

Inhibitory potential of 1,10-phenanthroline against *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is a Gram-negative bacterium associated with hospital-acquired infections and is frequently resistant to multiple antibiotics, making the search for new therapeutic strategies necessary. As iron plays an essential role in the survival and virulence of this pathogen, this study evaluated the antimicrobial activity of 1,10-phenanthroline and ethylenediaminetetraacetic acid (EDTA) against ten clinical isolates of *A. baumannii*, the antibiofilm and cytotoxic effects of 1,10-phenanthroline in Vero cells (BCRJ 0245) and the *in silico* physicochemical and pharmacokinetic profiles of 1,10-phenanthroline, EDTA, and ciprofloxacin. The minimum inhibitory concentration (MIC) of the two chelating agents ranged from 0.85 to 223.3 µg/mL, with 1,10-phenanthroline showing the most notable results (MIC ranging from 0.85 to 13.5 µg/mL). In addition, 1,10-phenanthroline inhibited biofilm formation, with percentages ranging from 24.4% to 88.9% among the evaluated isolates, and showed no cytotoxicity in Vero cells up to the highest concentration tested (1600 µg/mL). The *in silico* evaluation demonstrated the hydrophilic nature of EDTA and the lipophilicity of 1,10-phenanthroline, indicating greater potential for absorption and interaction with bacterial membranes, in contrast to the balanced profile of ciprofloxacin. Therefore, the results indicate that 1,10-phenanthroline exhibits antimicrobial activity, encouraging further investigations into its application against *A. baumannii*.

Introduction

Since 2017, carbapenem-resistant *Acinetobacter baumannii* has remained among the pathogens considered a critical priority by the World Health Organization, with an urgent recommendation for the development of new therapeutic alternatives [1]. In addition, together with five other bacterial species, it is among the microorganisms that comprise the ESKAPE group, due to its remarkable ability to resist antibiotics and disinfection/decontamination processes, particularly in hospital settings, as well as the numerous associated virulence mechanisms. Thus, it has been associated with a high burden of morbidity and mortality, especially related to a wide range of hospital-acquired infections, and is therefore characterized as a global health problem [2].

Infections caused by multidrug-resistant Gram-negative bacteria, such as *A. baumannii*, represent a significant risk to treatment efficacy. Consequently, the limited availability of effective antibiotics makes these bacteria a serious threat, requiring more complex therapies and care, contributing to prolonged hospital stays, increased costs to healthcare systems, greater spread of infection, and high mortality rates [1].

Iron is essential for several cellular activities and functions of *A. baumannii*, such as DNA synthesis and cellular respiration, in addition to playing a crucial role in the growth and survival of this bacterium. *A. baumannii* requires iron to persist within the host and complete the different stages of pathogenesis that culminate in infection and disease development; therefore, the iron acquisition mechanism is an important virulence factor in this pathogen. This microorganism is capable of employing different strategies for the acquisition of iron, even under conditions of low availability of this nutrient, such as the synthesis of siderophores. In addition, the amount of this element also directly or indirectly influences other virulence factors, such as bacterial biofilm production [3, 4].

Biofilms are organized communities of adherent bacteria surrounded by a polymeric matrix composed of polysaccharides, proteins, and DNA that provides protection against unfavorable conditions [5]. This matrix enables the persistence of this pathogen in hospital environments and facilitates the occurrence of new waves of infection. This structure provides the bacterium with the ability to naturally withstand adverse environmental conditions, such as nutrient deprivation. In addition, this aggregate provides greater tolerance to antibacterial agents, reducing antibiotic penetration into fully formed biofilms. As a consequence, bacteria organized in biofilms are more difficult to eliminate compared with planktonic bacterial cells, favoring the emergence and increase of antimicrobial resistance [6].

Given this scenario, there is a need to develop new strategies to treat infections caused by *A. baumannii* in order to reduce the impacts of this pathogen on global health and ensure effective treatment for patients. In this context, iron-chelating compounds have been reported to exhibit antibacterial activity against Gram-negative pathogens and may act *per se* or in combination with antimicrobials [7–9]. From this perspective, the aim of this study was to evaluate the activity of two synthetic iron-chelating compounds against clinical isolates of *A. baumannii*.

Materials and Methods

Clinical isolates of *A. baumannii*: This study included 10 clinical isolates provided by the University Hospital (HUSM) of the Federal University of Santa Maria (UFSM), phenotypically characterized using the Vitek® 2 automated system (bioMérieux, Marcy l'Etoile, France) as non-fermenting Gram-negative bacilli. This study followed the principles of Resolution 466/12 of the Brazilian National Health Council regarding research involving human subjects and was approved by the Research Ethics Committee of the Federal University of Santa Maria under CAAE number 38850614.4.00005346.

Molecular identification: Genomic DNA extraction was performed by thermal lysis in Tris-EDTA buffer (pH 8), followed by molecular identification through amplification of the *bla*_{OXA-51-like} gene, as described by Turton *et al.* [10], using the Forward (5'-TAA TGC TTT GAT CGG CCT TTG-3') and Reverse (5'-TGG ATT GCA CTT CAT CTT GG-3') *primers* and the standard strain *A. baumannii* ATCC 19606 as a control. The results were interpreted based on the detection of 369-base-pair (bp) amplicons by agarose gel electrophoresis (Ludwig Biotec, Brazil), using Blue Green Loading Dye I (LGC Biotechnology, United Kingdom).

Production and acquisition of iron-chelating compounds: In partnership with the Applied Physical Chemistry and Technology Laboratory of the School of Chemistry and Food (EQA) at the Federal University of Rio Grande (FURG), 1,10-phenanthroline, the compound with chelating potential was synthesized using the hydrothermal synthesis proposed by Oliveira *et al.* [11]. This compound was characterized by infrared spectroscopy using a Shimadzu IR PRESTIGE-21 spectrophotometer from 4000 cm⁻¹ to 400 cm⁻¹. Solid 1,10-phenanthroline samples were ground and mixed with spectroscopic-grade potassium bromide (KBr) for analysis by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). Ethylenediaminetetraacetic acid (EDTA) (Ludwig Biotec, Brazil) was commercially acquired. The compounds were maintained in 80% dimethyl sulfoxide (DMSO) at 10 mg/mL and refrigerated at 4°C until testing.

Antimicrobial susceptibility to iron-chelating compounds: The minimum inhibitory concentration (MIC) of the iron-chelating compounds, DMSO 80% and ciprofloxacin (positive control) was determined by broth microdilution assay with serial dilutions (1:2), according to the recommendations of the M100 document of the *Clinical and Laboratory Standards Institute* (CLSI) [12]. 1,10-phenanthroline was tested at concentrations ranging from 0.84 to 108.13 µg/mL, whereas EDTA was evaluated at concentrations ranging from 10.47 to 1,340.06 µg/mL. The assays were performed in 96-well microplates using resazurin as an indicator of cell viability. The MIC was defined as the lowest concentration capable of inhibiting microbial growth.

Antibiofilm activity: Initially, the biofilm-forming capacity of each isolate was determined according to the method described by Stepanovic *et al.* [13], with modifications. The strains were cultured on Mueller-Hinton (MH) agar at 37°C for 24 h and suspended in sterile saline solution, with the optical density (600 nm) adjusted to the 0.5 McFarland standard (≈10⁸ CFU/mL). Subsequently, the suspension was diluted at a ratio of 1:100 in MH broth, and 100 µL was inoculated and incubated at 37°C for 24 hours (h). After incubation, the contents were removed, the wells were washed with 1× PBS, and the adhered biofilms were fixed with methanol, stained with 0.4% crystal violet, and solubilized with ethanol. Absorbance was determined at 630 nm, allowing the strains to be classified as non-biofilm-forming, weak, moderate, or strong biofilm formers according to the optical density relative to the negative control. For the biofilm formation inhibition assays, only strains classified as strong biofilm formers and the standard strain ATCC 19606 of *A. baumannii* were selected. Based on

the minimum inhibitory concentration (MIC) results, according to the methodology described by Halicki *et al.* [14], bacterial suspensions adjusted to a concentration of 10^6 CFU/mL in standard MH medium were exposed to three different concentrations of the compounds: 2×MIC, 1×MIC, and 1/2×MIC. The samples were incubated for 24 h at 37°C and subsequently subjected to biofilm quantification using crystal violet, with the results compared with the positive control (bacterial inoculum only) and negative control (MH medium only).

Cytotoxicity: The cytotoxic activity was evaluated in the Vero cell line (Rio de Janeiro Cell Bank - BCRJ 0245), derived from kidney epithelial cells, at a concentration of 3.4×10^5 cells/mL, by means of a cell viability assay using resazurin after 24 h of exposure to different concentrations of the 1,10-phenanthroline compound (1600–100 µg/mL). Based on the results, the 50% inhibitory concentration (IC_{50}) was determined, defined as the concentration of the compound capable of reducing cell viability by 50%, according to the methodology described by Ahmed *et al.* [15].

In vitro assay analyses: All experiments were performed in triplicate, in independent assays. The obtained data were expressed as mean ± standard deviation. Graphs were constructed using GraphPad Prism 5.00 software.

In silico bioavailability test: The compounds were drawn using the Chem Sketch 2.0 chemical editor and analyzed using the SwissADME computational model (www.swissadme.ch), aiming to estimate effects that may impact the access of the molecules to the target through pharmacokinetic parameters related to absorption, distribution, metabolism, and excretion—ADME, according to Daina *et al.* [16]. Based on the results obtained, the following analyses were performed: Determination of the probability of bioavailability of an orally administered drug, following the guidelines of Lipinski's "Rule of Five" (RO5), with the following accepted values: molecular weight ≤ 500; $miLogP$ ≤ 5; number of hydrogen bond donors ≤ 5; number of hydrogen bond acceptors ≤ 10, in accordance with Lipinski [17]. Evaluation of pharmacokinetic descriptors according to the guidelines established by Veber [18] and Lovering [19], with the following accepted values: topological polar surface area (TPSA) ≤ 140 Å²; number of rotatable bonds ≤ 10; and fraction of sp³ carbon atoms (F_{sp3}) ≥ 0.47.

Results

The ten clinical isolates, previously characterized as non-lactose-fermenting Gram-negative bacilli and provided by HUSM, were subjected to the PCR technique and showed amplification of fragments corresponding to the *bla*_{OXA-51-like} gene, with 369 bp, thus being molecularly confirmed as *A. baumannii* (Fig. 1).

For the evaluation of compounds with potential activity against these pathogens, 1,10-phenanthroline was used, whose infrared spectrum was consistent with the expected structure. The spectrum showed characteristic peaks of out-of-plane C–H bond bending at 849.66 and 733.47 cm⁻¹, as well as a peak corresponding to the C=N double bond of the aromatic ring at 1590.46 cm⁻¹ (Fig. 2).

After confirmation of the isolates and characterization of the compound, antimicrobial susceptibility to the iron-chelating agents was evaluated. Both 1,10-phenanthroline and EDTA showed activity against the bacterial isolates tested, as demonstrated by the minimum inhibitory concentration (MIC) values presented in Table 1. The MICs of 1,10-phenanthroline against the clinical isolates ranged from 0.85 to 13.5 µg/mL, with four of the ten isolates showing an MIC of 6.8 µg/mL. In 80% of the evaluated isolates, 1,10-phenanthroline showed MIC values lower than those observed for ciprofloxacin, a broad-spectrum antibiotic used as a control, and against the standard strain *A. baumannii* ATCC 19606, it showed an MIC of 6.8 µg/mL. For EDTA, the MIC values against the clinical isolates ranged from 167.5 to 223.3 µg/mL. The control containing only 80% DMSO showed no antimicrobial activity against the tested strains, as evidenced by bacterial growth. This result indicates that this compound did not interfere with bacterial viability at the evaluated concentrations, ensuring that the observed activity is solely associated with the tested compounds.

Table 1. Minimum inhibitory concentration (MIC) of the synthetic iron-chelating compounds and the antibiotic ciprofloxacin (control), in µg/mL, against the analyzed clinical isolates (ISO) and the standard strain *A. baumannii* ATCC 19606.

	ISO 1	ISO 2	ISO 3	ISO 4	ISO 5	ISO 6	ISO 7	ISO 8	ISO 9	ISO 10	ATCC 19606
1,10-phenanthroline	6.8	6.8	0.85	6.8	13.5	13.5	13.5	6.8	0.85	0.85	6.8
EDTA	223.3	223.3	223.3	167.5	223.3	223.3	167.5	167.5	223.3	223.3	> 1,340.06
Ciprofloxacin	8	4	16	8	8	32	32	32	32	32	0.15

Considering the antimicrobial activity observed for 1,10-phenanthroline, its effect on biofilm formation was also evaluated. The isolates showed different capacities to form this structure, with 60% classified as weak biofilm formers (isolates 2, 5, 6, 7, 9, and 10), 20% as moderate biofilm formers (isolates 3 and 8), and 20% as strong biofilm formers (isolates 1 and 4). The standard strain *A. baumannii* ATCC 19606 was also classified as a weak biofilm former. Among the strong biofilm-forming isolates, at the concentration corresponding to 1/2×MIC (3.4 µg/mL), no inhibition of biofilm formation was observed. At the concentration corresponding to the MIC (6.8 µg/mL), among the clinical isolates, inhibition was observed only in isolate 4, with a percentage of 24.4%, while the standard strain *A. baumannii* ATCC 19606 showed 4.1% inhibition. When the concentration was increased to 2×MIC (13.6 µg/mL), greater antibiofilm activity was observed, with inhibition of 88.9% and 87.0% for isolates 1 and 4, respectively, while the standard strain showed an inhibition percentage of 27.9% (Fig. 3).

In addition to the antimicrobial and antibiofilm activities, the cytotoxicity of 1,10-phenanthroline was evaluated. The compound did not exhibit cytotoxic activity at any of the concentrations analyzed, ranging from 1600 to 100 µg/mL, maintaining 100% Vero cell viability even at the highest concentration tested (1600 µg/mL). Therefore, it was not possible to determine the IC₅₀ value within the evaluated range, since a 50% reduction in cell viability was not reached.

Complementary to the *in vitro* assays, *in silico* properties associated with the bioavailability of 1,10-phenanthroline, EDTA, and ciprofloxacin were analyzed. The physicochemical properties presented in Table 2 showed that the two chelating agents and the antimicrobial used as a control did not violate Lipinski’s Rule of Five (RO5).

Table 2. Physicochemical properties of ciprofloxacin, EDTA and 1,10-phenanthroline, to the RO5.

Compound	Molecular weight (g/mol)	MilogP	Hydrogen bond donors	Hydrogen bond acceptors
Ciprofloxacin	331.34	1.28	2	5
EDTA	292.24	-1.79	4	10
1,10-phenanthroline	180.21	1.86	0	2

Molecular weight ≤ 500; miLogP ≤ 5; number of hydrogen bond donors ≤ 5; number of hydrogen bond acceptors ≤ 10.

Regarding the other molecular descriptors presented in Table 3, and considering the criteria proposed by Veber [18] and Lovering [19], the topological polar surface area (TPSA) values were within the recommended range for 1,10-phenanthroline and ciprofloxacin, whereas EDTA showed a value above the recommended threshold. The fraction of sp³ carbon atoms (Fsp3) met the proposed criterion only for EDTA. With respect to the number of rotatable bonds, used as an indicator of molecular flexibility, the values were below 10 for 1,10-phenanthroline and ciprofloxacin, whereas EDTA

showed a value above the recommended limit. Blood-brain barrier permeability and gastrointestinal absorption were also estimated, and the results are presented in Table 3.

Table 3. Pharmacokinetic parameters of 1,10-phenanthroline, EDTA, and ciprofloxacin according to Veber and Lovering, followed by estimates of blood-brain barrier permeability and gastrointestinal absorption capacity.

Compound	TPSA (Å²)	Fraction Csp3	Rotatable bonds	Blood-brain barrier permeability	Gastrointestinal absorption
Ciprofloxacin	74.57	0.41	3	No	High
EDTA	155.68	0.60	11	No	Low
1,10-phenanthroline	25.78	0.00	0	Yes	High

Topological polar surface area (TPSA) ≤ 140 Å²; fraction of sp3 carbon atoms (Fsp3) ≥ 0.47; and number of rotatable bonds ≤ 10.

Discussion

Molecular confirmation of the ten clinical isolates as *A. baumannii*, through amplification of the *bla*_{OXA-51-like} gene, together with the characterization of 1,10-phenanthroline by infrared spectroscopy, was used as the basis for evaluating the biological activity of the iron-chelating compounds. Based on the antimicrobial susceptibility assays, both 1,10-phenanthroline and EDTA showed activity against the evaluated isolates, although differences were observed in the concentrations required for bacterial inhibition.

The 1,10-phenanthroline showed antimicrobial activity at low concentrations and, in most of the evaluated isolates, at concentrations even lower than those observed for EDTA, a compound with well-established chelating properties. Interestingly, all the evaluated clinical isolates were classified as resistant to ciprofloxacin. In addition, the MIC values of 1,10-phenanthroline were lower than those observed for ciprofloxacin. The variability in MIC values among the clinical isolates may be related to the individual characteristics of each isolate. Mechanisms such as overexpression of efflux pumps and alterations in membrane permeability may contribute to differences in susceptibility, while the intrinsic genetic diversity of clinical isolates may result in distinct responses to the compound [20]. The standard strain *A. baumannii* ATCC 19606 showed susceptibility similar to that observed for some of the clinical isolates, since its MIC corresponded to the value most frequently found among them.

The results obtained are consistent with Ventura *et al.* [21], who observed antimicrobial activity of phenanthroline-based compounds against MDR *A. baumannii* isolates, with activity at 12.7 µg/mL and a bactericidal effect at 33.2 µg/mL. The main mechanism attributed to 1,10-phenanthroline may be related to its chelating capacity, in addition to its action on planktonic cells and bacterial biofilms. 1,10-phenanthroline may also induce the production of reactive oxygen species and compromise the integrity of the bacterial cell membrane, favoring the entry of antimicrobial agents and enhancing their effects. Considering the scarcity of compounds with mechanisms of action distinct from those that have been used since the golden age of antibiotic development, for which bacteria do not yet have described resistance mechanisms, the different mechanisms of action proposed for 1,10-phenanthroline make it a promising compound for the development of new antimicrobial alternatives [22].

Like 1,10-phenanthroline, EDTA showed antimicrobial activity, as expected, against the clinical isolates of *A. baumannii*. This effect may be attributed to the ability of the compound to chelate metal ions essential for bacterial growth and metabolism. In addition, EDTA may increase the permeability of the outer membrane of Gram-negative bacteria through destabilization of lipopolysaccharides, favoring the entry of antimicrobial agents. Its ability to chelate divalent ions

present in the biofilm may also interfere with cell adhesion and limit the formation of this structure [23]. In this context, one of the strategies proposed to address resistance in these pathogens consists precisely of exploiting bacterial iron acquisition mechanisms by combining chelating compounds with antimicrobial agents and facilitating their entry when iron acquisition processes are active [24–25].

The importance of metal chelation becomes particularly evident when the antimicrobial results are related to antibiofilm activity. Among the evaluated isolates, the strong biofilm formers showed higher inhibition percentages than those observed for the standard strain ATCC 19606, which was classified as a weak biofilm former. At a concentration of 13.6 µg/mL (2×MIC), the reduction in biofilm formation was greater than 85% in both strong biofilm-forming isolates, whereas the standard strain showed 27.9% inhibition.

These results are relevant, since biofilm formation by *A. baumannii* is directly associated with infection persistence and greater tolerance to antimicrobial agents, as the polymeric matrix can hinder drug diffusion and favor the exposure of bacterial cells to subinhibitory concentrations. More than half of infectious diseases are associated with bacteria capable of forming biofilms, structures that favor bacterial survival in hostile environments [26–27]. Even antibiotics already used clinically may require high concentrations for the eradication of these structures, which often becomes unfeasible because of the associated toxic and adverse effects [27–29].

Ventura *et al.* [21] also demonstrated that biofilm formation by clinical isolates of *A. baumannii* was significantly affected by the presence of 1,10-phenanthroline, with a reduction observed from 35.2 µM, approximately 7.4 µg/mL. This behavior is consistent with the role played by iron in biofilm formation on surfaces. Studies indicate that this metal may act as a regulatory signal in the process and that, at concentrations adequate to support the bacterial life cycle, it may induce the production of the extracellular polymeric matrix [30–32].

Thus, the results obtained demonstrate that the activity of 1,10-phenanthroline is not restricted to planktonic cells, but also extends to biofilm formation. In the present study, inhibition was more pronounced in the strong biofilm-forming isolates when compared with the standard strain, which had a weak biofilm-forming capacity. This finding is particularly relevant considering that more developed biofilms contain a greater amount of extracellular matrix. Although the mechanism of action of 1,10-phenanthroline on this structure has not yet been fully elucidated, its ability to inhibit biofilm formation, together with the antibacterial activity observed against planktonic cells, demonstrates that its activity was not compromised by the high biofilm-forming capacity of the evaluated isolates.

In addition to antimicrobial and antibiofilm activity, evaluation of the safety of these compounds is an important aspect for advances in the preclinical stages of new antimicrobial development. In the literature, gaps remain regarding the evaluation of the cytotoxicity of 1,10-phenanthroline alone. Cirino *et al.* [33] evaluated the cytotoxicity of phendione (1,10-phenanthroline-5,6-dione) and derived metal complexes in Vero cells (ATCC CCL-81), reporting an IC₅₀ of 200 µM, approximately 42 µg/mL, for phendione and the copper and silver complexes. In the present study, on the other hand, 1,10-phenanthroline showed no cytotoxicity toward Vero cells (BCRJ 0245), even at a concentration of 1600 µg/mL, approximately 38 times higher than the concentration corresponding to the IC₅₀ reported for the derivatives evaluated by Cirino *et al.* [33]. Although differences between the compounds and experimental models do not allow a direct comparison, the maintenance of cell viability at the evaluated concentrations indicates low toxicity of 1,10-phenanthroline in this model and could translate into a selectivity index (IC₅₀/MIC) far above the cutoff proposed by Pavan *et al.* [34] regarding the pharmacological safety of promising compounds for the development of new antimicrobial agents.

The characteristics observed in the biological assays may also be related to the physicochemical properties obtained in the *in silico* analysis. 1,10-phenanthroline, EDTA, and ciprofloxacin showed no violations of the RO5, which is used to estimate the probability of oral bioavailability of compounds based on physicochemical properties. However, the absence

of rule violations alone does not guarantee oral bioavailability or appropriate pharmacokinetic behavior, since other factors are also involved in these processes [35].

Hydrogen bond donor and acceptor groups influence both the interaction of molecules with water and their permeability through lipophilic membranes. Although these interactions favor molecule hydration in an aqueous medium, an excessive increase in polarity may limit permeation through biological membranes [17]. miLogP, in turn, is a descriptor used to estimate the lipophilicity of a compound, that is, its affinity for oils and lipids [36], whereas TPSA represents the polar surface area of the molecule and helps estimate its interaction with water and its ability to permeate membranes. The fraction of sp³ carbon atoms represents the degree of saturation of the molecule and may also be related to its solubility and lipophilicity properties [37].

EDTA, despite its ability to destabilize the outer membrane of Gram-negative bacteria, increasing its permeability and potentially enhancing the action of other agents, showed a high number of hydrogen bond donor/acceptor groups, a negative miLogP, and high polarity, with a TPSA of 155.68 Å². These results characterize a highly hydrophilic molecule and are compatible with a lower capacity for gastrointestinal absorption and membrane permeation. Its saturated molecular structure and high Csp³ fraction (0.60) reinforce this hydrophilic character and may limit its cellular permeation. However, because of its chelating activity, EDTA may act as an adjuvant, facilitating the action of antimicrobial agents and also interfering with the ability to form bacterial biofilms [38].

On the other hand, 1,10-phenanthroline showed a positive miLogP associated with a low number of hydrogen bond donor/acceptor groups and low polarity, with a TPSA of 25.78 Å². This set of characteristics indicates greater lipophilicity and is compatible with better gastrointestinal absorption and greater capacity for passive diffusion through membranes. Its planar aromatic structure, together with the *in silico* results, reinforces its lipophilic character and provides support for the characteristics observed in the biological assays. Ciprofloxacin, used as a control, showed more balanced properties between lipophilicity and polarity, consistent with its oral bioavailability and broad tissue distribution.

EDTA showed antimicrobial activity against the *A. baumannii* isolates, an effect that may be related to its chelating properties and possible interference with bacterial membrane permeability and biofilm formation. 1,10-phenanthroline, in turn, showed antimicrobial activity at lower concentrations, high inhibition of biofilm formation in strong biofilm-forming isolates, absence of cytotoxicity at the evaluated concentrations, and physicochemical properties compatible with greater permeation capacity. Therefore, the results, together with data described in the literature, suggest that metal-chelating capacity may contribute to the observed effects. Thus, the findings highlight the potential of the evaluated compounds, particularly 1,10-phenanthroline, as alternatives to be explored against *A. baumannii*.

Conclusions

The evaluated compounds showed antimicrobial activity against clinical isolates of *A. baumannii*, with 1,10-phenanthroline being the compound that showed the most promising results under the conditions studied. In addition to its activity against bacterial cells, 1,10-phenanthroline was able to reduce biofilm formation, including in strong biofilm-forming isolates, without showing cytotoxicity in Vero cells at the concentrations evaluated. The *in silico* analyses indicated pharmacokinetic parameters favorable to the bioavailability of 1,10-phenanthroline. Taken together, these findings support further investigation of iron-chelating compounds as a potential strategy for the development of antimicrobial agents against this important hospital pathogen.

Declarations

CRediT Contribution Statement

C. O. N.: Investigation, Methodology, Data presentation design, Data analysis, Writing – original draft;
M. O. G.: Writing – review and editing;
S. L. L.: Investigation, Methodology;
D. C. R.: Investigation, Methodology;
R. Q.: Methodology;
J. R. M. V.: Investigation, Methodology;
D. F. R.: Conceptualization, Supervision, Project administration, Data analysis, Writing – review and editing.

Competing Interests

The authors declare that there are no conflicts of interest.

Ethics Approval

This study was approved by the Research Ethics Committee of the Federal University of Santa Maria (CAAE 38850614.4.00005346).

Data availability statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures



Figure 1

Agarose gel electrophoresis of the *blaOXA-51-like* gene amplified by PCR. Lane 1: 100 bp molecular ladder (Ludwig Biotec, Brazil); Lane 2: negative control; Lane 3: 369 bp positive control (*A. baumannii* ATCC 19606 genomic DNA); Lanes 4–10: *A. baumannii* isolates showing a 369 bp amplicon

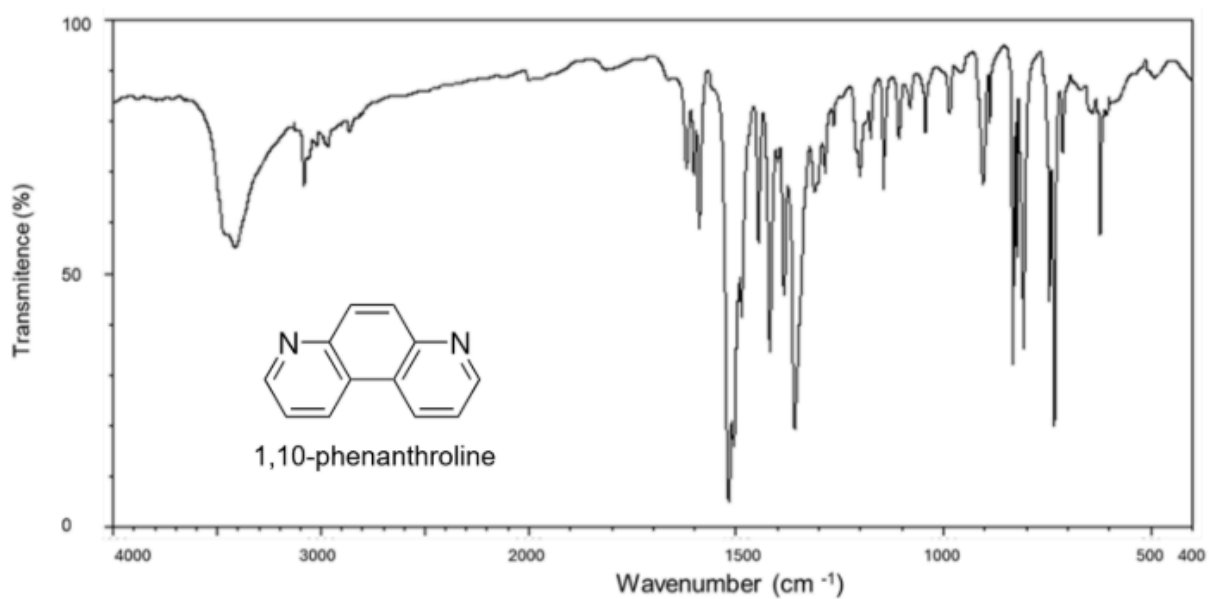


Figure 2

Infrared spectroscopy of the 1,10-phenanthroline compound, showing characteristic absorption bands at wavenumbers between 849.66 and 733.47 cm^{-1} and at 1590.46 cm^{-1} .

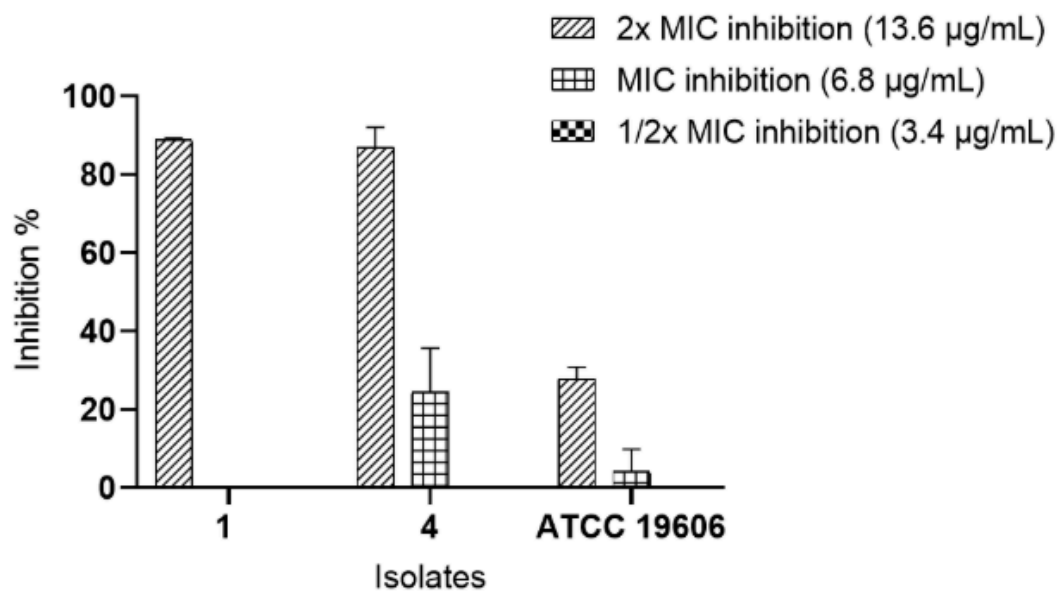


Figure 3

Percentage inhibition of biofilm formation by 1,10-phenanthroline in clinical isolates that exhibited a strong biofilm-forming profile and in the standard strain *A. baumannii* ATCC 19606, at different concentrations corresponding to the minimum inhibitory concentration (MIC) assay. The bars represent mean $\pm$ standard deviation.