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Biociências

Antimicrobial effect and inhibition of biofilm formation by phenolic acids on multi-drug resistant *klebsiella pneumoniae* isolates from a Public Hospital from Pernambuco, Brazil

Efeito antimicrobiano e inibição da formação de biofilme por ácidos fenólicos em isolados de Klebsiella pneumoniae *multirresistentes em um Hospital Público de Pernambuco, Brasil*

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Abstract: *Klebsiella* pneumoniae is an opportunistic pathogen related to several cases of healthcare-associated and community-acquired infections worldwide, especially in Brazil. Numerous studies have shown that isolated secondary metabolites, such as phenolic acids, have the potential to act against this problem. This study aimed to investigate the inhibitory potential associated with phenolic acids on growth and biofilm formation in clinical isolates of Multidrug-Resistant and Extensively Drug-Resistant *K. pneumoniae (MDR/XDR-KP).* Four clinical isolates from a public hospital in Recife, Pernambuco, Brazil, and a sensitive standard strain were used. The initial identification of the samples was carried out using VITEK®2 and BD-PhoenixTM 100 automation equipment, as well as the characterization of the resistance profile. The samples were then confirmed using the MALDI-TOF/MS technique. The Crystal Violet method was used to assess biofilm formation capacity. Four phenolic acids (gallic, trans-ferulic, caffeic, and 4-hydroxybenzoic) were used to evaluate the antimicrobial and biofilm-forming activities. The isolates were confirmed as *K. pneumoniae* species with MALDI-TOF/MS scores ranging from 2.459-2.083. The samples showed both MDR and XDR resistance profiles, and biofilm formation with different intensities. Of all the compounds tested, caffeic and *trans*-ferulic acids were the most effective, with growth and biofilm inhibition values of 70-85% and 70-90% using a concentration of 2 mg/mL, respectively. Notably, *K. pneumoniae* belongs to a group considered by the WHO to be a critical public health priority to be combated. In this context, the results showed that phenolic acids had a great potential impact on both bacterial growth and the biofilm-forming capacity of MDR/XDR-KP clinical isolates. This leads us to recognize the use of phenolic acids as a possible alternative in the fight against infections caused by MDR, XDR, and biofilm-forming bacterial species.
Keywords: *Klebsiella pneumoniae,* Phenolic compounds, Enterobacteriaceae, Antimicrobial Action, Biofilm.
Resumo: *Klebsiella pneumoniae* é considerada uma espécie de patógeno oportunista relacionada a diversos casos de infecções associadas, tanto à assistência à saúde quanto adquiridas na comunidade, mundialmente e principalmente no Brasil. Inúmeros estudos têm demonstrado que metabólitos secundários, isolados têm, como os ácidos fenólicos, o potencial de atuar contra este problema. Este estudo teve como objetivo investigar o potencial inibitório do crescimento e formação de biofilme associados aos ácidos fenólicos em isolados clínicos de *K. pneumoniae* Multidroga Resistente e Extensivamente droga resistente (MDR/XDR-KP). Foram utilizados quatro isolados clínicos provenientes de um hospital público de Recife, Pernambuco, Brasil, e uma cepa padrão sensível foram utilizados. A identificação inicial das amostras foi feita através dos equipamentos de automação VITEK®2 e BD-PhoenixTM 100, bem como a caracterização do perfil de resistência. Posteriormente as amostras foram confirmadas pela técnica de MALDI-TOF/MS. E para a avaliação da capacidade de formação de biofilme foi empregado o método do Cristal Violeta. Para avaliação das atividades antimicrobianas e antiformação de biofilme foram utilizados 4 ácidos fenólicos (gálico, trans-ferúlico, cafeico e 4-hidroxibenzóico). Os isolados tiveram sua identidade confirmada para espécie *K. pneumoniae* com MALDI-TOF/MS score variando entre 2.459-2.083. As amostras apresentaram perfis de resistência tanto MDR quanto XDR, e formadores de biofilme; porém com intensidades diferentes. Dentre todos os compostos testados, os ácidos cafeico e trans-ferúlico foram mais eficazes, com valores de inibição de crescimento e biofilme de 70-85% e 70-90% utilizando a concentração de 2 mg/mL, respetivamente. Diante dos resultados observou-se que os ácidos fenólicos, apresentaram grande potencial de impacto tanto no crescimento bacteriano quanto na capacidade de formação de biofilme dos isolados clínicos MDR/XDR-KP. Assim, leva-nos a reconhecer o uso de ácidos fenólicos como uma possível alternativa no combate a infecções causadas por espécies bacterianas MDRs e XDRs e formadoras de biofilme.
Palavras-chave: Ação antimicrobiana, Biofilme, Compostos Fenólicos, Enterobactérias, *Klebsiella pneumoniae*.

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INTRODUCTION

K. pneumoniae is a Gram-negative opportunistic pathogen that causes healthcare-associated infections (respiratory, urinary tract, and bloodstream infections). This species can be found in natural environments such as soils, plants, and animals, and colonises the human gastrointestinal tract (Martin & Bachman, 2018). Furthermore, it has a high capacity to form biofilms and can exhibit a Multidrug and Extensively Drug-Resistance profile (MDR/XDR), which makes it the second most common bacterium responsible for bacteremia in immunocompromised patients, with a high mortality rate (27.4-37.0%) (Zheng et al., 2018). Moreover, the World Health Organisation (WHO) has identified *K. pneumoniae* belonging to the ESKAPE group as requiring priority attention to control or eradicate (World Health Organization [WHO], 2017).

In the environment, bacteria can be found as planktonic cells or in a biofilm structure. Planktonic bacteria exhibit high mobility and increased susceptibility to environmental stresses and antimicrobial agents. While in biofilm form, they have greater resilience and survival in different environmental conditions, against multiple antimicrobial agents and the host defense system (Shakibaie, 2018). They have a 90% survival rate compared to planktonic cells, which is considered an evolutionary survival strategy (Freire et al., 2018).

Bacteria need to attach to surfaces to form a biofilm (Vert et al., 2012). This ability to attach allows bacteria to colonize, for example, medical devices such as catheters and surgical implants. Despite many efforts to maintain the sterility of implantable and prosthetic medical devices, biofilm contributes to 50-70% of implant-associated infections (Khatoon et al., 2018; Paharik & Horswill, 2016; VanEpps & Younger, 2016). In fact, according to the National Institute of Health, biofilm formation has been linked to 80% of microbial infections such as endocarditis, cystic fibrosis, periodontitis, chronic non-healing wounds, meningitis, renal infections, prosthetic and implantable device infections (Gupta et al., 2016; Jamal et al., 2018). As a result, the treatment of biofilm-associated infections is a notorious challenge due to the difficulty of diagnosis and the lack of appropriate biomarkers (Floyd et al., 2017).

Therefore, new strategies to prevent or eradicate bacterial biofilms have been studied extensively (Alara et al., 2021; Lu et al., 2019; Song et al., 2018). In recent years, medicinal plants have received attention for their biological properties, including antioxidant and anti-inflammatory (Unuofin & Lebelo, 2020), anticancer (Mani et al., 2021), antimicrobial and antibiofilm formation (Ribeiro et al., 2019). Some of these properties are related to the presence of phenolic compounds in their chemical composition, The existing types of phenolic compounds are mainly differentiated by the positions of their hydroxyls and carbon atoms. There are approximately 8,000 known types of phenolic compounds involved in the defence metabolism of various plants (Arciola et al., 2018; Cristea et al., 2017; G. Li et al., 2019). Even though the mechanism of action needs to be further elucidated, some studies have shown that some isolated phenolic compounds have activity on biofilm formation associated with several bacterial species, including *K. pneumoniae* (Strejcek et al., 2018; Wang et al., 2021).

Therefore, the present study aimed to investigate the influence of 4 phenolic acids (gallic acid, GA; trans-ferulic acid, TFA; caffeic acid, CA; 4-hydroxybenzoic acid, PHBA) on the growth and biofilm formation of multiresistant clinical isolates of *K. pneumoniae*.

MATERIALS AND METHODS

Bacterial isolates and culture condition

K. pneumoniae isolates were acquired from a public hospital in Recife, Pernambuco, Brazil from November to December 2021, upon approval by the Research Ethics Committee (REC) of UFPE, through protocol number: 2.986.708. Four clinical isolates were collected from different hospital sectors sectors (neurosurgery, Intensive Care Unite – ICU) and infection sites (rectal swabs, urine, and abscess samples). The isolate *K. pneumoniae* (UFPEDA 396), multidrug susceptible, was obtained from the *Department of Antibiotics at the Federal University of Pernambuco* collection (UFPEDA) and used as a control for the subsequent experiments. All isolates were stored in Brain and Heart Infusion (BHI) agar with mineral oil (24°C) and BHI broth with 15% glycerol at -20°C.

The initial identification and resistance profile of the isolates were carried out using VITEK®2 compact (BioMérieaux) and BD-Phoenix™ 100 (Becton Dickinson-BD) automation equipment. Resistance classification was performed according to Magiorakos et al. (2012) related to *Enterobacteriales* species, in which the organisms can be classified as MDR (Multi-Drug Resistant), XDR (Extensively Drug Resistant), and PDR (Resistant to All drugs). The evaluated antimicrobial agents were from the following classes: Aminoglycosides (amikacin, gentamicin), carbapenems (ertapenem, imipenem, meropenem), first and second-generation cephalosporins (cefazolin), third and fourth generation cephalosporins (ceftriaxone, ceftazidime, and cefepime), cephamycin (cefoxitin), fluoroquinolone (ciprofloxacin), penicillin, penicillin plus β-lactamase inhibitors (ampicillin-sulbactam), polymyxins (colistin).

MALDI-TOF mass spectrometer

K. pneumoniae isolates were identified by MALDI-TOF/MS technique. Bacterial colonies were suspended in 300 µL of Milli-Q water, and 900 µL of absolute ethanol was added. The suspensions were centrifuged at 15,600 g for 2 min. The supernatant was removed, and the pellet was dried out in SpeedVac for 20 min. After this, 50 µL of formic acid (70%) and 50 µL of acetonitrile were added into the samples. The resulting mixture was homogenized on a vortex shaker and centrifuged at 15,600 g for 2 min. The supernatant was transferred to a new microtube. The matrix was prepared with alpha-cyano-4-hydroxycinnamic acid (10 mg/mL), 50% acetonitrile, and 0.3% trifluoroacetic acid. Then, it was added to the MALDI plate containing the samples at room temperature (18°C) for crystallization. MS spectra were acquired in positive linear mode (acceleration voltage: 20 kV and detection range – m/z: 2,000 – 20,000) using the Flex Control Version 3.0 program on the MALDI-TOF Autoflex III mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The obtained scores were compared with data existing data in MALDI Biotyper Version 3.1 database.

Investigation of phenolic acid effect on bacterial growth and biofilm formation

Compounds acquisitions

The following compounds: gallic (Purity – ≥98.0% – Formula: C7H6O5 MW: 170.12 g/mol), *trans*-ferulic (Purity – ≥99% – Formula: C10H10O4 MW: 194.18 g/mol), caffeic (Purity – ≥98.0% – Formula: C9H8O4 MW: 180.16 g/mol), and 4-hydroxybenzoic acids (Purity – ≥98.0% – GC – Formula: C7H6O3 MW: 138,12 g/mol) were used in the present study and purchased commercially from Sigma-Aldrich Company.

Bacterial growth inhibition

Bacterial growth inhibition was evaluated through the Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC). The tests were performed using a 96-well microtiter plate, according to the European Committee on Antimicrobial Susceptibility Testing (Eucast, 2018) and with some adaptations from Liu *et al. (2020).* There added 160 µl of BHI broth to each well. The compounds were diluted to a final concentration of 2000, 1000, 500, 250, and 125 µg/mL per well. The microbial density was adjusted to 1.5 x 10^8 CFU/mL, and there inoculated 20 µL into each well. MIC of all compounds was determined as the lowest concentration where 90% of growth inhibition was detected, and MBC as the lowest concentration where no bacterial growth was observed.

Capacity of biofilm formation

Biofilm formation was evaluated by a method described by Stepanović et al. (2007) under different conditions: two culture media, BHI and Luria-Bertani broth (LB); in addition, 10% glucose was added or not. Biofilm formation was quantified using optical density at 570 nm (OD_{570}), and isolates were classified as weak (ODcontrol < ODsample < 2x ODc), moderate (2x ODc < ODs ≤ 4x ODc), and strong (4x ODc < ODs) biofilm producers, and/or non-biofilm producers.

Phenolic acids effect on biofilm formation

The effect of all compounds on the biofilm formation of *K. pnemoniae* isolates was evaluated through the method described by Trentin et al. (2011) with some modifications. Inoculum density was adjusted to 1.5 x 10^8 CFU/mL. There added 20 µL of all compounds to a 96-well microtiter plate, along with 20 µL of inoculum and 160 µL of BHI broth. The 96-well plate was incubated for 24 h. After the incubation period, the contents were discharged and washed three times with saline solution at 0.9%. For biofilm fixation, the 96-well plate was incubated at 55 °C for 60 min. The anti-biofilm effect was determined by the crystal violet method, 0.4% crystal violet was used for 15 min. After that, the 96-well plate was washed three times with distilled water, and absolute ethanol was added for 40 min. The 96-well plate was carried to an Enzyme-Linked Immunosorbent Assay (ELISA) reader, at the wavelength of 570 nm to see the results. A control with only culture medium and bacteria was used as 100% growth. Values above 100% were considered stimulatory and inhibitory below them.

Statistical analysis

All tests were performed in quadruplicate and three independent experiments. The mean and standard deviation were calculated and the graphs were produced using GraphPad Prism (version 5.0)**.**

RESULTS

Bacterial identification

The MDR/XDR-KP isolates were identified as *K. pneumoniae* by the automated method and confirmed by the MALDI-TOF/MS, in which they presented score values between 2,083 and 2,459. A score between 2.3 and 3.0 indicates reliable species identification; between 2.000 and 2.299 indicates reliable genus identification and probable species identification; between 1.700 and 1.999 indicates probable genus identification; and a score below 1.700 indicates no reliable identification. (Table 1).

Bacterial epidemiology

According to the resistant profiles classification proposed by Magiorakos et al. (2012). One isolate presented an MDR and three XDR profiles (Table 2). The most frequent sectors of the hospital were the Intensive Care Unit (ICU), followed by Neurosurgery and Medical Clinic with 50%, 25%, and 25%, respectively. Regarding the colonization sites in the two hospitals, most MDR-KP isolates were collected from rectal swabs (50%), urine (25%), and abscesses (25%).

Table 1
Characteristics of *K. pneumoniae* isolates collected from the public hospital

Strain identification	MALDI-TOF/MS Score	Infection site	Ward of isolation	Resistance profile	Biofilm Formation			
					BHI	BHI + G	LB	LB + G
653.12	2.113	Rectal swab	Neurosurgery	XDR	+++	++	++	+
672.12	2.083	Urine	ICU	MDR	+++	++	+++	++
686.12	2.342	Rectal swab	ICU	XDR	+++	++	+++	++
829.12	2.459	Abscess	Medical Clinic	XDR	+++	+++	+++	+++

Note. ICU – Intensive Care Unit; MDR – Multidrug-Resistant; BHI – Brain and Heart Infusion broth; G – Glucose; LB – Luria Bertani broth; **+++ –** Strong; **++ –** Moderate; **+ –** Weak biofilm producers.

Table 2
Resistant profile of *K. pneumoniae* clinical isolates.

Antimicrobial classes	Antibiotics	Resistance profile			
		653.12	672.12	686.12	829.12
Aminoglycoside	AMK	S	S	S	S
	GEN	R	R	R	R
Carbapenem	ETP	R	R	R	R
	IMP	R	R	R	R
	MER	R	R	R	R
Cephalosporin	CFZ	R	R	R	R
	CRT	R	R	R	R
	CAZ	R	R	R	R
	CPM	R	R	R	R
Cephamycin	CFO	R	R	R	R
Fluoroquinolone	CIP	R	I	R	R
Penicillin + β-lactamase inhibitors	AMS	R	R	R	R
Polymyxin	COL	S	S	S	S

Note. AMK – Amikacin; GEN – Gentamicin; ETP – Ertapenem; IMP – Imipenem; MER – Meropenem; CFZ – Cefazolin; CRT – Ceftriaxone; CAZ – Ceftazidime; CPM – Cefepime; CFO – Cefoxitin; CIP – Ciprofloxacin; TGC – Tigecycline; AMS – Ampicillin-Sulbactam; COL – Colistin; R – Resistant; S – Susceptible, I – Susceptible Increased Exposure.

Inhibition

Biofilm formation assay

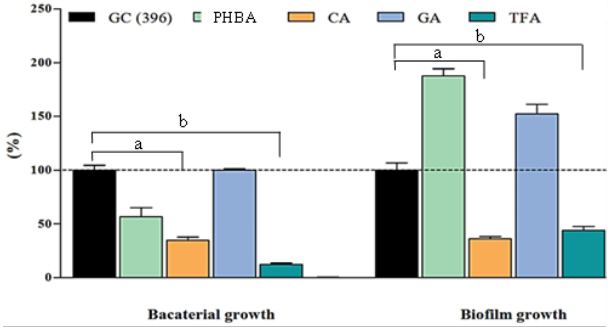
In this study, almost all isolates were able to form a biofilm, as shown in Table 1. For all culture media and conditions tested (BHI, BHI + G, LB, and LB + G), it was observed that most isolates were strong biofilm producers in BHI (50%), followed by BHI + G (44%), LB (41%) and LB + G (12%) (Table 1).

Bacterial growth inhibition and antibiofilm formation assays

To assess the phenolic acids, their capacity to inhibit bacterial growth and biofilm formation was tested. All phenolic acids were at 2000 µg/mL. An initial test was performed with a non-resistant bacterium (UFPDA 396).

There observed that the phenolic acids inhibited bacterial growth and biofilm formation as seen in Figure 1. Thereby, the compound PHBA showed growth inhibition (50%) and stimulant to biofilm formation (94%) at the concentration tested.

Figure 1
Inhibitory effect of the tested phenolic acids on bacterial growth and biofilm formation of UFPDA 396



Note. Growth control (GC), 4-Hydroxybenzoic acid (PHBA), caffeic acid (CA), gallic acid (GA), trans-ferulic acid (TFA) in planktonic cells and biofilm from UFPDA 396. Error bars indicate standard deviation. 'a' means statically significant between GC and CA; 'b' means statically significant between GC and TFA.

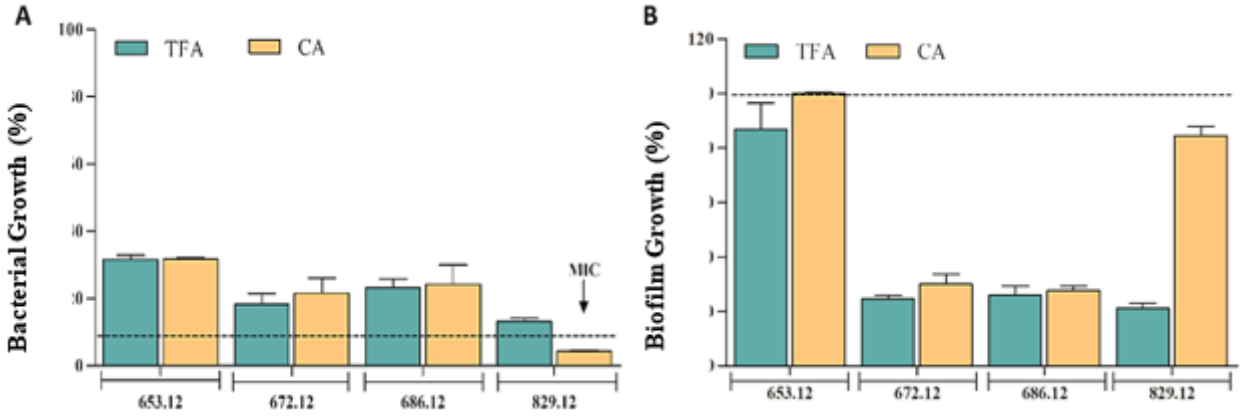
Trans-ferulic acid had an inhibitory effect of 80% and 50% on bacterial growth and biofilm formation, respectively. While caffeic acid obtained 65% for both assays acids. Gallic acid was the only compound that did not show growth and biofilm-inhibiting activity but increased biofilm production by 50%.

Compounds that performed better in both assays were selected to test whether the phenolic acids also inhibited bacterial growth and biofilm formation in resistant isolates. As shown in Figure 1, trans-ferulic and caffeic acids were selected.

Figure 2 shows the bacterial growth inhibition and antibiofilm formation effects of *trans*-ferulic and caffeic acids. Both compounds were also at 2000 µg/mL. For antibiofilm activity, caffeic acid was at 1000 µg/mL (figure 2B) since it demonstrated MIC value at the initial concentration tested. For the test, *trans*-ferulic and caffeic acids showed an average of 75% inhibition for the two activities tested. Independently, for isolate 653.12, in which neither *trans*-ferulic nor caffeic acids did not show antibiofilm activity. For isolate 829.12, in which caffeic acid showed MIC, but at the subinhibitory concentration (1000 µg/mL) the compound did not show antibiofilm formation activity.

Figure 2

Inhibitory effect on bacterial growth (A) and biofilm formation (B) of trans-ferulic and caffeic acids



Note. Trans-ferulic acid (TFA) and caffeic acid (CA). Error bars indicate standard deviation. 'a' and 'b' mean statically significant.

DISCUSSION

Currently, it is known that biofilm-forming bacteria pose significant challenges in the treatment of infections (Di Domenico et al., 2020), and the need for new potent antimicrobial agents against microorganisms in the biofilm state is one of the current strategies to combat these infections. This study also investigated whether some phenolic acids exert inhibitory activity on the growth and biofilm formation of MDR/XDR-KP isolates.

K. pneumoniae is known as one of the main microorganisms related to the cause of Healthcare-Associated Infections in compromised patients (Ferreira et al., 2018). Therefore, precise and quick identification of this pathogen, as well as other species of clinical interest, is necessary to conduct a better treatment strategy for compromised patients (Pasternak, 2012).

Emerging technologies like MALDI-TOF/MS (Matrix-assisted laser desorption ionization-time of flight mass spectrometry) could be an alternative for microbial diagnosis in clinical routine (Y. Li et al., 2019). The technique identified the isolates as *K. pneumoniae*, highlighting its significance for bacterial identification studies. Although there are other precise molecular techniques, such as sequencing; the literature shows that MALDI-TOF can be used for various purposes, such as epidemiological studies and detection of antibiotic resistance, and blood and urinary tract pathogens. This technique can be useful in taxonomy, epidemiology, and related fields (Singhal et al., 2015).

It is worth noting, there is still a significant challenge in MDR/XDR-KP infection treatment (Sales et al., 2014). The ICU is considered an epicentral site of infections with a high prevalence of MDR bacteria revealed by Sales et al. (2014); which has been observed worldwide (Flores et al., 2016).

Other studies have found a significant incidence of MDR/XDR bacteria in ICUs worldwide, including Brazil (Bassetti et al., 2018; Cadavid et al., 2018; Conci Campos et al., 2017). Previous research has shown a high incidence of enterobacterial infections during neurosurgery, particularly from carbapenem-resistant isolates (Trentin et al., 2011). Additionally, there has been an increase in meningitis cases linked to MDR-KP, rising from 25% to 40% in adults (Ku et al., 2017). Patrial et al. (2019) documented two cases of post-neurosurgery infections related to MDR-KP in Brazil.

Rectal swabs were the most commonly used collection method (50%) for hospital isolation facilities. They are a useful tool for public health professionals to screen for MDR/XDR enteric bacteria in hospitals during routine epidemiological surveillance. Urine and abscess samples were the second most common collection sites (25% for both). Studies by Cristea et al. (2017) and G. Li et al. (2019) have shown that

MDR/XDR-KP isolates are more frequently found in urine samples than in other types of infection sites.

Hospital-isolated MDR/XDR-KP were found capable of biofilm formation. Singh et al. (2019), reported that BHI is the medium of choice for biofilm formation. In addition, the same authors mentioned that the BHI medium has the necessary nutrients to produce fimbriae and pili, which are essential for initial adhesion to the substrate. Furthermore, it was reported to be the best medium for bacterial growth, adherence and biofilm formation for other bacterial species (Wijesinghe et al., 2019). Concerning this ability, some studies have shown higher mortality in immunosuppressed patients infected with biofilm-forming *K. pneumoniae* (Chen et al., 2020; Di Domenico et al., 2020; Khalil et al., 2019).

Relating to the antimicrobial and antibiofilm effects, of the four compounds in the concentration tested (2,000 µg/mL), Gallic acid and 4-Hydroxybenzoic acid showed the opposite effect on the UFPEDA 396 bacteria. Gallic acid and 4-Hydroxybenzoic acid increased by 0.5 times and one time, respectively, the biofilm production by UFPEDA 396. According to Plyuta et al. (2013), depending on the concentration, compounds can act as stimulating or reducing biofilm formation. It is worth noting that in a study published by Lin et al. (2022), it was observed that Gallic acid at 1,700 µg/mL had an inhibitory effect on capsule production by a hypervirulent *K. pneumoniae*. Thus, is needed further investigation to elucidate this issue.

In contrast, *trans*-ferulic acid and caffeic acid showed an inhibitory effect on growth and biofilm formation. The same study by Plyuta et al. (2013), reported a stimulant action of Ferulic acid on *P. aeruginosa* biofilm, depending on the concentration tested. However, our data did not show stimulating activity on UFPEDA396 and MDR/XDR-KP biofilm formation by *trans*-Ferulic and Caffeic acid.

Kot et al. (2015), reported the inhibitory effect of 0.5% Ferulic acid on biofilm formation by the clinical isolate *Escherichia coli* in a urinary catheter. Kot et al. (2019) demonstrated the antimicrobial activity of Ferulic and Caffeic acids against fish bacteria isolates; however, a higher concentration (3,120 µg/mL) was needed to achieve this activity. Wijesundara and Rupasinghe (2019) pointed out the anti-biofilm formation in *Staphylococcus pyogenis* from plant ethanol extracts, the author related the presence of Ferulic and Caffeic acids, among others.

Muñoz-Cazares et al. (2017), in their review, argue that Ferulic and Caffeic acid had properties of antibiofilm activity. Furthermore, they pointed to the first report on the antibiofilm effect against *S. epidermis* promoted by Caffeic acid and the ability of Ferulic acid to control biofilm formation in *E. coli* and *Listeria monocytogenes*.

Furthermore, as it is known to form a biofilm, the bacteria need to adhere to a surface (Vert et al., 2012). Thus, Gato et al. (2020), demonstrated the anti-adhesive activity of *Vaccinium corymbosum* (Blueberry) extract on MDR-KP in the HT-29 colorectal cells. They confirmed the presence of Caffeic acid in the extract, which in the study, were capable of reducing the adherence of MDR-KP to the colorectal cell.

Regarding *trans*-Ferulic acid, Šuran et al. (2021), demonstrate de presence of *trans*-Ferulic acid, as the second most concentrated compound in propolis extract. The extract could inhibit the bacterial growth of gram-negative bacteria (*P. aeruginosa* and *Acinetobacter baumannii*), even on eradication of biofilm from them. Rezaeiroshan et al. (2022), pointed out the enhancement of niosomal nanoparticles containing *trans*-Ferulic acid to ciprofloxacin antimicrobial activity. It reduced the MIC values of the antibiotic on *K. pneumoniae* ATCC among the other species tested.

CONCLUSION

The conclusion of this study demonstrated the high incidence and dissemination of obtaining biofilm producers and multidrug-resistant *K. pneumoniae* in different hospital sectors of one public hospital in Recife-PE. Resistance and biofilm formation profiles were observed in *K. pneumoniae* indicating that conventional therapy is still ineffective in infection treatment caused by these bacteria. Thus, continuous monitoring of laboratory practices and epidemiological surveillance must be carried out to minimize dissemination in hospital environments. As a therapeutic alternative to minimize a biofilm-associated infection, was demonstrated the potential of some phenolic compounds, and Caffeic and *trans*-ferulic acid were the most relevant

when correlated with the capacity to reduce biofilm formation in MDR/XDR-KP. Furthermore, our data are the first related to *trans*-Ferulic acid promoting antibiofilm activity on MDR/XDR-KP strongly biofilm producers. Consequently, these acids could be used for future medical formulations to treat or prevent biofilm formation. Notwithstanding, this suggests that future trials are needed to increase the reliability of their use in the control of biofilms.

CONCURRENT STATEMENT OF INTEREST

The authors declare that they have no competing interests.

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