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BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY - RESEARCH PAPER

Antimicrobial activity of cinnamaldehyde against multidrug-resistant *Klebsiella pneumoniae*: an in vitro and in vivo study

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Abstract
The emergence and spread of multidrug-resistant (MDR) *Klebsiella pneumoniae* strains have increased worldwide, posing a significant health threat by limiting the therapeutic options. This study aimed to investigate the antimicrobial potential of cinnamaldehyde against MDR-*K. pneumoniae* strains in vitro and in vivo assays. The presence of resistant genes in MDR-*K. pneumoniae* strains were evaluated by Polymerase Chain Reaction (PCR) and DNA sequencing. Carbapenem-resistant *K. pneumoniae* strains show the bla_{KPC-2} gene, while polymyxin-resistant *K. pneumoniae* presented bla_{KPC-2} and alterations in the *mgrB* gene. Cinnamaldehyde exhibited an inhibitory effect against all MDR-*K. pneumoniae* evaluated. An infected mice model was used to determine the in vivo effects against two *K. pneumoniae* strains, one carbapenem-resistant and another polymyxin-resistant. After 24 h of cinnamaldehyde treatment, the bacterial load in blood and peritoneal fluids decreased. Cinnamaldehyde showed potential effectiveness as an antibacterial agent by inhibiting the growth of MDR-*K. pneumoniae* strains.

Keywords Cinnamaldehyde · Multidrug-resistant · Gram-negative · Infection

Introduction

Antimicrobial resistance represents a major threat to global health [1] since it is directly associated with delays in initiating the appropriate antimicrobial therapy, long-term hospitalization with increased medical expenditure costs, and high morbidity and mortality rates [2]. The resistance genes generally reside in the transposons transported by plasmids, which increases their dissemination possibility causing frequent hospital outbreaks. *Klebsiella pneumoniae* is a leading cause of nosocomial infections, and the emergence and spread of multidrug-resistant (MDR) strains have become a major public health concern worldwide [3] Infections caused by MDR *K. pneumoniae* have limited therapeutic options, resulting in increased use of polymyxin B as a therapeutic option of last resort, despite its nephrotoxicity and neurotoxicity potential [4].

The antimicrobial resistance of *K. pneumoniae* is primarily driven by the acquisition of mobile genetic elements carrying genes that encode antibiotic resistance, particularly to beta-lactams and carbapenems. Horizontal gene transfer, particularly via plasmids, has played a significant role in the spread of these resistance determinants [3]. However, for polymyxin resistance, the mechanism is mainly attributed to changes in the structure of lipopolysaccharide (LPS), mutations or insertion sequences in the *mgr*B gene, missense mutations, and the acquisition of mobile genetic elements that carry resistance genes, such as *mcr* [5]. The emergence of MDR bacteria also resistant to polymyxin B reduces available therapeutic options, demonstrating the

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urgent need for developing alternative strategies to prevent its rapid spread [6].

Efforts to control infections caused by multidrug-resistant (MDR) strains of bacteria are hindered by the limited number of antibiotics currently available. According to the World Health Organization (WHO), only two out of the 50 antibiotics under study are currently being investigated specifically for use against MDR strains [7]. Therefore, more cumulative efforts are required to develop new and effective drug therapies. The bioactive potentials of various essential oils have been increasingly explored as a potential therapeutic strategy for fatal diseases caused by MDR bacteria [8]. Interestingly, cinnamon spices obtained from trees of the genus *Cinnamomum* contain a high amount of cinnamaldehyde (85.3–90.5%) [9] used as a natural flavorant and fragrance agent in kitchen and industry which has been identified as an active component with antimicrobial properties [10]. However, to the best of our knowledge, no study has described the activity of cinnamaldehyde against MDR *K. pneumoniae* to date. In this study, we investigated the antimicrobial performance of cinnamaldehyde in vitro and in vivo against *K. pneumoniae* strains resistant to carbapenem and polymyxin B along with the evaluation of the acute toxicity displayed by cinnamaldehyde.

Materials and methods

Reagents

Several antibiotics like amikacin (Lot 01774), aztreonam (Lot MKBW 2997 V), ceftazidime (Lot 1,004,245), ertapenem (Lot T024320) imipenem (Lot 1238–1), meropenem (Lot 037M4713), polymyxin B sulfate (Lot 027M400), tigecycline hydrate (Lot 0000023817), and cinnamaldehyde ≥ 95% (Lot MKBV4784V), were purchased from Sigma-Aldrich, St Louis, MO, USA.

Bacterial identification and antimicrobial susceptibility testing

The strains included in this study were previously isolated from infection sources of patients admitted to an intensive care unit of a tertiary hospital in Dourados, Mato Grosso do Sul, Brazil, and the resistance determinants were characterized as previously described [11, 12].

Bacterial species identification was performed using the BD Phoenix 100® automated system (BD Diagnostic Systems) and confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using the Microflex Spectrometer LT (Bruker Daltonics, Massachusetts, USA) [13]. The minimal inhibitory concentrations (MIC) were determined by broth microdilutions

according to the medical laboratory standards of the Clinical and Laboratory Standards Institute (CLSI) [14], whereas the preliminary screening for carbapenemases was performed by ertapenem hydrolysis assay using mass spectrometry [11].

Molecular characterization of resistance genes

The presence of resistance genes ($bla_{CTX-M-1-like}$, $bla_{CTX-M-2-like}$, $bla_{CTX-M-8-like}$, $bla_{CTX-M-14-like}$, $bla_{GES-like}$, $bla_{GIM-like}$, bla_{IMP-10}, $bla_{IMP-like}$, bla_{KPC-2}, $bla_{NDM-like}$, bla_{OXA-23}, $bla_{OXA-48-like}$, $bla_{SHV-like}$, $bla_{SIM-like}$, $bla_{SME-like}$, $bla_{SPM-like}$, $bla_{TEM-like}$, $bla_{VIM-like}$, and $mcr-1$) was evaluated by polymerase chain reaction (PCR) and DNA sequencing. The nucleotide sequences of $pmrA$, $pmrB$, $phoP$, $phoQ$, $crrB$, and $mgrB$ genes were amplified and sequenced to investigate the innate chromosomal polymyxin-resistance mechanism [12].

Antibacterial activity of cinnamaldehyde

Antimicrobial susceptibility tests were performed by a microdilution method using 96-well polystyrene microtiter plates in Mueller Hinton Broth (MHB) [14]. The cinnamaldehyde concentrations ranged from 72 to 0.035 mg/mL (supplemented with a final concentration of 0.5% Tween 80 v/v). The inoculum was adjusted to correspond to 0.5 McFarland standard (1×10^8 CFU/mL), using spectrophotometry, with an optical density ranging between 0.08 and 0.10. Each well was inoculated with a final bacterial concentration of 5×10^5 colony-forming units (CFU)/mL. The plates were incubated at 37 °C for 18–24 h. Two positive (*Escherichia coli* ATCC® 25,922 and *K. pneumoniae* ATCC® 700,603) and negative (MHB and Tween) controls were used. The tests were performed in triplicate.

Time-kill assay

The time-kill method was performed using the broth macrodilution technique [15] with approximately 5×10^5 CFU/mL in a final volume of 3.2 mL and verified with a spectrophotometer by Vitek® 2 (bioMerieux). The bacterial suspension was cultured at 37 °C, and the samples were obtained after 0, 2, 4, 6, and 12 h of incubation. At every designated sample time, 1 μL was withdrawn from each tube using a sterile loop and seeded in MHB agar plates. The plates were incubated for 24 h at 37 °C followed by the determination of the colony counts. MHB and saline were used as a negative and as a sterility control, respectively. Polymyxin B (Poly) and amikacin (Amik) were used as positive controls. Bacteria count log_{10} values were calculated as the mean of triplicate experiments.

Animals

Healthy female Swiss mice 8–12 weeks old and weighing 20–30 g were used for all in vivo experiments. The animals were kept in polypropylene cages with controlled temperature (22 ± 3 °C), humidity (40–60%), and light (12 h light–dark cycles), receiving standard commercial feed and water ad libitum. In vivo tests were performed according to the National Council for the Control of Animal Experimentation (CONCEA) [16]. This study was conducted with the approval of the Research Ethics Committee from Universidade Federal da Grande Dourados (UFGD) (number 877.292/2014 and 4.014.325/2020) by the Ethics Committee on Animal Use of UFGD (number 25/18) and the Centro Universitário da Grande Dourados (Unigran) (number 080/18).

The median lethal dose test

Determination of the median lethal dose (LD_{50}) was performed as described previously [17], with few modifications. In brief, two independent experiments were carried out in the animals, one using carbapenem-resistant *K. pneumoniae* strain and another polymyxin-resistant *K. pneumoniae* strain. Seven groups of six animals were injected intraperitoneally (i.p.) using a needle in the right lower abdomen with bacterial suspensions (0.1 mL) of $2,8 \times 10^8$, 3.8×10^8, 4.8×10^8, 5.8×10^8, 6.8×10^8, 7.8×10^8, and 8.8×10^8 CFU/mL. After 24 h, the number of survivors was assessed.

Antimicrobial therapy

The in vivo antibacterial activity of cinnamaldehyde was performed as described previously by Toledo et al. [17] with modifications. For this, two independent experiments were performed, one using carbapenem-resistant *K. pneumoniae* strain and the other with polymyxin-resistant *K. pneumoniae* strain. All animals were injected with a 0.1-mL i.p. aliquot of LD_{50} of each respective bacterial strain. Five groups with eight animals each experiment was used that were identified as follows: Naive (without infection to assess the baseline indexes), Control (infected without treatment), cinnamaldehyde at a dosage of 30 mg/kg (CEO 30), and cinnamaldehyde at a dosage of 100 mg/kg (Ceo 100). Polymyxin B at 2 mg/kg (Poly B) and amikacin at 7.5 mg/kg (Amik) were used as positive controls administered i.p. every 12 h, for carbapenem-and polymyxin-resistant *K. pneumoniae* strains, respectively. Treatments were performed 1 h after the bacterial inoculations. In both the tests, the cinnamaldehyde was administered by gavage after every 8 h. All surviving animals were anesthetized with a combination of xylazine and ketamine (10 and 60 mg/kg, i.p., respectively). After the euthanization of animals by exsanguination, organs were collected for analysis. Blood and pleural fluid samples were collected to assess the bacterial culture, followed by utilization of the blood for hematological analysis using an automated hematological analyzer (Sysmex Poch-100iV Diff). Peritoneal fluid was obtained by washing the incision, performed using the aseptic technique, with a Milli-Q lab water system.

Acute toxicity

The acute toxicity of cinnamaldehyde was determined according to the Bruce procedure adopted by the Organization for Economic Co-operation and Development (OECD) [18]. The test was performed by administering a single dose of cinnamaldehyde (2000 mg/kg) by intra-gastric gavage in only one animal based on its body weight. After the survival of the animal, within 48 h, four additional animals were dosed sequentially to obtain a total of five animals for the test. The animals were observed individually for the first 30 min after being dosed, followed by periodical observation for the first 24 h with particular attention provided during the first 4 h. After that, they were closely observed for 14 days for gross morphological, physiological, behavioral changes, and mortality. After the test was completed, the survived animals were weighed and then euthanized. The organs (heart, lung, spleen, liver, and kidney) were removed, weighed, and examined macroscopically.

Statistical analysis

The data were expressed as a percentage and mean $\pm$ standard error (SE). The differences among the groups were assessed using one-way and two-way analysis of variance (ANOVA). The significance test was performed using the GraphPad Prism software, considering the value of $p < 0.05$ (version 3.02; Graph-Pad Software Inc., San Diego, CA, USA).

Results

Susceptibility and molecular assays of MDR-K. pneumoniae strains

Five MDR-*K. pneumoniae* strains were evaluated. The results of antimicrobial susceptibility tests showed that carbapenem-resistant *K. pneumoniae* strains were resistant to amikacin (MIC, 64 mg/L), aztreonam (MIC > 32 mg/L), ceftazidime (MIC > 256 mg/L), imipenem (MIC > 16 mg/L), meropenem (MIC > 16 mg/L), and ertapenem (MIC, > 32 mg/L), though they were sensitive to polymyxin B and tigecycline (MIC < 0.5 mg/L) (Supplementary Table 1). While carbapenemase production was confirmed

by MALDI-TOF MS, PCR and sequencing results displayed that the bla_{KPC-2} coding gene was present in carbapenem-resistant strains of *K. pneumoniae* that were resistant to polymyxin B and other antibiotics and sensitive to amikacin (MIC < 2 mg/L) and tigecycline (MIC < 0.5 mg/L) (Table 1). The presence of the bla_{KPC-2} gene and mutations in the *mgrB* gene were identified in polymyxin-resistant strains. In the carbapenem-resistant strains of *K. pneumoniae* studied, bla_{OXA-48}, bla_{NDM}, and $bla_{CTX-M-8}$ were each detected in separate strains. The genes like $bla_{CTX-M-1-like}$, $bla_{CTX-M-2-like}$, $bla_{CTX-M-14-like}$, $bla_{GES-like}$, $bla_{GIM-like}$, bla_{IMP-10}, $bla_{IMP-like}$, $bla_{NDM-like}$, bla_{OXA-23}, $bla_{SHV-like}$, $bla_{SIM-like}$, $bla_{SME-like}$, $bla_{SPM-like}$, $bla_{TEM-like}$, $bla_{VIM-like}$, and *mcr-1*, on the other hand, were not detected.

Antibacterial activity of cinnamaldehyde

Cinnamaldehyde exhibited significant inhibitory effects in the evaluated five MDR-*K. pneumoniae* strains, with MIC values ranging from 0.035 to 0.28 mg/mL (Table 1). In the time-kill assay, the minimum bactericidal concentration (MBC) was performed using *K. pneumoniae* strains, as described in Table 1. A decrease in the viable cell counts over a while was observed, while the total inhibition effects were displayed after 8 h of cinnamaldehyde treatment. Polymyxin B (1 µg/mL) and amikacin (64 µg/mL) were considered as positive controls, with successful inhibitory effects after 4 h. No bacterial growth was observed in the control MHB (Fig. 1).

In vivo *antibacterial assay of Cinnamaldehyde*

LD50 survival curves were observed for 24 h in mice. LD_{50} value was 3.8×10^8 CFU/mL in both the evaluated strains. The in vivo antimicrobial activity of cinnamaldehyde was evaluated for 24 h using mice infections with carbapenem-resistant and polymyxin-resistant *K. pneumoniae* strains. Half of the control group (untreated) died within 24 h after getting infected (50% mortality). In the experiment using LD_{100}, both *K. pneumoniae* strains presented 100% mortality in the Inf group (without treatment), while those treated with the reference drugs (poly B and Amik) had 100% survival. Treatment with 100 mg/kg cinnamaldehyde showed a 50% and 37% survival rate in carbapenem and polymyxin-resistant *K. pneumoniae* strains, respectively (Fig. 2A, B).

Treatment with cinnamaldehyde (100 mg/kg) showed a significant reduction in the log_{10} values of bacterial CFU/mL in the bloodstream (Figs. 3A, 4A) and pleural fluid (Figs. 3B, 4B) in mice infected with carbapenem-resistant and polymyxin-resistant *K. pneumoniae* strains. Mice infected with carbapenem-resistant *K. pneumoniae* strains and treated with Ceo (30 and 100 mg/kg, $p < 0.001$) showed a decrease in total leukocyte and neutrophil count (Fig. 3C, D). Regarding the

Table 1 Antimicrobial resistance profiles of the carbapenem- and polymyxin-resistant *K. pneumoniae* strains

Number	Bacterial strains	Genes	MIC of antibiotics (mg/mL)										Cinnamaldehyde	
			FEP	CTX	CTZ	AZT	IMP	MER	ERT	AMIK	POLY	TGC	MIC (mg/mL)	MBC (mg/mL)
KP01	*K. pneumoniae*	bla_{KPC-2}	>256	>256	>254	>32	>16	>16	>32	64	<0.5	<0.5	0.07	0.14
KP03	*K. pneumoniae*	bla_{OXA-48}	>256	>256	>254	>32	>16	>16	>32	64	<0.5	<0.5	0.14	0.28
KP03	*K. pneumoniae*	bla_{NDM}	>256	>256	>254	>32	>16	>16	>32	>64	<0.5	<0.5	72	144
KP04	*K. pneumoniae*	$bla_{CTX-M-8}$	>256	>256	>254	>32	>16	>16	>32	>64	<0.5	<0.5	0.28	0.28
KP05*	*K. pneumoniae*	altered *mgrB*/bla_{KPC-2}	>254	>256	>254	>32	>16	>16	>32	<2	>4	<0.5	0.14	0.14

Abbreviations: *KP, Klebsiella pneumoniae; FEP,* cefepime; *CTX,* cefotaxime; *CTZ,* ceftazidime; *AZT,* aztreonam; *IMP,* imipenem; *MER,* meropenem; *ERT,* ertapenem; *AMIK.* amikacin; *POLY,* polymyxin B; *TGC,* tigecycline; *MIC,* minimal inhibitory concentrations; *MBC,* minimal bactericidal concentrations

*Polymyxin-resistant

Fig. 1 Time-kill curves of MDR-*K. pneumoniae* strains evaluated in this study. Carbapenem-resistant strains (**A**) polymyxin-resistant strains; (**B**) CR, carbapenem-resistant; PR, polymyxin-resistant; MHB, Mueller Hinton Broth. Differences among the groups were analyzed by a two-way ANOVA test, followed by the comparison of ***$p < 0.001$ and **$p < 0.01$ with the saline group. SD ± media
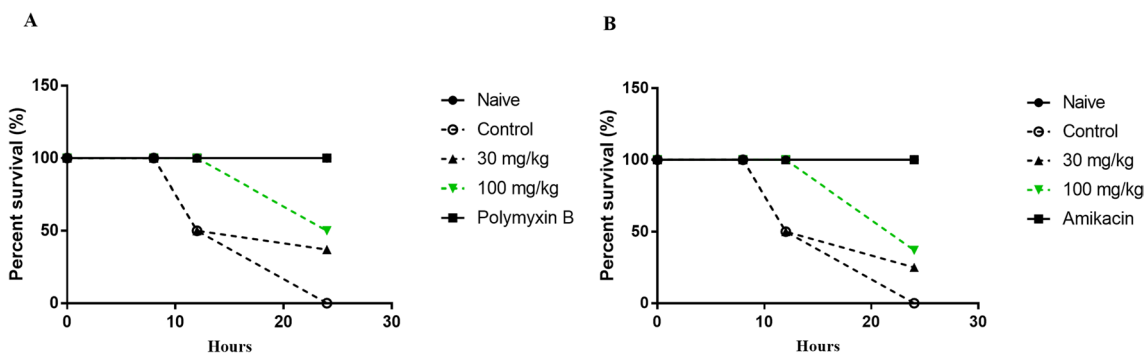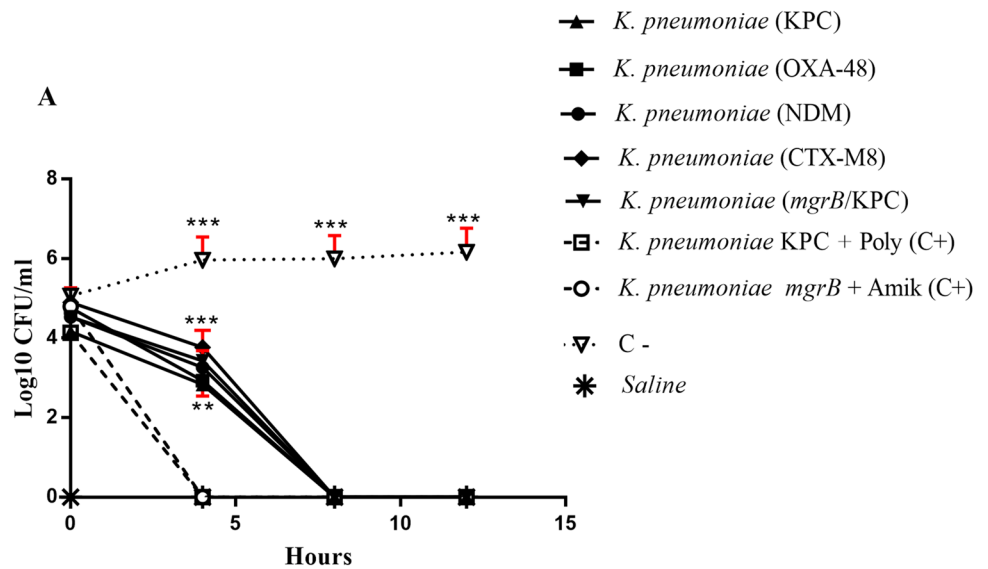

Fig. 2 Survival curves of mice infected with MDR *K. pneumoniae* and treated with cinnamaldehyde. Carbapenem-resistant *K. pneumoniae* (**A**) polymyxin-resistant *K. pneumoniae* (**B**) polymyxin B and untreated (control) were used as positive and negative controls, respectively

response against polymyxin-resistant *K. pneumoniae* strain, all groups treated with Ceo (30 and 100 mg/kg), and amikacin had a significant decrease in total leukocyte count (Fig. 4C). A statistical difference in neutrophils was not observed in the Ceo-treated groups (Fig. 4D).

Acute toxicity

The animals did not show any signs of toxicity or death immediately after an experiment with cinnamaldehyde (2000 mg/kg) or during the observation period. No changes were observed in their behavior or water and food consumption. The macroscopic analysis showed no changes and differences in the organ weight.

Discussion

Due to the overuse of antibiotics, the emergence of resistant bacteria is now posing a serious public health threat worldwide. Therefore, the search for newer drugs to combat this grave situation is greatly needed to meet future demands [19]. This study described the inhibitory effect of cinnamaldehyde against *K. pneumoniae* strains resistant to carbapenem and polymyxin. Antibacterial assays showed the potential of action of cinnamaldehyde against carbapenem and polymyxin-resistant *K. pneumoniae* strains (MIC = 0.035–0.28 mg/mL). Similar results have been described for *Cinnamomum cassia L.* essential oil

Fig. 3 Effects of cinnamaldehyde in the number of cells of CFU in mice infected with MDR *K. pneumoniae* after 24 h of treatment. Blood (**A**) and pleural fluid (**B**) in animals infected with carbapenem-resistant *K. pneumoniae*. Total leukocytes (WBC) (**C**), neutrophils (**D**), and lymphocytes (**E**) in animals infected with carbapenem-resistant *K. pneumoniae*. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$ as compared to the control group (Inf). Differences among the groups were analyzed by one-way ANOVA followed by the Newman-Keuls test. Among the groups were analyzed by one-way ANOVA followed by the Newman-Keuls test
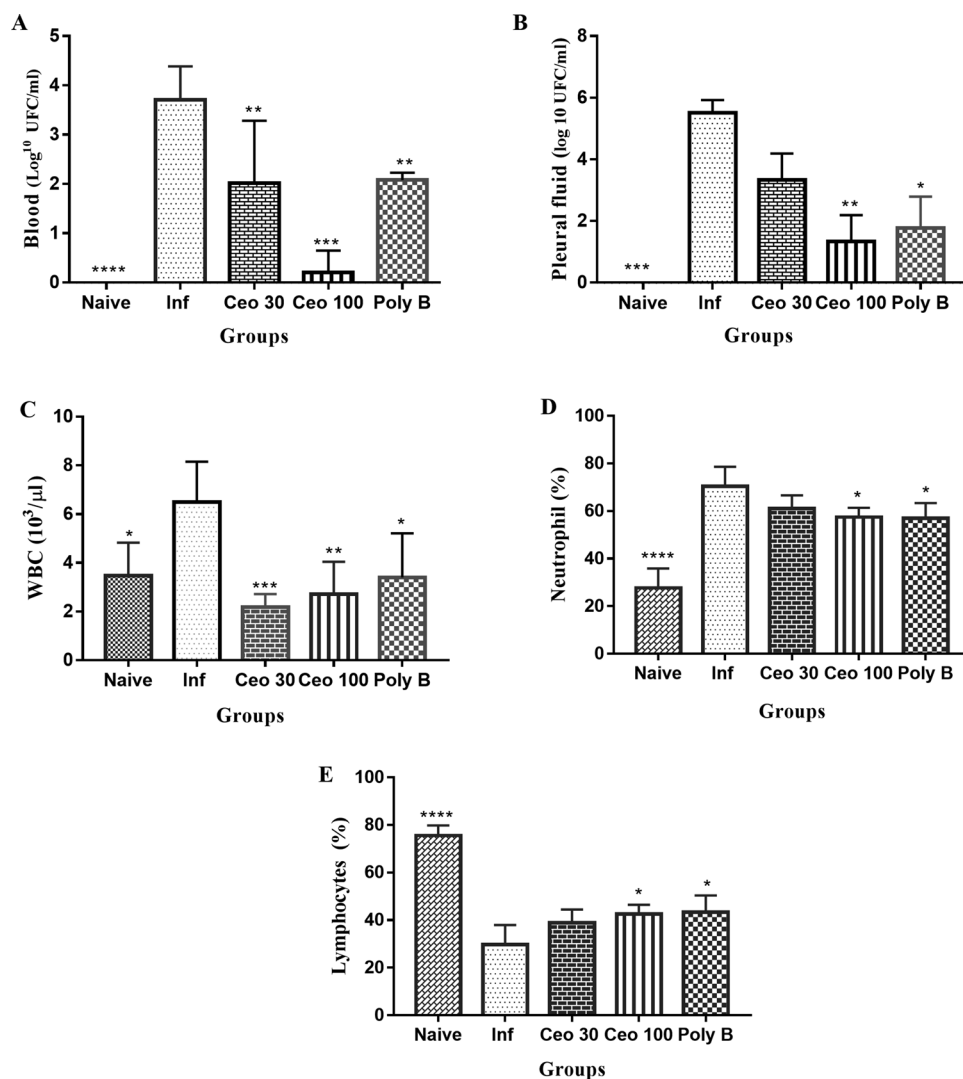
alone and in combination with antibiotics against carbapenemase-producing *K. pneumoniae* and *Serratia marcescens* [20].

Antibacterial action of cinnamaldehyde and its in vitro antibacterial effects against polymyxin-resistant *Klebsiella aerogenes* [21], MDR-*Pseudomonas aeruginosa* [10], and fluoroquinolone-resistant extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) were described [22]. Its antimicrobial activity involves the disruption of the bacterial cell membrane's integrity by increasing the cell membrane permeability, thereby causing seepage of intracellular contents [23]. In *K. aerogenes*, the cinnamaldehyde carbonyl group binds to proteins preventing the decarboxylase activity [24, 25] and getting access to the periplasmic space as well as the inner cellular contents of Gram-negative bacteria, through the outer membrane of porous proteins [26].

Although antimicrobial effects of cinnamaldehyde against MDR-*K. pneumoniae* has been described in previous studies

[10, 21, 27, 28], to our knowledge, in vivo antimicrobial activity of cinnamaldehyde has not been reported to date. Our study described the models of peritoneal inoculums of carbapenem and polymyxin-resistant *K. pneumoniae* strains to achieve a median-lethal dose of sepsis. Our results revealed that cinnamaldehyde treatment significantly increased the survival rate of mice infected with carbapenem and polymyxin-resistant *K. pneumoniae* strains as compared to the control group (untreated). Additionally, a significant decrease in the number of CFU in blood and pleural fluid with cinnamaldehyde (100 mg/kg) was observed. These results were similar to the commercial drugs used as a positive control.

It was evident that only tigecycline, polymyxin, and some aminoglycosides had favorable antibacterial activities against MDR bacterial strains. However, these drugs have varied side effects that limit their usage. According to the Food and Drug Administration (FDA), tigecycline is not indicated for treating diabetic foot infection, hospital

Fig. 4 Effects of cinnamalde-
hyde in the number of cells
of CFU in mice infected with
polymyxin-resistant *K. pneu-
moniae* after 24 h of treatment.
Blood (**A**) and pleural fluid (**B**)
in animals infected with carbap-
enem-resistant *K. pneumoniae*.
Total leukocytes (WBC) (**C**),
neutrophils (**D**), and lympho-
cytes (**E**) in animals infected
with polymyxin-resistant *K.
pneumoniae*. ****$p < 0.0001$,
***$p < 0.001$, **$p < 0.01$, and
*$p < 0.05$ as compared to the
control group (Inf). Differ-
ences among the groups were
analyzed by one-way ANOVA
followed by the Newman-Keuls
test. Among the groups were
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followed by the Newman-Keuls
test
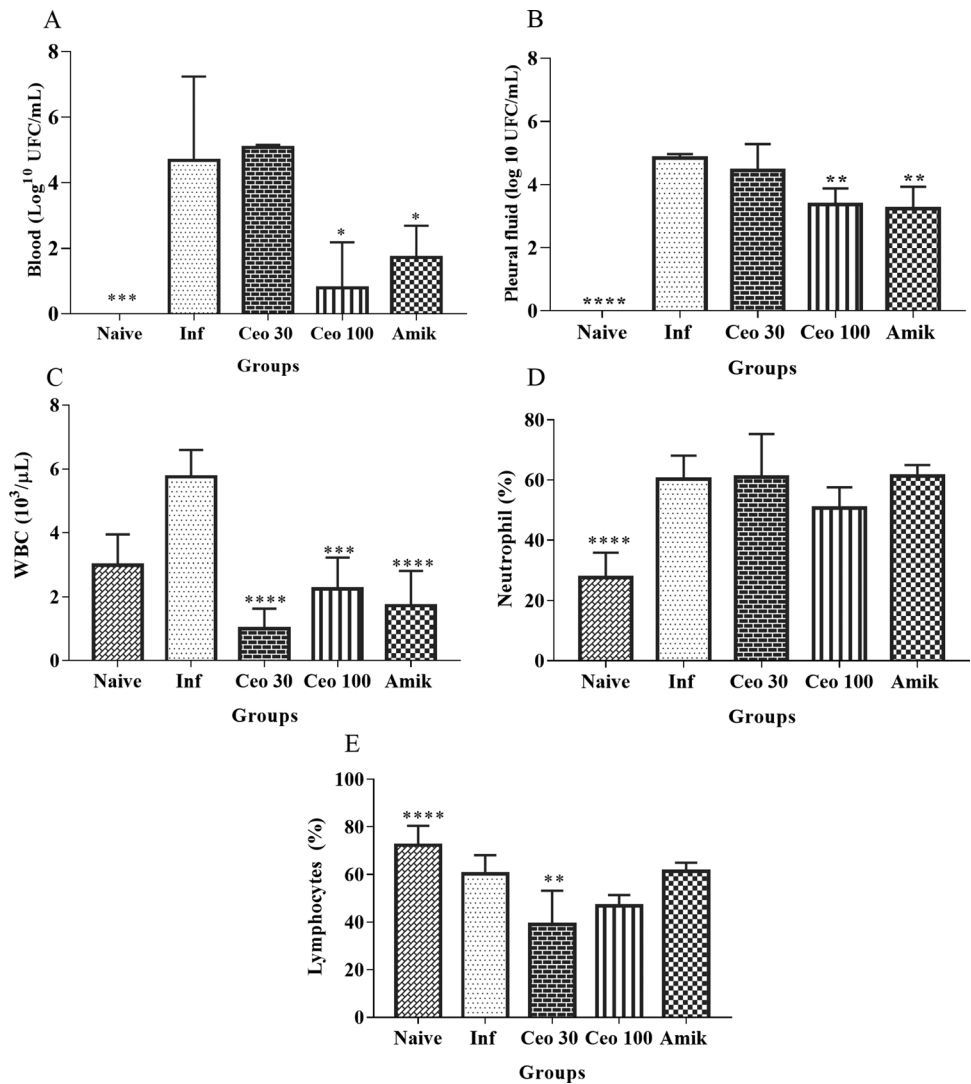
pneumonia, or mechanical ventilation [29]. Moreover, the global spread of carbapenem-resistant *K. pneumoniae* has increased the use of polymyxin B with an inevitable risk of emerging drug resistance [30]. As polymyxins have several neurotoxic and nephrotoxic effects, and their pharmacoki-netics are still poorly understood [4] such therapies resulted in higher mortality of patients when compared to other described antibiotics [31]. Unfortunately, aminoglycosides are also known to cause ototoxicity [32]. Thus, alternative therapies to replace these antibiotics for combating the increased antibiotic resistance are the need of the hour for which cinnamaldehyde might be considered as a promising candidate.

During the spread of infection, the leukocytes initiate the immune cascade by enhancing the expression of immuno-suppressive pathways, such as interleukin 10 (IL-10), and early activation of innate inflammatory response systems [33]. In our study, untreated animals had a higher total leu-kocyte count as compared to the treated and non-infected

groups (NAIVE); thus, suggesting a functional infectious or inflammatory process in these animals. Our results depicted that the groups treated with cinnamaldehyde showed a decrease in total leukocyte count, suggesting that it was efficient in controlling the initial surge of infection, being the first defense against the pathogens [34]. Another study reported a similar result in mice infected by KPC-producing *K. pneumoniae* and treated with carvacrol [35].

The concentration of the neutrophils in infected mice with *P. aeruginosa* was higher than in the control animals without infection [36]. In our study, differences in the neutrophil count between the two evaluated *K. pneumoniae* strains were observed. The group treated with cinnamaldehyde (100 mg/kg) displayed a statistically significant decrease in neutro-phils, similar to the group treated with the reference drug. However, groups treated with cinnamaldehyde and infected by polymyxin-resistant strain did not show any statistical differences. Despite the fact that *K. pneumoniae* strains may vary in genome size, capsule composition, and expression of

resistance genes, it is still unclear whether immunological defenses against different *K. pneumoniae* strains may differ according to their genetic characteristics [30]. Although the strains evaluated in this study had different resistance mechanisms, it is difficult to predict that this reason might be responsible for the observed difference in neutrophils. Thus, more experimental studies are required to have a lucid understanding of the contribution of cinnamaldehyde in initiating the immune response during the infection of MDR *K. pneumoniae*. Hence, it might provide additional helpful information regarding the severity of the disease and its acuity or effective response to anti-inflammatory treatment.

In our study, animals treated with cinnamaldehyde at a dose of 2000 mg/kg did not exhibit any signs of acute toxicity. This was further supported by the absence of clinical findings in toxicological screening and the earlier reported mortality [22]. The acute toxicity and oral LD_{50} values for cinnamaldehyde in rats and guinea pigs have been reported as 2220 mg/kg and 1160 mg/kg, respectively [37].

There was no evidence of increased unscheduled DNA synthesis when rats were administered cinnamaldehyde 500 mg/kg of the body weight by intraperitoneal injection [38]. Cinnamaldehyde is completely absorbed in mice and humans, rapidly converted to cinnamic acid, and eliminated from the body [39]. Essential oils and their primary compounds, which are considered flavoring substances by the European Regulation 1999/217/CE and Generally Recognized As Safe (GRAS) substances by the Food and Drug Administration (FDA), have been extensively studied for their antimicrobial properties [40]. The lower dose of cinnamaldehyde (100 mg/kg), demonstrated in our study, which was able to inhibit the growth of the evaluated MDR strains, is lower than the levels described by previous studies as safe concentrations of cinnamaldehyde for animals.

However, it is important to recognize the limitations of our study, including the fact that we evaluated only a limited number of *K. pneumoniae* strains. Future studies should expand the range of microorganisms being tested, as well as its pharmacokinetics and pharmacodynamics. Furthermore, exploring new techniques such as cytometry assay and scanning electron microscopy may provide more information about cinnamaldehyde's mechanism of action and its potential as a clinical treatment. On the other hand, the in vitro and in vivo antimicrobial activity of cinnamaldehyde were promising, without toxicity. Thus, its therapeutic potential may be more explored.

Conclusion

Our results provided evidence on the potential of cinnamaldehyde as a promising antimicrobial agent against multidrug-resistant *K. pneumoniae*, a clinically important Gram-negative bacteria. This study provides a starting point for further research on cinnamaldehyde as an antimicrobial agent. Thus, this findings contribute to ongoing efforts to identify new treatments for bacterial infections resistant to conventional antibiotics.

Declarations

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