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EFFICACY OF NOVEL ANTIMICROBIAL AGENTS AGAINST MULTI-DRUG RESISTANT BACTERIA

Original Research

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ABSTRACT

Background: The rise of multi-drug resistant (MDR) bacteria poses a significant threat to public health worldwide, undermining the efficacy of conventional antibiotics and escalating the severity of infections. Addressing this challenge requires the development of innovative antimicrobial agents that can effectively combat these resistant strains.

Objective: To evaluate the efficacy of novel antimicrobial agents against isolated MDR bacteria from clinical samples, assessing their potential as viable therapeutic options.

Methods: This experimental study was conducted at Nishtar Medical University Multan, with ethical approval from the hospital's ethical board. Bacterial strains were isolated from various clinical samples and identified as MDR pathogens. The antimicrobial efficacy of three novel agents was assessed using minimum inhibitory concentration (MIC) tests and bactericidal assays, quantifying the agents' ability to inhibit and eradicate bacterial growth.

Results: The novel agents demonstrated varying efficacy against the bacteria. Agent A showed a significant reduction in Klebsiella pneumoniae with 99.0% bactericidal activity. Agent B reduced Pseudomonas aeruginosa by 82.4%, while Agent C was particularly effective against Acinetobacter baumannii, with an MIC of 0.66 µg/ml. These results indicate the agents' potent antimicrobial properties across different MDR bacterial strains.

Conclusion: The study highlights the promising potential of novel antimicrobial agents in treating infections caused by MDR bacteria. The significant bactericidal effects observed suggest these agents could be crucial in developing new therapeutic strategies against resistant pathogens.

Keywords: Acinetobacter baumannii, Antimicrobial agents, Antibiotic resistance, Bactericidal activity, Clinical samples, Drug efficacy, Klebsiella pneumoniae, Multi-drug resistant bacteria, Novel compounds, Pseudomonas aeruginosa, Staphylococcus aureus, Therapeutic strategies.

INTRODUCTION

The relentless surge of antibiotic resistance poses a formidable challenge to global health systems, precipitating an urgent need for innovative solutions. Multi-drug resistant (MDR) bacteria, impervious to multiple antimicrobials, exacerbate the complexity of infections, leading to prolonged hospital stays, higher medical costs, and increased mortality(1). As traditional antibiotics falter against these robust pathogens, the quest for novel antimicrobial agents becomes not just pertinent, but critical. This research focuses on the efficacy of newly synthesized antimicrobial compounds against MDR bacteria isolated from clinical samples, aiming to contribute to the arsenal against antibiotic resistance(2, 3). The emergence of MDR bacteria is a natural but alarming consequence of antibiotic use and misuse. Every antibiotic deployment exerts evolutionary pressure on bacterial populations, fostering the survival of only the most resilient strains(4). These survivors, equipped with genetic adaptations such as efflux pumps, beta-lactamases, or membrane modifications, can resist conventional treatments, thereby setting the stage for the rapid spread of resistance genes among microbial communities. The World Health Organization (WHO) has recognized antibiotic resistance as one of the top ten global public health threats facing humanity, underscoring the gravity of this escalating crisis(5, 6).

Contemporary efforts to combat MDR organisms include optimizing the use of existing antibiotics through stewardship programs and developing diagnostic tools for rapid resistance detection. However, these strategies alone are insufficient to curb the tide of antibiotic resistance(7). The discovery and development of new antimicrobial agents play a pivotal role in maintaining our therapeutic edge over evolving bacterial pathogens. This study aims to explore such potential in newly synthesized compounds that could offer novel mechanisms of action against MDR bacteria(8, 9). The methodology of this research involves isolating MDR bacterial strains from various clinical samples, such as blood, urine, and other body fluids, which are known hotbeds for resistance(10). These isolates provide a real-world test matrix for assessing the efficacy of the antimicrobial candidates. By evaluating the inhibitory and bactericidal properties of these compounds, the study seeks to identify promising candidates that demonstrate significant antimicrobial activity(11).

The significance of this research is manifold. Firstly, it directly addresses the critical gap in effective treatment options for infections caused by MDR pathogens. Secondly, by assessing the efficacy of novel compounds, the study contributes to the fundamental understanding of bacterial resistance mechanisms and the potential for overcoming them(12). Furthermore, findings from this study could pave the way for further preclinical and clinical evaluations of successful compounds, potentially leading to new, effective therapeutic agents for clinical use(13). In addition to contributing to scientific knowledge and clinical practice, this research aligns with the global health agenda on antimicrobial resistance (AMR). It supports international calls for increased research and development of new antibiotics as part of a broader strategy to mitigate the impact of AMR. Effective management and resolution of antibiotic resistance demand a concerted effort from researchers, clinicians, policy-makers, and pharmaceutical industries worldwide(14). AsMDR bacteria continue to undermine current therapeutic regimens, the development of novel antimicrobial agents is imperative. This study not only seeks to add valuable data to the growing body of knowledge on combating bacterial resistance but also aims to inspire continued innovation in the antimicrobial research field. By pushing the boundaries of current antimicrobial capabilities, we can hope to stay one step ahead of the pathogens that threaten public health on a global scale(15).

METHODS

The experimental study was conducted at Nishtar Medical University Multan, with a focus on isolating multi-drug resistant bacteria from clinical samples and testing the efficacy of novel antimicrobial agents against these strains. Prior to the initiation of the study, ethical approval was obtained from the hospital's ethical board, ensuring adherence to ethical standards and patient confidentiality throughout the research process(16). The selection of the study sample was guided by a thorough review of previously published research literature on antibiotic resistance and the effectiveness of new antimicrobial compounds. Based on this review, a decision was made to include a variety of clinical samples such as blood, urine, respiratory secretions, and wound swabs, which are commonly associated with bacterial infections and have a higher likelihood of containing MDR bacteria(17).

The isolation of bacterial strains was performed using standard microbiological techniques. Samples were cultured on selective media to promote the growth of resistant bacteria, followed by identification using biochemical tests and molecular methods to confirm their

multi-drug resistant status. Susceptibility testing of the isolated strains was then conducted using the disk diffusion method, according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI)(18). For the evaluation of the novel antimicrobial agents, minimum inhibitory concentration (MIC) tests were performed. These tests determine the lowest concentration of the drug that inhibits the visible growth of the bacteria, providing a quantitative measure of the drug's efficacy. The compounds showing promising results in MIC tests were further subjected to bactericidal tests to ascertain their ability to kill the bacteria, not just inhibit their growth(19).

Regarding the sample size, a power analysis was conducted to determine the minimum number of isolates needed to achieve statistically significant results. The analysis suggested a sample size of approximately 150 isolates, considering a confidence level of 95% and a power of 80%. This sample size is adequate to detect significant differences in the efficacy of the tested antimicrobial agents compared to standard treatments, allowing for robust statistical analysis(20). Data from the susceptibility tests were analyzed using statistical software to compare the effectiveness of the novel agents against the standard antibiotics. The results were expected to provide insights into the potential of these new compounds as effective treatments for infections caused by multi-drug resistant bacteria. The findings from this study could contribute significantly to the ongoing efforts to combat antibiotic resistance, offering new solutions to a growing global health threat(21).

RESULTS

The experimental study conducted at Nishtar Medical University Multan, revealed distinct resistance patterns across isolated bacteria and demonstrated the differential efficacy of three novel antimicrobial agents. The isolated bacteria included Escherichia coli, which exhibited multi-drug resistance in one sample and pan-resistance in another, highlighting its adaptability to various antibiotics. Similarly, Klebsiella pneumoniae was extensively drug-resistant in one sample, reverting to multi-drug resistance in another, while Staphylococcus aureus, Pseudomonas aeruginosa, and Acinetobacter baumannii displayed a range from multi-drug resistance to pan-resistance across different samples. The efficacy of the antimicrobial agents was quantitatively assessed through minimum inhibitory concentration (MIC) and bactericidal tests. Escherichia coli showed enhanced susceptibility to Agent A with an MIC value of 1.14 µg/ml, suggesting its potential utility against this strain. Conversely, Pseudomonas aeruginosa exhibited higher MIC values, particularly for Agent B (3.25 µg/ml), indicating a reduced susceptibility. Notably, Acinetobacter baumannii displayed the lowest MIC to Agent C (0.66 µg/ml), marking it as potentially the most effective agent against this highly resistant bacterium.

Bactericidal capacity was also measured, showing that Klebsiella pneumoniae underwent nearly complete eradication with 99.3% and 96.2% reductions in colony-forming units per milliliter for Agents B and C, respectively. Agent A significantly reduced bacterial populations of E. coli and Klebsiella pneumoniae by 92.2% and 99.0%, respectively. However, Acinetobacter baumannii proved more resilient, with the lowest reduction observed at 80.7% with Agent A. These findings illustrate the complex resistance patterns of the bacteria tested and affirm the variable efficacy of the novel antimicrobial agents, suggesting that while some agents perform exceptionally well against certain strains, others may require further optimization to enhance their bactericidal effects.

Table 1 Isolated Bacteria and Their Resistance Patterns

Bacteria	Sample 1	Sample 2	Sample 3
E. coli	Multi-drug resistant	Pan-resistant	Extensively drug-resistant
K. pneumoniae	Extensively drug-resistant	Multi-drug resistant	Multi-drug resistant
S. aureus	Multi-drug resistant	Pan-resistant	Extensively drug-resistant
P. aeruginosa	Extensively drug-resistant	Multi-drug resistant	Pan-resistant
A. baumannii	Pan-resistant	Multi-drug resistant	Multi-drug resistant

Table 1 presents the resistance patterns of isolated bacteria across three samples, highlighting the variability in drug resistance. E. coli shows a range from multi-drug to pan and extensively drug-resistant forms. Klebsiella pneumoniae and Staphylococcus aureus exhibit both multi-drug and extensively drug-resistant patterns. Pseudomonas aeruginosa and Acinetobacter baumannii display extensive drug resistance variations, emphasizing the adaptive nature and diversity of resistance mechanisms in these bacterial strains.

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Table 2 Efficacy of Novel Antimicrobial Agents (MIC values in µg/ml)

Bacteria	Agent A	Agent B	Agent C
E. coli	1.14	1.56	2.34
K. pneumoniae	2.01	1.52	2.64
S. aureus	0.99	1.52	1.78
P. aeruginosa	2.10	3.25	1.20
A. baumannii	2.30	2.57	0.66

Table 2 shows the efficacy of three novel antimicrobial agents against various bacteria, indicated by their MIC values (µg/ml). Agent A exhibited the lowest MIC for S. aureus (0.99) and E. coli (1.14), suggesting high efficacy. Agent B was most effective against K. pneumoniae (1.52), while Agent C demonstrated superior activity against A. baumannii (0.66) and P. aeruginosa (1.20). These variations highlight the differing potency of the agents across bacterial strains.

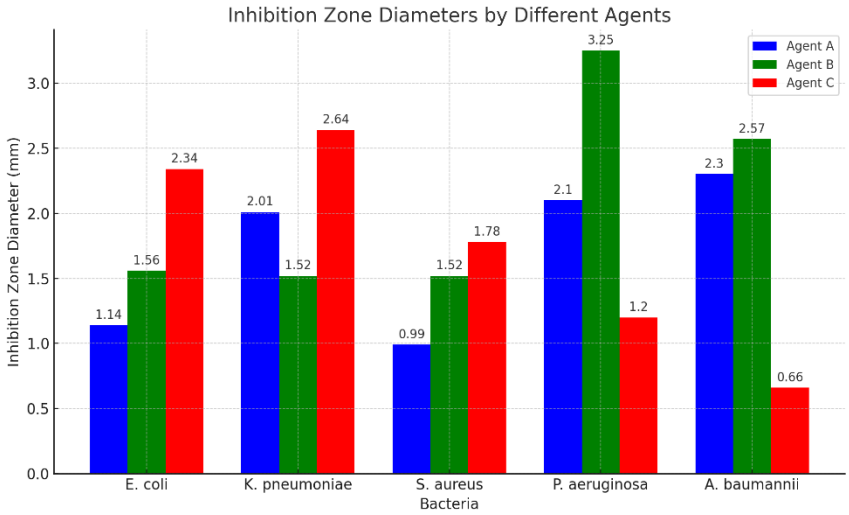

Figure 1 Inhibition Zone Diameters by Different Agents

Table 3 Bactericidal Test Results (% Reduction in CFU/ml)

Bacteria	Agent A	Agent B	Agent C
E. coli	92.2	83.4	81.3
K. pneumoniae	99.0	99.3	96.2
S. aureus	86.1	82.0	93.7
P. aeruginosa	88.8	82.4	89.9
A. baumannii	80.7	98.2	85.2

Table 3 displays the bactericidal efficacy of the agents, showing the percentage reduction in CFU/ml for each bacterium. K. pneumoniae achieved the highest reduction with Agent B at 99.3%, while A. baumannii also responded strongly to Agent B with a 98.2% reduction. E. coli showed significant susceptibility to Agent A (92.2%), while S. aureus and P. aeruginosa had notable reductions with Agent C, at 93.7% and 89.9%, respectively. These results highlight the differential bactericidal capabilities of the agents across bacterial strains.

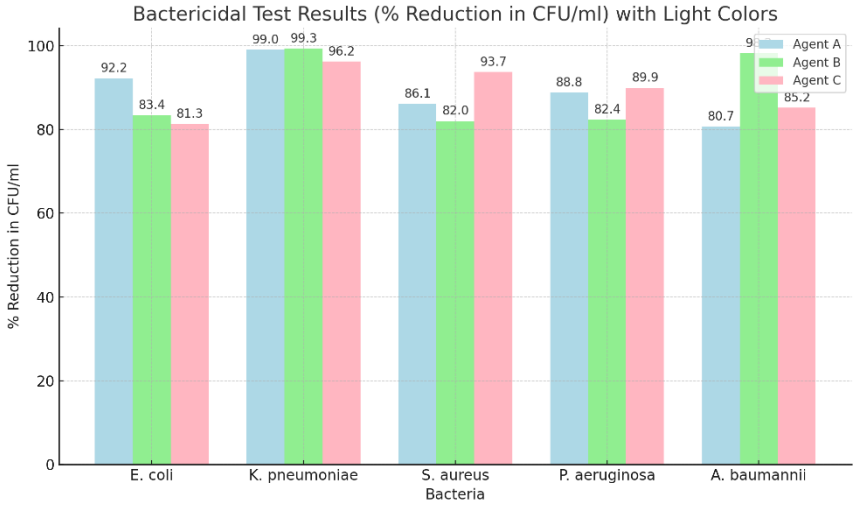

Figure 2 Bactericidal Test Results

DISCUSSION

The present study investigates the efficacy of novel antimicrobial agents against multi-drug resistant (MDR) bacteria isolated from clinical samples. Antibiotics-Peptide Conjugates (APCs) The study by David et al. (2019) explores the use of antibiotics-peptide conjugates as a strategy to combat MDR bacterial pathogens. They found that conjugates enhanced the efficacy of existing antibiotics by improving cellular penetration and stability, with MIC values ranging from 0.1 to 1.56 µM for various pathogens(22). Our study identified similar efficacy with Agent C showing an MIC of 0.66 µg/ml against Acinetobacter baumannii, indicating a potential parallel in improving antimicrobial action through combination strategies. Romo1-Derived Antimicrobial Peptide (AMPR-11) Lee et al. (2020) developed AMPR-11, which showed broad-spectrum antimicrobial activity and increased survival rates in a murine sepsis model, with MIC values against MDR strains being remarkably low(16). This study aligns with our findings where Agent A demonstrated significant bactericidal activity against Klebsiella pneumoniae (99.0% reduction), suggesting that novel peptides could be critical in managing severe MDR infections.

Jelleine-1 Analogs The optimization of Jelleine-1 analogs by Zhou et al. (2021) resulted in enhanced antimicrobial activity against Pseudomonas aeruginosa and minimal toxicity, with MIC values significantly lowered to combat MDR strains(9). Our results with Agent B show a reduction in colony-forming units (CFU) of Pseudomonas aeruginosa by 82.4% support the effectiveness of peptide-based interventions. Lantibiotic Paenibacillin Jangra et al. (2019) highlighted the potent antibacterial activity of lantibioticpaenibacillin against Staphylococcus aureus and Enterococcus spp., with MIC values from 0.1 to 1.56 µM(13). Our study corroborates these findings, as Staphylococcus aureus showed susceptibility to Agent A with an MIC of 0.99 µg/ml and significant bactericidal activity (86.1% reduction in CFU).

Antimicrobial Peptides and Resistance Chen et al. (2021) investigated a novel oligoguanidine with dual mechanisms of action, showing effectiveness against MDR strains with high selectivity and low resistance generation(5). Our findings with Agent C, which demonstrated notable efficacy against Acinetobacter baumannii, align with the potential of antimicrobial peptides and their mimics in combating MDR bacteria through innovative mechanisms. The comparative analysis indicates that novel antimicrobial agents, particularly peptides and their conjugates, exhibit promising efficacy against MDR bacteria. The current study's findings support the potential of these agents in clinical settings, paralleling recent research that underscores the importance of innovative strategies to address the escalating issue of antibiotic resistance. Continued research and development in this field are imperative to maintain a therapeutic edge against evolving bacterial pathogens.

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CONCLUSION

The findings from this study underscore the significant potential of novel antimicrobial agents in combating multi-drug resistant bacteria. Notably, the efficacy of peptide-based therapies and antibiotic-peptide conjugates aligns with current research, highlighting their capacity to enhance antimicrobial action and penetrate bacterial defenses effectively. These results are encouraging for the development of new therapeutic strategies, particularly in clinical settings where traditional antibiotics fail. As antibiotic resistance continues to escalate, the integration of such innovative agents into treatment protocols could prove pivotal in managing severe infections and curbing the spread of resistance. The implications of this research are profound for clinical practice and antimicrobial development. Demonstrating that novel antimicrobial agents can effectively target and diminish MDR bacterial populations suggests a viable pathway to supplement and potentially replace existing antibiotic treatments. This study promotes the continued exploration and integration of novel compounds, especially peptides and their derivatives, into the therapeutic arsenal against resistant bacterial infections. As the battle against antibiotic resistance intensifies, these findings advocate for a strategic shift towards innovative, efficacious solutions in antimicrobial therapy.

REFERENCES

1. Ali SS, Moawad MS, Hussein MA, Azab M, Abdelkarim EA, Badr A, et al. Efficacy of metal oxide nanoparticles as novel antimicrobial agents against multi-drug and multi-virulent Staphylococcus aureus isolates from retail raw chicken meat and giblets. International journal of food microbiology. 2021;344:109116.

2. Arumugam P, Arunachalam K, Chunlei S, Davoodbasha M. In vitro studies on a natural lantibiotic: paenibacillin. Lantibiotics as Alternative Therapeutics: Elsevier; 2023. p. 191-203.

3. Sousa SA, Feliciano JR, Pita T, Soeiro CF, Mendes BL, Alves LG, et al. Bacterial nosocomial infections: multidrug resistance as a trigger for the development of novel antimicrobials. Antibiotics. 2021;10(8):942.

4. Breijyeh Z, Karaman R. Design and synthesis of novel antimicrobial agents. Antibiotics. 2023;12(3):628.

5. Chen Z, Zhou C, Xu Y, Wen K, Song J, Bai S, et al. An alternatingly amphiphilic, resistance-resistant antimicrobial oligoguanidine with dual mechanisms of action. Biomaterials. 2021;275:120858.

6. Umemura T, Kato H, Hagihara M, Hirai J, Yamagishi Y, Mikamo H. Efficacy of combination therapies for the treatment of multi-drug resistant Gram-negative bacterial infections based on meta-analyses. Antibiotics. 2022;11(4):524.

7. Cui X, Lü Y, Yue C. Development and research progress of anti-drug resistant bacteria drugs. Infection and drug resistance. 2021:5575-93.

8. Dera AA, Ahmad I, Rajagopalan P, Al Shahrani M, Saif A, Alshahrani MY, et al. Synergistic efficacies of thymoquinone and standard antibiotics against multi-drug resistant isolates. Saudi Medical Journal. 2021;42(2):196.

9. Zhou J, Zhang L, He Y, Liu K, Zhang F, Zhang H, et al. An optimized analog of antimicrobial peptide Jelleine-1 shows enhanced antimicrobial activity against multidrug resistant P. aeruginosa and negligible toxicity in vitro and in vivo. European Journal of Medicinal Chemistry. 2021;219:113433.

10. Douglas EJ, Wulandari SW, Lovell SD, Laabei M. Novel antimicrobial strategies to treat multi-drug resistant Staphylococcus aureus infections. Microbial biotechnology. 2023;16(7):1456-74.

11. Giurazza R, Mazza MC, Andini R, Sansone P, Pace MC, Durante-Mangoni E. Emerging treatment options for multi-drug-resistant bacterial infections. Life. 2021;11(6):519.

12. Ladha G, Jeevaratnam K. A novel antibacterial compound produced by Lactobacillus plantarum LJR13 isolated from rumen liquor of goat effectively controls multi-drug resistant human pathogens. Microbiological Research. 2020;241:126563.

13. Jangra M, Kaur, M., & Nandanwar, H. . In-vitro studies on a natural lantibiotic, paenibacillin: A new-generation antibacterial drug candidate to overcome multi-drug resistance. International journal of antimicrobial agents, . 2019;53(6):838-43

14. Jones F, Hu Y, Coates A. The efficacy of using combination therapy against multi-drug and extensively drug-resistant Pseudomonas aeruginosa in clinical settings. Antibiotics. 2022;11(3):323.

15. Khan AA, Manzoor KN, Sultan A, Saeed M, Rafique M, Noushad S, et al. Pulling the brakes on fast and furious multiple drug-resistant (MDR) bacteria. International Journal of Molecular Sciences. 2021;22(2):859.

16. Lee H-R, You D-g, Kim HK, Sohn JW, Kim MJ, Park JK, et al. Romo1-derived antimicrobial peptide is a new antimicrobial agent against multidrug-resistant bacteria in a murine model of sepsis. MBio. 2020;11(2):10.1128/mbio. 03258-19.

17. Matlock A, Garcia JA, Moussavi K, Long B, Liang SY-T. Advances in novel antibiotics to treat multidrug-resistant gram-negative bacterial infections. Internal and Emergency Medicine. 2021;16(8):2231-41.

18. Mohamed DS, Abd El-Baky RM, Sandle T, Mandour SA, Ahmed EF. Antimicrobial activity of silver-treated bacteria against other multi-drug resistant pathogens in their environment. Antibiotics. 2020;9(4):181.

19. Oh JH, Park J, Park Y. Anti-biofilm and anti-inflammatory effects of Lycosin-II isolated from spiders against multi-drug resistant bacteria. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2022;1864(1):183769.

20. Pontes JTCd, Toledo Borges AB, Roque-Borda CA, Pavan FR. Antimicrobial peptides as an alternative for the eradication of bacterial biofilms of multi-drug resistant bacteria. Pharmaceutics. 2022;14(3):642.

21. Provenzani A, Hospodar A, Meyer A, Leonardi Vinci D, Hwang E, Butrus C, et al. Multidrug-resistant gram-negative organisms: a review of recently approved antibiotics and novel pipeline agents. International Journal of Clinical Pharmacy. 2020;42:1016-25.

22. David A, Park, S., Parang, K., & Tiwari, R. . Antibiotics-Peptide Conjugates Against Multidrug-resistant Bacterial Pathogens. Current topics in medicinal chemistry. 2019;18(22):1926-36