DOI: 10.5433/1679-0367.2025v46n2p120

Antimicrobial and antibiofilm activity of antidepressants against *Staphylococcus aureus*

Atividade antimicrobiana e antibiofilme de antidepressivos frente à *Staphylococcus aureus*

Giulianna Oliveira Lodetti[1], Luís Henrique Nunes de Souza[2], Liliana Scorzoni[3], Luiz Eduardo Nunes Ferreira[4], Priscila Luiza Mello[5]

Abstract

Antimicrobial resistance represents a major health challenge, particularly due to the ability of *Staphylococcus aureus* to form biofilms and persist under antimicrobial pressure. In this context, drug repurposing has emerged as a promising strategy to identify alternative therapeutic options from approved drugs. This study aimed to evaluate the antimicrobial, antibiofilm, and cytotoxic activities of the antidepressants fluoxetine, paroxetine, and duloxetine against planktonic and biofilm-forming *Staphylococcus aureus* strains. Antibacterial activity was determined by broth microdilution assays to establish the minimum inhibitory concentration and minimum bactericidal concentration against *Staphylococcus aureus* ATCC 33591 and ATCC 29213. Antibiofilm activity was assessed in mature biofilms using cell viability assays and biomass quantification. Cytotoxicity was evaluated in RAW 264.7 macrophages using a cell viability assay, with calculation of mean effective concentration values. Fluoxetine and duloxetine exhibited lower inhibitory and bactericidal concentrations compared to paroxetine. In biofilm assays, fluoxetine and duloxetine significantly reduced biofilm cell viability and biomass, whereas paroxetine showed a less consistent effect. Cytotoxicity analysis revealed similar EC_{50} values among the evaluated drugs, and the concentrations associated with relevant antibacterial and antibiofilm effects were approximately 20-fold higher than the EC_{50} values determined in macrophages, indicating that, in this in vitro model, antimicrobial and antibiofilm activity occur in a concentration range accompanied by measurable cytotoxicity. Overall, the results indicate that antidepressants, particularly fluoxetine and duloxetine, exhibit *in vitro* antimicrobial and antibiofilm activity against *Staphylococcus aureus*, reinforcing the potential of these compounds for future drug repurposing studies.

Keywords: microbial resistance; drug repurposing; gram-positive bacteria; cytotoxicity; bacterial biofilms.

1 Bachelor's degree in Biological Sciences from Universidade Guarulhos – UNG, Guarulhos, São Paulo, Brazil. *E-mail:* giuliannalodetti.oliveira@gmail.com *Orcid:* https://orcid.org/0009-0007-7646-1486

2 Master's in Nursing from the University of Guarulhos (UNG), Guarulhos, São Paulo, Brazil. *E-mail:* nunessouzalh@gmail.com *Orcid:* https://orcid.org/0000-0003-4544-9701

3 Ph.D. in Biosciences and Biotechnology Applied to Pharmacy from Universidade Estadual Paulista – UNESP, Botucatu, São Paulo, Brazil. Professor of the Graduate Program in Nursing at Universidade Guarulhos – UNG, Guarulhos, São Paulo, Brazil. *E-mail:* liliscorzoni@yahoo.com.br *Orcid:* https://orcid.org/0000-0002-0178-6653

4 Ph.D. in Dentistry (Pharmacology) from Universidade Estadual de Campinas – UNICAMP, Piracicaba, Brazil. Professor at the Graduate Program in Nursing (Stricto Sensu) at Universidade Guarulhos – UNG, Guarulhos, São Paulo, Brazil. *E-mail:* luiz.enferreira@gmail.com *Orcid:* https://orcid.org/0000-0002-4882-5402

5 Ph.D. in General and Applied Biology Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, Botucatu, São Paulo, Brazil. Faculty member at Universidade Guarulhos – UNG, Guarulhos, São Paulo, Brazil. *E-mail:* priscila_mello@msn.com *Orcid:* https://orcid.org/0009-0002-2864-3633

Resumo

A resistência antimicrobiana representa um importante desafio em saúde, especialmente devido à capacidade de *Staphylococcus aureus* formar biofilmes e persistir sob pressão antimicrobiana. Nesse contexto, o reposicionamento de fármacos surge como uma estratégia promissora para a identificação de alternativas terapêuticas a partir de medicamentos já aprovados. O objetivo deste estudo foi avaliar a atividade antimicrobiana, antibiofilme e a citotoxicidade dos antidepressivos: fluoxetina, paroxetina e duloxetina frente a cepas planctônicas e formadoras de biofilme de *Staphylococcus aureus*. A atividade antibacteriana foi determinada por microdiluição em caldo para a obtenção da concentração inibitória mínima e da concentração bactericida mínima frente às cepas *Staphylococcus aureus* ATCC33591 e ATCC 29213. A atividade antibiofilme foi avaliada em biofilmes maduros por ensaio de viabilidade celular e quantificação de biomassa. A citotoxicidade foi avaliada em macrófagos RAW 264.7 por ensaio de viabilidade celular, com cálculo dos valores de concentração efetiva média. A fluoxetina e a duloxetina apresentaram menores concentrações inibitórias e bactericidas em comparação à paroxetina. Nos ensaios em biofilme, fluoxetina e duloxetina promoveram redução significativa da viabilidade celular e da biomassa do biofilme, enquanto a paroxetina apresentou efeito menos consistente. Os ensaios de citotoxicidade demonstraram valores de EC_{50} semelhantes entre os fármacos, e as concentrações associadas à atividade antibacteriana e antibiofilme foram aproximadamente 20 vezes superiores aos valores de EC_{50} obtidos em macrófagos, indicando que, neste modelo in vitro, tais efeitos ocorrem em uma faixa de concentração acompanhada de citotoxicidade mensurável. Os resultados indicam que antidepressivos, especialmente fluoxetina e duloxetina, apresentam atividade antimicrobiana e antibiofilme *in vitro* frente a *Staphylococcus aureus*, reforçando o potencial desses compostos para estudos futuros de reposicionamento farmacológico.

Palavras-chave: resistência microbiana; reposicionamento de medicamentos; bactérias gram-positivas; citotoxicidade; biofilmes bacterianos

Introduction

Antimicrobial resistance constitutes an evolutionary response of bacteria to exposure to antimicrobial agents introduced into their environment and currently represents one of the major global public health challenges [1]. Antibiotic resistance occurs when microorganisms acquire or develop mechanisms capable of neutralizing, avoiding, or circumventing the action of these drugs, thereby making infections caused by resistant pathogens more difficult to treat [2]. As highlighted by Harbarth et al. [3], the recurrent use of antibiotics exerts selective pressure on bacterial populations, favoring the emergence of mutations or the exchange of genetic material, which directly contributes to the development of antimicrobial resistance.

According to the 2024 World Health Organization Priority Bacterial Pathogens List [4], methicillin-resistant *Staphylococcus aureus* (MRSA) remains classified as a high-priority pathogen, representing a problem of global relevance. It is estimated that approximately 50% of reports attributed to antimicrobial resistance are associated with two main pathogens, *Staphylococcus aureus* and *Escherichia coli*, which are directly related to increased morbidity, mortality, and healthcare costs resulting from potentially severe and difficult-to-treat infections.

Staphylococcus aureus is a Gram-positive bacterium that has emerged as one of the leading causes of hospital-acquired infections. Infections caused by resistant strains, such as MRSA, present greater therapeutic complexity, particularly due to the ability of this microorganism to form biofilms on both abiotic and biotic surfaces [5]. Microbial biofilms consist of organized communities of cells embedded in a self-produced extracellular polymeric matrix composed mainly of polysaccharides,

proteins, and nucleic acids. This structure provides protection against adverse environmental conditions, including ultraviolet radiation, extreme variations in temperature and pH, nutrient limitation, and exposure to antimicrobial agents [6].

Gram-positive bacteria, such as *S. aureus*, exhibit multiple barriers to eradication when organized in biofilms, directly impacting different sectors, including healthcare settings and the food industry. In hospital environments, biofilm-producing microorganisms can colonize tissues of patients with chronic diseases as well as medical devices such as catheters and prostheses. S. aureus expresses several virulence factors, including toxins, enzymes, and adhesion proteins, which promote colonization, immune evasion, and dissemination within host tissues [7]. Despite therapeutic advances, the treatment of biofilm-associated bacterial infections remains challenging, even when strategies based on pharmacokinetic and pharmacodynamic profiles are applied [8]. Given the high level of resistance exhibited by *S. aureus*, particularly related to biofilm formation, controlling infections caused by resistant strains, including MRSA, has become a priority in healthcare settings, reinforcing the need for studies aimed at identifying effective therapeutic alternatives.

In this context, drug repurposing, also referred to as drug repositioning, has emerged as a promising strategy in response to the high costs, long timelines, and elevated failure rates associated with the development of new drugs [9]. This approach involves identifying new therapeutic applications for medications that have already been approved for other clinical indications [10]. As emphasized by Breckenridge and Jacob [11], drug repurposing offers relevant advantages, including cost reduction, lower toxicity risk, and shorter development timelines. However, economic factors and the need for dose adjustments or additional clinical trials still represent barriers to the broader implementation of this strategy [17].

Since the first description of antimicrobial activity of a psychotropic drug, chlorpromazine, in 1959 [12], several studies have demonstrated that certain antidepressants exhibit antimicrobial activity against different groups of microorganisms, particularly Gram-positive bacteria [5,13,20]. Among these drugs, paroxetine, a selective serotonin reuptake inhibitor, has been reported to exhibit antimicrobial activity against a wide range of microorganisms, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, among others [4,13]. Fluoxetine, the first antidepressant of this class to achieve widespread clinical efficacy and a well-established safety profile, has also been reported to exhibit antimicrobial activity in addition to its consolidated use in the treatment of depression [8,14]. Similarly, duloxetine, classified as a serotonin and norepinephrine reuptake inhibitor, has demonstrated promising results against resistant bacteria [18].

Although the precise mechanisms underlying the antibacterial activity of antidepressants have not been fully elucidated, evidence from the literature suggests that these compounds may alter bacterial membrane integrity, modulate efflux pumps, and disrupt cellular energy metabolism, among other stress-response pathways [4,5,12,18,20]. Such pleiotropic effects could contribute to their activity against both planktonic and biofilm-associated cells, particularly in Gram-positive species such as *S. aureus* [16,21,23]. Although previous studies have reported antimicrobial effects of antidepressants against Gram-positive bacteria, comparative analyses integrating planktonic susceptibility, antibiofilm activity, and cytotoxicity within the same experimental design remain limited. Furthermore, differences between non-biofilm-forming and biofilm-forming *S. aureus* strains, including MRSA isolates, remain poorly explored in the context of antidepressant drug repurposing.

Therefore, the present study aims to systematically evaluate the antimicrobial, antibiofilm, and cytotoxic activities of the antidepressants fluoxetine, paroxetine, and duloxetine against planktonic and biofilm-forming *S. aureus* strains, including a methicillin-resistant *S. aureus* (MRSA) strain. By simultaneously assessing antibacterial and antibio-

film effects and cytotoxicity at the same concentrations, this work seeks to contribute to a better understanding of the potential and limitations of these drugs as candidates for therapeutic repurposing in the context of antimicrobial resistance.

Materials and Methods

Study design

This study consisted of a quantitative, non-clinical experimental assay. The experimental design was conceived to correlate the concentrations associated with antibacterial and antibiofilm activity with their corresponding effects on host cell viability, an essential aspect in the early evaluation of drug repurposing candidates.

Study location

The experiments were conducted at the Microbiology Laboratory and the Inflammation and Immunology Laboratory of Universidade Guarulhos (UNG), Brazil.

Materials

For bacterial culture, Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India), Mueller–Hinton (MH) broth (HiMedia, Mumbai, India), and a 0.5 McFarland standard (equivalent to 1.5×10^8 colony-forming units per milliliter) were used. For cell culture, RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA), glutamine (4 mM), glucose (4500 mg/L), sodium pyruvate (1 mM), sodium bicarbonate (1500 mg/L), penicillin (100 U/mL), streptomycin (100 µg/mL), and gentamicin (10 µg/mL) was employed.

The solutions used included 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA), 0.9% saline solution, phosphate-buffered saline (PBS), and dimethyl sulfoxide (DMSO) (Synth, Diadema, SP, Brazil).

Bacterial and cell strains

Staphylococcus aureus strains obtained from the American Type Culture Collection were used: ATCC 33591, a methicillin-resistant *S. aureus* (MRSA) strain with low biofilm-forming capacity, and ATCC 29213, a methicillin-susceptible *S. aureus* strain with high biofilm-forming capacity.

Drug solubilization

Paroxetine, fluoxetine, and duloxetine were solubilized in the respective assay media with the aid of DMSO and prepared in serial dilutions starting from 1000 µM.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method, according to the recommendations of the Clinical and Laboratory Standards Institute[16]. Ciprofloxacin was used as a reference antimicrobial agent for comparison. Bacterial inocula were prepared in saline solution and standardized to a 0.5 McFarland turbidity standard. Ninety-six–well microplates containing the drugs (1000 to 0.1 µM/mL) and bacterial inocula were incubated (N = 4/group) at 37 ± 2 °C for 24 h. The MIC was defined as the lowest drug concentration capable of completely inhibiting visible bacterial growth after incubation. The positive growth control consisted of BHI broth with bacterial inoculum (5 µL), while sterility controls contained only culture medium.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined according to the method described by the National Committee for Clinical Laboratory Standards[17]. After MIC determination, approximately 10 µL from wells without visible

bacterial growth and from the last well showing visible growth were plated onto Mueller–Hinton agar and incubated at 37 ± 2 °C for 48 h. The MBC was defined as the lowest concentration at which no bacterial growth was observed.

Evaluation of antimicrobial activity against biofilms

Drugs presenting bactericidal activity (MBC values) were selected for evaluation of antimicrobial activity against *in vitro* monospecies biofilms.

Biofilm formation

Biofilms were formed at the bottom of 96-well plates using bacterial inocula adjusted to 10^7 CFU/mL in BHI broth (N = 12/group). Plates were incubated at 37 ± 2 °C for 24 h. After incubation, wells were washed twice with saline solution, the culture medium was replaced, and plates were incubated for an additional 24 h, totaling 48 h of biofilm formation. Subsequently, biofilms were treated with drug concentrations previously determined based on MIC and MBC values and incubated for a further 24 h. Saline solution was used as the negative control, with 200 µL added per well for all treatments.

MTT assay for biofilm cell viability

After treatment, wells were washed with saline solution and subjected to the MTT assay to assess biofilm cell viability. A volume of 200 µL of MTT solution (0.3 mg/mL) was added to each well, followed by incubation in the dark for 3–4 h at 37 ± 2 °C. After incubation, the solution was removed and 100 µL of absolute ethanol was added to solubilize formazan crystals. Absorbance was measured at 570 nm using a microplate reader, and results were expressed as percentage of cell viability.

Biofilm biomass quantification by crystal violet assay

Adhered biofilm biomass was quantified using the crystal violet method. After treatments, 200 µL of methanol was added per well for 20 min to fix the biofilm. Methanol was removed, and plates were incubated at 37 ± 2 °C for 24 h to dry. Subsequently, 200 µL of 1% (v/v) crystal violet solution was added for 5 min. The dye was removed, wells were washed with sterile saline solution, and the retained dye was solubilized with absolute ethanol. Absorbance was measured using a microplate reader, and values were used to quantify biofilm biomass.

Cell culture

RAW 264.7 cells were cultured in RPMI medium in culture flasks with a growth area of 75 cm² and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Experiments were performed at approximately 80% confluence.

Cytotoxicity analysis by MTT reduction assay

RAW 264.7 cells (5×10^5 cells/mL) were seeded in 96-well plates and exposed to fluoxetine, paroxetine, and duloxetine at increasing concentrations for 24 h. RPMI medium was used as the control. Cytotoxicity was assessed using the MTT reduction assay. After incubation, wells were washed with PBS (pH 7.4), and 200 µL of RPMI containing MTT (0.3 mg/mL) was added. Following 4 h of incubation, the medium was removed and 100 µL of absolute ethanol was added to dissolve the formazan crystals. Absorbance was measured at 570 nm, and cell viability was expressed as a percentage relative to the control.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test ($p \leq 0.05$), using GraphPad Prism version 5.0.Parte superior do formulário

Results

Antimicrobial activity against Staphylococcus aureus

For the *S. aureus* ATCC 33591 strain, fluoxetine and duloxetine exhibited MIC_{90} values ≥ 500 µM, whereas paroxetine showed higher MIC_{90} values (≥ 1000 µM). All tested drugs presented minimum bactericidal concentration (MBC) values ≥ 1000 µM, indicating that, at higher concentrations, each compound was capable of inhibiting bacterial growth in this strain.

For the *S. aureus* ATCC 29213 strain, all drugs exhibited MIC_{90} values ≥ 500 µM, with comparable MBC values, except for paroxetine, which showed an MBC ≥ 1000 µM. These results suggest a relatively uniform antimicrobial profile among the compounds, with slight variation observed for paroxetine.

After completion of the MIC and MBC assays, the results obtained are summarized as follows:

Table 1. Minimum inhibitory concentration (MIC_{90}) and minimum bactericidal concentration (MBC) of antidepressant drugs against *Staphylococcus aureus*.

Drug	*S. aureus* (ATCC 33591)		*S. aureus* (ATCC 29213)	
	MIC_{90} (µM)	**MBC (µM)**	**MIC_{90} (µM)**	**MBC (µM)**
Fluoxetine	500	1000	500	500
Paroxetine	1000	1000	500	1000
Duloxetine	500	1000	500	500

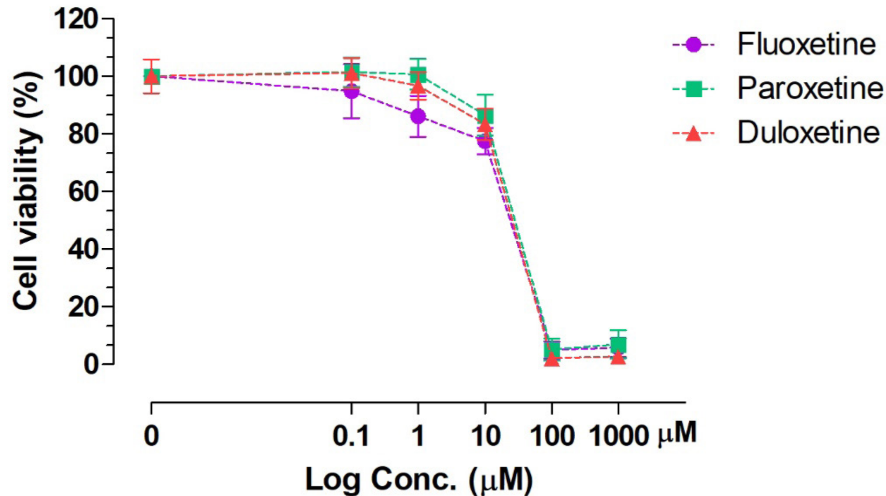
Legend: MIC_{90}, minimum inhibitory concentration required to inhibit 90% of bacterial growth; MBC, minimum bactericidal concentration.
Source: The authors.

Fluoxetine and duloxetine exhibited greater efficacy than paroxetine in reducing the viability of the tested *Staphylococcus aureus* strains. The data indicate that fluoxetine and duloxetine showed lower MIC_{90} and MBC values compared to paroxetine, reflecting higher antibacterial activity under the experimental conditions evaluated.

Cytotoxicity assessment in RAW 264.7 cells

The cytotoxicity of the drugs was evaluated in immortalized RAW 264.7 macrophages using the MTT reduction assay after 24 h of exposure to different concentrations. At the lowest concentrations tested (0.1–1 µM), all drugs exhibited cell viability close to 100%, indicating low cytotoxicity within this range. As shown in Figure 1, fluoxetine and duloxetine induced a gradual, dose-dependent reduction in cell viability starting at 10 µM, with a statistically significant decrease observed at 100 µM and 1000 µM compared with the untreated control ($p < 0.05$). Paroxetine also reduced cell viability at higher doses, but at 100 µM and 1000 µM it maintained significantly higher percentages of viable cells than fluoxetine and duloxetine at the same concentrations ($p < 0.05$).

Figure 1. Cell viability of RAW 264.7 macrophages after 24 h exposure to fluoxetine, paroxetine, and duloxetine. Results are expressed as mean ± SD. Cell viability is shown as percentage relative to the untreated control. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; $p < 0.05$ was considered statistically significant for comparisons between treated groups and the control, as well as between different drugs at the same concentration.



Legend: Cell viability (mean ± SD) of RAW 264.7 cells after 24 h of exposure to different concentrations of fluoxetine, paroxetine, and duloxetine. The y-axis represents the percentage of cell viability relative to the control group. The x-axis represents the logarithm of drug concentration (μM). *n* = 17.
Source: The authors.

EC_{50} values were calculated using nonlinear regression analysis (log[inhibitor] vs. normalized response, variable slope model). As shown in Table 2, no significant differences were observed in EC_{50} values among the tested drugs ($p > 0.05$), indicating similar cytotoxicity profiles under the evaluated conditions. Considering these results together with the MIC_{90} and MBC data, the concentrations required to achieve antibacterial activity were approximately 20-fold higher than the EC_{50} values determined in macrophages, indicating that antimicrobial and antibiofilm activity occurs at concentrations above those affecting cell viability, with cytotoxic effects restricted to the highest concentration ranges.

Table 2. EC_{50} values (µM) of the antidepressants tested in RAW 264.7 cells after 24 h.

Drug	EC_{50} (µM) (±DP)
Fluoxetine	20.33 (0.040)
Paroxetine	24.71 (0.036)
Duloxetine	19.96 (0.036)

Source: The authors.

No significant differences were observed in cytotoxicity values among the tested drugs, indicating similar cellular profiles under the evaluated conditions. Accordingly, the concentration required to achieve antibacterial activity was approximately 20-fold higher than the EC_{50} values determined in macrophages, indicating that antimicrobial activity occurs at concentrations above those affecting cell viability, with cytotoxic effects restricted to the highest concentration ranges.

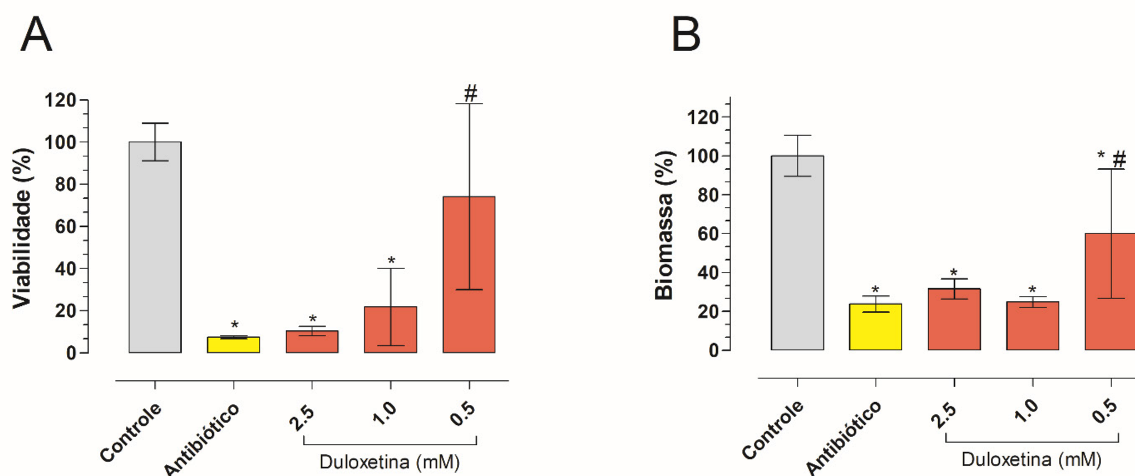
Antibiofilm activity against S. aureus ATCC 29213

The antibiofilm activity of fluoxetine, paroxetine, and duloxetine against mature biofilms of *Staphylococcus aureus* ATCC 29213 was evaluated using cell viability (MTT) assays and biomass quantification by the crystal violet method after 24 hours of exposure to the treatments (Figure 2A–F).

The MTT assay results demonstrated that all drugs promoted a significant reduction in biofilm cell viability at higher concentrations compared with the control group (0 mM) (Figure 2A, 2C, 2E). Duloxetine and fluoxetine exhibited a more pronounced effect on biofilm viability reduction, whereas paroxetine showed a less consistent effect, particularly at intermediate concentrations.

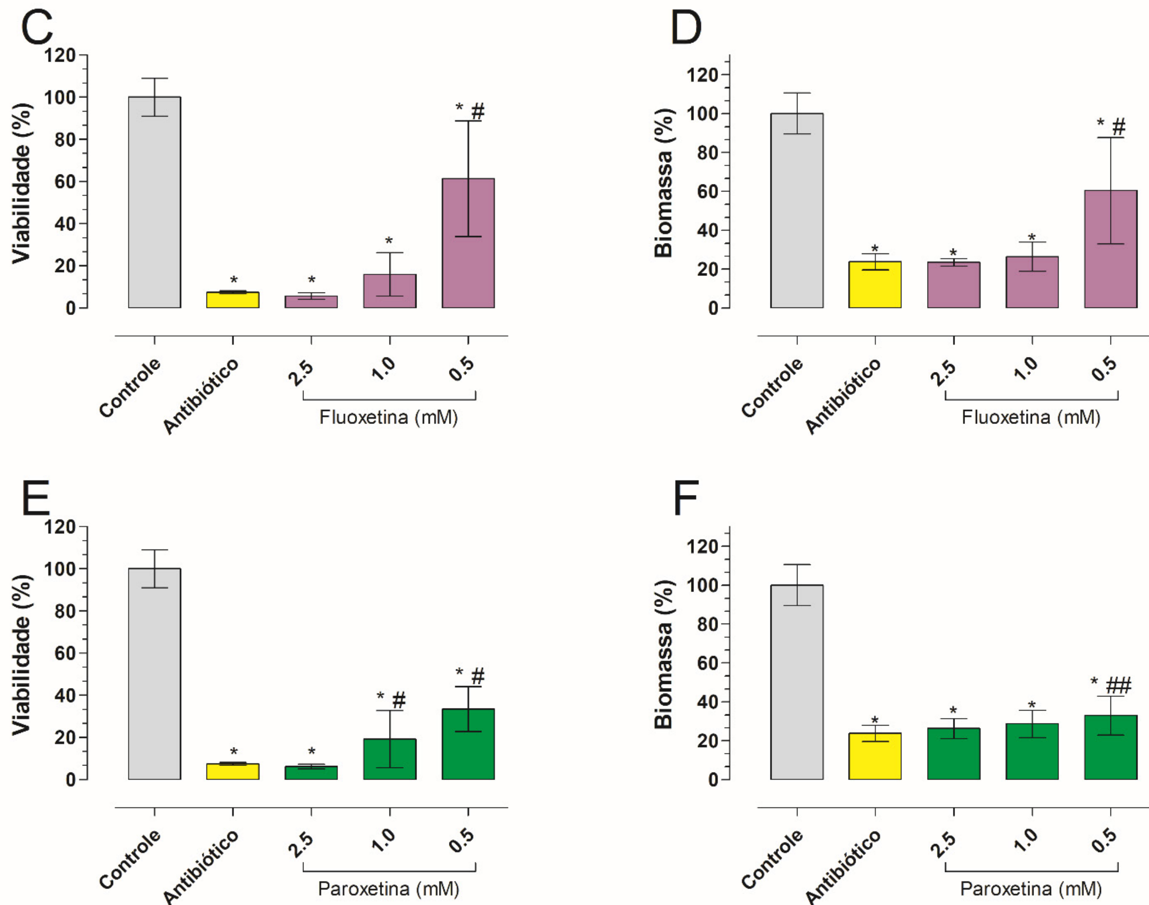
Complementarily, the crystal violet assay revealed a significant reduction in biofilm biomass following treatment with the antidepressants, corroborating the findings obtained in the cell viability assay (Figure 2B, 2D, 2F). Biomass reduction was more evident in the groups treated with fluoxetine and duloxetine, indicating a greater ability of these compounds to interfere with the structure and maintenance of the bacterial biofilm.

Figure 2. Antibiofilm activity of duloxetine, fluoxetine and paroxetine against *Staphylococcus aureus* ATCC 29213**.**



(continued on the next page)

(figure continuation)



Legend: Cell viability of biofilm-associated cells was assessed by the MTT reduction assay (A, C, E), and biofilm biomass was quantified by the crystal violet method (B, D, F) after 24 h of exposure to the treatments. Panels A–B correspond to duloxetine, C–D to fluoxetine and E–F to paroxetine. Results are expressed as mean ± standard deviation (SD). The y-axes represent the percentage of cell viability or biofilm biomass relative to the untreated control (Control), and the x-axes represent drug concentration (mM). * Indicates a statistically significant difference compared with the untreated control (0 mM), $p < 0.01$, one-way ANOVA followed by Tukey's post hoc test. # Indicates a statistically significant difference compared with the antibiotic group (penicillin [100 U/mL], streptomycin [100 µg/mL] and gentamicin [10 µg/mL]), $p < 0.01$, one-way ANOVA followed by Tukey's post hoc test. ## Indicates a statistically significant difference compared with the antibiotic group, $p < 0.05$, one-way ANOVA followed by Tukey's post hoc test. $n \geq 8$.
Source: The authors.

Discussion

Antimicrobial resistance represents one of the most pressing contemporary challenges in public health, compromising the effectiveness of available antibiotics and significantly increasing morbidity and mortality rates associated with bacterial infections. In the face of the scarcity of new antimicrobial classes in the pharmaceutical pipeline, drug repurposing has emerged as a promising strategy by enabling the identification of new therapeutic applications for already approved drugs with previously established pharmacokinetic and safety profiles [3,17].

In this context, antidepressants belonging to the selective serotonin reuptake inhibitors and se-

rotonin–norepinephrine reuptake inhibitors classes have attracted growing interest due to their demonstrated antimicrobial and antibiofilm activities in different experimental models. Evidence from the literature indicates that these drugs exhibit *in vitro* antimicrobial activity, possibly related to alterations in bacterial membrane integrity, modulation of efflux pumps, and disruptions in cellular energy metabolism [4,5,13,18].

The present study was not designed to elucidate the molecular mechanisms underlying the observed antimicrobial or antibiofilm effects. Therefore, any mechanistic interpretations should be considered exploratory and based on previously published evidence, as such mechanisms were not experimentally investigated herein. The primary contribution of this study lies in the phenotypic characterization of antimicrobial and antibiofilm activity combined with cytotoxicity assessment, a critical step in early-stage drug repurposing studies.

The results demonstrated that fluoxetine and duloxetine exhibited lower MIC_{90} and MBC values compared to paroxetine against *Staphylococcus aureus* ATCC 33591 and ATCC 29213 strains. These findings corroborate previously reported data indicating greater antibacterial activity of certain antidepressants against clinically relevant Gram-positive bacteria. Cabral *et al.* reported antimicrobial activity of paroxetine against a wide range of microorganisms, including *S. aureus*, often at relatively high concentrations, which is consistent with the higher MIC_{90} and MBC values observed in the present study [4]. In contrast, fluoxetine has been consistently described as one of the selective serotonin reuptake inhibitors with the greatest antimicrobial potential, exhibiting bactericidal activity against both susceptible and multidrug-resistant *S. aureus* strains [8,21].

Duloxetine, although classified as a serotonin–norepinephrine reuptake inhibitor, has also been associated with relevant antibacterial effects, particularly against resistant bacteria **[18]**. The activity observed in this study supports the hypothesis that antidepressants with higher lipophilicity and enhanced ability to cross cellular membranes may exhibit superior antimicrobial efficacy, possibly related to direct interactions with bacterial structures.

Importantly, the antibacterial activity observed in the present study was comparable between the methicillin-resistant strain (*S. aureus* ATCC 33591) and the methicillin-susceptible strain (*S. aureus* ATCC 29213), particularly for fluoxetine and duloxetine, which showed lower MIC_{90} and MBC values than paroxetine against both isolates (Table 1). This finding suggests that the mechanisms underlying the activity of these antidepressants are not directly affected by methicillin resistance and may bypass classical resistance pathways associated with β-lactam antibiotics. Considering that MRSA remains a high-priority pathogen in the global antimicrobial resistance agenda [22], the maintenance of antibacterial activity against the MRSA strain is a relevant aspect of the potential repurposing of fluoxetine and duloxetine.

Cytotoxicity assessment in RAW 264.7 cells demonstrated that the tested antidepressants presented EC_{50} values lower than the concentrations required for antimicrobial activity, with no statistically significant differences among the drugs. These findings indicate that, although antimicrobial activity occurs at concentrations higher than those affecting cell viability, the observed cytotoxic effects were dose-dependent and restricted to higher concentrations, which imposes limitations on direct extrapolation to the clinical context. Previous studies have highlighted that, despite *in vitro* antimicrobial activity, the effective concentrations of antidepressants often exceed those systemically achieved under conventional therapeutic regimens **[8]**. In this regard, alternative strategies such as topical application, local formulations, or combination therapy with conventional antimicrobials may represent more feasible approaches.

Biofilm formation by *S. aureus* constitutes a critical virulence factor, contributing to infection persistence and antimicrobial resistance. In the present study, fluoxetine, duloxetine, and par-

oxetine exhibited measurable antibiofilm activity, evidenced by reductions in both cellular viability and biofilm biomass. Fluoxetine and duloxetine demonstrated more consistent effects, whereas paroxetine showed less pronounced activity, particularly at intermediate concentrations. These findings are consistent with studies reporting interference of these drugs with biofilm structural organization and bacterial metabolic activity **[16,21]**.

Although our biofilm assays were conducted using the high biofilm-forming strain *S. aureus* ATCC 29213, the ability of fluoxetine and duloxetine to reduce biomass and metabolic activity in mature biofilms reinforces their potential relevance in the context of persistent infections caused by both MRSA and methicillin-susceptible strains, in which biofilm formation represents a major therapeutic challenge [16,21,23].

Despite the promising results, this study presents limitations inherent to the *in vitro* experimental model, which does not fully reproduce the complexity of pharmacokinetic and immunological interactions observed *in vivo*. Therefore, future investigations should explore strategies to enhance the antimicrobial efficacy of these drugs, including controlled-release systems, topical formulations, combination therapy with conventional antimicrobials, and evaluation in *in vivo* models, as well as detailed elucidation of the mechanisms of action involved.

From a public health perspective, the continuous rise of antimicrobial resistance reinforces the need to investigate alternative and adjuvant strategies to conventional antibiotics, particularly against pathogens associated with persistent and difficult-to-eradicate infections. In this context, the findings of the present study contribute to the broader effort to identify non-conventional agents with potential relevance in infection control **[22]**.

Conclusion

The results of this study demonstrate that the evaluated antidepressants exhibited *in vitro* antibacterial activity against *Staphylococcus aureus*, with fluoxetine and duloxetine showing superior performance compared to paroxetine under the experimental conditions adopted. However, the concentrations associated with relevant antibacterial and antibiofilm effects were approximately 20-fold higher than the EC_{50} values determined in macrophages, indicating that the antibacterial/antibiofilm doses tested in this in vitro model are accompanied by measurable cytotoxicity.

Additionally, assays performed on mature biofilms demonstrated a reduction in both metabolic viability and biofilm biomass, with more consistent effects observed for fluoxetine and duloxetine. These findings reinforce the potential of these drugs as candidates for therapeutic repurposing. However, further studies are required to elucidate their mechanisms of action and to evaluate their feasibility in more complex experimental models, particularly in scenarios that may allow local or topical administration or combination with conventional antimicrobials to minimize systemic toxicity.

References

1. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov. 2004 Aug;3(8):673–683. doi:10.1038/nrd1468.

2. Avershina E, Shapovalova V, Shipulin G. Fighting antibiotic resistance in hospital-acquired infections: current state and emerging technologies in disease prevention, diagnostics and therapy. Front Microbiol. 2021;12:707918. doi:10.3389/fmicb.2021.707918.

3. Breckenridge A, Jacob R. Overcoming the legal and regulatory barriers to drug repurposing. Nat Rev Drug Discov. 2018 Aug;17(8):559–560. doi:10.1038/nrd.2018.92.

4. Cabral VPF, et al. Antibacterial activity of paroxetine against *Staphylococcus aureus* and possible mechanisms of action. Future Microbiol. 2023;18(7). doi:10.2217/fmb-2022-0232.

5. Cederlund H, Mårdh PA. Antibacterial activities of non-antibiotic drugs. J Antimicrob Chemother. 1993 Sep;32(3):355–365. doi:10.1093/jac/32.3.355.

6. Christaki E, Marcou M, Tofarides A. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. J Mol Evol. 2020 Jan;88(1):26–40. doi:10.1007/s00239-019-09914-3.

7. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 9th ed. Wayne: CLSI; 2012. CLSI document M07-A9.

8. Foletto VS, et al. Repositioning of fluoxetine and paroxetine: study of potential antibacterial activity and its combination with ciprofloxacin. Med Chem Res. 2020;29(3):495–503. doi:10.1007/s00044-020-02507-6.

9. Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health. 2017 Jul-Aug;10(4):369–378.

10. Harbarth S, et al. Antimicrobial resistance: one world, one fight! Antimicrob Resist Infect Control. 2015;4:49. doi:10.1186/s13756-015-0091-2.

11. Hengzhuang W, Høiby N, Ciofu O. Pharmacokinetics and pharmacodynamics of antibiotics in biofilm infections of *Pseudomonas aeruginosa in vitro* and in vivo. Methods Mol Biol. 2014;1147:239–254. doi:10.1007/978-1-4939-0467-9_17.

12. Kowalska M, et al. Paroxetine-overview of the molecular mechanisms of action. Int J Mol Sci. 2021 Feb;22(4):1662. doi:10.3390/ijms22041662.

13. Muñoz-Bellido JL, Muñoz-Criado S, García-Rodríguez JA. Antimicrobial activity of psychotropic drugs: selective serotonin reuptake inhibitors. Int J Antimicrob Agents. 2000 May;14(3):177–180. doi:10.1016/S0924-8579(99)00154-5.

14. National Committee for Clinical Laboratory Standards (NCCLS). Methods for determining bactericidal activity of antimicrobial agents. Wayne: NCCLS; 1999. Approved guideline M26-A.

15. Perez-Caballero L, et al. Fluoxetine: a case history of its discovery and preclinical development. Expert Opin Drug Discov. 2014 May;9(5):567–578. doi:10.1517/17460441.2014.907790.

16. Preda VG, Săndulescu O. Communication is the key: biofilms, quorum sensing, formation and prevention. Discoveries (Craiova). 2019;7(1):e91. doi:10.15190/d.2019.13.

17. Pushpakom S, et al. Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov. 2019 Jan;18(1):41–58. doi:10.1038/nrd.2018.168.

18. Souza VA, Silva CM, Pimentel EF, et al. Antibacterial activity of duloxetine against multidrug-resistant bacteria. *Microbial Pathogenesis*. 2018;123:135–141. doi:10.1016/j.micpath.2018.07.040.

19. Rodriguez-Tudela JL. EUCAST definitive document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. Clin Microbiol Infect. 2008 Apr;14(4):398–405. doi:10.1111/j.1469-0691.2007.01935.x.

20. Rukavishnikov G, et al. Antimicrobial activity of antidepressants on normal gut microbiota: results of the *in vitro* study. Front Behav Neurosci. 2023;17:1132127. doi:10.3389/fnbeh.2023.1132127.

21. Smith K, et al. Influence of tigecycline on expression of virulence factors in biofilm-associated cells of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2010 Jan;54(1):380–387. doi:10.1128/AAC.00155-09.

22. World Health Organization. Bacterial priority pathogens list: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: WHO; 2024.

23. Yin W, et al. Biofilms: the microbial "protective clothing" in extreme environments. Int J Mol Sci. 2019 Jul;20(14):3423. doi:10.3390/ijms20143423.

Received on: October 5, 2025
Accepted on: December 20, 2025