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HYPOTHESIS | OPEN ACCESS

A HYPOTHESIS ABOUT THE VARIATION WITHIN B-LACTAMASES: AN EPIGENETIC-LIKE MECHANISM

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ABSTRACT

β-lactamase has been well studied as an enzyme responsible for microbial surviving against various antibiotics and the spreading of the resistance. It could be existed in different microbes with 100% identity. Or, it could be existed in the same species as well as in different species in identities not equal to 100%. The question is, did the differences and the similarities between the β-lactamase is due to mutations, host adaptation, its mobility, all of that or something else. This study aims to investigate different β-lactamase belonging to one class (class C) to deep our understand to such differences. Our hypothesis is that β-lactamases gain their differences due to both of mutation and host adaptation. The differences between thirty different β-lactamases have been evaluated using different point of investigation including the protein and the DNA sequences and the β-lactamases protein 3D structure models. The study suggests that host adaptation might be forced such kind of changes. And that changes might explain why different β-lactamases existed in the same strain? That because of a second expected transformation from the recipient to the original host after such modification has been happened. This study is a single step toward the understanding of the confusing fact that β-lactamase could be different within single strains and similar within different ones. As well as it, explain the global differences within the microbial strains. Our hypothesis might not absolutely correct but it should be considers as a material for further investigation and judgment..

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KEY WORDS

β-Lactamase; antibiotics; resistance; protein model

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[I] INTRODUCTION

The elevation of resistance to a new antibiotic is a painful action happened due to incorrect attitude and the misuse of the different types of antibiotics. Antibiotics, which could be on our side if used correctly, might be source of problems if they subjected to misuse. In a previous study, we investigated that, a single strain, (*E. coli* ATCC 8739) found in the protein database have different *β*-lactamases [1]. By investigating the BLAST protein, database for the existence of the *E. coli* ATCC 8739 *β*-lactamase the results showed that this protein could be found in hundreds of different microbes with 100% identity [1]. Treating patients with broad-spectrum antibiotics induce resistant [2, 3]. The resistance to antibiotics happened mainly due to the acquiring of R-factor or due to new mutation(s) in old but useless existing resistance gene(s), which upon being mutated become extra-resistant [4-6].

Such useless resistant gene becomes effective due to the new changes in its protein's amino acid constituents. Antibiotic resistance reduces the chance of the patient recovery. Amara (2011); Amara and Hussain (2006); Hussain and Amara (2006) reported that those mutations could induce microbial variation under the strain level [7-9]. TEM *β*-lactamase is the most prevalent one in Gram-negative enteric bacteria [10, 11]. Venkatachalam et al., (1994) introduce amino acid substitutions in the active site pocket of the *β*-lactamase [10]. The experiments have been identified in natural isolates with increased resistance to extended-spectrum cephalosporins, such as cefotaxime and ceftazidime. Mutants were selected for 100-fold more ceftazidime resistance than wild-type. All mutants had a serine substitution at position 238, a lysine or arginine at position 240, and a small amino acid at position 241. The role of each substitution was investigated by constructing individual G238S, E240K, and R241G mutants as well as the G238, SE240K double mutant. The G238S mutant increases catalytic efficiency for both ceftazidime and cefotaxime. However, to achieve significant increases in catalytic efficiency, both G238S and the E240K mutants are required. The R241G mutant results in a small increase in catalytic efficiency for only ceftazidime. This is an example has been done in lab however, nature is more dynamic and the probability that similar or more forms can be happened is very high. The existence of another protective mechanism in certain microbes can give the chance and the time for the resistance to be happened, acquired and established. Spore forming bacteria can produce spore for protecting the microbes against antimicrobial agents until the condition become more suitable for germinating a vegetative

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cell [12-17]. Hyperdization with resistant microbes can also exist naturally [7]. Such hyperdization might happen also with the genome of the dead resistant microbes. Transformation can transfer R-factor harboring plasmid or integrate it into the genomic DNA (by transposing elements) and stable new gene or genes acquired [18-21]. The studies done on the different microbes have been neglecting the role of the microbial community in the resistance elevation except in issues such as R-factor transformation. β-lactamase which is a subject of many studies is proved wide diverse due to mutagenesis which induced resistance. However, this study investigate a new concept about the β-lactamase gene differences within microbes, which is based upon that such differences might be due to adaptation rather than mutagenesis or evolutionary concepts. In simple words, β-lactamases faces some sort of changes due to their existence in new host strains and due to the forces of their location in such new system. Such kind of changes is similar to the epigenetic concept while the new host should have different food and metabolic pathways, which by one or another way must effect on the newly acquired β-lactamase genes [22, 23]. Such Epigenetic-Like change might solve the paradigm that the mother host strain carry different β-lactamases genes.

[II] MATERIALS AND METHODS

2.1. The used protein sequences

Thirty protein sequences have been collected from BLAST (NIH) protein database and represent the amino acids constituents from the genus *Escheichia, Citrobacter, Enterobacter, Klebsiella, Salmonella, Proteus, Lelliottia. Kluyvera* and *Peantoea*. The complete name, gene bank number and the amino acids constituents can be found in the protein alignment in **Figure–1**, a, b, and c. The amino acids sequences are adjusted to FASTA format to enable various types of analysis using the different software used in this study [24-27].

2.2. The software used in this study

Several software were used in this study to do various sequences analysis. Clustal W v. 1.7 has been used for alignment both of the amino acids and the nucleotides used in this study to generate BOOTSTRAP N-J tree. MEGA v. 5.1 has been used to generate a comparative analysis of the twelve amino acid sequences and the phylogenic tree as in **Table– 1** and **Figure– 1** and **2**. The PAST statistical package has been used to do clustering of the different numeric data as in **Figure– 3**. MODELLER v 9v8 has been used in protein models generation for the five amino acid sequences used in this study against four published β-lactamase models as in **Figure– 4**. In addition, for calculating the % of the similarity of each protein sequence with the four used models as in **Table– 2** and **Figure– 4** [28-35].

2.3. Generating amino acids Profiles

For each of the thirty different proteins of the β-lactamase enzymes, an amino acids profile was generated. For each profile, each amino acid has been given as % and the overall data has been summarized in **Table– 1**. For that, the software OMGA 5.1 was used to analyze the sequences collected for each protein individually and for all of the thirty used sequences collectively. An average for each of the twenty amino acids for the thirty sequences have been also calculated and given as an average %. OMGA 5.1 enables calculating the % of each amino acid in each protein. The average of each amino acid % for each of the thirty proteins was summarized in **Table– 1**.

2.4. Generating amino acids Phylogenic Trees

Alignments and Phylogenic trees for the protein primer sequences of amino acids have been generated **[Figure– 1]**. The sequence alignment and the phylogenic trees have been generated using Clustal W version 1.7 and MEGA 5.1. The software does alignment for both of the amino acids and the nucleotides used in this study and generate a BOOTSTRAP N-J tree for each.

2.5. Generating β-lactamases protein models

A model for each of the five selected β-lactamases has been generated using the software MODELLER v 9.8 **[Figure– 4]**. Four published β-lactamase models have been used to build the hypothetical model for each of the five β-lactamase using MODELLER v 9.8. The four β-lactamase amino acids sequences are: 27542960 *Enterobacter aerogenes*, 495596866 *Citobacter sp*. A1, 15804744 *E. coli* o157:H7-str. EDL933, 210061213 *Klebsiella pneumoniae* and 21213049 *Lelliottia nimipra*. The models have been built using four published β-lactamases models, they are 2WZX (*Pseudomonas aeruginosa*) [Amp-C β-lactamase (*Pseudomonas aeruginosa*) in complex with compound M-02] [36], 2WZZ (*Pseudomonas aeruginosa*) AMP-C β-lactamase (*Pseudomonas aeruginosa*) in complex with compound M-03] [37], 3S1Y (*Pseudomonas aeruginosa*) [AMP-C β-lactamase (*Pseudomonas aeruginosa*) in complex with a β-lactamase] [38], and 2ZC7 [Crystal structure of class C β- lactamase ACT-1] [39].

[III] RESULTS AND DISCUSSION

This study concern with investigating thirty of β-lactamases related to class C amino acids sequences. The study contains protein sequences from each of the following genus: *Escheichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Proteus*, *Lelliottia*, *Kluyvera* and *Peantoea*. The study aims to map the similarities and the differences between such proteins to evaluate the mobility of β-lactamase in different microbial strains. Recently Amara, (2011); Amara et al., (2012), have been published a study about the existence of single β-lactamase in different microbial strains. The study reports the existence of a single type of β-lactamase in hundreds of microbial strains with 100% identity. Amara et al., (2012) postulated different mechanisms for the distribution of the β-lactamase resistance genes, particularly due to the microbial ecosystem community in the presence of strains able to produce such biopolymers. The biofilm production and the spore formation are interfere with the antimicrobial activity and enable surviving of the different microbes from the correct killing dosage of most of the antimicrobial compounds particularly the disinfectants. Such escaping from the different exposure to antimicrobial compounds causes the elevation of new β-lactamase mutants or the acquiring of new resistant genes, which were not existed before. Amara (2011) describe in details a study about the different mechanisms might responsible for the formation of the resistant [39]. Such mechanisms might contain transferring complete microbial genome to intact or ghost of a bacterial cell. Exopolysaccarid formation is another system for the protection [39]. Alginate can cause mechanical protection by coating or immobilizing the microbial cells [39]. Another hypothesis about

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the distribution of a single resistance gene within the microbes has been described [1]. This study concern with the analysis of thirty *β*-lactamase protein sequences. The alignment of the different protein sequences in general show high level of similarity within the difference *β*-lactamases. However, one could observe that there is some similarity within the primer structure between some sequences. For example, the three first sequences are nearly similar to each other but different to the other sequences. Out of the thirty used sequences, twenty-two of them consist of 380 aa. Apparently, its seams that 380 aa is the correct constituents of the *β*-lactamases. Only one sequence carries 379 amino acids which is clear that the sequence might has a loss for one amino acid. The rest has 378 amino acids sequences. The amino acid profiles visualize the distribution of the different amino acids in the proteins backbones. Even the differences in the amino acids number is not a significant factor could effect on the function, particularly if the differences located in only three amino acids. By combining between each of the data in **Table– 1**, the sequences alignment and the phylogenic tree one could follow the changes, which have been happened for the *β*-lactamase gene. But, the most critical point which prove our hypothesis is that strains from the same species are located in different groups such as *E. coli* and *Klebsiella pneumonia* as in **Figure– 2**. A protein model for the homology modeling was done using MODELLER v 9v8 and four *β*-lactamase pdb files. The selected *β*-lactamases protein sequences for the five selected *β*-lactamases protein sequence have been generated. The different models have been generated using the MODELLER v 9v8 software. The five models have been subjected to alignment to show the similarity within their structure. The evolutionary history was inferred using the Neighbor-Joining method [40]. The optimal tree with the sum of branch length = 1.07534597 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method [41] and are in the units of the number of amino acid substitutions per site. The analysis involved thirty amino acid sequences. All positions containing gaps and missing data were eliminated. There were 373 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [42]. The distribution of the amino acids as show in **Figure– 1**.

The distribution of the amino acids within the thirty sequences also prove that *β*-lactamases in the same genus have more similarity than that existed within the different genus. The distribution of the amino acids within the thirty sequences has been summarized in **Table– 1**. The amino acids have been summarized as a % for each sequence and the overall % of the thirty sequences have been calculated using the option in the MEGA 5.1 software. The distribution of the amino acids % in the thirty sequence have been analyzed using the statistical software PAST where the data has been analyzed using the clustel analysis option in the PAST software. The different amino acids could be ranked from the lower to the higher % as in **Table– 1**. Cys was the lowest one according to its % and followed by His, Phe, Met, Trp, Arg, Asp, Asn, Tyr, Glu, Ile, Ser, Lys, Thr, Gln, Pro, Val, Gly, Leu, Ala. The amino acids % ranked from 0.5% till 11.1%. His, which is an important residue in the *β*-lactamases active site, has been ranked a number 2. Active amino acids have less number in the protein sequence backbone. The **Table–1** of the amino acids distribution has been rearranged after the MEGA 5.1. Where the amino acids have been ranked from the lower to the higher %. In the amino acids % Table, the sequences have been rearranged according to the phylogenic tree which obtained MEGA 5.1 [43-45]. For each of the five-clustered groups as shown in the phylogenic tree, one amino acids sequence has been used to generate protein 3D model. The selected *β*-lactamases for model generating are 27542960 *Enterobacter aerogenes*, 495596866 *Citobacter sp*. A1, 15804744 *E. coli* o157:H7-str. EDL933, 210061213 *Klebsiella pneumoniae* and 21213049 *Lelliottia nimipra*. The different modles have been generated using Modller 9v8 software. The models have been built using four published *β*-lactamases models, they are 2WZX (Pseudomonas aeruginosa), 2WZZ (*Pseudomonas aeruginosa*), 3S1Y (*Pseudomonas aeruginosa*), and 2ZC7. The built models show high similarities to the four template used models. The similarity % ranked from 72.14% and 97.21%. Even the template *β*-lactamase models are originally from *P. aeruginosa* but *Klebsiella pnumoniae* give 97.21% similarity, which another proves about the similarity of *β*-lactamase within different genus and species. All the five generated models have been successfully alignment to each other as in **Figure– 4**.

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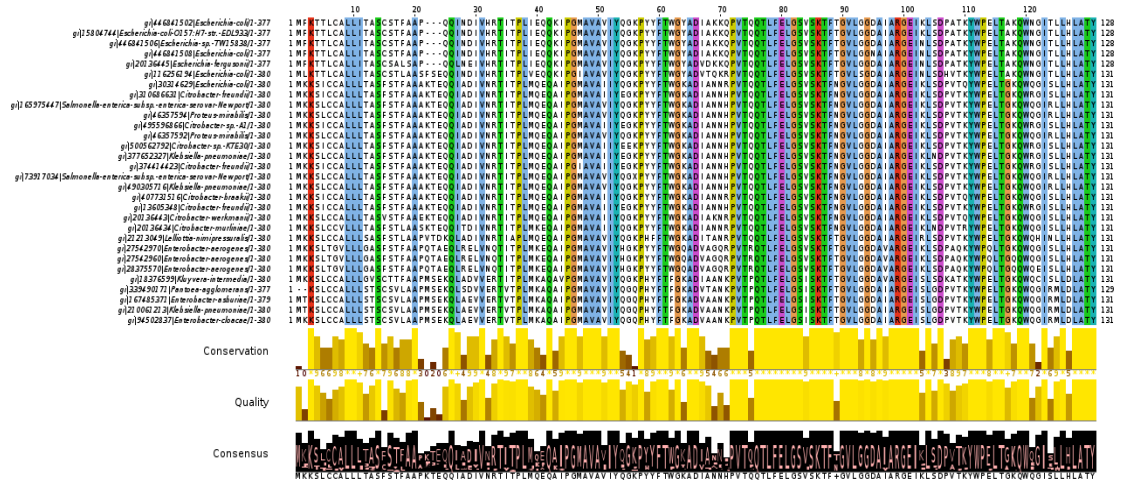

Fig: 1.a

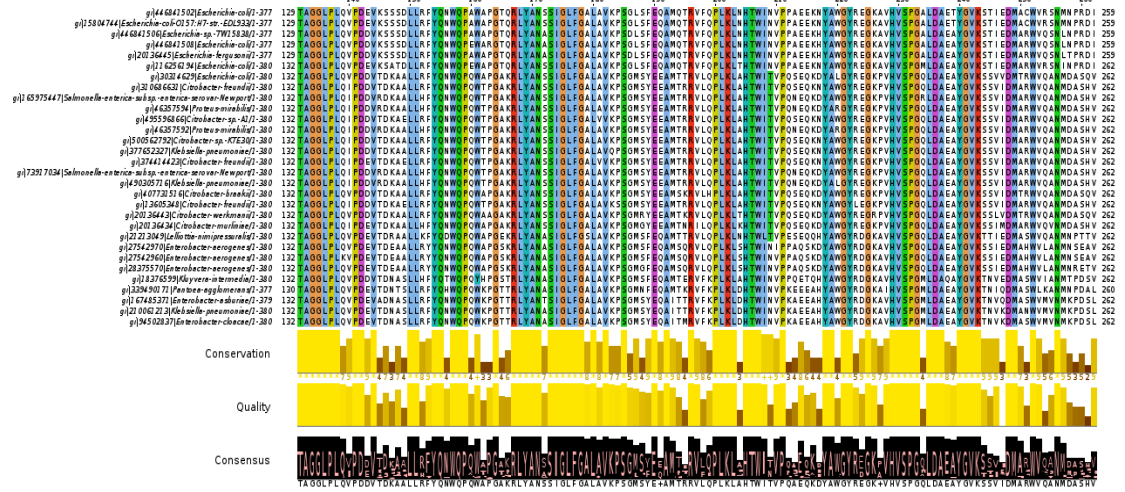

Fig: 1.b

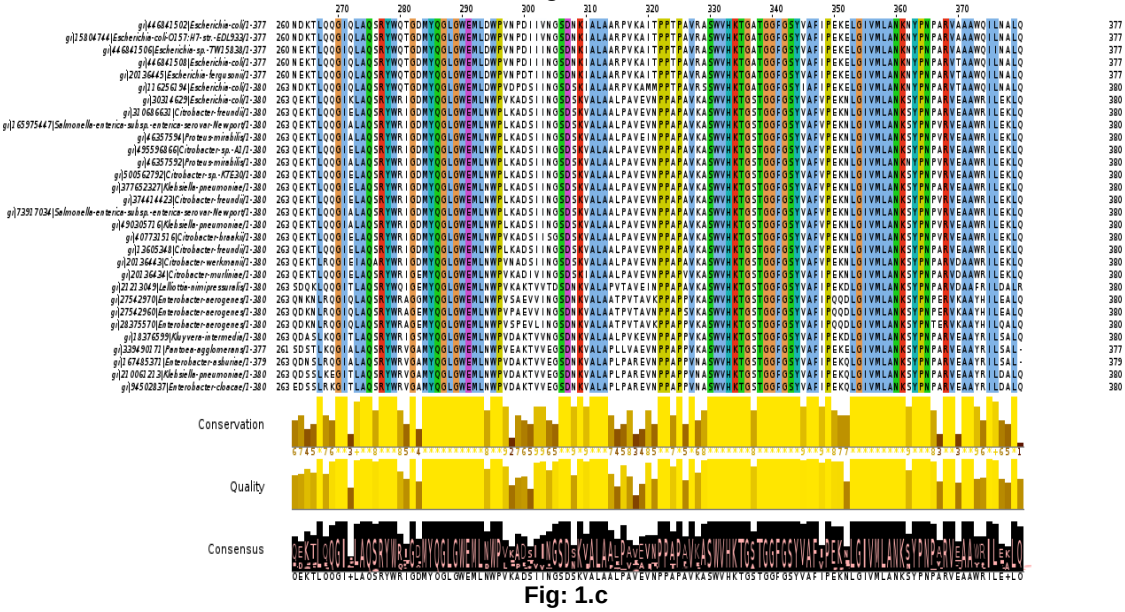

Fig: 1.c

Fig: 1. a), b) and c) Multiple alignment of the primary sequences of the thirty *β*-lactamases

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Table: 1. *β*-lactamases different amino acids % and an average for each amino acid of the thirty tested *β*-lactamases

β-lactamases bacterial host	Amino acids %																				
	Cys	His	Phe	Met	Trp	Arg	Asp	Asn	Tyr	Glu	Ile	Ser	Lys	Thr	Gln	Pro	Val	Gly	Leu	Ala	**Total**
gi|495596866|*Citrobacter sp.*-A1	0.5	1.6	2.4	2.6	3.4	3.4	3.4	3.7	3.9	5.0	5.5	6.3	6.3	5.5	5.8	6.1	6.8	7.9	8.9	10.8	380
gi|374414423|*Citrobacter-freundii*	0.5	1.6	2.4	2.4	3.4	3.4	3.2	3.7	3.9	5.3	5.8	6.3	6.3	5.5	5.8	6.1	7.1	7.9	8.9	10.5	380
gi|136053488|*Citrobacter-freundii*	0.5	1.6	2.4	2.6	3.4	3.2	3.4	3.7	3.9	5.0	5.5	6.6	6.3	5.5	5.8	5.8	6.6	7.9	9.2	11.1	380
gi|310686631|*Citrobacter-freundii*	0.5	1.6	2.4	2.6	3.4	3.4	3.4	3.7	3.9	4.7	5.5	6.3	6.3	5.5	5.8	6.1	6.6	7.9	8.9	11.3	380
gi|500562792|*Citrobacter sp.*-KTE30	0.5	1.6	2.4	2.6	3.4	3.4	3.4	3.7	3.9	5.0	5.5	6.3	6.3	5.5	5.8	6.1	6.8	7.6	8.9	11.1	380
gi|377652327|Klebsiella pneumoniae	0.5	1.6	2.4	2.6	3.4	3.4	3.4	3.9	3.9	4.7	5.3	6.1	6.1	5.5	6.1	6.1	6.6	7.9	9.2	11.3	380
gi|739170347|Salmonella-enterica-subsp.-enterica-serovar-Newport	0.5	1.8	2.4	2.6	3.4	3.4	3.7	3.7	4.0	4.2	5.0	5.8	6.1	5.3	6.3	6.1	7.1	7.9	9.2	11.3	379
gi|165975447|Salmonella-enterica-subsp.-enterica-serovar-Newport	0.5	1.8	2.4	2.6	3.4	3.7	3.7	3.7	3.9	4.2	5.0	5.8	6.3	5.3	6.1	6.1	7.1	7.9	9.2	11.3	380
gi|490305716|Klebsiella pneumoniae	0.5	1.8	2.4	2.6	3.2	3.4	3.7	3.7	3.9	4.2	5.0	5.8	6.3	5.3	6.3	6.1	7.1	7.9	9.5	11.3	380
gi|46357592|Proteus-mirabilis	0.5	1.8	2.4	2.6	3.2	3.7	3.7	3.9	3.9	4.2	5.0	5.5	6.3	5.3	6.3	6.1	7.1	7.9	9.2	11.3	380
gi|463575947|Proteus-mirabilis	0.5	1.8	2.4	2.6	3.2	3.7	3.7	3.7	3.9	4.2	5.3	5.8	6.3	5.3	6.3	6.1	6.8	7.9	9.2	11.3	380
gi|407731516|Citrobacter-braakii	0.5	1.3	2.4	2.6	3.4	3.2	3.7	3.2	3.9	4.5	5.3	6.8	6.3	5.3	6.6	6.1	7.4	7.9	8.7	11.1	380
gi|303146291|Escherichia-coli	0.5	1.1	2.4	2.6	3.2	3.2	3.7	3.4	3.9	4.2	5.0	6.3	6.3	5.8	6.8	6.1	7.4	7.9	8.9	11.3	380
gi|201364433|Citrobacter-werkmanii	0.5	1.1	2.1	2.6	3.4	3.9	3.9	3.4	3.9	4.5	5.0	5.8	6.1	5.8	6.6	5.8	7.4	7.9	8.9	11.3	380
gi|201364344|Citrobacter-murliniae	0.5	1.6	2.1	2.9	3.4	3.2	3.4	3.9	3.9	4.7	6.1	5.8	6.6	5.5	6.1	6.1	6.8	8.2	9.2	10.0	380
gi|275429600|Enteroba	0.0	1.8	2.4	2.6	3.2	3.2	3.2	3.4	4.2	4.2	3.4	6.6	5.3	5.0	7.4	6.6	7.9	8.7	9.5	11.6	380

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cter aerogenes																					
gi|28375570|Enterobacter aerogenes	0.0	1.8	2.4	2.6	3.2	3.4	3.2	3.2	4.2	4.2	3.4	6.1	5.3	5.5	7.9	6.6	7.6	8.7	9.7	11.1	380
gi|27542970|Enterobacter aerogenes	0.0	1.8	2.4	2.6	3.2	3.2	2.9	3.4	4.2	3.9	3.7	6.6	5.5	5.0	7.4	6.6	7.6	8.9	9.5	11.6	380
gi|21213049|Lelliottia-nimipressuralis	0.5	1.8	2.9	2.6	3.2	3.2	4.5	3.2	3.2	3.9	4.2	5.5	5.8	7.4	6.3	6.3	7.6	7.4	9.5	11.1	380
gi|18376599|Kluyvera-intermedia	1.1	1.8	2.9	3.2	2.6	2.4	4.2	3.2	3.9	3.4	3.7	6.6	6.3	6.6	5.0	6.8	8.2	7.9	8.9	11.3	380
gi|339490171|Pantoea-agglomerans	0.8	1.6	2.7	3.2	2.9	2.9	3.7	3.4	3.7	4.8	3.7	6.1	6.4	6.4	4.2	6.9	7.7	8.2	9.8	10.9	377
gi|167485371|Enterobacter-asburiae	0.8	1.3	2.4	3.4	2.9	3.4	3.7	3.7	4.0	4.2	3.4	6.1	5.5	6.1	4.7	7.1	8.4	8.2	9.2	11.3	379
gi|210061213|Klebsiella pneumoniae	0.8	1.3	2.4	3.4	2.9	3.2	3.9	3.7	3.9	4.5	3.4	5.8	6.1	6.3	4.5	7.1	8.4	8.2	9.2	11.1	380
gi|94502837|Enterobacter-cloacae	0.8	1.3	2.4	3.7	2.9	3.4	3.9	3.4	3.9	4.5	3.4	6.1	6.3	5.8	4.2	7.1	8.4	8.2	9.2	11.1	380
gi|116256194|Escherichia-coli	0.5	1.6	2.6	2.4	3.4	3.7	3.9	3.9	3.9	4.2	6.1	6.1	5.0	7.6	6.3	6.3	6.3	7.4	8.9	9.7	380
gi|158047444|Escherichia-coli-O157:H7-str.-EDL933	0.8	1.3	2.9	2.1	3.4	3.2	3.7	4.5	4.0	3.7	6.1	5.0	5.6	7.2	6.4	6.9	6.1	7.7	8.5	10.9	377
gi|446841502|Escherichia-coli	0.8	1.3	2.9	2.1	3.4	3.2	3.7	4.5	4.0	3.7	6.1	5.0	5.6	7.2	6.4	6.9	6.1	7.7	8.5	10.9	377
gi|446841508|Escherichia-coli	0.5	1.6	2.9	1.9	3.4	3.4	3.4	4.8	4.0	4.0	6.4	4.8	5.3	7.2	6.6	6.6	5.8	7.7	8.8	10.9	377
gi|446841506|Escherichia-sp.-TW15838	0.5	1.6	2.9	1.9	3.4	3.2	4.0	4.5	4.0	3.7	6.6	4.8	5.3	6.9	6.6	6.9	5.8	7.4	8.5	11.4	377
gi|201364455|Escherichia-fergusonii	0.5	1.6	2.7	1.9	3.4	3.2	4.0	4.2	4.0	4.0	5.6	5.0	5.3	7.4	6.6	6.9	6.1	7.4	9.3	10.9	377
Average %	0.5	1.6	2.5	2.6	3.3	3.3	3.6	3.7	3.9	4.3	5.0	5.9	6.0	6.0	6.1	6.4	7.1	7.9	9.1	11.1	379.33

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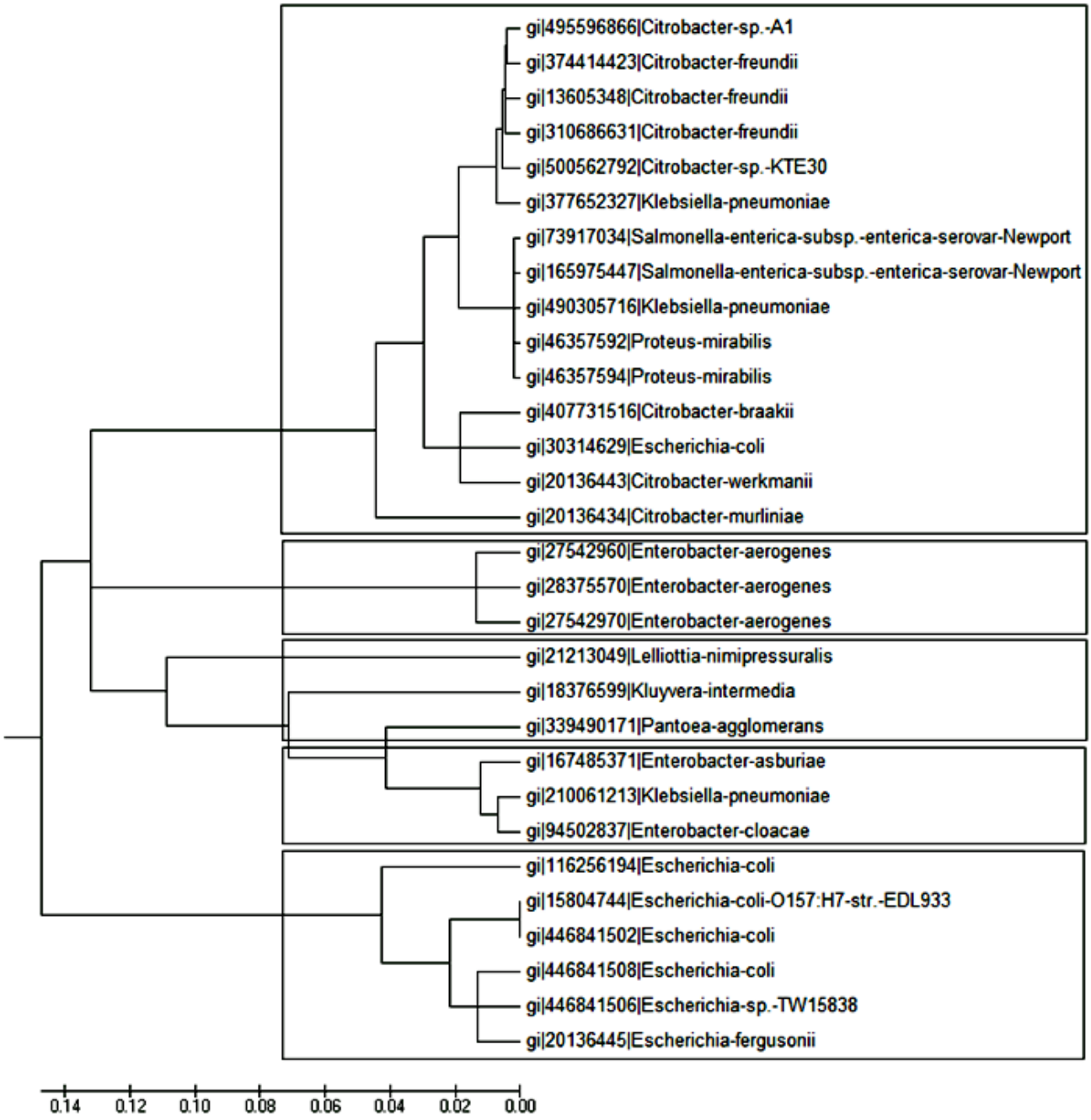

Fig: 2. Phylogenic tree for the thirty used *β*-lactamase

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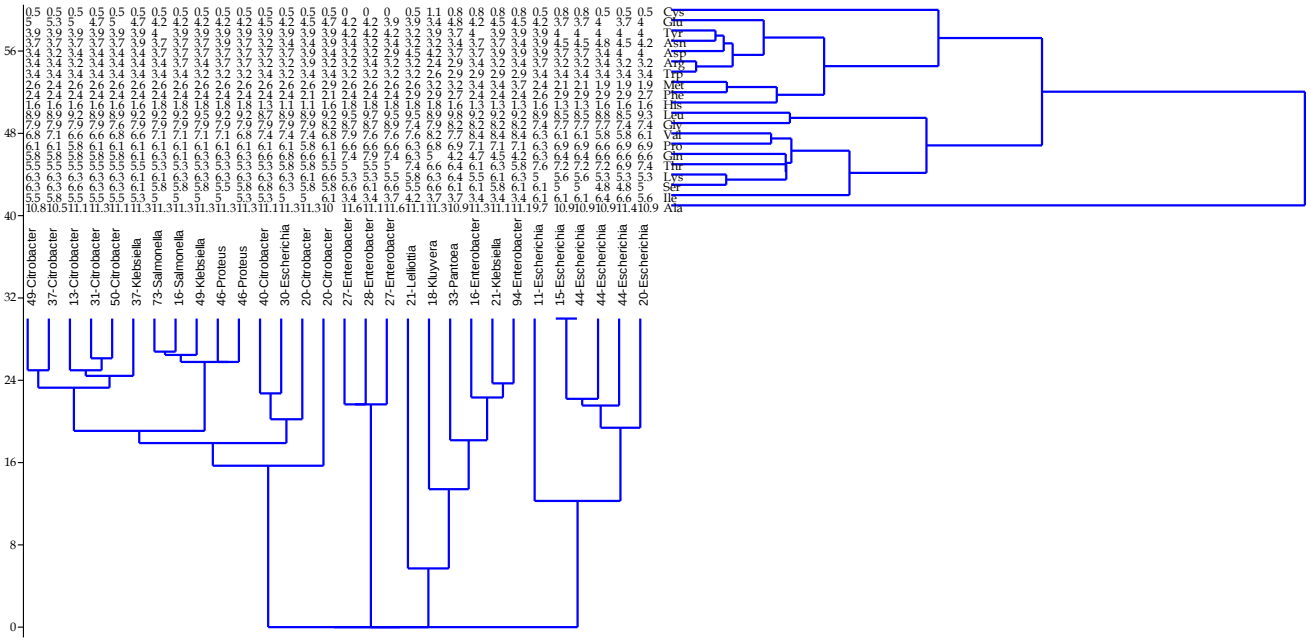
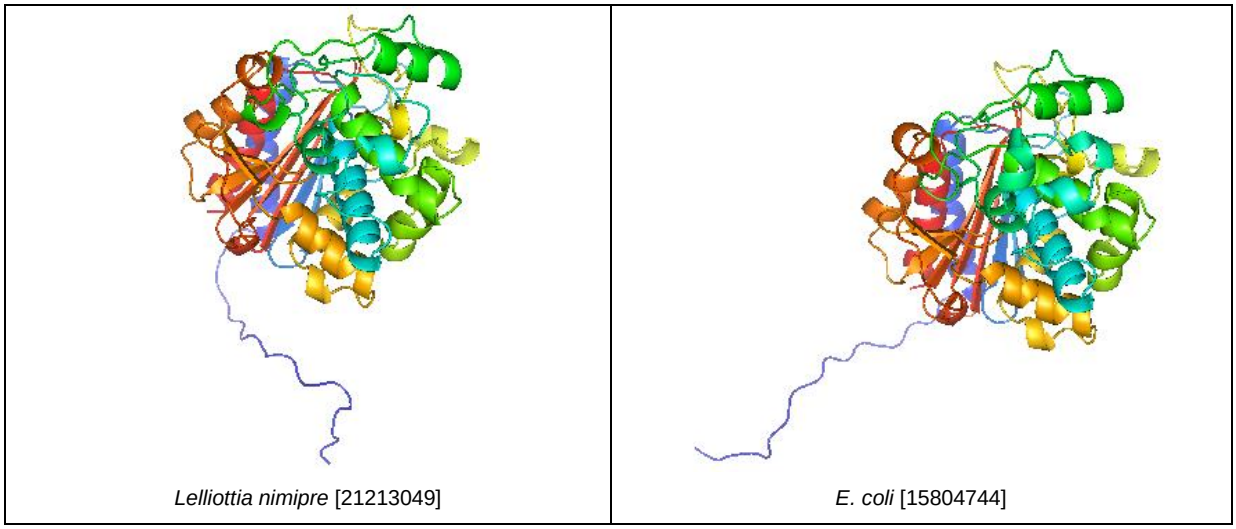


Fig: 3. Cluster analysis for the amino acids %



Lelliottia nimipre [21213049]

E. coli [15804744]

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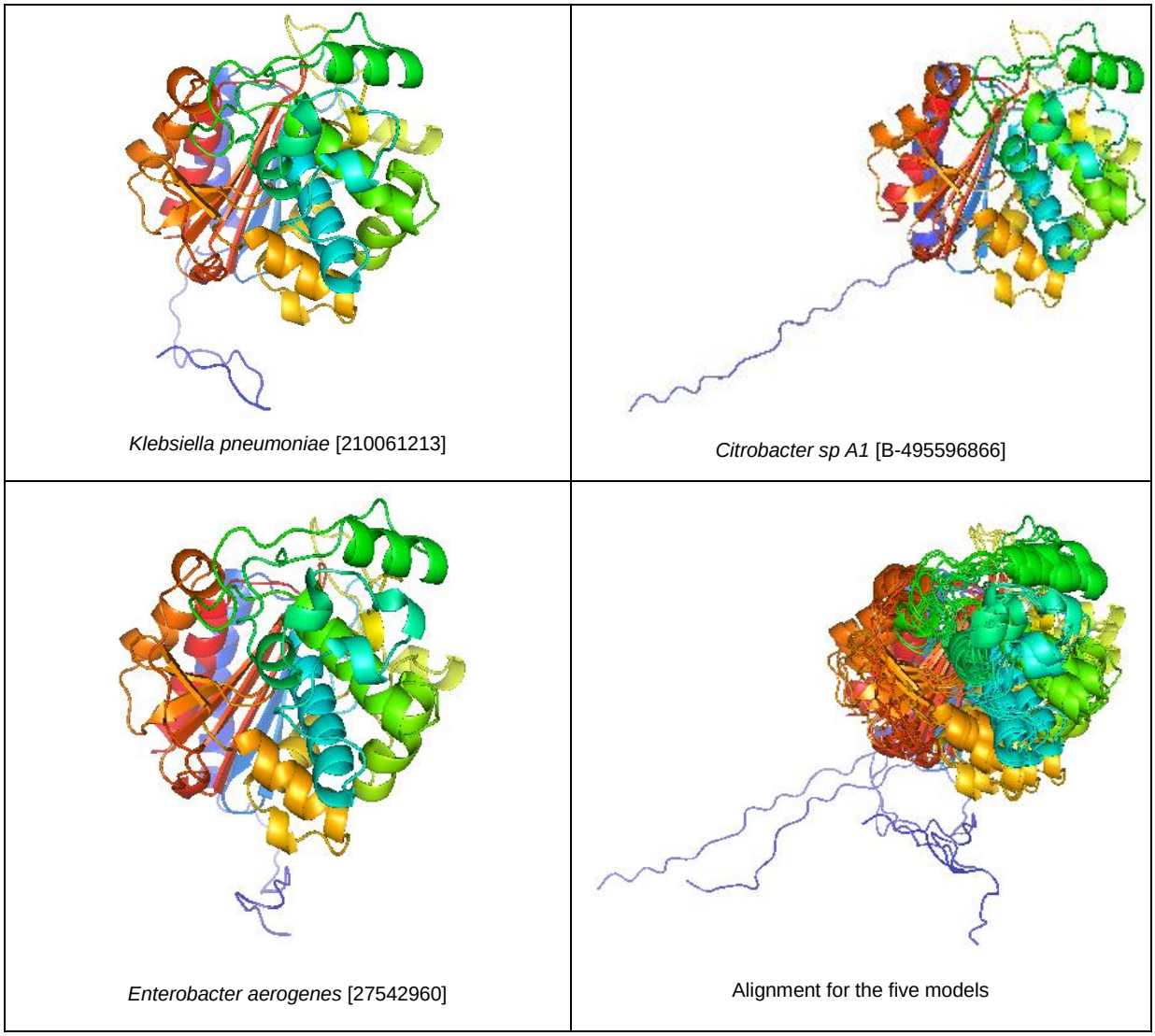

Fig: 4. Five *β*-lactamase models and an alignment for them

Table:2. Different *β*-lactamases similarity % to 2WZX, 2WZZ, 3S1Y and 2ZC7

Bacterial Names	% of similarity	Rank
***E. coli* 15804744**	72.14%	1
***Enterobacter aerigenes* 27542960**	74.93%	2
***Citrobacter sp* A1 495596866**	75.20%	3
***Lelliottia nimipre* 21213049**	78.27%	4
***Klebsiella pneumoniae* 210061213**	97.21%	5

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[IV] CONCLUTION

This study hit β-lactamase thirty sequences from different points to map the similarity and the differences aiming to point any linkage between the differences of the β-lactamases within the different species and the similarity of the β-lactamases within the same genus. The thirty sequences show a clear similarity within the same genus as proved by the sequence alignment, the phylogenic tree and the cluster analysis of the amino acids profile %. Particularly the phylogenic tree of the multiple alignment gives the same cluster analysis of the amino acids % and can be divided into five major groups based on the clustering profile and the genus which existed within. One species represent each group from the five clustered groups has been selected and a protein model for the five sequences have been built using the MODELLER software. The five built modules have been subjected to alignment to show the overall 3D similarity. The thirty selected sequences of the β-lactamases are highly similar as shown from the amount of the amino acids conserved region in **Figure– 1 a, b and c**. Even so, successfully the amino acids have been arranged in groups could be divided to five groups as described above. Even similar but more similar within the same genus. This is a prove for our agent that β-lactamases might subjected to host adaptation rather than mutagenesis or evolution concept. The study postulates the probability that that β-lactamase is changed due Epigenetic-Like mechanism. Such change happened during its transformation between different bacterial species. That explains its variation. In addition, it can be either similar within other different species, which have no, such effect, or that the effect needs time and special environmental conditions to be happened.

CONFLICT OF INTEREST

The authors have no conflict of interests to declare

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