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Evaluation of Antibiofilm Activity of Daphnetin (Coumarin derivative) against *Pseudomonas Aeruginosa*

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ABSTRACT

Nowadays, most *P.aeruginosa* isolates are multidrug resistant (MDR), so the need for a new drug intervention is increasing. This study aimed to investigate the effect of a natural coumarin derivative, Daphnetin, against *P.aeruginosa,* particularly its biofilm. Also, the incidence of biofilm formation and the antibiotic susceptibility pattern among isolates were detected. A cross sectional study was conducted in intensive care unit (ICU) involving fifty patients that showed signs of infection and after taking a proper history for the participants the samples taken underwent the following: Sample culture, identification of isolates by colony morphology, Gram stain, biochemical tests, antibiotic susceptibility testing by Kirby-Bauer method then detection of biofilm formation by Micro titer plate assay (MPA) after that evaluation of the effect of Daphnetin as following: preparation of Daphentin (DAP) stock solution, making serial dilutions of DAP (0.055-1.781mg/ml), assessing the bacterial growth treated with these different concentrations using optical density (OD_{600}). The results showed that *P.aeruginosa* was the most prevalent pathogen common in burn swabs (42.9%), followed by endotracheal aspirates (28.5%). Most isolates were biofilm producers (71.4%), 28.6% of isolates were sensitive to amikacin, ciprofloxacin, meropenem, and imipenem, and 42.9% of isolates were sensitive to piperacillin, tazobactam, and ceftazidime. At the effective DAP dosage (0.445–1.781 mg/mL), the amount of biofilm progressively declined. It was concluded that *P.aeruginosa* is an important opportunistic pathogen. Management of biofilm-forming *P.aeruginosa* is more difficult. DAP plays a consequential role in the inhibition of biofilm formation

Keywords: Antibiotics, Biofilm, Daphnetin, Multi-drug resistance *Pseudomonas aeruginosa*.

1. Introduction

Pseudomonas aeruginosa is classified as a Gram-negative pathogenic bacterium. It has the potential to provoke hospital-acquired infections, particularly among immunocompromised individuals (Maita and Boonbumrung, 2014; Pachori et al., 2019). Furthermore, this bacterium has rapidly developed antibiotic resistance that can subsequently lead to recurrent, chronic infections (Reynolds and Kollef, 2021). *P. aeruginosa* is renowned for its capacity to build biofilms during infections caused by diverse virulence factors, resulting in persistent infections (Arciola et al., 2012; Azam and Khan, 2019). Biofilm is viewed as a distinct microenvironment that comprises diverse regions with varying concentrations of nutrients and bacterial secretions. This variability results in varying degrees of bacterial resistance (Wang et al., 2022). Consequently, considerable effort was devoted to understanding the process of biofilm formation. *P. aeruginosa* quorum-sensing (QS) systems are essential to its capacity to form biofilms. These systems coordinate many functions, including the formation of several virulence factors, bioluminescence, and plasmid conjugation (Fan et al., 2022; Zeng et al., 2023). There are different kinds of QS systems produced by *P. aeruginosa*, including the Las system (Brexó and Sant'Ana, 2018). The genes encoding virulence agents, e.g., those regulating biofilm formation, are governed by the collaborative function of these quorum-sensing circuits, facilitating the dissemination of these genes among organisms (Senturk et al., 2012; Soto-Aceves et al., 2023). The emergence of antimicrobial-resistant *P. aeruginosa* constitutes a substantial threat, carrying profound implications for healthcare. Especially in critical care settings, resistant strains represent about 21% in the intensive care units (ICU) (Eid et al., 2025). This high prevalence could be attributed to excessive antibiotic use and inappropriate antimicrobial stewardship. Patients in the ICU have an elevated susceptibility to infections owing to compromised host defences, a protracted immune response, and the utilisation of several treatments and invasive devices (Pachori et al., 2019), which in turn increases morbidity and mortality. The existing antibiotic regimen for *P.aeruginosa* is diminishing in effectiveness due to the rising incidence of drug resistance. Consequently, it is imperative to identify medications or natural compounds that possess antimicrobial and anti-biofilm properties, which can render infections more susceptible to antibiotics.

Natural substances are receiving more and more attention these days because of their exceptional antimicrobial properties and uncommon adverse effects (Swetha et al., 2019). Besides overcoming resistance, medications made from Chinese herbs make bacteria more vulnerable to many non-natural medications (Qi et al., 2022). Because of the widespread development of antibiotic resistance brought on by antibiotic misuse, it is now crucial to employ non-antibiotic agents, like harmless natural chemicals, in addition to or instead of conventional antibiotics to control harmful microorganisms (Ye et al., 2020). Coumarins are a class of phenolic chemicals originating from botanical sources. Their antimicrobial properties have garnered significant attention (El-Sawy et al., 2024). Consequently, coumarins are becoming noteworthy contenders for the advancement of next-generation antimicrobial agents. The coumarin family possesses significant biological characteristics, notably their role as effective anti-virulent agents and inhibitors of the formation of biofilms in a diverse array of pathogens (Reen et al., 2018; Qais et al., 2021). Preventing the development of a bacterial biofilm is a useful strategy to strengthen the antibacterial activity of medications and raise the susceptibility of infections to medicines (Thakur et al., 2020, Fatima et al., 2023). Daphnetin (DAP) is a coumarin derivative utilised in traditional Chinese herbal medicine, demonstrating efficacy in anticoagulation, neuroprotection (Zhang et al., 2020), anti-inflammation, and anticancer (Mohamed et al., 2014, Wu et al., 2019). Additionally, *Ralstonia solanacearum's* antibacterial activity and anti-biofilm impact were demonstrated by DAP (Yang et al., 2016, Yang et al., 2021). It is crucial to recognise that the decrease in biofilm formation by this coumarin derivative, in contrast to antibiotics that focus on inhibiting cell growth, stems from its specific antibiofilm activity rather than its antimicrobial properties (Luciardi et al., 2020). Therefore, the risk of drug resistance may be mitigated by specific coumarins that function as biofilm inhibitors but do not impede bacterial growth (Qiu et al., 2025). This study focused on the evaluation of the effect of DAP on biofilm formation, detection of the patterns of antibiotic sensitivity and resistance among various isolates, and detection of biofilm formation by *P. aureginosa*.

Materials and methods

Drugs and chemicals

Daphnetin (DAP) was purchased in powder form from Enzo Biochem, Inc, USA (Catalog no.: ALX-270-281)

Study design and testing

A cross-sectional study, which involved patients in the ICU at Tanta University Hospitals, was conducted in the Medical Microbiology, Immunology, and Pharmacology Departments at the Faculty of Medicine, Tanta University. All research participants provided their written consent before beginning the study. The ethics and research committee of Tanta University's Faculty of Medicine granted ethical permission for this work. The protocol's approval code is: 36264PR956/11/24.

Every patient underwent the following:

1. Taking a history that includes the patient's age, sex, underlying condition, onset, progression, and length of illness, and an antibiotic-based treatment plan.
2. Clinical examination: this includes a comprehensive examination with a focus on looking for infection-related symptoms such as fevers greater than 38 °C, chills, rigors, erythema, edema, and tenderness in SSI, chest infection.

Criteria for inclusion

Patients who exhibit signs of infection, such as burns, pneumonia, UTIs, etc., upon admission to Tanta University Hospital

Criteria for exclusion

Individuals at various outpatient clinics.

Microbiological study

Sampling: Fifty samples were obtained in a strictly aseptic environment. The samples were labeled and sent to the Department of Microbiology and Immunology laboratory. The samples included urine samples, endotracheal aspirates, wound swabs, and more. After that, the collected samples were cultured on MacConkey (Oxoid, UK), nutritional agar, and blood agar before being incubated for 24 hours at 37 °C (Huang et al., 2009, Sandman and Ecker, 2014). Gram-stained film, colony morphology, and biochemical processes (oxidase test, TSI) were used to identify the isolates (Cobos-Trigueros et al., 2017, Quan et al., 2023). The Kirby-Bauer disk diffusion method over Mueller-Hinton agar was used to test the *P.aeruginosa* isolates for antibiotic sensitivity (Hombach et al., 2013; Institute, 2024). Regarding the biochemical identification of the *P.aeruginosa* isolates, the oxidase test was done by oxidase detection strips (Oxoid, UK).

***P. aeruginosa* biofilm formation detection using the Microtiter plate assay (MPA)**

Bacterial adherence to an antibiotic surface can be observed using the microtiter plate experiment for biofilm formation research. The dye crystal violet (CV) was used to quantify the degree of biofilm development, and the ELISA plate reader was used to measure each well's optical density (λmax = 551 nm) (Stepanović et al., 2007; Nasirmoghadas et al., 2018).

Determination of DAP minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of DAP against *P.aeruginosa* was designated using the Nature method (Belanger and Hancock, 2021). Briefly, Preparation of DAP stock solution: According to the following equation (C= M/V, where C= concentration of DAP, M=drug mass, V= volume of DMSO), Determination of dilution factor (DF): To make lower concentrations from our starting stock concentration (3.562mg/ml) using the following formula: DF =C stock/C desired, 5 µL of the bacterial solution was added to the test well, the optical density (OD_{600})was measured to determine the amount of bacterial growth in each well, the MIC was assessed using the turbidity of the culture.

Detection of biofilm inhibition activity of DAP against *P.aeruginosa*

DAP and bacterial suspensions were mixed at several doses (0.055–1.781 mg/mL). OD_{600} was used to assess the bacterial growth of *P.aeruginosa* treated with different dosages of DAP (Haney et al., 2021).

3. Results

Distribution of studied subjects according to epidemiological factors

Fifty samples were compiled from patients in the ICU of Tanta University Hospital. The studied cases had the following criteria: males (n=30,60%), females (n=20,40%). The predominant age group was between forty and fifty years, representing fifty percent of cases. Forty cases (80% of cases) had a positive history of previous antibiotic intake and had underlying chronic disease, as shown in Table 1.

Table 1. Distribution of studied subjects according to epidemiological factors

Epidemiological factor	No.	%
Age		
20-30 years	10	20.0%
40-50 years	25	50.0%
60-70 years	15	30.0%
Sex		
Male	30	60.0%
Female	20	40.0%
Previous antibiotic intake		
Positive	40	80.0%
Negative	10	20.0%
Underlying chronic disease		
Present	40	80.0%
Absent	10	20.0%

Distribution of studied subjects according to the isolated pathogen (n=50)

Thirty-five (70%) of the collected samples that underwent culture on MacConkey plate showed non-lactose fermenter colonies (NLF) of *P. aeruginosa* and were oxidase positive. Other organisms that were detected during our study, representing 30% of the cases studied, were *CONS, Staphylococcus aureus, and Acinetobacter baumanii,* respectively, as shown in Table 2.

Antibiotic susceptibility profile of *P. aeruginosa* isolates according to CLSI (2024)

The thirty –five samples of *P.aeruginosa* were cultured on Muller –Hinton agar for detection of antibiotic susceptibility pattern, the results were as following 28.6% (n=10) of isolates were sensitive to amikacin, ciprofloxacin, meropenem and imipenem, 42.9% (n=15) of isolates were sensitive to piperacillin, tazobactam and ceftazidime indicated the presence of marked antibiotic resistance among the Pseudomonas isolates as shown in Table 3.

Table 2. Distribution of studied subjects according to the isolated pathogens

Isolated pathogen	No	%Percentage
P. aeruginosa	35	70.0%
Coagulase-negative Staphylococci (CONS)	7	14.0%
S.aureus	5	10.0%
Acinetobacter	3	6.0%

Distribution of *P.aeruginosa* isolates according to the type and sources of specimens

The specimens were collected from different sources, as shown in Table 4. *P.aeruginosa* isolates were more common in burn swab (42.9%, n=15), followed by endotracheal aspirate (28.5%, n=10), then wound and urine (14.3%, n=5).

Table 3. Antibiotic susceptibility profile of *P. aeruginosa* isolates

Drug	Disc content (µg)	Susceptible	%
Amikacin	30	10	28.5%
Cefepime	30	5	14.3%
Cefoperazone-sulbactam	30	5	14.3%
Ceftazidime	30	15	42.8%
Ciprofloxacin	5	10	28.6%
Imipenem	10	10	28.6%
Levofloxacin	5	5	14.3%
Meropenem	10	10	28.6%
Piperacillin-tazobactam	110	15	42.9%

Table. 4. Distribution of Pseudomonas isolates according to the type and sources of specimens

Type of specimen	No. of isolates	%
Burn	15	42.9%
Wound	5	14.3%
Endotracheal aspirate	10	28.5%
Urine	5	14.3%

Distribution of isolated *P.aeruginosa* according to Biofilm formation by Microtiter plate assay (MPA)

Twenty-five samples of *P.aeruginosa* isolates were biofilm producers that represented 71.4% of the *Pseudomonas* isolates, as shown in Table 5.

Table. 5. Distribution of isolated P. aeruginosa according to Biofilm formation by MPA

Number of isolates	Biofilm producer	Non-biofilm producer
35	25 (71.4%)	10(28.6%)

Effect of daphnetin (DAP) at different concentrations on *P.aeruginosa*

Effect of DAP in different concentrations on biofilm formation by Pseudomonas, at the effective dosage (0.445–1.781 mg/mL), the amount of biofilm formation progressively declined, and the difference was substantially significant ($p< 0.05$) in comparison to the positive control group, as shown in Table 6 and Fig.1.

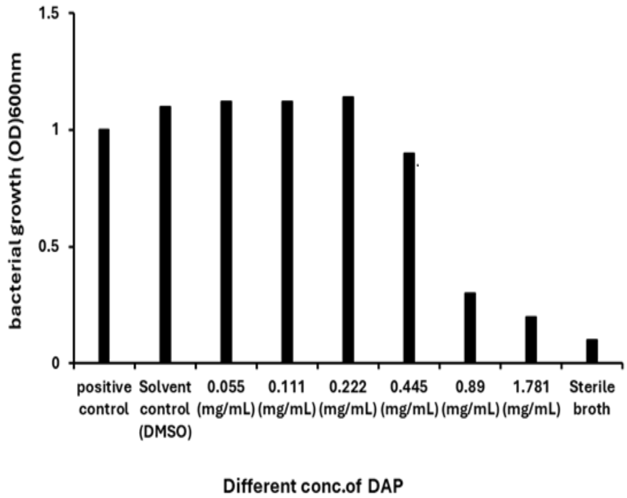

Fig. .1. OD_{600} was used to assess the bacterial growth treated with different dosages of DAP

Table 6. Effect of different concentrations of DAP solution on *P.aeruginosa*

Different concentrations of DAP	Turbidity
positive control	++
Solvent control (DMSO)	++
0.05 (mg/mL)	++
0.11 (mg/mL)	++
0.22 (mg/mL)	++
0.445 (mg/mL)	++
0.89 (mg/mL)	-
1.781 (mg/mL)	-
Sterile broth	-

(++) represented noticeable turbidity, and (-) a clear solution.

4. DISCUSSION

One common bacterium that forms biofilms is *P. aeruginosa* (Lima et al., 2018). Coordinating gene expression and QS systems can secrete virulence factors and establish drug resistance. Since *P. aeruginosa* biofilm formation, drug resistance, virulence agent expression, and motility are all attributed to the QS system (Pang et al., 2019). Creating biofilm-inhibiting drugs may provide a novel approach to antibacterial and antibiofilm therapy. According to recent research, Certain botanical substances have anti-biofilm properties (D'almeida et al., 2017). An extract from plants called daphnetin (DAP) has been shown to have antimicrobial and anti-biofilm properties, and showed a significant decrease in *Ralsonia solanacearum* pathogenicity (Yang et al., 2021). Our study showed that DAP could prevent the production of biofilms by *P. aeruginosa,* similar to the results of a former literature study that reported that DAP reduced biofilm formation (Luo et al., 2017).

Our study involved fifty patients in the ICU, most of whom were elderly, had chronic underlying diseases, and had a positive history of previous antibiotic intake. These factors favor colonization and infection by opportunistic pathogens such as *Pseudomonas*. The epidemiological factors identified in our investigation were corroborated by additional research (Chalise et al., 2008). As regards the distribution of the isolated pathogens throughout our investigation, *Pseudomonas aeruginosa* was the most frequently isolated bacterium. This could be attributed to the nature of patients involved in the study and the source of specimens, which is in concordance with other studies (Gupta et al., 2015, Reddy et al., 2025). The antibiotic profile of the *P.aeruginosa* isolates revealed marked antibiotic resistance that may be due to the constitutive release of several enzymes. This bacterium exhibits inherent resistance to many classes of antibiotics, further enhanced by chromosomal mutations or the acquisition of extrachromosomal DNA. Additionally, biofilms are crucial for antibiotic resistance (Nasirmoghadas et al., 2018, Ribeiro et al., 2019). A further investigation revealed that isolates exhibiting both MDR and non-MDR phenotypes did not reveal substantial changes regarding the virulence determinants examined, such as biofilm formation and various motilities, most probably because of the microorganism's ability to adapt, expressing its virulence factors solely in circumstances essential for survival, or it could be that the in vitro methodologies employed were not sufficiently advanced to identify its association (Kritsotakis et al., 2017; Gajdács et al., 2021). Regarding the kinds and origins of *Pseudomonas aeruginosa* samples that were isolated during our investigation, burn swabs had the highest prevalence of *P.aeruginosa* isolates. *This* can be explained by the fact that the organism is an opportunistic, commonly biofilm-forming bacterium, and burn patients are more prone to infection due to being an immuno-compromised host, which is supported by Sharma et al. (2015).

Sharma et al. (2015), and Roy et al. (2024) concerning the distribution of *P.aeruginosa* isolates based on biofilm formation, the majority of isolates were identified as biofilm producers via MTP**.** This is consistent with another study performed in Hungary and Cameroon (Kádár et al., 2010; Chimi et al., 2024). Regarding the effect of DAP at different concentrations on biofilm formation by *P.aeruginosa*, our results indicated that DAP could prevent *P.aeruginosa* biofilm formation, and this inhibitory effect grew steadily as the concentration increased. Biofilm development was not inhibited by DAP when the concentration was less than 0.445 mg/mL. Additionally, the medication solvent (DMSO diluted 100-fold) did not influence biofilm genesis, and the sterile broth group did not exhibit any biofilm formation. Also, each group's OD600 matched the DAP MIC of 0.890 mg/mL, as well as demonstrating that when DAP concentration was less than 0.890 mg/mL, it had no direct bactericidal or bacteriostatic impact on *P. aeruginosa***.** This is supported by another study performed in China concerning the inhibitory effect of DAP on biofilm formation and motility of *P.aeruginosa* (Ye et al., 2022). These findings are consistent with other studies that support anti biofilm of properties of DAP against other organisms as *R. solanacearum* by marked reduction of extracellular polysaccharides (EPS) production and inhibiting the expression of EPS biosynthesis genes (epsA, epsP, epsB, epsC, wecC, epsE, and epsF) and the transcriptional regulator factor (xpsR), delaying tobacco bacterial wilt disease progression and reducing bacterial density in the stem and antifungal properties against some candida species by suppressing the formation of elongated fungal forms, the growth rate of established biofilms, and biofilm formation on the surface of coverslips (Lemos et al., 2020, Li et al., 2022).

Conclusions

Pseudomonas aeruginosa represents a significant opportunistic pathogen associated with numerous nosocomial infections, especially in immunocompromised individuals. Pseudomonas also has marked antibiotic resistance to several classes of antibiotics. Several factors contribute to colonization by *P.aeruginosa*, including the presence of a catheter and patients on a ventilator. Biofilm-forming Pseudomonas are more difficult to manage and cause more severe infections than non-biofilm producers.

Biofilm contributes to the emergence of antibiotic resistance in *P.aeruginosa* isolates. Various non-antibiotic (natural) herpetic agents can significantly contribute to the control of bacterial resistance, such as DAP.

DAP is a natural herpetic agent with antibacterial activity against *Pseudomonas,* making it a promising agent in the management of such infections, particularly in antibiotic-resistant strains, but it requires further research to be used in human therapy and clinical trials.

Limitations of the study:

DAP demonstrated antimicrobial effects against *P. aeruginosa* at high concentrations and anti-biofilm effects at concentrations that do not inhibit bacterial growth. The QS system plays an essential role in regulating P. aeruginosa biofilm formation. Still, the mechanism underlying the anti-QS system of DAP was not explored, which is a limitation of the study. Therefore, further studies are needed to investigate this mechanism. Moreover, further research is needed to clarify the pharmacokinetics and pharmacodynamics of DAP in animals infected with *P. aeruginosa* biofilms before it can enter clinical trials for future human use.

Declaration and Conflicts of interest

The authors have no conflicts of interest.

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References

Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW, 2012. Biofilm formation in *Staphylococcus* implant infections: a review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials*. 33: 5967-5982.

Azam MW, Khan AU, 2019. Updates on the pathogenicity status of *Pseudomonas aeruginosa*. *Drug Discov Today*. 24: 350-359.

Belanger CR, Hancock RE, 2021. Testing physiologically relevant conditions in minimal inhibitory concentration assays. *Nat Protoc*. 16: 3761-3774.

Brexó RP, Sant'Ana AS, 2018. Microbial interactions during sugar cane must fermentation for bioethanol production: does quorum sensing play a role? *Crit Rev Biotechnol*. 38: 231-244.

Chalise P, Shrestha S, Sherpa K, 2008. Epidemiological and bacteriological profile of burn patients at Nepal Medical College Teaching Hospital. *Nepal Med Coll J*. 10: 233-237.

Chimi LY, Noubom M, Bisso BN, Singor Njateng GS, Dzoyem JP, 2024. Biofilm formation, pyocyanin production, and antibiotic resistance profile of *Pseudomonas aeruginosa* isolates from wounds. *Int J Microbiol*. 2024: 1207536.

Cobos-Trigueros N, Zboromyrska Y, Morata L, Alejo-Cancho I, Calle CD, Vergara Gómez A, Cardozo Espinola C, Arcas MP, Soriano Viladomiu A, Marco Reverte F, 2017. Time-to-positivity, type of culture media and oxidase test performed on positive blood culture vials to predict *Pseudomonas aeruginosa* in patients with Gram-negative bacilli bacteraemia. *Rev Esp Quimioter*. 30(1): 9-13.

D'Almeida RE, Molina RDI, Viola CM, Luciardi MC, Peñalver CN, Bardón A, Arena ME, 2017. Comparison of seven structurally related coumarins on the inhibition of quorum sensing of *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. *Bioorg Chem*. 73: 37-42.

Eid R, Dabar G, Hanna L-R, Saliba G, Riachy M, Choucair J, Saliba R, 2025. Comparison of antimicrobial resistance in *Pseudomonas aeruginosa* from intensive care and non-intensive care units and its impact on treatment decisions. *Sci Rep*. 15: 11288.

El-Sawy ER, Abdel-Aziz MS, Abdelmegeed H, Kirsch G, 2024. Coumarins: quorum sensing and biofilm formation inhibition. *Molecules*. 29: 4534.

Fan Q, Wang H, Mao C, Li J, Zhang X, Grenier

D, Yi L, Wang Y, 2022. Structure and signal regulation mechanism of interspecies and interkingdom quorum sensing system receptors. *J Agric Food Chem*. 70: 429-445.

Fatima M, Amin A, Alharbi M, Ishtiaq S, Sajjad W, Ahmad F, Ahmad S, Hanif F, Faheem M, Khalil AAK, 2023. Quorum quenchers from *Reynoutria japonica* in the battle against methicillin-resistant *Staphylococcus aureus* (MRSA). *Molecules*. 28: 2635.

Gajdács M, Kárpáti K, Nagy ÁL, Gugolya M, Stájer A, Burián K, 2021. Association between biofilm-production and antibiotic resistance in *Escherichia coli* isolates: a laboratory-based case study and a literature review. *Acta Microbiol Immunol Hung*. 68: 217-226.

Gupta S, Mahmood U, Gurung S, Shrestha S, Kushner A, Nwomeh BC, Charles A, 2015. Burns in Nepal: a population-based national assessment. *Burns*. 41: 1126-1132.

Haney EF, Trimble MJ, Hancock RE, 2021. Microtiter plate assays to assess antibiofilm activity against bacteria. *Nat Protoc*. 16: 2615-2632.

Hombach M, Zbinden R, Böttger EC, 2013. Standardisation of disk diffusion results for antibiotic susceptibility testing using the SIRscan automated zone reader. *BMC Microbiol*. 13: 225.

Huang C-Y, Hsieh S-P, Kuo P-A, Jane W-N, Tu J, Wang Y-N, Ko C-H, 2009. Impact of disinfectant and nutrient concentration on growth and biofilm formation for a *Pseudomonas* strain and mixed cultures from a papermachine system. *Int Biodeterior Biodegradation*. 63: 998-1007.

Institute CLS, 2024. Performance standards for antimicrobial susceptibility testing; 34th informational supplement. *CLSI document*, Wayne PA.

Kádár B, Szász M, Kristóf K, Pesti N, Krizsan G, Szentandrássy J, Rókusz L, Nagy K, Szabó D, 2010. In vitro activity of clarithromycin in combination with other antimicrobial agents against biofilm-forming *Pseudomonas aeruginosa* strains. *Acta Microbiol Immunol Hung*. 57: 235-245.

Kritsotakis EI, Flora K, Eirini A, Maria R, Eleni I, Gikas A, 2017. Prevalence, incidence burden, and clinical impact of healthcare-associated infections and antimicrobial resistance: a national prevalent cohort study in Greece. *Infect Drug Resist*. 10: 317-328.

Lemos AS, Florencio JR, Pinto NC, Campos LM, Silva TP, Grazul RM, Pinto PF, Tavares GD, Scio E, Apolonio ACM, 2020. Antifungal activity of the natural coumarin scopoletin against planktonic cells and biofilms of multidrug-resistant *Candida tropicalis*. *Front Microbiol*. 11: 1525.

Li S, Yang L, Ran Y, Ding W, 2022. A epsB mutant of *Ralstonia solanacearum* as novel biocontrol agent of tobacco bacterial wilt via activating salicylic acid signalling. *Physiol Mol Plant Pathol*. 119: 101834.

Lima JLC, Alves LR, Jacome PRLA, Bezerra JP, Maciel MAV, Morais MMC, 2018. Biofilm production by clinical isolates of *Pseudomonas aeruginosa* and structural changes in LasR protein of non-biofilm producers. *Braz J Infect Dis*. 22: 129-136.

Luciardi MC, Blázquez MA, Alberto MR, Cartagena E, Arena ME, 2020. Grapefruit essential oils inhibit quorum sensing of *Pseudomonas aeruginosa*. *Food Sci Technol Int*. 26: 231-241.

Luo J, Dong B, Wang K, Cai S, Liu T, Cheng X, Lei D, Chen Y, Li Y, Kong J, 2017. Baicalin inhibits biofilm formation, attenuates quorum sensing-controlled virulence and enhances *Pseudomonas aeruginosa* clearance in mice. *PLoS One*. 12: e0176883.

Maita P, Boonbumrung K, 2014. Association between biofilm formation of *Pseudomonas aeruginosa* clinical isolates versus antibiotic resistance and related genes. *[Journal details missing]*.

Mohamed MR, Emam MA, Hassan NS, Mogadem AI, 2014. Umbelliferone and daphnetin ameliorate carbon tetrachloride-induced hepatotoxicity in rats via Nrf2-mediated HO-1 expression. *Environ Toxicol Pharmacol*. 38: 531-541.

Nasirmoghadas P, Yadegari S, Moghim S, Esfahani BN, Fazeli H, Poursina F, Hosseininassab SA, Safaei HG, 2018. Evaluation of biofilm formation and frequency of multidrug-resistant *Pseudomonas aeruginosa* from burn patients in Isfahan. *Adv Biomed Res*. 7:

61.
Pachori P, Gothalwal R, Gandhi P, 2019. Emergence of antibiotic-resistant *Pseudomonas aeruginosa* in intensive care units: a critical review. *Genes Dis*. 6: 109-119.
Pang Z, Raudonis R, Glick BR, Lin T-J, Cheng Z, 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative strategies. *Biotechnol Adv*. 37: 177-192.
Qais FA, Khan MS, Ahmad I, Husain FM, Khan RA, Hassan I, Shahzad SA, Alharbi W, 2021. Coumarin exhibits antibiofilm and antiquorum sensing activity against Gram-negative bacteria: in vitro and in silico investigation. *ACS Omega*. 6: 18823-18835.
Qi C, Peng X, Yuan S, Zhang M, Xu X, Cheng X, 2022. Evaluation of antibacterial and anti-inflammatory effects of a natural products-containing toothpaste. *Front Cell Infect Microbiol*. 12: 827643.
Qiu N, Liu W, Zhang X, 2025. Based on quorum sensing: reverse effect of traditional Chinese medicine on bacterial drug resistance. *Front Cell Infect Microbiol*. 15: 1582003.
Quan Y, Zeng Y, Li J, Fu T, Sun Q, Xiu H, Cui X, 2023. Evaluation of the accuracy and stability of oxidase test in microbiology teaching. *Exp Sci Technol*. 21: 122-126.
Reddy RK, Murari A, Ali A, Kumar R, Lutunaika L, Choudhari A, Devi S, Kulsum F, 2025. Burns injury characteristics and outcomes at Lautoka Hospital, Fiji. *Burns Open*. 9: 100384.
Reen FJ, Gutierrez-Barranquero JA, Parages ML, O'Gara F, 2018. Coumarin: a novel player in quorum sensing and biofilm inhibition. *Appl Microbiol Biotechnol*. 102: 2063-2073.
Reynolds D, Kollef M, 2021. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections: an update. *Drugs*. 81: 2117-2131.
Ribeiro ÁCS, Crozatti MTL, Silva AAD, Macedo RS, Machado AMO, Silva ATA, 2019. *Pseudomonas aeruginosa* in the ICU: prevalence, resistance profile, and antimicrobial consumption. *Rev Soc Bras Med Trop*. 53: e20180498.
Roy S, Mukherjee P, Kundu S, Majumder D, Raychaudhuri V, Choudhury L, 2024. Microbial infections in burn patients. *Acute Crit Care*. 39: 214.
Sandman K, Ecker C, 2014. *Pseudomonas* isolation and identification: challenges of polyphasic taxonomy. *J Microbiol Biol Educ*. 15: 287-291.
Senturk S, Ulusoy S, Bosgelmez-Tinaz G, Yagci A, 2012. Quorum sensing and virulence of *Pseudomonas aeruginosa* during urinary tract infections. *J Infect Dev Ctries*. 6: 501-507.
Sharma NP, Duke JM, Lama BB, Thapa B, Dahal P, Bariya ND, Marston W, Wallace HJ, 2015. Descriptive epidemiology of unintentional burn injuries admitted to a tertiary-level hospital in Nepal. *Asia Pac J Public Health*. 27: 551-560.
Soto-Aceves MP, Diggle SP, Greenberg EP, 2023. Microbial primer: LuxR-LuxI quorum sensing. *Microbiology*. 169: 001343.
Stepanović S, Vuković D, Hola V, Bonaventura GD, Djukić S, Ćirković I, Ruzicka F, 2007. Quantification of biofilm in microtiter plates: testing conditions and recommendations for staphylococci. *APMIS*. 115: 891-899.
Swetha TK, Pooranachithra M, Subramenium GA, Divya V, Balamurugan K, Pandian SK, 2019. Umbelliferone impedes biofilm formation and virulence of methicillin-resistant *Staphylococcus epidermidis*. *Front Cell Infect Microbiol*. 9: 357.
Thakur S, Ray S, Jhunjhunwala S, Nandi D, 2020. Insights into coumarin-mediated inhibition of biofilm formation in *Salmonella Typhimurium*. *Biofouling*. 36: 479-491.
Wang Y, Bian Z, Wang Y, 2022. Biofilm formation and inhibition mediated by bacterial quorum sensing. *Appl Microbiol Biotechnol*. 106: 6365-6381.
Wu Z, Wu H, Li C, Fu F, Ding J, Shao S, Li K, Yu X, Su Y, Liang J, 2019. Daphnetin attenuates LPS-induced osteolysis and RANKL-mediated osteoclastogenesis via ERK and NFATc1 pathways. *J Cell Physiol*. 234: 17812-17823.
Yang L, Ding W, Xu Y, Wu D, Li S, Chen J, Guo B, 2016. Antibacterial activity of hydroxycoumarins against *Ralstonia solanacearum*. *Molecules*. 21: 468.
Yang L, Wei Z, Li S, Xiao R, Xu Q, Ran Y, Ding W, 2021. Plant secondary metabolite daphnetin reduces EPS production and virulence of *Ralstonia solanacearum*.

Pestic Biochem Physiol. 179: 104948.

Ye L, Zhang J, Xiao W, Liu S, 2020. Efficacy and mechanism of action of natural antimicrobial drugs. *Pharmacol Ther*. 216: 107671.

Ye Z, Ye L, Li D, Lin S, Deng W, Zhang L, Liang J, Li J, Wei Q, Wang K, 2022. Effects of daphnetin on biofilm formation and motility of *Pseudomonas aeruginosa*. *Front Cell Infect Microbiol*. 12: 1033540.

Zeng X, Zou Y, Zheng J, Qiu S, Liu L, Wei C, 2023. Quorum sensing-mediated microbial interactions: mechanisms, applications, challenges and perspectives. *Microbiol Res*. 273: 127414.

Zhang X, Yao J, Wu Z, Zou K, Yang Z, Huang X, Luan Z, Li J, Wei Q, 2020. Chondroprotective and antiarthritic effects of daphnetin in osteoarthritis models. *Life Sci*. 240: 116857.