the most probable sites for phosphorylation. The beta lactamase does not have any probable acetylation sites as amino acids Serine, Threonine, Glycine and Alanine are not present at positions 1-3. The Beta lactamase enzyme is a Serine beta lactamase enzyme as itsactive site or substrate binding site is composed of Serine amino acid. Serine is involved in the formation of the acyl-ester intermediate complex which is important for the catalysis.

Beta Lactamase enzymes from *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* have certain sequences which are aligned due to their similar domain fold structure or motif. The Beta lactamase enzymes from both different bacterial strains are said to be Orthologous i.e, theenzymes are doingsame catalytic reaction, having some structural homology but their mode of action is different and is found in different bacterial strains, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis*. The sequences which are aligned in alpha carbon backbone are composed of amino acids of same property. The beta lactamases from *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* have certain drug (ligand) binding pockets, which can be utilised for the treatment against these microbial pathogens. 10 drug (ligand) binding pockets makes up the structure of beta lactamase from *Mycobacterium tuberculosis* and 13 drug (ligand) binding pockets makes up the structure of beta lactamase from *Klebsiella pneumoniae* (data not shown).Further study of alignment between the drug binding pockets of these two enzymes followed by screening of small molecule library may be helpful in finding out common inhibitors of beta lactamase from both type of organisms.

Conclusion

The current study reveals that beta lactamase isolated from different organisms may vary widely if their secondary and tertiary structure is concerned. Despite of this wide difference they may possess same or similar ligand binding pockets that may helpful in establishing newer and common drug that can inhibit the beta lactamase activity.

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