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Original article

Antibacterial effect of probiotics against colistin-resistant *Klebsiella pneumoniae* clinical isolates: an *in vitro* study

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ABSTRACT

Background: The rise of colistin-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) represents a significant challenge to antimicrobial therapy, necessitating the search for alternative or adjunctive therapies. This study aimed to assess the antibacterial effects of *Lactobacillus* spp. cell-free supernatants (CFS) against colistin-resistant *K. pneumoniae* isolates and investigate potential synergy between CFS and colistin. **Methods:** A total of 187 *K. pneumoniae* isolates were collected from hospitalized patients. Colistin resistance was determined using the broth microdilution method, identifying 25 colistin-resistant isolates (13.4%). Antimicrobial susceptibility was assessed using the Kirby-Bauer method. The antibacterial activity of selected *Lactobacillus* strains was evaluated using the agar-well diffusion method, while a modified Kirby-Bauer assay was used to assess the potential synergistic effect of colistin and CFS. **Results:** All 25 colistin-resistant isolates were multidrug-resistant (MDR), with 56% resistant to all tested antibiotics. *Lactobacillus* CFS exhibited significant antibacterial activity, with *L. helveticus* producing the largest inhibition zones, showing a statistically significant difference compared to other strains. However, rather than enhancing antibacterial activity, colistin reduced the inhibitory effects of *Lactobacillus* CFS against colistin-resistant *K. pneumoniae*. **Conclusion:** *Lactobacillus* CFS demonstrated significant antibacterial activity against colistin-resistant *K. pneumoniae*, highlighting its potential as a viable alternative antimicrobial approach. However, colistin did not enhance this effect, indicating a lack of synergy. Further in vivo studies are required to validate the clinical applicability of *Lactobacillus* in combating MDR-*K. pneumoniae* infections.

Introduction

The worldwide surge in multidrug-resistant (MDR) Gram-negative bacteria is becoming a growing healthcare challenge [1]. *Klebsiella pneumoniae (K. pneumoniae)* is a major hospital-acquired pathogen, accounting for nearly one-third of Gram-negative infections, including pneumonia, urinary tract infections, meningitis, and bloodstream infections [2]. Over recent years, *K. pneumoniae* has rapidly evolved into an MDR pathogen by acquiring resistance to multiple antimicrobial classes, with carbapenem resistance becoming particularly prevalent due to the spread of carbapenemase enzymes [3,4].

Carbapenem-resistant *K. pneumoniae* severely limits treatment options for critical infections. As a result, polymyxins (colistin and polymyxin B) have been reintroduced as last-resort therapies due to the scarcity of novel antimicrobials [5]. However, colistin overuse in clinical settings,

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particularly in low- and middle-income countries, has contributed to the emergence of resistance [6]. Furthermore, its widespread use in animal husbandry for infection control and growth promotion has facilitated the dissemination of colistin-resistant *K. pneumoniae* across clinical, veterinary, and environmental settings [7,8]. This increasing prevalence is especially concerning, given the already limited treatment options and the high mortality rates associated with colistin-resistant infections [9].

Managing colistin-resistant *K. pneumoniae* remains challenging. Combination antibiotic therapy has been shown to be more effective than monotherapy in both in vitro and in vivo studies, offering several advantages: (i) enhanced antimicrobial activity at lower concentrations, (ii) reduced treatment costs, and (iii) minimized toxicity, particularly nephrotoxicity and neurotoxicity [10]. Given the urgent need for alternative and safer strategies to combat antibiotic resistance, optimizing antimicrobial combinations is a critical priority [11]. Beyond conventional antibiotic-based approaches, non-traditional strategies such as probiotics and their metabolites have gained attention for their potential to enhance bacterial susceptibility when used alongside conventional antibiotics [12].

Among these, *Lactobacillus* species are widely recognized as biological therapeutics with immune-modulating properties and are classified as generally recognized as safe (GRAS) [13]. *Lactobacillus* exerts its antimicrobial effects through multiple mechanisms, including competition for nutrients, secretion of antimicrobial substances, immune activation, and competition for adhesion sites [13,14]. These strategies enable *Lactobacillus* to inhibit a range of bacterial pathogens, including *Acinetobacter* spp., *Escherichia coli*, and *K. pneumoniae* [11,15–17]. Additionally, a recent study demonstrated that combining polymyxin E with the cell-free supernatant (CFS) of certain probiotic *Bacillus* strains enhanced its antimicrobial activity against *Acinetobacter* spp. isolates [11]. However, to our knowledge, the potential synergy between probiotics and colistin against colistin-resistant *K. pneumoniae* remains unexplored. Therefore, this study aimed to evaluate, in vitro, the antibacterial effects of *Lactobacillus* CFS alone and in combination with colistin against clinical isolates of colistin-resistant *K. pneumoniae*.

Methods

This research obtained ethical clearance from the Research Ethics Committee of the Faculty of Medicine, Cairo University (N-100-2024). All procedures complied with ethical standards of the 1964 Declaration of Helsinki.

Bacterial strains:

The study was carried out over six months, from May to November 2024, at the Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University. A total of 187 *K. pneumoniae* isolates were obtained from various clinical samples of hospitalized patients. These samples were cultured on MacConkey agar and blood agar (Oxoid, UK) and incubated aerobically at 37°C for 48 hours. Bacterial identification was conducted using standard microbiological methods, including colony morphology assessment, Gram staining, and biochemical testing [18].

Determination of MIC of colistin using the broth microdilution method

The broth microdilution (BMD) method was employed to determine the minimum inhibitory concentration (MIC) of colistin for each *K. pneumoniae* isolate. The testing was performed using colistin sulfate powder (ADWIA Pharmaceuticals Co., Egypt) and cation-adjusted Mueller-Hinton broth (CA-MHB) (Liofilchem, Italy). Isolates with an MIC $\geq$ 4 μg/mL were classified as colistin-resistant, in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines [19]. Colistin-resistant isolates were further analyzed as follows:

Anti- microbial susceptibility testing

The antimicrobial susceptibility of colistin-resistant *K. pneumoniae* isolates was evaluated using the Kirby-Bauer disk diffusion method. A bacterial suspension, standardized to a 0.5 McFarland turbidity level, was evenly inoculated onto Mueller-Hinton agar (MHA) plates (Oxoid, England). The tested antimicrobial agents included amoxicillin-clavulanate (30 μg), cefoxitin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), imipenem (10 μg), meropenem (10 μg), ciprofloxacin (5 μg), trimethoprim-sulfamethoxazole (25 μg), amikacin (30 μg), and gentamicin (10 μg). Antimicrobial discs were purchased from Oxoid Limited (Basingstoke, Hampshire, England). *E. coli* ATCC 25922 served as the quality control strain for susceptibility testing.

The diameters of inhibition zones were recorded for each antibiotic, and isolates were defined as MDR if they exhibited resistance to at least one antibiotic in three or more antimicrobial categories. The results were interpreted in accordance with the CLSI 2024 guidelines [19].

In Vitro Antibacterial Activity of Lactobacillus spp. Alone and in Combination with Colistin:

Lactobacilli strains

The study utilized *Lactobacillus acidophilus* (LA-5®) from Chr. Hansen's dairy culture collection (Hørsholm, Denmark), along with *Lactobacillus casei*, *Lactobacillus helveticus*, and a blend of *Lactobacillus rhamnosus* and *Lactobacillus paracasei*, generously supplied by the Dairy Science Department, Faculty of Agriculture, Cairo University. All Lactobacillus strains were standardized to an optical density of 0.5 at 600 nm (OD600), corresponding to approximately 10^8 CFU/mL before use in experiments.

Preparation of cell-free supernatant

The CFS was prepared using de Man, Rogosa, and Sharpe (MRS) broth (Sigma-Aldrich). Following incubation at 37°C for 24 hours, cultures were centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatants were then sterilized by filtration through a 0.22-µm cellulose acetate filter (Millipore, Billerica, MA, USA) and stored at -80°C until further use. [20].

Evaluation of the Antibacterial Effect of Lactobacillus spp. via the Agar-Well Diffusion Assay

The antibacterial activity of *Lactobacillus* spp. was evaluated using the agar-well diffusion assay. Colistin-resistant *K. pneumoniae* isolates were standardized to a 0.5 McFarland turbidity level and evenly spread onto MHA plates with a sterile cotton swab. Wells (10 mm in diameter) were created using a sterile cork borer, and 100 µL of CFS from each *Lactobacillus* strain was dispensed into them. Plates were then incubated at 37°C for 24 hours. The inhibition zones diameters were measured in millimeters to determine antimicrobial activity [20]. Quality control strain *K. pneumoniae* (ATCC 35657) was used for comparison with CFS of different Lactobacillus strains [15].

Combination of colistin with CFS of Probiotics

The antimicrobial combination assay was conducted using the modified Kirby-Bauer disc diffusion method, as previously described [21]. Overnight cultures of *K. pneumoniae* isolates in brain heart infusion (BHI) broth were diluted and adjusted to adjusted to a 0.5 McFarland turbidity standard. The standardized bacterial suspension was streaked onto MHA plates in three directions. Three different discs were prepared: one containing only the antibiotic, another with the antibiotic infused with the tested bacterial CFS, and a third with only the bacterial CFS. A blank, untreated disc was used as a negative control. Quality control strain *K. pneumoniae* (ATCC 35657) was used for comparison with CFS of different Lactobacillus strains [15]. The discs were then placed on the inoculated agar surface and left for 30 minutes to facilitate diffusion before being incubated at 37°C for 24 hours. The inhibitory zones' diameters were measured and recorded after incubation.

Statistical analysis:

We utilized SPSS version 25 for data analysis. Numerical variables, such as inhibition zone diameters, were expressed as means and standard deviations, while categorical data were summarized as frequencies and proportions. Differences in inhibition zones among the four Lactobacillus strains were assessed using one-way ANOVA, followed by Tukey's post hoc test for pairwise comparisons. The antibacterial effect of *Lactobacillus* CFS versus its combination with colistin was evaluated using a paired t-test. Statistical significance was set at $p < 0.05$.

Results:

Antimicrobial susceptibility testing of the tested isolates:

Out of the 187 *K. pneumoniae* isolates, 25 (13.4%) were resistant to colistin as determined by the broth microdilution method. The antimicrobial susceptibility profile of tested isolates is illustrated in Figure 1. A significant proportion (96%, 24/25) were non-susceptible to all tested third- and fourth-generation cephalosporins, as well as gentamicin. Carbapenem resistance (non-susceptibility to both meropenem and imipenem), was observed in 76% of isolates. Notably, 14 isolates (56%) demonstrated resistance to all tested antibiotics, including colistin. All 25 isolates were categorized as MDR, showing resistance to at least one antimicrobial agent in three or more drug classes.

Antibacterial activity of Lactobacillus strains alone and in combination with colistin

The antibacterial activity of Lactobacillus-derived CFS was assessed against 25 colistin-resistant *K. pneumoniae* isolates. All four tested

Lactobacillus CFS exhibited notable antibacterial effects, with *L. helveticus* showing the largest inhibition zones, followed by *L. acidophilus* (LA-5) and the combination of *L. paracasei* + *L. rhamnosus*, while *L. casei* demonstrated slightly lower activity. Statistical analysis revealed significant differences in inhibition zones among the tested Lactobacillus strains (p = 0.0408). Tukey's post-hoc test for multiple comparisons between the different strains showed that *L. helveticus* had significantly larger inhibition zones than other strains. However, no significant differences were detected among the other three Lactobacillus strains (Table 1).

When comparing the inhibition zones of CFS alone to those of CFS combined with colistin, colistin significantly reduced the antibacterial activity of all tested Lactobacillus strains (Figure 2 & Table 2). Paired *T*-tests showed statistically significant reductions in inhibition zones (Table 3). These findings challenge our initial assumption that colistin may act synergistically with Lactobacillus-derived CFS against colistin-resistant *K. pneumoniae*. Instead, the results suggest that colistin may interfere with the antibacterial activity of *Lactobacillus* metabolites.

Table 1. Tukey's HSD Post-hoc test for multiple comparisons between the different *Lactobacillus* strains.

Comparison	**Mean difference**	**95% confidence interval (CI)**	***P-value***
L. Helviticus vs. LA-5	1.20	(0.02-2.37)	0.010
L. helviticus vs. L. casei	1.92	(0.75-3.10)	0.000
L. helviticus vs. L. paracasei + L. rhamnosus	1.24	(0.07-2.41)	0.008
L. casei vs. L. paracasei + L. rhamnosus	-0.68	(-1.85-0.49)	0.314
L. casei vs. LA-5	-0.72	(-1.90-0.45)	0.284
LA-5 vs. L. paracasei + L. rhamnosus	0.04	(-1.12-1.21)	0.990

Table 2. Antibacterial effect of CFS from tested *Lactobacilli* strains alone and in combination with colistin.

Lactobacilli strain	**CFS alone (Mean ± SD) (mm)**	**Colistin alone (Mean ± SD) (mm)**	**CFS + Colistin (Mean ± SD) (mm)**
L. casei	13.76 ± 1.4	9.24 ± 1.62	12.16 ± 1.41
L. acidophilus (LA-5)	14.48 ± 1.6	9.24 ± 1.62	12.76 ± 1.79
L. heliviticus	15.68 ± 3.2	9.24 ± 1.62	11.28 ± 0.98
L paracasei+L rahmnosus	14.44 ± 2.6	9.24 ± 1.62	11.76 ± 1.01

Table 3. Results of paired *t*-Tests to compare the inhibition zones of each *Lactobacillus* strain alone vs. in combination with colistin.

Strain	**Mean Difference**	**95% confidence interval (CI)**	***p-value***
L. casei* vs. colistin+*L.casei	−1.60 mm	(−2.33 to −0.87)	0.00015
L. acidophilus (LA-5) vs. colistin+LA5	−1.72 mm	(−2.40 to −1.04)	0.000024
L. helviticus* vs. colistin+ *L. helviticus	−4.40 mm	(−5.89 to −2.91)	0.000003
L. paracasei + L. rhamnosus* vs. Colistin + *L. paracasei + L. rhamnosus	−2.68 mm	(−3.86 to −1.50)	0.00009

Figure 1. Antimicrobial susceptibility pattern of *K. pneumoniae* isolates.

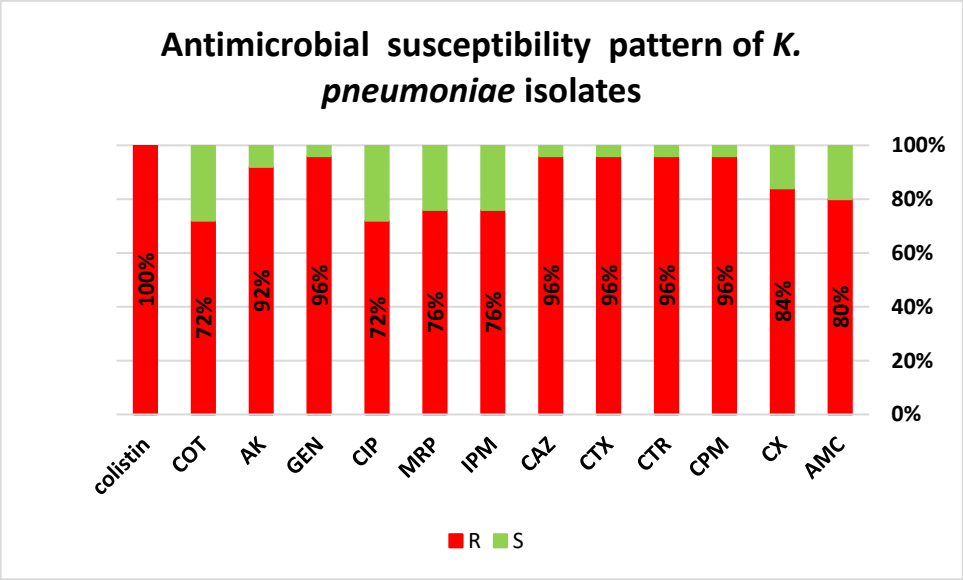
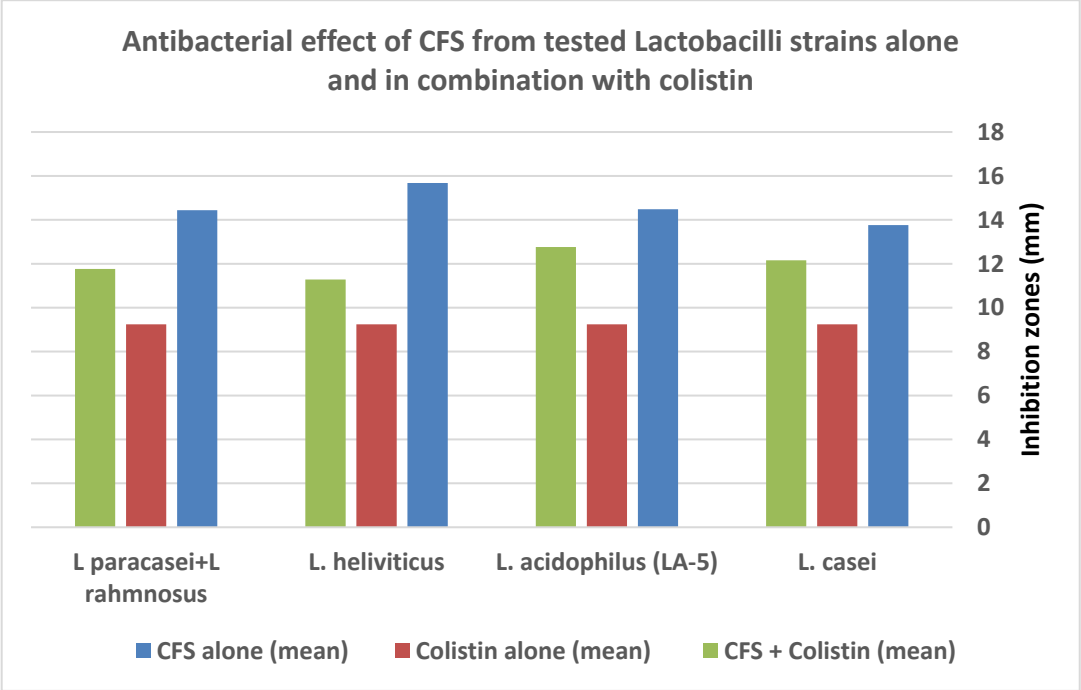


Figure 2. Antibacterial effect of CFS from tested *Lactobacilli* strains alone and in combination with colistin.



Discussion

K. pneumoniae is a major opportunistic pathogen responsible for severe nosocomial infections [22]. The widespread and improper use of antibiotics has significantly contributed to the escalation of antimicrobial resistance, complicating the selection of effective therapeutic options [23]. Due to renal and neurological toxicity, colistin was largely abandoned in the 1970s in favor of less toxic alternatives. However, it has recently reused as a last-resort therapy—alone or in combination—for carbapenem-resistant and MDR Gram-negative infection [24]. This renewed reliance on colistin has, in turn, led to the emergence and dissemination of colistin-resistant *K. pneumoniae* strains [25]. In the present study, colistin resistance was detected in 13.4% of *K. pneumoniae* isolates. *Makled et al.* reported a similar resistance rate of 11.1% among ICU isolates from Menoufia University Hospitals

[26], while *Abozahra et al.* observed a higher rate (39%) in Egypt [27]. In contrast, *Zafer et al.* identified a lower prevalence (4.9%) among cancer patients [28]. Additionally, *Abdelhamid et al*. reported that all 50 studied isolates in their investigation were susceptible to colistin [29]. The observed discrepancies in colistin resistance rates across different studies in Egypt may be attributed to regional variations in antimicrobial stewardship practices, differences in patient populations, or methodological variations in susceptibility testing.

In the present study, 56% of *K. pneumoniae* isolates exhibited resistance to all tested antibiotics. Similarly, *Abozahra et al.* reported a 53.6% resistance rate among their *K. pneumoniae* isolates [27]. Resistance rates ranged from 80% to 96% for β-lactams, 76% for carbapenems, and 92% to 96% for aminoglycosides, aligning with previous reports [27]. Ciprofloxacin resistance was observed in 72% of isolates, comparable to the 80% resistance rate reported by *Karimi et al.* [30]. The increasing prevalence of MDR and XDR *K. pneumoniae* represents a major public health threat, demanding immediate intervention. Strengthening antimicrobial stewardship, improving microbiology surveillance (through rapid identification, susceptibility testing, and systematic reporting), and reinforcing infection control measures are essential in combating resistance to last-line antibiotics.

Novel antimicrobial treatments and alternative therapeutic strategies are urgently needed to combat colistin-resistant infections [31]. One promising alternative to conventional antibiotics is the use of non-antibiotic therapies, such as probiotics. The selection of probiotics in this study aligns with the recommendations of international health and agriculture authorities. In this study, four Lactobacillus strains were evaluated for their antimicrobial effectiveness against clinical strains of colistin-resistant *K. pneumoniae.* The agar well diffusion assay was employed to evaluate Lactobacillus CFS antibacterial activity due to its simplicity, reproducibility, and suitability for screening multiple strains under standardized conditions. This method offers a rapid, visual assessment of antimicrobial effects and is widely used for probiotic-derived compounds. While quantitative methods like broth microdilution and time-kill assays provide detailed interaction dynamics, they are labor-intensive and typically assess a single agent at a time. In contrast, the agar well diffusion assay enables simultaneous testing of multiple substances against a single microorganism, allowing for efficient comparative analysis through easily interpretable inhibition zones [32]. Notably, all four strains' CFS exhibited substantial antibacterial effects, highlighting their potential as adjunctive or alternative therapeutic options. The antibacterial activity of Lactobacillus CFS is primarily mediated by antimicrobial metabolites such as hydrogen peroxide, lactic acid, and bacteriocins, which lower pH, disrupt bacterial membranes, and inhibit bacterial growth. Additionally, CFS may interfere with pathogen colonization by depleting essential nutrients and altering adhesion site availability. Some Lactobacillus-derived metabolites have also been linked to immunomodulatory effects, further enhancing their antimicrobial potential [16]. These mechanisms may explain the strong inhibition zones observed in our study.

As members of the revised *Lactobacillus* genus, *L. helveticus* and *L. acidophilus* share a high degree of genetic similarity and are phylogenetically linked to gut-associated bacteria, enabling their survival in both intestinal and dairy environments [33]. In our study, both strains demonstrated notable antibacterial activity against *K. pneumoniae*, with *L. helveticus* exhibiting significant large inhibition zones when compared to other strains with mean inhibition zone of 15.68 ± 3.2 mm while *L. acidophilus* (LA-5) showed a mean inhibition zone of 14.48 ± 1.6 mm. These results align with those of *Abelhalim et al.,* who reported a mean inhibition zone of 13.3 mm for *L. helveticus* CFS against MDR *K. pneumoniae* [15]. Similarly, *Mokhtar et al.* investigated a CFS mixture dominated by *L. acidophilus*, observing strong antibacterial effects against ESBL-producing *K. pneumoniae*, with mean inhibition zones of 17 ± 2.4 mm [34]. The antimicrobial activity of *L. helveticus* and *L. acidophilus* are likely attributed to their ability to produce organic acids, bacteriocins, and various bioactive substances. Notably, *L. acidophilus* has been shown to secrete antimicrobial substances that not only suppress bacterial growth but also interfere with biofilm development in *K. pneumoniae* [35]. Similarly, *L. helveticus* is believed to release bacteriocins into the CFS, interfering with biofilm development by preventing cellular aggregation [36]. These findings highlight the potential of *L. helveticus* and *L. acidophilus* as promising antimicrobial agents against MDR *K. pneumoniae*,

likely through a multifaceted mechanism involving acidification, bacteriocin secretion, bioactive peptide release, and biofilm inhibition. However, further in vivo studies are warranted to evaluate their therapeutic potential and clinical applications in managing MDR *K. pneumoniae* infections.

L. paracasei and L. rhamnosus exhibited inhibition zones of 11–22 mm, with a mean inhibition zone of 14.44 ± 2.6 mm. These results align with earlier studies that have documented strong antibacterial activity of these strains. *Abelhalim et al.* reported a mean inhibition zone of 14.32 mm for *L. rhamnosus* CFS against MDR *K. pneumoniae* [15]. Similarly, *Chen et al.* demonstrated that both *L. paracasei* and *L. rhamnosus* displayed strong antibacterial activity against carbapenem-resistant *K. pneumoniae*, each producing mean inhibition zones exceeding 15 mm [20]. The antimicrobial activity observed in both strains is likely attributable to the production of organic acids, bacteriocins, and other antimicrobial peptides. *L. rhamnosus*, in particular, is known to secrete lactic acid and antimicrobial compounds. *De Keersmaecker et al.* demonstrated that its potent antimicrobial activity against *Salmonella* was driven by the accumulation of lactic acid [37]. On the other hand, *Shahverdi et al.* reported a weaker antibacterial effect of *L. paracasei* CFS against a pathogenic *K. pneumoniae* strain, with a mean inhibition zone of 8.3 ± 0.8 mm [38]. This discrepancy may explain why the combination of *L. paracasei* and *L. rhamnosus* in our study exhibited inhibition zones comparable to those of *L. rhamnosus* alone. However, our study was limited by the inability to assess the antibacterial activity of each strain independently, which warrants further investigation.

While our findings are based on in vitro experiments, existing clinical evidence suggests a potential therapeutic role for probiotics. *Morrow et al*. documented that administration of *L. rhamnosus* significantly reduced ventilator-associated pneumonia rates in ICU patients colonized with MDR Gram-negative bacteria [39]. These findings highlight the necessity for additional *in vivo* research to assess the clinical efficacy of *Lactobacillus* strains in combatting colistin-resistant *K. pneumoniae* infections.

In the present study, *L. casei* exhibited relatively lower antibacterial activity, with a mean inhibition zone of 13.76 ± 1.4 mm. This aligns with the results of *Abelhalim et al.*, who reported a weak inhibitory effect of *L. casei* against carbapenem-resistant *K. pneumoniae*, with inhibition zones ranging from 0 to 10 mm [15]. However, a more recent study observed a stronger antibacterial effect, reporting a mean inhibition zone of 20 mm for *L. casei* against *K. pneumoniae* isolates [40]. Limited studies have specifically evaluated the antimicrobial activity of *L. casei* against *Klebsiella* species. However, other studies have reported that *L. casei* can exert antibacterial effects against other Gram-negative bacilli. For example, *Soltani et al.* reported a 15 mm inhibition zone for *L. casei* against *E. coli* [17]. Likewise, *Shaaban et al.* demonstrated that CFS from *L. casei* effectively inhibited *Proteus mirabilis* biofilm formation, highlighting its potential as an antimicrobial agent [41].

To assess potential synergy between Lactobacillus CFS and colistin, we used the modified Kirby-Bauer disk diffusion method due to its simplicity, reproducibility, and visual representation of bacterial inhibition. While broth-based methods like the checkerboard assay provide quantitative FIC indices, they require extensive preparation and prolonged incubation. Given the exploratory nature of this study, disc diffusion was chosen for its ease, cost-effectiveness, and ability to generate preliminary interaction data [21]. Notably, if synergy had been observed, future studies could incorporate checkerboard assays for precise quantification.

Unexpectedly, colistin addition reduced inhibition zones compared to lactobacilli alone. This contrasts with a prior study on Lactobacillus CFS and polymyxin E against *Acinetobacter spp*., where synergy enhanced bacterial inhibition [11]. A key distinction between that study and ours is that their *Acinetobacter* strains were not polymyxin-resistant, whereas our *K. pneumoniae* isolates exhibited colistin resistance. This suggests that colistin resistance mechanisms may impair potential synergy with *Lactobacillus* CFS. In *K. pneumoniae*, colistin resistance is primarily driven by lipid A modifications via *mcr* genes or chromosomal mutations (*pmrAB, mgrB*), which alter the outer membrane and may disrupt probiotic interactions. Specifically, these membrane changes could reduce susceptibility to bioactive peptides or bacteriocins in *Lactobacillus* CFS, thereby limiting synergy. This hypothesis warrants further investigation to determine whether colistin resistance provides cross-protection against probiotic-derived antimicrobial compounds [42]. Several studies have

examined the antimicrobial potential of probiotic-derived CFS in combination with antibiotics, with mixed findings. *Abelhalim et al.* reported no additive effect when combining *Lactobacillus* CFS with cefoperazone against MDR *K. pneumoniae* [15]. Conversely, *Aminnezhad et al*. observed a significant increase in inhibition zones when *L. plantarum* CFS was combined with antibiotics against *Pseudomonas aeruginosa* [43]. Similarly, *Isayenko et al.* reported an enhanced inhibitory effect against *Acinetobacter baumannii* when metabolic complexes of *Lactobacillus* were combined with antibiotics [44]. These discrepancies underscore the influence of bacterial species, resistance mechanisms, and probiotic strain selection on synergistic outcomes. Our findings suggest that *Lactobacillus* strains exert antibacterial effects independent of colistin. Future investigations should assess whether alternative probiotic strains, different bacterial targets, or alternative antibiotic combinations could yield enhanced synergistic effects.

As far as we know, this research is the first to look into the antimicrobial effects of *Lactobacillus* against colistin-resistant *K. pneumoniae*. While *in vitro* results do not always reflect *in vivo* effectiveness, our findings suggest that *Lactobacillus* strains may play a role in preventing or treating colonization and infections caused by colistin-resistant *K. pneumoniae*. Although our *in vitro* findings highlight the antibacterial potential of Lactobacillus strains, there *in vivo* efficacy may be influenced by host immune responses, gut microbiota interactions, and the stability of probiotic-derived compound [45]. Animal studies are needed to assess their therapeutic potential against colistin-resistant *K. pneumoniae*, while clinical trials will be essential to evaluate safety, tolerability, and efficacy in humans. Future research should also explore alternative probiotic strains, diverse bacterial targets, and novel antibiotic combinations to enhance synergy and expand therapeutic applications [20].

Conclusion

This study demonstrates the antibacterial potential of *Lactobacillus* strains against colistin-resistant *K. pneumoniae*, highlighting their possible role as an alternative or adjunctive strategy to combat antimicrobial resistance. Among the tested strains, *L. helveticus* exhibited the strongest inhibitory effects, while the combination of *L. paracasei, L. rhamnosus,* and *L. acidophilus* also showed substantial activity. However, combining colistin with *Lactobacillus* strains resulted in an indifferent effect, indicating a lack of synergy between their antimicrobial mechanisms and colistin. Despite these promising findings, several limitations should be acknowledged. First, this study was conducted *in vitro,* which may not fully reflect *in vivo* conditions. Second, the specific mechanisms underlying *Lactobacillus* antimicrobial activity were not explored. Third, the potential effects of probiotics on biofilm formation and host immune modulation were not assessed. Additionally, the sample size of 25 colistin-resistant isolates, while offering preliminary insights, may limit statistical power and generalizability. Larger-scale studies with a more diverse bacterial collection are needed to confirm these findings. Further clinical studies are essential to validate the therapeutic potential of *Lactobacillus* strains in managing MDR *K. pneumoniae* infections and to assess their role in infection prevention and treatment.

Conflicts of intereset:

None declared.

Financial disclosure:

None declared.

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