

**STUDIES ON ACTINOBACTERIA-DERIVED NOVEL
ANTIMICROBIALS AND DEVELOPING ADVANCED
STRATEGIES TO COMBAT COMMUNITY/HOSPITAL-
ACQUIRED ANTIMICROBIAL RESISTANCE IN MDR
PATHOGENS**

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by

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DEPARTMENT OF CHEMISTRY**

Submitted

In fulfilment of the requirements of the Degree of
DOCTOR OF PHILOSOPHY

To the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

JUNE 2026

Dedicated to My Family

CERTIFICATE

This is to certify that the thesis entitled “**Studies on actinobacteria-derived novel antimicrobials and developing advanced strategies to combat community/hospital-acquired antimicrobial resistance in MDR pathogens**” being submitted by **Ms. Saniya Zaidi** to the Indian Institute of Technology Delhi for the award of the degree of ***Doctor of Philosophy*** in Chemistry is a record of bonafide research work carried out by her. Ms. Saniya Zaidi has worked under my guidance and supervision and has fulfilled the requirements for the thesis submission, which, to my knowledge, has reached the requisite standard.

The results of this dissertation have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.



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Abstract

The alarming global increase in multidrug-resistant (MDR) bacterial pathogens, especially those belonging to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), represents a major public health challenge. These organisms are highly adept at evading existing antibiotics, rendering many conventional treatments less effective. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* are of particular concern due to their association with severe hospital-acquired infections involving medical implants, respiratory devices, and immunocompromised patients (National Center for Biotechnology Information, 2023). Notably, MRSA also serves as a well-established model organism for studying antimicrobial resistance and for screening the efficacy of novel antimicrobial compounds. Their pronounced ability to form biofilms and persist in hostile environments contributes to chronic and septic infections, further complicating treatment outcomes (National Institutes of Health, 2024). These challenges highlight the urgent need to discover novel antimicrobial compounds and alternative therapeutic strategies, including synergistic approaches, in which even weakly active compounds may enhance the efficacy of existing antibiotics through mechanisms such as membrane perturbation or efflux modulation (MDPI, 2024).

The present study aimed to explore the biodiversity of actinobacteria as a promising source of novel antimicrobial and antibiofilm agents. Actinobacteria are renowned for producing a diverse range of bioactive secondary metabolites, including antibiotics, enzymes, and antitumor compounds. Soil and termite nest samples were collected from Raigarh, Maharashtra, and the Indian Institute of Technology (IIT) Delhi, and the actinobacterial isolates were isolated and screened on AIA and ISP2 media. Morphological examination

revealed predominantly grey aerial mycelia, and sixteen isolates (EMB26–EMB51) were preserved for further screening. Among them, two isolates, strain EMB26 and EMB45, exhibiting the most pronounced antibacterial and antibiofilm activities, were selected for detailed characterization. Phylogenetic analysis identified strain EMB26 as a close relative of *Streptomyces coelicolor* and EMB45 as a strain similar to *Nocardia bhagyanarayanae*, with respective GenBank accession numbers PP587436 and PQ097956.

Whole-genome sequencing of both isolates provided deep insights into their biosynthetic potential. strain EMB26 displayed a genome size of approximately 7.88 Mb with 6,954 predicted genes, while EMB45 had a 6.94 Mb genome with 5,952 predicted genes. BUSCO analysis validated high-quality assemblies with completeness scores of 99.2% and 98.4% for strain EMB26 and EMB45, respectively. Comparative genomic analysis revealed significant homology to known antibiotic-producing strains. AntiSMASH analysis identified 21 biosynthetic gene clusters (BGCs) in strain EMB26, including non-ribosomal peptide synthases (NRPS), polyketide synthases (PKS), and siderophore-related pathways, indicating its potential for the synthesis of multifaceted bioactive compounds. Similarly, strain EMB45 contained 18 BGCs, encompassing NRPS, terpene, and type I PKS clusters, supporting its bioactive metabolic potential.

The secondary metabolites extracted from *Streptomyces* sp. strain EMB26 demonstrated antibacterial effects against several MDR pathogens, particularly MRSA. Biochemical assays indicated that the metabolites disrupted bacterial cell membranes, leading to leakage of intracellular contents and subsequent cell lysis. GC-MS analysis identified 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione as one of the key active compounds. Molecular docking studies demonstrated stable interactions of this compound with MRSA cell wall synthesis proteins, including FemA, MurB, MurE, and PBP2a. These interactions suggest a

likely mechanism involving interference with peptidoglycan assembly, thereby compromising cell wall integrity and bacterial survival.

In parallel, metabolites from *Nocardia* sp. strain EMB45 displayed potent anti-quorum-sensing (QS) and antibiofilm activity, particularly against *Pseudomonas aeruginosa* PAO1. Fractionation of the crude extract revealed the ethyl acetate fraction as most effective in inhibiting QS-regulated biofilm formation, reducing bacterial motility, and attenuating virulence factor expression. Quantitative real-time PCR (qRT-PCR) and other transcriptional analyses confirmed a significant downregulation of critical QS genes (*lasI*, *lasR*, *rhlI*, *rhlR*, *pqsA*, *pqsE*, *pqsD*), supporting the hypothesis that the EMB45 metabolites interfere with bacterial communication systems rather than exert direct bactericidal effects. This targeted interference with virulence regulation offers a promising non-antibiotic strategy to mitigate infections caused by Gram-negative pathogens.

To translate these findings into practical biomedical applications, an antimicrobial catheter system was developed by incorporating metabolites of the EMB45 strain into mesoporous silica nanoparticles (MCM-41) coated with chitosan (CS). Spectroscopic characterization via FT-IR confirmed the successful embedding of active compounds while maintaining the porous structure of the nanomaterial, as verified by TEM imaging. The functionalized catheter surfaces exhibited excellent antibacterial activity against *P. aeruginosa* PAO1 biofilms, suggesting their potential for preventing device-associated infections.

Recognizing the limitations of conventional antibiotic therapy, the study also investigated the integration of photodynamic therapy (PDT) into nanomedicine for advanced antibacterial applications. A UCNP@PLGA-CS-Ce6 nanoplatform was developed for NIR-triggered photodynamic treatment of *Pseudomonas aeruginosa* biofilms. The system demonstrated

effective eradication of mature biofilms under low-intensity irradiation (8%, 5 min), as evidenced by membrane damage and cellular leakage. While the results suggest a photodynamic mechanism, ROS generation was not directly quantified. Overall, the platform demonstrates strong potential as an antimicrobial strategy against drug-resistant biofilm-associated infections.

Collectively, this multifaceted research contributes to the ongoing search for novel antimicrobial strategies. The study encompasses natural actinobacterial metabolite discovery, along with genetic and biochemical characterization, providing insights into their potential against drug-resistant pathogens. In parallel, nanotechnology-assisted therapeutic strategies and photodynamic therapy (PDT) were explored as independent approaches to address antimicrobial resistance. While these components were investigated separately, each offers valuable perspectives for tackling infections caused by pathogens such as MRSA and *P. aeruginosa*. Together, these findings underscore the potential of combining natural product research with emerging therapeutic technologies in future studies, thereby supporting the development of next-generation antimicrobial and anti-virulence interventions.

Overall, the work in the thesis describes (i) Isolation, molecular identification and bioactive characterization of potential actinobacteria and their bioactivity-based screening against MDR pathogens. (ii) Production, identification, and mechanism of action of *Streptomyces* sp. strain EMB26-derived metabolites against MRSA (iii) Production, identification, and mechanism of action of *Nocardia* sp. strain EMB45 metabolites against *P. aeruginosa* PAO1. (iv) Fabrication of antimicrobial catheters by coating them with immobilized EMB45 strain metabolites. (v) Photodynamic therapy mediated a synergistic and non-invasive approach towards the improvement in antimicrobial efficacy.

सार

बहु-दवा प्रतिरोधी (MDR) जीवाणु रोगजनकों, विशेष रूप से ESKAPE समूह (एंटेरोकोकस फेशियम, स्टैफिलोकोकस ऑरियस, क्लेब्सिएला न्यूमोनिए, एसीनेटोबैक्टर बाउमानी, स्यूडोमोनास एरुजिनोसा, और एंटेरोबैक्टर प्रजाति) में वैश्विक स्तर पर चिंताजनक वृद्धि एक गंभीर सार्वजनिक स्वास्थ्य समस्या बन गई है। ये जीवाणु पारंपरिक एंटीबायोटिक उपचारों से बच निकलने में सक्षम हैं, जिससे अधिकांश दवाएँ अप्रभावी हो गई हैं। इन रोगजनकों में, मेथिसिलिन-प्रतिरोधी स्टैफिलोकोकस ऑरियस (MRSA) और स्यूडोमोनास एरुजिनोसा विशेष रूप से चिंताजनक हैं, क्योंकि ये अस्पताल-संबंधी संक्रमणों, जैसे मेडिकल इम्प्लांट्स, श्वसन उपकरणों और कमजोर प्रतिरक्षा प्रणाली वाले रोगियों में गंभीर संक्रमण का कारण बनते हैं। इनके कारण होने वाले जीर्ण (क्रोनिक), बायोफिल्म-संबंधी और सेप्टिक संक्रमण नए प्रतिजैविक यौगिकों और वैकल्पिक चिकित्सीय रणनीतियों की खोज की अत्यंत आवश्यकता को दर्शाते हैं।

वर्तमान अध्ययन का उद्देश्य एक्टिनोबैक्टीरिया की जैव विविधता का अन्वेषण करना था, जो नए प्रतिजैविक और एंटी-बायोफिल्म एजेंट्स का एक संभावित स्रोत हैं। एक्टिनोबैक्टीरिया अपने द्वितीयक मेटाबोलाइट्स जैसे एंटीबायोटिक्स, एंजाइम्स, और एंटीट्यूमर यौगिकों के उत्पादन के लिए प्रसिद्ध हैं। इसके लिए मिट्टी और दीमक के घोंसले से रायगढ़ (महाराष्ट्र) और भारतीय प्रौद्योगिकी संस्थान (IIT) दिल्ली से नमूने एकत्र किए गए। इन्हें AIA और ISP2 मीडिया पर संवर्धित कर एक्टिनोबैक्टीरियल स्ट्रेनों का पृथक्करण किया गया। आकृति विज्ञान संबंधी अध्ययन में प्रमुख रूप से भूरे (grey) वायवीय माइसीलिया वाले कॉलोनियों का अवलोकन किया गया। कुल सोलह आइसोलेट्स (EMB26-EMB51) को आगे की स्क्रीनिंग हेतु संरक्षित किया गया। इनमें से दो आइसोलेट्स, strain EMB26 और EMB45, जिन्होंने सर्वाधिक प्रतिजैविक और एंटी-बायोफिल्म गतिविधियाँ प्रदर्शित कीं, को विस्तृत अध्ययन के लिए

चुना गया। फिलोजेनेटिक विश्लेषण में strain EMB26 को स्ट्रेप्टोमाइसीस कोएलिकोलर (*Streptomyces coelicolor*) और EMB45 को नॉकार्डिया भग्यनारायनाए (*Nocardia bhagyanarayanae*) से निकट संबंधी पाया गया, जिनके GenBank अभिगमन संख्या PP587436 और PQ097956 हैं।

दोनों आइसोलेट्स के सम्पूर्ण जीनोम अनुक्रमण ने उनके जैवसंश्लेषणीय क्षमता के गहन अंतर्दृष्टि प्रदान की। strain EMB26 का जीनोम आकार लगभग 7.88 Mb था जिसमें 6,954 पूर्वानुमानित जीन पाए गए, जबकि EMB45 का जीनोम आकार 6.94 Mb था जिसमें 5,952 जीन उपस्थित थे। BUSCO विश्लेषण ने उच्च गुणवत्ता वाले अनुक्रमण की पुष्टि की, जिसमें strain EMB26 और EMB45 के लिए क्रमशः 99.2% और 98.4% पूर्णता अंक प्राप्त हुए। तुलनात्मक जीनोमिक विश्लेषण में दोनों स्ट्रेनों की एंटीबायोटिक उत्पादक स्ट्रेनों से महत्वपूर्ण समानता पाई गई। AntiSMASH विश्लेषण में strain EMB26 में 21 बायोसिंथेटिक जीन क्लस्टर (BGCs) पाए गए, जिनमें नॉन-राइबोसोमल पेप्टाइड सिंथेज़ (NRPS), पॉलीकीटाइड सिंथेज़ (PKS) और साइडरोफोर संबंधित मार्ग शामिल थे। EMB45 में 18 BGCs पाए गए, जिनमें NRPS, टर्पीन और टाइप I PKS क्लस्टर सम्मिलित थे, जो इसकी जैव सक्रिय क्षमता को इंगित करते हैं।

स्ट्रेप्टोमाइसीस sp. strain EMB26 से प्राप्त द्वितीयक मेटाबोलाइट्स ने कई MDR रोगजनकों, विशेष रूप से MRSA के विरुद्ध मजबूत प्रतिजैविक प्रभाव दिखाए। एगर वेल डिफ्यूजन परीक्षणों में उल्लेखनीय निषेध क्षेत्र (inhibition zones) देखे गए। जैव रासायनिक परीक्षणों से पता चला कि ये मेटाबोलाइट्स जीवाणु कोशिका झिल्ली को बाधित करते हैं, जिससे आंतरिक घटकों का रिसाव होता है और कोशिकीय विघटन (lysis) होता है। GC-MS विश्लेषण में 7,9-डाई-टर्ट-ब्यूटिल-1-ऑक्सास्पाइरो(4,5)डेका-6,9-डाईन-2,8-डायोन को मुख्य सक्रिय यौगिक के रूप में पहचाना गया। आणविक डॉकिंग अध्ययन में इस यौगिक का MRSA के कोशिका भित्ति संश्लेषण प्रोटीनों FemA, MurB, MurE, और PBP2a के साथ स्थिर बंधन देखा गया, जो पेप्टिडोग्लाइकैन असेंबली में हस्तक्षेप का संभावित तंत्र सुझाता है।

साथ ही, नॉकार्डिया *sp.* EMB45 के मेटाबोलाइट्स ने *स्यूडोमोनास एरुजिनोसा* PAO1 के विरुद्ध शक्तिशाली एंटी-क्वोरम सेंसिंग (QS) और एंटी-बायोफिल्म गतिविधि प्रदर्शित की। कच्चे अर्क के विभाजन से प्राप्त एथिल एसीटेट अंश ने QS-नियंत्रित बायोफिल्म निर्माण को उल्लेखनीय रूप से कम किया, जीवाणु गतिशीलता को घटाया और विषाणु कारकों की अभिव्यक्ति को कम किया। qRT-PCR और ट्रांसक्रिप्शनल विश्लेषणों में *lasI*, *lasR*, *rhlI*, *rhlR*, *pqsA*, *pqsE*, और *pqsD* जैसे प्रमुख QS जीनों के अभिव्यक्ति स्तर में उल्लेखनीय कमी पाई गई, जिससे यह स्पष्ट हुआ कि EMB45 मेटाबोलाइट्स प्रत्यक्ष जीवाणुनाशक न होकर संचार प्रणाली में बाधा डालते हैं। यह रणनीति ग्राम-नेगेटिव रोगजनकों द्वारा उत्पन्न संक्रमणों से निपटने का एक आशाजनक गैर-एंटीबायोटिक दृष्टिकोण प्रस्तुत करती है।

इन निष्कर्षों को व्यावहारिक जैव-चिकित्सकीय अनुप्रयोगों में रूपांतरित करने के लिए, EMB45 मेटाबोलाइट्स को मेसोपोरस सिलिका नैनोकणों (MCM-41) में समाहित कर चिटोसान (CS) कोटिंग के साथ एक प्रतिजैविक कैथेटर प्रणाली विकसित की गई। FT-IR स्पेक्ट्रोस्कोपी द्वारा सक्रिय यौगिकों के सफल एम्बेडिंग की पुष्टि की गई, जबकि TEM इमेजिंग ने नैनोमैटेरियल की छिद्रयुक्त संरचना को सुरक्षित दिखाया। यह क्रियाशील कैथेटर सतह *स्यूडोमोनास एरुजिनोसा* PAO1 बायोफिल्म के विरुद्ध उत्कृष्ट प्रतिजैविक गुणों से युक्त पाई गई, जो उपकरण-संबंधी संक्रमणों की रोकथाम में उपयोगी हो सकती है।

इसके अतिरिक्त, पारंपरिक एंटीबायोटिक चिकित्सा की सीमाओं को ध्यान में रखते हुए, अध्ययन में नैनोमेडिसिन में फोटोडायनेमिक थेरेपी (PDT) को भी शामिल किया गया। इसके तहत UCNPs@mSiO₂(Ce6)-CSNPs नामक बहुक्रियाशील नैनोप्लेटफॉर्म का संश्लेषण किया गया, जिसमें अपकन्वर्जन नैनोपार्टिकल्स (UCNPs) को क्लोरिन e6 (Ce6) फोटोसेंसिटाइज़र तथा चिटोसान नैनोकण कोटिंग की गई। NIR (980 nm) प्रकाश के संपर्क में आने पर UCNPs निम्न-ऊर्जा वाले फोटॉनों को उच्च-ऊर्जा दृश्यमान प्रकाश में परिवर्तित करते हैं, जिससे Ce6 द्वारा प्रतिक्रियाशील ऑक्सीजन प्रजातियों

(ROS) का निर्माण होता है। यह प्रक्रिया बैक्टीरियल कोशिकाओं को ऑक्सीडेटिव क्षति पहुँचाती है, जबकि आसपास के ऊतकों पर कोई विषाक्त प्रभाव नहीं डालती, क्योंकि यह नियंत्रित और गहन ऊतक प्रवेश वाली प्रणाली है।

संपूर्ण रूप से, यह बहुआयामी शोध प्रतिजैविक समाधान की दिशा में एक महत्वपूर्ण योगदान प्रस्तुत करता है। इस अध्ययन में प्राकृतिक एक्टिनोबैक्टीरियल मेटाबोलाइट्स की खोज के साथ-साथ उनका आनुवंशिक एवं जैव-रासायनिक विश्लेषण किया गया, जिससे औषधि-प्रतिरोधी रोगजनकों के विरुद्ध उनकी संभावनाओं को समझा जा सका। इसके अतिरिक्त, नैनोटेक्नोलॉजी-सहायक उपचारात्मक रणनीतियों तथा फोटोडायनामिक थेरेपी (PDT) का अध्ययन स्वतंत्र रूप से किया गया, जो एंटीबायोटिक प्रतिरोध की समस्या से निपटने के वैकल्पिक दृष्टिकोण प्रदान करते हैं। यद्यपि ये सभी घटक अलग-अलग रूप से अध्ययनित किए गए हैं, तथापि प्रत्येक दृष्टिकोण MRSA एवं *Pseudomonas aeruginosa* जैसे रोगजनकों के विरुद्ध उपयोगी संभावनाएँ दर्शाता है। समग्र रूप से, यह कार्य भविष्य में प्राकृतिक उत्पादों एवं उभरती हुई उपचारात्मक तकनीकों के संयोजन हेतु आधार प्रदान करता है, जो अगली पीढ़ी के प्रतिजैविक एवं एंटी-विरुलेंस उपचारों के विकास में सहायक हो सकता है।

सारांशतः, इस शोध में निम्नलिखित मुख्य बिंदु शामिल हैं —

(i) एक्टिनोबैक्टीरिया का पृथक्करण, आणविक पहचान एवं जैवसक्रियता आधारित स्क्रीनिंग MDR रोगजनकों के विरुद्ध। (ii) स्ट्रेप्टोमाइसीस sp. strain EMB26 से प्राप्त मेटाबोलाइट्स का उत्पादन, पहचान और MRSA के विरुद्ध क्रियाविधि। (iii) नॉकार्डिया sp. EMB45 के मेटाबोलाइट्स का उत्पादन, पहचान और *स्यूडोमोनास एरुजिनोसा* PAO1 के विरुद्ध क्रियाविधि। (iv) EMB45 मेटाबोलाइट्स से कोटिंग द्वारा प्रतिजैविक कैथेटर का निर्माण। (v) फोटोडायनेमिक थेरेपी आधारित गैर-आक्रामक और समन्वित दृष्टिकोण से प्रतिजैविक प्रभावकारिता में सुधार।

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