Indigenous Oral and Gut Phages Defeat the Deadly NDM-1 Superbug

Bioinformatics and Biology Insights
Volume 17: 1–4

DOI: 10.1177/11779322231182767

Pradeep Kumar Yadalam[1], Raghavendra Vamsi Anegundi[1], Ramya Ramadoss[2], M Saravanan[3], AshokKumar Veeramuthu[4] and Artak Heboyan[5]

[1]Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. [2]Department of Oral Pathology & Oral Biology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. [3]Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. [4]Centre for Transdisciplinary Research, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. [5]Department of Prosthodontics, Faculty of Stomatology, Mkhitar Heratsi Yerevan State Medical University, Yerevan, Armenia.

ABSTRACT

AIM: Antibiotics treat various diseases by targeting microorganisms by killing them or reducing their multiplication rate. New Delhi Metallo-beta-lactamase-1 (NDM-1) is produced by bacteria possessing the resistance gene blaNDM-1, the enzyme that makes bacteria resistant to beta-lactams. Bacteriophages, especially Lactococcus, have shown their ability to break down lactams. Hence, the current study computationally evaluated the binding potential of Lactococcus bacteriophages with NDM using Molecular docking and dynamics.

METHODS: Modelling of NDM I-TASSER for Main tail protein gp19 OS=Lactococcus phage LL-H or Lactobacillus delbrueckii subsp. lactis after downloading from UNIPROT ID- Q38344. Cluspro tool helps in Understanding cellular function and organization with protein-protein interactions. MD simulations(19) typically compute atom movements over time. Simulations were used to predict the ligand binding status in the physiological environment.

RESULTS: The best binding affinity score was found -1040.6 Kcal/mol compared to other docking scores. MD simulations show in RMSD values for target remains within 1.0 Angstrom, which is acceptable. The ligand-protein fit to receptor protein RMSD values of 2.752 fluctuates within 1.5 Angstrom after equilibration.

CONCLUSIONS: Lactococcus bacteriophages showed a strong affinity to the NDM. Hence, this hypothesis, supported by evidence from a computational approach, will solve this life-threatening superbug problem.

KEYWORDS: Lactococcus, protein, bacteriophage, oral biology, oral microflora

RECEIVED: January 13, 2023. **ACCEPTED:** May 30, 2023.

TYPE: Short Report

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHORS: Pradeep Kumar Yadalam, Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India. Email: yadalam1980@gmail.com

Artak heboyan, department of prosthodontics, faculty of stomatology, mkhitar heratsi yerevan state medical university, 2 koryun street, yerevan 0025, armenia. Email: heboyan.artak@gmail.com

Introduction

Antibiotics treat various diseases by targeting different microorganisms by killing them or reducing their multiplication rate. [1] Antibiotics are classified into several categories based on how they eradicate microorganisms. Beta-lactam antibiotics are the most common antibiotic used to treat common bacterial infections.[2] When beta-lactam antibiotics are no longer effective, another class of antibiotics called carbapenems is often used as a last option. A bacterium is said to resist an antibiotic if it no longer kills it.[3] Few or no treatments are available for some bacteria because they are highly resistant to antibiotics which are referred to as Superbugs. There is currently no treatment available for these superbugs.[4]

Highlights

- New Delhi metallo-beta-lactamase 1 (NDM-1) makes bacteria resistant to beta-lactams. The NDM-1 can be contagious and spread from person to person through contamination.
- The current study identifies an indigenous bacterium, Lactococcus bacteriophage LL-H, as a potential inhibitor of the NDM-1 Strain.
- As it is commonly available in the oral and gut, this finding might have a tremendous impact, if found successful, in combating antibiotic resistance.

New Delhi metallo-beta-lactamase 1

The NDM-1 is produced by bacteria possessing the resistance gene blaNDM-1. The enzyme NDM-1 makes bacteria resistant to beta-lactams. Antibiotics such as carbapenems are widely used to treat antibiotic-resistant bacterial infections. The gene for NDM-1 can be transmitted from one strain of bacteria to another via horizontal gene transfer.[5] Gram-negative bacteria

such as *Escherichia coli* and *Klebsiella pneumonia* are the most common secretors of this enzyme.[4]

The NDM-1 is of significant public concern. The emergence of antibiotic resistance is disastrous as most antibiotics will no longer be effective in infection management. The NDM-1 can be contagious and spread from person to person through contact with contaminated hands or objects.[6] Already, this strain has been found in various countries, including the United States, Great Britain, Sweden, Canada, Australia, and Japan, and the number is reportedly increasing. If this pandemic emerges as a serious concern, it could be lethal because most health care systems would be paralyzed by antibiotic inefficiency.[7]

Lactococcus bacteriophage LL-H

The first Lactococcus bacteriophage (BP) LL-H genome to be fully sequenced was found in 1996. Four of the 52 open reading frames in the 34.6-kb LL-H double-stranded DNA genome can be identified on the complementary strand. Sugar fermentation and decarboxylation generate buffered conditions for lactic acid bacteria (LAB) to thrive in the human digestive tract.[8] The LAB and the host have complex molecular interactions. The phage lytic cycle begins with a powerful interaction between phage proteins at the tail tip and specific cell wall–binding receptors. After successful attachment, phage nucleic acid enters the cell, multiplies within it, and generates phage progeny. In *E coli* and other gram-negative bacteria, phage adhesion to cell surface receptors has been extensively studied.[9] Researchers identified and characterized phages from various species of bacteria from oral biofilms. Only a few studies have demonstrated the presence of Lactococcus phage colonies in oral plaque and the gut.[10]

Bacteriophages are viruses that can infect and kill bacteria without harming human or animal cells. Hence, they may be used to treat bacterial infections alone or in combination with antibiotics. Researchers are rethinking that BPs can be used after the emergence of multiple life-threatening bacterial-resistant infections.[11] Bacteriophage has been studied in vitro in animals and humans in the United States and Europe. They live in soil, seas, oceanic and terrestrial surfaces, and harsh settings, such as extreme temperatures. It is impressive that lytic phages can kill bacteria without disrupting the beneficial microbiota, eliminating antibiotic-resistant superinfections or fewer drug responses.[8] Researchers are exploring using BPs to treat bacterial infections due to the exponential growth of multi-drug-resistant bacteria and a fall in developing and producing novel antibacterial medicines. However, current knowledge does not allow BPs in the clinical setup. Bacteriophages, especially Lactococcus, have shown their ability to break down lactams; however, their use has not been tried against NDM-1 clinically.[12] Besides, Lactococcus is the most common biological entity in nature, safe, and has been shown to effectively fight and destroy multi-drug-resistant bacteria. Hence, this study provides evidence for targeting NDM-1 with *Lactobacillus delbrueckii* BP using a computational approach.

Methodology

Desmond, a software from Schrödinger LLC, was used to study 100 ns molecular dynamics (MD) simulations.[13] In MD simulation, receptor-ligand docking was the first valuable step because it provides a static view of the molecule's binding position at the protein's active site.[14] The MD simulations generally predict ligand binding status in physiological milieu by incorporating Newton's classical equation of motion.[15,16] Receptor-ligand complex was preprocessed (optimization and minimization) using Maestro's Protein Preparation Wizard. This step removed steric clashes, bad contacts, and distorted geometries. System Builder tool was used to build all systems, while TIP3P (Transferable Intermolecular Interaction Potential 3 Points), an orthorhombic box, was used as a solvent model with OPLS_2005 force field.[17] Counterions were used to neutralize the models and 0.15 M sodium chloride (NaCl) added to simulate physiological conditions with 300 K temperature and 1 atm pressure throughout the simulation period. Before simulation, models were loosened. For inspection, trajectories were stored after every 100 ps and protein-ligand stability was confirmed by root-mean-square deviation (RMSD) over time.

Modeling of Lactococcus Phage Tail Protein Using I TASSER

I-TASSER (Iterative Threading ASSEmbly Refinement)[18] is a hierarchical approach to protein structure prediction and structure-based function annotation. It first identifies structural templates from the PDB by the multiple threading approach. The continuous fragments excised from the PDB templates are reassembled into full-length models by replica-exchange Monte Carlo simulations with the unaligned threading regions (mainly loops) built by ab initio modeling. The fragment assembly simulation is performed again in the third step, starting from the SPICKER cluster centroids. The spatial restraints collected from both the LOMETS templates and the PDB structures by TM-align are used to guide the simulations. The crystal structure of ndm-1 was obtained from PDB id-5ypl.

To model, the structure of ligand I-TASSER is used. Below is the given FASTA sequence and downloaded from UNIPROT ID-Q38344:

>tr|Q38344|Q38344_BPLLH Main tail protein gp19 OS=Lactococcus phage LL-H or *Lactobacillus delbrueckii* subsp. lactis phage OX=12348 GN=g17 PE=4 SV=1

MALQTTQPFFPHNWQTGYFIDTKGGLDPKATT-SATFASLAAFVTGVTPAPNDVNDTSIYF

ADEDNASPEKTGTAYTWAVTGHVLVGDPACNY-ILNLYQTHATGNAAKTLLKIVYPNGKTL

ITRVVVQSIVVGGGNGNAKQTLTFTLAESGK-GILSDTVGA

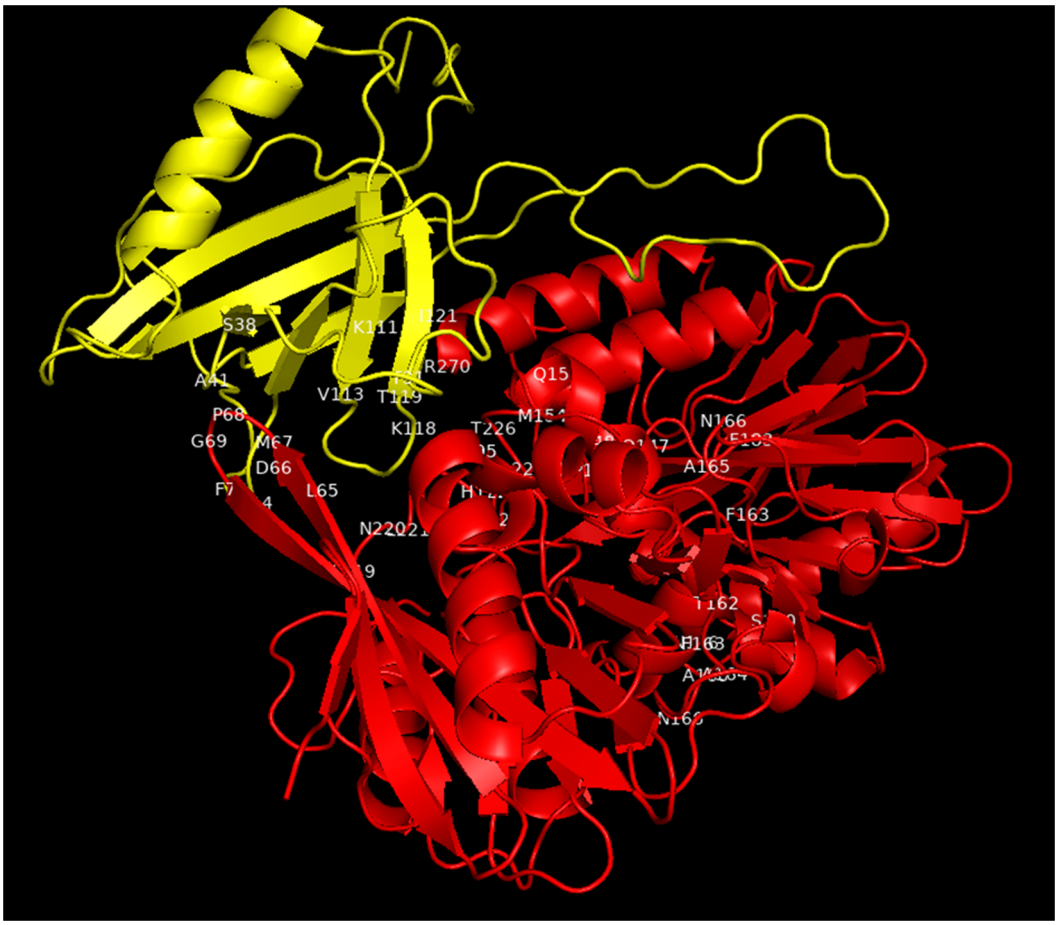

Figure 1. Binding interaction of Lactococcus tail protein (Q38344) with metallo-β-lactamases (5ypl) protein of New Delhi strain. Red shows receptors, and yellow shows ligand proteins. Interaction interface residues are shown as a single letter in white color.

Molecular Docking

Protein-protein docking

The Cluspro[19] tool helps in understanding the cellular function and organization with protein-protein interactions. Direct and template-based docking approaches can be distinguished. Direct techniques, based on thermodynamics, seek to determine the structure of the target complex located at the lowest Gibbs free energy in conformational space and hence necessitate a computationally feasible free energy evaluation model and efficient minimization algorithms.

MD simulation

For 100 ns, Desmond, a software from Schrödinger LLC, was used to model MD. The earliest phase of receptor and ligand complexes for MD simulation was docking experiments. Molecular docking studies can predict ligand-binding states in static situations. Docking is helpful because it provides a static view of a molecule's binding pose at the active site of a protein. By integrating Newton's classical equation of motion, MD simulations typically compute atom movements over time. Simulations were used to predict the ligand binding status in the physiological environment.

The receptor-ligand complex was preprocessed using Protein Preparation Wizard of Maestro, which included complex optimization and minimization. All the systems were prepared using the System Builder tool. TIP3P, a solvent model with an orthorhombic box, was chosen. The OPLS 2005 force field was used. To make the models neutral, counterions were introduced; 0.15 M NaCl was added to mimic physiological conditions. The NPT (isothermal-isobaric ensemble) ensemble with 300 K temperature and 1 atm pressure was chosen for the entire simulation. The models were relaxed before the simulation. The trajectories were saved for examination after every 100 ps, and the simulation's stability was verified by comparing the protein and ligand's RMSD over time.

Results

Using Cluspro, this ligand protein was docked with the receptor metallo-β-lactamases (5ypl) protein of the New Delhi strain (5ypl) and analyzed for binding energy and protein-protein interactions. The best binding affinity score was found at –1040.6 Kcal/mol compared with other docking scores. (Figure 1)

The outcomes of MD demonstrate how the RMSD values for the C-alpha atoms of the protein-protein complex changed over time. In Figure 2, the plot demonstrates that the complex stabilizes at 20 000 ps. Following that, changes in RMSD values

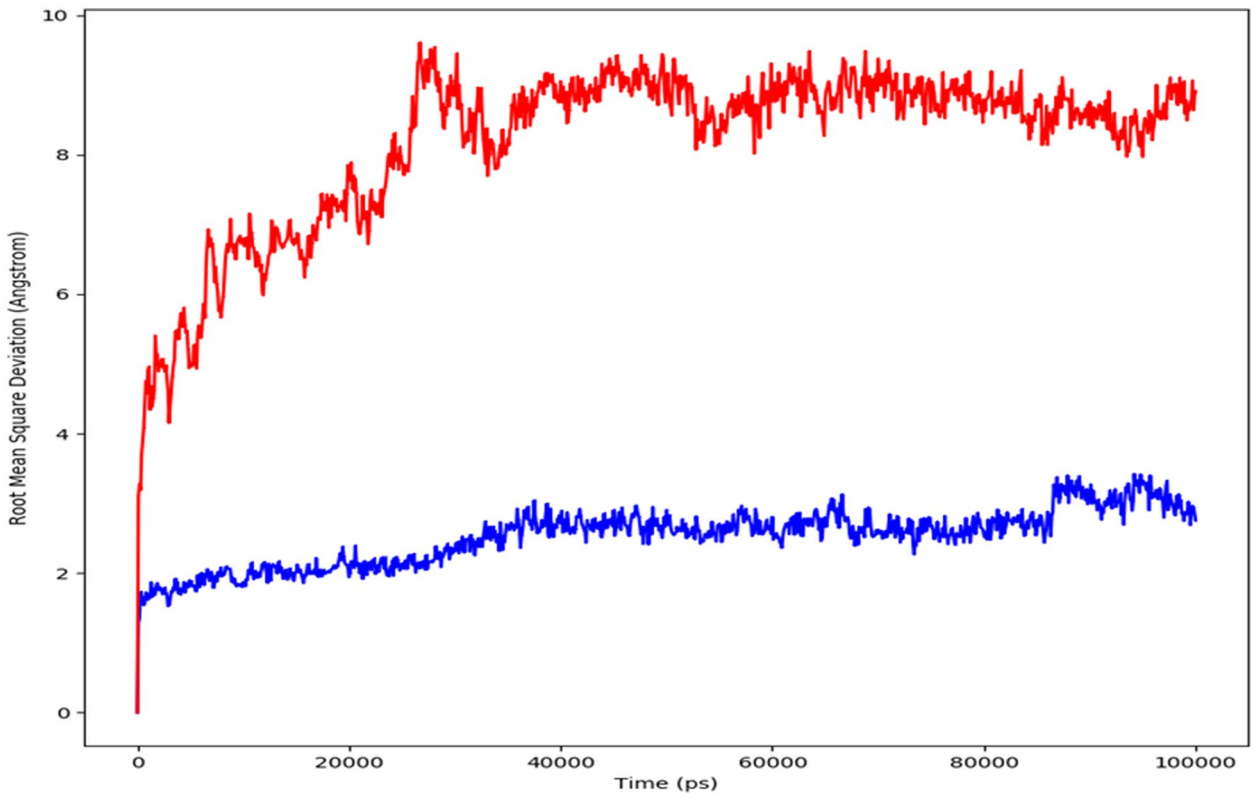

Figure 2. The change in root mean square deviation (RMSD) between the receptor and ligand's C-alpha atoms with time. The receptor and ligand-protein RMSD fluctuation over time is depicted on the left Y-axis. Blue indicates the RMSD of receptor protein, and red indicates the RMSD of ligand protein.

for the target remain within 1.0Å units, which is acceptable for the duration of the simulation. After equilibration, the RMSD values of the ligand-protein fit to the receptor protein, which are 2.752, vary within 1.5Å. These results suggest that the ligand protein remained tightly bound to the receptor's binding site during the simulation. The molecular mechanics with generalized born and surface area solvation (MM-GBSA) technique is commonly used to assess ligand binding energy in protein molecules. As a result, the MM-GBSA estimates produced from the MD simulation trajectories well validated the binding energy derived from the docking data.

Conclusion

To date, no treatment method has been successful in combating this superbug. The superbug NDM-1 possesses all the characteristics to become a potential threat to public health. Hence, this hypothesis, supported by evidence from a computational approach, will solve this life-threatening superbug problem. As Lactococcus BP is indigenous and readily available, this could be a promising approach. On the other hand, future in vivo and well-designed clinical trials are required to equip us to fight this potentially life-threatening pandemic.

REFERENCES

1. Murray CJ, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399:629-655.
2. Pandey N, Cascella M. *Beta Lactam Antibiotics*. Treasure Island, FL: StatPearls Publishing; 2022.
3. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem*. 2014;6:25-64.
4. Moellering RC Jr. NDM-1—a cause for worldwide concern. *N Engl J Med*. 2010;363:2377-2379.
5. Khan AU, Maryam L, Zarrilli R. Structure, genetics and worldwide spread of New Delhi metallo-β-lactamase (NDM): a threat to public health. *BMC Microbiol*. 2017;17:101.
6. Ranjan R, Thatikonda S. β-lactam resistance gene NDM-1 in the aquatic environment: a review. *Curr Microbiol*. 2021;78:3634-3643.
7. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis*. 2010;10:597-602.
8. Vasala A, Välkkilä M, Caldentey J, Alatossava T. Genetic and biochemical characterization of the Lactobacillus delbrueckii subsp. lactis bacteriophage LL-H lysin. *Appl Environ Microbiol*. 1995;61:4004-4011.
9. Mahony J, Cambillau C, Van Sinderen D. Host recognition by lactic acid bacterial phages. *FEMS Microbiol Rev*. 2017;41:S16-S26.
10. Szafrański SP, Winkel A, Stiesch M. The use of bacteriophages to biocontrol oral biofilms. *J Biotechnol*. 2017;250:29-44.
11. Golkar Z, Bagasra O, Pace DG. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *J Infect Dev Ctries*. 2014;8:129-136.
12. Al- Jassim N, Mantilla-Calderon D, Scarascia G, Hong P-Y. Bacteriophages to sensitize a pathogenic New Delhi metallo β-lactamase-positive Escherichia coli to solar disinfection. *Environ Sci Technol*. 2018;52:14331-14341.
13. Bowers KJ, Chow DE, Xu H, et al. Scalable algorithms for molecular dynamics simulations on commodity clusters; 2006. https://ieeexplore.ieee.org/document/4090217.
14. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. *Molecules*. 2015;20:13384-13421.
15. Hildebrand PW, Rose AS, Tiemann JKS. Bringing molecular dynamics simulation data into view. *Trends Biochem Sci*. 2019;44:902-913.
16. Rasheed MA, Iqbal MN, Saddick S, et al. Identification of lead compounds against Scm (fms10) in Enterococcus faecium using computer aided drug designing. *Life*. 2021;11:77.
17. Shivakumar D, Williams J, Wu Y, Damm W, Shelley J, Sherman W. Prediction of absolute solvation free energies using molecular dynamics free energy perturbation and the OPLS force field. *J Chem Theory Comput*. 2010;6:1509-1519.
18. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. *Nat Protoc*. 2010;5:725-738.
19. Kozakov D, Hall DR, Xia B, et al. The ClusPro web server for protein–protein docking. *Nat Protoc*. 2017;12:255-278.