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Institute of Integrative Omics and Applied Biotechnology Journal

Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

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HYPOTHESIS | OPEN ACCESS

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

Amro Abd Al Fattah Amara[1], Atef Mohamed Shibl[2]

[1]*Protein Research Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technological Applications, Universities and Research Centre District, New Borg El-Arab, EGYPT*
[2]*Division of Microbiology, Pharmaceutics Department, College of Pharmacy at KSU Riyadh, Kingdom of Saudi Arabia*

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

***Corresponding author:** Email: amroamara@web.de Tel: +00201010777905; Fax: +2034593423

[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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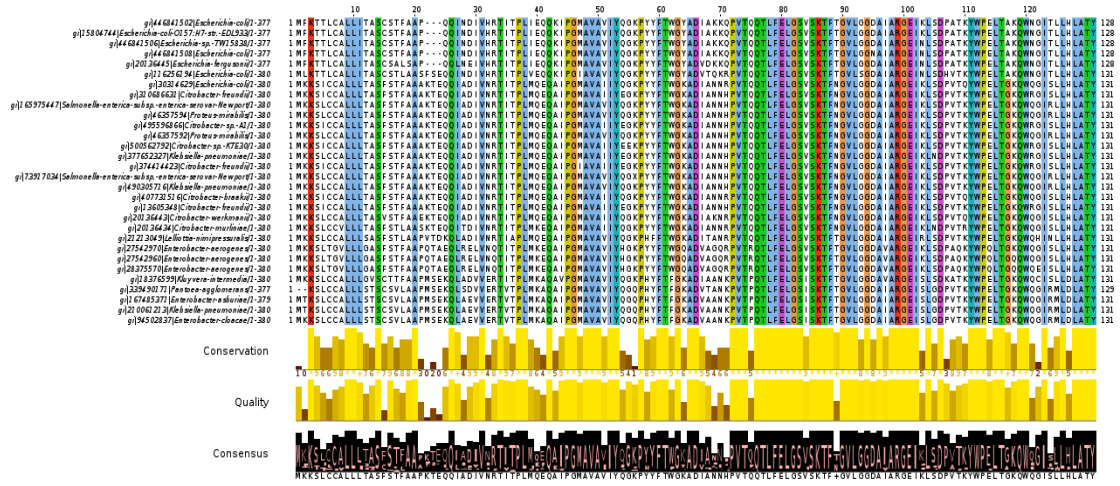

Fig: 1.a

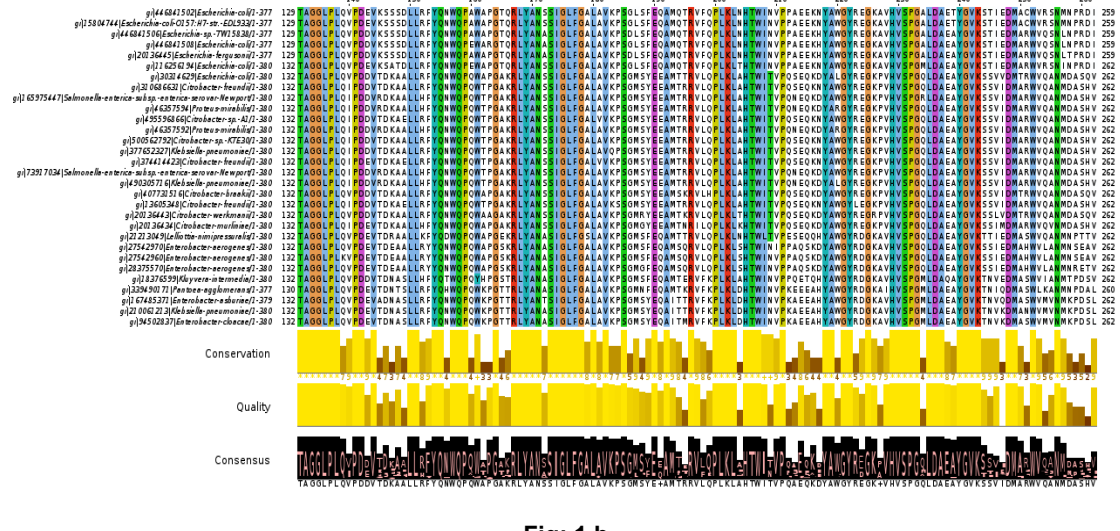

Fig: 1.b

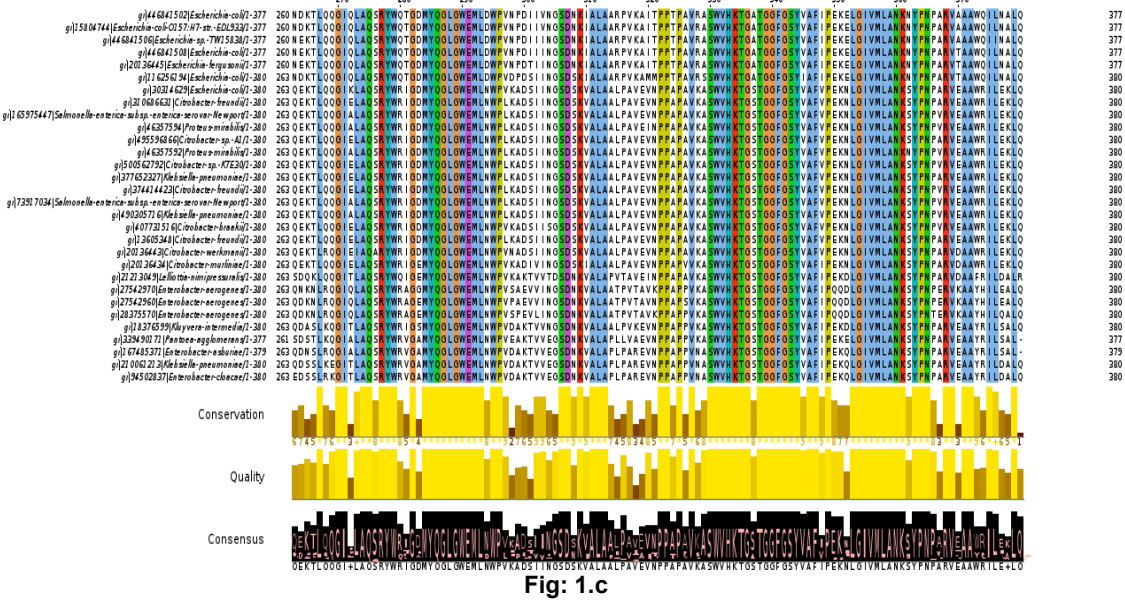

Fig: 1.c

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|1360534 8|*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|739170 34|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|463575 92|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|463575 94|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|303146 29|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|201364 43|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|201364 34|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|275429 60|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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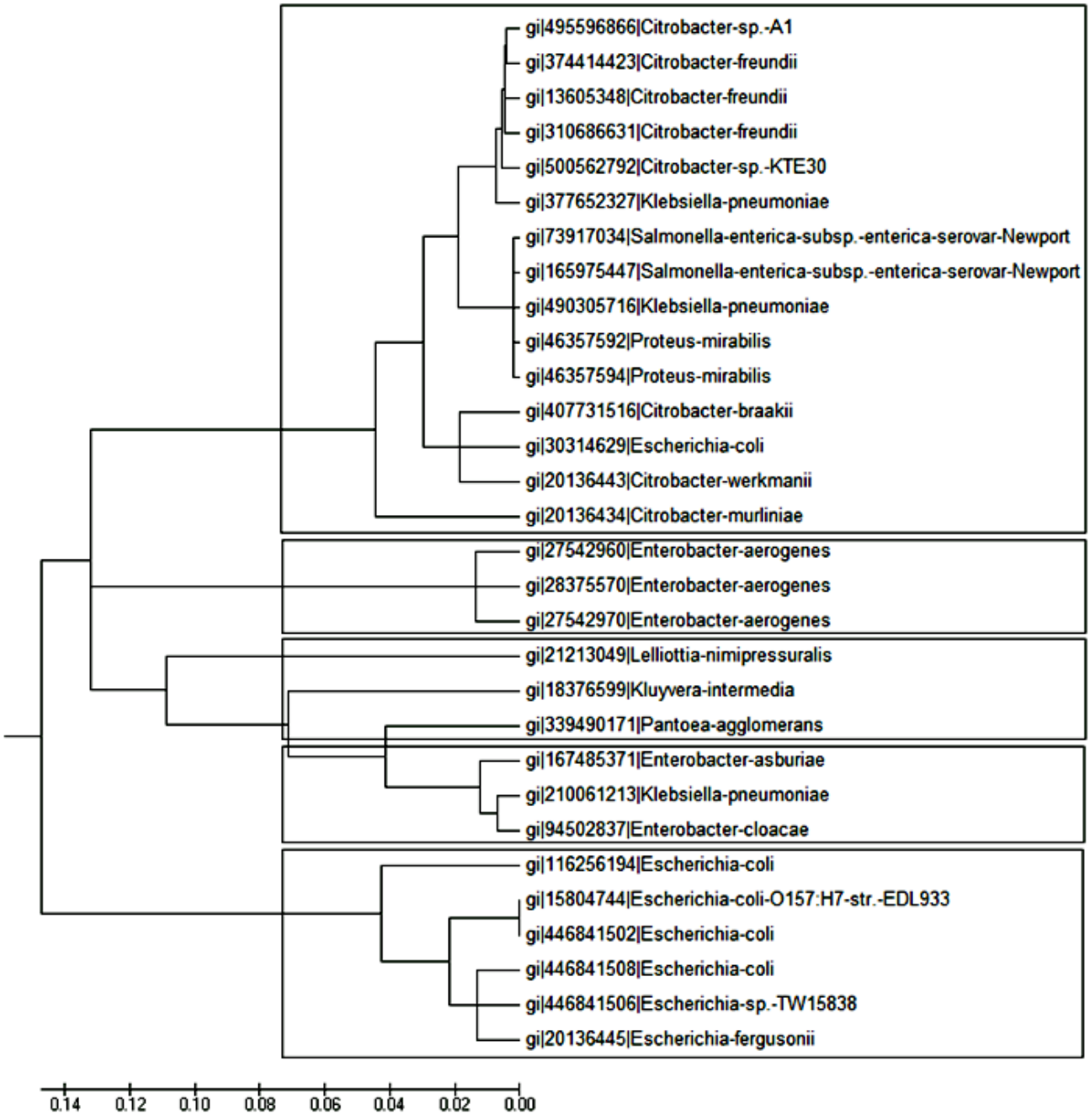

Fig: 2. Phylogenic tree for the thirty used *β*-lactamase

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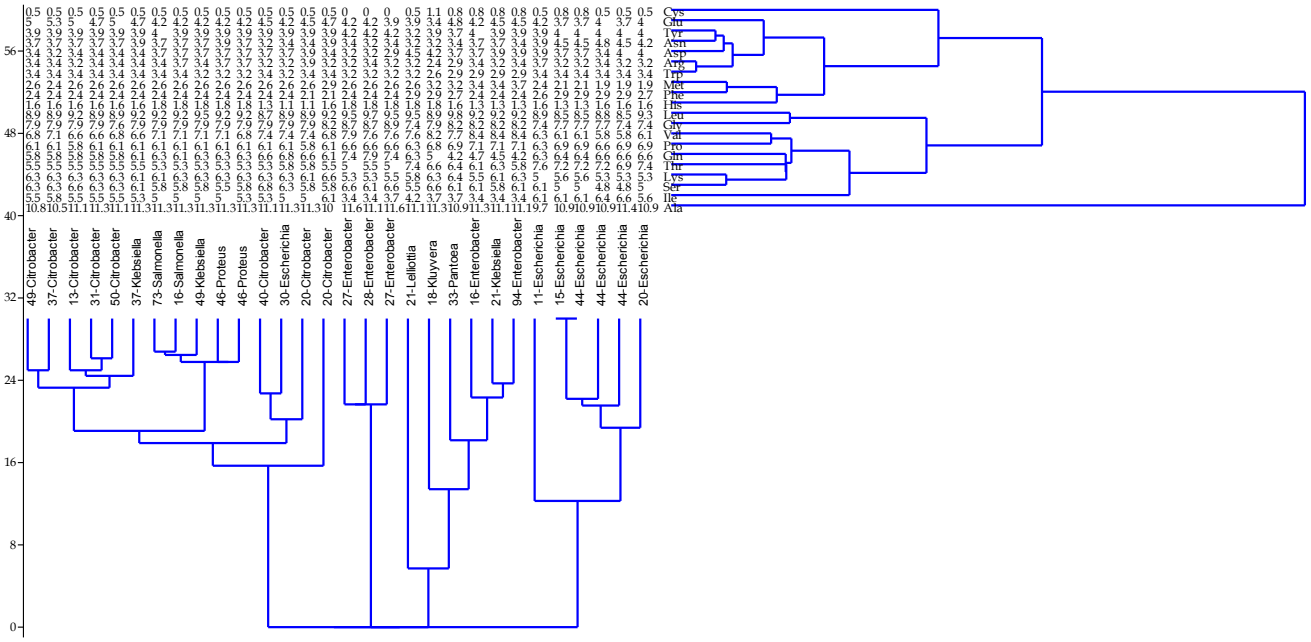
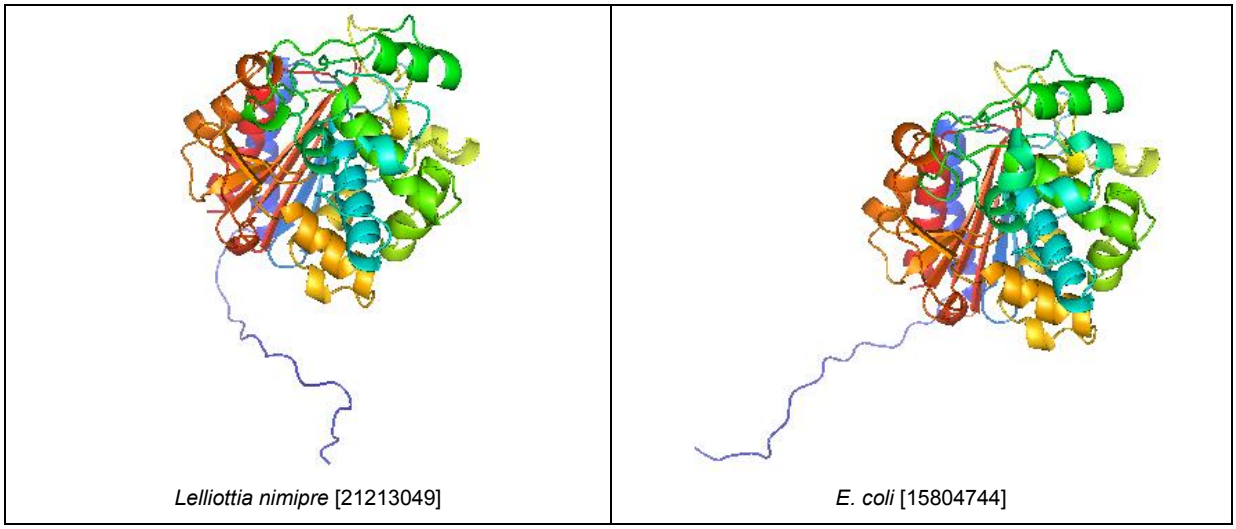


Fig: 3. Cluster analysis for the amino acids %



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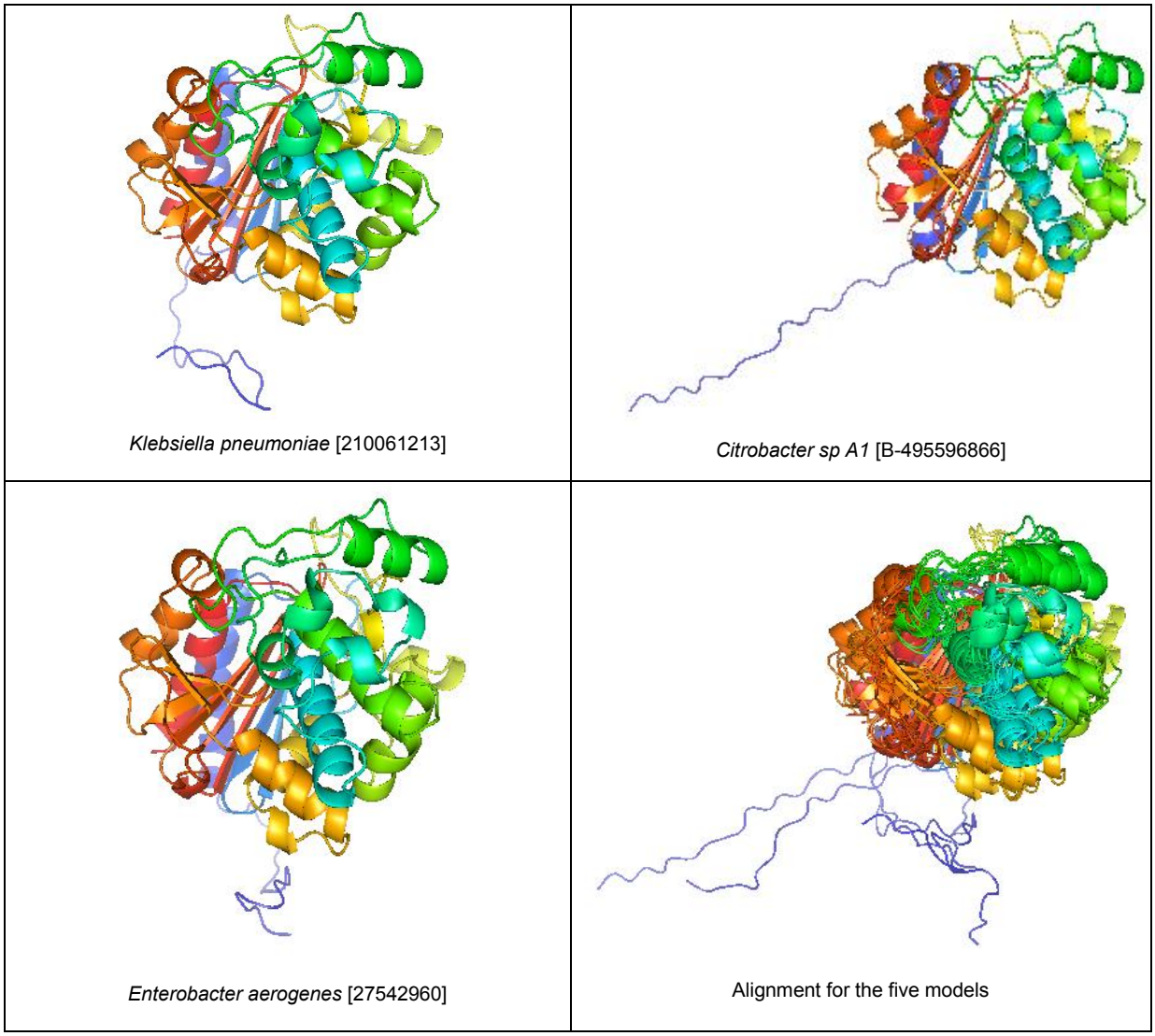

Fig: 4. Five β-lactamase models and an alignment for them

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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ABOUT AUTHORS

Prof. Dr. Amro Abd-Al-Fattah Amara is working in the field of molecular microbiology and biotechnology. He works as the head of the protein research department in (Mubarak) City for scientific research and, technological applications. He is interested in linking science to the real life to find simple solutions for problems.

Prof. Dr. Atef Mohamed Shibl a member of the Pharmaceutics Department, College of Pharmacy, King Saud University. Dr. Atef has a long history on the research concern with the antibiotics and the antibiotics resistance.

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MEMBRANE AND NON-MEMBRANE PROTEINS – A COMPARISON

Rajneesh K. Gaur*

Department of Biotechnology, Ministry of Science and Technology New Delhi–110003, INDIA

ABSTRACT

Membrane and non-membrane proteins (MPs & nMPs) constitute the total cellular protein content. The correlation between the amino acid composition of these two classes of proteins within the same and different major class of organism is interesting to know. Amino acid composition analysis of two classes of proteins indicates that the prokaryotic and eukaryotic MPs and nMPs are unique. Furthermore, the composition analysis of essential amino acids in prokaryotic and eukaryotic MPs and nMPs shows the occurrence of high overall percentage frequency of essential amino acids in pkMPs. The high occurrence of essential amino acids in pkMPs may be exploited for medicinal purpose.

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KEY WORDS

Membrane Proteins; MPs; non-Membrane Proteins; nMPs; Amino acid composition; Prokaryotes; Eukaryotes, Essential amino acids

***Corresponding author:** Email: meetgaur@gmail.com; Tel: +91-9990290384; Fax: +91-11-24360295

[I] INTRODUCTION

Proteins are broadly classified as membrane (integral part of either cellular or organelle membrane i.e. MPs) and non-membrane (outside the membrane; nMPs) depending upon their cellular location [1]. Proteins are polymers of amino acids and all the organisms uselimited repertoire of twenty amino acids for synthesis of MPs & nMPs. Simultaneously, MPs and nMPs of prokaryotes (pk) and eukaryotes (ek) work in a fundamentally different environment. The cellular working environment of MPs & nMPS may have an influence on the overall amino acid composition of these proteins e.g. the arrangement of hydrophobic amino acids helps in distinguishing MPs from nMPs [2]. The amino acid composition have been explored separately for different purposes such as determination of sequence length [3], identification of conserved sequences [4]; prediction of structural class [5], discrimination of intra- and extra cellular proteins [6], prediction of sub-cellular location [7]. To find out the contrasting features between MPs & nMPS of different as well as same class of organism, the overall amino acid composition analysis may be helpful.

The amino acids are classified as essential and non-essential depending upon whether they are absorbed or metabolically synthesized. It is also interesting to know the frequency distribution of essential amino acids between the two class of MPs & nMPs. The contrasting features of MPs and nMPs may be utilized to improve and develop prediction models or for either pharmaceutical or diagnostic purposes.

[II] MATERIALS AND METHODS

Membrane protein sequences were taken from PDBTM [8] and OMP [9] databases. Mostly, the chosen sequences possess corresponding structures in PDB. The dataset for non-membrane proteins was curated manually from the sequences extracted from PSORT [10], eSLDB [11] and RefSeq [12] databases. Protein sequences flagged as putative, potential uncharacterized, hypothetical and similar to the predicted protein are deleted from the initially downloaded RefSeq sequences. For both dataset, the amino acid composition was calculated as reported by Gaur *et. al.* (2010) [13]. The calculated amino acid composition is compiled in **Table–1**.

[III] RESULTS AND DISCUSSION

The amino acid composition of the prokaryotic and eukaryotic MPs and nMPs are shown in **Table –1**. The composition analysis shows that hydrophobic amino acids such as 'L', 'V', 'A', 'G' etc. occurs in higher proportion than hydrophilic amino acids as they are responsible for forming the core of proteins [14]. On broader scale, though many details are known about the proteins, there are still several questions remains unanswered such as what is the percentage of amino acid compositional similarity/difference in proteins of two major class of organism i.e. prokaryote and eukaryote as well as two major class of proteins i.e. MPs and nMPs.

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Table: 1. Amino acid composition of prokaryotic and eukaryotic MPs and nMPs.

Amino acid residues	Amino acid composition (%)			
	Prokaryotes		Eukaryotes	
	MPs	nMPs	MPs	nMPs
F	5.59	3.60	5.23	3.78
I	6.57	5.26	6.12	4.98
W	2.28	1.35	1.68	1.14
L	10.39 [A]	10.40 [A]	11.13	9.01
V	7.63	7.05	7.19	6.34
M	**2.83**	2.44	**2.74**	2.36
Y	3.96	2.60	3.55	2.91
C	0.59	1.15	1.67	2.21
A	9.54	10.3	7.89	6.32
T	6.04	5.43	5.73 [B]	5.81 [B]
H	1.70	2.16	2.26	2.55
G	9.46	7.65	7.16	6.01
S	5.69	5.98	6.86	8.23
Q	**3.29**	4.10	**3.26**	4.42
R	4.12	6.83	4.92	5.41
K	3.56	4.07	4.59	6.38
N	3.94	3.33	3.48	4.65
E	4.32	5.98	5.03	6.76
P	4.12	4.87	5.37 [B]	5.37 [B]
D	4.38	**5.43**	4.15	**5.35**

The amino acids similar in their composition distribution between prokaryotic and eukaryotic MPs and nMPs are highlighted in bold.The superscript 'A' indicates the amino acids composition similarity between prokaryotic MPs and nMPs, while the superscript 'B' indicates the amino acids composition similarity between eukaryotic MPs and nMPs. The amino acids are arranged in decreasing order of hydrophobicity [19]. The total of the overall amino acid composition may not be 100% as the figures are rounded off to the second place of decimal.

Firstly, we compare the amino acid composition MPs and nMPs between two separate class of organism i.e. prokaryotes and eukaryotes **[Table–1]**. Amino acid composition of MPs of prokaryotes and eukaryotes revealed that out of 20 amino acids, only two amino acids i.e. 'M' (Hydrophobic; ~ 2.8% of total amino acid composition) & 'Q' (Hydrophilic; ~3.3% of total amino acid composition) is similar between each other. The MPs have similar cellular environment in prokaryotes and eukaryotes but they are unique in their amino acid composition distribution. The existing difference may be attributed towards cellular functional requirement [15]. In contrast to MPs, amino acid composition analysis of prokaryotic and eukaryotic nMPs indicates the existence of only one residue similarity i.e. 'D' (Hydrophilic; ~5.4% of total amino acid composition). The observed similarity for the 'D' residue may be explained due to the role of this residue in the stability of the protein's active site as well as their structure as a whole [16]. The analysis shows that nMPs of prokaryotes and eukaryotes are also unique in their distribution of amino acids.

Secondly, we compare the amino acid composition MPs and nMPs with in each class of organism i.e. prokaryotes and eukaryotes **[Table–1]**. The compositional analysis between pkMPs and pknMPs shows the similar compositional distribution of only single amino acid i.e. 'L' (Hydrophobic; ~10.4% of total amino acid composition), while comparison of ekMPs and eknMPs indicate the similar distribution of 'T' (hydrophilic; ~5.8% of total amino acid composition) and 'P' (Hydrophobic; ~5.3% of total amino acid composition).Therefore, the MPs and nMPs are different from each other even within the same class of organism in their amino acid composition.

Thirdly, since both prokaryotes and eukaryotes are dependent on an external supply of essential amino acids (i.e. F, I, W, L, V, M, T, H, K), it is interesting to compare their overall composition between MPs and nMPs. The overall essential amino acid comparison shows that pkMPs & ekMPs possess 46.59% & 43.93%, while pknMPs and eknMPs possess 41.07% & 42.35% of essential amino acids content respectively **[Table–1]**.The slightly high percentage of essential amino acids in pkMPs may be explained as a result of difference in evolution of metabolic pathways [17]. The high percentage of essential amino acids in pkMPs may be utilized for pharmaceutical advantages. pkMPs & ekMPs have relatively high percentage of 'I' in comparison to respective nMPs. The compositional percentage of 'H' & 'K' is low in pkMPs with respect to remaining types of proteins under consideration, while eknMPs is rich in 'K' in comparison to pkMPs, pknMPs & ekMPs **[Figure–1]**. 'K' residue more often involved in post-translational modifications of proteins, which explain its slightly high frequency distribution in eknMPs [18].

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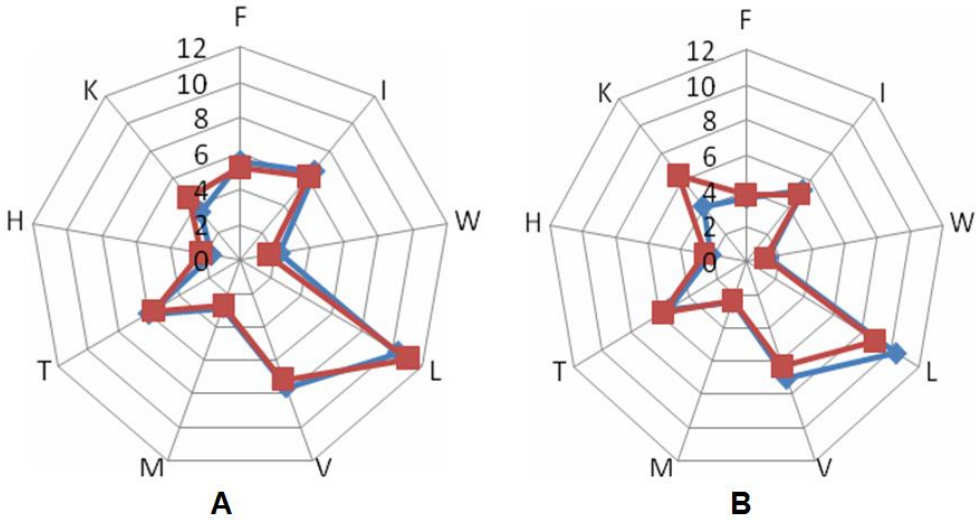

Fig: 1. Radar diagram presents the comparison of essential amino acids distribution between (A) prokaryotic and eukaryotic MPs. (B) prokaryotic and eukaryotic nMPs.

[IV] CONCLUTION

In conclusion, depending upon the amino acid composition, MPs and nMPs are unique to prokaryotes and eukaryotes as well as significantly different within the same class of organism. Furthermore, the comparison of essential amino acid content shows the occurrence of high percentage of these amino acids in pkMPs.

ABBREVIATIONS: MPs - **M**embrane **P**roteins, nMPs - **n**on-**M**embrane **P**roteins, pknMPs – **P**ro**k**aryotic **n**on-**M**embrane **P**roteins, eknMPs – **E**u**k**rayotic **n**on-**M**embrane **P**roteins, pkMPs – **P**ro**k**aryotic **M**embrane **P**roteins, ekMPs – **E**u**k**rayotic **M**embrane **P**roteins

CONFLICT OF INTEREST

The author declares having no competing interests.

FINANCIAL DISCLOSURE

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ABOUT AUTHOR

Dr. Rajneesh Kumar Gaur is currently associated with Department of Biotechnology, Ministry of Science and Technology, New Delhi, India. He is interested in analyzing the biological data available as Structural Biology and Bioinformatics based databases.

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CASE REPORT OPEN ACCESS

ENDODONTIC TREATMENT OF MANDIBULAR CANINE WITH TWO CANALS – A CASE REPORT

Aniket Jadhav
Dental College & Hospital, Bharati Vidyapeeth Deemed University, Pune, INDIA

ABSTRACT

The mandibular canine is usually considered a single-rooted tooth with a single root canal. However, two canals and more rarely two roots may also occur. This paper reports the case of a patient with bilateral mandibular canines with two roots and two root canals. The initial periapical radiographs of the mandibular right and left canines for endodontic treatment revealed the presence of two root canals in each tooth. After coronal opening, the cervical third was prepared with a SX file of the ProTaper system and root canal length was confirmed using Root ZX mini electronic apex locator. Root canal preparation was completed with the series of ProTaper instruments and the root canal was filled with gutta-percha and AH plus sealer. The final radiographs showed two well-obturated canals ending at the electronically located apices. Clinicians should always consider the presence of anatomical variations in the teeth during endodontic treatments. Despite the low prevalence, variations may occur in the number of roots and root canals of mandibular canines, as demonstrated in this case report.

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KEY WORDS

Mandibular Canine; Two Roots; Two root canals

***Corresponding author:** Email: aniketjadhav24@gmail.com Tel: +919890100866; Fax: +91 24373266

[I] INTRODUCTION

The aim of endodontic treatment is the elimination of infection from root canal system and prevention of re-infection. The anatomy of root canal system determines the parameters under which endodontic treatment will be accomplished and also directly affects the success of the root canal treatment.[1] Anomalous root and root canal morphology can be found associated with any tooth with varying degree and incidence.[1-4] Knowledge of the root canal anatomy is the basic prerequisite for successful completion and outcome of endodontic treatment.[5] Numerous studies done by various others revealed a wide variation in the number of roots and canal pattern in mandibular canines. The occurrence of two roots and even more two root canals is rare, ranging from 1% to 5%. [6, 7] This paper reports the case of a patient with mandibular canines having two roots and two root canals.

[II] CASE REPORT

A 32-year-old male patient reported to the Endodontology department with severe pain in right and left mandibular canine region. The diagnostic radiographs showed deep proximal caries in relation to 33 and 43. Also it showed two roots for both 33 and 43. Endodontic treatment was performed under local anesthesia. The rubber dam kit was used for isolation. Access cavity preparation for both 33 and 43 revealed buccal and lingual canals (**Figure-1 and -2**). The cervical third was prepared with a SX file of the ProTaper system and root canal length was confirmed using Root ZX mini apex locator. Root canal preparation was completed with series of ProTaper instruments. 5% Sodium hypochlorite was used for irrigation. Final obturation was performed with 20 number 6% gutta percha and AH plus sealer was used.

[III] DISCUSSION

The complex nature of root canal morphology of canines should be thoroughly understood. Good quality of radiographs are taken at two different horizontal angulations are very helpful in providing the clues about the number of root canals a tooth can have. Interpretation of radiographs is equally important. During radiographic examination, a careful interpretation of periodontal ligament space could suggest the presence of an extra root or canal. Additional root canals if not detected, are a major reason for failure. [4] Nevertheless, manual exploration of root canal system with an endodontic file or explorer is a reliable way to identify the exact configuration of root canal, especially the number of foramina.[1] Care should be taken at access opening because exploration and location of canal orifices helps to navigate the canal. Practice of extension of access cavity buco-lingually, is mandatory to find extra and hidden canals. Efforts should be made to locate the point where the root or the canals divide. The more apically a root canal divides, the more difficult

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here [4]. In this case, root canals were divided immediately below the pulp chamber, so, it became easy to carry out further treatment.

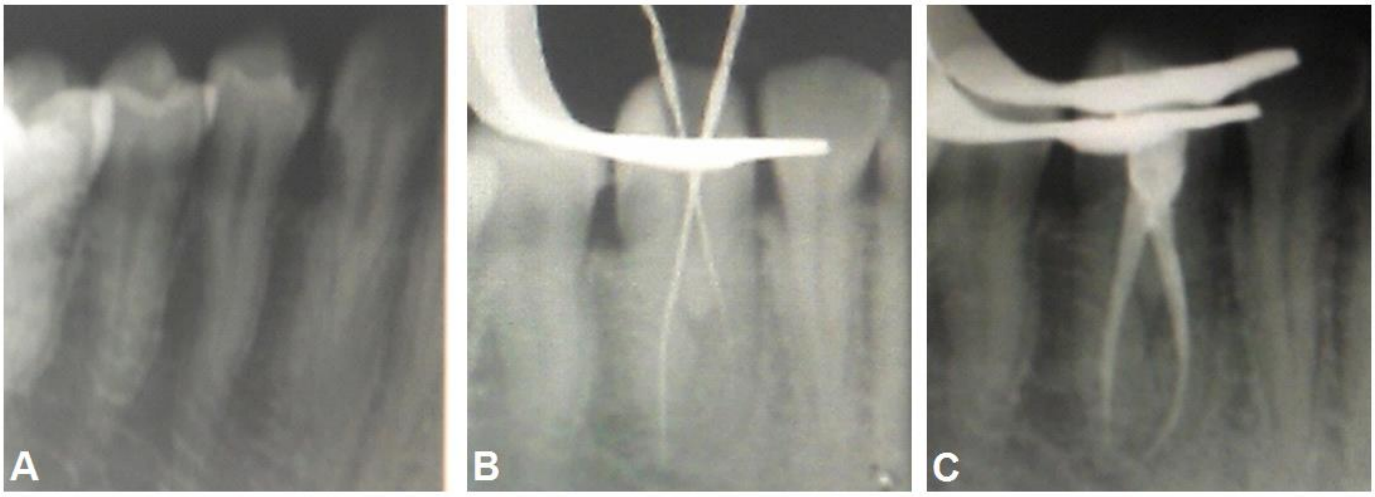

Fig.1: Radiographs of tooth 33. A) Preoperative radiograph, B) Working length radiograph, and C) Post obturation radiograph

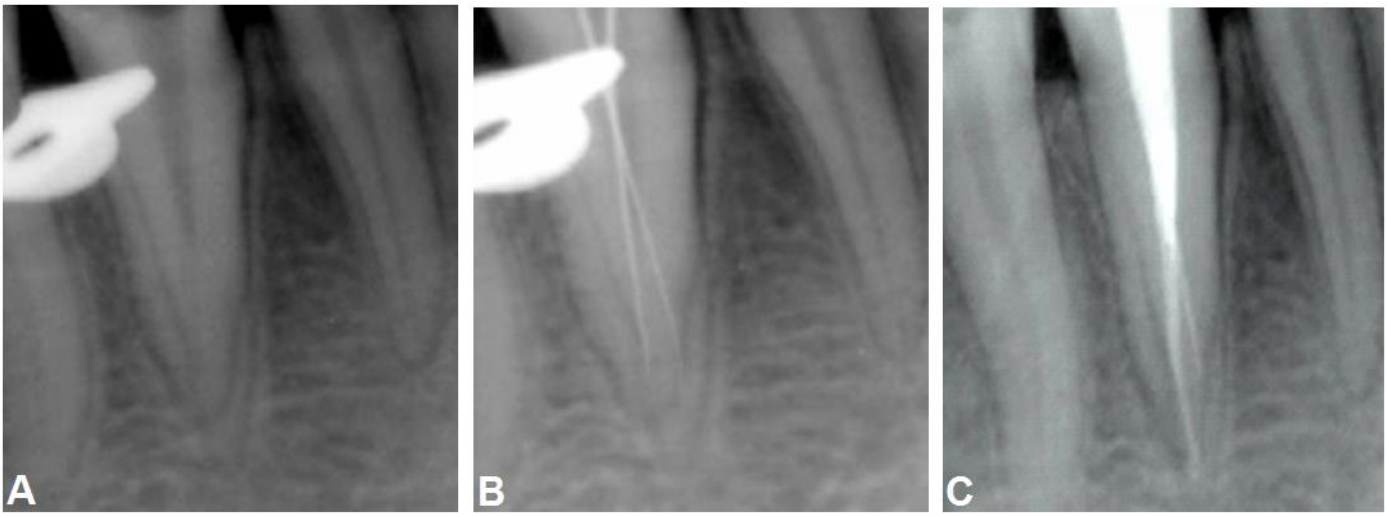

Fig.2: Radiographs of tooth 43. A) Preoperative radiograph, B) Working length radiograph, and C) Post obturation radiograph

[IV] CONCLUSION

Clinicians should be aware of anatomical variations in the teeth they are managing, and should never assume that canal systems are simple. Even though the most common anatomy of mandibular canines comprises a single root and a single root canal, clinicians should consider the possible variations and always search for the second root canal in teeth with either one or two roots.

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CONFLICT OF INTEREST
No competing interests in relation to the work.

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ABOUT AUTHOR

Dr. Aniket Jadhav *is an Assistant Professor at Bharati Vidyapeeth Deemed University,Dental College & Hospita, Pune.*

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