Distribution of Biofilm-associated Genes among Acinetobacter baumannii by in-silico PCR

A. Aldrin Joshua a, A. S. Smiline Girija b*, P. Sankar Ganesh c and J. Vijayashree Priyadharsini c

a Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences
Saveetha University, Chennai - 600077, Tamil Nadu, India.

b Department of Microbiology, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India.

c Department of Microbiology, Saveetha Dental College and Hospitals, SIMATS, Saveetha University, Chennai-600 077, Tamil Nadu, India.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Background: Acinetobacter baumannii is a coccobacillus that is Gram negative, non motile, non fermentative and oxidase negative. It is the most common and successful nosocomial pathogen recognised by WHO. This dreadful pathogen causes urinary tract infections, ventilator associated pneumonia (VAP), bacteremia, etc. These infections are most common in hospital wards especially Intensive Care Unit (ICU). The infections are due to biofilm formation by the virulent genes of A. baumannii, and the common biofilm-associated genes of A. baumannii were bap, csuE, fimH, epsA, bfmS, ptk, pgaB, ompA, blaPER-1. Among these, bap, epsA and ompA genes are highly prevalent among the clinical strains of A. baumannii.

Aim: To detect the three vital biofilm-associated genes of A. baumannii by in-silico PCR analysis.

Materials and Methods: 19 isolates of A. baumannii were selected and 3 target genes, namely epsA, ompA and bap gene were used for the amplification process through in-silico PCR simulation tools. Evolutionary analysis was done for the ompA gene.

Results: The epsA gene was expressed in 10.52% of the total strains selected with the highest occurrence of ompA gene as 57.89%. bap gene was not observed from the study strains included. From evolutionary analysis based on ompA distributed strains, the Acinetobacter baumannii SDF

*Corresponding author: E-mail: smilinegirja.sdc@saveetha.com;
and Acinetobacter baumannii BJJAB0715 might be the parental strains where the evolution of strains would have started. Through successive generations, the Acinetobacter baumannii MDR-ZJ06 and Acinetobacter baumannii TYTH-1 had become the multidrug resistant strains present in the environment.

**Conclusion:** The findings of the study confirms the distribution of epsA and ompA genes among the 19 different strains of A. baumannii. The study suggests periodical monitoring of biofilm based virulence genes among the clinical strains and to curtail the A. baumannii infections.

**Keywords:** Acinetobacter baumannii; biofilm; innovative in-silico PCR; novel ompA gene; epsA gene; bap gene; environmental strains.

1. **INTRODUCTION**

*Acinetobacter baumannii* is a coccobacillus that is Gram negative, non motile, non fermentative and oxidase negative. It is the most common and successful nosocomial pathogen as recognised by the World Health Organization (WHO). This dreadful pathogen causes urinary tract infections, ventilator associated pneumonia (VAP), bacteremia and even soft tissue, abdominal, CNS infections. These microorganisms also develop infections in the urinary catheter and cause severe recalcitrant infections. These infections are most common in hospital wards especially in the Intensive Care Units (ICU) [1]. The infections are attributed to the biofilm formation by the virulent genes of *A. baumannii*, and the common biofilm-associated genes of *A. baumannii* were *bap, csuE, fimH, epsA, bfmS, ptk, pgaB, ompA, bla<sub>PER</sub>* [2]. Among these, *bap, epsA* and *ompA* genes are highly prevalent among the clinical strains of *A. baumannii*. Biofilm formation occurs in three stages, early development, matrix formation and maturation where more iron uptake causes biofilm formation by *A. baumannii* [3].

*ompA* gene is the abundant surface protein responsible for serum resistance and biofilm formation. The AIS-0316 gene encodes *ompA* for a putative transcription factor, they act as anti-repressor on the promoter region which inhibits AbH-NS protein for transcriptional regulation [4]. They have enhanced mortality due to abundant beta-barrel protein which is encoded by the outer membrane and induces mitochondrial fragmentation for its pathogenesis [5]. Most of the gene belongs to the usher chaperone assembly system, where its products form pious bundle structure in the bacterium and form an important factor in biofilm formation [6].

*bap* gene codes for the development of three-dimensional biofilm towers and channels on both biotic and abiotic surfaces [7]. *fimH* gene was the most virulent gene detected in 74% of blood samples which was collected from nosocomial infections [8]. *A. baumannii* produces exopolysaccharide or EPS encoded by *epsA* which accumulates on the cell surface and renders protection for the cell against harsh environment [9]. *epsA* gene from EPS is a sticky cell formed in the biofilm which is located in the extracellular matrix of the mitochondria. It is responsible for 50 to 90% of total organic carbon found in biofilm, which helps in bacterial survival and resistance. The production of EPS plays a vital role in aggregation of bacteria in the biofilm formation, in which the P1-P8 genome decreases the expression of genes in the amplification process. *bfmS* regulates cell envelope structure for virulence and biofilm formation, acts as sensor kinase and is one of the most important virulence genes [10]. The distribution of *ptk* gene was found to be 95% which was collected from 100 isolates [11] from immunocompromised patients in ICU. The most common pattern of presence of biofilm genes was found to be *bfmS-csuE-epsA-bap-kpsMT-ompA-pta-pgaB*. The previous work for drug resistant *A. baumannii* was conducted as clinical studies from urine samples collected from urinary tract infected patients [12–14] and through conventional analytical tests and also by PCR [15]. Through most of the research works, it is confirmed that *A. baumannii* isolates colonise more on the respiratory tract, urinary tract causing severe infections. Many studies documenting the drug resistant isolates, not many studies are related to the co-occurrence of biofilm genes among the drug-resistant strains. In-silico PCR analysis is a useful tool for ensuring primer specificity and identifies mismatches in primer binding sites. It avoids amplification of unwanted amplicons and is useful in detection of any pathogens, gene discovery and molecular diagnosis [16,17]. So, the aim of the study was to detect the three vital biofilm-associated genes of *A. baumannii* by in-silico PCR analysis.
2. MATERIALS AND METHODS

Study Setting: The present study was an observational \textit{in-silico} study done in the Department of Microbiology, Saveetha Dental College and Hospital. Institutional approval for the research was obtained and the SRB approval number is IHEC/SDC/UG-1906/21/147.

2.1 Strains Used in the Present Study

Genome sequences of the \textit{Acinetobacter baumannii} strains used in the study are provided under Table 1. The analysis of genomic strains was done in \textit{in-silico} simulation tools where 19 isolates of \textit{A. baumannii} were selected from a select default tool and used for distribution of the biofilm-associated genes [23].

2.2 Primers Used in the Study

3 most common biofilm-associated genes, namely \textit{ompA}, \textit{epsA}, \textit{bap} gene were opted for the study [24] and the primers used for detection of selected biofilm-associated genes are given in Table 2.

2.3 \textit{in-silico} PCR Amplification

The amplification of biofilm-associated genes which was characterised were performed using \textit{in-silico} PCR simulation tools (http://insilico.ehu.es/PCR/). There are a high variety of web servers useful in designing primer sequences and computational optimisation which is used for conditioning the PCR. The primers are validated successfully for GC clamp, self annealing and hairpin formation using the ‘PCR Primer stat’ program. The web server used was \textit{insilico}.ehu.es and the genus was selected as \textit{Acinetobacter}. Selected primers were given as input in the forward and reverse primers column and the command of amplify was performed. The bands generated were analyzed for the frequency of its presence among the selected strains. The presence of genes in different strains of \textit{A. baumannii} were provided through this tool in a few seconds [25,26].

2.4 Evolutionary Analysis by Maximum Likelihood Method

The evolutionary history was concluded through the Maximum Likelihood method, using the Tamura Nei model. The evolutionary analysis included 14 nucleotide sequences. It was conducted in Mega X software. The bands from \textit{in-silico} PCR amplification were used and placed in this software. The evolutionary analysis was done for all the three biofilm genes, where only \textit{ompA} gene associated strains was selected for phylogenetic analysis and was given in Fig. 3.

3. RESULTS

From the results analysed from \textit{in-silico} PCR simulation tool, it is inferred that \textit{epsA} gene was present in 2 strains, \textit{ompA} gene was present in 11 strains and \textit{bap} gene was not present in any of the strains. The \textit{epsA} gene was present in strains namely \textit{A. baumannii} AB307-0294, \textit{A. baumannii} AYE and not present in other strains (Fig. 1). The \textit{epsA} gene showed the highest annealing temperature of 60°C with the DNA amplicon size as 451bp. There was no need for phylogenetic analysis for \textit{epsA} associated strains as it was present in only 2 strains. The \textit{epsA} gene was expressed in 10.52% of the total genome of \textit{A. baumannii} which is shown in Fig.4.

\textit{ompA} gene was present in 11 strains from 19 selected isolates, namely \textit{Acinetobacter baumannii} 1656-2 chromosome, \textit{Acinetobacter baumannii} ACICU, \textit{Acinetobacter baumannii} ATCC 17978, \textit{Acinetobacter baumannii} BJAB07104, \textit{Acinetobacter baumannii} BJAB0715, \textit{Acinetobacter baumannii} BJAB0868, \textit{Acinetobacter baumannii} MDR-TJ, \textit{Acinetobacter baumannii} MDR-ZJ06, \textit{Acinetobacter baumannii} TYTH-1, \textit{Acinetobacter baumannii} ZW85-1, \textit{Acinetobacter baumannii} SDF (Fig. 2). The \textit{ompA} gene showed an annealing temperature of 58°C and DNA amplicon size of 531bp. The phylogenetic tree for \textit{ompA} associated strains was provided in Fig. 3. The \textit{ompA} gene was expressed in 57.89% of the total genome of \textit{A. baumannii} which is shown in graph 1. \textit{bap} gene was not associated with these 19 selected strains as there may be presence of other virulent genes found in them.

The phylogenetic tree shows that the \textit{Acinetobacter baumannii} SDF and \textit{Acinetobacter baumannii} BJAB0715 might be the parental strains where the evolution of strains would have started (Fig. 3). Through successive generations, the \textit{Acinetobacter baumannii} MDR-ZJ06 and \textit{Acinetobacter baumannii} TYTH-1 had become the multidrug resistant strains present in the environment. The isolates possessed \textit{ompA} gene in higher frequency which might be the reason for multidrug resistance, biofilm formation and causative of severe infections (Fig. 4).
Table 1. Strains used for the in-silico PCR analysis and the detection of epsA, OmpA and bap genes

| S.No | Strains of *Acinetobacter* | epsA gene | ompA gene | bap gene |
|------|---------------------------|------------|-----------|----------|
| 1    | *Acinetobacter* DR1       | -          | -         | -        |
| 2    | *Acinetobacter* ADP1      | -          | -         | -        |
| 3    | *Acinetobacter* baumannii SDF | -     | +         | -        |
| 4    | *Acinetobacter* baumannii TCDC-AB0715 | -     | -         | -        |
| 5    | *Acinetobacter* baumannii 1656-2 chromosome | - | +       | -        |
| 6    | *Acinetobacter* baumannii ACICU | - | +       | -        |
| 7    | *Acinetobacter* baumannii MDR-TJ | - | +       | -        |
| 8    | *Acinetobacter* baumannii BJAB07104 | - | +       | -        |
| 9    | *Acinetobacter* baumannii MDR-JJ06 | - | +       | -        |
| 10   | *Acinetobacter* baumannii BJAB0868 | - | +       | -        |
| 11   | *Acinetobacter* baumannii TYTH-1 | - | +       | -        |
| 12   | *Acinetobacter* baumannii BJAB0715 | - | +       | -        |
| 13   | *Acinetobacter* baumannii ATCC 17978 | - | +       | -        |
| 14   | *Acinetobacter* baumannii AYE | + | -       | -        |
| 15   | *Acinetobacter* baumannii AB307-0294 | + | -       | -        |
| 16   | *Acinetobacter* baumannii AB0057 | - | -       | -        |
| 17   | *Acinetobacter* baumannii ZW85-1 | - | +       | -        |
| 18   | *Acinetobacter* baumannii D1279779 | - | -       | -        |
| 19   | *Acinetobacter* pittii PHEA-2 | - | -       | -        |

Fig. 1. In-silico PCR amplification showing the amplicons of epsA with an amplicon size of 451 bp
Fig. 2. In-silico PCR amplification showing the amplicons of \textit{ompA} with an amplicon size of 531bp

Fig. 3. Phylogenetic tree constructed based on the \textit{ompA} gene identified in different strains of \textit{A. baumannii}
Table 2. Primers and PCR conditions used for the detection of the three target genetic determinants

| Target Genes | Primers sequences (5’–3’) | Annealing Temperature(°C) | DNA amplicon Size (bp) |
|--------------|---------------------------|--------------------------|-----------------------|
| epsA         | AGCAAGTGTTATCCAATCG    | 60                        | 451                   |
|              | ACCAGACTCACCCATTACAT  |                          |                       |
| ompA         | CGCTTCTGCTGGTGCTGAAT | 58                        | 531                   |
|              | CGTCACGTAGCGTTAGGGTA  |                          |                       |
| bap          | TACTTCCAATCCAATGCTAGGGGCTACGACAAATGCAGTAGGCTACGTTAGGGTA | 55                        | 1225                  |
|              | TTATCCACCTCCAATGATCAGCAACAAACCCTACGCTACGTTAGGGTA       |                          |                       |

Fig. 4. Graph showing the distribution of biofilm genes among 19 strains of *A. baumannii*. X-axis denotes the biofilm genes detected in the clinical isolates and the Y-axis represents the strains among which the biofilm genes were detected. *ompA* gene was frequently distributed at 57.89% followed by *epsA* gene at 10.52% among the strains under study. No *bap* gene was detected among the selected strains.

4. DISCUSSION

The present investigation documents the distribution of biofilm-associated genes among the virulent strains of *A. baumannii*. Higher frequency was observed with *ompA* and *epsA* that correlates with an earlier study [22]. *ompA* was present in 11 strains and *epsA* gene was present in 2 strains. The previous study analysed 17 genomes where the current study used 19 genomes and genotype 2 and 3 encoded for 1 to 15 and 6 to 10 genes for virulence factors. The *ompA* gene was present in 57.89% and *epsA* gene was present in 10.52% of the total strains. Another work by Eze et al., 2018 [23] concluded that out of 24 clinical isolates selected, 22 expressed *bap* gene which was 91.66% but in our study, *bap* gene was not associated with the selected strains. The *ompA* gene was expressed in 100% of the strains in the previous study but in the present study, it was expressed in 11 out of 19 selected strains which should be 57.89% and *epsA* gene was 10.52%.
The work by Russo et al., 2010 [24] documented on the *A. baumannii* AB 307-0294 strain possessing an *epsA* gene which was similar to present study where *epsA* gene was observed among our strains as well. The *bap* gene expressed none of the strains which is in contrast with the earlier study where *bap* gene was present in about 66% of total strains. The previous study also found that annealing temperature for *ompA* gene was 56°C where in present study it was 58°C. The DNA amplicon size for the genes was found to be decreased when compared with previous author's work.

Another research by Thummeepak et al., 2016 [25] reported on the presence of *ompA* gene in 84.4% of total genomic strains together with *bap* gene in 48% but in the present study there is a lesser distribution of selected biofilm genes. It may be due to the presence of other genes like *limH, csuE, pgaB*, etc., which makes the difference in distribution. Our study selected 3 target genes for 19 strains but in previous work, it was seven target genes. Yet in another study *bap* gene was present in 92% of total strains but in the current study, no distribution was found [26].

This type of research is the need of the hour in all the laboratories for epidemiological surveillance. The present study did not undergo any experimental verification which may be considered as the limitation of the same. In recent years, multidrug-resistant *A. baumannii* has become more prevalent in hospital wards, ICU and it has become a more dreadful pathogen causing severe infections. These genes could be further targeted for advanced therapeutic strategies such as gene therapy, as WHO had recognized this pathogen to be a critical priority for the need of antibiotics through research and development, and this study forms a platform for future researchers and can be taken up by geneticists for their research to provide an efficient treatment strategy for *A. baumannii* associated infections. Though there is much research undergoing in the microbial field related to the bioinformatics analysis [27–35]. Pertained to dentistry many studies are in association with covid-19 pandemic, dental materials and oro-dental infections [36–40] in-silico based tools and databases are promising to evaluate the virulence factors. The computational approach also holds good to evaluate and analyze the factors associated with systemic diseases and also to detect compounds from natural sources [41], [42], [43].

Computational detection of resistant determinants using the tools holds good for all the bacterial pathogens (44) and also to evaluate novel compounds from both marine and plant compounds (45, 46). The limitation of the study is that it was carried out as a computational approach, and thus the future prospects are set to evaluate the same using the clinical strains for the frequency of the genetic determinants.

5. CONCLUSION

The present investigation documents the role of various genetic determinants that contribute to the pathogenesis of *A. baumannii* in health-care settings. Among the various virulence factors, the findings of the study confirms the distribution of *epsA* and *ompA* genes among the 19 different strains of *A. baumannii*. In addition, the study recommends the in-silico PCR tool as an efficient methodology for the preliminary selection of the virulence determinants in the pathogenic bacteria. It also suggests the need of periodical monitoring of biofilm based virulence genes in all the health care settings to curtail the *A. baumannii* infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

FUNDING SOURCE

The present study was supported by Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha Dental College and Hospitals, Saveetha University, Chennai and was funded by Sanitary Officers association.

REFERENCES

1. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother 2007;51:3471–84.


2. Bardbari AM, Arabestani MR, Karami M, Keramat F, Alikhani MY, Bagheri KP. Correlation between ability of biofilm formation with their responsible genes and MDR patterns in clinical and environmental Acinetobacter baumannii isolates. Microb Pathog 2017;108:122–8.

3. Azizi O, Etal SMR. Class 1 integrons in non-clonal multidrug-resistant Acinetobacter baumannii from Iran, description of the new bla IMP-55 allele in In1243. Journal of Medical Microbiology 2016;65:928–36. https://doi.org/10.1099/jmm.0.000315.

4. Oh K-W, Kim K, Islam MM, Jung H-W, Lim D, Lee JC, et al. Transcriptional Regulation of the Outer Membrane Protein A in Acinetobacter baumannii. Microorganisms 2020;8. https://doi.org/10.3390/microorganisms8050706.

5. Tiku V, Kofoed EM, Yan D, Kang J, Xu M, Reichelt M, et al. Outer membrane vesicles containing OmpA induce mitochondrial fragmentation to promote pathogenesis of Acinetobacter baumannii. Sci Rep 2021;11:618.

6. Azizi O, Shahcheraghi F, Salimizand H, Modarresi F, Shakibaie MR, Mansouri S, et al. Molecular Analysis and Expression of bap Gene in Biofilm-Forming Multi-Drug-Resistant Acinetobacter baumannii. Rep Biochem Mol Biol 2016;5:62–72.

7. Luo TL, Rickard AH, Srinivasan U, Kaye KS, Foxman B. Association of blaOXA-23 and bap with the persistence of Acinetobacter baumannii within a major healthcare system. Front Microbiol 2015;6:182.

8. Montaz H, Seifati SM, Tavakol M. Determining the prevalence and detection of the most prevalent virulence genes in Acinetobacter baumannii isolated from hospital infections. International Journal of Medical Laboratory 2015;2:87–97.

9. Thummeepak R, Kongthai P, Leung tongkam U, Sithisak S. Distribution of virulence genes involved in biofilm formation in multi-drug resistant Acinetobacter baumannii clinical isolates. Int Microbiol 2016;19:121–9.

10. Kim SY, Kim MH, Kim SI, Son JH, Kim S, Lee YC, et al. The sensor kinase BtmS controls production of outer membrane vesicles in Acinetobacter baumannii. BMC Microbiol 2019;19:301.

11. Zeighami H, Valadkhani F, Shapouri R, Samadi E, Haghi F. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. BMC Infect Dis 2019;19:629.

12. Girija A, Priyadharsini JV, A P. Prevalence of Acb and non-Acb complex in elderly population with urinary tract infection (UTI). Acta Clin Belg 2021;76:106–12.

13. Girija SA, Jayaseelan VP, Arumugam P. Prevalence of VIM- and GIM-producing Acinetobacter baumannii from patients with severe urinary tract infection. Acta Microbiol Immunol Hung 2018;65:539–50.

14. Smiline Girija AS, Others. CLSI based antibiogram profile and the detection of MDR and XDR strains of Acinetobacter baumannii isolated from urine samples. Med J Islam Repub Iran 2019;33:3.

15. Smiline ASG, Vijayashree JP, Paramasivam A. Molecular characterization of plasmid-encoded blaTEM, blaSHV and blaCTX-M among extended spectrum β-lactamases [ESBLs] producing Acinetobacter baumannii. Br J Biomed Sci 2018;75:200–2.

16. Yu B, Zhang C. In silico PCR analysis. Methods Mol Biol. 2011;760:91–107.

17. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach. Acta Virol. 2020;64(1):93–9.

18. Priyadharsini JV, Girija ASS, Paramasivam A. An insight into the emergence of Acinetobacter baumannii as an oro-dental pathogen and its drug resistance gene profile--An in silico approach. Heliyon. 2018;4(12):e01051.

19. Girija SA, Priyadharsini JV, Paramasivam A. Prevalence of carbapenem-hydrolyzing OXA-type β-lactamases among Acinetobacter baumannii in patients with severe urinary tract infection. Acta Microbiol Immunol Hung. 2019 Dec 9;67(1):49–55.

20. Usanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reseprine. Nat Prod Res. 2021 Jun;35(11):1893–8.

21. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as
antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441–8.

22. Priyadharshini JV, Vijayashree Priyadharshini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Vol. 94, Archives of Oral Biology. 2018. p. 93–8.

23. Eze EC, Chenia HY, El Zowalaty ME. Acinetobacter baumannii biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. Infect Drug Resist. 2018 Nov 15;11:2277–99.

24. Russo TA, Luke NR, Beanan JM. The K1 capsular polysaccharide of Acinetobacter baumannii strain 307-0294 is a major virulence factor. Infect Immun. 2010 Sep;78(9):3993–4000.

25. Thummeepak R, Kitti T, Kunthalert D, Sitthisak S. Enhanced Antibacterial Activity of Acinetobacter baumannii Bacteriophage ØABP-01 Endolysin (LysABP-01) in Combination with Colistin. Front Microbiol. 2016 Sep 7;7:1402.

26. Fallah A, Rezaee MA, Hasani A, Barghagi MHS, Kafil HS. Frequency of bap and cpaA virulence genes in drug resistant clinical isolates of Acinetobacter baumannii and their role in biofilm formation. Iran J Basic Med Sci. 2017 Aug;20(8):849–55.

27. Priyadharshini JV, Vijayashree Priyadharshini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Vol. 94, Archives of Oral Biology. 2018. p. 93–8.

28. Paramasivam A, Vijayashree Priyadharshini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020 Feb;43(2):153–4.

29. Vijayashree Priyadharshini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of Acinetobacter baumannii as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. Heliyon. 2018 Dec;4(12):e01051.

30. Paramasivam A, Vijayashree Priyadharshini J. Novel insights into m6A modification in circular RNA and implications for immunity. Cell Mol Immunol. 2020 Jun;17(6):668–9.

31. Paramasivam A, Priyadharssini JV, Raghnandhakumar S. Implications of m6A modification in autoimmune disorders. Cell Mol Immunol. 2020 May;17(5):550–1.

32. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoVID-19) Leading to Acute Respiratory Distress Syndrome? Front Immunol. 2020 May 27;11:1206.

33. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. Hypertens Res. 2020 Jan;43(1):74–5.

34. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from Porphyromonas gingivalis with the bioactive compounds from Rosmarinus officinalis. Asian Biomed. 2019 Oct 1;13(5):197–203.

35. Kumar SP, Girija ASS, Priyadharssini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from Ganoderma lucidum: A computational study. pharmaceutical-sciences [Internet]. 2020;82(2).

36. Samuel SR, Kuduruthullah S, Khair AMB, Shayeob MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021 May;31(3):436–41.

37. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285–6.

38. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021 Jun;126:105132.

39. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? J Dent Sci. 2020 Dec;15(4):562–3.

40. Reddy P, Kritikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. Oral Health Prev Dent. 2020 Apr 1;18(1):379–86.

41. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. Hypertens Res. 2020 Dec;43(12):1459–61.
42. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of A. baumannii and targeting with essential oil compounds from Azadirachta indica. Journal of King Saud University - Science. 2020 Dec 1;32(8):3380–7.

43. Girija AS. Fox3 (+) CD25 (+) CD4 (+) T-regulatory cells may transform the nCoV's final destiny to CNS! Journal of medical virology, 2020:9:5623-27.

44. S Sivakumar, ASS Girija, JV Priyadharsini. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in Streptococcus sp., using computational. Journal of King Saud University-Science 32 (1), 904-909.

45. Smiline Girija A.S, Pandi Suba.K. BEHP - a phthalate derivative characterized from the south Indian squid and its anti-HCV like property: An in-vitro and in-silico analysis. International Journal of Pharma and Bio Sciences 6 (1B), 401 – 41.

46. VP Vaishnavi S, Preetha Chaly, Smiline Girija AS, Raghuraman R, Pandi Suba. Antimicrobial activity of Gotukola leaves and neem leaves: A comparative in-vitro study. Int J of Ayurveda and Holisitc Medicine 3 (3), 11-15.

© 2021 Joshua et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.