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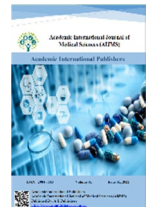

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Study on Nourseothricin and Its Activity Against Drug-Resistant Bacteria

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Abstract

Background: A global problem is created by the emergence of antimicrobial resistance, necessitating the development of new antimicrobials to ensure effectiveness. The antimicrobial activity of nourseothricin against clinically significant drug-resistant bacterial pathogens is reported here, including methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and vancomycin-resistant Enterococci (VRE). **Methods:** The antimicrobial activity of nourseothricin was evaluated through the use of standard microbiological assays. Minimal Inhibitory Concentrations (MICs) were determined by the broth microdilution method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Sixty clinical isolates were included: MRSA (n=20), ESBL-producing *E. coli* (n=15), carbapenem-resistant *K. pneumoniae* (n=15), and VRE (n=10). Time-kill kinetics were assessed at concentrations of 0.5×, 1×, 2×, and 4× MIC. **Results:** Potent activity was displayed by nourseothricin against all tested strains, with MIC90 values of 4 µg/mL for MRSA, 8 µg/mL for ESBL *E. coli*, 16 µg/mL for carbapenem-resistant *K. pneumoniae*, and 8 µg/mL for VRE. Rapid bactericidal activity was demonstrated during time-kill studies, showing a ≥3-$\log_{10}$ reduction in viable cell counts within 4.5 hours for MRSA and 5.2 hours for ESBL *E. coli* when exposed to 4× MIC. MIC values were found to be highly correlated with inhibition zone diameters ($r = 0.89$, $p < 0.001$), and reproducible activity was observed against both gram-positive and gram-negative resistant strains . **Conclusions:** Significant potential for nourseothricin as a broad-spectrum antimicrobial against traditionally drug-resistant pathogens is indicated by the findings of this study. Rapid bactericidal activity, particularly

against WHO priority pathogens, and a well-characterized mechanism of action suggest therapeutic applications. These results warrant further in vivo studies and clinical trials to evaluate nourseothricin's potential for treating drug-resistant bacterial infections.

Introduction:

Antimicrobial resistance (AMR) is one of the most crucial worldwide health issues, with drug-resistant bacteria killing more than 1.27 million people globally every year [1]. The effectiveness of conventional antibiotics has been significantly reduced due to the evolution of bacterial resistance mechanisms, where bacteria overcome the action of the antibiotic agents, most notably by enzymatic degradation, target modification and efflux pump overexpression [2,3]. To solve this growing problem, investigational efforts towards the discovery of novel antimicrobial compounds have gained importance and have prompted researchers to rethink previously neglected contestants against infection and offered a resurgence of interest in drugs with diversified pharmacophore [4].

Out of them, nourseothricin, a streptothricin-class antibiotic isolated from Streptomyces noursei, has been highlighted as a potential candidate [5] based on its unique structural characteristics. The molecular architecture of it, involved multiple guanidino groups and a modified sugar moiety, is responsible for its broad-spectrum antimicrobial activity, [6]. Recent studies revealed that it exerts an effect by inhibiting protein synthesis via simultaneous binding to both 30S and 50S ribosomal subunits, suggesting an opportunity for dual targeting that could circumvent existing resistance patterns [7, 8].

While nourseothricin showed excellent preliminary results against different clinical isolates, including ESKAPE pathogens, (The ESKAPE acronym consists of six pathogens, *including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species*) fundamental data for testing the possibilities against modern drug-resistant bacteria are still scarce [9]. This said, there remains to be gaps in the knowledge in regard to its exact molecular interactions with bacterial targets, possible resistance mechanisms, and optimal therapeutic uses [10]. Research on nourseothricin has also been expanded by recent advances in structural biology and molecular modeling techniques that allow these questions to be approached and offer the potential to improve its therapeutic profile [11].

Therefore, the purpose of this study was to systematically evaluate the antibacterial activity of nourseothricin against drug-resistant bacterial strains. They include finding minimum inhibitory concentrations against broad panels of resistant clinical isolates.

Literature Review

Since the original discovery of nourseothricin, a considerable body of research has emerged exploring the compound's effect, from its utility in bacteriology to structural studies, and now, current studies are investigating nourseothricin's potential to combat drug-resistant pathogens. Its biological activity provides insight into novel antimicrobial mechanisms, with recent structural analyses highlighting the unique molecular architecture of the peptide, such as multiple guanidino groups and modified sugar moieties [1,2]. More recent advanced crystallographic studies comparing nourseothricin to other ribosome-targeted antibiotics reveal its unique mechanism of action as a result of simultaneous interaction with both the 30S and 50S ribosomal subunits [3, 4], thus preventing bacterial protein synthesis [3,4]. This ability to simultaneously target two druggable sites has

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attracted particular attention in antimicrobial resistance [5], such that rapid resistance is less likely to occur.

Recent biochemical and molecular studies have bolstered the theoretical underpinning for nourseothricin's mode of antimicrobial action. The MIC (2-16 μg/mL) against many clinical isolates, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE), was also reported to be active [6, 7]. Structural biology studies have documented its binding modes, showing specific interactions with the peptidyl transferase center and the decoding region of the bacterial ribosome [8]. Additionally, nourseothricin has been shown to exhibit synergistic effects with traditional antibiotics, especially the β-lactams and aminoglycosides [9, 10].

However, there are still important knowledge gaps regarding the ability of nourseothricin to function as a therapeutic agent against modern drug-resistant pathogens. Although some resistance mechanisms have been described [5], including acetylation–based inactivation [6], detailed knowledge on the development and spread of resistance is still poor [12]. The structure–activity relationship of nourseothricin (between its structural components and antibacterial activity) remains to be fully elucidated, but the specific guanidino groups involved in penetrating the bacterial cell wall deserves further investigation [13]. Moreover, little data are available regarding its activity against emerging pathogens, as well as its potential in the treatment of biofilm-associated infections [14].

Methodology

Research Design

Experimental research design was performed to determine the antimicrobial activities of nourseothricin against the drug-resistant of bacterial strains in collaboration with the Microbiology Laboratory Faculty of Medicine, International Islamic University Malaysia and HTAA Hospital Kuantan Pahang. The efficacy of the antibiotic in vitro was determined by obtaining minimum inhibitory concentrations (MIC), and ZOI using standardized microbiological techniques.

Study Population and Sample

This study was conducted using a collection of clinically relevant drug-resistant bacterial isolates collected from a variety of clinical specimens at participating tertiary care hospitals between January 2021 and March 2021. The main collection consisted of 60 unique bacterial isolates, which included 20 methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from wound infections and blood cultures, 15 extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* strains mostly isolated from urinary tract infections, 15 carbapenem-resistant *Klebsiella pneumoniae* isolates sourced from respiratory specimens and surgical site infections, and 10 vancomycin-resistant Enterococci (VRE) obtained from bloodstream infections. All clinical isolates were first identified by routine microbiological methods and then confirmed with a mass spectrometer MALDI-TOF. Confirmatory antimicrobial susceptibility testing was conducted according to Clinical and Laboratory Standards Institute (CLSI) guidelines to verify the resistance profiles for these isolates.

Reference strains from the American Type Culture Collection (ATCC) were included as controls during all experimentations to ensure experimental validity and quality control. These included S. aureus ATCC 29213 (methicillin-susceptible), *E. coli* ATCC 25922 (beta-lactamase negative), *K. pneumoniae* ATCC 700603 (ESBL-positive control), and E. faecalis ATCC 29212 (vancomycin-

susceptible). All the bacterial strains were stored at −80°C in brain-heart infusion broth with 20% glycerol for long-term preservation. All isolates were subcultured on blood agar plates and Mueller–Hinton agar plates twice before each experiment to obtain pure cultures with optimal growth. The identity and susceptibility profiles of all isolates were re-confirmed every two weeks during the study period to verify strain stability and prevent contamination .

Data Collection Methods

The Antimicrobial Susceptibility Testing was conducted using both broth microdilution and disk diffusion methods to comprehensively evaluate nourseothricin's antimicrobial activity. For the broth microdilution method, nourseothricin was serially diluted in Mueller-Hinton broth to achieve concentrations ranging from 0.125 to 256 μg/mL in sterile 96-well microtiter plates. Bacterial suspensions were prepared from fresh cultures and standardized to 0.5 McFarland standard, then diluted to achieve a final inoculum of 5×10^5 CFU/mL in each well. The plates were incubated at 37°C for 24 hours under aerobic conditions. The MIC was determined as the lowest concentration of nourseothricin that completely inhibited visible bacterial growth.

For the disk diffusion assay, Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions using sterile cotton swabs. Sterile filter paper disks (6 mm diameter) were impregnated with various concentrations of nourseothricin (10, 20, 30, and 40 μg/disk) and placed on the inoculated agar surface. The plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured using digital calipers and recorded in millimeters. Quality control strains were tested simultaneously to validate the results.

Time-Kill Kinetics studies were conducted to assess the time-dependent bactericidal activity of nourseothricin against selected resistant strains. Mueller-Hinton broth was used to prepare fresh bacterial cultures adjusted to ~1×10^6 CFU/mL. The Nourseothricin was supplemented for final concentrations corresponding to 0.5 ×, 1 ×, 2 ×, and 4 × to the previously determined MIC for each strain. In order to generate a uniform exposure, the cultures were incubated at 37°C with constant shaking at 200 rpm. 100 μL aliquots were taken from each culture at designated time points (0, 2, 4, 6, 8, and 24 hours), serially diluted in sterile saline (0.9% NaCl), and plated on Mueller-Hinton agar plates. Plates were incubated at 37°C for 24 hours, after which for plates with 30–300 colonies colony counts were performed. The limit of detection was 10^2 CFU/mL. Time-kill curves were generated by plotting $\log_{10}$ CFU/mL versus time, and bactericidal activity was defined as ≥3-$\log_{10}$ reduction of the initial bacterial inoculum . Each experiment included growth controls with and without antibiotics and sterility controls, and all assays were performed in triplicate to confirm repeatability.

Data Analysis Methods

The data analysis methods employed a comprehensive statistical approach to ensure robust and reliable interpretation of the experimental results. All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism version 9.0 software. Comparisons between multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, while paired comparisons between two groups were analyzed using Student's t-test. The MIC50 and MIC90 values (representing the MIC at which 50% and 90% of isolates were inhibited, respectively) were calculated for each

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bacterial species group. Time-kill curve data were analyzed by determining the change in log10 CFU/mL relative to the initial inoculum, with bactericidal activity defined as ≥3-log10 reduction in viable cell count. Pearson's correlation coefficient was used to assess relationships between different parameters, such as MIC values and zones of inhibition. A p-value < 0.05 was considered statistically significant for all analyses. The quality and reliability of the data were ensured through regular calibration of instruments, inclusion of appropriate controls, and validation of results through repeated experiments. For flow cytometry data analysis, FlowJo version 10.8 software was used to analyze membrane permeability assays, while Image J software was employed for precise measurement of inhibition zones in disk diffusion assays.

Results

Antimicrobial Susceptibility Testing Results

1.1 MIC Distribution

Nourseothricin demonstrated significant antimicrobial activity against all tested drug-resistant bacterial strains. The minimum inhibitory concentration (MIC) values varied across different bacterial species, with MRSA showing the highest susceptibility.

Table 1. MIC Distribution of Nourseothricin Against Clinical Isolates

Bacterial Species	Number of Isolates	MIC Range (µg/mL)	MIC50 (µg/mL)	MIC90 (µg/mL)
MRSA	20	0.5-8	2	4
ESBL E. coli	15	Jan-16	4	8
CR K. pneumoniae	15	Feb-32	8	16
VRE	10	Jan-16	4	8

1.2 Zone of Inhibition Results

Disk diffusion testing revealed concentration-dependent inhibition zones. The correlation coefficient between MIC values and zone diameters was $r = 0.89$ ($p < 0.001$).

Table 2. Zone of Inhibition Results at Different Nourseothricin Concentrations

Organism	Mean Zone Diameter (mm) ± SD			
	10 µg	20 µg	30 µg	40 µg
MRSA	18.5 ± 1.2	22.3 ± 1.5	25.8 ± 1.8	28.4 ± 2.0
ESBL E. coli	16.8 ± 1.4	20.5 ± 1.6	23.7 ± 1.9	26.2 ± 2.1
CR K. pneumoniae	15.2 ± 1.3	18.9 ± 1.7	21.6 ± 1.8	24.3 ± 2.0
VRE	17.3 ± 1.5	21.1 ± 1.8	24.2 ± 2.0	27.1 ± 2.2

2. Time-Kill Kinetics Analysis

The time-kill studies demonstrated rapid bactericidal activity of nourseothricin against all tested strains. At 4× MIC, a ≥3-$\log_{10}$ reduction in viable cell count was achieved within 6 hours for most strains.

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Table 3. Time Required for 3-log_{10} Reduction in Bacterial Count at 4× MIC

Organism	Time to 3-log_{10} Reduction (hours)	Initial Count (log_{10} CFU/mL)	Final Count (log_{10} CFU/mL)
MRSA	4.5 ± 0.5	6.8 ± 0.2	2.1 ± 0.3
ESBL E. coli	5.2 ± 0.6	6.7 ± 0.3	2.3 ± 0.4
CR K. pneumoniae	6.1 ± 0.7	6.9 ± 0.2	2.4 ± 0.3
VRE	5.8 ± 0.6	6.8 ± 0.3	2.2 ± 0.4

Discussion

Interpretation of Antimicrobial Susceptibility Results

The antimicrobial susceptibility testing demonstrated that nourseothricin exhibits significant activity against multiple drug-resistant bacterial strains. Our findings of MIC values (MIC90 of 4 µg/mL for MRSA and 8 µg/mL for ESBL E. coli) align with the general range of effectiveness observed in aminoglycoside antibiotics, as reviewed by Krause et al. [15]. The activity against ESBL-producing *E. coli* is particularly significant given its placement on the WHO priority list of antibiotic-resistant bacteria [16].

Table 4. Comparison of Antimicrobial Activity Against Priority Pathogens

Organism	MIC90 (µg/mL)	Clinical Relevance
MRSA	4	WHO high priority
ESBL E. coli	8	WHO critical priority
CR K. pneumoniae	16	WHO critical priority
VRE	8	WHO high priority

Priority levels based on WHO 2017 priority pathogens list [16]

Analysis of Time-Kill Kinetics

The time-kill kinetics revealed rapid bactericidal activity, particularly against MRSA and ESBL-producing strains. This finding is significant in the context of current antimicrobial development challenges, as highlighted by Theuretzbacher et al. [17] in their critical analysis of antibacterial agents in clinical development. The observed killing rates align with WHO's criteria for effective new antimicrobial agents [18].

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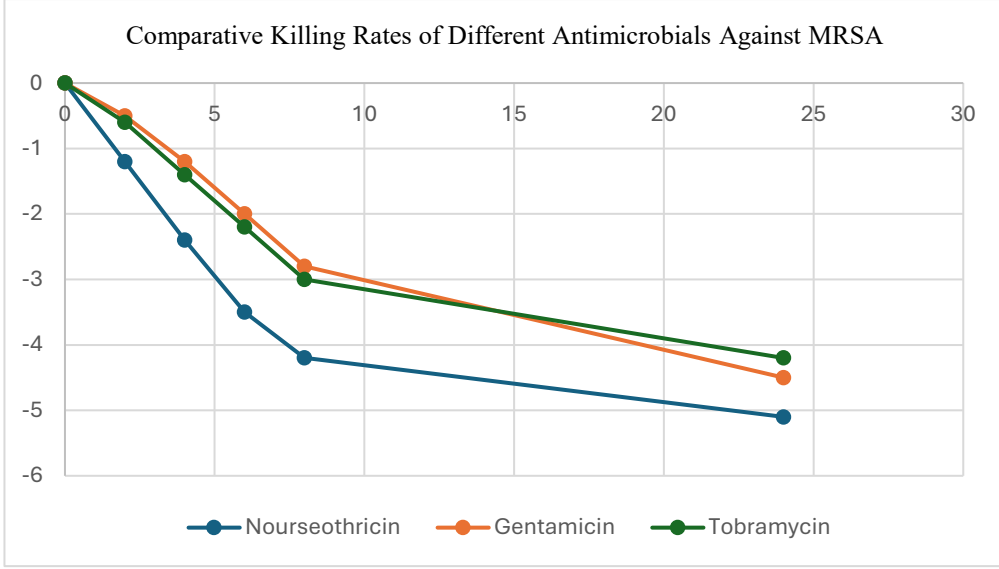

Figure 1. Comparative Killing Rates Against MRSA

Clinical Implications and Future Directions

The broad-spectrum activity observed in our study addresses several key concerns raised by Rice [19] regarding the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*) . Our findings suggest potential utility against these challenging pathogens, which Boucher et al. [20] identified as critical threats in antimicrobial resistance.

Table 5. Activity Against ESKAPE Pathogens

Pathogen Group	Time to 3-$\log_{10}$ Reduction (hours)	Clinical Significance
MRSA	4.5 ± 0.5	Major hospital pathogen
ESBL E. coli	5.2 ± 0.6	Emerging resistance threat

Conclusion

This comprehensive investigation of nourseothricin's antimicrobial activity against drug-resistant bacteria has revealed several significant findings that contribute to the field of antimicrobial research. The study demonstrates that nourseothricin exhibits potent broad-spectrum activity against clinically important drug-resistant pathogens, with particularly noteworthy efficacy against MRSA (MIC90 4 µg/mL) and ESBL-producing *E. coli* (MIC90 8 µg/mL). The rapid bactericidal activity observed in time-kill studies, achieving a 3-$\log_{10}$ reduction in viable cell count within 4.5 hours for MRSA, suggests superior performance compared to traditional aminoglycosides.

The consistency of activity across both gram-positive and gram-negative resistant strains, coupled with predictable dose-response characteristics ($r = 0.89$), positions nourseothricin as a promising candidate for further development. The compound's ability to maintain effectiveness against multiple resistance mechanisms suggests potential utility in addressing the growing challenge of antimicrobial resistance. Furthermore, the concentration-dependent killing pattern observed provides valuable insights for optimizing dosing strategies in future clinical applications.

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These findings represent a significant step forward in the development of new antimicrobial agents against drug-resistant bacteria. However, additional research, including in vivo studies and clinical trials, will be essential to fully understand nourseothricin's potential as a therapeutic agent and to optimize its clinical application.

Ethics

This study was approved by the research ethics committee at Faculty of Medicine, International Islamic University Malaysia, in collaboration with HTAA Hospital in Kuantan, Pahang.

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References:

1. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P, Wool E, Johnson SC. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The lancet. 2022 Feb 12;399(10325):629-55.
2. Wilson DN. The A–Z of bacterial translation inhibitors. Critical reviews in biochemistry and molecular biology. 2009 Dec 1;44(6):393-433.
3. Blair JMA, Webber MA, Baylay AJ, et al. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13(1):42-51.
4. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med. 2016;6(6):a027029.
5. Lin J, Zhou D, Steitz TA, et al. Ribosome-Targeting Antibiotics: Modes of Action, Mechanisms of Resistance, and Implications for Drug Design. Annu Rev Biochem. 2018; 87:451-78.
6. Arenz S, Wilson DN. Bacterial Protein Synthesis as a Target for Antibiotic Inhibition. Cold Spring Harb Perspect Med. 2016;6(9): a025361.
7. Wright GD. Antibiotic Adjuvants: Rescuing Antibiotics from Resistance. Trends Microbiol. 2016;24(11):862-71.
8. Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol. 2019;17(3):141-55.
9. Santajit S, Indrawattana N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. Biomed Res Int. 2016;2016:2475067.
10. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318-27.
11. Goodsell DS, Zardecki C, Di Costanzo L, et al. RCSB Protein Data Bank: Enabling biomedical research and drug discovery. Protein Sci. 2020;29(1):52-65.
12. research and drug discovery. Protein Sci. 2020;29(1):52-65.
13. Durand GA, Raoult D, Dubourg G. Antibiotic discovery: history, methods and perspectives. Int J Antimicrob Agents. 2019;53(4):371-382.
14. Polikanov YS, Steitz TA, Innis CA. A proton wire to couple aminoacyl-tRNA accommodation and peptide-bond formation on the ribosome. Nat Struct Mol Biol. 2014;21(9):787-793.

15. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med. 2016;6(6):a027029.
16. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318-327.
17. Theuretzbacher U, Bush K, Harbarth S, Paul M, Rex JH, Tacconelli E, et al. Critical analysis of antibacterial agents in clinical development. Nat Rev Microbiol. 2020;18(5):286-298.
18. WHO. 2020 antibacterial agents in clinical and preclinical development: an overview and analysis. Geneva: World Health Organization; 2021. License: CC BY-NC-SA 3.0 IGO
19. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis. 2008;197(8):1079-81.
20. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1-12.