Indian J Microbiol
https://doi.org/10.1007/s12088-024-01280-z

ORIGINAL RESEARCH ARTICLE

Antimicrobial Proficiency of Amlodipine: Investigating its Impact on *Pseudomonas spp.* in Urinary Tract Infections

Pooja Sharma[1] · Aakanksha Kalra[2] · Abhay Dev Tripathi[3] · Vivek K. Chaturvedi[4] · Bharti Chouhan[1]

Received: 14 January 2024 / Accepted: 4 April 2024

Abstract Antibiotic resistance in urinary tract infections (UTIs) is a growing concern due to extensive antibiotic use. The study explores a drug repurposing approach to find non-antibiotic drugs with antibacterial activity. In the present study, 8 strains of *Pseudomonas spp.* were used that were clinically isolated from UTI-infected patients. Amlodipine, a cardiovascular drug used in this study, has shown potential antimicrobial effect in reducing the various virulence factors, including swimming and twitching motility, biofilm, rhamnolipid, pyocyanin, and oxidative stress resistance against all the strains. Amlodipine exhibited the most potent antimicrobial activity with MIC in the range of 6.25 to 25 µg/ml. Significant inhibition in biofilm production was seen in the range of 45.75 to 76.70%. A maximum decrease of 54.66% and 59.45% in swimming and twitching motility was observed, respectively. Maximum inhibition of 65.87% of pyocyanin pigment was observed with the effect of amlodipine. Moreover, a significant decrease in rhamnolipids production observed after amlodipine treatment was between 16.5 and 0.001 mg/ml as compared to the control. All bacterial strains exhibited leakage of proteins and nucleic acids from their cell membranes when exposed to amlodipine which suggests the damage of the structural integrity. In conclusion, amlodipine exhibited good antimicrobial activity and can be used as a potential candidate to be repurposed for the treatment of urinary tract infections.

Keywords Amlodipine · Urinary tract infections · Drug repurposing · Antibacterial activity

✉ Bharti Chouhan
drsbharti.chouhan@gmail.com

[1] Department of Zoology, University of Rajasthan, Jaipur, Rajasthan 302004, India

[2] Dr. B. Lal Institute of Biotechnology, University of Rajasthan, Jaipur, Rajasthan 302017, India

[3] School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi 221005, India

[4] Department of Gastroenterology, Institute of Medical Sciences (BHU), Varanasi 221005, India

Introduction

Urinary tract infections (UTIs) are widespread bacterial infections affecting individuals globally, presenting a significant public health concern. The advancements of multidrug-resistant (MDR) strains of *Pseudomonas spp*., particularly *Pseudomonas aeruginosa*, have added a complex layer to the management of UTIs. These MDR pathogens have evolved intricate resistance mechanisms that defy traditional antibiotics, creating a pressing need for innovative treatment strategies. MDR *Pseudomonas spp*. pose a formidable challenge in healthcare due to their exceptional resistance to multiple antibiotic classes. Their resistance mechanisms, including efflux pumps, antibiotic-modifying enzymes, and membrane alterations, render them impervious to many antimicrobial agents. The proliferation of MDR strains has led to prolonged hospital stays, increased healthcare costs, and elevated morbidity and mortality rates associated with UTIs. Conventional antibiotics, historically the cornerstone of UTI treatment, are becoming increasingly ineffective against MDR Pseudomonas strains, necessitating a reevaluation of treatment approaches. This situation has catalyzed the exploration of innovative strategies, one of which is drug repurposing – the reevaluation of existing non-antibiotic drugs for potential utility in treating bacterial infections. Drug repurposing offers a promising avenue for addressing MDR *Pseudomonas* UTIs. It leverages the existing library of

Published online: 18 April 2024

drugs with established safety profiles, potentially expediting the availability of new treatments while minimizing the risks and costs of developing new antibiotics [1]. This approach has particular relevance given the slow pace of antibiotic development, which can take many years and substantial resources [2].

Non-antibiotic drugs, initially designed for various therapeutic purposes, often possess mechanisms of action distinct from traditional antibiotics. This diversity is a critical advantage. Bacteria primarily develop resistance through genetic mutations or resistance gene exchange. Non-antibiotic drugs, with novel mechanisms of action, can evade these resistance mechanisms, making it challenging for bacteria to evolve resistance. Moreover, when used in combination with antibiotics, non-antibiotic drugs can enhance treatment outcomes and reduce the likelihood of resistance emergence.

Amlodipine is among the repositioned medications that have been consolidated in the literature to be used with new therapeutic indications. These medications include cardiovascular, psychotropic, antihistamines, anti-inflammatory, local anesthetics, tranquilizers, and anti-hypersensitive [3]. Due to its hydropyridine and calcium channel blocker properties, amlodipine is frequently the first-choice medication for the scientific indication or treatment of arterial hypertension, angina, and cardiac arrhythmias. Investigation on amlodipine has suggested its antifungal properties [4] and parasite-fighting properties [5]. Moreover, it exhibits antineoplastic and antibacterial properties. These properties are not entirely understood, although it is hypothesized that the antibacterial properties arise from the compound's action on macrophages or inhibition of the efflux pump [6]. Antineoplastic effects can be caused via inducing apoptosis, changing the permeability of cells, and suppressing the G1 phase of the life cycle [1]. We propose amlodipine redirection as a successful treatment for a variety of diseases, particularly infections brought on by bacteria that are resistant to drugs, as reported in literature [7].

In conclusion, MDR *Pseudomonas* UTIs represent a significant healthcare challenge, necessitating novel treatment approaches. Drug repurposing of non-antibiotic drugs emerges as a promising alternative, addressing the urgent need to combat antibiotic resistance, expediting treatment development, and harnessing diverse mechanisms of action.

This paper explores the significance of drug repurposing, delving into its mechanisms with respect to the inhibition of swimming and twitching motilities and biofilm production. The paper contributes to the amlodipine effects on the production of various virulence factors, including rhamnolipid and phycocyanin, and challenges, with a specific focus on its role in managing UTIs caused by *Pseudomonas spp.* It aims to contribute to our understanding of this innovative approach and its potential in addressing this critical global health concern.

Materials & Methods

Materials

Dimethyl sulfoxide (DMSO), Luria–Bertani (LB) agar and broth, Mueller–Hinton-broth, Mueller Hinton agar, and Tryptone soya broth agar were bought from Sigma (St. Louis, USA). Amlodipine was bought from Dhamtech Pharmaceuticals in Maharashtra, India. All compounds were of pharmacological quality. A total of 8 UTI *Pseudomonas* strains were taken for study, including *Pseudomonas aeruginosa*, *Pseudomonas Stutzeri*, *Pseudomonas fluorescens,* and *Pseudomonas putida* and strains were clinically isolated and provided by the Dr. B.Lal Clinical Laboratory, Jaipur, India.

Estimation of MIC (Minimum Inhibitory Concentration)

MIC determination was done by antibacterial susceptibility testing [8]. The assay was performed with the collaboration of broth microdilution test with resazurin dye, which is based on the principle that the reduction of blue resazurin to the pink product resorufin is only done by metabolically active cells. The number of cells visible in pink color is metabolically active and is proportionate to this reduction [9]. Minimum inhibitory concentration (MIC) was determined as the lowest concentration in the resazurin test that showed no live cells, as indicated by a blue color. Bacterial colonies were isolated from the agar plates, which were then placed in Luria broth medium and left to grow overnight. To meet McFarland's criterion of 0.5, the bacterial suspension was adjusted. The cells were diluted with medium to a concentration of 1×10^6 cfu/ml. Amlodipine's antimicrobial properties were examined using the broth microdilution method. To dissolve amlodipine, DMSO was utilized. The chemical was synthesized at two-fold concentrations in the range of 0 µg/ml to 200 µg/ml from the stock solution. As a standard antibiotic, streptomycin was taken. A total of 200 µl each well of a 96-well plate was made by adding 5 µl of test substance and 5 µl of cells to 190 µl of LB medium. The plate was then incubated for 24 h. To ascertain the vitality of the cells, resazurin dye solution was added after a 24-h incubation period [10]. Each well received a volume of 20 µl of Resazurin, which was then incubated for two hours. Where pink showed the presence of living cells, dark purple indicated the presence of dead cells. The absorbance was measured with a microplate reader at 570 and 590 nm. The vitality of cells was calculated and expressed in percentage.

Evaluation of Biofilm Inhibition

Microtiter biofilm inhibition assay was performed to assess the capacity of amlodipine drug to suppress biofilm

development by crystal violet method with a slight modification of Abbas et.al. [11]. Aliquots containing 10 µl of each bacterial solution were roughly adjusted to an OD600 of 0.4 and combined with 1 ml of both drug-free and fresh TSB. 200 µl TSB aliquots, either drug-free or with medication, were added to the microtiter plate wells and incubated for the whole night at 37ºC. After the non-adhered planktonic cells were removed, the wells were air-dried and cleaned with phosphate buffer saline (PBS). Following a one-hour air drying period and a 25-min methanol fixation, the adhering cells were stained for fifteen minutes using 0.1% crystal violet. The wells underwent three PBS washes before being redissolved in either glacial acetic acid (33%), or 95% ethanol. Using a micro-titer reader, the OD absorbance at 590 nm analysis was determined three times. The biofilm inhibition percentage was determined according to the formula.

$$\text{Biofilm Inhibition\%} = \frac{\text{OD590(Control)} - \text{OD(Drug Treated Sample)}}{\text{OD590(Control)}} * 100$$

Swimming and Twitching Motilities Inhibition Assay

Modified from the technique of Abbas et al., the impact of amlodipine on twitching and swimming was observed[12]. To conduct the swimming experiment, swimming agar plates containing 1% tryptone,0.3% agar, and 0.5% NaCl were produced, both with and without amlodipine. A 24-h incubation period at 37 °C was followed by the insertion of 5 µl into the middle of the agar plates after the preparation and dilution of an overnight culture of *Pseudomonas spp*. in tryptone broth. The zone of swimming was measured.

In order to perform the twitching experiment, 2 µl of the generated culture was stab-inoculated into 1% LB agar plates containing amlodipine and control plates. The plates were then incubated for 48 h at 37 °C. After air-drying, the plates were subjected to crystal violet staining following agar removal. Subsequently, the dried plates underwent a water wash to eliminate any residual dye. The measurement of twitching zones was then conducted.

Pyocyanin Inhibition Assay

Pyocyanin is one prominently discovered *P. aeruginosa* virulence factor. The MIC level of pyocyanin synthesis was measured both with and without amlodipine drug. The test was employed to evaluate the effects of amlodipine on *P. aeruginosa* that was treated with MIC and that was not. The synthesis of pyocyanin by *Pseudomonas* strains was measured as previously mentioned by Das and Manefield [13]. Specific controls pertaining to drug-treated samples were employed for individual strains. After correcting to an OD600 of 0.5, the strains were cultured for a night before being utilized in the experiment. In MIC, 1 ml of broth containing and excluding amlodipine was mixed with 10 µl aliquots of the bacterial suspensions. After being centrifuged for 10 min at 11,000 rpm, the tubes were incubated for 48 h at 37º C. Pyocyanin synthesis in the supernatant was estimated using a wavelength of 691 nm. The experiment was conducted thrice, and the extent of inhibition was assessed by comparing the drug's inhibitory impact on pyocyanin production with the respective strain-specific controls.

Oxidative Stress Sensitivity Test

The potential of amlodipine to reduce resistance to oxidative stress was assessed using the revised hydrogen peroxide disc test method developed by Hassett et.al. [14]. The diameters of the inhibition zones that developed around hydrogen peroxide-loaded discs on LB agar plates streaked with *Pseudomonas species* bacteria and with amlodipine drug were measured at its minimum inhibitory concentration (MIC). *Pseudomonas* cultures in LB were produced overnight, and aliquots of 0.1 ml were evenly distributed on the LB-agar plates surface that contained amlodipine. Sterile paper discs (6 mm) were placed on LB agar plates, and 10 µl of 1.5% H_2O_2 was administered onto the discs. The same procedures were used to create control plates devoid of amlodipine. Following a 24-h incubation period at 37 °C, the inhibitory zones were identified on the plates.

Rhamnolipid Assay

The rhamnolipid production was assessed using a colorimetric orcinol-sulfuric acid test procedure [15]. To put it briefly, 1 volume of supernatant and 9 volumes of recently made 0.19% orcinol in 53% sulfuric acid were combined. After 30 min of 80ºC incubation, 15 min of RT cooling, and an OD measurement at 421 nm wavelength, the sample was examined. A standard curve between rhamnose and OD421 with a range of 0–50 µg/ml was created using rhamnose. According to the OD value and the standard curve, the rhamnolipid concentration was determined to be three times the rhamnose concentration [16]. In a medium containing 2% glucose or higher as a supplement, the orcinol-sulfuric acid test will state the observed rhamnolipid concentration in the supernatant [17].

Assessment of Amlodipine's Impact on Bacterial Protein Permeability

The approach employed by Kamurai et al. [1] was utilized to determine the possible mode of action that amlodipine employs to inhibit bacterial growth, its effect on protein leakage was investigated. The cells were treated with a

concentration of amlodipine at its MIC. To determine how much protein seeped out of bacterial cells after they were exposed to amlodipine, the Bradford technique was utilized. Cells suspended in a 0.9% saline solution at an optical density of 600 (OD600) equal to 1.5, were subjected to incubation at 37ºC with 120 rpm shaking following the addition of amlodipine at its Minimum Inhibitory Concentration (MIC) for 120 min. Afterward, the cell solution (500 µl) was centrifuged at 7000 rpm for two minutes. Protein quantification was executed using Bradford's method. In a brief procedure, 50 µl of the supernatant were mixed with 950 µl of Coomassie Brilliant Blue G-250. The absorbance at 590 nm was then measured with a spectrophotometer after a ten-minute color development period. Controls for comparison included untreated cells and 0.1% SDS. Bovine serum albumin (BSA) served as the standard for determining protein content.

Loss of 260 nm Absorbing Material

Cell lysis can be monitor by the release of UV-absorbing substances. The examination of cytoplasmic membrane leakage was made possible by monitoring the discharge of cellular constituents, including ions, metabolites, and nucleic acids, into the bacterial suspensions at 260 nm by UV spectrophotometer [18]. *Pseudomonas spp.* broth cultures in the tryptone-broth medium were left overnight and adjusted to OD600. The cells were collected by centrifuging them for 15 min at 400 rpm, discarding the supernatant, and then washing and resuspending the pellet in 1X 7.4 pH PBS (phosphate buffer saline). A MIC of amlodipine was added to the suspension of cells. The positive control was streptomycin (8 mg/ml). Three sets of experiments were conducted. As a control, drug-free cells were used. Every sample underwent a 60-min incubation period at 37 °C. Following treatment, the cell solution was centrifuged for 15 min at 13,400 rpm to extract the supernatant, and the OD 260 value was recorded to determine the proportion of extracellular UV-absorbing components that were released. Three duplicates of each measurement were taken.

Results

Analysis of MIC Values for the Bacterial Strains

The eight UTI clinical isolates of *Pseudomonas* that were used in this study were subjected to MIC testing using both the antibiotic (streptomycin) and nonantibiotic drug amlodipine (Table 1). Therapeutically significant concentrations of the non-antibiotic medication amlodipine were selected in the range of 3.12 to 200 µg/ml to assess the minimum inhibitory concentrations in order to assess the antimicrobial property of amlodipine against these microorganisms. The compound's compositions are displayed in Fig. 1. A part of the antibiotics was prepared in 10% DMSO and diluted to 0.05% DMSO for testing purposes. The positive control was streptomycin. Amlodipine's MIC ranged from 6.25 to 25 µg/ml. According to Table 1, of the eight test microorganisms, four strains *(P. aeruginosa (PA41), P. stutzeri (BE4), P. fluorescence (BE6), and P. putida (Ppt)* have demonstrated the MIC at 12.5 µg/ml; three strains *(P. aeruginosa (PA9), P. aeruginosa (PA46), and P. stutzeri (BE5)* have shown the MIC at 6.25 µg/ml, while *P. aeruginosa (PA5)* has demonstrated the highest MIC of 25 µg/ml among all.

Assay of Biofilm Inhibition

The measurement of biofilm inhibition demonstrated that the studied medication, amlodipine, could prevent biofilm development at MIC (Fig. 2). The significance of the mean difference between MIC values of amlodipine-treated and untreated *Pseudomonas* isolates in this triplicate study was determined using the one-way ANOVA. Statistical-significance was inferred for p-values below 0.05. The presented data in Table 2 represent the mean ± standard error, including the percent change from the untreated *Pseudomonas* control for each parameter. Maximum inhibition was seen in *P. putida, P. fluorescens* (BE6), and *P. stutzeri,* at 76.70%, 69.82%, and 57.33%, respectively, although *P. aeruginosa* showed less inhibition when compared to other strains.

Inhibition of Swimming and Twitching Motilities Assay

The dimensions of *Pseudomonas spp.* swimming and twitching zones on LB agar plates were assessed both in the presence and absence of amlodipine at its Minimum Inhibitory Concentration (MIC). The experiments were replicated three times for accuracy (Fig. 3). Amlodipine substantially decreased the swimming motilities of *P. aeruginosa* (PA41) to a maximum of 54.66% and a minimum of 41.90% (PA5). In contrast, to control strains, *P. fluorescence (BE6)* showed

Table 1 Distribution of minimum inhibitory concentration of Amlodipine against the isolated strains

S.no	Bacterial strains	MIC 50 (µg/ml)	MIC 90 (µg/ml)
1	*P. aeruginosa (PA5)*	25	50
2	*P. aeruginosa (PA9)*	6.25	25
3	*P. aeruginosa (PA41)*	12.5	25
4	*P. aeruginosa (PA46)*	6.25	25
5	*P.stutzeri (BE4)*	12.5	50
6	*P.stutzeri (BE5)*	6.25	12.5
7	*P. fluorescens (BE6)*	12.5	50
8	*P.putida (Ppt)*	12.5	50

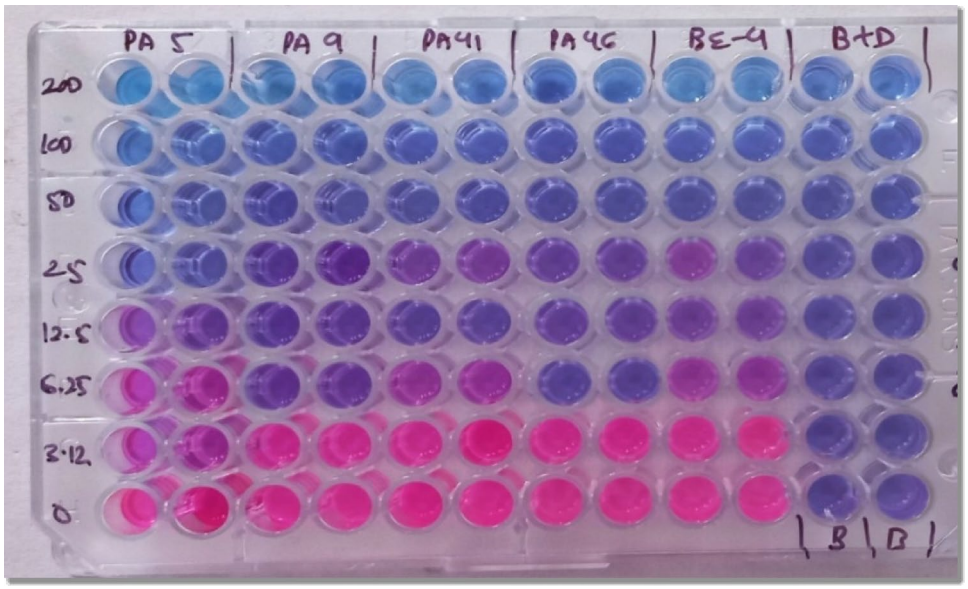

Fig. 1 Determination of MIC of Amlodipine against *Pseudomonas spp.* Strains via broth microdilution in combination with resazurin assay: The pink color represents live cells, and the blue color represents dead cells

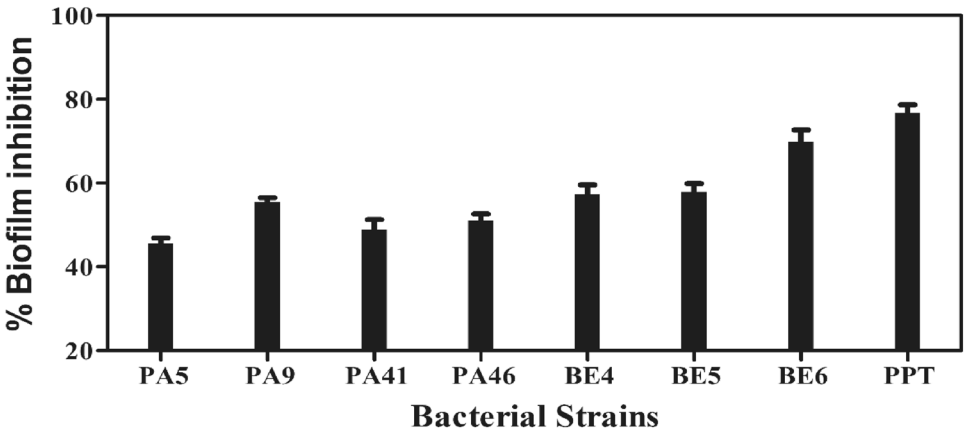

Fig. 2 Biofilm inhibitory activity of Amlodipine against all test strains

Table 2 Effect of Amlodipine on tested strains in biofilm inhibition

S.no	Bacterial strains	Percent Biofilm inhibition
1	*P. aeruginosa (PA5)*	45.75 ± 1.3210
2	*P. aeruginosa (PA9)*	55.53 ± 1.1034
3	*P. aeruginosa (PA41)*	48.75 ± 2.3782
4	*P. aeruginosa (PA46)*	51.72 ± 1.6219
5	*P.stutzeri (BE4)*	57.24 ± 2.3112
6	*P.stutzeri (BE5)*	57.93 ± 1.9870
7	*P. fluorescens (BE6)*	69.86 ± 2.8110
8	*P.putida (Ppt)*	76.70 ± 2.0212

the highest level of twitching motility inhibition at 59.45%, *while P. aeruginosa (PA5)* showed the lowest level of inhibition at 48.57%.

Pyocyanin Inhibition Assay

One significant *P. aeruginosa* virulence component that has surfaced is pyocyanin. The MIC level of pyocyanin synthesis was measured both with and without amlodipine drug. The study was conducted thrice, and the one-way ANOVA test was employed to evaluate the effects of amlodipine on *P. aeruginosa* that was treated with MIC and that was not. The significance level of the data was set at *p*-values less than 0.05. The findings were displayed as mean standard error of percentage change from untreated *P. aeruginosa* controls (Fig. 4a). Amlodipine shown a notable capacity to diminish pyocyanin synthesis and effectively reduce pyocyanin pigment production. There was no phycocyanin production seen in *P. fluorescens* and *P. stutzeri*, whereas amlodipine showed significant inhibition of phycocyanin production in different strains of *P. aeruginosa* and *P. putida.* The percentages of inhibition of pyocyanin pigment in the presence of

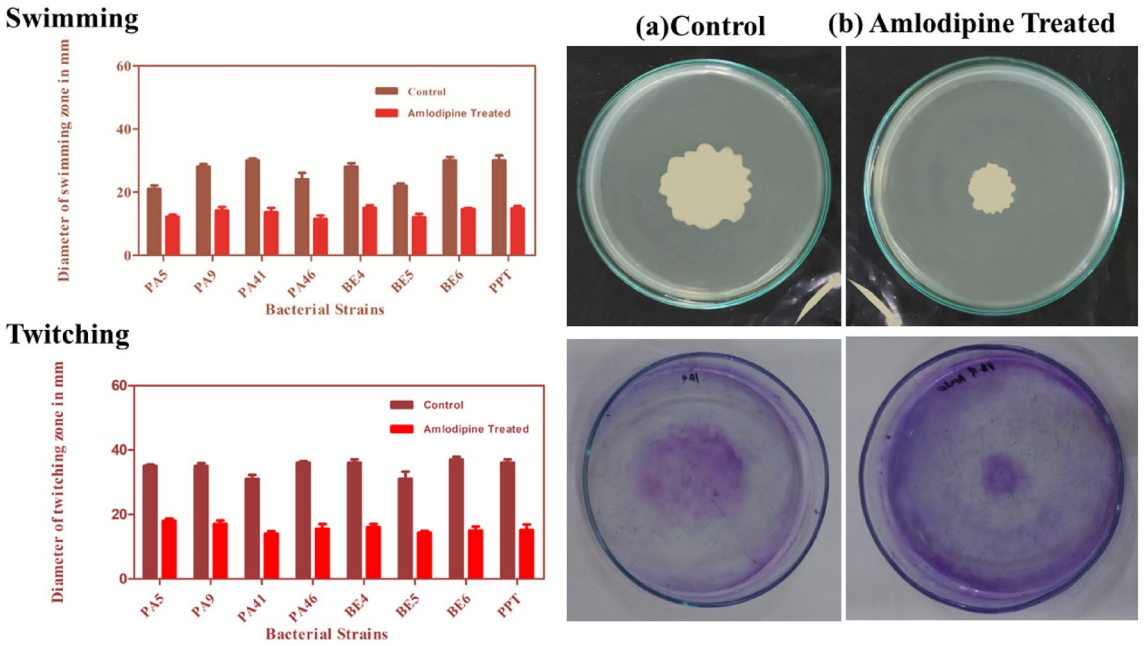

Fig. 3 Inhibition of Swimming and twitching motility by sub-MIC of amlodipine. The results are expressed as mean ± SD of three independent experiments. **a** control **b** amlodipine treated

amlodipine were seen maximum in *P. aeruginosa* and *P. putida,* respectively, were 61.25% and 65.87%.

Sensitivity to Oxidative Stress

P. aeruginosa's capacity to survive and proliferate inside cells depends critically on its resistance to oxidative stress. Measurements were made of the widths of the inhibition zones that formed around hydrogen peroxide-loaded discs on LB agar plates that were streaked with bacterial culture of *pseudomonas spp.* and contained amlodipine drug at its MIC. Amlodipine significantly increased the widths of inhibitory zones in plates, indicating that these medications can reduce *Pseudomonas spp.* resistance to oxidative stress. However, there was no discernible effect seen in *P. stutzeri* and *P. fluorescens* with amlodipine, whereas a considerable effect was seen in *Pseudomonas* resistance to oxidative stress. The experiment was conducted in triplicate. The results showed the mean ± standard error of inhibitory zones in millimeters for both *Pseudomonas* controls treated with amlodipine drug and those who were not. (Fig. 5). Amlodipine considerably enhanced the widths of inhibitory zones in plates, indicating that these medications can lessen *Pseudomonas spp*. resistance to oxidative stress. However, there

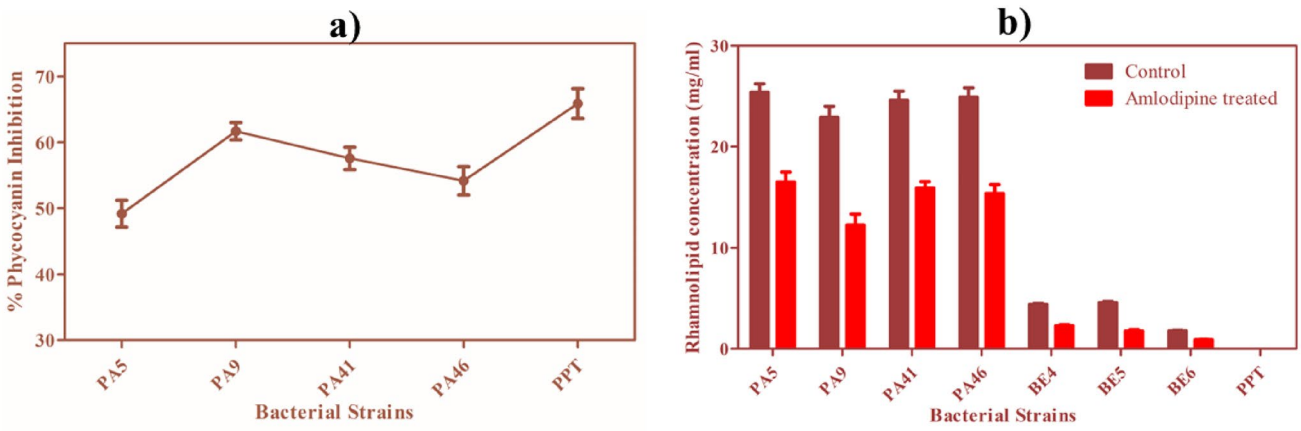

Fig. 4 **a** Effect of amlodipine on Inhibition of Phycocyanin production. **b** Effect of amlodipine on inhibition of Rhamnolipid production

was no discernible effect was seen in *P. stutzeri* and *P. fluorescens* with amlodipine, whereas a considerable effect was seen in *P. aeruginosa* and *P. putida* with 64.70% and 54.94% inhibitory activity, respectively.

Assessment of the Impact of Amlodipine on Bacterial Protein Permeability

In order to ascertain the potential mechanism of action utilized by amlodipine to impede bacterial growth, its impact on protein leakage was examined. Amlodipine concentration at its MIC was applied to the cells. The Bradford method was used to calculate the protein amount that leaked out of bacterial cells following their exposure to amlodipine. It was demonstrated that when bacteria were treated with amlodipine, the released protein concentration was raised as compared to control, and there was an increase in protein leakage (Fig. 6a). As per the data obtained, the extracellular protein released in control of *P. aeruginosa* strains was in the range of 82.76 to 90.55 µg/ml; for *P. stutzeri*, it was in the range of 87.63 to 103.21 µg/ml, and 94.44 µg/ml, 105.16 µg/ml for *P. fluorescens* and *P. Putida* respectively. Whereas the amlodipine-treated cells of *P. aeruginosa* strains were in the range of 589.07 to 687.43 µg/ml, for *P. stutzeri*, it was in the range of 535.54 to 556.96 µg/ml, and 600.77 µg/ml, 421.61 µg/ml for *P. fluorescens* and *P. putida* respectively Data obtained from amlodipine treated cells demonstrated the increase in extracellular protein released by cells as compared to control that was a clear indication of membrane damage with the effect of the drug.

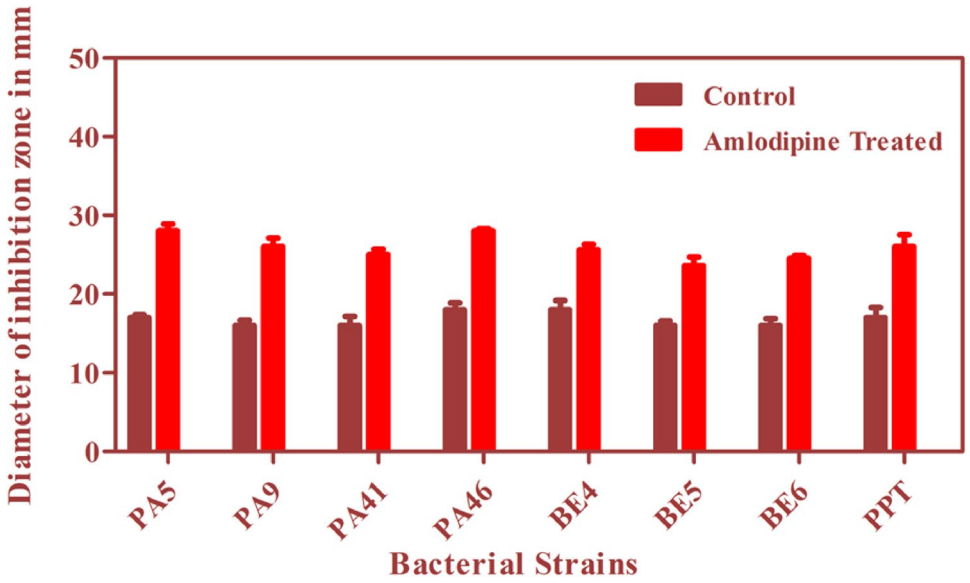

Fig. 5 Effect of amlodipine on resistance of oxidative stress on *Pseudomonas spp.* The results are expressed as mean ± SD of three independent experiments

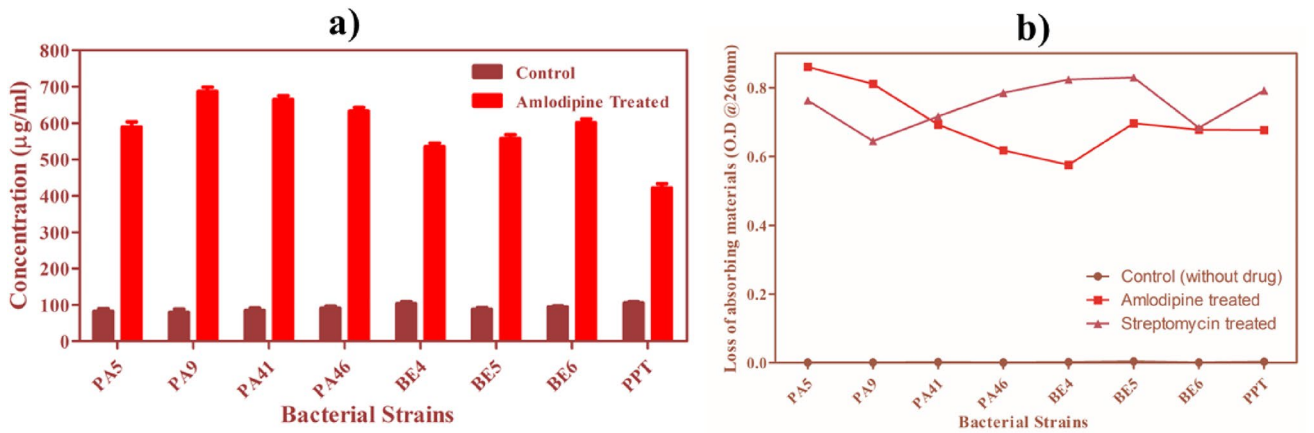

Fig. 6 **a** Effect of amlodipine on protein leakage **b** loss at 260 nm with the effect of amlodipine

Rhamnolipid Assay

It is evident from data derived from control strains of *Pseudomonas spp.* that each strain has the production of rhamnolipids (Fig. 4b). Of all the strains utilized in this study, *P. aeruginosa* produced the highest amount of rhamnolipids, ranging from 22.9 to 25.4 mg/ml. This is quite similar to the previously published work of Chong et al. [19] and Soberón-Chávez et al. [20]. Rhamnolipid produced by control strains is in the range of 25.6 to 0.005 mg/ml, significant decrease in rhamnolipid production observed after amlodipine treatment was noted between 16.5 and 0.001 mg/ml, according to the data gathered which indicates that amlodipine has the great potential in inhibition of biosynthesis of rhamnolipid that leads to decrease in the virulence of bacterial strains.

Loss of 260 nm absorbing material-

According to Zhou et al. [21], the measurement of UV-absorbing material release serves as an indicator of cell lysi. Analyzing the release of cellular components, such as ions, metabolites, and nucleic acids, into the bacterial suspensions at 260 nm allowed for the analysis of cytoplasmic membrane leakage (Bajpai et al. [22]). As indicated by Table 3 and Fig. 6b, the OD dramatically increased to 0.861 from 0.00 (P value < 0.05) following treatment with amlodipine at MIC. It appears from these findings that amlodipine causes damage to the cytoplasmic membrane, which allows intracellular components to leak out.

Discussion

The main objective of this investigation was to determine which non-antimicrobial drugs would have had the most antibacterial effects against pathogenic *Pseudomonas* strains that cause urinary tract infections. With its intricate drug resistance mechanism, *Pseudomonas* is a frequent opportunistic disease found in hospitals that poses a major risk to public health. Therefore, a popular area of study right now is investigating novel anti-infective therapeutic approaches. It was observed in a previously published study that a class of cardiovascular medicines offers intriguing research about drug redirection. Amlodipine was found to have strong antibacterial action on bacteria [23, 24]. Since amlodipine is an antihypertensive medication, it works by blocking long-lasting L-type channels, which are responsible for allowing calcium ions to enter cardiac and vascular muscle cells. Its structure has an incomplete phenothiazine ring and a halogen (chlorine) attached, both of which are essential to the compound's antibacterial action [25].A study by Yan et al. concluded that CCB might be utilized to treat a bacterial infection and can limit the development of *Pseudomonas spp.* and the expression of its virulence factors. In this existing study, Amlodipine (AML) had MIC in the range of 6.12 μg/ml to 25 μg/ml and exhibited potent antimicrobial activity against all the pathogenic strains of *Pseudomonas*. The results of this study support the previously published findings that indicate amlodipine has good antibacterial activity [26]. It also appears to be the most potential dihydropyridine Ca^{2+} channel blocker, which is used to treat hypertension. Amlodipine also demonstrated a remarkable range of MIC90 values of 10–50 µg/ml in vitro against a variety of bacterial strains [24].There can be following reasons behind the mechanism of inhibiting the growth of bacteria by amlodipine. It can be done by Inducing iron starvation response by regulating the calprotectin and by using the CCB for iron channel protein PfeA that directly affects the quorum sensing mechanism and flagellated locomotion in *Pseudomonas* to exert an antibacterial effect [27]. It may function as antagonizing the Ca^{2+} by changing the structure of drug efflux membrane protein that includes (OprN, OprM, OprP, and OpdQ) which results in an increase in the time of the drug in bacteria hence providing antibacterial activity[28].This molecule is proposed to possess potential efficacy against bacteria, including *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium, Bacillus cereus, Pseudomonas aeruginosa, and Acinetobacter baumannii* [24, 28–31]. The factors that contribute to the virulence of *Pseudomonas* include quorum-sensing, biofilm,

Table 3 Effect of amlodipine at MIC on membrane integrity in *Pseudomonas spp.* Measured by components release (from cell) of UV absorbing components at 260 nm

Strain	Without drug		Amlodipine treated		Streptomycin treated	
	Mean	SD	Mean	SD	Mean	SD
P.aeruginsa(PA5)	0.0012	±0.001	0.861	±0.002	0.763	±0.0031
P.aeruginsa (PA9)	0.001	±0.002	0.811	±0.0024	0.645	±0.0034
P.aeruginsa(PA41)	0.0022	±0.001	0.693	±0.0031	0.717	±0.0014
P.aeruginsa(PA46)	0.0011	±0.003	0.618	±0.0014	0.785	±0.0015
P.Stutzeri (BE4)	0.0024	±0.002	0.576	±0.005	0.824	±0.004
P.Stutzeri (BE5)	0.004	±0.004	0.697	±0.0021	0.83	±0.0022
P.fluorescens (BE6)	0.0013	±0.001	0.678	±0.003	0.684	±0.0041
P.putida (PPT)	0.0031	±0.003	0.677	±0.0014	0.792	±0.0018

and flagella, which provide bacterial communications and drug resistance, as well as pilli, LPS, and other processes that help in bacterial adherence and colonization to the host. Moreover, effectors and poisons are introduced into the host by secretion systems [26, 32–36]. *P. aeruginosa* frequently produces a biofilm in order to initiate a persistent infection; this is related to extracellular polysaccharide and bacterial motility [37]. The creation of biofilms has the potential to multiply bacterial drug resistance hundreds of times [38]. *P. aeruginosa's* pathogenicity is largely dependent on the synthesis of virulence factors and the synthesis of biofilms [39]. Growth at high Ca^{2+} levels increases biofilm formation in *Pseudomonas* and triggers the manufacture of many secreted virulence components, including alginate, extracellular proteases, and pyocyanin, according to research done by Guragain et al. [40–42]. According to findings of present study, Amlodipine considerably reduced the biofilm production by *Pseudomonas spp*. This decrease in biofilm activities can be related to the effect of amlodipine, a calcium channel blocker, in decreasing the Ca2 + uptake, which would lead to pathogenicity and defective cell physiology as calcium is characterized as a regulator of biofilm formation [43], with similar effect of amlodipine was also reported in findings of Gupta et al. 2016 [4]. Another reason that can be considered is the inactivation of the EstA gene that affects cellular motility, which includes swimming, twitching motility, and swarming, as well as rhamnolipid deficiency that directly affects biofilm production. *P. aeruginosa* secretes a number of lipolytic enzymes, one of these, EstA, is an autoinducer protein found in the outer membrane [44]; it helps give bacteria their virulence function [45]. EstA is a potential enzyme for these kinds of downstream processes. Wilhelm et al.'s study shows that swimming, swarming, and twitching of P. aeruginosa EstA are necessary for biofilm formation in addition to rhamnolipid synthesis since the biofilm generated by the estA mutant differed from the wild-type biofilm. *P. aeruginosa* forms biofilms by a very complicated process in which the generation of rhamnolipids, feeding, quorum sensing, and cell motility all contribute to the development of mature biofilms [46–49]. Motility is associated with bacterial adhesion and the establishment of biofilm [50]. MIC amlodipine decreased both twitching and swimming motility in our investigation, which is in line with findings that salicylic acid [51] and azithromycin [52]. Amlodipine showed a significant motility inhibitory activity against all the strains in the range of 48.57 to 59.45% and 41.90 to 54.66% in twitching and swimming motility, respectively, and this may also relate to interference with impairment of bacterial adhesion and biofilm development, which ultimately leads to decrease in virulence.

Pyocyanin is characterized by pigment secreted by *P. aeruginosa* responsible for virulence and has the capacity to oxidize and decrease other molecules, therefore eliminating competing microorganisms and mammalian cells [53]. Through a type II secretion system, *P. aeruginosa* secretes PCN into the surrounding environment [54]. It is thought that PCN's zwitter ionic characteristics and low molecular weight make it easy for the toxin to pass across cell membranes [55–57]. Because of this, reporting on PCN levels is restricted to the compartments that are directly linked to infection. It has been demonstrated that PCN intercalates with DNA via an oxidative stress-dependent process. This facilitates cell-to-cell contacts between *P. aeruginosa* cells by modulating their physicochemical interactions and cell surface characteristics. Therefore, by encouraging eDNA, it has been proposed that PCN might help in the production of biofilms and related to virulence [13]. In order to augment the virulence of *P. aeruginosa* in the present study, amlodipine inhibited the pyocyanin production in the range of 49.7 to 61.25%, although *P. aeruginosa* strain (PA5) showed lesser inhibition of phycocyanin as compared to other *P. aeruginosa* strains (PA9, PA41, PA46, BE6) this might be because of more resistance of the strain which can be related to higher MIC and lesser biofilm inhibition. Results of current findings are near about similar to the study done by Kim et al. and Rasamiravaka et al. [58, 59], whereas a higher percentage (78%) of phycocyanin inhibition was seen with the effect of aspirin [42]. We have reported the lesser inhibition (33.33%) of phycocyanin at sub-MIC of paracetamol as compared to amlodipine used in the present study [58]. As per the previous research conducted, it was reported that a multitude of pathogens produces enzymes such as catalase and superoxide dismutase (SOD) to combat the lethal effects of reactive oxygen species. For the treatment of persistent *P. aeruginosa* infections, disruption of superoxide dismutase activity may be able to overcome multidrug resistance and strengthen the effects of existing bactericidal drugs [59]. In this investigation, the presence of amlodipine's minimum inhibitory concentration (MIC) (42.22–64.70%) increased the effect of H_2O_2 and decreased tolerance to oxidative stress.

Rhamnolipid is considered an important virulence determinant of *Pseudomonas* [60]. Moreover, it plays a variety of functions, including cell lysis [61], swarming motility [62], and biofilm formation [47]. It is also a heat-stable hemolysin [63]. As per our results, all strains showed production of rhamnolipid in the range of 0.005 to 25.4 mg/ml (control values), but it was seen after treatment with amlodipine, there was a drastic reduction (0.001 to 16.5 mg/ml) in rhamnolipid supported by the Fig. 4b**,** justifying the role of amlodipine in inhibition of rhamnolipid production which is ultimately related to decrease in other virulence factors of *Pseudomonas* like biofilm production. It indicates that amlodipine has some function in inhibition of biosynthesis of rhamnolipids and has shown a greater suppression of rhamnolipid synthesis. As per the data, rhamnolipid

inhibition was observed in the range of 35 to 80% for all the strains, where maximum inhibition 80% was observed in *P. putida* and 35 to 46.72% was observed by *P. aeruginosa* which is higher as compared to sub-MIC of paracetamol reported by Saleemet al. and in range with the study done by Kim et al. on 6-gingerol [64, 65].

The current work used a protein leakage experiment to investigate the mechanism of action of amlodipine in inhibiting the growth of *Pseudomonas spp.* This was carried out in order to ascertain how amlodipine affected the bacterial membrane's integrity. One structural element that might be harmed by the antibacterial activity of amlodipine is the bacterial membrane. Bacteria classified as Gram-negative have an exterior membrane that protects their inner membrane or cell wall from antibiotics and drugs, which makes them more resistant. The cytoplasmic membrane serves as a diffusion barrier, and a compromise in its permeability leads to the leakage of proteins and nucleic acids. Targeting the membrane represents a critical strategy in the formulation of antimicrobial medications [66]. Therefore, pharmaceuticals having the capacity to disrupt membranes might be employed as antimicrobial drugs, to combat bacteria that cause chronic infections [67]. Amlodipine treatment releases protein out of bacterial cells, which is a sign of membrane damage. Based on the quantity of liberated cellular components, the extent of damage may be estimated [68]. As a sign of membrane integrity, the discharge of intracellular components. When tiny ions like potassium and phosphate are treated with the right antimicrobial agent, large molecules like DNA, RNA, and other materials seep out. The term "260-nm absorbing materials" refers to the substantial UV absorption of certain materials at 260 nm. This method is widely used to determine membrane integrity measures (Denyer et.al. 1990; Hugo and Snow, 1981). The results of this investigation suggest that amlodipine may influence *Pseudomonas* species by perforating the bacterial plasma membrane. The leakage of intracellular components supports this. Regarding NSAIDs, Ahmed et al. obtained similar findings [69].

Conclusion

Repurposing FDA-approved drugs is a viable approach to address the issue of rising anti-microbial resistance. In addition to demonstrating the possible suppression of virulence by the cardiovascular drug Amlodipine, our investigation clarified the substantial influence of this drug on biofilm formation, swarming motility, and several other virulence features shown in clinical isolates of *Pseudomonas spp*. Therefore, treating bacterial infections brought on by MDR strains of bacteria may benefit from the use of amlodipine in addition to antibiotics. It can be concluded that amlodipine has potential and can be further explored for its characteristics for UTI treatments.

Future Prospect

This study clearly demonstrates that the cardiovascular medication amlodipine has a promising antibacterial action, and it may be repurposed for antimicrobial therapy. Further research might support the hypothesis that amlodipine acts as an assistant compound by concentrating within the macrophage, which promotes intracellular killing and prevents mutation responses that lead to resistance because it may boost the antibacterial action. Research on the synergistic combination of amlodipine with other antibiotic and non-antibiotic drugs can greatly expand the scope of prolonged antibiotic therapy in bacterial infections, particularly for drug-resistant bacteria.

Acknowledgements The authors would like to acknowledge the University of Rajasthan and Dr. B. Lal Institute of Biotechnology for providing technical and lab support. ADT gratefully acknowledge DBT, India for the fellowship support (DBT award no. DBT/JRF/BET-19/I/2019/AL/140). VKC gratefully acknowledges to DHR-MoHFW, Govt of India for support through Young Scientist fellowship Grant R.12014/56/2022-HR.

Author Contributions All authors contributed to the study. PS: writing original draft, data collection, and literature search; AK: data collection, literature search, editing. ADT and VKC: editing and revision. BC: concept idea, data analysis, and revision. The final paper was reviewed and approved by all contributors.

Funding Not available.

Availability of Data and Materials Not applicable.

Declarations

Conflict of interests No financial or non-financial interests are reported.

References

1. Kamurai B, Mombeshora M, Mukanganyama S, Khamesipour F (2020) Repurposing of Drugs for Antibacterial Activities on Selected ESKAPE Bacteria Staphylococcus aureus and Pseudomonas aeruginosa. Int J Microbiol 2020:1–9. https://doi.org/10.1155/2020/8885338
2. Foletto VS, da Rosa TF, Serafin MB et al (2021) Repositioning of non-antibiotic drugs as an alternative to microbial resistance: A systematic review. Int J Antimicrob Agents 58:106380
3. Bansal KK, Goyal R, Sharma A et al (2023) Repurposing of Drugs for the Treatment of Microbial Diseases. Drug Repurposing for

Emerging Infectious Diseases and Cancer. Springer, Cham, pp 347–394
4. Gupta P, Chanda R, Rai N et al (2016) Antihypertensive, amlodipine besilate inhibits growth and biofilm of human fungal pathogen Candida. Assay Drug Dev Technol 14:291–297
5. Reimão JQ, Mesquita JT, Ferreira DD, Tempone AG (2016) Investigation of calcium channel blockers as antiprotozoal agents and their interference in the metabolism of Leishmania (L.) infantum. Evid-Based Complement Alternat Med. https://doi.org/10.1155/2016/1523691
6. Laudy AE, Kulińska E, Tyski S (2017) The impact of efflux pump inhibitors on the activity of selected non-antibiotic medicinal products against Gram-negative bacteria. Molecules 22:114
7. Coelho SS, da Rosa TF, Rampelotto RF et al (2021) Amlodipine repositioning: scientific studies and synergistic effects. Am J Ther 28:e772–e776
8. Hegazy WAH, Khayat MT, Ibrahim TS et al (2020) Repurposing anti-diabetic drugs to cripple quorum sensing in pseudomonas aeruginosa. Microorganisms 8:1285. https://doi.org/10.3390/microorganisms8091285
9. Lescat M, Poirel L, Tinguely C, Nordmann P (2019) A resazurin reduction-based assay for rapid detection of polymyxin resistance in acinetobacter baumannii and Pseudomonas aeruginosa. J Clin Microbiol. https://doi.org/10.1128/JCM.01563-18
10. Costa P, Gomes ATPC, Braz M et al (2021) Application of the resazurin cell viability assay to monitor Escherichia coli and Salmonella typhimurium inactivation mediated by phages. Antibiotics 10:974
11. Abbas HA, Hegazy WAH (2020) Repurposing anti-diabetic drug "Sitagliptin" as a novel virulence attenuating agent in Serratia marcescens. PLoS ONE 15:e0231625
12. Abbas HA, Elsherbini AM, Shaldam MA (2017) Repurposing metformin as a quorum sensing inhibitor in Pseudomonas aeruginosa. African Health Sci 17:808–819
13. Das T, Manefield M (2012) Pyocyanin promotes extracellular DNA release in Pseudomonas aeruginosa
14. Hassett DJ, Schweizer HP, Ohman DE (1995) Pseudomonas aeruginosa sodA and sodB mutants defective in manganese-and iron-cofactored superoxide dismutase activity demonstrate the importance of the iron-cofactored form in aerobic metabolism. J Bacteriol 177:6330–6337
15. Irorere VU, Tripathi L, Marchant R et al (2017) Microbial rhamnolipid production: a critical re-evaluation of published data and suggested future publication criteria. Appl Microbiol Biotechnol 101:3941–3951
16. Abalos A, Pinazo A, Infante MR et al (2001) Physicochemical and Antimicrobial Properties of New Rhamnolipids Produced by *Pseudomonas a eruginosa* AT10 from soybean oil refinery wastes. Langmuir 17:1367–1371. https://doi.org/10.1021/la0011735
17. Cheng T, Liang J, He J et al (2017) A novel rhamnolipid-producing Pseudomonas aeruginosa ZS1 isolate derived from petroleum sludge suitable for bioremediation. AMB Express 7:120. https://doi.org/10.1186/s13568-017-0418-x
18. Devi KP, Nisha SA, Sakthivel R, Pandian SK (2010) Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. J Ethnopharmacol 130:107–115
19. Chong H, Li Q (2017) Microbial production of rhamnolipids: opportunities, challenges and strategies. Microb Cell Fact 16:1–12
20. Soberón-Chávez G, González-Valdez A, Soto-Aceves MP, Cocotl-Yañez M (2021) Rhamnolipids produced by Pseudomonas: from molecular genetics to the market. Microb Biotechnol 14:136–146
21. Zhou K, Zhou W, Li P et al (2008) Mode of action of pentocin 31–1: An antilisteria bacteriocin produced by Lactobacillus pentosus from Chinese traditional ham. Food Control 19:817–822
22. Bajpai VK, Ajay Sharma AS, Baek KwangHyun BK (2014) Antibacterial mode of action of the essential oil obtained from Chamaecyparis obtusa sawdust on the membrane integrity of selected foodborne pathogens. Food Technol Biotechnol 52:109
23. Almeida HMD e S, Brandão LBS, de Melo TR, Ferreira SB (2022) Anti-Bacterial Perspective of Non-Antibiotic Drugs. In: Medical Sciences Forum. MDPI, p 22
24. Mazumdar K, Kumar KA, Dutta NK (2010) Potential role of the cardiovascular non-antibiotic (helper compound) amlodipine in the treatment of microbial infections: scope and hope for the future. Int J Antimicrob Agents 36:295–302
25. Kumar KA, Ganguly K, Mazumdar K et al (2003) Amlodipine: a cardiovascular drug with powerful antimicrobial property. Acta Microbiol Pol 52:285–292
26. Hauser AR (2009) The type III secretion system of Pseudomonas aeruginosa: infection by injection. Nat Rev Microbiol 7:654–665
27. Zygiel EM, Nelson CE, Brewer LK et al (2019) The human innate immune protein calprotectin induces iron starvation responses in Pseudomonas aeruginosa. J Biol Chem 294:3549–3562. https://doi.org/10.1074/jbc.RA118.006819
28. Yan Z, Xia L, Xu X et al (2023) Exploring calcium channel blocker as a candidate drug for Pseudomonas aeruginosa through network pharmacology and experimental validation. Chem Biol Drug Des 102:1353–1366
29. Kumar R, Kaur M, Bahia MS, Silakari O (2014) Synthesis, cytotoxic study and docking based multidrug resistance modulator potential analysis of 2-(9-oxoacridin-10 (9H)-yl)-N-phenyl acetamides. Eur J Med Chem 80:83–91
30. Hu C, Li Y, Zhao Z et al (2018) In vitro synergistic effect of amlodipine and imipenem on the expression of the AdeABC efflux pump in multidrug-resistant Acinetobacter baumannii. PLoS ONE 13:e0198061
31. Elkhatib WF, Haynes VL, Noreddin AM (2009) Microbiological appraisal of levofloxacin activity against Pseudomonas aeruginosa biofilm in combination with different calcium channel blockers in vitro. J Chemother 21:135–143
32. Veesenmeyer JL, Hauser AR, Lisboa T, Rello J (2009) Pseudomonas aeruginosa virulence and therapy: evolving translational strategies. Crit Care Med 37:1777
33. Poole K (2011) Pseudomonas aeruginosa: resistance to the max. Front Microbiol 2:65
34. Gellatly SL, Hancock REW (2013) Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis 67:159–173
35. Newman JW, Floyd RV, Fothergill JL (2017) The contribution of Pseudomonas aeruginosa virulence factors and host factors in the establishment of urinary tract infections. FEMS Microbiol Lett 364:fnx124
36. Liao C, Huang X, Wang Q et al (2022) Virulence factors of Pseudomonas aeruginosa and antivirulence strategies to combat its drug resistance. Front Cell Infect Microbiol 12:926758
37. Moradali MF, Ghods S, Rehm BHA (2017) Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol 7:39
38. Hall CW, Mah T-F (2017) Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev 41:276–301
39. Wang S, Feng Y, Han X et al (2021) Inhibition of virulence factors and biofilm formation by wogonin attenuates pathogenicity of Pseudomonas aeruginosa PAO1 via targeting pqs quorum-sensing system. Int J Mol Sci 22:12699
40. Sarkisova S, Patrauchan MA, Berglund D et al (2005) Calcium-induced virulence factors associated with the extracellular matrix of mucoid Pseudomonas aeruginosa biofilms. J Bacteriol 187:4327–4337

41. Patrauchan MA, Sarkisova SA, Franklin MJ (2007) Strain-specific proteome responses of Pseudomonas aeruginosa to biofilm-associated growth and to calcium. Microbiology (N Y) 153:3838–3851
42. Guragain M, Lenaburg DL, Moore FS et al (2013) Calcium homeostasis in Pseudomonas aeruginosa requires multiple transporters and modulates swarming motility. Cell Calcium 54:350–361
43. Kolodkin-Gal I, Parsek MR, Patrauchan MA (2023) The roles of calcium signaling and calcium deposition in microbial multicellularity. Trends Microbiol. https://doi.org/10.1016/j.tim.2023.06.005
44. Wilhelm S, Tommassen J, Jaeger K-E (1999) A novel lipolytic enzyme located in the outer membrane of Pseudomonas aeruginosa. J Bacteriol 181:6977–6986
45. Henderson IR, Nataro JP (2001) Virulence functions of autotransporter proteins. Infect Immun 69:1231–1243
46. Wilhelm S, Gdynia A, Tielen P et al (2007) The autotransporter esterase EstA of Pseudomonas aeruginosa is required for rhamnolipid production, cell motility, and biofilm formation. J Bacteriol 189:6695–6703
47. Davey ME, Caiazza NC, O'Toole GA (2003) Rhamnolipid surfactant production affects biofilm architecture in Pseudomonas aeruginosa PAO1. J Bacteriol 185:1027–1036
48. Klausen M, Aaes-Jørgensen A, Molin S, Tolker-Nielsen T (2003) Involvement of bacterial migration in the development of complex multicellular structures in Pseudomonas aeruginosa biofilms. Mol Microbiol 50:61–68
49. Klausen M, Heydorn A, Ragas P et al (2003) Biofilm formation by Pseudomonas aeruginosa wild type, flagella and type IV pili mutants. Mol Microbiol 48:1511–1524
50. Arora SK, Neely AN, Blair B et al (2005) Role of motility and flagellin glycosylation in the pathogenesis of Pseudomonas aeruginosa burn wound infections. Infect Immun 73:4395–4398
51. Guo M, Gamby S, Zheng Y, Sintim H (2013) Small molecule inhibitors of AI-2 signaling in bacteria: state-of-the-art and future perspectives for anti-quorum sensing agents. Int J Mol Sci 14:17694–17728. https://doi.org/10.3390/ijms140917694
52. Nalca Y, Jänsch L, Bredenbruch F et al (2006) Quorum-sensing antagonistic activities of azithromycin in Pseudomonas aeruginosa PAO1: a global approach. Antimicrob Agents Chemother 50:1680–1688
53. Mavrodi DV, Bonsall RF, Delaney SM et al (2001) Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from Pseudomonas aeruginosa PAO1. J Bacteriol 183:6454–6465
54. Hall S, McDermott C, Anoopkumar-Dukie S et al (2016) Cellular effects of pyocyanin, a secreted virulence factor of Pseudomonas aeruginosa. Toxins (Basel) 8:236
55. Turner JM, Messenger AJ (1986) Occurrence, biochemistry and physiology of phenazine pigment production. Adv Microb Physiol 27:211–275
56. Lau GW, Hassett DJ, Ran H, Kong F (2004) The role of pyocyanin in Pseudomonas aeruginosa infection. Trends Mol Med 10:599–606
57. Baron SS, Rowe JJ (1981) Antibiotic action of pyocyanin. Antimicrob Agents Chemother 20:814–820
58. El-Mowafy SA, Abd El Galil KH, El-Messery SM, Shaaban MI (2014) Aspirin is an efficient inhibitor of quorum sensing, virulence and toxins in Pseudomonas aeruginosa. Microb Pathog 74:25–32. https://doi.org/10.1016/j.micpath.2014.07.008
59. Martins D, McKay G, Sampathkumar G et al (2018) Superoxide dismutase activity confers (p) ppGpp-mediated antibiotic tolerance to stationary-phase Pseudomonas aeruginosa. Proc Natl Acad Sci 115:9797–9802
60. Reis RS, Pereira AG, Neves BC, Freire DMG (2011) Gene regulation of rhamnolipid production in Pseudomonas aeruginosa–a review. Bioresour Technol 102:6377–6384
61. Jensen PØ, Bjarnsholt T, Phipps R et al (2007) Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by Pseudomonas aeruginosa. Microbiology (N Y) 153:1329–1338
62. Deziel E, Lepine F, Milot S, Villemur R (2003) rhlA is required for the production of a novel biosurfactant promoting swarming motility in Pseudomonas aeruginosa: 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology (N Y) 149:2005–2013
63. Abdel-Mawgoud AM, Lépine F, Déziel E (2010) Rhamnolipids: diversity of structures, microbial origins and roles. Appl Microbiol Biotechnol 86:1323–1336
64. Seleem NM, Atallah H, Abd El Latif HK et al (2021) Could the analgesic drugs, paracetamol and indomethacin, function as quorum sensing inhibitors? Microb Pathog 158:105097
65. Kim H-S, Lee S-H, Byun Y, Park H-D (2015) 6-Gingerol reduces Pseudomonas aeruginosa biofilm formation and virulence via quorum sensing inhibition. Sci Rep 5:8656
66. Epand RM, Walker C, Epand RF, Magarvey NA (2016) Molecular mechanisms of membrane targeting antibiotics. Biochimica et Biophysica Acta (BBA) Biomembranes 1858:980–987
67. Mun S-H, Kim S-B, Kong R et al (2014) Curcumin reverse methicillin resistance in Staphylococcus aureus. Molecules 19:18283–18295
68. Wu Y, Bai J, Zhong K et al (2016) Antibacterial activity and membrane-disruptive mechanism of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a novel phenolic compound from pine needles of Cedrus deodara, against Staphylococcus aureus. Molecules 21:1084
69. Ahmed EF, El-Baky RMA, Ahmed ABF et al (2017) Antibacterial activity of some non-steroidal anti-inflammatory drugs against bacteria causing urinary tract infection. Am J Infect Dis Microbiol 5:66–73