An in Silico Approach for Molecular Analysis of β-Lactamases

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***Abstract*— The emergence of bacteria resistant to several important classes of antibiotics has become a major clinical problem in the last decade. β- Lactamases are the main cause for resistance against β-lactam antibiotics, because they inactivate the antibiotics by hydrolysis of the β-lactam ring. With the introduction of newer generations of β-Lactam antibiotics, β-Lactamases are also rapidly evolving and increasing their spectrum of activity. The present study aims to investigate the changes produced in the enzymes which allow the adaptation of the β- Lactamases to new targets. For this protein sequences of the TEM, CTX-M, and SHV types of β-lactamases are analyzed using Insilco tools of BLAST search, Clustal and phylogenetic analysis. Multiple sequence alignment of β-lactamase family enzymes would help in recognizing critically conserved residues that might contribute in β -lactam hydrolysis as conserved residues are vital for enzyme stability and/or function.**

The present work was aimed to identify new amino acid positions at which mutations can occur, to detect β-lactamases with a determination of mutation profile between essential and non-essential residues that can play a key role in understanding the molecular details of β –lactamase mediated antibiotic resistance.

***Keywords*— *β-Lactam antibiotics; β- Lactamases; TEM; CTX-M; SHV; Resistance*.**

I. INTRODUCTION

β -Lactam antibiotics are widely used anti-microbial agents over the last 60 years. Its continuous and indiscriminate use led to the evolution of resistance against them. β-Lactamase enzymes secreted by bacteria confer resistance against β-lactam as they cleave the amide bond in β-lactam ring, rendering the antibiotics ineffective against bacteria [1], [2].

β-lactamase enzymes are classified primarily based on structure such as conserved amino acids and protein sequence motifs [3] or the functional characteristics of the enzymes [4]. β-lactamases are classified into four molecular classes, A, B, C, and D. Ambler proposed two classes: class A, the active-site serine β-lactamases; and class B, the metallo-β-lactamases that require a bivalent metal ion, usually Zn2+, for activity. Later, two new classes of serine β -lactamase were discovered that shared a little sequence similarity to the known Class A enzymes and were designated as Classes C and D [5], [6]. Understanding of β -lactamase sequences and relationship between their structure and function is important to know the possible is important to know the possible spread and evolution of antibiotic resistance.

The TEM-1 enzyme is the best known member of the class A β- lactamases that efficiently hydrolyzes β-lactam antibiotics, particularly penicillins, and thus provides a mechanism of resistance against them [7], [8]. CTX-M enzymes belong to class A β-lactamases. CTX-M enzyme (derived its name from being highly active on CefoTaXime and isolated in Munich) exhibits greater hydrolytic activity against cefotaxime in comparison to ceftazidime [9]. However, it has been reported that some clinical isolates show a significant degree of resistance towards ceftazidime as well [10]. β-lactamases produced by the Enterobacteriaceae is the SHV family that belongs to Class A β- lactamases.

β-Lactamases are gaining more and more prominence with increase in the number of multi resistant strains of bacteria particularly those producing Extended spectrum of Beta-lactamases (ESBLs). ESBLs hydrolyze extended-spectrum cephalosporins with an oxyimino side chain. Thus ESBLs confer resistance to these antibiotics and related oxyimino-beta lactams. In typical circumstances, they derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases. The ESBLs are frequently plasmid encoded.

The recent advancements in bioinformatics and related tools provide means to analyze the evolution, phylogenetic and epidemiological relation in *in silico* research in biology. As *In silico* research has the potential to speed the rate of discovery by reducing the need for expensive lab work and clinical trials, in the present work tools like BLAST, ClustalX, and Phylogeny from bioinformatics are used.

II. METHODOLOGY

A. Protein Database Search Using BLAST

The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between protein or nucleotide sequences [11]. A BLAST search enables to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. BLAST can be used to analyze functional and evolutionary relationships between sequences as well as identify gene families.

Class A β –lactamases (TEM-1, TEM-10, CTX-M-2, CTX-M-14, SHV-5 and SHV-12 types selected for this study are the most commonly encountered and most wide spread representatives of β –lactamases . The protein sequences of class A β–lactamases were retrieved from NCBI (http://www.ncbi.nlm.nih.gov) and searched for sequence similarity using protein BLAST (BLASTP) [12].
(http: //blast.ncbi.nlm.nih.gov/Blast.cgi).

B. Multiple Sequence Alignments

A multiple sequence alignment (MSA) is a sequence alignment of more than three biological sequences such as protein, DNA or RNA. From the resulting MSA, sequence homology can be inferred and phylogenetic analysis can be done to assess whether the sequences shared any evolutionary origin or not.

Highly similar sequences after protein BLAST are retrieved from NCBI and subjected to multiple sequence alignment. Multiple sequence alignment was carried out for the representatives taken in this study by using ClustalX 2.1 software.

C. Phylogenetic Analysis

To study the evolutionary relationships among groups of organisms or species phylogenetic studies are needed. A phylogenetic tree based on the multiple sequence alignment was generated.

Phylogenetic analysis for TEM, CTX-M and SHV β-lactamases proteins and their evolutionary relationship with those obtained from database was done online by Clustal X version 2.1 software.

III. RESULTS AND DISCUSSION

We have retrieved protein sequences of beta-lactamases from NCBI that shows 85% to 100% conservation. The amino acids of TEM, CTX-M and SHV protein sequences were compared against protein databank using BLAST P.

Those sequences that are produced a significant alignment to TEM-1 β–lactamase of *E. coli* sequence ID (gb|AAR25033.1|), TEM-10 β–lactamase of *Morganella morganii* sequence ID (gb|AAC72362.1|), CTX-M-2 β–lactamase of *Klebsiella pneumoniae* sequence ID (gb| ACC77506.1|), CTX-M-14 β–lactamase of *E. coli* sequence ID (gb|YP_009069602.1|), SHV-5 β–lactamase of *Salmonella typhimurium* sequence ID (gb|AKA86687.1 |) and SHV-12 β–lactamase of *Klebsiella pneumoniae* sequence ID (gb| AFN82170.1|) with other types of TEM, CTX-M and SHV β -lactamases were downloaded in FASTA format and subjected to Multiple Sequence Alignment..

Highly similar sequences which were retrieved from NCBI are subjected to multiple sequence alignment. There was 3 amino acid substitutions detected when TEM-1 compared with other TEM type β -lactamases at highly conserved region Glutamic acid to Lysine (E-K) at 102 position, Glycine to Serine (G-S) at position 236 and Lysine to Glutamic acid (K-E) at position 32 as shown in Fig. 1. There was 2 amino acid substitutions detected when TEM-10 compared with other TEM type β -lactamases at highly conserved region Alanine to Threonine (A-T) at 290 position and Glutamic acid to Lysine (E-K) at position 237 as shown in Fig. 2. TEM-type β -lactamases that belongs to class A β -lactamases are most often found in *E. coli* and *K. pneumoniae*. They are also found in other species of Gram-negative bacteria with increasing frequency. Currently more than 140 TEM-type enzymes have been described. TEM-1, TEM-10, TEM-12, and TEM-26 are among the most common [13]. Initially ESBLs in this family evolved by Single amino acid substitutions at positions 104, 164, 238, and 240 but recent ESBLs have a broader spectrum of mutations with more than a single amino acid substitution.

In CTX-M-2 β–lactamases there was 2 amino acid substitutions detected at highly conserved region Lysine to Alanine (K-A) and Glutamic acid to glutamine (E-Q) at positions 102 and 124 respectively as shown in Fig. 3. CTX-M-14 enzyme when compared with other CTX-M type β–lactamases there were amino acid substitutions detected at highly conserved region Glutamic acid to Lysine (E-K) at positions 289 and Glutamine to Histidine (Q-H) at position 60 as shown in Fig. 4. CTX-M enzymes are not very closely related to TEM or SHV β-lactamases in that they show only approximately 40% identity with these two commonly isolated beta-lactamases. More than 80 CTX-M enzymes are currently known. CTX-M-14, CTX-M-3, and CTX-M-2 are the most widespread. CTX-M-15 is currently the most widespread type in *E. coli* and is widely prevalent in the community [14].

There was an amino acid substitution detected when SHV-5 compared with other SHV type β -lactamases at highly conserved region Glutamic acid to Lysine (E-K) at position 235 as shown in Fig. 5. There was 2 amino acid substitutions detected when SHV-12 compared with other SHV type β -lactamases at highly conserved region Alanine to Threonine (A-T) at 290 position and Glutamic acid to Lysine (E-K) at position 237 as shown in Fig. 6. The SHV-1enzyme belongs to the molecular class A serine β-lactamases and share extensive structural and functional similarity with TEM β-lactamases. SHV-1 shares 68 percent of its amino acids with TEM-1 and has a similar overall structure. The SHV-1 β-lactamase behaves as a typical penicillinase hydrolyzing penicillins and early generation cephalosporins. ESBLs in this family also have amino acid changes around the active site, most commonly at positions 238 and 240. The first reported SHV β-lactamase had a narrow spectrum of activity. By the accumulation of point mutations at sites that affect the active site of the enzyme, a family of derivatives of SHV-1 has evolved. Derivatives of SHV-1 either have an extended spectrum of activity, capable

of inactivating third-generation cephalosporins, or are resistant to β-lactamase inhibitors. This leads to the evolution and spread of the SHV family of β-lactamases [15].

The phylogenetic analysis of the TEM, CTX-M and SHV β – lactamases as shown in Fig. 8-Fig.14. Phylogeny represents the evolution. The horizontal lines are branches that represent evolutionary lineages that are changing over the time. In phylogram branch lengths are proportional to the distance.

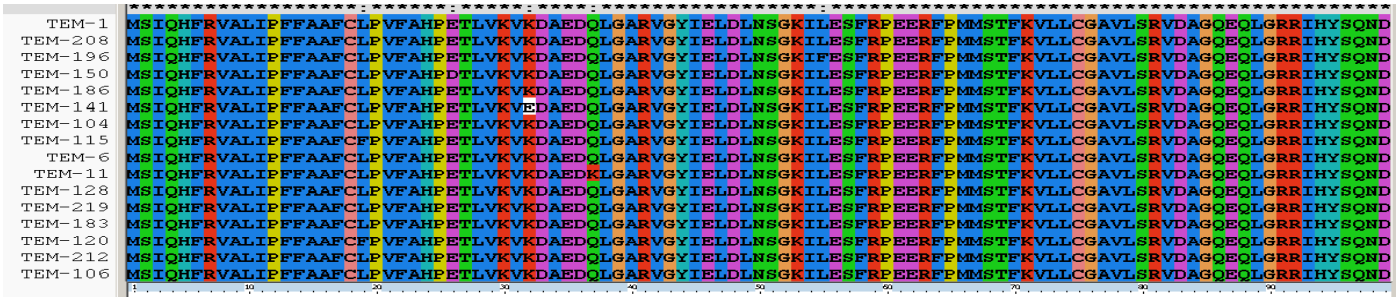

Fig. 1: Amino acid alignment of TEM-1 β-lactamase compared to other TEM β-lactamases from NCBI. The alignment was performed using Clustal X 2.1 multiple sequence alignment software.

Fig. 2: Amino acid alignment of TEM-10 β-lactamase compared to other TEM β-lactamases from NCBI. The alignment was performed using Clustal X 2.1 multiple sequence alignment software.

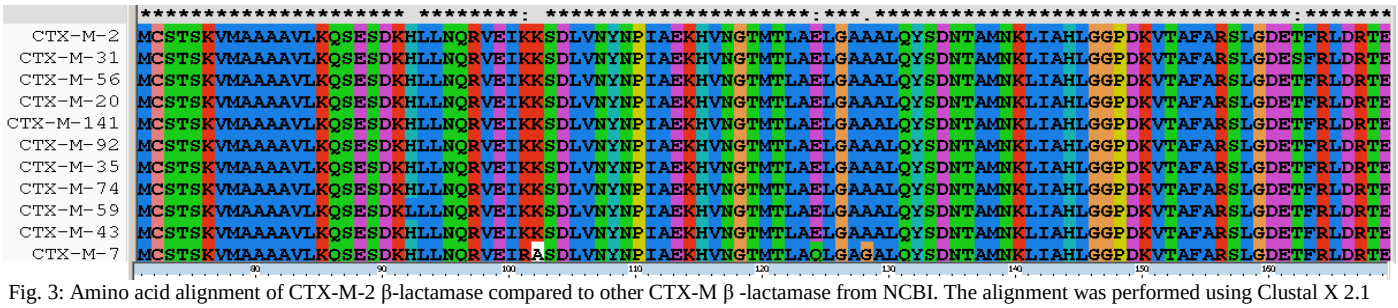

Fig. 3: Amino acid alignment of CTX-M-2 β-lactamase compared to other CTX-M β -lactamase from NCBI. The alignment was performed using Clustal X 2.1 multiple sequence alignment software.

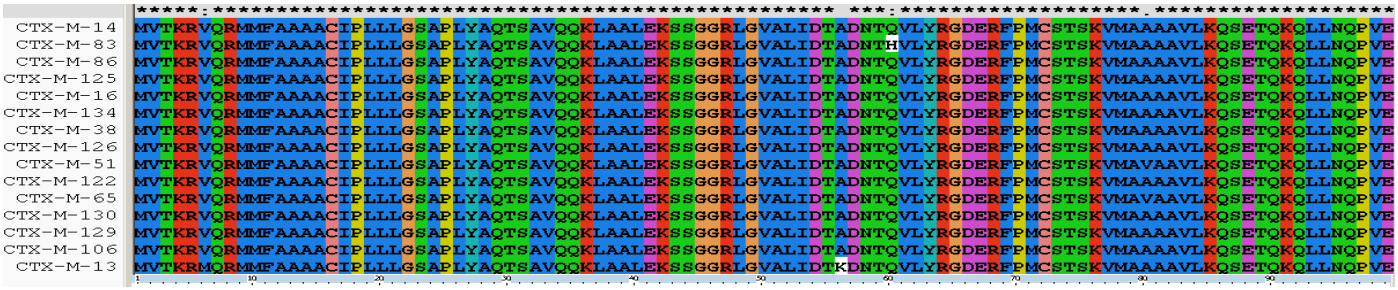

Fig 4: Amino acid alignment of CTX-M-14 β-lactamase compared to other CTX-M β -lactamases from NCBI. The alignment was performed using Clustal X 2.1 multiple sequence alignment software.

Fig 5: Amino acid alignment of SHV-5 β-lactamase compared to other SHV β -lactamases from NCBI. The alignment was performed using Clustal X 2.1 multiple sequence alignment software.

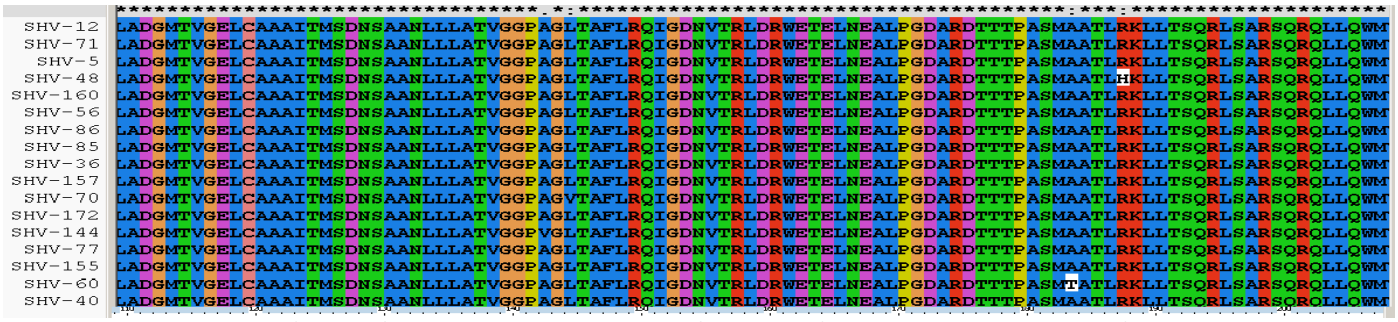

Fig 6: Amino acid alignment of SHV-12 β-lactamase compared to other SHV β -lactamases from NCBI. The alignment was performed using Clustal X 2.1 multiple sequence alignment software.

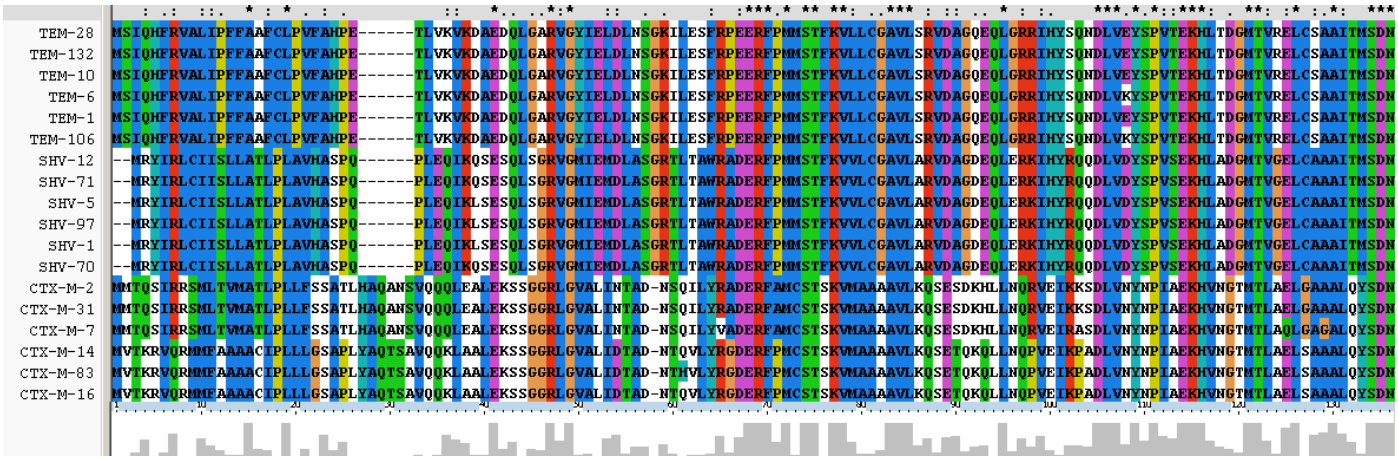

Fig 7: Amino acid alignment of TEM, CTX-M and SHV β-lactamases. The alignment was performed using Clustal X 2.1 multiple sequence alignment software

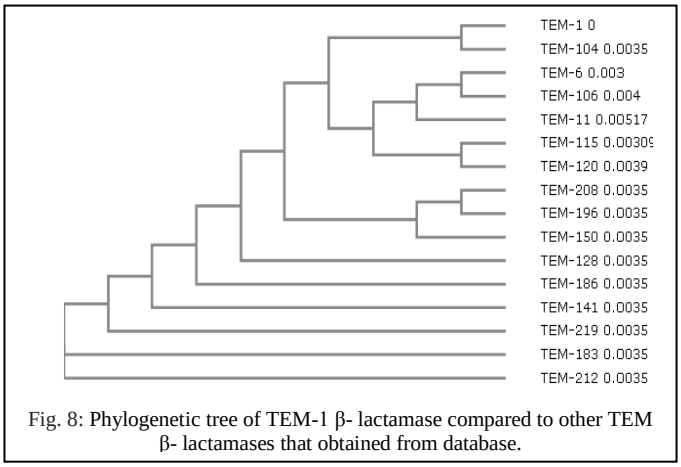

Fig. 8: Phylogenetic tree of TEM-1 β- lactamase compared to other TEM β- lactamases that obtained from database.

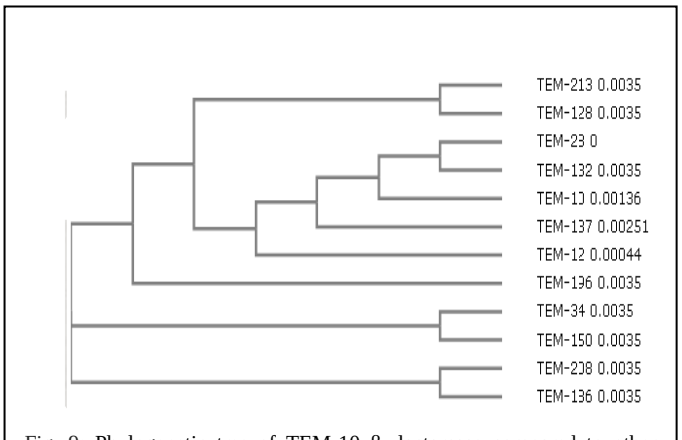

Fig. 9: Phylogenetic tree of TEM-10 ß- lactamase compared to other

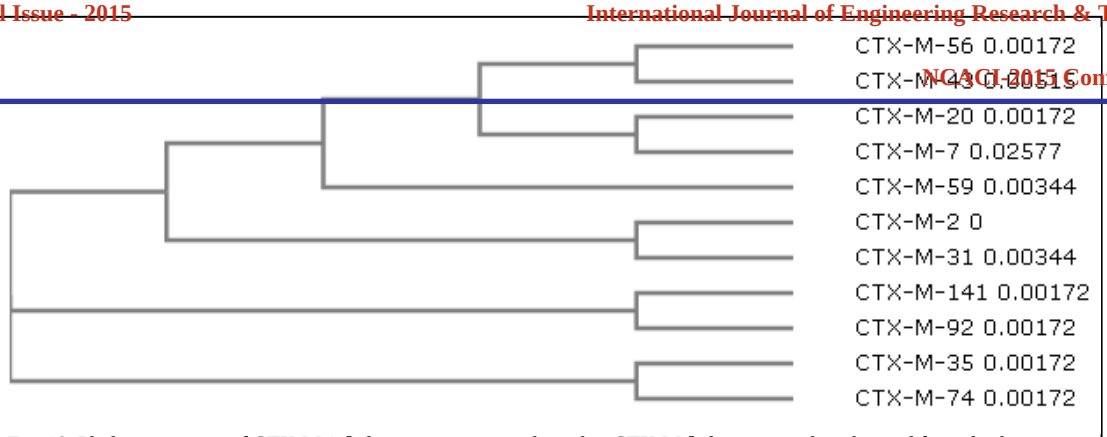

Fig. 10: Phylogenetic tree of CTX-M-2 β- lactamase compared to other CTX-M β- lactamases that obtained from database.

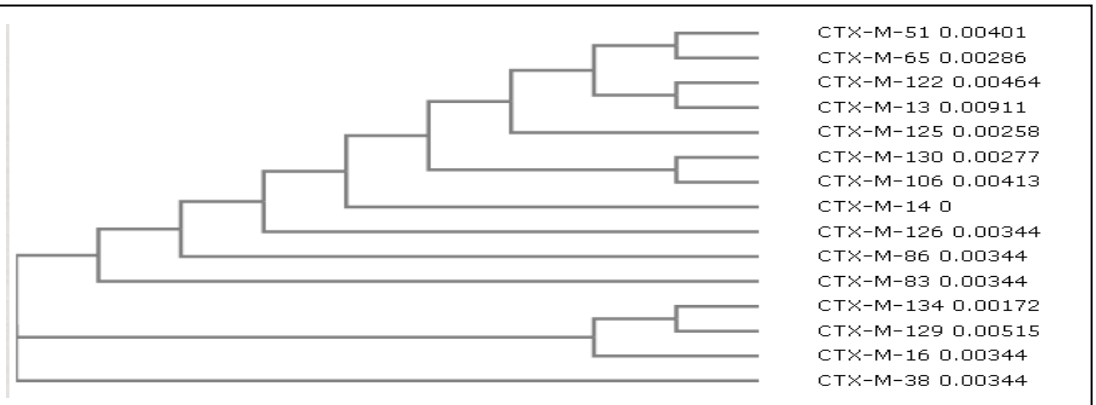

Fig. 11: Phylogenetic tree of CTX-M-14 β- lactamase compared to other CTX-M β- lactamases that obtained from database.

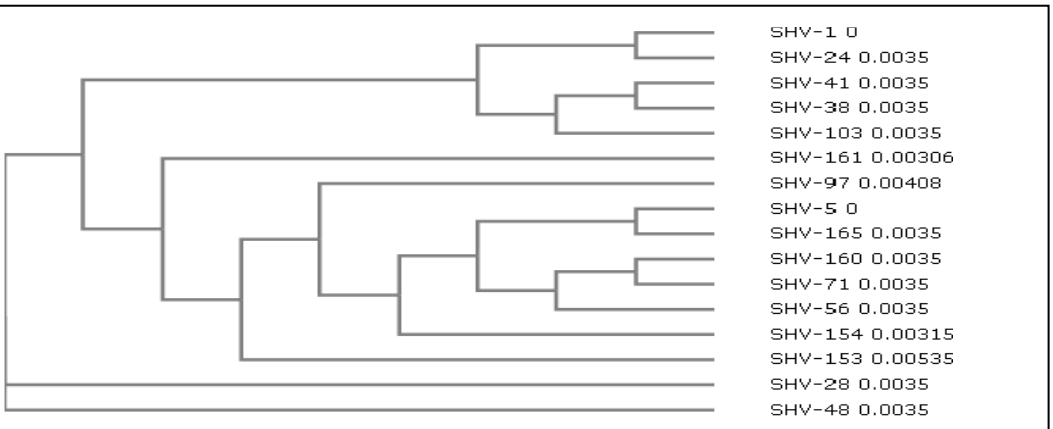

Fig. 12: Phylogenetic tree of SHV-5 β- lactamase compared to other SHV β- lactamases that obtained from database.

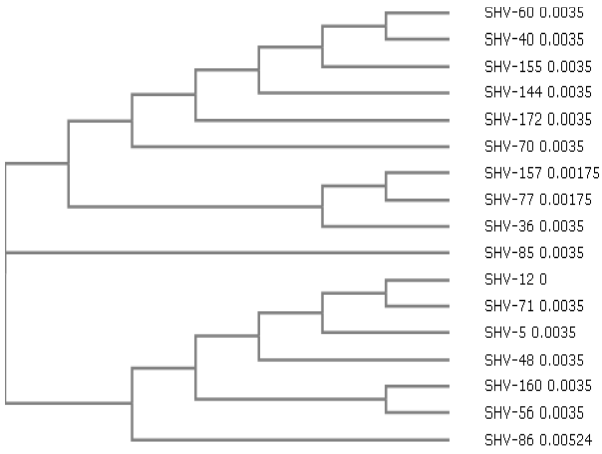

Fig. 13: Phylogenetic tree of SHV-12 β- lactamase compared to other SHV β- lactamases that obtained from database.

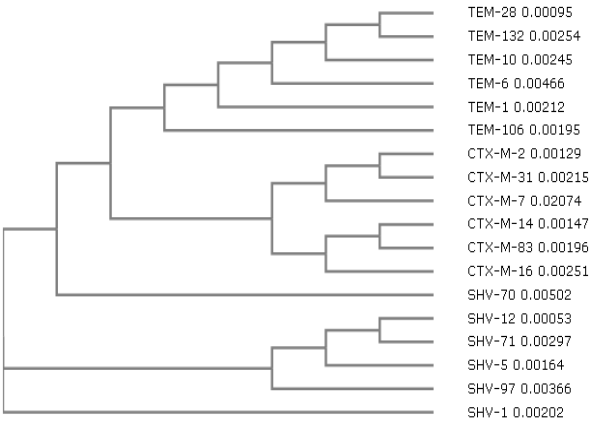

Fig. 14: Phylogenetic tree of TEM, CTX-M & SHV β- lactamases.

IV. CONCLUSION

β- lactamases are important group of clinically important enzymes conferring resistance to β- lactam antibiotics. There is a continuous race between the introduction of new generation of β- lactam antibiotics and evolution of newer β- lactam enzymes to counteract them. Hence analysis of the relationship in mutations and phylogenetic analysis is crucial to develop strategies to control the resistant organisms and studying their epidemiology. The present study focused on the identifying the important substitutions leading to the evolution of various classes of β- lactamases.

The list of variants in each family of β-lactamase indicates that β- lactamases are fast evolving family of proteins. Residues at certain positions are essential and therefore substitutions at these positions may result in a non-functional protein. However, other residue positions might be less important and thus substitutions at these positions will be more frequent. Therefore, identification and discrimination between essential and non-essential residues is helpful in understanding the molecular aspects of β- lactamase mediated antibiotic resistance.

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