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Original Article

Characterizing the antimicrobial activity of cisplatin against Gram-positive clinical strains: bridging existing gaps

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Abstract

Introduction: Multidrug resistant (MDR) bacteria are one of the most important current threats to public health. Therefore, serious concerns were raised about the effectiveness of traditional antibiotics. Cisplatin, a chemotherapeutic agent that is used mainly in cancer treatment, has recently attracted attention and popularity for its potential antibacterial properties. Our study aim is to evaluate the antibacterial properties of cisplatin against several common MDR and non-MDR Gram-positive strains, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), Methicillin-Susceptible *Staphylococcus aureus* (MSSA), Coagulase-Negative *Staphylococcus* (CoNS), and Vancomycin-Resistant *Enterococcus* (VRE).

Methodology: Cisplatin's Minimum Inhibitory Concentration (MIC) was calculated through standard microdilution methods.

Results: Our findings indicated that cisplatin had a minimum inhibitory concentration (MIC) of 256 µg/mL across all tested bacterial strains, reflecting its limited antibacterial efficacy in its currently available formulation. However, although the MIC values were high, this study explored cisplatin's activity against these bacterial strains which uncovers a potential gap about cisplatin clinical effectiveness and indicates that further enhancement is required.

Conclusions: These results confirm that cisplatin antibacterial activity has not been extensively explored against these resistant strains. Cisplatin alone shows limited efficacy based on the relatively high MIC values observed. However, combination therapy with other antibiotics may improve its therapeutic potential. Additionally, novel delivery systems—nanoparticle formulations, for instance—could enhance antibacterial activity while reducing toxicity. Future investigations should evaluate these approaches in more detail.

Key words: Cisplatin; Multidrug-resistant bacteria; MIC; MRSA; VRE.

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Introduction

Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi [1]. In April 2014, the World Health Organization announced that this serious threat is no longer a future prediction; it is happening right now in every region of the world [2]. Without urgent, coordinated action by many stakeholders, the world is headed for a post-antibiotic era, in which common infections and minor injuries that have been treatable for decades can once again kill [3]. In 2014, the Review on Antimicrobial Resistance estimated that antimicrobial resistance could cause 10 million deaths

a year by 2050, surpassing cancer [4,5]. Data show that AMR is spreading globally at different rates, outpacing all efforts to mitigate this crisis [6]. The rapidity of new antibiotics discovered appears to be outpacing the spread of antibiotic resistance, which has reversed the emergence and spread of antimicrobial resistance against antibacterial compounds are now outpacing the discovery and development of new antibiotics [3,7]. Without harmonized and immediate action on a global scale, the world is heading towards a post-antibiotic era in which common infections could once again kill [3,8].

Staphylococcus aureus causes a spectrum of diseases from simple skin and soft tissue infections (SSTIs) to more serious conditions such as endocarditis, osteomyelitis, bacteremia, meningitis, and pneumonia [4,5]. Methicillin-resistant *S. aureus* (MRSA) exemplifies how rapidly bacterial pathogens can acquire multidrug resistance. *Staphylococcus aureus* produces the PC1 β-lactamase, the product of the *blaZ* gene, which was the original mechanism of resistance to β-lactam antibiotics in this organism. β-lactamase hydrolyzes the β-lactam ring, rendering it inactive. Methicillin-resistant S. aureus has also acquired the *mecA* gene, which encodes PBP2a, enabling the bacteria to sustain cell-wall synthesis when other PBPs are inhibited by β-lactam antibiotics [5]. The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), which has a low affinity for β-lactam antibiotics [6,7].

CoNS colonize human skin and mucosal membranes, which is why they are considered harmless commensal bacteria [8-10]. CoNS act as opportunistic pathogens causing nosocomial infections among immunocompromised, immunosuppressed, long-term hospitalized, and critically ill patients. The use of various medical devices, including indwelling vascular catheters, cardiac pacemakers, prosthetic heart valves, chronic ambulatory peritoneal dialysis catheters, and prosthetic joints, has been accompanied by the ability of microorganisms to adhere to these devices [8-10]. Skin flora such as staphylococcal species, including *Staphylococcus aureus* and coagulase-negative *staphylococci* are the most common organisms to create biofilms on central venous catheters, prosthetic joints or orthopedic hardware, and prosthetic heart valves [8-10]. Bacteria containing *mecA* continue to synthesize cell wall as a result of the cooperation between transpeptidase domain of the PBP2A and the transglycosylase domain of the native staphylococcal PBP2. β-lactamase, which is encoded by a *blaZ* gene, hydrolyses only penicillin-rings, hence conferring narrow-spectrum β-lactam resistance [11-14].

Enterococci are common residents of the human gastrointestinal tract and the female genitourinary tract and act as opportunistic pathogens in severely ill patients. Glycopeptide-resistant *enterococci* (GRE), particularly *Enterococcus faecium*, have spread as multi-resistant opportunistic pathogens in hospitals worldwide. Vancomycin resistance in *E. faecalis* and *E. faecium* has been subdivided into phenotypes, *VanA* and *VanB* [15,16]. Glycopeptides inhibit bacterial cell wall synthesis by binding to the D-Ala-D-Ala dipeptide terminus of the peptidoglycan (PG) precursors, sequestering the substrate from transpeptidation and transglycosylation reactions [17]. The D-Ala-D-Ala complex with glycopeptides is stabilized by an array of hydrophobic van der Waals contacts and five hydrogen bonds lining the antibiotic binding pocket [17]. The production of modified peptidoglycan precursors ending in D-Ala-D-Lac or D-Ala-D-Ser associated with the elimination of D-Ala-D-Ala-ending precursors is responsible for resistance [15-17]. This replacement leads to diminished binding affinity of glycopeptides for their targets (1,000-fold lower and 7-fold lower for D-Ala-D-Lac and D-Ala-D-Ser precursors, respectively) [17]. Linezolid resistance was rare among *E. faecalis* (1/364, 0.3%) and *E. faecium* (2/344, 0.6%) [18]. However, linezolid-resistant VRE isolates have been reported [18].

The global spread of microbial resistance coupled with high costs and slow pace in the discovery of a new antibiotic have made drug repositioning an attractive and promising alternative in the treatment of infections caused by multidrug resistant (MDR) microorganisms [1,19]. Recently, drug repurposing has gained more attention as an alternative strategy to identify new antimicrobial agents [20]. There are several advantages to repurposing old drugs with known safety and pharmacokinetic profiles over de novo drug discovery. Examples are reductions in time, cost, and risks associated with the development of novel antibiotics. Several approved drugs for different ailments have been successfully repurposed as anti-infective agents [21,22]. The known safety of approved drugs minimizes the possibility of failure for adverse toxicology, making them attractive de-risked compounds for new applications with potentially lower overall development costs and shorter development timelines [21,22].

The chemotherapeutic agent cisplatin creates DNA crosslinks in the body. There is emerging evidence that it also exhibits antibacterial activity and can inhibit bacterial persister cells [23]. However, no research has been performed to evaluate its effectiveness in combating clinical strains of MRSA. Furthermore,

cisplatin has not been adequately studied for its effects on MSSA, CoNS, and VRE. In spite of efforts to develop new antimicrobials, Gram-positive pathogens such as S. *aureus,* CoNS*, and Enterococcus spp.* The challenges remain substantial. However, cisplatin's antibacterial efficacy against MRSA, MSSA, CoNS, and VRE remains poorly investigated despite its well-recognized anticancer properties. To our knowledge, no comprehensive in vitro assessment has yet compared cisplatin's antimicrobial activity across these clinically relevant isolates. This study intends to determine the antibacterial potential of cisplatin against Gram-positive clinical strains to fill this gap.

Methodology

Cisplatin was purchased from Sigma-Aldrich (USA). A stock solution was prepared by dissolving the compound in 5% dimethyl sulfoxide (DMSO), which was selected due to its solubility properties and compatibility with experimental conditions. Working concentrations were calculated using the standard dilution formula $C_1V_1 = C_2V_2$ to ensure accuracy. All solutions were prepared under sterile conditions to maintain experimental integrity and prevent contamination.

MRSA identification

Bacterial isolates were processed at the Molecular and Clinical Microbiology Laboratory, King Abdulaziz University Hospital (KAUH). Blood culture samples were analyzed using the BacT/Alert VIRTUO automated blood culture system (BioMérieux, USA) for the detection of microbial growth. Rapid identification of pathogens and antimicrobial resistance genes was performed using the BioFire BCID2 panel. Positive blood culture samples were subcultured onto 5% sheep blood agar plates and incubated at 35–37°C for 18–24 hours. Identification of Gram-positive cocci was carried out using the VITEK 2 system (BioMérieux, France). Confirmation of MRSA isolates was achieved by detection of the *mecA* gene using the GeneXpert system (Cepheid, USA). Antimicrobial susceptibility testing (AST) was performed using the VITEK 2 AST-GP panel (P580), which included vancomycin, linezolid, gentamicin, and rifampicin among other antibiotics [10]. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) M100 guidelines [24].

Detection of Coagulase-Negative Staphylococci (CoNS) Using the VITEK 2 System

Clinical samples suspected of harboring CoNS were processed and identified using the VITEK 2 system (BioMérieux, Marcy-l'Étoile, France). Isolates were subcultured onto blood agar plates and incubated at 35–37°C for 18–24 hours to ensure adequate growth and purity. The presence of Gram-positive cocci was confirmed by Gram staining. Species-level identification and antimicrobial susceptibility testing (AST) were subsequently performed using the VITEK 2 ID-GP and AST cards according to the manufacturer's instructions. The system employs colorimetric and fluorometric detection methods to provide automated, high-throughput identification, with results typically generated within 6–12 hours.

For antimicrobial susceptibility testing, isolates were inoculated into the AST-GP card, and minimum inhibitory concentrations (MICs) for multiple antibiotics were determined. Results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. This method facilitated rapid, accurate, and standardized identification of CoNS, thereby enabling effective antimicrobial stewardship and infection control measures.

Detection of Vancomycin-Resistant Enterococci (VRE) via VITEK 2

Identification and antibiotic susceptibility testing of VRE were performed using the VITEK 2 system (BioMérieux, Marcy-l'Étoile, France), an automated platform designed for microbial identification and susceptibility profiling. Clinical isolates suspected to be *Enterococcus* spp. were cultured on blood agar and incubated at 35–37°C for 18–24 hours. A bacterial suspension was prepared in 0.45% NaCl and adjusted to a McFarland standard of 0.5 to ensure optimal inoculum density for accurate analysis. The prepared suspension was loaded into the VITEK 2 GN or GP identification card, depending on the Gram stain result, and processed accordingly. Vancomycin resistance was assessed using the AST (Antimicrobial Susceptibility Testing) card, with results automatically interpreted based on predefined breakpoints.

The system provided minimum inhibitory concentration (MIC) values for vancomycin and other antibiotics tested. Isolates were classified as susceptible, intermediate, or resistant in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. The VITEK 2 system enabled rapid and automated detection of VRE, thereby facilitating timely infection control measures and antimicrobial stewardship [25].

MIC Assay

MICs were determined by the broth microdilution method according to CLSI guidelines. A stock solution of the test compound was prepared at 10 mg/mL and stored appropriately. Serial two-fold dilutions of the compound were prepared in 96-well microtiter plates using Mueller-Hinton broth (MHB). Bacterial inocula were prepared from overnight cultures and adjusted to a turbidity equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The inoculum was further diluted in MHB to achieve a final concentration of approximately 5×10^5 CFU/mL in each well.

The microtiter plates were incubated at 35 ± 2°C in ambient air for 18–20 hours. The MIC was defined as the lowest concentration of the compound that inhibited visible bacterial growth. Each plate included a growth control (inoculated broth without compound) and a sterility control (uninoculated broth). All experiments were performed in triplicate [26-28].

Ethical Statement

This study was conducted in strict accordance with ethical guidelines and regulations. Sample collection and research procedures were approved by the Research Ethics Committee (REC) of the Faculty of Medicine at King Abdulaziz University (Ethics Reference No. 301-24). All protocols complied with the Declaration of Helsinki, ensuring the protection of participants' rights, privacy, and confidentiality. The clinical isolates analyzed in this study were obtained as part of routine hospital diagnostic processes, without any additional risk to patients. Ethical oversight was maintained throughout, with careful handling, identification, and analysis of isolates, reinforcing the study's commitment to both scientific rigor and ethical responsibility.

Results

Antimicrobial Activity of Cisplatin Against Gram-Positive Clinical Isolates

The antimicrobial activity of cisplatin was evaluated against 132 Gram-positive clinical isolates. These included 60 MRSA, 10 MSSA, 60 CoNS and 2 VRE. Minimum inhibitory concentrations were determined by broth microdilution. Cisplatin exhibited uniform activity across all tested isolates. The MIC for MRSA isolates (n = 60) was 256 µg/mL. Similarly, the MIC for MSSA isolates (n = 10) was 256 µg/mL. CoNS isolates (n = 60) showed an MIC of 256 µg/mL. The two VRE isolates tested also demonstrated an MIC of 256 µg/mL. All MIC values are summarized in Table 1.

Discussion

This study represents the first in vitro evaluation of cisplatin against MRSA, MSSA, CoNS, and VRE. Our findings revealed that the MIC of cisplatin for these Gram-positive bacteria was 256 µg/mL, a notably high MIC compared to Gram-negative [23]. These results suggest that cisplatin requires substantially higher concentrations to inhibit the growth of Gram-positive organisms compared to Gram-negative bacteria.

Despite cisplatin exhibiting a relatively high MIC of 256 µg/mL against MRSA, MSSA, CoNS, and VRE, this study plays a crucial role in characterizing the antibacterial potential of cisplatin. By systematically evaluating cisplatin's antimicrobial properties, this research highlights the existing gap in knowledge regarding its direct efficacy against multidrug-resistant pathogens. The findings provide valuable insights into the limitations of cisplatin as a standalone antibacterial agent while simultaneously emphasizing its potential for further enhancement through drug repurposing strategies, which represent "a promising strategy in the therapy of bacterial infections.

Moreover, this study underscores the novelty of investigating cisplatin beyond its conventional role as a chemotherapeutic agent, paving the way for future strategies to improve its antimicrobial efficacy. These strategies may include combination therapy with established antibiotics to achieve synergistic effects. Antibiotic combination therapy, exploiting synergies, old-drug rejuvenation and resistance reduction could provide the solution to AMR, and when certain carefully selected drugs are combined in the correct formulation, the bactericidal effects are synergistic, with potential to kill the highly resistant bacteria, thereby lowering the required effective dose and minimizing potential toxicity [29]. Additionally, it has been suggested that the possibility of utilizing advanced

Table 1. Minimum Inhibitory Concentration (MIC) of Cisplatin Against Clinical Isolates.

Number of tested organisms	Organism	MIC (µg/mL)
60	MRSA	256
10	MSSA	256
60	CoNS	256
2	VRE	256

The table presents the MIC values (µg/mL) of cisplatin tested against various bacterial strains, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), Methicillin-Susceptible *Staphylococcus aureus* (MSSA), Coagulase-Negative Staphylococci (CoNS), and Vancomycin-Resistant *Enterococcus* (VRE). MIC values were determined using standard broth microdilution assays.

drug delivery systems. Nanoparticle drug delivery systems are designed to exploit the enhanced permeability and retention (EPR) effect, and encapsulation within polymeric NPs increases drug efficacy, specificity, and tolerability, thereby enhancing the therapeutic index [30]. By addressing these aspects, this research not only deepens understanding of cisplatin's antimicrobial activity but also lays the foundation for future developments to optimize its clinical applicability in combating resistant bacterial infections.

The relatively higher MIC of 256 µg/mL across all the tested Gram-positive bacteria (MRSA, MSSA, CoNS, and VRE) can be explained by several factors. One of the key factors is the difference in the cell wall structure between Gram-positive and Gram-negative bacteria. As described by Rohde *et al.* (2019), the peptidoglycan layer is substantially thicker in Gram-positive bacteria than in Gram-negative bacteria; the peptidoglycan forms around 40 to 90% of the cell wall's dry weight in Gram-positive bacteria but only around 10% of Gram-negative strains [31]. This thick peptidoglycan layer may limit the uptake of cisplatin. In contrast, Gram-negative bacteria possess an additional membrane layer, the outer membrane, and the major role of this membrane must usually be to serve as a permeability barrier to prevent the entry of noxious compounds [32]. Our data provide important insight into the potential limitations of cisplatin in treating Gram-positive infections and emphasize the importance of developing strategies to increase the efficacy of cisplatin against these pathogens. The observed MIC of 256 µg/mL serves as a benchmark for future studies investigating the therapeutic potential of cisplatin against Gram-positive organisms.

Despite testing cisplatin against both MRSA and MSSA, we observed no significant difference in the MIC between the two strains. This finding suggests that the resistance mechanisms typically associated with MRSA, such as altered penicillin-binding proteins (PBPs) or the presence of the *mecA* gene, do not appear to influence the efficacy of cisplatin. Consequently, this indicates that cisplatin's antimicrobial activity is not dependent on these specific mechanisms of resistance. Indeed, "despite the fact that cisplatin can bind a wide range of cellular components, including proteins, RNA, membrane phospholipids, microfilaments, and thiol-containing peptides, DNA is considered a major target for cisplatin. Furthermore, the success of cisplatin in cancer chemotherapy derives from its ability to crosslink DNA and alter DNA structure. Cisplatin appears to exert its antimicrobial actions via DNA binding or the disruption of intracellular processes [33]. These results highlight the need for further research into the precise molecular interactions that underpin cisplatin's antimicrobial properties, especially in the context of resistant strains.

We hypothesize that a higher MIC is needed to kill MRSA, CoNS, and VRE compared to Gram-negative bacteria due to differences in cell wall structure, drug permeability, and resistance mechanisms. Cisplatin exhibits a lower MIC against Gram-negative bacteria like *Escherichia coli* primarily because the outer membrane contains various protein channels, called porins, which are involved in the influx of various compounds, including several classes of antibiotics [34-36]. In contrast, Gram-positive bacteria lack an outer membrane and possess a thick peptidoglycan layer [37] demonstrated that the highly organized and tightly packed PG structure in *S. aureus* can facilitate the clogging of the PG pores, which prevents further drug penetration from reaching intracellular targets [37].

Furthermore, Gram-positive bacteria, such as S. *aureus,* may possess more robust DNA repair mechanisms, in particular nucleotide excision repair [38,39]. It may allow them to withstand cisplatin-induced DNA damage, necessitating higher drug concentrations to be bactericidal. It is also crucial to take into account resistance mechanisms. While *Pseudomonas aeruginosa* and *E. coli* have metal-ion efflux systems, cisplatin can still accumulate before being actively eliminated [40]. Because of alternative detoxification mechanisms, such as thiol-based inactivation, cisplatin may not be as potent against *S. aureus* and *Enterococcus* spp [41-42]. Gram-positive bacteria have highly cross-linked peptidoglycan layers, which may also hinder cisplatin from targeting intracellular targets [43].

Cisplatin releases reactive oxygen species (ROS), which are connected to oxidative stress response differences [44]. Gram-negative bacteria may be more vulnerable, whereas Gram-positive bacteria possess strong antioxidant defense systems, such as catalases and peroxidases, which offer enhanced protection [45].

One of the key limitations of this study is the high MIC values (256 µg/mL) observed for cisplatin against all tested Gram-positive bacterial strains (MRSA, MSSA, CoNS, and VRE), suggesting its limited standalone antibacterial efficacy, which may restrict its clinical application. Additionally, the study focused only on *in vitro* MIC determination without assessing the pharmacokinetics, toxicity, or therapeutic potential of cisplatin in an *in vivo* model, which is crucial for understanding its real-world applicability. The number

of tested MSSA (n = 10) and VRE (n = 2) isolates was relatively low, which may not fully represent the diversity of these bacterial strains, potentially limiting the generalizability of the results. Moreover, the study did not evaluate the potential synergistic effects of cisplatin in combination with other antibiotics, which could help enhance its antibacterial efficacy and overcome resistance mechanisms.

It should be noted that the MIC values observed for cisplatin (256 µg/mL) are significantly higher than therapeutically achievable concentrations, which limits its direct clinical applicability. Although we used only 5% DMSO as a solvent, previous studies have demonstrated that "DMSO reduced the activity of cisplatin. The presence of sulfur in DMSO may have partially reduced cisplatin activity, potentially affecting the results [46]. In future work, we plan to explore alternative, sulfur-free solvents to minimize this limitation and more accurately assess cisplatin's efficacy.

Cisplatin is associated with significant dose-limiting toxicities that restrict its clinical use. Indeed, the most serious and one of the more common presentations is acute kidney injury (AKI), which occurs in 20-30% of patients. Nephrotoxicity occurs in up to 70% of pediatric patients and 30–40% of adults, typically presenting as acute kidney injury characterized by elevated serum creatinine and blood urea nitrogen, proteinuria, glucosuria, cation wasting, and hypomagnesemia [47-49]. It has been demonstrated that cisplatin-mediated hearing loss essentially involves a robust generation of ROS in the cochlea, outer hair cells, spiral ganglia, stria vascularis, and the spiral ligament. Ototoxicity affects 40–60% of adults and up to 70% of children, manifesting as irreversible, bilateral, high-frequency sensorineural hearing loss, which may cause speech and language delays in children and social or cognitive impairments in adults [47-49]. These toxicities underscore the limitations of cisplatin in clinical practice.

Exploring combination therapies represents a particularly promising direction for future work. Cisplatin alone may not achieve clinically useful MICs against Gram-positive pathogens but pairing it with established antimicrobials could change this picture entirely. The logic here is straightforward: if two drugs hit different cellular targets, pathogens like MRSA and VRE—which have become notoriously difficult to treat—may be unable to mount effective resistance against both simultaneously. What makes this approach even more attractive. In addition to combination therapies, it is critical to investigate the role of biofilm formation in the resistance of Gram-positive bacteria to cisplatin. Since MRSA and VRE are known to form biofilms that act as a physical barrier to antibiotic penetration, anti-biofilm strategies could play a crucial role in enhancing the activity of cisplatin. Furthermore, a deeper understanding of how cisplatin interacts with the cell wall and intracellular structures of Gram-positive bacteria is essential. Investigating the mechanisms of resistance, such as the efflux of cisplatin by resistant strains, will provide valuable insights into how cisplatin can be used more effectively. Finally, pharmacokinetic studies in animal models and clinical trials are necessary to determine the optimal dosing regimens for cisplatin against Gram-positive organisms. This will help to identify safe and effective dosage strategies to maximize the drug's therapeutic potential, particularly for resistant strains like MRSA and VRE.

Conclusions

In conclusion, this study provides the first comprehensive *in vitro* evaluation of cisplatin against MRSA, MSSA, CoNS, and VRE, highlighting its limited antibacterial activity with an MIC of 256 µg/mL across all tested strains. Further translational research, including *in vivo* studies, combination therapy testing, biofilm targeting, and toxicity assessments, is essential before any potential clinical application can be considered.

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Conflict of interest

No conflict of interest is declared.

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