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EC MICROBIOLOGY
Research Article

One Percent of *Escherichia coli* O157:H7 Peptides May Contain Putative Beta-Lactamase Activity

Krithiga Gunalan[1,2], Celine QL Wong[1,2], Marilyn PY Neo[1,2], Maurice HT Ling[1,2,3]*

[1]*Department of Applied Sciences, Northumbria University, United Kingdom*

[2]*School of Life Sciences, Management Development Institute of Singapore, Singapore*

[3]*HOHY PTE LTD, Singapore*

***Corresponding Author:** Maurice HT Ling, School of Life Sciences, Management Development Institute of Singapore, Singapore.

Received: June 27, 2020; **Published:** July 31, 2020

Abstract

Beta-lactamases are enzymes conferring resistance to beta-lactam antibiotics, which has become a global challenge. Studies had suggested that beta-lactamases are primitive enzymes that existed before the antibiotic era, leading to the question on potential sources and emergence of beta-lactamases. This study examines the possibility of putative beta-lactamases in *Escherichia coli* O157:H7 by sequence comparison to known extended-spectrum beta-lactamases (ESBLs) from *E. coli*. Our results suggest that 57 peptides out of 5021 (1.14%) *E. coli* O157:H7 peptides have 64.7% probability of beta-lactamase activity. Phylogenetic analysis clustered the top 10 (by sequence similarity score) of these 58 peptides within known ESBLs. This suggests that these peptides may contain putative beta-lactamases activity and potentially be a source of putative beta-lactamase.

Keywords: *Escherichia coli O157:H7; Extended-Spectrum Beta-Lactamases (ESBLs)*

Introduction

Bacterial resistance against antimicrobial agents is a major challenge globally, according to World Health Organization [1]. Such resistances may be a result of drug uptake inhibition, antibiotics inactivation, and target sites alteration [2]. Beta-lactam antibiotics caused disruption of cell wall structure synthesis by acylation of penicillin-binding proteins [3] involved in the cross-linking peptide chains to form peptidoglycan, resulting in weakened cell wall and leading to cell death [4].

Beta-lactamases are enzymes that inactivate beta-lactam antibiotics by hydrolysing the beta-lactam ring [5]. Enzymatically, beta-lactamases can be categorized into serine beta-lactamases and metallo-beta-lactamases [5]. Serine beta-lactamases hydrolyses the substrates through the catalytic activity of serine by forming an acyl enzyme [6] while metallo-beta-lactamases facilitate hydrolysis by utilizing at least one zinc ion at the active site [7]. Penicillinase was the first beta-lactamases found in 1940 before the discovery of antibiotics [8]. Nevertheless, several phylogenetic analyses had suggested the existence of beta-lactamases to be more than 2 billion years, suggesting that beta-lactamases pre-dates the use of beta-lactams [6]. Further mutations of beta-lactamases [9] led to the emergence of extended spectrum beta lactamases (ESBLs). However, the question on the origins of beta-lactamases remains.

Several studies have either suggested the potential for *de novo* origins of beta-lactamases [10] or enzymes with potential beta-lactamase activities [5]. This study examines the possibility of putative beta-lactamases in *Escherichia coli* O157:H7. Our results suggest that

57 *E. coli* O157:H7 peptides may have beta-lactamase activity, suggesting that these peptides may be a potential source of putative beta-lactamase.

Materials and Methods

Data sets

ESBLs from 17 different strains of *E. coli* were identified from GenBank using the following search term, *("extended spectrum beta-lactamase"[title] AND complete[title]) AND "Escherichia coli"[porgn]*, and the peptide sequences were collated as known ESBLs from *E. coli*. Peptide of *E. coli* O157:H7 (Accession number NZ_CP041623) proteome was collated as *E. coli* O157:H7 peptides.

Sequence alignment

Two sets of pairwise alignments were performed using Smith-Waterman algorithm [11] from SeqProperties [12]. In the first set, pairwise alignments were performed for sequences within ESBLs from *E. coli* to identify the alignment scores characteristic of ESBLs. In the second set, pairwise alignments were performed for sequences between ESBLs from *E. coli* and *E. coli* O157:H7 peptides to identify putative ESBLs in *E. coli* O157:H7. The top 10 peptides with the highest minimum alignment score were chosen for multiple sequence alignment using Clustal Omega [13] to examine the sequence similarities between the identified peptide to the 17 ESBLs from *E. coli*.

Results and Discussion

Characterization of Known ESBLs from *E. coli*. ESBLs from 17 different strains of *E. coli* were obtained from Genbank. The strains are (i) BVT20 (protein accession AWH12117.1), (ii) BVT8 (protein accession AWH12075.1), (iii) CCT42 (protein accession AWH12101.1), (iv) CCT43 (protein accession AWH12102.1), (v) CCT64 (protein accession AWH12105.1), (vi) CCT7 (protein accession AWH12099.1), (vii) FSEC191 (protein accession ALB75298.1), (viii) FSEC224 (protein accession ALB75299.1), (ix) IBT13 (protein accession AWH12120.1), (x) KK31 (protein accession AHZ64982.1), (xi) KKA (protein accession AHZ64983.1), (xii) MBT42 (protein accession AWH12122.1), (xiii) MKT25 (protein accession AWH12093.1), (xiv) MKT3 (protein accession AWH12124.1), (xv) PA12 (protein accession AHZ64984.1), (xvi) SRT41 (protein accession AWH12111.1), and (xvii) WB6 (protein accession AHZ64981.1). These 17 peptides ranged from 286 to 292 amino acids and of pairwise alignments scores (Figure 1) ranged from 140 to 300 using Smith-Waterman algorithm [11].

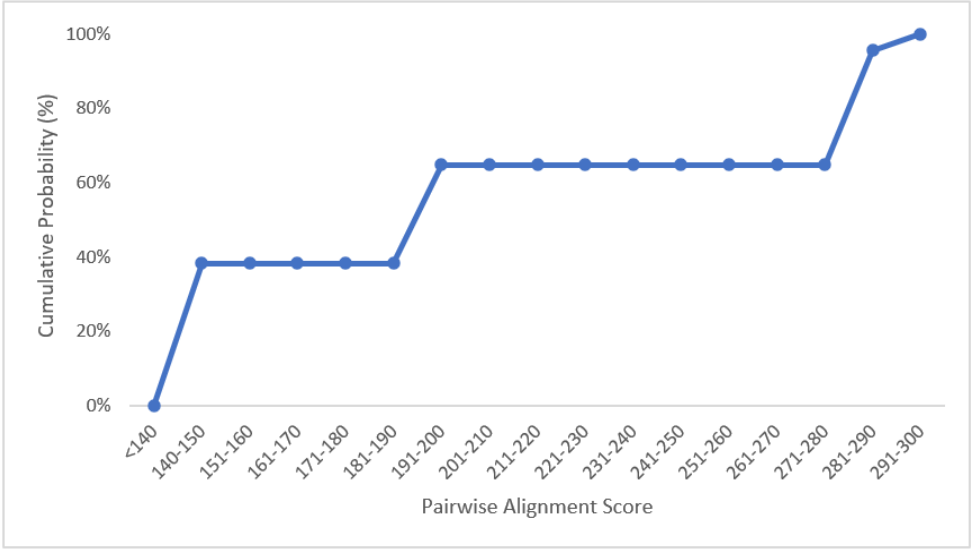

Figure 1: *Pairwise alignment scores of known ESBLs from E. coli.*

57 peptides with 64.7% probability of beta-lactamase activity

The minimum pairwise alignment scores between ESBLs from *E. coli* and *E. coli* O157:H7 peptides performed using Smith-Waterman algorithm [11] ranged from 0 to 247, suggesting an overlap of scores between the two sets of pairwise alignments. 260 peptides from *E. coli* O157:H7 proteome are above the minimum ESBL minimum score of 140. Of which, 57 peptides are above the minimum score of 190 (Table 1). Using similar arguments proposed in earlier studies [10,14-16]; as the range of pairwise alignment scores among baseline sequences represents the sequence diversity of ESBLs; therefore, if a random sequence is not likely a putative ESBL, then its minimum pairwise alignment score with known ESBLs (baseline sequences) should be lower than the minimum pairwise alignment score among known ESBLs. Furthermore, earlier studies [10,14-16] also suggest that the pairwise alignment scores of ESBLs can be used as probabilities for the likelihood of other peptides having similar functions. This is supported by previous studies correlating sequence similarity to function similarity [17-19]. Using this argument, our results suggests that the 57 peptides with minimum score above 190 have 64.7% probability of beta-lactamase activity. Interestingly, none of these 57 peptides (Table 1) suggest of beta-lactamase activity.

Protein Accession	Minimum Score	Locus Tag	Protein Name
WP_014714124.1	247	FNZ21_RS14550	Inverse autotransporter adhesin-like protein YeeJ
WP_000937974.1	243	FNZ21_RS02015	DEAD/DEAH box helicase
WP_000383109.1	236	FNZ21_RS02030	DEAD/DEAH box helicase
WP_001229642.1	229	FNZ21_RS02020	Class I SAM-dependent DNA methyltransferase
WP_000736260.1	226	FNZ21_RS11795	Alpha2-macroglobulin
WP_000114119.1	225	FNZ21_RS02250	DNA phosphorothioation-dependent restriction protein DptH
WP_001302074.1	221	FNZ21_RS20845	ATP-dependent helicase
WP_001289110.1	220	FNZ21_RS25445	Type IV secretion protein Rhs
WP_023436697.1	219	FNZ21_RS01880	Invasin
WP_021502145.1	219	FNZ21_RS13215	Alpha-2-macroglobulin family protein
WP_001310491.1	218	FNZ21_RS08135	Glutamate synthase large subunit
WP_070479907.1	213	FNZ21_RS06195	RHS repeat protein
WP_000014822.1	213	FNZ21_RS18855	RHS repeat protein
WP_000015262.1	213	FNZ21_RS24780	RHS repeat protein
WP_000014801.1	213	FNZ21_RS25670	RHS repeat protein
WP_000509132.1	212	FNZ21_RS00305	RHS repeat protein
WP_000653944.1	210	FNZ21_RS03755	DNA-directed RNA polymerase subunit beta
WP_000263098.1	209	FNZ21_RS03760	DNA-directed RNA polymerase subunit beta
WP_000014969.1	209	FNZ21_RS04025	RHS repeat protein
WP_000572668.1	209	FNZ21_RS23275	Chromosome partition protein MukB
WP_000077784.1	209	FNZ21_RS25330	Enterobactin non-ribosomal peptide synthetase EntF
WP_001033191.1	207	FNZ21_RS06265	Adhesin
WP_012779382.1	206	FNZ21_RS11115	Adhesin-like autotransporter YpjA/EhaD
WP_000970087.1	206	FNZ21_RS11610	Phosphoribosylformylglycinamidine synthase
WP_000139561.1	206	FNZ21_RS19085	ATP-dependent RNA helicase HrpA
WP_000060930.1	205	FNZ21_RS02495	Autotransporter assembly complex protein TamB
WP_001253587.1	205	FNZ21_RS07995	AsmA2 domain-containing protein

WP_001302260.1	205	FNZ21_RS15120	Trifunctional transcriptional regulator/proline dehydrogenase/ L-glutamate gamma-semialdehyde dehydrogenase
WP_010917813.1	205	FNZ21_RS18565	Autotransporter barrel domain-containing lipoprotein
WP_000040373.1	204	FNZ21_RS18795	Nitrate reductase subunit alpha
WP_000628197.1	203	FNZ21_RS19165	Pyruvate:ferredoxin (flavodoxin) oxidoreductase
WP_000096047.1	202	FNZ21_RS03600	Methionine synthase
WP_000515108.1	201	FNZ21_RS14860	Host specificity protein J
WP_001302008.1	201	FNZ21_RS16280	Transcription-repair coupling factor
WP_000032935.1	201	FNZ21_RS17510	Nitrate reductase subunit alpha
WP_001301864.1	200	FNZ21_RS10255	Exodeoxyribonuclease V subunit beta
WP_000515042.1	200	FNZ21_RS19210	Host specificity protein J
WP_000515426.1	199	FNZ21_RS24135	Host specificity protein J
WP_001263828.1	198	FNZ21_RS13190	AIDA-I family autotransporter adhesin YfaL/EhaC
WP_000515612.1	198	FNZ21_RS17100	Host specificity protein J
WP_001126371.1	197	FNZ21_RS01345	Carbamoyl-phosphate synthase large subunit
WP_001301578.1	197	FNZ21_RS12525	Acid-sensing system histidine kinase EvgS
WP_000356841.1	197	FNZ21_RS13770	DUF4132 domain-containing protein
WP_001197863.1	197	FNZ21_RS13980	Multidrug efflux RND transporter permease subunit MdtB
WP_001294772.1	196	FNZ21_RS00590	DNA polymerase III subunit alpha
WP_000514945.1	196	FNZ21_RS18340	Host specificity protein J
WP_000076999.1	196	FNZ21_RS23440	DNA translocase FtsK
WP_001240839.1	195	FNZ21_RS15235	Filamentous hemagglutinin N-terminal domain-containing protein
WP_000573920.1	194	FNZ21_RS25405	Cu(+)/Ag(+) efflux RND transporter permease subunit CusA
WP_001303798.1	193	FNZ21_RS00385	Type VI secretion system membrane subunit TssM
WP_000946911.1	193	FNZ21_RS10245	Exodeoxyribonuclease V subunit gamma
WP_038425866.1	193	FNZ21_RS17970	Host specificity protein J
WP_000177746.1	193	FNZ21_RS25875	Lymphostatin Efa1/LifA
WP_001082875.1	192	FNZ21_RS08825	Beta-galactosidase subunit alpha
WP_001301590.1	192	FNZ21_RS11650	DUF5107 domain-containing protein
WP_001304182.1	191	FNZ21_RS19860	Multidrug efflux RND transporter permease subunit
WP_001132452.1	191	FNZ21_RS25890	Multidrug efflux RND transporter permease subunit

Table 1: *57 peptides with 64.7% probability of beta-lactamase activity.*

Ten peptides with the highest minimum alignment score from 219 to 247 (Table 1) were chosen for multiple sequence alignment using Clustal Omega [13] to examine the sequence similarities between the identified peptide to the 17 ESBLs from *E. coli*. Multiple sequence alignments are commonly used to deduce the evolutionary history of a set of sequences [20] and had been used to infer functional and structural similarities [21,22]. Phylogenetic analysis showed that each of these 10 peptides are clustered within the known ESBLs rather than outgroup (Figure 2). This suggests that these 10 peptides may be evolutionarily and functionally close to several known ESBLs than other known ESBLs; hence, supporting our results from sequence alignments and suggesting a plausibility of putative beta-lactamase activity.

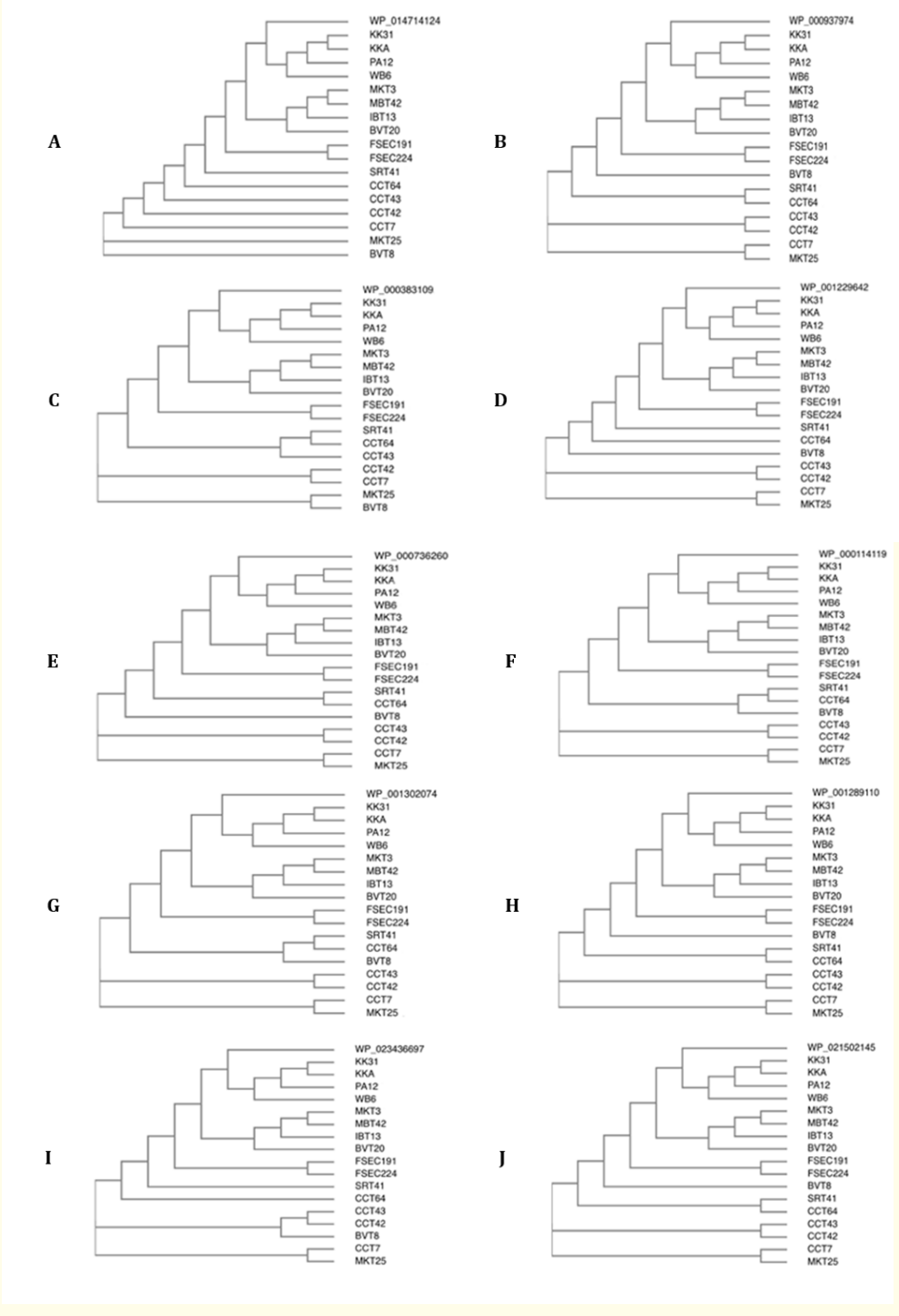

Figure 2: *Phylogenetic analysis top 10 peptides within known ESBLs. The top 10 peptides are (A) Inverse autotransporter adhesin-like protein YeeJ, (B) DEAD/DEAH box helicase, (C) DEAD/DEAH box helicase, (D) Class I SAM-dependent DNA methyltransferase, (E) Alpha2-macroglobulin, (F) DNA phosphorothioation-dependent restriction protein DptH, (G) ATP-dependent helicase, (H) Type IV secretion protein Rhs, (I) Invasin, and (J) Alpha-2-macroglobulin family protein. The minimum pairwise alignment scores and Locus Tags are given in table 1.*

Our results support both the possibility of natural resistance to antibiotics [23] and widespread genetic changes as a result of antibiotic stress [24-26]. Resistance to antimicrobial agents may not be surprising [27]; however, the current increase in resistance rates against the requisite antibiotics has been quite alarming by its accession by the bacteria. Moreover, it is unlikely that resistant strains will revert back to susceptibility [28,29] and putative functions may rapidly evolve [10,15,16]. Hence, it is very crucial to study the mechanism of bacteria becoming resistant to antibiotics in order to tackle any possible outbreak of bacterial resistance [30].

Conclusion

Beta-lactamase pre-dates the use of antibiotics but the question on its origins remains. This study suggests that about 1% of *E. coli* O157:H7 peptides may contain putative beta-lactamases activity and potentially be a source of putative beta-lactamase.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Munita JM and Arias CA. "Mechanisms of Antibiotic Resistance". *Microbiology Spectrum* 4.2 (2016): VMBF-0016-2015.
2. Khan S., *et al.* "Rapid optical determination of β-lactamase and antibiotic activity". *BMC Microbiology* 14.1 (2014): 84.
3. Reygaert WC. "An overview of the antimicrobial resistance mechanisms of bacteria". *AIMS Microbiology* 4.3 (2018): 482-501.
4. Bush K and Bradford PA. "β-Lactams and β-Lactamase Inhibitors: An Overview". *Cold Spring Harbor Perspectives in Medicine* 6.8 (2016): a025247.
5. Bush K. "Past and Present Perspectives on β-Lactamases". *Antimicrobial Agents and Chemotherapy* 10 (2018): e01076-e01018.
6. Hall BG and Barlow M. "Evolution of the serine beta-lactamases: past, present and future". *Drug Resistance Updates Review Comment Antimicrobial Anticancer Chemother* 7.2 (2004): 111-123.
7. Bush K and Jacoby GA. "Updated functional classification of beta-lactamases". *Antimicrobial Agents and Chemotherapy* 54.3 (2010): 969-976.
8. Ur Rahman S., *et al.* "The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases". *BioMed Research International* (2018): 9519718.
9. Bradford PA. "Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat". *Clinical Microbiology Reviews* 14.4 (2001): 933-951.
10. Kwek BZ., *et al.* "Random sequences may have putative beta-lactamase properties". *Acta Scientific Medical Sciences (ASMS)* 3.7 (2019): 113-117.
11. Smith TF and Waterman MS. "Identification of common molecular subsequences". *Journal of Molecular Biology* 147.1 (1981): 195-197.
12. Ling MHT. "SeqProperties: A Python Command-Line Tool for Basic Sequence Analysis". *Acta Scientific Microbiology* 3.6 (2020): 103-106.
13. Madeira F., *et al.* "The EMBL-EBI search and sequence analysis tools APIs in 2019". *Nucleic Acids Research* 47.1 (2019): W636-W641.
14. Thong-Ek C., *et al.* "Potential de novo origins of archaebacterial glycerol-1-phosphate dehydrogenase (G1PDH)". *Acta Scientific Microbiology* 2.6 (2019): 106-110.

15. Ardhanari-Shanmugam KD., *et al.* "De Novo Origination of Bacillus subtilis 168 Promoters from Random Sequences". *Acta Scientific Microbiology* 2.11 (2019): 07-10.

16. Usman S., *et al.* "Pseudomonas balearica DSM 6083T promoters can potentially originate from random sequences". *Journal of Proteomics and Bioinformatics* 8.2 (2019): 66-70.

17. Joshi T and Xu D. "Quantitative assessment of relationship between sequence similarity and function similarity". *BMC Genomics* 8.1 (2007): 222.

18. Wang J., *et al.* "Revealing and avoiding bias in semantic similarity scores for protein pairs". *BMC Bioinformatics* 11.1 (2010): 290.

19. Higdon R., *et al.* "Modeling sequence and function similarity between proteins for protein functional annotation". Proc Int Symp High Perform Distrib Comput (2010): 499-502.

20. Gotoh O. "Multiple sequence alignment: algorithms and applications". *Advances in Biophysics* 36 (1999): 159-206.

21. Shortridge MD and Powers R. "Structural and Functional Similarity between the Bacterial Type III Secretion System Needle Protein PrgI and the Eukaryotic Apoptosis Bcl-2 Proteins. Friedberg I, editor". *PLoS ONE* 4.10 (2009): e7442.

22. Fuertes MA., *et al.* "Evolutionary conserved compositional structures hidden in genomes of the foot-and-mouth disease virus and of the human rhinovirus". *Scientific Reports* 9.1 (2019): 16553.

23. Martínez JL. "Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials". *Frontiers in Microbiology* 3 (2012): 1.

24. Coculescu B-I. "Antimicrobial resistance induced by genetic changes". *Journal of Medicine and Life* 2.2 (2009): 114-123.

25. Blázquez J., *et al.* "Antibiotic-Induced Genetic Variation: How It Arises and How It Can Be Prevented". *Annual Review of Microbiology* 72.1 (2018): 209-230.

26. Laehnemann D., *et al.* "Genomics of rapid adaptation to antibiotics: convergent evolution and scalable sequence amplification". *Genome Biology and Evolution* 6.6 (2014): 1287-1301.

27. Aslam B., *et al.* "Antibiotic resistance: a rundown of a global crisis". *Infection and Drug Resistance* 11 (2018): 1645-1658.

28. Castillo CF and Ling MH. "Resistant Traits in Digital Organisms Do Not Revert Preselection Status despite Extended Deselection: Implications to Microbial Antibiotics Resistance". *BioMed Research International* (2014): 648389.

29. Castillo CF., *et al.* "Resistance Maintained in Digital Organisms Despite Guanine/Cytosine-Based Fitness Cost and Extended De-Selection: Implications to Microbial Antibiotics Resistance". *MOJ Proteomics Bioinforma* 2.2 (2015): 00039.

30. Kapoor G., *et al.* "Action and resistance mechanisms of antibiotics: A guide for clinicians". *Journal of Anaesthesiology Clinical Pharmacology* 33.3 (2017): 300-305.

Volume 16 Issue 8 August 2020