Thymoquinone Derived From Black Seed: Cytotoxicity and Inhibitory Effect Against MRSA and MSSA Infections

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ABSTRACT

Staphylococcus aureus (S. aureus) is known for its ability to colonize human body, with approximately 30% of individuals harbouring it asymptomatically. While it can cause benign skin infections, S. aureus can lead to severe diseases such as hospital-acquired pneumonia (HAP) and bacteremia, especially when methicillin-resistant strains (MRSA) are involved. MRSA has become a major challenge in healthcare setting due to its virulence and extensive antibiotic resistance, complicating treatment options and increasing patient morbidity and mortality. The mecA gene, which encodes for PBP2a, contributes to this resistance, leading to poor efficacy of most beta-lactam antibiotics. This phenomenon has created an urgent need for alternative therapies to combat resistant strains. Drug repositioning has emerged as a promising strategy, utilizing existing compounds for novel therapeutic applications. Thymoquinone (TQ), a bioactive compound derived from Nigella sativa (black seed), displays antimicrobial properties against various pathogens, potentially including MRSA. This study aims for the first time to investigate the differential effects of TQ on MRSA and methicillin-sensitive S. aureus (MSSA), focusing on its antibacterial activity, resistance profiles, and toxicity. A total of 40 isolates (20 MRSA and 20 MSSA) were analyzed using standardized methods. Preliminary results indicate a statistically significant (p value 0.0002) difference in MIC values between MRSA and MSSA, with MRSA exhibiting higher resistance. The cytotoxicity of TQ, evaluated with human breast cancer cells, demonstrates a direct correlation with TQ concentration. Overall, the findings of this study provided insights into TQ's therapeutic potential, establishing a foundation for developing effective strategies against MRSA and contributing to the ongoing fight against antibiotic resistance.

Keywords: Thymoquinone , AMR, MRSA, MSSA, Hospital-acquired pneumonia

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive, catalase-, and coagulase- positive bacterium known for its remarkable ability to colonize and infect human body[1,2]. Its virulence is attributed to a plethora of factors, including Protein A (which binds immunoglobulin G and evades immune detection), various toxins (such as alpha-toxin and Panton-Valentine leukocidin), a suite of enzymes (like coagulase and proteases), and adhesion factors (such as fibronectin-binding proteins) that facilitate tissue adherence and biofilm formation[3-5]. These mechanisms allow *S. aureus* to thrive as both a commensal organism and a potent pathogen[6,7]. Approximately 30% of individuals are colonized with *S. aureus*, most commonly in the nose, axilla, and perineum[6,8]. While asymptomatic carriage bacterial infection is frequent, the bacterium can cause a range of diseases, from superficial skin infections to life-threatening conditions such as bacteremia, pneumonia, and infective endocarditis[9].

HAP is defined as pneumonia occurring 48 hours or more after hospital admission, not present at the time of admission[10]. It is a major healthcare-associated infection, particularly in critically ill or mechanically ventilated patients[11]. Symptoms include fever, cough, purulent sputum production, dyspnea, and respiratory distress[10]. Complications such as sepsis, acute respiratory distress syndrome (ARDS), and multi-organ failure often accompany severe cases, especially when caused by methicillin-resistant *Staphylococcus aureus* (MRSA)[11,12]. MRSA has emerged as a dominant pathogen in HAP due to its virulence and extensive antibiotic resistance[13]. Risk factors include prolonged hospital stays, invasive procedures, broad-spectrum antibiotic use, and underlying immunosuppression[14,15]. MRSA's presence in healthcare settings poses significant challenges, as it increases morbidity, length of hospitalization, and mortality[16].

The history of MRSA resistance reflects the ongoing battle between antibiotic innovation and bacterial adaptation[13]. *S. aureus* rapidly developed resistance to penicillin through beta-lactamase production[17,18]. This prompted the introduction of methicillin into the market, which was quickly undermined by the emergence of MRSA strains harbouring the *mecA* gene[15,18]. This gene encodes penicillin-binding protein 2a (PBP2a), conferring resistance to virtually all beta-lactam antibiotics[19]. Currently, the therapeutic alternatives for MRSA are restricted to a few medications, namely vancomycin, daptomycin, and linezolid[20]. However, each of these has its own drawbacks. Vancomycin's nephrotoxicity and poor tissue penetration, daptomycin's inactivation in lung surfactant, and linezolid's potential for hematologic toxicity highlight the critical need for alternative therapies[1,2].

The development of antibiotics is a costly and time-consuming endeavour, often requiring billions of dollars and decades of research[22]. Despite these efforts, bacterial resistance has frequently outpaced drug development[23]. The rapid emergence of resistance has left pharmaceutical companies disillusioned, as the therapeutic lifespan of new antibiotics is increasingly short-lived[24,25]. This challenge underscores the urgent need for novel and cost-effective solutions to combat resistant pathogens like MRSA[25].

Drug repositioning has emerged as a promising strategy for addressing antimicrobial resistance[26]. This approach leverages existing drugs or compounds for new therapeutic uses, offering a faster, more cost-effective alternative to de novo drug development[27].Among natural compounds, TQ—a bioactive compound derived from *Nigella sativa* (black seed)— known for its antimicrobial properties[28]. TQ demonstrates bactericidal activity through mechanisms such as membrane disruption, reactive oxygen species (ROS) generation, and inhibition of bacterial enzyme systems[29,30].

To date, no comprehensive study has explored the differential effects of TQ on MRSA and methicillin-sensitive *S. aureus* (MSSA). This is a critical gap, as MRSA's distinct resistance mechanisms could influence its susceptibility to TQ. Furthermore, evaluating the toxicity profile of TQ is essential to assess its safety for potential clinical application. By investigating TQ antibacterial activity, resistance profiles, and toxicity, this study aims to provide valuable insights into its therapeutic potential. Such research could pave the way for innovative strategies to combat MRSA and other resistant pathogens effectively.

MATERIAL AND METHODS

The TQ employed in this study was purchased from Sigma-Aldrich (USA). For the preparation of the TQ solution, a precisely measured amount of the compound was carefully weighed using an analytical balance to ensure the accuracy of the calculations. The TQ was then suspended in dimethyl sulfoxide (DMSO) (5% DMSO), which was chosen as the solvent due to its compatibility and solubility properties. The concentration of the stock solution was carefully created based on the equation C1V1=C2V2C, where C1V1 represents the concentration and volume of the stock solution and C2V2 represents the required concentration and volume of the working solution. All preparations were carried out under sterile conditions to avoid contamination and ensure the integrity of the study.

Bacterial isolates: This study targeted the in-depth study of *S. aureus* isolates to investigate the differences between resistant and susceptible strains. A group of 40 isolates were examined, including 20 MRSA and 20 MSSA, to allow a direct comparison of their characteristics. The bacterial isolates were obtained from the microbiology culture collections housed in the clinical and molecular biology laboratories at King Abdulaziz University Hospital (KAUH) in Jeddah, Saudi Arabia. These facilities ensure the highest standards for the collection, identification and storage of clinically relevant bacterial pathogens.

After collection, the *S. aureus* isolates were maintained in sterile glycerol stocks and stored at -80 °C to preserve their viability and genetic integrity over extended periods of time. Prior to commencing the experiments, the frozen isolates were carefully thawed and subcultured onto sheep blood agar plates supplied by the Saudi Prepared Media Laboratory Company. This step ensured that the bacteria were revived and active and provided optimal growth and experimental conditions. The cultures were incubated under aerobic conditions at a temperature of 35-37°C for approximately 20 hours.

Ethical statement: The framework for this study was stringently upheld throughout the research. Sample collection and research activities (Sep 2024) adhered closely to the guidelines established by the Ethics and Research Committee at King Abdulaziz University (Reference No. 301-24), these procedures were consistent with the principles of the Declaration of Helsinki, which prioritizes ethical responsibility and the protection of participants' rights and privacy. Since the clinical isolates utilized in this study were collected during routine diagnostic processes at the hospital, the Ethics Committee approved a waiver for informed consent. This waiver was issued because no additional patient samples or interactions were necessary, thereby reducing ethical concerns and allowing the study to proceed efficiently. The careful approach taken in handling, cultivating, identifying the isolates, and ensuring ethical oversight highlights the study's dedication to both scientific integrity and ethical accountability.

Microbial identification of bacterial isolates: The Molecular and Clinical Microbiology Laboratory at KAUH processed all samples, both aerobic, using the BACT/Alert VIRTUO automated microbial

detection system (BioMérieux, Durham, NC, USA), which provides real-time results. These samples were stored in an incubator until a signal-positive alarm was triggered or a maximum of five days had passed. Positive samples were subjected to Gram staining, and the results were recorded in the system. BioFire (Biomerieux Inc., Durham, NC, USA) was utilized for the early detection and treatment of sepsis using molecular technology, specifically the BioFire Blood Culture Identification 2 (BCID2) panel, which can identify pathogens and antimicrobial resistance genes in positive blood cultures. This device can detect both *S. aureus* and resistant genes (mecA/mecC) within about one hour of positive blood culture results. Positive blood cultures were grown on 5% sheep blood agar (provided by Saudi Prepared Media Laboratories, Riyadh, Saudi Arabia). The blood agar and chocolate agar plates were placed in an incubator at 35-37 °C.

Confirmatory and screening tests for MRSA: The GeneXpert system (Cepheid; Sunnyvale, CA, USA) is a fast detection device that employs real-time polymerase chain reaction (PCR) and provides results within two hours. The specificity and sensitivity of GeneXpert MRSA were tested with a nasal or skin swab and a GeneXpert MRSA ID kit at 99% and 100%, respectively. The nasal swabs were prepared across mannitol salt agar with 4µg/ml oxacillin (Saudi Prepared Media Laboratories) to isolate MRSA. Isolates were subjected to antibiotic susceptibility using either the disk diffusion test or the VITEK-2 identification system.

Microdilution Assay: In this study, the broth microdilution method was employed to ascertain the MIC of the compounds tested. This widely recognized technique is lauded for its accuracy and reproducibility in assessing antimicrobial susceptibility. The process commenced with the preparation of a stock solution at a concentration of 512 micrograms per milliliter (µg/mL), chosen based on the known resistance profiles of *S. aureus*, which frequently exhibits elevated MICs to various antimicrobial agents. This concentration was meticulously calculated using the formula C1V1=C2V2, ensuring precise preparation by accurately weighing the compound and dissolving it in an appropriate solvent under sterile conditions. Such rigorous calculation was crucial for minimizing errors and guaranteeing consistent results.

The MIC assay utilized Mueller-Hinton Broth (MHB) as the growth medium, providing optimal conditions for bacterial proliferation and result reproducibility. Following the preparation of the stock solution, serial two-fold dilutions were conducted in a 96-well microtiter plate (Italy) to generate a gradient of decreasing concentrations. By transferring a defined volume of the stock solution to successive wells containing sterile broth medium, the concentration was halved at each step. This systematic dilution approach covered a wide range of concentrations, ensuring accurate identification of the MIC, even if the compound exhibited unexpected potency or resistance[31,32].

Following the serial dilution, a standardized bacterial inoculum, manufactured in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations, was put into each well. Each well comprised a total volume of 100 µL, consisting of 95 µL of broth and 5 µL of bacterial suspension, calibrated to a turbidity of 0.5 McFarland standards, approximately corresponding to 10^8 CFU/mL. The suspension was then diluted in the broth to attain the necessary inoculum density. The use of a consistent inoculum ensured consistency across wells, minimizing variability and improving result reliability.

The microtiter plates were covered and incubated at 35−37°C for 18-20 hours under aerobic conditions. After incubation, the MIC was assessed by visually evaluating the wells to identify the lowest concentration where no visible bacterial growth occurred, defining this point as the MIC—the minimum concentration needed to effectively inhibit bacterial growth.

To validate the MIC determination, results were compared against positive control wells, which contained bacteria without any test compound, and negative control wells with only the broth medium. The positive controls showed expected bacterial growth, confirming inoculum viability, while the negative controls verified the sterility of the broth and reagents used. All experiments, including the MIC determination and subsequent analyses, were performed in triplicate. Average values from the three independent replicates were calculated to ensure the reliability and reproducibility of the results. MIC was defined as lowest concentration of the antimicrobial agent can inhibit the growth of the bacteria[1].

Cytotoxicity Assessment: The cytotoxicity of TQ was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Solarbio-China). MRC7 is a human breast carcinoma cells were cultured in a 96-well plate. The cells were treated with increasing concentrations of TQ (16,32,64 µg/ml) and incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2. After the incubation period, the treatment media was carefully removed, and the cells were washed once with phosphate-buffered saline (PBS). MTT solution, prepared at a final concentration of 0.5 mg/ml. The cells were then incubated for an additional 3-4 hours at 37°C to allow for MTT reduction. After the incubation, the media was removed, and the resulting formazan crystals were solubilized using dimethyl sulfoxide (DMSO). The absorbance was measured at 590 nm using a microplate reader (BioTek 800 TS Absorbance Reader-United States) to quantify cell viability[33,34].

Statistical analysis: The collected data were first evaluated for completeness and consistency to ensure reliability. The normal distribution of data for both sensitive and resistant strains was assessed through skewness, kurtosis, outlier analysis, and the Shapiro-Wilk test, confirming normality ($p > 0.05$ for all groups). Differences between MSSA and MRSA clinical strains were analyzed using the t-test to compare means and determine statistical significance. A one-way ANOVA was also performed to assess differences among groups, with post-hoc comparisons to identify pairwise differences. Statistical significance was defined as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. All analyses were conducted using GraphPad Prism software (version 8).

RESULTS

Thymoquinone MIC Values against MSSA and MRSA

The antimicrobial susceptibility profiles demonstrated notable disparities between the MRSA and MSSA strains. Of the 20 MRSA isolates, a significant quantity exhibited a range of MIC values; 8 isolates had a MIC of 32 µg/mL, whereas 8 isolates displayed MIC of 16 µg/mL. Furthermore, two isolates had an elevated MIC of 64 µg/mL. Furthermore, two isolates had a heightened MIC of 128 µg/mL, signifying the presence of strong resistance mechanisms in these bacteria.

The 20 MSSA isolates displayed significantly (p value 0.0002) reduced MIC values, with 12 isolates at 8 µg/mL and 8 isolates at 4 µg/mL, indicating a high level of susceptibility to the investigated antimicrobial agent (Table 1). This considerable disparity underscores the diversity among resistant MRSA strains in contrast to the consistent susceptibility profile of the MSSA group.

Table 1. The antimicrobial susceptibility profiles of MRSA and MSSA strains, including the percentage distribution of the isolates based on their MIC values

Strain Type	MIC Value (µg/mL)	Number of Isolates	Percentage (%)
MRSA	32	8	40%
	16	8	40%
	64	2	10%
	128	2	10%
MSSA	8	12	60%
	4	8	40%

Effects of TQ on MRC-7 Breast Cancer Cell Viability

The results of the experiment are illustrated in Figure 1, which demonstrates the percentage viability of MRC-7 breast cancer cells following treatment with increasing concentrations of TQ (16 µg/ml, 32 µg/ml, and 64 µg/ml), compared to the untreated control group. The y-axis represents cell viability (%) while the x-axis represents the treatment groups.

The control group exhibited 100% cell viability, serving as the baseline for comparison. Upon treatment with 16 µg/ml TQ, a slight but statistically significant reduction in cell viability was observed compared to the control group ($p < 0.05$). This indicates mild cytotoxicity at this concentration. Treatment with 32 µg/ml TQ resulted in a marked reduction in cell viability, which was statistically highly significant ($p < 0.0001$). Finally, treatment with 64 µg/ml TQ caused the most pronounced cytotoxic effect, with cell viability dropping to its lowest observed level. The differences between the 64 µg/ml treatment group and all other groups were statistically highly significant ($p < 0.0001$).

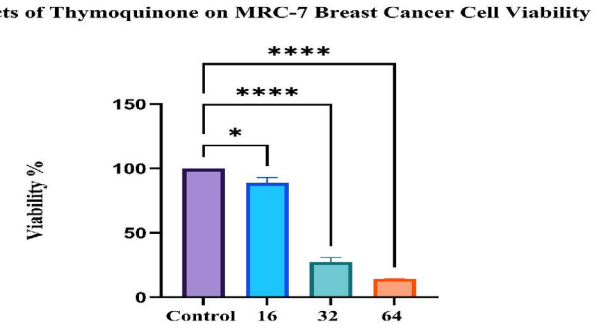

Figure 1: Effect of TQ on MRC-7 breast cancer cell viability.

Cell viability was measured as a percentage relative to the untreated control group (set to 100%). Cells were treated with TQ at 16 µg/ml, 32 µg/ml, and 64 µg/ml. Statistical analysis was performed using one-way ANOVA followed by post hoc pairwise comparisons. Statistical significance is denoted as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. Error bars represent the mean ± SEM of triplicate experiments.

DISCUSSION

The antimicrobial susceptibility profiles reveal a significant disparity between MRSA and MSSA strains. The data indicates that the MRSA strains exhibited higher MIC values, with 40% of the isolates demonstrating a MIC value of 32 µg/mL. This level of resistance is concerning, especially given that 10% of the MRSA isolates recorded MIC values as high as 128 µg/mL. In contrast, MSSA strains showed MIC values range from 4 µg/mL to 8 µg/mL, emphasizes the presence of robust resistance mechanisms in MRSA, such as efflux pumps and enzymatic degradation pathways. This fundamental difference in antimicrobial susceptibility underscores the need for healthcare systems to continuously monitor the prevalence and resistance profiles of these pathogens to ensure effective treatment outcomes. Moreover, the results indicate that elevated MIC values in MRSA may result from mechanisms including *mecA* expression, whereas the reduced MIC values in MSSA reflect an absence of this gene as resistance mechanism, rendering them more susceptible to antimicrobial therapy. These results underscore the necessity for continuous monitoring of antibiotic susceptibility trends in these therapeutically important diseases.

The findings from this study highlight a significant knowledge gap in understanding the underlying mechanisms driving the high resistance observed in MRSA. Unlike MSSA, whose susceptibility to antibiotics allows for a wider range of treatment options, MRSA's resistance complicates clinical management[35]. This difference not only affects treatment strategies but also stresses the importance of timely and accurate identification of these strains in diagnostic laboratories[36]. Furthermore, given the increasing prevalence of MRSA in clinical settings, the need for ongoing surveillance of these antimicrobial susceptibility patterns is more pressing than ever[37]. This research contributes to the existing body of knowledge, providing insights into the resistance mechanisms while pointing to a significant clinical challenge in effectively managing MRSA infections[38]. Moreover, the significant differences (p value 0.0002) in antimicrobial susceptibility and resistance mechanisms between MRSA and MSSA highlight the importance of accurate identification and appropriate treatment strategies in clinical settings. Monitoring these differences is crucial for effective management of infections caused by these pathogens.

When examining the efficacy of TQ against both sensitive and resistant strains in this study, preliminary findings indicates a notable difference in its effectiveness, paralleling the variations seen in antimicrobial susceptibility profiles. In the present study, TQ has demonstrated promising antimicrobial properties, suggesting it could serve as a potential treatment against MSSA strains. However, resistance profiles like those observed for traditional antibiotics could emerge in the MRSA strains, thereby presenting a novel area for future research[39]. This aspect could be pivotal since understanding TQ's mechanism of action against resistant strains could provide new strategies for combating resistant pathogens and filling a gap in current therapeutics. Similar patterns were observed in the *Pseudomonas aeruginosa* isolates (not published), which also displayed a significant disparity between resistant and sensitive strains in terms of antimicrobial susceptibility. The consistent finding of high MIC values in resistant *P. aeruginosa* strains indicates the presence of similar resistance mechanisms, such as biofilm formation and the production of beta-lactamases. These parallel results across MRSA and *P. aeruginosa* underscore a broader concern regarding antibiotic resistance among clinically relevant pathogens. Such consistency in resistance profiles further emphasizes the urgent need for healthcare providers to adopt targeted treatment strategies based on accurate microbial identification and susceptibility testing.

The TQ on MRC-7 breast cancer cell viability demonstrate a clear concentration-dependent cytotoxic effect, as evidenced by the experimental results in this study. The data illustrated in Figure 1 indicate that while the control group maintained full cell viability at 100%, even the lowest tested concentration of TQ at 16 µg/mL produced a statistically significant reduction in cell viability. This initial decrease suggests that TQ may initiate cellular mechanisms that compromise viability, although additional studies are needed to elucidate the underlying pathways involved in this early response. As

the concentration of TQ increased to 32 µg/mL, the reduction in MRC-7 cell viability became markedly pronounced,. This indicates that as TQ concentration rises, its cytotoxic effects become increasingly effective against breast cancer cells. The findings suggest that TQ may disrupt critical cellular processes, leading to altered growth dynamics and enhanced cell death at this dosage. Importantly, at the highest concentration tested, 64 µg/mL, the cytotoxic effect was maximized, resulting in the lowest observed cell viability, which again was statistically significant in comparison to all other treatment groups. This indicates a potent ability of TQ to induce cell death, making it a compelling subject for further investigation in therapeutic applications against breast cancer. Considering these findings, the significant cytotoxic effects observed with TQ raise the possibility of exploring its synergistic potential in combination with conventional antibiotics or chemotherapeutic agents. Synergistic interactions could allow for reduced dosages of both TQ and the partner drug, potentially minimizing side effects while enhancing overall therapeutic efficacy. The idea of combining TQ with other treatment protocols could pave the way for novel treatment strategies aimed at increasing patient response rates in breast cancer therapies. Exploration of such combinations may improve treatment outcomes while capitalizing on the unique mechanisms of action offered by TQ as it targets cancer cells.

Overall, the results of this study contribute valuable insights into the potential application of TQ, particularly in enhancing the effectiveness of existing treatment regimens. The compelling evidence pointing to its cytotoxicity against MRC-7 breast cancer cells highlights the need for further research to fully characterize its mechanisms of action and its potential role in multi-modal treatment approaches. Building on this, investigations into the benefits of TQ combined with other therapeutic agents could significantly impact clinical practice, offering new hope in the fight against this formidable disease.

The antimicrobial effect of TQ observed in this study can be attributed to several key mechanisms that collectively contribute to its effectiveness against bacterial pathogens. Initially, it is suggested that TQ possesses the capability to irreversibly alter bacterial morphology[29]. This morphological change can lead to structural weaknesses in the bacterial cell wall or membrane, ultimately compromising the overall integrity of the bacterial cells[2]. One of the primary ways in which TQ exerts its antimicrobial activity is by disrupting the integrity of the bacterial cell membrane[40]. The cell membrane plays a crucial role in maintaining the homeostasis of the cell, controlling the passage of ions and other molecules in and out of the cell[41]. When TQ disrupts this membrane integrity, it creates pores or destabilizes the membrane structure, leading to an uncontrolled influx of substances that the cell normally regulates[42]. This disruption can result in a loss of essential ions and other critical cellular components, ultimately compromising cellular function and viability[43]. Moreover, the treatment with TQ induces protein leakage from the bacterial cells, which can have detrimental effects on cellular metabolism[44]. By causing protein leakage, TQ may not only lead to a decrease in the concentration of essential proteins within the cell but may also result in the loss of vital cellular functions that are critical for bacterial survival and proliferation[45]. Additionally, TQ is believed to interfere with intracellular protein functions, potentially altering the normal metabolic pathways within the bacteria. This interference could disrupt key physiological processes vital for bacterial growth and replication, thereby enhancing the antimicrobial effect of TQ[46]. Given these speculative mechanisms of action, it is evident that TQ may operate through a multifaceted approach that synergistically compromises bacterial physiology at various levels. Despite the substantial evidence supporting these hypotheses regarding TQ's antibacterial mechanisms, further investigations are warranted to obtain a clearer and more detailed understanding of its precise mode of action. Future studies could incorporate advanced imaging techniques, molecular biology approaches, and biochemical assays to elucidate the specific cellular targets and pathways affected by TQ. This deeper insight will not only enhance our comprehension of TQ's antimicrobial properties but also potentially lead to the development of novel antibacterial therapeutics based on its mechanisms of action, providing valuable alternatives in the ongoing battle against antibiotic-resistant bacteria.

Additionally, evaluating the efficacy of the compounds against other pathogens, such vancomycin resistant *enterococcus* (VRE), *Klebsiella pneumonia* is important for understanding their antimicrobial spectrum. Time-kill assays can provide detailed insights into the drugs' bactericidal activity over time, while examining their effects on biofilm formation will offer valuable information on their potential use against chronic, biofilm-associated infections[47]. Moreover, it is vital to investigate the potential for resistance development against the new drugs[48]. Understanding the mechanisms through which bacteria might gain resistance will inform strategies to mitigate this risk, such as combining the drug with other agents or adjusting treatment regimens. This comprehensive approach will enhance the therapeutic application of these compounds and support their successful integration into clinical practice. The time kill assay will be instrumental in assessing the bactericidal effect of TQ, providing valuable insights into its killing kinetics against various pathogens. Additionally, the *in vivo* model will be crucial for evaluating the efficacy, pharmacokinetics, and pharmacodynamics of TQ, helping to further understand its therapeutic potential and behavior in a biological system. This comprehensive approach will enhance the translational potential of TQ in treating infections.

While this study provides valuable insights into the antibacterial activity of TQ against MRSA and MSSA, several limitations should be acknowledged. First, the study's scope was limited to *in vitro* analyses using only 40 isolates, which may not fully represent the diverse genetic and phenotypic variability of MRSA and MSSA strains encountered in clinical settings. Second, although the cytotoxicity of TQ was assessed using human breast cancer cells, its effects on other human cell types or tissues remain unexplored, limiting the broader understanding of its safety profile. Additionally, the study primarily focused on MIC values and did not evaluate the dynamic pharmacokinetics and pharmacodynamics of TQ, which are crucial for understanding its clinical efficacy. Lastly, the potential for resistance development during prolonged exposure to TQ was not investigated, which is an important consideration for its therapeutic application. Further studies, including in vivo models, are necessary to validate these findings and assess the broader applicability of TQ in clinical settings.

CONCLUSION

In summary, the demonstrated differences in susceptibility profiles of MRSA and MSSA not only reveal the critical challenges posed by antibiotic resistance but also open doors for investigating new therapeutic agents like TQ against these pathogens. The current study is aligned with past research that has highlighted similar trends, though these specific findings regarding TQ's efficacy against resistant strains remain unpublished. Thus, prioritizing such investigations could lead to valuable contributions to the field of antimicrobial resistance, identifying viable treatment options that could complement existing therapies and potentially revolutionize treatment protocols in the face of rising antibiotic resistance.

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Competing Interest: None

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