# International Conclave on AMR and Future of Antibiotics

## (ICAFA - 2023)

**SRM University, Andhra Pradesh, India. November 8-9, 2023**


AMR INSIGHTS
TOWARDS A WORLD FREE FROM AMR

Innovate UK

IFCAI
INFECTION CONTROL ACADEMY OF INDIA

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FEDERATION OF ASIAN BIOTECH ASSOCIATIONS

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**Prof. Jayaseelan Murugaiyan**
**Dr. Sutharsan Govindarajan**
**Prof. Anirban Chakraborty**

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# Message from the chairs, ICAFA 2023

**Prof. Jayaseelan Murugaiyan and Dr. Sutharsan Govindarajan**

Department of Biological Sciences, SRM University-AP, Andhra Pradesh, India

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**Dear Esteemed Guests, Distinguished Speakers, Honourable Delegates, and all participants of the "International Conclave on antimicrobial resistance (AMR) and Future of Antibiotics**,"

It is a great pleasure to extend our warmest welcome to the International Conclave on AMR and Future of Antibiotics, organized by the Department of Biological Sciences, SRM University - AP, Andhra Pradesh, in collaboration with The AMR Insights, The Netherlands, Infection Control Academy of India (IFCAI), Federation of Asian Biotech Associations (FABA), and NITTE (Deemed to be University), Mangaluru. This conference is scheduled to take place at SRM University AP, Andhra Pradesh, on November 8 and 9, 2023. The conference addresses antimicrobial resistance issues and discusses antibiotic alternatives, which are paramount in our global healthcare landscape.

During this conference, leading experts worldwide and across India will join hands to combat AMR and enlighten young participants on the direction of research, policies, and the significance of antibiotic alternatives. We aim to bring together experts from UK Innovate, AMR Insights (The Netherlands), Global AMR Hub (Berlin, Germany), leading Indian experts, innovators, and stakeholders of the Andhra Pradesh state action plan for the containment of AMR. Andhra Pradesh is the fourth state in India to acknowledge the danger of AMR and proactively initiate steps toward its containment by releasing the Andhra Pradesh Action Plan for the Containment of Antimicrobial Resistance. This conference will be held in a hybrid mode, with both in-person and virtual participants, facilitating the exchange of ideas, networking opportunities, education for the younger generation, and ensuring healthcare safety through policies, awareness, and action plans. Participants, both physical and online attendees, are now important social ambassadors in the fight against the surge of AMR.

This two-day conference will include keynote lectures, presentations by young scientists, panel discussions, poster and oral sessions, and interactive networking. The conference will comprise the following seven sessions: AMR in One-Health, AMR and Environment, AMR and Omics, AMR – Action Plans and Policy, Alternatives to Antibiotics, AMR and Pathogen Strategies, and the Young Scientist Session.

In addition to the main conference event, on November 9, 2023, three roundtables with the themes: 1) One-Health, 2) Therapeutics and Vaccines, and 3) Diagnostics and Therapeutics, each lasting 90 minutes, will include experts from UK Innovate, representatives from the Andhra Pradesh Government departments involved in the state action plan, leading researchers from AIIMS Mangalagiri and other universities, and industrial partners. These roundtables will discuss several actions to be taken forward and list "Ten Mantras to Contain AMR Pathogens" – emergency response and action protocols.

We quote, " நோய்நாடி நோய்முதல் நாடி அதுதணிக்கும் வாய்நாடி வாய்ப்பச் செயல்" . Kural 948– Thirukkural, which means Diagnosing the root cause of the disease, identifying the correct curing, and ensuring its success. Together, we can succeed in containing AMR. Once again, we are honored to welcome you to this unique conference, and let us work towards a safe future.

**Prof. Jayaseelan Murugaiyan**
Associate Dean in-charge (Sciences) SEAS Professor and Head
Department of Biological Sciences SRM University AP

**Dr. Sutharsan Govindarajan**
Assistant Professor
DBT-Wellcome Early Career Faculty SRM University AP

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## DAY 1- November 08th

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| 8:00 AM - 9:00 AM | **Registration and Breakfast** |
| | **Inaugural Session** |
| 9:00 AM – 9:25 AM | Inauguration |

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| | **SESSION 1: AMR in One-Health** |
| 9:25 AM – 10:00 AM | Plenary: **Dr Bhabatosh Das**<br>Translational Health Science and Technology Institute, Haryana<br>*Functional and Genetic Heterogeneity of Antimicrobial Resistance Among Multidrug-Resistant Gram-negative Pathogens* |
| 10:00 AM – 10:25 AM | D**Dr. Krishna Kumar B**<br>NITTE University Centre for Science Education and Research, Mangaluru<br>*Unifying Genomic Datasets: Leveraging Metadata Standardization for AMR Interpretation in a One-Health Context* |
| 10:25 AM – 10:50 AM | **Dr Usha Lamichhane**<br>Global AMR R&D Hub Secretariat, Germany<br>*The Global AMR R&D Hub: Global Knowledge Centre and Driving Force for Evidence-based AMR Advocacy* |
| 10:50 AM – 11:15 AM | **Dr Maarten van Dongen**<br>AMR Insights, Netherland<br>*Microbiome and Antimicrobial Resistance from a One-Health Perspective* |
| 11:15 AM – 11:30 AM | **TEA BREAK** |

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| | **SESSION 2: AMR and Environment** |
| 11:30 AM -11:55 AM | **Dr Simon Doherty**<br>Queen's University Belfast, UK<br>*AMR: Investing in Animal Health to Support One-Health* |
| 11:55 AM – 12:20 PM | **Prof. P Anand Kumar**<br>Sri Venkateswara Veterinary University, Andhra Pradesh<br>*Antimicrobial Resistance Surveillance and Containment Programmes in Andhra Pradesh* |
| 12:20 PM – 12:45 PM | **Dr K Pani Prasad**<br>ICAR-Central Institute on Fisheries Education, Mumbai<br>*Antimicrobial Resistance (AMR) Surveillance in Aquaculture* |
| 12:45 PM – 1:45 PM | **LUNCH** |

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| | **SESSION 3: AMR and Genomics** |
| 1:45 PM - 2:10 PM | **Prof. Niyaz Ahmed**<br>University of Hyderabad, Hyderabad<br>*Dynamics and Evolution of Multiple Drug-Resistant Bacteria in Human and Animal Settings: Implications for Global Infection Control Priorities* |
| 2:10 PM – 2:35 PM | **Dr Shijulal Nelson Sathi** Rajiv Gandhi Centre for Biotechnology, Trivandrum *Evolutionary Route of Antibiotic Resistance in Staphylococcus aureus* |
| 2:35 PM – 3:00 PM | **Prof. Burra V L S Prasad**<br>K L University, Guntur<br>*Innovative Strategies Against Antimicrobial Resistance (AMR): Advancing Tuberculosis Mitigation* |
| 3:00 PM – 3:15 PM | **TEA BREAK** |

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| | **SESSION 4: AMR – Action Plans and Policy** |
| 3:15 PM – 3:40 | PM Keynote: **Dr Phil Packer**<br>Innovate UK<br>*The UK Response to AMR: With Emphasis on PACE (Pathways to Antimicrobial Clinical Efficacy)* |
| 3:40 PM – 4:05 PM | **Dr. GVS Murthy**<br>The London School of Hygiene & Tropical Medicine, UK<br>*The Missing Link in AMR Control Activities: The Civil Society* |

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| 11:15 AM – 11:30 AM | **PANEL DISCUSSION** | |
| | **Prof. Jayaseelan Murugaiyan (Moderator)**<br>SRM University-AP | |
| | **Panelists** | |
| 4:05 PM – 5:30 PM | **Dr Courtney Soulsby**<br>Global Director, Healthcare and Life Sciences Sector | **Prof. Ranga Reddy Burri**<br>Infection Control Academy of India (IFCAI), India |
| | **Dr Joanna Wiecek**<br>CSO, CircaGene, UK | **Prof. Bipin Kumar G Nair**<br>Amrita Vishwavidyapeetham, Amritapuri |
| | **Dr Mandy Nevel**<br>Head of Animal Health and Welfare, AHDB, UK | **Dr Deepti Vepakomma**<br>AIIMS, Mangalagiri |
| | **Dr Peter Coombs**<br>LifeArc, UK | **Dr Mike Strange**<br>LifeArc, UK |
| | **Dr Robin Cohen**<br>aVaxiPen | |
| 5:30 PM – 6:30 PM | **Cultural Events** | |
| 6:30 PM – 7:30 PM | **Studentnet Working** | |
| 7:30 PM – 9:00 PM | **Dinner** | |

## DAY 2- November 09th

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| 8:00 AM - 9:00 AM | **Breakfast** |
| 9:00 AM - 9:25 AM | **SESSION 5: Alternative to Antibiotics** |
| 9:00 AM – 9:25 AM | **Prof. Geetha Kumar**<br>Amrita Vishwavidyapeetham, Amritapuri<br>*From Anti-virulence Strategies to Bacteriophages: Resisting Antimicrobial Resistance* |
| 9:25 AM – 9:50 AM | **Dr. Nagendra Hegde**<br>National Institute of Animal Biotechnology (NIAB), Hyderabad<br>*Bacteriophages in Mitigating AMR* |
| 9:50 AM – 10:15 AM | **Prof. Vikas Jain**<br>Indian Institute of Science Education and Research (IISER), Bhopal<br>*Molecular Designing of a Small Phage-derived Therapeutic against Drug-Resistant Mycobacterium tuberculosis* |
| 10:15 AM – 10:40 AM | **Dr. Arunasree**<br>University of Hyderabad, Hyderabad<br>*Host-directed Immunotherapy and Bacteriophages for AMR Infections* |
| 10:40 AM – 10:55 AM | **TEA BREAK** |
| 10:55 AM – 11:20 AM | **Dr T Ramamurthy**<br>ICMR-National Institute of Cholera and Enteric Diseases, Kolkata<br>*Perceiving the Role of Transcriptome in Antimicrobial Resistant Pathogens* |
| 9:00 AM - 9:25 AM | **SESSION 6: Young Scientist Session** |
| 11:20 AM – 11:35 AM | **Dr. Tridib Mahata**<br>Postdoctoral Researcher, Tel Aviv University, Israel<br>*T5 phage-encoded Antimicrobials* |
| 11:35 AM – 11:50 AM | **Dr Gyanendra P Dubey**<br>DBT-Ramalingaswami Fellow, University of Hyderabad, Hyderabad<br>*Decrypting the Edge of Bacterial Cell-to-cell and Gut Bacterial- intestinal Host Cell Interaction* |
| 11:50 AM – 12:05 PM | **Sezanur Rahman**<br>Senior Research Officer, International Centre for Diarrhoeal Disease Research, Bangladesh<br>*iPHaGe Study: Identifying Lytic Phages as Potential Alternatives to Antibiotics for Combating Multidrug-Resistant Enterobacteriaceae* |
| 12:05 PM – 12:20 PM | **Dr Vijaya Kumar Deekshit**<br>Associate Professor G-I, NITTE University Centre for Science Education and Research, NITTE, Mangaluru<br>*Role of In-vitro Gut Conditions in Modulating Antimicrobial Resistance and Biofilm Forming Ability of Gut Pathogens* |

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| 12:20 PM – 12:35 PM | **Dr Vidya Prabhakar Kodali**<br>Assistant Professor- Vikrama Simhapuri University, Nellore<br>*Antagonistic Activities of Pongamia pinnata against Biofilm Forming Bacteria* |
| 12:35 PM – 12:50 PM | **Dr. Chetana Baliga**<br>DBT-Ramalingawami Fellow, Ramaiah University of Applied Sciences, Bengaluru<br>*Expanding the Antimicrobial Activity of Peptide Antibiotics that Inhibit Translation Termination* |
| 12:50 PM – 1:05 PM | **Dr Roshan Naik**<br>Founder, Diagopreutic Pvt Ltd, Goa<br>*Same Day Visual Quantitative Antibiotic Sensitivity Assay Directly from Clinical Samples* |
| 1:05 PM – 1:15 PM | **Dr Sridharan**<br>Senior Manager- Products, Genotypic Technology Pvt Ltd., Bengaluru<br>*Antimicrobial Drug Resistance - Fast and Rapid Elucidation using Nanopore Sequencing* |
| 10:40 AM – 10:55 AM | **LAUNCH** |
| 9:00 AM - 9:25 AM | **SESSION 7: AMR and Pathogen Strategies** |
| 2:00 PM – 2:25 PM | **Prof Dipshikha Chakravortty**<br>Indian Institute of Sciences (IISc), Bangalore<br>*Right to Live - How Far We Can Go? Newer Strategies to Tackle AMR* |
| 2:25 PM – 2:50 PM | **Prof. Medicharla V Jagannadham**<br>University of Hyderabad, Hyderabad<br>*Functional Analysis of Membrane Vesicles of Bacteria* |
| 2:50 PM – 3:15 PM | **Dr Saravanan Matheshwaran**<br>Indian Institute of Technology (IIT), Kanpur<br>*Targeting Mycobacterial "SOS" Response- A Strategy to Toggle Antimicrobial Resistance (AMR)* |
| 3:15 PM – 3:30 PM | **TEA BREAK** |
| 3:30 PM – 5:00 PM | **Oral and Poster Presentations – Parallel Session** |
| 5:00 PM – 5:30 PM | **Valedictory** |

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# Functional and genetic heterogeneity of antimicrobial resistance among multidrug-resistant Gram-negative pathogens

**Bhabatosh Das**

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**ICAFA- 2023**
**SRM University**
**Andhra Pradesh, India**
**November 8-9, 2023**

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## Abstract

Most microbes are naturally competent and evolved rapidly by modifying their genomes through horizontal acquisition of mobile genetic elements (MGEs) linked with fitness traits or by accumulating spontaneous or indels mutations. Recently, we completed a multicentric study in India and collected clinical samples from different patients suffering from sepsis, urinary tract infections, and respiratory infections for isolating causative organisms and decoding their genome sequences to understand AMR functions, genetic heterogeneity, and dynamics of resistance traits. The genomic analysis identified several acquired AMR genes (ARGs) with a pathogen-specific signature. We observed that $bla_{CTX\text{-}M\text{-}15}$, $bla_{CMY\text{-}42}$, $bla_{NDM\text{-}5}$, and *aadA(2)* were prevalent in *Escherichia coli*, and $bla_{TEM\text{-}1B}$, $bla_{OXA\text{-}232}$, $bla_{NDM\text{-}1}$, *rmtB*, and *rmtC* were dominant in *Klebsiella pneumoniae*. In contrast, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* harbored $bla_{VEB}$, $bla_{VIM\text{-}2}$, *aph*(3'), *strA/B*, $bla_{OXA\text{-}23}$, *aph*(3') variants, and *amrA*, respectively. Regardless of the type of ARG, the MGEs linked with ARGs were also pathogen-specific. The sequence type of these pathogens was identified as high-risk international clones, with only a few lineages being predominant and region-specific. Whole-cell proteome profiling and analysis of multidrug-resistant *K. pneumoniae*, *A. baumannii*, *E. coli*, and *P. aeruginosa* strains revealed differential abundances of resistance-associated proteins in the presence and absence of different classes of antibiotics commonly used in clinical practice. The pathogen-specific resistance signatures and differential abundance of AMR-associated proteins identified in this study should add value to AMR diagnostics and the choice of appropriate drug combinations for successful antimicrobial therapy.

**Keywords:** Antimicrobial resistance genes, Mobile genetic elements, High-risk international clones

Citation. Das, B . 2023. Functional and genetic heterogeneity of antimicrobial resistance among multidrug-resistant Gram-negative pathogens. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 4. https://doi.org/10.51585/gtop.2023.2.0035

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# Unifying genomic datasets: Leveraging metadata standardization for AMR interpretation in a One-Health context

**Ballamoole K. Kumar**[1*], **Kattapuni S. Prithvisagar**[1], **Jayaseelan Murugaiyan**[2] and **Maarten B. M. van Dongen**[3]

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**Abstract**

Antimicrobial resistance (AMR) is a global health concern necessitating a holistic approach, integrating data across human, animal, and environmental health domains, known as the One-Health perspective. The advent of high-throughput genomics technologies has revolutionized the study of AMR, generating vast amounts of data that would provide the highest practicable level of structural detail on the individuating traits of an organism. In our comprehensive global study, genomic datasets of ESKAPE pathogens available from different continents were analyzed using different bioinformatics tools to identify AMR genetic traits and understand the global and region-wise spatiotemporal trends in antimicrobial resistance. The genomic data will also be correlated with other collected metadata and antibiotic consumption data to enable an important contribution to disease control and prevention, particularly in designing diagnostics and effective intervention strategies for controlling ESKAPE pathogens. A notable challenge was identified throughout our study—the lack of unified metadata in genomic datasets across public databases—which impedes the effective utilization of invaluable genomic information. During the presentation in this conclave, we will explore the critical role of metadata standardization in unifying genomic datasets and its leverage in maximizing AMR insights within the One-Health perspective. Researchers can enhance data interoperability, facilitate meaningful comparisons, and derive comprehensive insights into AMR dynamics across various sectors by employing consistent metadata standards. Such a unified approach should emphasize the necessity of a standardized metadata framework for effectively utilizing genomic data, advancing our understanding of AMR within the broader One-Health perspective.

**Keywords:** One-Health, ESKAPE pathogens, Antimicrobial resistance, Metadata

Citation. Kumar, B. K., Prithvisagar, K. S., Murugaiyan, J. and van Dongen, M. B. M. 2023. Unifying genomic datasets: Leveraging metadata standardization for AMR interpretation in a One-Health context. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 5. https://doi.org/10.51585/gtop.2023.2.0035

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# The Global AMR R&D Hub: Global knowledge center and driving force for evidence-based AMR advocacy

**Usha Lamichhane**

Global AMR R&D Hub, Berlin, Germany


**ICAFA- 2023**
**SRM University**
**Andhra Pradesh, India**
**November 8-9, 2023**

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**Abstract**

Antimicrobial resistance (AMR) is a significant global public health threat that extends to the global economy, healthcare costs, and productivity. However, the research and development pipeline for new antibacterials, particularly antibiotics, which are key infrastructure for health systems, is insufficient. The challenges to AMR are complex, including scientific, economic, and regulatory hurdles and spanning multiple sectors and disciplines. The Global AMR Research and Development (R&D) Hub (HUB) is a G20-born organization with the mandate to address these challenges and improve coordination and collaboration in AMR R&D. Through the Dynamic Dashboard, the Hub offers a platform to foster evidence-based global priority-setting and decision-making on allocating resources for AMR R&D in a One-Health context. The Hub is also committed to advocacy efforts at the highest political levels, including the EU, G7, and G20, to drive action on AMR. After a first initial report in May 2022, the HUB, in collaboration with WHO, has provided a progress update on incentivizing the development of new antibacterial treatments to the G7 Finance and Health Ministries in May 2023. At the G20 under the Indian Presidency, HUB provided interventions at the health working group and health ministers meetings highlighting the development and access crisis, the need for sustainable financing for R&D, and the need to establish feasible targets for AMR R&D informed by public health needs. The HUB continues to be a knowledge center and driving force addressing global priorities for AMR R&D and promoting high-level coordination and advocacy for AMR, with a focus on turning the political momentum generated so far into global target setting and action, especially as we head towards the "High-level Meeting on Antimicrobial Resistance at the United Nations General Assembly in 2024".

**Keywords:** One-Health, Antimicrobial resistance, Global AMR Research and Development Hub, G20

Citation. Lamichhane, U. 2023. The Global AMR R&D Hub: Global knowledge center and driving force for evidence-based AMR advocacy. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 6. https://doi.org/10.51585/gtop.2023.2.0035

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# Microbiome and antimicrobial resistance from a One-Health perspective

**Maarten B. M. van Dongen**

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**ICAFA- 2023**
**SRM University**
**Andhra Pradesh, India**
**November 8-9, 2023**

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## Abstract

The microbiome refers to the collection of genomes from the microbiota, the living microorganisms in a defined environment. The resistome represents the collection of antibiotic resistance genes (ARGs) in a defined microbiome. In his presentation, Dr. Maarten van Dongen will explain that the One-Health microbiome should take the central stage when considering antimicrobial resistance (AMR) and the holistic approach needed to tackle AMR as an escalating global threat. Based on an examination of recent literature, Dr. van Dongen will shed light on the human (gut) microbiome as an important source of ARGs and a diverse reservoir from which new resistance determinants can be recruited to pathogens. He will explain how antibiotic usage impacts the human microbiome at an individual and population level, in wastewater, and in the urban environment. Likewise, traveling and changes in diet impact AMR in the human microbiome. Also, the environmental dimension is discussed: in addition to the wastewater microbiome, the soil microbiome will be on the table. It will be explained how herbicides enhance ARGs in the soil microbiome. And there is also good news: the animal gut microbiome can be a valuable source for the research and development of novel antimicrobials. We need to take a much more holistic, One-Health approach to combating AMR globally: how can we contain resistant microorganisms and prevent new resistances from emerging?

**Keywords:** Microbiome, Resistome, One-Health, Antibiotic, Herbicide

Citation. van Dongen, M. B. M. 2023. Microbiome and antimicrobial resistance from a One-Health perspective. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 7. https://doi.org/10.51585/gtop.2023.2.0035

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# Antimicrobial resistance (AMR): Investing in animal health to support One-Health

**Simon Doherty**

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**ICAFA- 2023**
**SRM University**
**Andhra Pradesh, India**
**November 8-9, 2023**

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**Abstract**

One-Health recognizes the interconnectedness between people, animals, plants, and their shared environment, but the approach embraces the requirement for successful inter- and trans-disciplinary collaboration to bring about change. Antimicrobial resistance is recognized as one of the 'Global Grand Challenges' that can benefit from a One-Health approach, deriving its effectiveness from improved communication between the medical, veterinary, biopharma, and life sciences disciplines to co-create innovative solutions and drive replacement, reduction, and refinement ('3Rs') in the use of antimicrobial products. In this short presentation, veterinarian and One-Health advocate Dr. Simon Doherty will discuss the role of veterinarians in promoting the principles of good antimicrobial stewardship in domestic animals, affecting change in people and the environment, too. Using examples, he'll describe how innovative 3R approaches have dramatically reduced the use of antimicrobials in food-producing animals in the UK in the last decade through public-private partnerships involving various relevant stakeholders. He'll also describe how both government and assurance schemes can play a role in supporting change. Investment is key to stimulating innovation, though Dr. Doherty will describe his involvement with creating two reports launched in the Summer of 2023 outlining the criticality of Investing in Animal Health to support One-Health.

**Keywords:** One-Health, Antimicrobial resistance, Food producing animals

Citation. Doherty, S. 2023. Antimicrobial resistance (AMR): Investing in animal health to support One-Health. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 8. https://doi.org/10.51585/gtop.2023.2.0035

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# Antimicrobial resistance surveillance and containment programs in Andhra Pradesh

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**ICAFA- 2023**
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**November 8-9, 2023**

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## Abstract

Indo-Dutch pilot project on antimicrobial resistance (AMR) under One-Health was executed in the Krishna District of Andhra Pradesh during 2020-2021. *Escherichia coli* was isolated and characterized from the urine samples of humans, cloacal swabs of chickens, and water samples (environment). A total of 74 *E. coli* isolates from chicken cloacal swabs, 79 *E. coli* isolates from water samples, and 100 clinically significant *E. coli* isolates from human urine samples were isolated during this pilot project. All the *E. coli* isolates from human, animal, and environment sectors were tested for antibiotic resistance against the harmonized panel of antibiotics. The resistance to 3rd and 4th-generation cephalosporins is lesser in *E. coli* isolates from chicken cloacal and water samples than human urine samples. Higher resistance to tetracyclines, trimethoprim-sulfamethoxazole, ciprofloxacin, and chloramphenicol was observed in *E. coli* isolates of chicken cloacal swabs. The *E. coli* isolates from water samples showed higher resistance to Amoxicillin/Clavulanic acid. Presumptive Extended Spectrum $\beta$-Lactamase producing *E. coli* were also isolated from chicken cloacal swabs (14 isolates) and water samples (5 isolates). The AMR pilot project was conducted with a small sample size and needs a study with many samples from human, animal, and environmental settings for conclusive results. Taking a cue from the AMR pilot project, the Andhra Pradesh Action Plan for Containment of AMR (APAPCAR) under the One-Health approach was released by the Government of Andhra Pradesh in 2022 for implementation. Andhra Pradesh State AMR cell with a nodal officer, representatives from the stakeholder Departments, and subject experts was constituted for a periodic review of the progress of APAPCAR. National network AMR surveillance programs are in progress in some state medical and veterinary institutions. Programs for capacity building in AMR surveillance and containment strategies are also initiated.

**Keywords:** Antimicrobial resistance, One-Health, Antibiotics, *Escherichia coli*

Citation. Kumar, P. A. 2023. Antimicrobial resistance surveillance and containment programs in Andhra Pradesh. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 9. https://doi.org/10.51585/gtop.2023.2.0035

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# Antimicrobial resistance (AMR) surveillance in aquaculture

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## Abstract

The World Health Organization identifies antimicrobial resistance (AMR) as a critical 21st-century public health threat. India's fisheries and aquaculture sectors are vital to millions' food, income, and livelihood. However, intense antibiotic use in aquaculture contributes to AMR. To address this issue, the Indian Council of Agricultural Research, in collaboration with the FAO, established the Indian Network of Fisheries and Animal Antimicrobial Resistance (INFAAR). INFAAR conducts structured AMR surveillance in food animals, including aquatic species. Analyzing the data informs the development of policies and strategies to mitigate AMR's impact and prevent its transmission to humans. In this surveillance study, in aquaculture, the *Vibrio* species, *Staphylococcus* species, and *Escherichia coli* are analyzed and isolated from aquatic species from brackish water viz *Litopenaeus vannamei* shrimps and *Aeromonas* species, *Staphylococcus* species, and *Escherichia coli* from freshwater fishes. The antibiogram study of the isolated bacteria is analyzed using the antimicrobial surveillance software – WHONET and interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. In the study, shrimp farms of Maharashtra and Gujarat, initially, penicillin and erythromycin were resistant to the *Staphylococcus* species, but their resistance increased to aztreonam, amikacin, and ampicillin as the year progressed. The antibiotic such as ampicillin and erythromycin were resistant to the *E. coli*. However, amikacin has been immediately resistant to *E. coli* for the last two years. In the case of *Vibrio* species, ampicillin was resistant for the last five years, but a gradual increase in the resistance toward cefoxitin was observed over the last three years. These findings showed that microorganisms are developing resistance to more antibiotics as the year progresses.

**Keywords:** AMR surveillance, Aquaculture, Microbiology, Antibiotics

Citation.Kurcheti, P. P., Momaya, A., Jeena, K. and Pokanti, V. 2023. Antimicrobial resistance (AMR) surveillance in aquaculture. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 10. https://doi.org/10.51585/gtop.2023.2.0035

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# Dynamics and evolution of multiple drug-resistant bacteria in human and animal settings: Implications for global infection control priorities

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## Abstract

Molecular epidemiology of pathogenic bacteria has largely been carried out through functionally neutral or inert sequences, mostly entailing polymorphic gene loci or repetitive tracts spread throughout bacterial genomes. However, it is very important to engage functionally relevant markers to assign a valid epidemiological context to track pathogens, such as their acumen to acquire multiple drug resistance (MDR), their phenotypic diversity concerning clinical or community level dynamics of incidence/transmission, as well as their response or refractoriness to treatment. This presentation aims to discuss the above ideas in light of our works entailing high throughput genomics and functional epidemiology of multiple drug-resistant bacteria studied from different settings in South Asian neighbors, India, and Bangladesh. Further, it will also be possible to present and discuss our recent analyses based on an extensive trajectory of phenotypic resistance in clinical isolates over 14 years. It will also be interesting to discuss findings emanating from a knowledgebase of plasmids encompassing different compatibility groups entailing enteric and non-enteric pathogens, forming a deep landscape of their diversity and plasticity relevant to their propensity to acquire, confer, disseminate, and shuffle/shuttle fitness traits across different species and lineages of pathogenic and environmentally dwelling bacteria. Discussion of these findings would enlighten us about the sheer prowess of bacterial organisms to evolve and spread with new fitness advantages such as MDR phenotypes of different types. It is possible that approaches based on such multi-dimensional and multicentric strategies as mentioned above would likely successfully target the spread of MDR pathogens and guide the global infection control priorities and policies.

**Keywords:** Multiple drug resistance, Polymorphic gene, High throughput genomics, Functional epidemiology, Phenotypic resistance

Citation. Ahmed, N. 2023. Dynamics and evolution of multiple drug-resistant bacteria in human and animal settings: Implications for global infection control priorities. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 11. https://doi.org/10.51585/gtop.2023.2.0035

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# Evolutionary route of antibiotic resistance in *Staphylococcus aureus*

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## Abstract

Multidrug-resistant bacterial pathogens are a leading concern worldwide. However, resistant genes' origin and evolutionary route in gram-positive pathogens such as *Staphylococcus aureus* (*S. aureus*) remain largely unknown. We performed a detailed phylogenomic analysis of the completely sequenced *S. aureus* genome compared to non-*Staphylococcus aureus* reference bacterial genomes. The phyletic patterns of SCCmec-encoded resistant genes in *Staphylococcus* species significantly differ from that of its core genes, indicating frequent exchange of these genes between *Staphylococcus* species. Our in-depth analysis of SCCmec-resistant gene phylogenies reveals that the SCCmec element might have originated within the Bacillales lineage and assembled its current structural form in one of the early lineages of *Staphylococcus* species and later transferred to *S. aureus*. Interestingly, we found that at least 89% of SCCmec-encoded and 63% of non-SCCmec-encoded resistant genes were influenced by lateral gene transfers from both closely and distantly related species, which shaped the current distribution of resistant genes in *S. aureus*. In this talk, I will discuss the genome characteristics of multi-drug resistant *S. aureus* and the role of lateral gene transfers in shaping the resistance gene distribution in *S. aureus*.

**Keywords:** Multidrug-resistant *Staphylococcus aureus*, Lateral gene transfer, SCCmec-encoded resistant genes

Citation. Nelson-Sathi, S. 2023. Evolutionary route of antibiotic resistance in *Staphylococcus aureus*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 12. https://doi.org/10.51585/gtop.2023.2.0035

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# Innovative strategies against antimicrobial resistance (AMR): Advancing tuberculosis mitigation

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## Abstract

In the realm of global healthcare, the term AMR, standing for Antimicrobial Resistance, has become a stark reminder of the complex battle against infectious diseases. This phenomenon encapsulates the remarkable ability of microorganisms, spanning bacteria, viruses, fungi, and parasites, to evade the effects of medications, particularly antimicrobial drugs specifically crafted to combat these pathogens. AMR arises as microorganisms undergo evolutionary adaptations, rendering the drugs employed for treatment gradually becoming ineffective over time. The emergence of resistant strains poses a grave threat, allowing infections to persist and potentially spread, making AMR a pressing and immediate global health concern. This resistance is not solely a product of natural evolution but is exacerbated by the inappropriate and excessive use of antimicrobial drugs across human healthcare, veterinary practices, and agricultural sectors. Consequently, this escalating crisis comprises our ability to effectively treat common infections, leading to prolonged illnesses, heightened mortality rates, and a substantial increase in healthcare expenditure. In light of these challenges, addressing AMR necessitates a multifaceted approach encompassing responsible drug usage, enhanced infection prevention and control measures, and interdisciplinary pursuit of innovative strategies and novel agents to counter this resistance. This presentation offers an insightful overview of our lab's efforts over the past five years to confront one of the most resilient adversaries in infectious diseases: Tuberculosis (TB). Our research has been meticulously focused on designing and developing anti-TB MEBP vaccines and therapeutic agents. Central to our endeavors are the exploration and exploitation of novel targets, including Methyltransferases, and innovative strategies involving allosteric inhibitors. By delving into these intricate pathways and adopting novel techniques, we aim to forge a path toward effective treatments and preventive measures against TB. In the upcoming research phase, we are eager to embark on pre-clinical studies, marking a pivotal stride towards translating our comprehensive knowledge into tangible solutions in the fight against TB and the broader spectrum of AMR.

**Keywords:** Antimicrobial resistance (AMR), Tuberculosis (TB), 16S rRNA methyltransferases (16SrRMTs), RecA allosteric inhibitors, MEBP vaccines, Drug development, Allosteric inhibitors

Citation. Prasad, B. V. L. S. 2023. Innovative strategies against antimicrobial resistance (AMR): Advancing tuberculosis mitigation. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 13. https://doi.org/10.51585/gtop.2023.2.0035

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# The UK response to AMR with emphasis on Pathways to Antimicrobial Clinical Efficacy (PACE)

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## Abstract

The UK was one of the first countries to establish a National Action Plan (NAP) on AMR (even before the 2015 Global Action Plan on Antimicrobial Resistance (GAP)) with a strategy and action plan in place as early as 2000. The NAP highlights the requirements and deliverables needed to address AMR. UK Government departments, Industry, academics, and others reference this in developing their future AMR Research and Development programs. Innovate UK has joined forces with LifeArc and Medicines Discovery Catapult (MDC) to create PACE (Pathways to Antimicrobial Clinical Efficacy), a £30 million initiative supporting early-stage innovation against antimicrobial resistance (AMR). PACE will harness its unique expertise - catalyzing and working with the global AMR community to accelerate the speed of innovation to mitigate the risk of AMR. PACE will bring together the right funding, resources, and partnerships to help innovators progress their early-stage antimicrobial drug and diagnostics projects with greater speed, support, and confidence – giving the best AMR innovations the greatest chance of success. PACE has launched its first funding call to create and advance an exciting and diverse pipeline of early-phase antimicrobial projects to treat bacterial infections with high unmet needs. The intention is to provide up to £1M in non-diluted funding to 10 projects. There are many other important initiatives in the UK, including, but not restricted to, the Global AMR Innovation Fund (GAMRIF), which has invested a further £39M to support R&D development around the world to reduce the threat of antimicrobial resistance in low- and middle-income countries (LMICs). BactiVac is a bacterial vaccine network bringing together members in academia, industry, and policy sectors to accelerate the development of vaccines against bacterial infections in LMICs. LifeArc is making significant investments to accelerate the progression of affordable and accessible solutions to tackle infections worldwide, and icon is working to bridge the gap in the infection innovation system between industry, academia, and the NHS.

**Keywords:** Antimicrobial resistance, Pathways to Antimicrobial Clinical Efficacy, Global AMR Innovation Fund, BactiVac, LifeArc, Iicon

Citation. Packer, P. 2023. The UK response to AMR with emphasis on Pathways to Antimicrobial Clinical Efficacy (PACE). Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 14. https://doi.org/10.51585/gtop.2023.2.0035

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# The missing link in AMR control activities: The civil society

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## Abstract

The last few decades have shown the immense value that civil societies, through a community engagement process, can make to control health problems. This is particularly so in the case of infectious diseases, where civil society has played a major role in both elimination and control. The level of civil society engagement in polio eradication has been well documented. The same has been the case with dengue, where, across the globe, tremendous success has been observed due to community involvement. Recently, with COVID-19, community engagement and civil society response played a crucial role. The change in human behavior was the cornerstone of the success of these initiatives due to shared population behavior patterns and not just individual responses, which influence societal norms and responsibilities. The issue with AMR is more complex as there is a need to work at different ecosystem levels, which concerns humans and animals. Therefore, changing individual and community behaviors and reinforcing positive changes using the One-Health platform is needed. This may not translate into immediate success but needs persistent efforts over a long time. Patience and perseverance are needed. There is a need to strategize at different levels – Individuals, Communities, Civil Rights advocates, and institutions like schools encompassing the whole-of-society approach. We have to learn and adapt from successful initiatives across the globe. Challenges in the community include a lack of contextual understanding, mistrust of public initiatives, lack of interest in the health system to partner, and a poor appreciation of populations on what roles they can play. It is important to convince the communities to progress from a complete lack of interest through incremental steps to a position where they feel empowered to own the initiatives and influence policy and programs.

**Keywords:** One-Health, Antimicrobial resistance, Community awareness

Citation. Murthy, G. V. S. 2023. The missing link in AMR control activities: The civil society. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 15. https://doi.org/10.51585/gtop.2023.2.0035

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# From anti-virulence strategies to bacteriophages: Resisting antimicrobial resistance

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**Abstract**

Antimicrobial resistance (AMR) has been recognized as a serious global threat affecting various sectors, including healthcare systems worldwide, resulting in increased morbidity and mortality. Three of the WHO-classified critical priority pathogens, namely, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* have been implicated as being responsible for the majority of deaths associated with multidrug-resistant (MDR) infections. Therefore, research focused on innovative solutions to combat this crisis is the need of the hour. After screening a collection of different Natural Products, Clove bud oil (CBO) was selected as a Quorum Sensing inhibitor for further studies, which demonstrated that in addition to regulating virulence phenotypes of Gram-negative ESKAPE pathogens such as *Pseudomonas aeruginosa*, CBO can also function as a host immunomodulator, resulting in its effective use as an anti-infective in a whole animal model of pathogenesis. Isolation and characterization of lytic bacteriophages that can sensitize and kill MDR pathogens is another alternative strategy that can be successfully used to combat MDR infections that are not responsive to conventional antibiotics. We have successfully isolated and characterized novel bacteriophages specific to *Pseudomonas aeruginosa* PA01. Furthermore, we have demonstrated that these phage cocktails can successfully kill 96% of our collection of MDR clinical isolates of *Pseudomonas aeruginosa*. These observations have significant translational potential in combating the global AMR crisis.

**Keywords:** Antimicrobial resistance, Multidrug-resistant pathogens, Clove bud oil, Quorum sensing, Bacteriophages

Citation. Kumar, G. 2023. From anti-virulence strategies to bacteriophages: Resisting antimicrobial resistance. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 16. https://doi.org/10.51585/gtop.2023.2.0035

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# Bacteriophages in mitigating AMR

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**Abstract**

Antimicrobial resistance (AMR) has assumed pandemic proportions, and without a course correction, it is likely to burden healthcare systems worldwide heavily. Besides antimicrobial stewardship programs, several lines of investigation have been targeting outcome-oriented research into alternative antimicrobials to mitigate AMR. Ideally, such alternatives need to be broad-ranging yet focused so as not to affect normal microbiota. One such alternative is the use of bacteriophages. Although used for more than a century, the rise in resistance of microbes to various antimicrobials has reinvigorated research and development into the use of bacteriophages. We have begun to search for phages to target pathogens causing mastitis in livestock. Mastitis is an economically taxing disease of milk-producing animals and is primarily treated with antibiotics, often of higher class. We isolated 25-30 phages each against the principal bacterial pathogens, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* associated with bovine mastitis, and initially characterized them for tropism. By applying certain criteria such as broad strain tropism, morphology, latent period, growth kinetics and burst size, pH and temperature stability, and genetic characterization, half a dozen phages were selected for each bacterial pathogen. These are being further explored for *in-vitro* studies that mimic various situations of intra-mammary infection to streamline a process to translate the research into product development activities. Ultimately, it is envisaged that bacteriophages could replace or at least drastically reduce the application of antibiotics in treating mastitis in livestock. It is also anticipated that regulatory pathways for phage therapy will mature over time.

**Keywords:** Bacteriophage, Mastitis, Intra-mammary infection, Antibiotics, Phage therapy

Citation. Parida, S. and Hegde, N. R. 2023. Bacteriophages in mitigating AMR. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 17. https://doi.org/10.51585/gtop.2023.2.0035

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# Applying the Pareto principle for improved AMR containment outcomes within the framework of global, national, and State action plans

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## Abstract

AMR, a major global health concern, demands immediate action. It is an ongoing and evolving pandemic that impacts vulnerable populations everywhere. Its containment is intricate and requires robust policies. However, implementing these policies faces challenges due to suboptimal prioritization of plans and strategic objectives. While many National Action Plans (NAPs) share common strategic priorities (SPs), there is a noticeable disparity in the attention and resource allocation by governments and various funding agencies. These entities tend to favor high-cost, low-outcome interventions such as new antimicrobial research, leaving minimal resources for low-cost, high-impact interventions. This imbalance calls for a reassessment of priorities to ensure effective resource utilization. This conceptual approach proposes a review of the prioritization of strategic objectives and plans using the Pareto principle (80/20 rule). This rule suggests that 80% of consequences stem from 20% of causes. Applying this to AMR, we hypothesize that 80% of resistance is caused by 20% of the drivers and activities. We suggest revisiting and prioritizing strategic objectives and plans using the "vital few principles" for effective AMR containment. Infection prevention should be central to plans at every level, emphasizing surveillance-based prevention, timely diagnosis, and sustainable treatment options.

**Keywords:** OGAP, SAP, NAP, AMR containment action plans, Strategic priorities, Infection prevention, Timely diagnosis, Sustainable treatment options, Vital few principles

Citation. Burri, R. R. 2023. Applying the Pareto principle for improved AMR containment outcomes within the framework of global, national, and State action plans. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 18. https://doi.org/10.51585/gtop.2023.2.0035

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# Host-directed immunotherapy and bacteriophages for AMR infections

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## Abstract

Despite successful eradication, prevention, and control efforts, infectious diseases caused by bacteria, viruses, fungi, and other parasites represent a major global problem in human health. Among these infectious diseases, hospital-acquired infections (nosocomial infections, HAI) affect the patient and public health in general. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Klebsiella pneumoniae*, vancomycin-resistant Enterococcus (VRE), etc are some of the bacteria posing a great challenge in the treatment of HAIs. The use of antibiotics to treat infections has been a common medical practice. However, developing antimicrobial resistance (AMR) to antibiotics by microbes led to the combination therapy of two or more antibiotics. The emergence of "superbugs" that are resistant to almost all antibiotics has raised the alarm for immediate and effective treatment strategies to overcome AMR infections. The advent of every new antibiotic is invariably associated with developing resistance to that antibiotic. Host-directed immunotherapy and bacteriophages have gained importance in recent years as alternatives to antibiotic-based therapies. Anti-inflammatory drugs such as corticosteroids, interferons, and IL6 receptor antagonists have successfully treated TB and COVID-19. However, these drugs have their limitations in terms of adverse effects and dosage normalization. Although bacteriophage-based therapy has not yet reached the clinic to its full potential, it is of renewed interest due to the dried-up antibiotic drug discovery pipeline and increasing AMR infections. We have demonstrated the use of non-steroidal anti-inflammatory (NSAIDs) drugs as alternatives to steroid-based drugs to combat AMR bacterial infections. The *in-vitro*, *in-vivo*, and *ex-vivo* studies showed that celecoxib was able to improve the immune response of the host, and in bacteria, it increased the permeability of the bacterial membrane, allowing entry of more antibiotics. We have isolated *E. coli* and *S. aureus*-specific bacteriophages showing broad activity against clinically isolated human and veterinary strains.

**Keywords:** Nosocomial infections, Superbugs, Bacteriophage, Non-steroidal anti-inflammatory drugs, Celecoxib

Citation. Kalle, A. M. 2023. Host-directed immunotherapy and bacteriophages for AMR infections. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 19. https://doi.org/10.51585/gtop.2023.2.0035

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# Perceiving the role of the transcriptome in antimicrobial-resistant pathogens

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## Abstract

Many bacterial pathogens exhibit resistance to antimicrobials, mostly by having an efflux system, gene mutations, plasmids, and horizontally transferred genes. Antimicrobials can affect bacteria at many levels in addition to their direct effects on the biological function. These include impacts on their stress response, survival, metabolism, gene expression, and mutations. Antimicrobial-resistant (AMR) bacteria have many genetic alterations that significantly change the functionally associated genes that influence the gene networks. RNA is a key molecule that translates the genetic information encoded in cellular DNA into functional cellular products. Transcriptomics has identified the role of RNA in controlling essential housekeeping activities and survival strategies adopted by the bacteria, including resistance to antimicrobials. Metatranscriptomics has identified several antimicrobials that induce several other functions of the bacteria. Transcriptomic analysis has been established as insightful and indispensable to functionalizing many genes not associated with the AMR and identifying new biological pathways. Most altered transcriptions were associated with biofilm formation, efflux system, membrane function, regulation of ions, secretion system, increase in virulence, etc. Computational analysis of transcriptions, including gene ontology and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis, has identified metabolism, nucleotide transport, intracellular trafficking, secretion, and signal transduction mechanisms. These metabolic network models have shown several important metabolic activities of pathogens and hosts during infections. Using the genome-scale transcriptomic and multiomics model provides a fundamental understanding of the complex metabolic responses to antimicrobials at the systems level. Such models may significantly impact the understanding of the AMR mechanism in clinical/pharmacology research and help identify new targets to reduce antimicrobial resistance.

**Keywords:** Antimicrobial resistance, Metatranscriptomics, Gene ontology, KEGG pathway, Multiomics model

Citation. Ramamurthy, K. 2023. Unifying genomic datasets: Antimicrobial resistance, Metatranscriptomics, Gene ontology, KEGG pathway, Multiomics model. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 20. https://doi.org/10.51585/gtop.2023.2.0035

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# Right to live - how far we can go? Newer strategies to tackle AMR

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**Abstract**

Mankind and Infectious diseases caused by bacteria are on an interesting path, where both having the "Right to live" are turning into battlefields. When Alexander Flemming discovered Penicillin in 1928, and mentioned, "*I did not invent penicillin. Nature did that. I only discovered it by accident*". Further, as early as 1945, Sir Alexander Fleming raised the alarm regarding antibiotic overuse when he warned that the "public will demand [the drug and] . . . then will begin an era . . . of abuses." The overuse of antibiotics drives the evolution of resistance. Wish we had paid attention to his words and taken precautions. The emergence of drug-resistant pathogens has proven to be a grave public health problem. Worldwide, 5.3 million deaths occur annually due to antibiotic-resistant infections. This number can be expected to increase severalfold over time. Globally, a third of all ICU patients develop drug-resistant infections, which substantially increase patient mortality and health care costs. The multidrug-resistant ESKAPE pathogens, namely, *Enterococcus faecium*, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, *Pseudomonas aeruginosa*, and *Enterobacter* spp., have emerged as the leading causes of nosocomial infections. In this talk few strategies will be discussed, which must be kept in mind while tackling bacterial infectious diseases.

**Keywords:** Drug-resistant pathogens, Nosocomial infections, ESKAPE pathogens, Antibiotics

Citation. Chakravortty, D. 2023. Right to live - how far we can go? Newer strategies to tackle AMR. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 21. https://doi.org/10.51585/gtop.2023.2.0035

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# Functional analysis of membrane vesicles of bacteria

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## Abstract

Bacteria release outer membrane vesicles (OMVs) throughout all growth stages, serving various functions such as intracellular signaling, horizontal gene transfer, and defensive mechanisms. Recent research highlights the crucial role of OMVs in bacterial multidrug resistance. Studies indicate that OMVs from *E. coli* shield bacteria against membrane-active antibiotics like colistin and melittin and protect other bacterial strains, including *P. aeruginosa* and *A. radioresistens*. However, the protection against antibiotics differs depending on the situation, and the mechanism varies for different antibiotics. OMVs were found to sequester and degrade portions of antibiotics, leading to reduced effective concentrations and potentially promoting antibiotic resistance. The proteome analysis of *E. coli*'s OMVs reveals the presence of peptidases and proteases, which can degrade antibacterial peptides like melittin. Further investigations on OMV proteomes from various bacterial strains show correlations to biofilm augmentation, quorum sensing, and cytotoxicity. Comparative studies of drug-sensitive and resistant strains of *Acinetobacter baumannii* demonstrate differences in the number of antibiotic-conferring proteins present in their OMVs, impacting bacterial responses to antibiotics. Additionally, an indole derivative was found to decrease the production of OMVs and subsequently affect antibiotic efficacy. Understanding the intricate role of OMVs in drug resistance is essential for a comprehensive approach to tackling this problem effectively.

**Keywords:** One-Health, ESKAPE pathogens, Antimicrobial resistance, Metadata

Citation. Jagannadham, M. V. 2023. Functional analysis of membrane vesicles of bacteria. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 22. https://doi.org/10.51585/gtop.2023.2.0035

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# The T5 phage-encoded antimicrobials

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**Abstract**

As we rapidly approach the post-antibiotic era with the advent of multidrug-resistant bacteria, an alternative to present-day antibiotics becomes the need of the hour. Bacteriophages have coevolved with bacterial hosts and developed multiple means to target host metabolic pathways. Studying these phage-encoded gene products may lead to identifying new targets and novel molecular tools for manipulating host bacteria. We have identified that T5.008 and T5.015 inhibit bacterial cell growth. A whole-genome DNA-seq-based method identified the *E. coli* cell division protein, FtsZ, and the DNA repair protein, uracil DNA glycosylase (Ung), as the interacting proteins with T5.008 and T5.015, respectively. We found that T5.008 disrupts the FtsZ ring and inhibits cell division. This provides a selective advantage to T5, maximizing its progeny number by preventing the escape of daughter cells. We also found that T5.015 binds Ung, and they selectively nick dUMP-containing DNA together. The endonucleolytic activity of T5.015 leads to replication blockage, followed by the blocking of cell division. Thus, by inhibiting replication and cell division, T5.015 may enhance the utilization of host cell resources. Our studies thus reveal two phage proteins that may be used as antimicrobials through unique mechanisms we have elucidated.

**Keywords:** Multidrug-resistant Bacteria, Lateral gene transfer, Antimicrobials

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# Decrypting the edge of bacterial cell-to-cell and gut bacterial-intestinal host cell interaction

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**Abstract**

It is evident that bacteria directly communicate with their encounter parts in an 'inter-and-intra species' manner and interact with their host. We have described that, when bacteria reside close by, they interconnect with each other via membranous nanotubes and exchange their cytoplasmic molecules, notably resistance to the antibiotics both in a transient and hereditary manner. I will discuss how bacteria build these nano-tubular networks and their molecular architectures, remarkably independent of classical conjugation. Such bacterial attributes are highly prominent in the host microbiota, where bacteria communicate with each other and interact with the host cells. In germ-free mice models, we grew the premier gut symbiotic bacteria, Segmented Filamentous Bacteria (SFB), in an *in-vitro* environment with intestinal cells and *in-vivo* conditions. Utilizing higher-resolution cell biology tools, we found that only the single-celled intracellular offspring (IOs) harbor flagella. The IOs flagella are specific to their holdfast, which attaches with host epithelium, thus regulating almost all the immune systems and host pathophysiology. I believe that such fascinating bacterial physiology is likely to change our view on how molecular cross-talk and ongoing <<war-and-peace>> between bacteria and host cells results in the emergence of symbiosis and pathogenesis.

**Keywords:** Gut Symbiotic Bacteria, Antibiotics

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# iPHaGe Study: Identifying Lytic Phages as Potential Alternatives to Antibiotics for Combating Multidrug-Resistant *Enterobacteriaceae*

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## Abstract

The emergence of multidrug-resistant (MDR) bacteria is a grave global health issue, and the time for studying the problem has passed; finding solutions is now crucial. Lytic bacteriophage or phage has been proposed as a promising alternative to antibiotics for combatting MDR. Selling antibiotics without a prescription, improper dosing, incomplete courses, and the use of generic antibiotics are common practices in Bangladesh, contributing to the development of MDR. Ongoing MDR surveillance at icddr, b reveals that over 90% of MDR pathogens circulating in the Bangladeshi community and hospitals are *E. coli* and *Klebsiella pneumoniae*. Comprehensive characterization at the icddr, b Genome Centre (iGC) confirms the diversity of these pathogens (n=3000), with 267 MLST of *E. coli* and 156 MLST of *Klebsiella pneumoniae* identified. With an established biobank of MDR bacteria, iGC initiated the iPHaGe (icddr, b Phage Hunting and Genomics) study, aiming to discover therapeutic phages and to create a phage biobank with potential therapeutic applications. Preliminary findings identified 83 phages from 11 hospital effluents against 13 different *Enterobacteriaceae* host strains. These phages were further sequenced using NGS methods and subjected to host range analysis. Two notable phages, iPHaGe-KPN-11i and iPHaGe-KPN-12i, exhibited potential against extended-spectrum cephalosporin-resistant *K. pneumoniae*. Both phages belong to the *Straboviridae* family, possessing a single endolysin gene (phage_ lysozyme; PF00959.22) capable of lysing at least 12 distinct *Enterobacteriaceae in-vitro*. Unlike many host-specific phages, these two demonstrated a broader lytic spectrum, enabling the opportunity to use them in phage cocktails for host range expansion with further evaluations and could be a beacon of hope in the looming darkness of the AMR crisis.

**Keywords:** Multidrug-Resistant Bacteria, Host-specific phages, *Klebsiella pneumoniae*, *E. coli*

Citation. Rahman, S., Khan, T. , Hossain, Md. M., Faysal, Md. N. I. , Haider, A. , Mahamud, I., Akther, J., Mondal, S. I. , Begum, A., Biswas, S. K. , Jubair, M. and Rahman, M. 2023. iPHaGe Study: Identifying Lytic Phages as Potential Alternatives to Antibiotics for Combating Multidrug-Resistant *Enterobacteriaceae*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 25. https://doi.org/10.51585/gtop.2023.2.0035

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# Role of *in-vitro* gut conditions in modulating antimicrobial resistance and biofilm-forming ability of gut pathogens

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## Abstract

Antimicrobial resistance and biofilm formation by gut pathogens is a serious concern worldwide, leading to outbreaks resulting in high mortality rates. In the present study, clinical and environmental strains of *E. coli* were subjected to biofilm-forming ability under in-vitro gut conditions. The integrity of preformed biofilm on exposure to ciprofloxacin was studied. Similarly, the biofilm-forming ability of multidrug-resistant and susceptible nontyphoidal *Salmonella* isolates on exposure to bile was also studied. A high degree of resistance was observed in clinical *E. coli* isolates with a concomitant prevalence of bla TEM. Bile, pH, and low temperature enabled the *E. coli* biofilm to resist the effect of ciprofloxacin. Clinical isolates of *E. coli* formed strong biofilms in in-vitro gut conditions following exposure to high concentrations of ciprofloxacin. The expression of biofilm genes varied between different gut conditions, viz., the presence of bile, pH, and low temperature, included in this study. Among *Salmonella* isolates, the relative gene expression study of the selected serovars for eight different genes showed a striking difference in the expression levels, supporting the hypothesis that the presence of bile triggers biofilm formation in food-associated strains of nontyphoidal *Salmonella* by upregulation of genes involved in biofilm production. This study demonstrates the importance of *pap*C and *csg*A for maintaining the biofilm integrity upon antibiotic exposure in *E. coli*. While *gcp*A plays a very important role in biofilm formation in *Salmonella* isolates. *E. coli* and *Salmonella* form biofilm as a survival strategy to adapt to the conditions in their environment irrespective of their drug resistance status. Mobile genetic elements such as plasmids, integrons, and transposons are important in drug resistance among gut pathogens. These pathogens can form biofilms irrespective of their resistance pattern. Further, the proximity of the bacteria within the biofilm can facilitate the transfer of antibiotic resistance and virulence genes through mobile genetic elements. Bile enhances the process of biofilm formation by turning on the genes responsible for biofilm production. Hence, it is important to elucidate various mechanisms of drug resistance and biofilm formation by gut pathogens such as *E. coli* and *Salmonella*.

**Keywords:** Drug Resistance, Antimicrobial Resistance, *Escherichia coli*, Salmonella

Citation. Deekshit, V. K., Kotian, A. and Aditya, V. 2023. Role of *in-vitro* gut conditions in modulating antimicrobial resistance and biofilm-forming ability of gut pathogens. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 26. https://doi.org/10.51585/gtop.2023.2.0035

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# Antagonistic activities of *Pongamia pinnata* against biofilm-forming bacteria

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## Abstract

*Pongamia pinnata* is a medium-sized glabrous tree that grows in the coastal regions of South Eastern Asia and Australia. All parts of the plant have been used as crude drugs for the treatment of tumors, piles, skin diseases, wounds, and ulcers. Extracts of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory, and analgesic activities. Previous phytochemical investigation of this plant indicated the presence of abounding prenylated flavonoids such as furanoflavones, furanoflavonols, chromenoflavones, furanochalcones, and pyranochalcones. In our preliminary studies, *P. pinnata* showed bacterial growth inhibition in a dose dose-dependent manner; The Microdilution method was used to test antibacterial activity). It was noted that *P. pinnata* extract decreased the violacein production and biofilm formation compared to the negative control. Among all three plant extracts (PP-DW, PP-M, and PP-E), distilled water extract showed the highest percentage of inhibition. It was observed that there was a significant positive result with the agar disc diffusion method. Biofilm formation was decreased with the increasing concentration of *P. pinnata* leaf extract on cover glasses incubated in broth (By light microscopic analysis of biofilm formation). All these experiments prove that the leaf extract of *P. pinnata* inhibited the biofilm formation capacity of the tough pathogens.

**Keywords:** *Pongamia pinnata*, Antibiofilm activity, Antagonistic activity

Citation. Kodali, V. P., Mekala, I., Valluru, N. and Vadlapudi, M. 2023. Antagonistic activities of *Pongamia pinnata* against biofilm-forming bacteria. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 27. https://doi.org/10.51585/gtop.2023.2.0035

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# Expanding the antimicrobial activity of peptide antibiotics that inhibit translation termination

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## Abstract

Several important antibiotics act by targeting various steps of bacterial protein synthesis. The antibacterial peptide Apidaecin (Api), produced by honeybees, is the first known antibiotic that inhibits translation termination. Api enters Gram-negative bacteria via inner membrane transporters, such as SbmA. Following the release of the newly synthesized protein, Api binds in the ribosome's nascent peptide exit tunnel (NPET), where its C-terminal residues interact with the release factor and arrest the ribosome at the stop codon, causing cell death. Recently, we showed that Drosocin (Dro), another Proline-rich antimicrobial peptide (PrAMP), also acts in an Api-like manner and inhibits translation termination. The unique mechanisms of action of Api and Dro make them attractive candidates for developing new antibiotics, but the dependence on transporters for uptake limits their antimicrobial activity. Therefore, we explored strategies for expanding the antimicrobial spectrum of Api action by designing Api-derived peptides with alternate entry mechanisms into bacterial cells. By conditional expression of the engineered Api gene in *Escherichia coli*, we produced the peptide directly in the cytosol of the bacterium and eliminated the need for import. Analysis of a comprehensive library of endogenously expressed single-site substitution mutants of Api highlighted the essentiality of the C-terminal residues for on-target activity. Interestingly, the N-terminal region of Api showed high tolerance towards substitutions and deletions. Building on these results, we designed hybrid peptides by coupling bacterial cell-penetrating peptides (CPPs) to the Api segment critical for activity upon the ribosome. These CPP-Api hybrids had improved antimicrobial activity compared to Api against *E. coli* and MDR strains of a clinically relevant pathogen, *Acinetobacter baumannii*. This 'missile-warhead' style hybrid peptide approach can be used for developing more potent antimicrobial peptides that can target a larger repertoire of bacteria while retaining Api's unique mode of action.

**Keywords:** Alternatives to small molecule antibiotics, Ribosome targeting, Translation termination, Hybrid antimicrobial peptides

Citation. Baliga, C., Zhu, W., Vazquez-Laslop, N. and Mankin, A. 2023. Expanding the antimicrobial activity of peptide antibiotics that inhibit translation termination. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 28. https://doi.org/10.51585/gtop.2023.2.0035

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# Same-day visual, quantitative antibiotic sensitivity assay directly from clinical samples

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## Abstract

Urinary tract infections (UTIs) account for 150 million cases annually worldwide, with a prevalence rate of 33.54% in India. Prescription without diagnosis is common during UTI treatment as reliable diagnosis takes 2-3 days, and not getting the right treatment contributes to antimicrobial resistance (AMR) and recurrent UTIs in 20-30% of women. AMR continues to be a global public health challenge due to improper and excessive use of antibiotics in human health and animal welfare, leading to severe antimicrobial-resistant infections, disease complications, and mortality. The current diagnosis of urinary tract infections (UTIs) involves a qualitative 2-minute dipstick analysis with a 25-75% sensitivity and 94-100% specificity, but exact identification of microorganisms and the right antibiotics requires urine culture, which takes 2-3 days, trained personnel and misses out nearly 48% UTI cases. We have developed a point-of-care visual, quantitative antibiotic sensitivity assay (ESKaPE$^{TM}$ kit, patent pending), which provides information on the right antibiotic in under 6 hours with clinical urine samples. The kit also provides information on significant bacteriuria and helps partially identify bacteria. The test has been validated in a pilot clinical evaluation with human urine samples with 91.6% sensitivity, 93.5% specificity, 84.6% PPV, 96.6 % NPV, and 93% accuracy. The kit has been validated in a NABL-accredited lab to provide results under 6 hr for urine, throat swabs, and blood samples. We currently provide veterinarians with antibiotic sensitivity information, which fits well with the WHO One-Health initiative.

**Keywords:** Urinary Tract Infection, Antimicrobial Resistance, One-Health

Citation. Naik, R. R. and Naik, L. S. 2023. Same-day visual, quantitative antibiotic sensitivity assay directly from clinical samples. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 29. https://doi.org/10.51585/gtop.2023.2.0035

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# Antimicrobial drug resistance - Fast and rapid elucidation using Nanopore sequencing

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## Abstract

In the relentless fight against antimicrobial resistance (AMR), Next-Generation Sequencing (NGS) technologies have become essential tools for surveillance and research. Genotypic, India's pioneering genomics company, is leading by embracing Nanopore sequencing for identifying pathogens and their associated antibiotic resistances and sensitivities. With its ability to provide fast, cost-effective, and portable NGS sequencing capabilities, Nanopore technology facilitates comprehensive genomic surveillance of pathogenic microorganisms, especially those carrying AMR genes. Here, we present a few case studies on the synergy between Nanopore sequencing and AMR research, underscoring how this transformative technology equips us to promptly pinpoint, monitor, and comprehend the genetic components driving antibiotic resistance. Amidst global threats AMR poses, whether in *Mycobacterium tuberculosis* (MTB) or malaria, Nanopore sequencing stands out as a dependable solution. Explore the innovative offerings from Genotypic in this pivotal pursuit: Genotypic Products.

**Keywords:** Antimicrobial resistance, NGS sequencing, *Mycobacterium tuberculosis*

Citation. Jegadeesan, S. 2023. Antimicrobial drug resistance - Fast and rapid elucidation using Nanopore sequencing. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 30. https://doi.org/10.51585/gtop.2023.2.0035

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# Computational approach towards repurposing of FDA approved drug molecules: Strategy to combat antibiotic resistance conferred by *Pseudomonas aeruginosa*

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## Abstract

*Pseudomonas aeruginosa* is one of the leading causes of hospital-acquired infections like ventilator-associated pneumonia, cystic fibrosis, diabetic foot ulcers, and delayed wound healing due to the pervasiveness of the multiple drug-resistant strains. In the study, 862 unique antimicrobial-resistant genes were retrieved from the NDARO database hosted by NCBI, followed by which we searched for the protein-protein interaction using STRING V9.0 database and retrieved interaction for 45 genes, which was then visualized and analyzed in Cytoscape 3.9.0. Among the 45 genes, the top 10 that manifested the maximum interactions are *opr*M, *mex*A, *mex*B, *mex*R, *mex*T, *opr*N, *nfx*B, *nal*C, *nal*D, and *gyr*B. Further, *mex*B was used in our further screening study to identify a potential inhibitor. One thousand six hundred two clinically approved drugs were screened virtually against MexB to understand their ability to inhibit the MexB protein. Amongst them, the top 5 drug molecules were selected based on the binding energies for analyzing their physio-chemical and toxicity properties. Lomitapide was found to have the maximum negative binding energy followed by Nilotinib, whereas Nilotinib's number of hydrogen bonds was higher than that of Lomitapide. A Pharmacokinetics study revealed that all five drug molecules had limited aqueous solubility and inadequate bioavailability scores for Lomitapide and Venetoclax, while Nilotinib, Eltrombopag, and Conivaptan demonstrated higher potential for therapeutic levels. Molecular dynamic simulation of Nilotinib on *mex*B protein was carried out for 20 nanoseconds. The Root Mean Square deviations, Root mean square fluctuations, hydrogen bond formation, and radius of gyration demonstrated high stability of the protein drug complex and lesser distortions, thereby making Nilotinib a potential inhibitor to be used against MexB of *Pseudomonas aeruginosa* .

**Keywords:***Pseudomonas aeruginosa*, Lomitapide, Venetoclax, MexB protein

Citation. Sivashanmugam, K. and Chatterjee, D. 2023. Computational approach towards repurposing of FDA approved drug molecules: Strategy to combat antibiotic resistance conferred by *Pseudomonas aeruginosa*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 31. https://doi.org/10.51585/gtop.2023.2.0035

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# A tale of two targets: Phage encoded factor as a dual morphogenetic regulator of bacteria

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**Abstract**

Bacteriophages encode various host takeover factors deployed to hijack the host cell machinery. Some notable mechanisms of host takeover include manipulation of transcription, translation, metabolism, and respiration. However, the role of phage-encoded factors in exploiting bacterial cell organization is not well explored. In this study, we aim to address this question by focusing on KilR, a toxic protein encoded by the Rac prophage of *E. coli*. We show that KilR, when expressed in low concentrations, disrupts bacterial cell division by preventing Z-ring formation, consistent with previous studies. Intriguingly, when expressed at higher amounts, KilR completely abolished the rod shape of the cell, suggesting that it also affects the bacterial cytoskeletal system. Using a functional mCherry fluorescent fusion, we show that KilR-mCherry/mCherry-KilR is diffusively localized in the cytoplasm. However, when co-expressed with the MreBCD cytoskeletal proteins, KilR-mCherry form filaments co-localized with fluorescently tagged MreB, suggesting the MreB system as the elongasome target of KilR. While FtsZ expression can completely abolish KilR toxicity, expression of MreBCD does not alleviate KilR-associated growth defect. mCherry-KilR phenotype resembles Wildtype KilR, whereas KilR-mCherry cells didn't turn around even upon overexpression of KilR. To determine the function, we have deleted 12 amino acids from the C-terminal region of KilR, and it is no longer toxic to the cells, and the phenotype was restored to normal. Currently, we are employing pulldown and Co-IP to uncover the mechanistic basis of KilR interactions with the divisome and the elongasome proteins. Our findings suggest that KilR is a phage-encoded dual morphogenetic regulator of bacterial cell shape. We will suggest a possible model for the physiological importance of phage-mediated modulation of host cell shape..

**Keywords:** Bacteriophage, FtsZ, MreB, Cell shape, Cell division

Citation. Marepalli, A., Nandhakumar, M. and Govindarajan, S. 2023. A tale of two targets: Phage encoded factor as a dual morphogenetic regulator of bacteria. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 32. https://doi.org/10.51585/gtop.2023.2.0035

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# Systemic exploration of bacterial pigments against antimicrobial-resistant *Acinetobacter baumannii*

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## Abstract

Bacterial pigments comprise a range of secondary metabolites that confer fitness advantages to the producers under diverse conditions. Since most of such chromogenic metabolites are produced in response to quorum sensing (QS) mediated expression of biosynthetic gene clusters, exploring the possible impact of the pigments on QS appears exigent. Thus, a systemic screening of bacterial pigments for QS-inhibition combined with antibiofilm and antimicrobial action against *Acinetobacter baumannii* might offer viable alternatives against the priority pathogen. Major bacterial pigments are classified (clustered) based on their physicochemical properties, and representatives of the clusters are screened for QS inhibition. The screen highlighted two bacterial pigments (PCN and PDG) as potent quorum quenchers. In silico analysis involving molecular docking and MD-simulation have highlighted potential interaction with major QS-regulators AbaI and AbaR and impaired biofilm formation, a major QS-dependent event in the pathogen. The pigments also altered the composition of extracellular polymeric substances (EPS) and affected biofilm-associated attributes like surface motility. An intricate gene expression analysis revealed modulation of QS-associated genes upon pigment treatment. Both the pigments augmented antibiotic action against *A. baumannii* biofilms. Cell viability analysis revealed the pigments to be modestly cytotoxic against HEK293, a non-cancer human cell line. Antibiotic-resistant clinical isolates demonstrated varied responsiveness against the pigments, with several resistant strains demonstrating collateral sensitivity. To further explore the antimicrobial action of the pigments, resistant lines were generated, and whole genome sequencing of the mutants indicated relevant genomic variations. The results underpin the prospect of a bacterial-pigment-based therapeutic strategy in combating *A. baumannii* infection.

**Keywords:** *Acinetobacter baumannii*, Biofilm, Quorum Sensing, QS-associated genes

Citation. Acharya, K. and Bhattacharya, A. 2023. USystemic exploration of bacterial pigments against antimicrobial-resistant *Acinetobacter baumannii*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 33. https://doi.org/10.51585/gtop.2023.2.0035

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# Influence of temperature on biofilm formation and correlation with AMR among *A. baumannii* isolated from environment and veterinary settings

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## Abstract

Once considered a rare opportunistic organism, *Acinetobacter baumannii* (*A. baumannii*) has emerged as a multidrug-resistant, even extensively drug-resistant pathogen. The clinical samples isolated from animals and humans share identical clones, suggesting that the animals may act as reservoirs. Thus, understanding the prevalence of *A. baumannii* in environmental and veterinary settings is crucial to establishing *A. baumannii's* interplay between environment and animals. Investigating the biofilm formation ability under varying temperatures is important to understand the survival ability of the pathogen in harsh environments and the zoonotic nature of the pathogen. Exploratory research was undertaken to investigate the distribution of *A. baumannii* among healthier animals (large ruminants, small ruminants, and poultry) and water bodies (Krishna River and local ponds). The selective media was used to isolate and characterize *A. baumannii* and the species identification was confirmed by MALDI TOF MS identification. The biofilm assay was carried out using a crystal violet assay. And antimicrobial resistance profile of the selected strains was also performed using the Kirby Bauer Disc diffusion assay. The minimum inhibitory concentration was determined to correlate AMR and biofilm-forming ability. At 27°C, the environmental isolates appeared to produce the biofilm and were susceptible to 80% of the antibiotics tested. On the other hand, at 37°C, the isolates from the veterinary settings appear to form a thick biofilm with varying AMR profiles. However, this study reflects the influence of temperature on biofilm phenotype; further research is needed to ascertain the presence and expression of genes associated with biofilm production and AMR.

**Keywords:** *Acinetobacter baumannii*, Antimicrobial resistance, Biofilm, MALDI TOF MS

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# Development of an oral bivalent vaccine delivery method for prevention of *Streptococcus agalactiae* and *Streptococcus iniae* diseases in finfish aquaculture

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## Abstract

Globally, there is a lack of protein sources for the increasing global population. Aquaculture plays a promising role in food production, and it is estimated that up to 70% of supplementary protein will be required from animal sources by 2050 (WOAH). Finfish are the most cultivated fish in marine, brackish, and freshwater; in freshwater finfish culture, Tilapia is the second largest cultivated fish worldwide. However, emerging disease outbreaks and Antimicrobial Resistance (AMR) pose challenges to sustainable aquaculture, potentially impacting rural economic states, food security, and human health. The COVID-19 pandemic has raised concerns about animal-borne infectious diseases. *Streptococcus agalactiae* and *Streptococcus iniae*, like AMRs, cause economic losses in low- and middle-income countries. Vaccines have been developed as a potential solution for AMR, but their immunogenic activity is often questioned. This study aims to develop a divalent whole vaccine using naturally derived lectin in *Orechromis niloticus*. Multiplex PCR has confirmed pure cultures for both bacterial species and quantified by qPCR. Experimental infections were carried out in various groups using lectin as an adjuvant via the oral route. Vaccine-challenging studies have been carried out with fingerling-sized *O. niloticus*. On the 25th day of post-vaccination, animals were examined for Interleukin-1, Interleukin-8, and LBP (lipopolysaccharide binding protein) immune gene in the head, kidney, and liver by RT-qPCR shows the promising result of a 2-fold up-regulation of all three genes compared with the controlled group. 25$^{th}$ day post-oral vaccinated with two booster-dosed animals were challenged with bacterial inoculation, with 78 and 81% RPS against *S. agalactiae* and *S. iniae*, respectively. These promising results on immune gene expression and RPS show that lectin is the better property of an adjuvant and efficient system to enhance immunity. The bivalent vaccine approach will reduce the burden on finfish aquaculture systems.

**Keywords:** qPCR, Immune gene, *Orichromis niloticus*, *S. agalactiae*, *S. iniae*

Citation. Nandhakumar, K., Byadgi, O. V., Chen, S. and Elumalai, P. 2023. Development of an oral bivalent vaccine delivery method for prevention of *Streptococcus agalactiae* and *Streptococcus iniae* diseases in finfish aquaculture. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 35. https://doi.org/10.51585/gtop.2023.2.0035

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# Anti-quorum sensing activity of selected cationic amino acids against *Chromobacterium violaceum* and *Pseudomonas aeruginosa*

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## Abstract

Antimicrobial resistance (AMR) has emerged as a significant and urgent public health concern that poses significant challenges to effectively preventing and managing chronic illnesses. The misuse and overuse of various antibacterial agents have a major impact on healthcare systems. N-Acyl homoserine lactones, also known as autoinducers, is a unique type of signaling molecule used by the bacterial community that passively permeates and regulates the bacterial population of Gram-negative bacteria like *Pseudomonas aeruginosa* and *Chromobacterium violaceum* that causes fatal infections when exposed to humans. The selected cationic amino acids histidine, arginine, and ornithine anti-quorum sensing activity were assessed against *P. aeruginosa* and *C. violaceum*. The MIC and sub-MIC concentrations were determined by the broth dilution method for the selected amino acids. Both the cultures have been treated with MIC and sub-MIC levels, and virulence factors like rhamnolipid, elastase, protease, pyoverdine, pyocyanin for *P. aeruginosa* and violacein, chitinase for *C. violaceum* is quantified. The quantification of exo-polysaccharides and the biofilm and visualization of biofilm by confocal laser scanning microscope, swarming, and swimming motility have been performed for both organisms. The genes activated for all these virulence factors were quantified by RT-qPCR and expressed in fold change. The results show that the histidine inhibited both the organisms at MIC and sub-MIC more than the percentage of virulence factors production when treated with arginine and ornithine. The selected cationic amino acids are an effective compound in antimicrobial therapeutics to reduce opportunistic bacterial infections.

**Keywords:** Amino acids, Cationic amino acids, Anti-quorum sensing activity, Biofilm, Emerging pathogen

Citation. Abinaya K., Gopinath, S. C. B., Kumarevel, T. and Raman, P. 2023. Anti-quorum sensing activity of selected cationic amino acids against *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 36. https://doi.org/10.51585/gtop.2023.2.0035

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# Identification of new lead molecules for targeting DAH7PS as anti-tuberculosis agents based on Pharmacophore modeling

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## Abstract

Tuberculosis (TB) is one of the deadliest infectious diseases caused by the bacterial pathogen *Mycobacterium tuberculosis* (Mtb). The shikimate pathway is one of the important biochemical cascades for producing aromatic compounds, which are incorporated in the biosynthesis of proteins, cofactors, and siderophores. The initial committed step is catalyzed by the enzyme 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAH7PS), a stereospecific aldol-like condensation reaction. The gene disruption studies related to the shikimate pathway have confirmed that Mtb is not viable if the pathway is not operational. These outcomes mark the DAH7PS as an attractive target for designing the drugs. The following list of molecules was selected from the various literature sources for pharmacophore modeling: 3-pyridine carboxaldehyde, Chlorogenic Acid, Rutin, Negundiside, VS16, VS18, and VS25. The following compounds were chosen because of their high affinity and good binding to the receptor site. The Pharmacophore model was generated using the ligand-based approach with the help of the Pharmacist webserver and subjected to virtual screening of the Zinc database using the Zincpharmer server. Based on the RMSD value, the top 100 molecules were selected for further screening with drug-likeness, ADME, and toxicity filters. Later, molecular docking was performed for the 30 compounds on Mtb DAH7PS enzyme (PDB ID: 2B7O) using the Glide program of the Schrodinger suite. It was found that many molecules have shown docking scores better than the co-crystallized ligand. The top 3 compounds were subjected to molecular dynamic simulations to prove their stability with the target enzyme and check their efficacy over 100 ns using Desmond with MM-GBSA calculations. It was found that the selected three compounds have formed a complex with an enzyme, which has better stability throughout the runtime. These compounds can be further explored *in-vitro* and *in-vivo* to prove their efficacy of antitubercular activity.

**Keywords:** AHAS, PyRx, Glide, Machine learning, In-silico anti-tb

Citation. Alavala, R. R. 2023. Identification of new lead molecules for targeting DAH7PS as anti-tuberculosis agents based on Pharmacophore modeling. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 37. https://doi.org/10.51585/gtop.2023.2.0035

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# Exploration of potential acetohydroxyacid synthase (AHAS) inhibitors through SBDD, molecular docking, molecular dynamics, and *in-silico* anti-tubercular screening by graph-based signature approach

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## Abstract

The advent of MDR, XDR, and TDR-TB has led to the failure of orthodox therapy regimens, and hence, the task of combating TB becomes more challenging day by day. Therefore, the need arises for identifying and isolating new drug targets and subsequent design of specific inhibitors. Bacterial Acetohydrxyacid Synthase (AHAS) was identified as a unique and novel enzyme catalyzing the branched-chain amino acid synthesis in bacteria, including *M. tuberculosis*. *M. tuberculosis* AHAS is very similar to *E. coli* AHAS and has similar functions. This necessitates the design of specific AHAS inhibitors against M.tb that would consequently decrease the BCAA supply in bacteria, and thus, an effective bacteriostasis can be achieved. More than 100 potential AHAS inhibitors were developed based on scaffolds and structural features, and their antitubercular activity was evaluated. Firstly, structure-based drug design studies were performed to explore the efficacy and potential binding interactions by PyRx. The findings were further enriched through the Glide algorithm. The selected compounds were projected to molecular dynamics simulation studies. In-silico anti-tb potential of the ligands was further evaluated by implementing a machine-learning algorithm. Concluding from the above studies, the selected AHAS inhibitors can serve as potential antitubercular agents. This study will be useful for developing a safe and effective drug against TB.

**Keywords:** AHAS, PyRx, Glide, Machine learning, In-silico anti-tb, Antitubercular agents

Citation. Gokhale, K. 2023. Exploration of potential acetohydroxyacid synthase (AHAS) inhibitors through SBDD, molecular docking, molecular dynamics, and in-silico anti-tubercular screening by graph-based signature approach. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 38. https://doi.org/10.51585/gtop.2023.2.0035

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# Interplay of selected polyphenolics, vitamins, and essential amino acids on the antibacterial activity of ciprofloxacin

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## Abstract

Antibiotic resistance is a major challenge to clinicians in treating various diseases. It is well established that *E. coli* is a common bacterium that often gets resistant to antibiotics. There is an emerging need to evaluate the antibacterial activity of natural compounds and dietary supplements, commonly taken with medication, serve as an alternative to antibiotics, and can be combined with antibiotics to combat resistance. The present study was conducted to determine the antibacterial activity of selected polyphenolics, vitamins, and essential amino acids alone and in combination with ciprofloxacin against *E. coli*. The antibacterial activity was assessed by microbroth dilution assay as per the CLSI guidelines, and MIC was used as an endpoint to compare the antibacterial efficacy of test compounds. MIC of ciprofloxacin alone against *E. coli* is 0.03$\mu$g/ml. Among the selected vitamins, ascorbic acid, thiamine, and pyridoxine, pyridoxine showed a MIC of 0.06mg/ml. Ascorbic acid and thiamine, essential amino acids tryptophan and phenylalanine alone do not exhibit any antibacterial activity but in combination with ciprofloxacin enhanced the antibacterial activity of ciprofloxacin by improvement in MIC by 2 to 4-fold. The polyphenolics ellagic acid, syringic acid, and shikimic acid alone exhibited MIC ranging from 2.5 to 0.06mg/ml. The combined MIC values were enhanced when compared with individual MIC values. Based on the results obtained, it is evident that a positive drug interaction was observed with the polyphenolics, vitamins, and essential amino acids ***in-vitro*** against *E. coli,* which can be used as a strategy to overcome antibiotic resistance.

**Keywords:** Antibiotic resistance, Ciprofloxacin, Polyphenolics, *E. coli*

Citation. Gullapudi, S., Anjaneyulu, P., Saiprathyusha, G., Metta, M. and Rao, S. 2023. Interplay of selected polyphenolics, vitamins, and essential amino acids on the antibacterial activity of ciprofloxacin. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 39. https://doi.org/10.51585/gtop.2023.2.0035

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# Peptidomimetics as new antimicrobials for treating fungal infections caused by *Candida* species

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## Abstract

Antimicrobial diseases are a significant global health concern, with nearly 20% of the world's population succumbing to secondary infections caused by microorganisms. Fungal infections, particularly those caused by *Candida* species, pose a substantial challenge due to their increasing resistance to common antifungal drugs like fluconazole and echinocandins. *Candida* infections can affect various body parts, including the bloodstream (known as candidemia), with mortality rates ranging from 15% to 49%, depending on factors such as the patient's overall health and the timeliness of treatment. Resistance rates vary across *Candida* species and geographic regions. This research focused on five *Candida* species: *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis* and *Candida krusei*. We aimed to identify key virulence-related proteins or pathways in these species. Our study involved generating structure-based pharmacophores to understand better the molecular interactions involved. Additionally, we conducted a virtual screening of a library containing 9,144 Asinex Peptidomimetic compounds to assess their pharmacokinetic properties, ADMET, TOPKAT, and oral drug-likeness. Subsequently, we performed ligand mapping to identify compounds that interact effectively with our target proteins. The compounds with the highest frequency of effective interactions were further examined for receptor-ligand interactions. Our findings indicate that certain Peptidomimetic compounds possess broad-spectrum activity against *Candida* species, potentially offering a promising approach to combat and mitigate the virulence of these infections.

**Keywords:** *Candida* species, Structure-based pharmacophore, Asinex Peptidomimetics, ADMET, Receptor-ligand interactions

Citation. Shanmugarajan, D., Battula, M., Jakkireddy, M. and David, C. 2023. Peptidomimetics as new antimicrobials for treating fungal infections caused by *Candida* species. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 40. https://doi.org/10.51585/gtop.2023.2.0035

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# *PathCRISP*: Revolutionizing early detection of antimicrobial resistance infections with molecular diagnostics

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## Abstract

The emergence of antimicrobial resistance (AMR), especially in bacterial pathogens, is a critical and pressing concern worldwide. Screening for ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter*) spp and any associated AMR is critical for treatment-related decisions of hospital-acquired infections. However, present diagnostic tools for detecting AMR require 18 hours or longer. The infection might rapidly increase during this waiting period, leading to sepsis and more complications. Hence, usually, a broad spectrum of antibiotics is administered, which would, in turn, lead to Multiple drug resistance (MDR). Therefore, early detection is the key element that can help guide appropriate therapy and bring down AMR-related concerns severely. We propose a CRISPR-based point-of-care detection tool called *PathCrisp* to overcome these limitations. Currently, our tool targets the most notorious AMR gene group - the Carbapenem resistance genes: NDM, OXA, VIM, IMP, and KPC. The guides designed in our test show high specificity to the target DNA. Our initial testing on hospital samples shows promising assay accuracy and sensitivity. Based on initial studies, the *PathCrisp* tool kit is sensitive to low copy numbers and accurately detects the five carbapenem resistance markers. Furthermore, our proposed tool kit reduces the waiting period to 2 hours, leading to early and accurate infection treatment. Shortly, we plan to multiplex this assay to include more resistance markers, including those for ESBLs and MRSA.

**Keywords:** Antimicrobial resistance, ESKAPE pathogens, *PathCRISP*, Multiple drug resistance

Citation. Patil, S. R, Mallur, D., Gupta, V., Chandru, V. and Arora, R. 2023. *PathCRISP*: Revolutionizing early detection of antimicrobial resistance infections with molecular diagnostics. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 41. https://doi.org/10.51585/gtop.2023.2.0035

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# *In-silico* molecular docking analysis for repurposing of approved macrolide antibiotics by siderophore conjugation

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**Abstract**

Antimicrobial resistance (AMR) is a developing concern for global health as numerous pathogens have evolved mechanisms to resist antibiotics. Especially Gram-negative bacteria are resistant to most antibiotics because they have a double membrane that currently available antibiotics cannot penetrate. Therefore, it is necessary to accelerate research on therapeutic strategies that afford potent and selective delivery of antibiotics to these Gram-negative resistant bacteria. These bacteria have specific outer membrane transporters which translocate ferric ions via siderophores into the bacterial cell. Antibiotics can be covalently linked to siderophores to deliver them across the bacterial membrane selectively. Macrolides are antibiotics that target bacterial ribosomes and certain enzymes and are highly susceptible to efflux. However, they do not cross the polar Gram-negative outer membrane. Still, enhancing the potency of macrolide antibiotics against these bacteria has been unexplored. In this project, screening of siderophore-conjugated macrolide antibiotics was performed using in-silico analysis. The binding pocket and binding affinity of siderophore-conjugated antibiotics exhibited an excellent binding score with FhuA, the receptor for ferrichrome-iron (PDB:1FCP) and periplasmic protein FhuD (PDB:1ESZ). The conjugate specifically binds to macrolide phosphotransferases (PDB: 3FRQ, 7W15), macrolide glycosyltransferases (PDB:2IYF), and peptidyl transferase (PDB: 1JZY) enzymes. These are some essential bacterial enzymes required for bacterial metabolism; inhibiting these enzymes would be detrimental to the bacteria. Thus, these results provide insight into the rational design of novel siderophore-drug conjugates against problematic AMR pathogens and could potentially be used in treating AMR.

**Keywords:** Antimicrobial resistance, Gram-negative resistant bacteria, In-silico analysis, Siderophore-conjugated antibiotics

Citation. Lakshminarayan, K., Murugan, D. and Rangasamy, L. 2023. *In-silico* molecular docking analysis for repurposing of approved macrolide antibiotics by siderophore conjugation. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 42. https://doi.org/10.51585/gtop.2023.2.0035

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# Uncovering the hidden connection of *DegP* serine protease and the *MreB* cytoskeletal system of bacteria: A transcriptomic study

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## Abstract

*MreB* is a bacterial cytoskeleton ubiquitously conserved in all rod-shaped bacteria. *MreB* monomers polymerize to form filaments and fibers, which are crucial for maintaining bacterial shape. Inhibition of *MreB* activity resulted in swelling of rod-shaped cells and eventual lysis. Additionally, the function of *MreB* is associated with other functions like cell wall biosynthesis, cell division, and chromosome segregation. Thus, *MreB* is a promising drug target owing to its cruciality and conserved nature. However, inhibition of *MreB* is not effective, and higher concentrations of inhibitors exhibited cytotoxicity. Here in this study, we attempt to explore the factors essential for the survival of bacteria during *MreB* inhibition. Transcriptome analysis of cells lacking functional *MreB* (through A22 treatment and Δ*MreB* cells) exhibited elevated expression of six genes, which includes *DegP*, a gene code for periplasmic serine protease. Relative viability analysis of *DegP* mutants exhibits a hypersensitive phenotype to A22 but not other antibiotics. In the absence of *DegP*, cells bulge and lose their shape even at lower concentrations of A22. Additionally, we found that A22 treatment enhanced the intracellular protein aggregation, which corroborates with the sensitive phenotype of A22. These findings highlight the role of *DegP* in cellular survival during bacterial cytoskeletal inhibition.

**Keywords:** A22, Bacterial cytoskeleton, *DegP*, *MreB*, Protein aggregation

Citation. Nagarajan, T., Kannan, B., Magar, S., Shanmugapriya, K.Amster-Choder, O. and Govindarajan, S. 2023. Uncovering the hidden connection of *DegP* serine protease and the *MreB* cytoskeletal system of bacteria: A transcriptomic study. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 43. https://doi.org/10.51585/gtop.2023.2.0035

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# Transfer-messenger RNA (tmRNA) of bacteria and its importance in survival during DNA damage

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## Abstract

DNA integrity in bacteria is regulated by various factors that act on the DNA. *trans*-translation has previously been shown to be important for the survival of *Escherichia coli* cells exposed to certain DNA-damaging agents. However, the mechanisms underlying this sensitivity are poorly understood. This study explored the involvement of the *trans*-translation system in the maintenance of genome integrity using various DNA-damaging agents and mutant backgrounds. Relative viability assays showed that SsrA-defective cells were sensitive to DNA-damaging agents, such as nalidixic acid (NA), ultraviolet radiation (UV), and methyl methanesulfonate (MMS). The viability of SsrA-defective cells was rescued by deleting *sulA*, although the expression of SulA was not more pronounced in SsrA-defective cells than in wild-type cells. Live cell imaging using a Gam-GFP fluorescent reporter showed increased double-strand breaks (DSBs) in SsrA-defective cells during DNA damage. We also showed that the ribosome rescue function of SsrA was sufficient for DNA damage tolerance. DNA damage sensitivity can be alleviated by partial uncoupling of transcription and translation using a sub-lethal concentration of ribosome-inhibiting antibiotic (tetracycline) or by mutating the gene coding for RNase H (*rnhA*). This study explores the basic understanding of DNA damage hypersensitivity of tmRNA defective cells. The lethality of tmRNA defective cells upon DNA damage portrays tmRNA as novel drug targets.

**Keywords:** Methyl methanesulfonate, Nalidixic acid, Ultraviolet radiation, Gam-GFP fluorescent reporter, *Escherichia coli*

Citation. Nagarajan, T., Munavar, M. M. and Govindarajan, S. 2023. Transfer-messenger RNA (tmRNA) of bacteria and its importance in survival during DNA damage. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 44. https://doi.org/10.51585/gtop.2023.2.0035

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# Comparison of Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for interpreting Micronaut automated system results of *Acinetobacter baumannii* isolates of the environment and veterinary settings

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**Abstract**

Rapid detection and accurate interpretation of antimicrobial resistance (AMR) is paramount. Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are the two most popular guidelines and breakpoints worldwide for deciding antibiotic susceptibility. CLSI is based on subscription, largely influenced by companies/FDA, while EUCAST is freely accessible and transparent in setting up the breakpoints. Both CLSI and EUCAST define "susceptible" (S), "susceptible increased dose" (I), and "resistant" (R). In general, EUCAST breakpoints appeared to result in lower susceptibility rates. There remains uncertainty on how to address the discrepancies between these two guidelines. In India, the Indian Council of Medical Research recommends CLSI guidelines for interpreting antimicrobial susceptibility. This study compared CLSI and EUCAST guidelines concerning *Acinetobacter baumannii* isolated from the environment and veterinary settings. The MICRONAUT automated system and MICRONAUT-S MDR MRGN screening plates containing 12 antibiotic groups, a commercial microdilution method was used to determine minimum inhibitory concentration (MIC) values of 123 *Acinetobacter baumannii* isolates. Interpretation using CLSI 2022 and EUCAST 2022 guidelines displayed the following: 1) one sample displayed a comparable result for colistin. Otherwise, 122 isolates were sensitive at increasing dose (I) according to CLSI and were sensitive (S) according to EUCAST, 2) for ciprofloxacin – CLSI guidelines displayed concordant results for 50 isolates, S for 72 isolates, I for one isolate, while EUCAST resulted in I for 72 isolates and R for one isolate. 3) for ceftazidim, comparable results for 89 isolates, S for 34 isolates as per CLSI, while EUCAST resulted in I for 32 and R for two isolates, respectively. 4) for trimethoprim/sulfamethoxazole, comparable results were obtained for 97 isolates; 25 and one were R and S, respectively, according to CLSI, and 26 isolates were I, according to EUCAST. 5) for piperacillin/Tazobactam, 113 isolates were comparable; CLSI resulted in 6, 3, and 1 with S, I, and R, respectively, while EUCAST indicated 6 and 4 were I and R, respectively. The results indicate that the susceptibility interpretation of *Acinetobacter baumannii* isolates was influenced by CLSI and EUCAST breakpoints discrepancies. In general, clinicians regard I as R, resulting in antimicrobial usage. Therefore, there is an urgent need for harmonizing the breakpoints across the guidelines.

**Keywords:** Antimicrobial resistance, Antimicrobial Susceptibility Testing CLSI, EUCAST, *Acinetobacter baumannii*

Citation. Adukkadukkam, S., Kozytska, T., Wareth, W. and Murugaiyan, J. 2023. Comparison of Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for interpreting Micronaut automated system results of *Acinetobacter baumannii* isolates of the environment and veterinary settings. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 45. https://doi.org/10.51585/gtop.2023.2.0035

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# Antimicrobial resistance, *Acinetobacter baumannii*, Livestock, Prevalence

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**Abstract**

The ubiquitous pathogen *Acinetobacter* (*A.*) *baumannii*, which was considered a neglected pathogen until the 2000s, has now turned into a critical nosocomial pathogen and is listed among the priority pathogen list released by WHO. *A. baumannii* accounts for 600,000 to 1,400,000 infections globally per year and is one of the six leading nosocomial infections. The alarming fact about *A. baumannii* is its emergence of resistance to new antimicrobials, which alarms the fact that there is no effective antibiotic to treat the infection. The clinical samples isolated from animals and humans share identical clones, suggesting that the animals may act as reservoirs. However, data from animal origin, especially from healthier ones, are rarely available to establish *A. baumannii* interplay between environment, animals, and humans. And there is no study reported on these aspects from India. Therefore, an exploratory research study investigated the distribution of *A. baumannii* among healthier animals (large ruminants, small ruminants, and poultry) in the rural region of Guntur, India. Samples representing six different systems (integumentary, digestive, respiratory, excretory, and urogenital) resulted in 51 non-duplicated isolates, with a 49% prevalence. No isolates were obtained from the salivary samples. The species identification was carried out using microbiological analysis and MALDI TOF MS. The Kirby Bauer disc diffusion assay using 16 antibiotics representing eight different classes, indicated that these isolates, except those isolated from the excretory system (n=10, ∼20%), possess comparable antimicrobial profiles: resistant to penicillin, aminoglycoside, cephalosporin and lincosamide, intermediate resistance to fluoroquinolone, meropenem (carbapenem) and vancomycin (glycopeptide) and susceptible to tetracycline. As far as our knowledge is concerned, this is the first study of its kind from India.

**Keywords:** Antimicrobial resistance, *Acinetobacter baumannii*, Livestock, Prevalence

Citation. Das, V., Adukkadukkam, S., Chandrasekhar. S., Kumar, P. A. and Murugaiyan, J. 2023. Prevalence of *Acinetobacter baumannii* in livestock in Guntur, India. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 46. https://doi.org/10.51585/gtop.2023.2.0035

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# Qualitative and quantitative biofilm assay of wild-type *Acinetobacter baumannii*

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**Abstract**

Rapid detection and accurate interpretation of antimicrobial resistance (AMR) is paramount. Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are the two most popular guidelines and breakpoints worldwide for deciding antibiotic susceptibility. CLSI is based on subscription, largely influenced by companies/FDA, while EUCAST is freely accessible and transparent in setting up the breakpoints. Both CLSI and EUCAST define "susceptible" (S), "susceptible increased dose" (I), and "resistant" (R). In general, EUCAST breakpoints appeared to result in lower susceptibility rates. There remains uncertainty on how to address the discrepancies between these two guidelines. In India, the Indian Council of Medical Research recommends CLSI guidelines for interpreting antimicrobial susceptibility. This study compared CLSI and EUCAST guidelines concerning *Acinetobacter baumannii* isolated from the environment and veterinary settings. The MICRONAUT automated system and MICRONAUT-S MDR MRGN screening plates containing 12 antibiotic groups, a commercial microdilution method was used to determine minimum inhibitory concentration (MIC) values of 123 *Acinetobacter baumannii* isolates. Interpretation using CLSI 2022 and EUCAST 2022 guidelines displayed the following: 1) one sample displayed a comparable result for colistin. Otherwise, 122 isolates were sensitive at increasing dose (I) according to CLSI and were sensitive (S) according to EUCAST, 2) for ciprofloxacin – CLSI guidelines displayed concordant results for 50 isolates, S for 72 isolates, I for one isolate, while EUCAST resulted in I for 72 isolates and R for one isolate. 3) for ceftazidim, comparable results for 89 isolates, S for 34 isolates as per CLSI, while EUCAST resulted in I for 32 and R for two isolates, respectively. 4) for trimethoprim/sulfamethoxazole, comparable results were obtained for 97 isolates; 25 and one were R and S, respectively, according to CLSI, and 26 isolates were I, according to EUCAST. 5) for piperacillin/Tazobactam, 113 isolates were comparable; CLSI resulted in 6, 3, and 1 with S, I, and R, respectively, while EUCAST indicated 6 and 4 were I and R, respectively. The results indicate that the susceptibility interpretation of *Acinetobacter baumannii* isolates was influenced by CLSI and EUCAST breakpoints discrepancies. In general, clinicians regard I as R, resulting in antimicrobial usage. Therefore, there is an urgent need for harmonizing the breakpoints across the guidelines.

**Keywords:** *Acinetobacter baumannii* Biofilm assay, Congo red agar assay, Crystal violet assay, Tube assay

Citation. Puchakayala, T. P., Adukkadukkam, S. and Murugaiyan, J. 2023. Qualitative and quantitative biofilm assay of wild-type *Acinetobacter baumannii*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 47. https://doi.org/10.51585/gtop.2023.2.0035

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# A comparative analysis of the AMR profile of *Acinetobacter baumannii* isolated from environmental and veterinary setting

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## Abstract

Multidrug-resistant *Acinetobacter baumannii* (*A. baumannii*), an opportunistic nosocomial bacterium, is of great global health concern due to the fast emergence of antimicrobial resistance and limited treatment options. Understanding the diversity and strain level differences is crucial for the "One-Health" perspective. A survey on the distribution of *A. baumannii* in various samples and antimicrobial resistance profiling will reflect the scenario in detail. Samples were collected from the environment (Krishna River and local pond) and veterinary (small and large ruminants, poultry) to compare the AMR profile of the isolated strains. The bacteria were isolated using selective growth media Hichrome Acinetobacter agar base, and species identification was carried out using biochemical methods. A total of 16 antibiotics belonging to eight antibiotic classes, Aminoglycosides (streptomycin, gentamycin), cephalosporins (ceftriaxone, cefepime), Tetracyclines (tetracyclines, doxycycline), penicillin (ampicillin, amoxicillin), fluoroquinolones (ciprofloxacin, levofloxacin), a carbapenem (ertapenem, meropenem), lincosamides: (clindamycin, lincomycin) and glycopeptides (vancomycin, teicoplanin) were chosen to perform antibiotic disc diffusion experiment on the effectiveness against isolated *A. baumannii* infections and the results were compared with the CLSI standard. The prevalence of *A. baumannii* among environmental samples was 73%, showing 46% for veterinary isolates. The antimicrobial activity of *A. baumannii* isolated from local ponds displayed variations from the CLSI standard. All the isolates were 100% susceptible to ciprofloxacin, levofloxacin, and meropenem. And 100% resistance towards clindamycin, lincomycin, vancomycin, and teicoplanin. The Krishna River isolates were detected as Multidrug-resistant strains with carbapenem resistance. The veterinary isolates showed resistance to penicillin, aminoglycoside, cephalosporin, glycopeptide, ertapenem (carbapenem), and lincosamide, except for the excretory system isolates. They showed a different AMR resistance profile towards tetracyclines, glycopeptides, and lincomycin (lincosamide) and were sensitive to carbapenems. This study could be taken further to analyze the AMR gene expression level.

**Keywords:** *Acinetobacter baumannii*, Antimicrobial resistance, Nosocomial pathogen

Citation. Palpandi, K., Adukkadukkam, S., Chandrasekhar. S., Kumar, P. A. and Murugaiyan, J. 2023. A comparative analysis of the AMR profile of *Acinetobacter baumannii* isolated from environmental and veterinary setting. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 48. https://doi.org/10.51585/gtop.2023.2.0035

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# Giant viruses of prokaryotes and their intracellular infection strategies

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**Abstract**

Bacteriophages are bacterial viruses that target specific bacteria. During infection, bacteriophage injects their DNA into the host cells for replication. The injected DNA is targeted by various host immune systems like the Restriction Modification and the CRISPR-Cas enzymes. Several phages utilize immune evasion strategies like anti-CRISPRs to protect their DNA. Jumbo-bacteriophages with large genomes were recently shown to protect their DNA through a novel mechanism called the phage shell. The phage shell is a proteinaceous compartment formed in the cytoplasm of the infected cell and is made by a self-organizing protein called Chimallin. The shell compartmentalizes the replicating phage DNA and provides pan-protection against many hosts' immune systems. Interestingly, the phage shell exhibits functional similarity to the eukaryotic nucleus and is localized by PhuZ, a tubulin homolog. The shell and the PhuZ interplay are at the heart of genome protection by jumbo-bacteriophages. However, not all jumbo-phages encode genes for the shell and the PhuZ, and the fact is that non-shell-forming jumbo-phages can protect their genome via unknown mechanisms. In light of this background, we aim to address a fundamental question *What are the infection strategies of Jumbo phages?* We use Live cell imaging, CRISPR Cas studies, co-immuno precipitation, and mass spectrometry techniques to address this question. Here, we show the different infection strategies employed by jumbo phages like Goslar, PhiKZ, and 121Q, and also, we show the prevalence of Shell/PhuZ/Anti CRISPR among jumbo phages, implying the presence of alternative strategies employed by jumbo phages to protect itself from host immune systems. Overall, this research will lead to a better understanding of novel viral defense mechanisms using a simple model system like bacteriophages and will improve our ability to use phages for biotechnological applications.

**Keywords:** Bacteriophages, Jumbo-bacteriophages, CRISPR Cas, PhuZ

Citation. Barath, S., Magar, S. and Govindarajan, S. 2023. Giant viruses of prokaryotes and their intracellular infection strategies. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 49. https://doi.org/10.51585/gtop.2023.2.0035

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# A lightning strike: Studies of light-activated photosensitizers against drug-resistant bacterial pathogens

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## Abstract

Antimicrobial resistance has emerged as a global threat to treat infectious diseases. Antibacterial photodynamic therapy (aPDT) is a promising alternative and highly suitable approach for treating cutaneous bacterial infections through topical applications. aPDT relies on light-responsive compounds called photosensitizers (PS), which generate reactive oxygen species (ROS) when induced by light, thereby killing bacterial cells. Despite several studies, the molecular details of targeting and cell death mediated by PS are poorly understood. In this study, we investigated the antibacterial properties of two water-soluble Sn (IV) porphyrins (**1** and **2**) previously described by our group. We demonstrate that Sn (IV)-porphyrins can be induced by blue light (427 nm LED) and exhibit various levels of bactericidal activity against Gram-positive and Gram-negative bacterial strains. Using localization studies through fluorescence microscopy, we show that **2** targets the bacterial membrane more effectively compared to **1** and exhibits comparatively higher aPDT activity. Using multiple fluorescence reporters, we demonstrate that photoactivation of **1** and **2** results in extensive collateral damage to the bacterial cells, including DNA cleavage, membrane damage, and delocalization of central systems necessary for bacterial growth and division. In summary, this investigation provides deep insights into the mechanism of bacterial killing mediated by PS compounds. Moreover, our approach offers a new method for evaluating the activity of PS, which may inspire the discovery of new PS with enhanced aPDT activity.

**Keywords:** Antibacterial photodynamic therapy, Photosensitizers, aPDT, Sn (IV)-porphyrins

Citation. Gayathri, MP., Nagarajan, T., Govindarajan, S. and Babu, B. 2023. A lightning strike: Studies of light-activated photosensitizers against drug-resistant bacterial pathogens. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 50. https://doi.org/10.51585/gtop.2023.2.0035

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# Exploring the role of PA4406.1: A novel sRNA of *P. aeruginosa*

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**Abstract**

Small regulatory RNAs (sRNA) are 50-500 nucleotide long, highly structured, non-coding RNA molecules. These sRNAs play a critical role in gene regulation via numerous mechanisms, including binding to protein targets, modification to mRNA targets, and regulation of gene expression. These interactions can be exploited to adversely affect bacterial growth and combat the issue of bacterial resistance. Our work aims to characterize the structure and function of a previously uncharacterized non-coding sRNA PA4406.1 of *Pseudomonas aeruginosa*, a critical priority pathogen. Interestingly, PA4406.1 is located immediately next to the *ftsZ* gene, a highly conserved cell division gene in most bacteria. This project aims to understand whether the non-coding sRNA PA4406.1 plays a regulatory role in *P. aeruginosa*. We cloned the sRNA PA4406.1 in the pHERD30T shuttle vector and over-expressed the sRNA to evaluate its effects on growth, motility morphology, and other properties of *P. aeruginosa*. In summary, our project attempts to understand the functional properties of a previously uncharacterized sRNA in a major human pathogen such as P. *aeruginosa*.

**Keywords:** Small regulatory RNAs, *P. aeruginosa, ftsZ,* sRNA

Citation. Vishnumolakala, J. D., Barath, S. and Govindarajan, S. 2023. Exploring the role of PA4406.1: A novel sRNA of *P. aeruginosa*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 51. https://doi.org/10.51585/gtop.2023.2.0035

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# Seeing is believing: Phage-bacteria interaction at single-cell resolution

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## Abstract

Bacterial infections are widespread and found to be the second largest cause of death globally, where, according to ReAct reports in 2019, 7.7 million deaths around the world were due to bacterial infections. One of the major causes of this global threat is the ability of such bacteria to develop the mechanism of standing the effect of antibiotics. *Pseudomonas aeruginosa* is now considered one of the most concerning infectious agents frequently associated with nosocomial infections that are no longer effectively treated by antibiotics. One way to overcome this health burden is to find alternative therapeutics. Phage therapy is a promising alternative strategy for addressing this threat. Our research sheds light on the untapped potential of bacteriophages as a novel strategy against MDR *P. aeruginosa* infections. In this project, we report the isolation of 7 bacteriophages from various environmental samples against the laboratory *P. aeruginosa* PA01. We screened their ability to infect and kill *P. aeruginosa* strains and evaluated their effectiveness in controlled laboratory settings and against clinical strains. The isolated phages exhibited potent inhibitory effects, suggesting the potential of bacteriophages as a plausible alternative therapeutic approach. Additionally, we endeavored to gain insights into the structural and genetic characteristics of the isolated bacteriophages. Through comprehensive characterization studies, we elucidate their structure, morphology, and genome size. These findings enrich our understanding of isolated bacteriophages and offer valuable information for future research and applications. The outcomes of this study underscore the efficacy of bacteriophages in inhibiting MDR strains and present a step forward in addressing the global challenge of antibiotic resistance. By elucidating the structural and genomic attributes of the isolated bacteriophages, we contribute to the broader field of phage-based therapeutics.

**Keywords:** Bacterial infections, Bacteriophages, *P. aeruginosa*, Antibiotics, MDR strains

Citation. TUYUBAHE, H., Barath, S. and Govindarajan, S. 2023. Seeing is believing: Phage-bacteria interaction at single-cell resolution. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 52. https://doi.org/10.51585/gtop.2023.2.0035

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# Development of a simple filter-based method for isolation of Jumbo bacteriophages

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## Abstract

Jumbo bacteriophages, characterized by their exceptionally large genome sizes (>180 kb), have recently emerged as intriguing players in the realm of bacterial viruses. However, their isolation and study have been challenging due to their scarcity in existing databases and the difficulties associated with their large size. This study introduces a new method for isolating these jumbo phages from any environmental samples. Traditionally, people employ a 0.2 $\mu$m filter-based technique to isolate bacteriophages, where the principle relies on the retention of phages by the filter due to their size. Notably, jumbo phages often evade detection using this conventional approach as they become entrapped on the upper side of the filter, making their isolation a cumbersome task. To overcome this limitation, we employed a simple strategy in which the 0.2 $\mu$m filter is inverted, allowing the jumbo phages to be eluted effectively from the filter's surface. This new approach successfully isolated two jumbo phages from a chicken fecal sample. Following their isolation, we conducted whole-genome sequencing and comprehensive genome characterization. The genome of one phage is >180 kb, making it a jumbo phage. In contrast, another phage genome size is predicted to be in the range of 160-170kb based on the genome similarity with other phages and is a partial genome. The results of our study demonstrate the effectiveness and feasibility of this method for isolating jumbo bacteriophages, offering valuable insights into their biology and potential applications in various fields. This technique promises to enhance the study of these viral giants and expand our understanding of the virosphere.

**Keywords:** Jumbo bacteriophage, Isolation, Whole genome sequencing

Citation. Magar, S., Kumar S, R., Parmar, A. and Govindarajan, S. Development of a simple filter-based method for isolation of Jumbo bacteriophages. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 53. https://doi.org/10.51585/gtop.2023.2.0035

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# Antibiotic resistome dynamics in an Urban community

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## Abstract

Effectively managing antibiotic resistance genes (ARGs) is crucial to protect public health. However, obtaining precise data on AMR across a wide range of populations is a substantial challenge. Wastewater-based monitoring (WBE) emerges as a promising solution, to comprehensively evaluate AMR in diverse and healthy human cohorts. WBE of AMR often depends on culture-based methods, which can be deceptive and lead to underestimated results. Therefore, integrating molecular techniques like PCR and advanced resistome profiling is crucial. In this regard, the current study was undertaken to track the abundance and diversity of AMR. This was achieved using quantitative real-time PCR and a high-throughput sequencing-based metagenomic approach. Additionally, to comprehend resistance patterns in the community, this study investigates how seasonal variations influence resistance dynamics. Water samples will be analyzed for 125 ARGs and 13 MGEs using PCR, and ARG-pathogen connections were determined through shotgun metagenomics and the Comprehensive Antibiotic Resistance Database (CARD). Our findings unveil essential and unique resistome components and prevalent ARG trends over the sampled period. This enhanced understanding of ARGs and their host microbiota holds potential for infection control and minimizing pathogen release into the environment. This study acts as an early alert system and contributes data to the repository of AMR density, diversity, and dynamic characteristics.

**Keywords:** Antibiotic resistome burden, Bacterial hosts, Mobilome, Next generation sequencing, One-Health, Wastewater-based epidemiology

Citation. Javvadi, Y. and Mohan, S. V. 2023. Antibiotic resistome dynamics in an Urban community. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 54. https://doi.org/10.51585/gtop.2023.2.0035

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# Determination of antibiotic resistance profile of bacterial community from environmental water using antibiotic-resistant bacterial contamination detection (ABCD) kit

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## Abstract

As described in the present work, developing antibiotic-resistant bacterial contamination detection (ABCD) kits represents an innovative and efficient approach to assessing antibiotic resistance in environmental bacterial communities. Analyzing origins, clinical significance, and occurrences of antibiotic resistance in ambient bacterial communities requires quick and simple monitoring techniques. In the present work, antibiotic-resistant bacterial contamination detection (ABCD) kits were developed. *Escherichia coli*, *Staphylococcus arlettae*, *Enterococcus faecalis*, and *Aerococcus viridans* strains with defined antibiograms were used to standardize the procedure, along with six clinically significant antibiotics. The technique was successfully tested on various water sources with various physicochemical properties. To detect the presence of resistant bacteria, only 1 mL of sample water must be combined with an optimized concentration of the antibiotic solution and incubated for 6 hours. After adding the bacterial detection PVDF membrane, a color change to pink may be seen in a certain amount of time. If there are no populations of bacteria that are resistant to antibiotics, there is no color change. In addition, the rate of color change is inversely proportional to the number of communities resistant to antibiotics. This is the first report that, as far as we are aware, can identify the antibiotic-resistance profile of any water source by observing only color change within a maximum of 7 hours (6 hours for the co-culture of bacteria and antibiotics + 1 hour for color change detection) without the assistance of any microbiology laboratories or skilled labor.

**Keywords:** Antibiotic Resistant Susceptible Kit, Environmental samples, PVDF membrane

Citation. Behere, M. J., Shinde, A. H. and Haldar, S. 2023. Determination of antibiotic resistance profile of bacterial community from environmental water using antibiotic-resistant bacterial contamination detection (ABCD) kit. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 55. https://doi.org/10.51585/gtop.2023.2.0035

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# Interaction of symbiotic gut bacteria, segmented filamentous bacteria, with intestinal cells

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## Abstract

The Clostridia-related anaerobe Segmented Filamentous Bacteria, SFB, is found in many vertebrate species and has emerged as a key member of the gut microbiota. SFB attaches to the ileal epithelium, promotes colonization resistance both in and outside the gut, and exerts a complex adjuvant effect on systemic immune responses. Much of the life cycle of SFB remains unexplored as SFB is difficult to culture and is routinely propagated in germ-free mice. We recapitulated SFB co-culturing with host intestinal cells *in-vitro* and generated SFB monocolonized mice *in-vivo*. Using transmission electron microscopy (TEM), we show that mouse and rat SFB are flagellated at the concave point of the unicellular intracellular offspring (IO) stage only and not in the filamentous stage. We show that flagellation occurs before full IO differentiation and IOs release from SFB filaments. We demonstrated flagellin proteins of the IOs interact with TLR5 molecules of the host cell, thus regulating its immune system. This finding provides new milestones to investigate the impact of SFB-host and other gut bacteria on immune systems.

**Keywords:** Gut Microbiota, Segmented Filamentous Bacteria, SFB, Unicellular intracellular offspring

Citation. Dubey, G. P. and Bhattacharjee, A. 2023. Interaction of symbiotic gut bacteria, segmented filamentous bacteria, with intestinal cells. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 56. https://doi.org/10.51585/gtop.2023.2.0035

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# Antifungal susceptibility testing and mutation analysis in dermatophytes causing recalcitrant *Tinea corporis* with high MIC for terbinafine

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## Abstract

Tinea or dermatophytosis is the most common skin infection affecting millions of people worldwide, with the most common causative agent being dermatophytes. There is increased prevalence and change in disease presentation, severity, treatment response, and relapse. Studies suggest that the emergence of *Trichophyton mentagrophytes* as principal causative organisms and high terbinafine resistance could cause changing patterns of the disease and response. The present study aimed at isolating and identifying dermatophytes from patients with tinea corporis, determining antifungal susceptibility patterns. Eighty-three skin samples collected from patients were subjected to direct microscopic examination, culturing, phenotypic identification, genotypic identification targeting ITS region, antifungal susceptibility testing, and mutation analysis. As a result, 65 samples were positive on direct KOH mounting, and 57 showed fungal growth identified as *T. mentagrophytes* in 42 samples, *Microsporum gypseum* in 11 samples, and *T. rubrum* in four samples. Antifungal susceptibility testing showed that 26 isolates were resistant to fluconazole, 12 were resistant to terbinafine, and eight were resistant to itraconazole (MIC >0.5 mg/l). Twenty-six isolates were completely sensitive to terbinafine, 15 to itraconazole, and eight to fluconazole (MIC <0.0625 mg/l). Six representative isolates showing the highest MIC were subjected to sequencing the squalene epoxidase gene, out of which only four isolates showed a mutation in the squalene epoxidase gene at P397L, and one isolate each showed mutation at P415I and P415S. In conclusion, *T. mentagrophytes* was the predominant causative agent. Recalcitrant dermatophytes show clinical resistance to the antifungal agents; however, phenotypically, they are sensitive to these antifungal agents. Mutation was not observed in one isolate with high MIC. There is a need to study the altered mechanism for *in-vivo* drug resistance of these *in-vitro* drug-sensitive isolates.

**Keywords:** Dermatophytosis, Antifungal Susceptibility, *Trichophyton mentagrophytes*

Citation. Kenjar, A. R., Raj, J. R. M., Girisha, B. S. and Karunasagar, I. 2023. Antifungal susceptibility testing and mutation analysis in dermatophytes causing recalcitrant *Tinea corporis* with high MIC for terbinafine. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 57. https://doi.org/10.51585/gtop.2023.2.0035

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# Prevalence of antibiotic resistance and biofilm-forming ability in *Pseudomonas aeruginosa* isolates from canine samples

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## Abstract

*Pseudomonas aeruginosa* is the most frequently isolated opportunistic Gram-negative bacilli from dogs' recurrent otitis and chronic suppurative infections. The ability to produce biofilm further protects the bacteria and thus enables them to become multi-drug resistant. The present study was conducted to detect the prevalence of antibiotic resistance in *P. aeruginosa* from canine samples. A total of ninety-seven (n=97) samples were collected from both otitis infections and chronic wounds of pet dogs. On microscopic, cultural, and biochemical examination, presumptively *P. aeruginosa* was identified in 35 samples. All 35 samples were confirmed by Polymerase Chain Reaction (PCR) as *P. aeruginosa* using species-specific 16S rRNA primers that yielded a specific PCR product of size 956 bp. The antibiotic sensitivity was detected using the Kirby-Bauer disc diffusion method with a panel of 10 different antibiotics. The results indicated that the organism was multi-drug resistant, with the highest resistance to tetracycline (97.14%) and the lowest resistance to imipenem (11.42%). None of the positive isolates had a MAR index of <0.2, indicating the resistance severity. The genetic determinants of Extended Spectrum Beta-Lactamase (ESBL) detected using multiplex-PCR revealed the prevalence of $bla_{\text{TEM}}$, $bla_{\text{OXA}}$, and $bla_{\text{SHV}}$ to be 20% (7/35), 31.42% (11/35) and 25.71% (9/35), respectively. Biofilm gene studies targeting ppyR and pslA indicated the presence of *ppy*R in 85.71% (30/35) and pslA in 77.14% (27/35). In conclusion, this study reported the prevalence of multi-drug resistance and biofilm-forming ability in *P. aeruginosa* among canine samples.

**Keywords:** Antibiotic Resistance genes, Biofilm, Multi-drug resistant, *P. aeruginosa*

Citation. Gogana, S., Deepika, K. G., Bindu, K. Ch., Swaroop T. and Kumar, P. A. 2023. Prevalence of antibiotic resistance and biofilm-forming ability in *Pseudomonas aeruginosa* isolates from canine samples. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 58. https://doi.org/10.51585/gtop.2023.2.0035

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# Prevalence of antibiotic resistance in *Mannheimia* isolates from sheep and goats with respiratory illness by qualitative and genetic methods

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## Abstract

*Mannheimia* species, a Gram-negative bacterium, is one of the major pathogens implicated in respiratory diseases of small ruminants. In Sheep and Goats, respiratory illness is a seasonal problem that causes huge economic losses to the farmers. To overcome the losses, in most affected areas, livestock keepers may resort to indiscriminate use of antibiotics, which poses a potential threat of increased antibiotic resistance. The present study evaluated the prevalence of antibiotic resistance in *Mannheimia* species isolated from respiratory illness cases of small ruminants. A total of 30 *Mannheimia* species have been isolated from 92 clinical cases of respiratory illness. *Mannheimia* isolates were confirmed in a PCR test targeting the 16S rRNA gene using genus-specific primers. The antibiotic resistance profile of the isolates was evaluated by the Kirby-Bauer disc diffusion method with eight selected antibiotics and the antibiotic resistance genes such as strA, $bla_{\text{TEM}}$, $bla_{\text{OXA}}$, and $bla_{\text{SHV}}$ with a product size of 506, 800, 546, and 713 bps, respectively were detected in multiplex PCR. The prevalence of high resistance was noted towards the drugs Ampicillin (80%) followed by Gentamicin (50%), Co-Trimoxazole (43%), Tetracycline (40%), Amoxicillin-Clavulanic acid (40%), Ceftriaxone (36.6%), Streptomycin (36.6%), and Enrofloxacin (26.6%). The prevalence of antibiotic resistance genes in the isolates was found to be the strA gene (70%), $bla_{\text{OXA}}$ (13.3%), $bla_{\text{TEM}}$ (10%), and $bla_{\text{SHV}}$ (10%). Analyzing the multiple antibiotic resistance index (MAR), out of 30 samples, none revealed a MAR index of less than 0.2. This indicates that there is a potential threat of AMR transmission. In conclusion, this study furnished obvious indications of the prevalence of antibiotic resistance patterns among respiratory illness-affected small ruminants.

**Keywords:** Antibiotic resistance genes, Multidrug resistance, *Mannheimia*, Respiratory Illness

Citation. Swaroop T., Sivarama K. G., Supriya, A. R., Swathi G. and Kumar, P. A. 2023. Prevalence of antibiotic resistance in *Mannheimia* isolates from sheep and goats with respiratory illness by qualitative and genetic methods. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 59. https://doi.org/10.51585/gtop.2023.2.0035

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# Exploring predatory *Bdellovibrio*: A potential biocontrol against multidrug-resistant *Escherichia coli* strains

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## Abstract

Extensive use and abuse of antimicrobial agents have led to increasing concern due to the problem of bacterial resistance. The treatment of infections caused by the biofilm-forming bacteria is a major challenge. The use of *Bdellovibrio* as a biocontrol might constitute a viable alternative. *Bdellovibrio* is a unique predatory bacterium that can prey on Gram-negative bacteria in the planktonic form and biofilms, reducing the prey population. The present study aimed to isolate *Bdellovibrio* strains from various environments using nitrofurantoin-resistant Escherichia coli of environmental origin. A total of 40 water samples were collected from different environmental sources like lakes (n=7), rivers (n=3), tap water (n=2), garden water (n=2), mangrove waters (n=2), and hospital effluent (n=24) and used for *Bdellovibrio* isolation. Six *Bdellovibrio* stains were identified by various morphological tests and further characterization by molecular methods and Transmission Electron microscopy. Predation activity of the *Bdellovibrio* was studied using a reduction in prey cell viability and kinetic lysis of *Bdellovibrio*. This approach involving natural predators can be seen as a unique way to potentially control or mitigate the growth of drug-resistant *E. coli* strains in various settings.

**Keywords:** Biofilm Forming bacteria, *Bdellovibrio*

Citation. Rai, D. and Mithoor, D. 2023. Exploring predatory *Bdellovibrio*: A potential biocontrol against multidrug-resistant *Escherichia coli* strains. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 60. https://doi.org/10.51585/gtop.2023.2.0035

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# Next-generation sequencing-based resistome analysis of *Escherichia coli* and *Pseudomonas* multi-drug resistant pathogens from sewage water

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## Abstract

An increasing number of antimicrobials (antibiotics, etc.), antimicrobial-resistant bacteria (ARB), and antimicrobial resistance genes (ARG) have been identified in aquatic environments, raising serious concerns about the potential emergence of antibiotic-resistant pathogens and even superbugs. A holistic 'One-Health' strategy for AMR surveillance is necessary to assess and manage health risks and the safety of humans, animals, plants, and the environment. These research findings aimed to analyze the problem of antimicrobial resistance (AMR) from an environmental perspective to quantify the pathways and transmission of antimicrobial resistance in the river, marine, and sewage wastewater reservoirs of the South Gujrat Region, Gujrat, India, with the effective use of genomic approaches that can monitor antimicrobial resistance (AMR) gene and mutation mobilization, persistence, and abundance in microbial populations. The isolates obtained from these samples were screened for bacterial identification and antimicrobial susceptibility testing (AST), followed by whole genome sequencing (WGS), which was carried out using an Illumina sequencing platform to understand multi-drug resistance (MDR) characteristics. After annotating the assembled draft genomes, sequence analysis was performed with the use of BV-BRC PATRIC (The Bacterial and Viral Bioinformatics Resource Centre, PAThosystems Resource Integration Centre) and CARD (The Comprehensive Antibiotic Resistance Database) bioinformatics software tools. These results reveal genetic characteristics and variants of potential multi-drug resistance (MDR) *E. coli* and *Pseudomonas* isolates. By monitoring environmental antimicrobial resistance (AMR), whole genome sequencing helps in resistance mechanisms, drug targets, epidemiological investigations, and the diagnosis of these multi-drug resistance (MDR) bacteria. A comprehensive whole genome sequencing (WGS) investigation of multi-drug resistance (MDR) strains indicated different antimicrobial resistance genes (ARG) and their precise resistance mechanisms.

**Keywords:** Antimicrobial resistance genes, Whole genome sequencing, Multi-drug resistance bacteria, Pathogen surveillance, BV-BRC PATRIC

Citation. Patel, R. and Patel, R. 2023. Next-generation sequencing-based resistome analysis of *Escherichia coli* and *Pseudomonas* multi-drug resistant pathogens from sewage water. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 61. https://doi.org/10.51585/gtop.2023.2.0035

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# Development of cost-effective, eco-friendly selenium nanoparticle-functionalized cotton fabric for antimicrobial and antibiofilm activity

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## Abstract

Selenium, a micronutrient, is an essential trace element in the human body. Recently, Selenium in nano forms emerged as a new member of nanomedicines because of its excellent antimicrobial activity and low cytotoxicity. This study synthesized selenium nanoparticles in situ on alkali-activated cotton fabric using guava leaf extract as a reducing agent. In healthcare settings, these nano-finished fabrics are crucial for preserving the wearer's health from nosocomial infections. The synthesis was monitored by a change in the fabric's color from white to light brick red. The UV-DRS analysis confirms the coating of Se-NPs on cotton. The XRD, FT-IR, and SEM-EDX characterization techniques were used to analyze the nanoparticles on cotton fabric. The peak at 788 $cm^{-1}$ in FT-IR confirms the formation of Se-NPs on cotton fabric. The XRD analysis confirms that the average crystallite size of the as-prepared nanoparticle is ∼17 nm. SEM-EDX analysis shows the successful coating of Se-NPs on coated fabric. ICP-OES studies confirm that 3.65 mg/g of selenium nanoparticles were present on the fabric. The Se-coated fabric showed a larger zone of inhibition against Gram-positive *S. aureus* (32 mm) compared to Gram-negative strains of *E. coli* (16 mm) and *K. pneumoniae* (26 mm). The fabric was also tested against the fungi *C. glabrata* (45 mm), *C. tropicalis* (35 mm), and *C. albicans* (35 mm), and results indicate it is more effective against fungi compared to bacterial strains. The coated fabric inhibits the biofilm formation of *C. albicans* (99%), *S. aureus* (78%), and *E. coli* (58%). The results demonstrated excellent antibacterial, antifungal, and antibiofilm activities of the Se-coated fabric. The prepared fabric is cost-effective, eco-friendly, and has the potential to be used in medicinal applications like wound cleaning, wound dressing, and surgical aprons.

**Keywords:** Cotton fabric, Selenium nanoparticles, Green synthesis, Guava, Antimicrobial, Biofilm

Citation. Mirza, K. and Sardar, M. 2023. Development of cost-effective, eco-friendly selenium nanoparticle-functionalized cotton fabric for antimicrobial and antibiofilm activity. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 62. https://doi.org/10.51585/gtop.2023.2.0035

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# Involvement of outer membrane proteins in the development of antibiotic resistance in *Acinetobacter baumannii*

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## Abstract

*Acinetobacter baumannii* is an opportunistic pathogen responsible for several nosocomial infections like pneumonia, wound sepsis, meningitis, and urinary tract infections. Although their natural habitats are soil and water, they are often isolated from blood, urine, and respiratory fluids from patients. It is one of the important ESKAPE pathogens mentioned in the World Health priority list. The outer membrane proteins (OMPs) are $\beta$ barrel integral proteins in the outer membrane of Gram-negative organisms. They allow the passage of antibiotics through them. Hence, protein profile and expression analysis could provide valuable information regarding their role in antibiotic resistance. In this study, the isolates were characterized by antibiotic susceptibility test and growth kinetics followed by the detection of antibiotic determinants to group them into antibiotic-sensitive and resistant strains. Further, total OMPs from the two groups of *A. baumannii* were extracted using the N-lauryl sarcosine method and analyzed using sodium dodecyl-sulfate–polyacrylamide gel electrophoresis. The gene expression of selected OMPs was then analyzed. The clinical isolates were mainly multidrug-resistant, while the environmental isolates were primarily sensitive. The antibiotic-resistant determinants detected in the study correspond to the phenotypic resistance exhibited by the isolates. The profile analysis showed differences in the banding pattern and varied band intensity between the antibiotic-sensitive and resistant isolates. The distinct bands were analyzed and categorized into major and minor OMP groups based on approximate sizes. The presence/absence of bands could be due to the difference in antibiotic susceptibility. The OMP genes of resistant isolates showed significant differential expression compared to sensitive isolates. Hence, this study provides information on the involvement of OMPs in antibiotic resistance.

**Keywords:** *A. baumannii*, Antibiotic resistance, OMP genes

Citation. Nayak, S., Akshay, S. D., Deekshit, V. K., Raj, J. R. M. and Maiti, B. 2023. Involvement of outer membrane proteins in the development of antibiotic resistance in *Acinetobacter baumannii*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 63. https://doi.org/10.51585/gtop.2023.2.0035

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# Application of *Citrus aurantifollia* on bacteria and yeast: An *in-vitro* study

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## Abstract

Anti-drug resistance and the failure to develop new antibiotics have emerged as problems in the current situation. Therefore, it is critical to identify the ideal adjuvant quickly. Contrary to antibiotics, natural products have several advantages. It is also a concern and affects the immune system that some gram-negative bacteria develop antibiotic resistance over time. The remarkable health benefits of ascorbic acid, also known as vitamin C, and the improvement of macrophage survival are largely due to its potent antibacterial, anthelmintic, and antifungal properties. Therefore, the WHO suggests that adults consume between 30 and 180 mg of vitamin C daily. With a specific product that is both inexpensive and widely available on the market, the current study aimed to evaluate the antimicrobial resistance of vitamin C using protein assays. While the fungi required an incubation period of 6 hours at a pH of around 7.5 for optimal growth, the various bacteria required an incubation period of 24 hours at 37 °C. To determine the minimum bactericidal and fungicidal concentrations (MBC and MFC), 20 $\mu$L of the MICs (minimum inhibitory concentrations) were plated on MHA for bacteria. *Citrus aurantifollia*, which is widely available, was discovered to be effective against *Candida albicans, Escherichia coli, and Staphylococcus aureus*. Given what is known now, the study enlists the help of medical professionals who could aid in replacing these antibiotics with natural products with no side effects.

**Keywords:** Ascorbic acid, *Citrus aurantifollia*, Antimicrobial resistance, Antidrug resistance, Macrophage, Immune system

Citation. Nayek, S., Ghosh, A. and Bhattacharya, M. 2023. Application of *Citrus aurantifollia* on bacteria and yeast: An *in-vitro* study. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 64. https://doi.org/10.51585/gtop.2023.2.0035

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# Determination of efflux-mediated drug resistance in *Escherichia coli*

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## Abstract

The *Enterobacteriaceae* family contains more than 95% of clinically important pathogens causing various illnesses. According to the WHO, by 2050, 10 million deaths might occur due to antibiotic resistance in human pathogens, and GDP could decline by 2.5% to 3.5% annually. Although the bacteria develop resistance through various mechanisms, the efflux pump remains the first line of defense against different antibiotic classes. The present study aims at determining the efflux-mediated drug resistance in *Escherichia coli (E. coli)*. The antibiotic susceptibility of *E. coli* isolates was determined using the Kirby-Bauer method, and efflux pump inhibitor PA$\beta$N was used to measure efflux pump activity. Out of 100 clinical isolates screened, 92% were resistant to $\beta$-lactams, fluoroquinolones, carbapenems, tetracycline, chloramphenicol, and cotrimoxazole, and harbored genes conferring resistance to these antibiotics. Significant efflux pump genes from the RND family of bacterial multidrug transporters were confirmed in 8 *E. coli* MDR isolates showing efflux activity. They showed basal expressional variation in the drug efflux pump genes *acr*A, *acr*B,*acr*D, *acr*E, and *tol*C in the presence of antibiotic and efflux pump inhibitors. The obtained data reveals the presence of active antibiotic efflux pumps in resistant isolates as a main mechanism of resistance. It is essential to take the influence of efflux pumps into account while developing new antibiotics to maximize the efficacy of current and future antibiotics.

**Keywords:** Antibiotic resistance, Antibiotics, *E.coli*

Citation. Barani D. T., Aditya, V., Kini, S. and Deekshit, V. K. 2023. Determination of efflux-mediated drug resistance in *Escherichia coli*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 65. https://doi.org/10.51585/gtop.2023.2.0035

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# Bioengineered magnesium oxide nanoparticles using *Vigna mungo* extract: *In-vitro* antioxidant and antimicrobial activity

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## Abstract

The recent era is dedicated to the realm of nanotechnology, which has captured the attention of many researchers to synthesize metal oxide nanoparticles. In the current study, we used the seed extract of *Vigna mungo*, commonly called black gram, which belongs to the Fabaceae family and is a prominent source of protein, for the synthesis of magnesium oxide nanoparticles (MgONPs). Green synthesis is an economical, sustainable, and promising method for synthesizing MgONPs. In addition, MgONPs exhibit potential antibacterial, antifungal, anticancer, antioxidant, and anti-diabetic activity. The biologically synthesized MgO NPs were characterized using various analytical techniques, including UV-visible spectroscopy, X-ray diffraction, Differential Scanning Calorimetry, and Fourier Transform Spectroscopy. Furthermore, we assessed the antioxidant activity of MgONPs using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and radical scavenging methods. Antimicrobial activity was evaluated using the agar well diffusion method. The MgONPs extracted from *Vigna mungo* exhibited antibacterial activity against *E. coli, L. pumilis, S. aureus,* and *S. pneumonia.* This study contributes to a better understanding of the current knowledge on the green synthesis of MgONPs from *Vigna mungo* and their antioxidant and antimicrobial activities against different species.

**Keywords:** Antioxidant activity, Antimicrobial activity, Green synthesis, MgONPs, *Vigna mungo*

Citation. Rekha, G. S. S., Saimanogna, K., Raju, B. D. P. and Sushma, N. J. 2023. Bioengineered magnesium oxide nanoparticles using *Vigna mungo* extract: *In-vitro* antioxidant and antimicrobial activity. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 66. https://doi.org/10.51585/gtop.2023.2.0035

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# *Botyrococcus braunii* microalgae-derived peptides as promising therapeutics against drug-resistant *Pseudomonas aeruginosa*

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## Abstract

Antimicrobial resistance (AMR) is one of the significant concerns for global public health. *Pseudomonas aeruginosa* has been declared as one of the critical pathogens by the World Health Organization due to its resistance to currently available antibiotics. One of the alternatives to overcome the AMR is antimicrobial peptides (AMP). The present study explores the microalga *Botryococcus braunii* as a potential source of antimicrobial peptides (AMPs) against drug-resistant *Pseudomonas aeruginosa*. The workflow includes the identification of AMPs from *B. braunii* based on physiochemical properties using various prediction and activity tools. The PBIT tool was used to identify non-homologous protein targets of *P. aeruginosa* PA01. Subsequently, potential AMPs were subjected to docking studies and validated through molecular dynamics (MD) simulations. These molecular predictions provide that two identified AMPs exhibit high docking scores of -29.6 and -26 kcal/mol, respectively, for the protein responsible for prenylated Flavin Mononucleotide (FMN) synthesis, likely to have a central role in bacterial electron transport and metabolic reactions. The molecular dynamic simulation determined the interaction stabilities between the AMPs and the protein at the binding site. Thus, the high binding affinity and insights from the molecular interaction signify that the identified AMPs from *B. braunii* can serve as potential alternative treatments against drug-resistant *P. aeruginosa*.

**Keywords:** Antimicrobial Peptides, Antimicrobial resistance, Gram Negative pathogens, *Botryococcus braunii, Pseudomonas aeruginosa*

Citation. Pujeri, S. S., Bhavan, G. K., Narayana, J. L. and Prasanna, A. 2023. *Botyrococcus braunii* microalgae-derived peptides as promising therapeutics against drug-resistant *Pseudomonas aeruginosa*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 67. https://doi.org/10.51585/gtop.2023.2.0035

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# *In-Silico* approach to identify candidates against MDR *Enterococcus faecalis* clinical isolates

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## Abstract

*Enterococcus faecalis* is a prevalent opportunistic pathogen and causes nosocomial infections. The growing resistance of *E. faecalis* to commonly used antibiotics has developed the need to identify alternate effective antibacterial medications. In this context, phytochemicals have been found as promising antibacterial agents. The present study aims to identify potential antibacterial phytoligands against *E. faecalis*. The methodology involves retrieval of the proteome sequence of the virulent *E. faecalis* V583 strain from UniProt. PBIT pipeline builder was used to identify non-homologous proteins in *E. faecalis* analyzed against human proteome and gut microbiota. DNA replication is an essential pathway for the survival of bacteria; therefore, the non-homologous proteins in the DNA replication pathway were identified as drug targets. AlphaFold2 predicted the 3D structure of these proteins, and validated models were docked using PyRx-0.8 against the screened 43 phytoligands. The result of molecular docking revealed that the first lowest vina score was observed in Quercetin (-8.1 kcal/mol) of *Macrotyloma uniflorum lam*(Horse gram), the second lowest vina score was observed in Ellagic acid(-7.8 kcal/mol) of *Punica gratum*(Pomegranate) and the third lowest vina score was observed in Kaempferol(-7.7 kcal/mol) of *Ricinus communis*(Castor bean) against the protein Adenine methyltransferase. Based on the vina score and binding interaction, Quercetin, Ellagic acid, and Kaempferol inhibit DNA replication, chromosome segregation, and repair, thus being potential antibacterial agents against *E. faecalis* infections.

**Keywords:** Multidrug resistance, AutoDock vina, PyRX, Phytoligands

Citation. Bhavyashree, V., Hamsaveni, S. M., Indupriya, M., Sahana, M., Karunakar, P. and Prasanna, A. 2023. *In-Silico* approach to identify candidates against MDR *Enterococcus faecalis* clinical isolates. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 68. https://doi.org/10.51585/gtop.2023.2.0035

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# ESKAPE explore: The interactive dashboard for exploratory genome analysis of ESKAPE pathogens

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**Abstract**

The advanced, affordable genome sequencing has led to a rise in genomic data for various pathogens worldwide; however, these datasets were not leveraged to enable meaningful comparisons and insights into AMR dynamics. Hence, global genomic datasets of ESKAPE pathogens were analyzed using various bioinformatics tools to identify AMR genetic traits, and their trends were visualized using an interactive dashboard (iDB) to understand the global and region-wise spatiotemporal trends in antimicrobial resistance. In this study, we used a total of 1,20,700 publicly available genomes comprising 21,649 (*E. faecium*), 27,718 (*S. aureus*), 39,157 (*K. pneumoniae*), 15,046 (*A. baumannii*), 12,448 (*P. aeruginosa*), and 4,682 (*Enterobacter spp*) and their associated metadata were collected from different segments of One-Health. The metadata was curated to make information uniform to all the genomes, viz., collection year (YYYY), location (UN's Geo-scheme Region), and isolation source (Clinical, Animal, Plant, Environmental, and Source-Unknown). Using ABRicate, the draft genome data was analyzed for the mass screening of AMR genes, and a presence-absence matrix summary file for each pathogen with corresponding metadata was produced. For the iDB, the dynamic web pages were created using the Bootstrap framework in the front end, while the backend database was created using MySQL, JavaScript-jQuery, Hypertext-Preprocessor, and JavaScript. For each pathogen, data visualization consisted of two primary parts: "Explore" to see the total data and "Analyze" to perform user-defined subset analysis. The distribution of draft genomes for each disease across geography, isolation source, and temporal trend is described in the "Explore" section. Analysis-based visualization includes Drug-class-based resistance patterns across the regions, Drug-class-based AMR distribution among different isolation sources, Drug-class v/s isolation source-based time-trend, and AMR gene distribution patterns among different isolation sources. The developed dashboard would become a global platform for the effective utilization of genomic data for effective intervention strategies, advancing our understanding of AMR within the broader One-Health perspective.

**Keywords:** Antimicrobial resistance, Bioinformatics, ESKAPE pathogens

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# From structure to resistance: 16S rRNA methyltransferases in tuberculosis and the challenge of AMR

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**Abstract**

Antimicrobial Resistance (AMR) poses a formidable global health challenge, exemplified by Extensively Drug-Resistant Tuberculosis (XDR TB), Multi-Drug-Resistant Tuberculosis (MDR TB), and Totally Drug-Resistant Tuberculosis (TDR TB). Current antimicrobial strategies primarily target essential bacterial proteins, aiming to disrupt critical biological processes and impede the emergence of drug-resistant strains. The 16S rRNA, an integral part of bacterial ribosomes, undergoes post-translational and post-transcriptional modifications, leading to diverse disease states, including Antimicrobial Resistance (AMR). Our research concentrates on targeting 16S rRNA methyltransferases (16SrRMTs) to achieve "two birds with one stone," i.e., a) inhibition of the translation process with the b) consequence of losing the ability to methylate, which is responsible for the development of drug-resistant strains, especially in prokaryotes. We have been studying the structure, function, and activity relationship of RsmD - an N2 G966 16SrRMT, specifically emphasizing its role in Mtb survival. The 16S rRNA exhibits distinct domains (I, II, III & IV), with more than two-thirds of methylation sites localized to the IV domain (3' minor domain), prompting an in-depth exploration of its functional implications. Our research investigates the potential functional and structural roles of seemingly deterministic, non-random, and non-uniform distribution of methylation sites. We will discuss the structural studies, analysis, and construed role of the 21 r-proteins in methyl transfer mechanisms within the 30S subunit and our observations regarding the crucial base-flipping phenomenon in successful methyl transfer to the respective substrates. We will also discuss the distribution of 16S rRMT methylation sites and their accessibility. In addition to this, we are trying to understand the structural dynamics leading the 16S rRMTs to the deeply buried inaccessible methylation sites. A possible explanation emerges that under stress, delayed assembly of ribosomal subunits and labile head domain could be responsible for 16SrRMTs to access respective methylation sites resulting in subsequent structural alterations in the 30S subunit. These changes, in turn, impact antibiotic binding sites, potentially leading to antibiotic resistance. With a comprehensive understanding of these intricate processes, we aim to develop targeted drugs against 16SrRMTs, enhancing the effectiveness of TB treatment while preventing the emergence of drug-resistant strains-two birds with one stone. These efforts represent a significant step forward in antimicrobial resistance research, aligning with global initiatives to improve TB treatment outcomes and curtail the menace of AMR.

**Keywords:** Antimicrobial Resistance (AMR), Tuberculosis (TB), Drug Resistance Mechanisms

Citation. Kadam, M. S. and Prasad, B. V. L. S. 2023. From structure to resistance: 16S rRNA methyltransferases in tuberculosis and the challenge of AMR. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 70. https://doi.org/10.51585/gtop.2023.2.0035

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# Computational Docking Examination of R47H-TREM2 mutant variant against Potential Amyloid-$\beta$ (A$\beta$) ligands with implications in the pathophysiology of Alzheimer's disease

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## Abstract

Alzheimer's disease (AD), the second-to-none neurodegenerative disorder, is the chief pathophysiological attribution of dementia. Recent studies emphasize the role of Microglial TREM2 receptors in the phagocytic amelioration of A$\beta$ plaques, which is believed to be the hallmark of AD pathophysiological character. The current work is a part of the major objective of distinguishing TREM2 binding efficacy with R47H-TREM2 mutant variant against a family of A$\beta$ ligands. The R47H-TREM2 mutant variant was reported to increase the AD risk by impairing the TREM2 receptor binding ability with that of A$\beta$. The current computational docking study examined the binding ability of R47H-TREM2 against a family of A$\beta$ ligands. I-TASSER server was employed for the 3D structure prediction of R47H- TREM2. For protein–protein interaction, the ClusPro server was employed. Coordinates of 6 varied A$\beta$ ligands: A$\beta$ 6, A$\beta$ oligomer, A$\beta$ 40, A$\beta$ 42, A$\beta$ 42A, and A$\beta$ 42B were imported from the PDB database. ClusPro, a rigid body docking algorithm, utilizes the PIPER program and ranks the generated docking models based on the cluster size (members). ClusPro results suggest that A$\beta$ 6 has the highest binding affinity with the R47H-TREM2 variant with the members of clusters - 411. The cluster size members of other top-ranked docking models of examined ligands were A$\beta$ 40 - 164, A$\beta$ oligomer - 124, A$\beta$ 42B - 85; A$\beta$ 42 - 68, and A$\beta$ 42A - 66, respectively. The results suggest that the computational interaction of R47H-TREM2 – A$\beta$ ligands needs further research and has pathophysiological implications in AD.

**Keywords:** Alzheimer's disease, R47H TREM2, A$\beta$ ligands, Microglia

Citation. Basha, SK C., Ramaiah, Mekala J. and Kosagisharaf, J. R. 2023. Computational Docking Examination of R47H-TREM2 mutant variant against Potential Amyloid-$\beta$ (A$\beta$) ligands with implications in the pathophysiology of Alzheimer's disease. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 71. https://doi.org/10.51585/gtop.2023.2.0035

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# Distribution of multidrug resistance genes among *E. Coli* isolated from chickens

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## Abstract

Anti-drug resistance and the failure to develop new antibiotics have emerged as problems in the current situation. Therefore, it is critical to identify the ideal adjuvant quickly. Contrary to antibiotics, natural products have several advantages. It is also a concern and affects the immune system that some gram-negative bacteria develop antibiotic resistance over time. The remarkable health benefits of ascorbic acid, also known as vitamin C, and the improvement of macrophage survival are largely due to its potent antibacterial, anthelmintic, and antifungal properties. Therefore, the WHO suggests that adults consume between 30 and 180 mg of vitamin C daily. With a specific product that is both inexpensive and widely available on the market, the current study aimed to evaluate the antimicrobial resistance of vitamin C using protein assays. While the fungi required an incubation period of 6 hours at a pH of around 7.5 for optimal growth, the various bacteria required an incubation period of 24 hours at 37 °C. To determine the minimum bactericidal and fungicidal concentrations (MBC and MFC), 20 $\mu$L of the MICs (minimum inhibitory concentrations) were plated on MHA for bacteria. *Citrus aurantifollia*, which is widely available, was discovered to be effective against *Candida albicans, Escherichia coli, and Staphylococcus aureus*. Given what is known now, the study enlists the help of medical professionals who could aid in replacing these antibiotics with natural products with no side effects.

**Keywords:** Ascorbic acid, *Citrus aurantifollia*, Antimicrobial resistance, Antidrug resistance, Macrophage, Immune system

Citation. Bothe, H. and Kamble, L. 2023. ADistribution of multidrug resistance genes among *E. Coli* isolated from chickens. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 72. https://doi.org/10.51585/gtop.2023.2.0035

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# Development of novel lectin-based rENO (recombinant $\alpha$-enolase) subunit vaccine against *Streptococcus iniae* for Tilapia (*Orechromis* spp.)

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## Abstract

Aquaculture is a highly economic food and livelihood source among the agricultural sectors. Tilapia (*Oreochromis sp.*) is the second most predominant farmed fish species globally and a major source of income for middle-income countries (LMIC). Intensification of tilapia farming has promoted severe disease outbreaks, resulting in high mortalities and economic loss, with *Streptococcus agalactiae* and *Streptococcus iniae* being major pathogens in tilapia culture. The zoonotic potential of *Streptococcus* raises additional difficulties regarding the spread of AMR and human health. Vaccines can be used as a safe and targeted therapeutic against zoonotic multidrug-resistant streptococcus in the emerging tendency to lessen the danger of AMR. This project brings together scientists from India and Taiwan to develop a vaccine that will be cross-protective against different *S. agalactiae serotypes* and *S. iniae* infections found in tilapia farming systems in Southeast Asia. The present study focused on developing a novel lectin-based subunit vaccine against *S. iniae*. Surface localized $\alpha$ Enolase of other streptococcus were recognized as immunodominant antigens, suggesting that this also could be true for *S. iniae* in tilapia. The genomic DNA will be isolated, and the $\alpha$-Enolase gene will be amplified by PCR following TA cloning into the pMD19 vector and subcloning to the pET32a vector for protein overexpression and purification. Expression of rENO will be examined and checked by nanodrop purity check and SDS-PAGE. Lectin adjuvanted purified rENO will be used for the initial immunization of fish, followed by a 21-day post-booster dose as an intraperitoneal injection. Fish will be challenged with an LD50 dose of virulent *S. iniae* after 35 days of initial immunization. Fish blood and tissue samples shall be tested at fixed intervals for histopathological alterations, antibody titers, and immune gene expression. Regulation in IL-1$\beta$, TNF-$\alpha$, MHC I$\alpha$, MHC II$\beta$, CD4-L2, CD8$\alpha$, and IFN-$\gamma$ gene expressions after post-vaccination will imply the efficacy of the vaccine. Commercialization of this vaccine would be needful for aquaculture industries and farmers. Moreover, the study emphasizes a safe potential approach to reduce the distribution of AMR by employing novel vaccines to protect fish against *Streptococcus*.

**Keywords:** Aquaculture, AMR, Lectin, *Streptococcus iniae*, Subunit vaccine

Citation. Guha, R., Byadgi, O. V., Chen, S. and Elumalai, P. 2023. Development of novel lectin-based rENO (recombinant $\alpha$-enolase) subunit vaccine against *Streptococcus iniae* for Tilapia (*Orechromis* spp.). Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 73. https://doi.org/10.51585/gtop.2023.2.0035

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# Identification and physiological significance of Arrestin Related Trafficking (ART) protein in *Candida albicans*

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## Abstract

Opportunistic pathogen *Candida albicans* are found majorly in the epithelial surface of human skin, oral region, gut, and vagina without causing any harm to the host individual, but in immunocompromised, they convert into the pathogenic form and cause infection. Protein turnover of the transporter in the plasma membrane depends on environmental factors. Endosomal trafficking of the N-acetyl glucosamine transporter (Ngt1) helps maintain the constant transporter level on the plasma membrane. In this process, it is also important to know about the proteins involved in this pathway, such as Snf1 (protein kinase), RSP5 (ubiquitin ligase protein), and ART-domain (arrestin-related trafficking adaptor) containing protein Rod1. In humans and yeast, arrestins are a family of Rod1 proteins that mediate selective nutritional transportation of ubiquitin-mediated endocytosis with the Rsp5. ART protein recruits RSP5 for ubiquitination of Ngt1. In this pathway, Snf1 inactivates the RSP5-ART complex by phosphorylation, thus inhibiting the endocytosis of Ngt1. To identify the endosomal significance of an ART-domain-containing protein, we have identified another ART domain-containing protein, i.e., orf19.4887, which has putative ubiquitin ligase binding activity. In our study, we prepared the null mutant of this gene and checked the physiological significance in Candida's growth and development. Our study revealed that the null mutant showed compromised growth compared to the wild type in inducing conditions, further indicating the significant role orf19.4887 in *C. albicans*.

**Keywords:** *Candida albicans*, N-acetyl glucosamine transporter, ART-domain, Snf1, RSP5

Citation. Haseena, S., Hanumantha, K. and Sharma, S. 2023. Identification and physiological significance of Arrestin Related Trafficking (ART) protein in *Candida albicans*. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 74. https://doi.org/10.51585/gtop.2023.2.0035

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# Nitroisobenzofuranone, a small molecule inhibitor of multidrug-resistant *Staphylococcus aureus*, targets peptidoglycan biosynthesis

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**Abstract**

Antimicrobial resistance is a global challenge, causing more than 10 million infections yearly. The World Health Organization (WHO) invested several million dollars to develop therapies, including biologics and small molecules, to combat drug resistance in bacteria. We synthesized a library of covalent small molecules and screened them in a phenotypic screening against *Staphylococcus aureus* bacteria to identify IITK2001 as the best inhibitor of multi-drug resistant *S. aureus* growth (4 µg/mL). Subsequent chemical reactivity analysis revealed the *in-situ* formation of IITK2020 as the mechanism by which IITK2001 functions. Biomolecular mechanistic evaluation using molecular docking, functional enzyme assays using 31 P NMR spectroscopy, competitive-activity-based protein profiling (ABPP), membrane polarization, and atomic force microscopy (AFM) analysis indicated the cell wall biosynthesis enzymes MurA and MurZ as the potential mechanistic targets of our lead molecules. Identification of this small molecule is an ideal starting point for us to explore additional structure-activity relationship analysis to improve efficacy.

**Keywords:** Antimicrobial resistance, *Staphylococcus aureus*, 31 P NMR spectroscopy, Competitive-activity-based protein profiling, MurA, MurZ

Citation. Rawat, V., Tiwari, S., Khanna, S., Gupta, U., Sridhar, S. N. C., Yadav, D. K., Kaul, G., Akhir, A., Saxena, D., Matheshwaran, S., Chopra, S. and Allimuthu, D. 2023. Nitroisobenzofuranone, a small molecule inhibitor of multidrug-resistant *Staphylococcus aureus*, targets peptidoglycan biosynthesis. Proceeding of International Conclave on AMR and Future of Antibiotics (ICAFA), SRM University, Andhra Pradesh, India. November 8-9, 2023. GMPC TOP. 3(2). pp. 75. https://doi.org/10.51585/gtop.2023.2.0035

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# Prophylactic effects of dietary *Biophytum sensitivum* and *Chromolaena odorata* extracts on the control of *Streptococcus agalactiae* infection, growth, and immunity in Nile tilapia (*Oreochromis niloticus*)

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## Abstract

Aquaculture is the fast-growing food-producing sector, and globally, it ensures food security for millions of people. Antimicrobial resistance (AMR) in aquaculture profoundly threatens public health and nutritional reliability. The intensified aquaculture practices have resulted in several new disease outbreaks and increased antibiotic dependency. Thus, the aquaculture production systems are considered "genetic factors" or "hotspots" of AMR genes. Medicinal plants as natural immunostimulants are promising alternative antibiotics to reduce AMR in aquaculture. This study explores the efficacy of Indian medicinal plants as phytotherapeutics to manage fish health. The herb *Biophytum sensitivum* hasn't got attention as an immunostimulant in aquaculture. Tilapia is the third most major finfish species produced through aquaculture. The plant extracts will be subjected to preliminary screening to test their antioxidant and antimicrobial activity, followed by incorporation into fish feed, which will be prepared in the laboratory. A total of 300 fish will be used for the experiment and each group with triplicate. Five experimental groups, including positive, negative, and three test groups, will be fed with formulated feed and treated for a while. It will be analyzed for growth and various specific and non-specific immune parameters, followed by the challenge test to be carried out with *Streptococcus agalactiae*. The blood and tissue samples will be collected and subjected to analyze growth and various hematology, biochemistry, and histology parameters. Studies on gut microbiota will be done, followed by the expression studies of innate immune-related genes will be performed. The effects of the dietary mixture of *Biophytum sensitivum* and *Chromolaena odorata* extracts in fish have not been performed yet. This polyherbal formulation will enhance Nile tilapia's growth and immune parameters. The herbal immunostimulants will be a promising and emerging alternative to antibiotics in aquaculture.

**Keywords:** Aquaculture, Phytotherapeutics, *Biophytum sensitivum*, Nile tilapia

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# Polymeric films with antibiotics for potential wound healing applications

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## Abstract

Wounds are injuries that result in the formation of openings in the skin or underlying tissues; they may range from superficial cuts to severe lacerations. A wide range of aerobic and anaerobic microorganisms have been isolated from the surfaces adjacent to and on the wounds, which are thought to initiate infection near the wound site. This microbial colonization of wounds causes a delay in wound healing and, in some situations, can result in morbidity and fatality. Antibiotics are valuable tools for preventing and controlling infections that delay wound healing. Antimicrobial resistance (AMR) is a looming wound care and management threat. In wounds, AMR can undermine the efficacy of antibiotics and antimicrobial treatments. Emerging techniques, such as nanoparticles, small molecules, and bacteriophages, have addressed the growing concern of AMR. Among these approaches, zinc oxide (ZnO) nanoparticles have garnered significant attention due to their potent antibacterial mechanisms. The antibacterial action of these nanoparticles is believed to stem from several factors, including the release of $Zn^{+2}$ ions, the generation of reactive oxygen species, or interactions between the nanoparticles and bacterial cell walls. This study aims to optimize the formulation of polymeric films loaded with nanoparticles and antibiotics (amoxicillin and cefprozil), exploring their potential synergistic effects in combating AMR. Various cross-linked polymeric film compositions have been developed, and their mechanical properties, antibacterial efficacy, and hemocompatibility were investigated. It was found that the prepared hydrogel films were swollen by more than 700% in PBS, pH 6.8. The films were strong and flexible and showed antibacterial activity against Gram-positive and Gram-negative. The results indicate that the developed hydrogel films could be potential candidates for wound healing applications.

**Keywords:** AMR, Antibiotics, Hydrogel, Wound healing, ZnO

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