Microbes and Infectious Diseases 2025; 6(4): 6649-6659

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Evaluation of the antimicrobial activity of *Lactobacillus* strains against multidrug resistant *Klebsiella and Pseudomonas* species

Mona Gamal Nada, Maha Mahmoud Kotb *

Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Egypt.

ARTICLE INFO

Article history:
Received 8 January 2025
Received in revised form 20 February 2025
Accepted 25 February 2025

Keywords:
Cell-free supernatants
Probiotics
inhibitory activity
Gram negative bacilli
biofilm

ABSTRACT

Background: The rise of multidrug-resistant (MDR) pathogens has encouraged more research to identify harmless and a potential substitute to antibiotics, such as probiotics. The current study aims to evaluate the inhibitory activity of cell-free supernatants (CFS) of *Lactobacillus acidophilus (L. acidophilus*) and *Lactobacillus fermentum (L. fermentum*) against MDR *Klebsiella* species and *Pseudomonas* species. **Methods:** The current study included a total of 52 (26 each) clinical isolates *of Klebsiella and Pseudomonas*, confirmed to be MDR by Kirby-Bauer disc diffusion method. Anti-bacterial activities of the CFS of *L. acidophilus (*DSM 20079) *and L. fermentum (*ATCC 9000338) against the MDR isolates were assessed by agar well diffusion. Detection of biofilm formation by test pathogens was done by the tissue culture plate (TCP) method and the CFS ability to interfere with biofilm production was studied. **Results:** The agar well diffusion test showed that the mean value of inhibition zone diameter of *L. fermentum* and *L. acidophilus* against *Klebsiella* were 14.3 mm *and* 13.82 mm respectively. While the mean value of inhibition zones of *L. fermentum* and *L. acidophilus* against *Pseudomonas* were 14.29 mm and 15.45 mm respectively. Biofilm production was identified in 73.1% of the isolates. *L. fermentum* and *L. acidophilus* revealed reductions of 46% and 47.2%, respectively, in biofilm production in *Klebsiella.* While *L. acidophilus* was more effective (61%) in inhibiting the formation of biofilm in *Pseudomonas* than *L. fermentum* (46.5%). **Conclusions**: *L. fermentum* and *L. acidophilus* have an important anti-bacterial effect against MDR *Klebsiella* species and *Pseudomonas* species.

Introduction

The rapid rise in antimicrobial resistance (AMR) constitutes a major danger to world health and necessitates the establishment of alternative strategies for combating dangerous illnesses. Severe nosocomial infections are caused by *Klebsiella* and *Pseudomonas.* These diseases include MDR and extensively drug-resistant (XDR) bacteria, which have the capability of evading the bactericidal effects of many antimicrobial drugs [1, 2]. However, the availability of effective antibiotics to treat such infections is declining every year, suggesting that fewer antibiotics will be available in the future and will most likely become ineffective shortly [3].

Therefore, the development of safe, all-natural antibiotic alternatives, like probiotics, phages and phytomedicines is critically important to treat infections caused by these kinds of microorganisms [4, 5, 6]. Higher-order antibiotics with adverse effects and potentiality to microbiome disruption are often required for biofilm-related

DOI: 10.21608/MID.2025.351516.2452
* *Corresponding author:* Maha Mahmoud Kotb
E-mail address: *kotbmaha@kasralainy.edu.eg*

disorders that are resistant to common antibiotics. Probiotic therapy has been recognized as an encouraging strategy for the prevention and cure of numerous infections, as well as a complement to antibiotic medication, due to its numerous advantages and intrinsic safety [2]. Since *Lactobacillus* produces and secretes certain chemicals into CFS, including bacteriocins, hydrogen peroxide and organic acids, CFS can be considered as a safe and effective substitute for synthetic antibiotics in biofilm inhibition [7]. The study aims to assess the CFS's inhibitory and anti-biofilm effects of *L. acidophilus* and *L. fermentum* against *Klebsiella and Pseudomonas* species.

Materials and Methods

The current study was performed as an *in vitro* experimental research between November 2023 and May 2024. A total of 52 (26 each) clinical isolates *of Klebsiella* species *and Pseudomonas* species were obtained from the Strain Bank, Medical Microbiology & Immunology Department, Faculty of Medicine, Cairo University and confirmed to be MDR were included in this study. These isolates were previously recovered from various clinical samples from hospitalized patients at Kasr Al Ainy University Hospitals. *L. acidophilus (*DSM 20079) *and L. fermentum (*ATCC 9000338) used in this study were obtained from the Faculty of Agriculture, Ain Shams University, Egypt. Approval for this research was obtained from the Research Ethics Committee of the Institutional Review Board (code: N-459-2023), Faculty of Medicine, Cairo University.

Bacterial isolates

The isolates were identified using colony morphology and standard microbiological assays(Gram stain, oxidase test, Triple Sugar Iron test (TSI), citrate test, urease test, Lysine Decarboxylase test (LDC) and Motility Indole Ornithine (MIO) test) [10].

Antibiotic susceptibility testing

All test pathogens ; *Klebsiella* species *and Pseudomonas* species were evaluated for antibiotic susceptibility by disc diffusion Kirby-Bauer method, using the antibiotic discs (Hi Media, India and Oxoid,UK); Piperacillin/Tazobactam (110µg TZP) , Amoxicillin–clavulanic acid (AMC 30µg), Gentamycin (CN 30µg), Ceftazidime (CAZ 30µg) , Imipenem (IMP; 10 µg), Meropenem (MEM; 10µg), Cefepime (CPM; 30µg) , Amikacin (AK; 30µg), Ciprofloxacin (CIP; 5µg), Aztreonam (AT 30µg), Cefoperazone (CPZ;75 µg**) .** Strains were considered MDR if non-susceptible to at least one antibiotic of ≥3 different categories [11]. Results were interpreted according to CLSI standard inhibition zone diameters [12]

Preparation of cell-free supernatants of *Lactobacilli*

The *Lactobacillus* strains were grown anaerobically in Man Rogosa Sharpe (MRS) broth (Sigma Aldrich, USA) for 48 hours at 37˚C. After incubation, centrifugation of samples done at 4000 rpm for 15 mins and filter-sterilization of the supernatants performed through a 0.22 um filter (Millipore Inc., Billerica, USA) and used freshly [7].

Agar well diffusion inhibitory activity of *Lactobacillus* strains against bacterial pathogens

The inhibitory effect of the CFS isolated from *L. acidophilus (*DSM 20079) *and L. fermentum (*ATCC 9000338) were tested separately against each MDR test isolate by agar well diffusion test. Adjustment of the isolates was done to 0.5 MacFarland suspensions in sterile tubes. With a sterile cotton swab, each isolate was swabbed separately on the surface of sterile Muller Hinton agar plates (Oxoid,UK). One hundred microliters of CFS was placed into wells with diameter 10 mm that were cut into agar plates with incubation at 37 °C for 24 h. The diameter of the inhibitory zone was determined in determined in millimeters and interpreted. All isolates were done in duplicates [13, 14].

Biofilm formation testing

MDR pathogens were screened for biofilm production using tissue culture plate method (TCP) which is often applied standardized technique for detection of biofilm formation [15, 16, 17]. The isolates from fresh agar plates were inoculated in 5 ml of Trypticase soy broth and were incubated at 37°C for 24 h. Further dilution of the bacterial suspensions 1:100 using fresh medium. A sterile polystyrene tissue culture plate with 96 flat bottom wells was used, and 200 µl of the produced bacterial solution was added to each well. Following incubation at 37°C for 24 h, gentle tapping was applied to remove contents from plates. After two steps of washing with 200 µL of phosphate buffer saline, the plates were incubated for an hour at 37°C.200 µL of ethanol was used to fix the biofilm developed, and it was left for 15 minutes. The plates staining was performed with 200 µL of 0.1% crystal violet for 10 min. Removal of excess stain was done

by washing twice with deionized water and the plates were left for drying. Finally, 200 µl of 33% glacial acetic acid was added to the wells. The optical densities (OD) of stained bacterial biofilms were measured at 570 nm**.** The test was done in triplicates. Results were interpreted as follows [16, 17]:

- Average OD values > 0.68 are considered as strong positive biofilm-producers
- Average OD values 0.35 to 0.68 are considered as moderate positive biofilm-producers.
- Average OD values 0.17 to 0.34 are considered as weak positive biofilm-producers.
- Average OD value <0.17 are considered as negative biofilm producers

Inhibitory effect of CFS of *L. fermentum (*ATCC 9000338) and *L. acidophilus* (DSM 20079) on biofilm-producing MDR strains

Tested strains were cultivated for 24 hours at 37 °C on TSB. After adjusting the broth's turbidity to meet the 0.5 McFarland standard, fresh TSB was added and the mixture was diluted to 1:100. A 96-well microtiter plate was filled with 100 µL of the bacterial suspension and 100 µL of CFS of *Lactobacillus* strains, and it was incubated for 24 hours at 37°C. Following incubation, the medium was discarded from every well, rinsed with PBS three times. Biofilm formation was fixed with 200 µL of ethanol and left for 15 minutes. Staining was done with 0.1% crystal violet for 5 minutes, followed by washing and drying. The final step involved adding 200 µL of 33% glacial acetic acid. Then optical density was measured at 570 nm. The anti-biofilm activity (%) was calculated using the following formula: (Control OD570 nm − Test OD570 nm / Control OD570 nm) × 100, where 'Control' represents the optical density values with unchallenged pure culture of test pathogen, and 'Test' represents the values under treatment conditions[18,7].

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Comparisons between quantitative variables were made using the non-parametric Mann-Whitney test. For comparison of serial measurements within each patient the non-parametric Friedman test and Wilcoxon signed rank test were used. For comparing categorical data, Chi square (X2) test was performed. Exact test was used instead when the expected frequency is less than 5. P-values less than 0.05 were considered statistically significant.

Results

Antimicrobial susceptibility testing

The susceptibility patterns among the 26 *Klebsiella* isolates varied with 25 isolates representing 96.2% showed resistance to both CAZ & AMC, 24 isolates (92.3%) showed resistance to CIP, while 23 of *Klebsiella* isolates (88.5 %) were resistant to CPZ, CPM & AT, only 20 isolates representing 76.9% showed resistance to TZP & CN, where 18 isolates (69.2%) were resistant to MEM & IMP and finally12 isolates (46.2%) were resistant to AK as illustrated in **figure (1).**

The susceptibility patterns among the 26 *Pseudomonas* isolates varied with all of the 26 isolates showing 100% resistance to CAZ, while 25 isolates (96.2%) showed resistance to both MEM &CIP, 92.3% representing 24 isolates showed resistance to CPM, 22 isolates (84.6%) were resistant to IMP, 20 isolates representing 76.9% were resistance to TZP and finally only 18 isolates (69.2%) showed resistance to AT **(Figure 2).**

Inhibitory activity of *Lactobacilli* CFSs by agar well diffusion

The CFS *of L. fermentum* and *L. acidophilus* showed good growth-inhibiting impact on the tested MDR *Klebsiella* isolates with the mean inhibition zones of *L. fermentum* CFS (14.3 mm) *and* for *L. acidophilus* CFS is (13.82 mm) **(Figure 3).**

The CFS *of L. fermentum* and *L. acidophilus* showed promising inhibitory effect on growth of the tested MDR *Pseudomonas* isolates with the mean inhibition zones of *L. fermentum* CFS (14.29 mm) *while* in *L. acidophilus* CFS, the mean inhibition zones were *(*15.45 mm) **(Figure 4).**

Formation of biofilm:

Based on the findings of the TCP method for biofilm formation, 38 isolates representing 73.1% of total isolates were biofilm forming. Where, 22 isolates were strong positive (42.3%) while 9 isolates were moderately positive (17.3%) and only 7 isolates were weak positive (13.5%), whereas the remaining 14 isolates (26.9%) did not produce biofilms **(Figure 5)**.

Anti-biofilm effect of *Lactobacilli* CFSs

Regarding the inhibiting impact of CFS of *L. fermentum on* 20 biofilm producing *Klebsiella* isolates, the inhibitory effect for the *Klebsiella* biofilm producer strains was 46 % , while inhibiting impact of CFS of *L. acidophilus* on the same *Klebsiella* biofilm producer strains revealed 47. % reduction rate, with statistically significant *p-value*. The efficacy of CFS of *L. fermentum and L. acidophilus* on biofilm producer *Klebsiella* isolates were almost the same (mean reduction is 46 % and 47.2 %, respectively) as shown in **table (1).**

The inhibitory impact of *L. fermentum's* CFS on the 18 biofilm-producing Pseudomonas isolates was 46.5%, while the inhibitory action of CFS of *L. acidophilus on* the same *Pseudomonas* biofilm producer strains revealed 61% reduction rate, with statistically significant *p value.* A slight difference was detected regarding the efficacy of CFS of *L. fermentum and L. acidophilus* on biofilm producer *Pseudomonas* isolates, where the mean reduction is 46.5% and 61% respectively as illustrated in **table (2).**

Table 1. The anti-biofilm impact of CFS of *L. fermentum* and *L. acidophilus on Klebsiella* isolates.

	Klebsiella		
	Mean OD before	**Anti-biofilm impact of CFS of *L. Fermentum***	**Anti-biofilm impact of CFS of *L.acidophilus***
Mean OD	0.81	0.35	0.37
SD	0.56	0.26	0.28
P value*		< 0.001	< 0.001
Mean % reduction		46.01	47.21
P value* between both *lactobacilli* strains		0.877	

* P-value is significant if <0.05

Table 2. The anti-biofilm impact of CFS of *L. fermentum* and *L. acidophilus on Pseudomonas* isolates.

	Pseudomonas		
	Mean OD before	**Anti-biofilm impact of CFS of *L.Fermentum***	**Anti-biofilm impact of CFS of *L.acidophilus***
Mean OD	1.03	0.53	0.39
SD OD	0.63	0.46	0.31
P value*		< 0.001	< 0.001
Mean % reduction		46.51	61.06
P value* between both lactobacilli strains		0.732	

*P value is significant if <0.05

Figure 1. Disk diffusion susceptibilities for *Klebsiella* isolates.

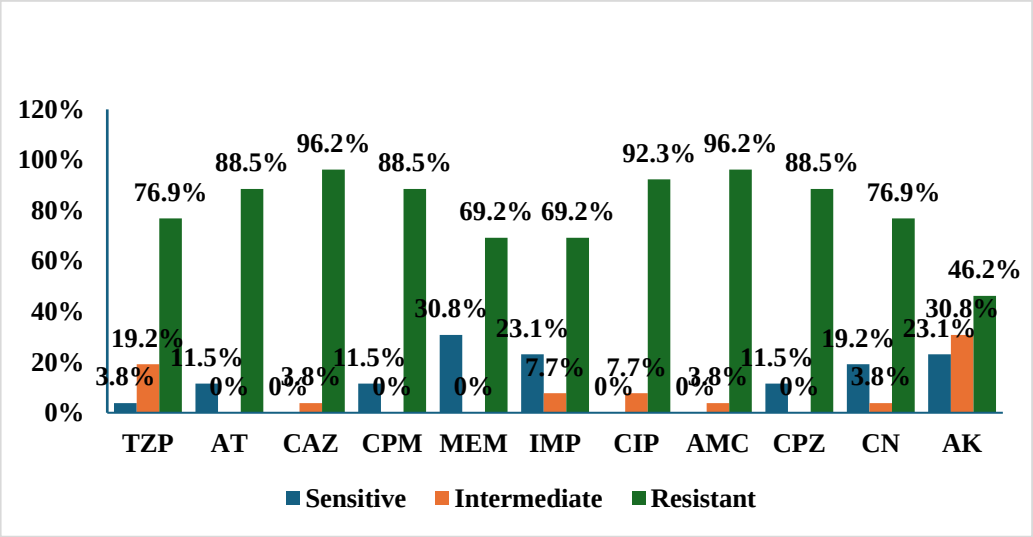


Figure 2. Disk diffusion susceptibilities for *Pseudomonas* isolates.

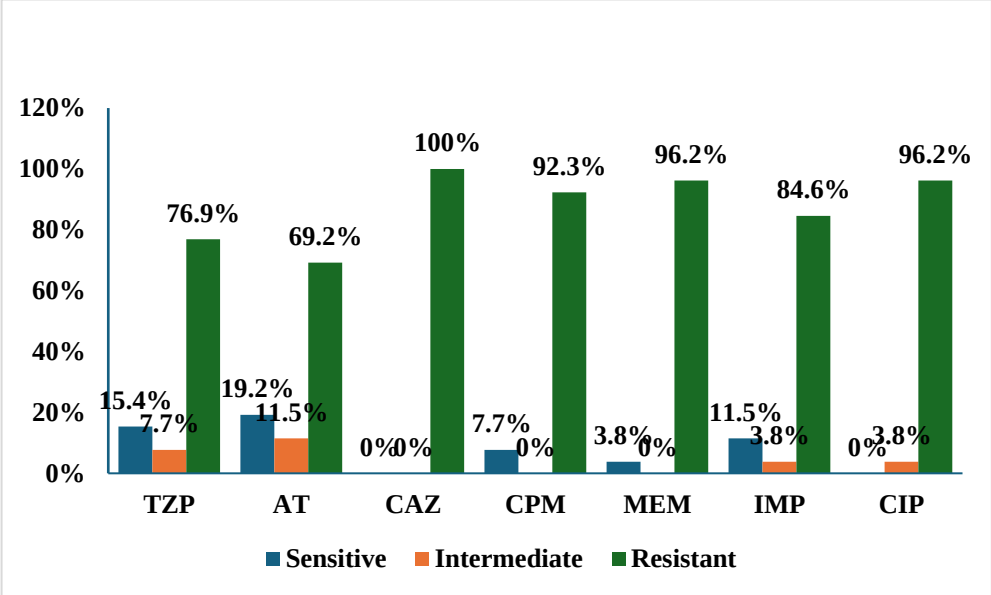


Figure 3. Inhibitory activity of *L. fermentum* and *L. acidophilus* against MDR *Klebsiella* isolates.

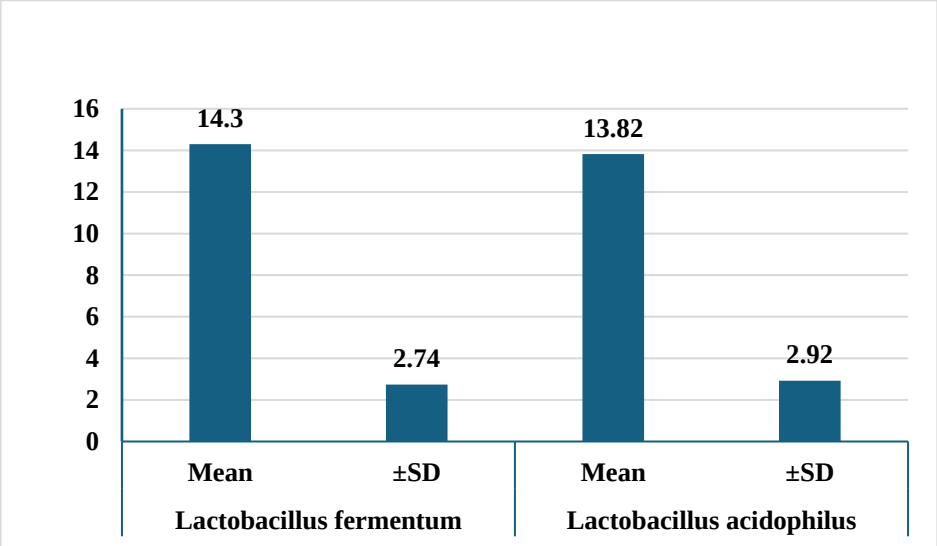


Figure 4. Inhibitory activity of *L. fermentum* and *L. acidophilus* against MDR *Pseudomonas* isolates.

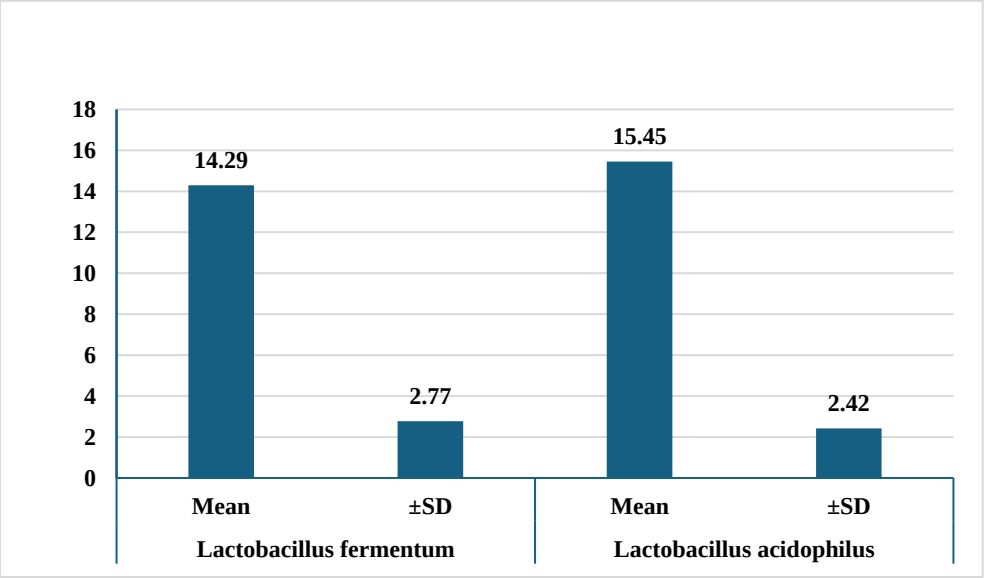
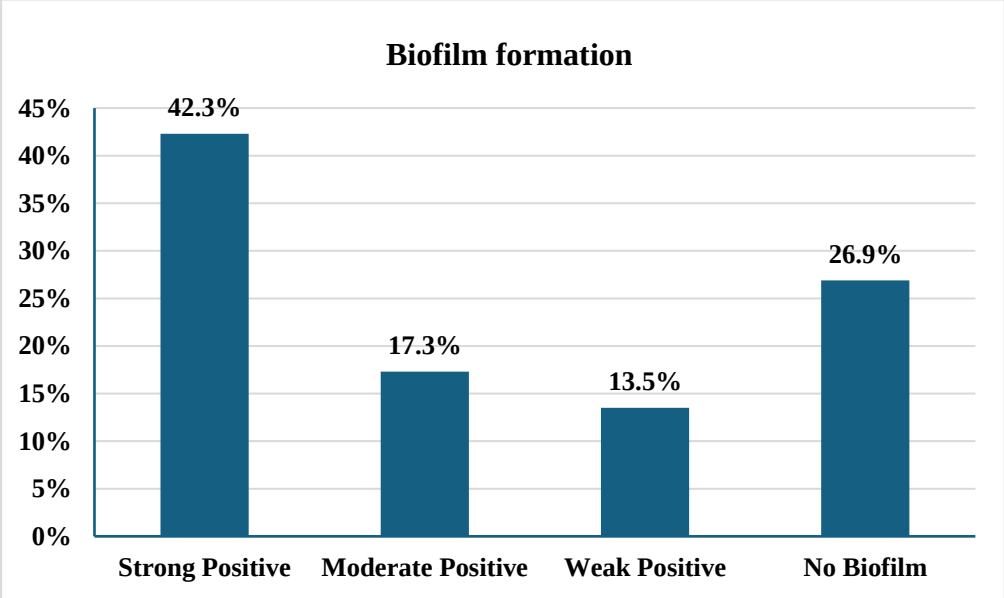


Figure 5. Formation of biofilm by TCP method.



Discussion

Lactobacilli are widely recognized probiotics that are generally considered to be safe natural treatment options and is additionally considered as an immune booster. There are several ways that lactobacilli could demonstrate their antibacterial activity, includes the synthesis of inhibitory substances. , activation of immunity, competing with harmful microbes for receptor binding and nutrition. *Lactobacilli* produce various inhibiting substances such as; lactic acid, formic acid, and acetic acid; bacteriocins; hydrogen peroxide; and peptides which are antibacterial [19, 20, 21]. *Klebsiella pneumoniae and P. aeruginosa* represent a major concern in nosocomial infections. Gram-negative opportunistic bacteria that cause serious infections in hospitals, such as urinary tract infections, pneumonia, and bacteremia, particularly in immunocompromised individuals [22]. Both have several virulence factors contributing to their pathogenicity and antimicrobial resistance. Probiotics, phytomedicine, phages, and other novel approaches are examples of non-antibiotic therapy against MDR pathogens. [6].

In our research, we studied the inhibitory activities of *L. acidophilus* and *L. fermentum* CFS against MDR *Klebsiella* and *Pseudomonas* isolates. Using the agar well diffusion test, the CFS showed

a significant antibacterial activity with diameter of inhibition zone greater than 13 mm against both pathogens. *L. acidophilus* showed mean inhibition zones of 13.82 mm against *Klebsiella,* and mean inhibition zones against *Pseudomonas* isolates were 15.45 mm, while *L. fermentum* produced mean inhibition zones of 14.3 mm and 14.29 mm against *Klebsiella and Pseudomonas* respectively. Considering that all tested *Klebsiella* and *Pseudomonas* isolates were MDR, accordingly, CFS is considered an excellent approach for elimination of such problematic pathogens.

This result was in line with another Egyptian study conducted at the Faculty of Medicine, Assiut University, by El-Mokhtar et al. who stated that by using agar well diffusion assays against ESBL producing *K. pneumoniae and P. aeruginosa* isolates, the zone of inhibition diameters was greater than 13 mm [22]. Another research by Shokri et al. reported that, employing the well diffusion method, the CFS of two *L. fermentum* strains out of 57 lactobacillus strains demonstrated inhibitory zones against 80 *P. aeruginosa* strains with a diameter of 12–20 mm. [18]. Similarly Al-Malkey et al. reported high inhibition zone diameters greater than 13 mm were detected by CFS *of L. acidophilus* by applying the technique of well diffusion against *P. aeruginosa* isolates [23]. Additionally, Hossain, et al. assessed the antimicrobial activity of CSF using the agar diffusion technique of both *L. fermentum* and *L. brevis,* where the two strains showed significant antimicrobial action against several bacteria, such as *K. pneumoniae and P. aeruginosa*. The *L.fermentum* strain, proved to be capable to efficiently inhibit all the target pathogens, with larger inhibition-zone formation in comparison to the *L. brevis* strain, which also inhibited all pathogens successfully [24].

In another study, Abdelhalim et al. evaluated the antibacterial action of CFS of different *Lactobacillus* spps. to wards10 isolates *of MDR Klebsiella pneumoniae* and reported that *L.acidophilus* had no inhibitory effect against MDR isolates, Yet, the most widely recognized growth-inhibiting effects were exhibited by *Lactobacillus rhamnosus* B-445 and *Lactobacillus helveticus.* [25].

Pathogen biofilm formation promotes the adaptation in adverse settings. Biofilms are three-dimensional complex structures generated on both biotic and abiotic surfaces, composed of bacteria which are enclosed in a polysaccharide shell with extracellular DNA. Slower rates of metabolism and replication are presented by bacteria comprising these communities. The polysaccharide extracellular matrix, the structure compactness, and limited strains' metabolic activity , all contributes to increased antibiotic resistance [26]. When treating infections caused by biofilms, the current antibiotics are ineffective due to the protective layers that the cells in the biofilm create. [27, 28].

The use of probiotics and their derivatives to combat biofilms is growing in popularity. [29]. Antimicrobial compounds present in the cell free supernatant had been considered to inhibit the pathogens growth and even induce cell death, resulting in the failure of cell aggregation to form biofilm. Finding LAB strains with antibiofilm activity is therefore essential in order to incorporate them as options to biofilm control. *Lactobacilli* have been evaluated in a number of experimental and clinical investigations for their potential utility in the prevention or treatment of bacterial biofilm-induced infections. Biofilm development is reduced or even disrupted by CFS from LAB. [30, 22].

In the current study, we screened for biofilm formation using TCP. Out of 52 MDR isolates, 38 isolates were biofilm-producing, representing 73.1% of total isolates that were biofilm-forming. Where 22 isolates were strong positive (42.3%), while 9 isolates were moderately positive (17.3%) and only 7 isolates were weak positive (13.5%), whereas the remaining 14 isolates (26.9%) were non-biofilm-forming.

According to a research study by Nasirmoghadas et al., biofilm formation rates were greater, indicating that 93% of tested isolates developed biofilm, where 67 samples reported weak biofilm producers, 22 samples were moderate, and only four samples were strong [31]. A research conducted by Jabalameli et al. revealed that over 96% of the isolates produced biofilm. with 22.9% producing weak biofilm , 26% were moderate producers, and 47% were strong [32].According to Ghadaksaz et al., 50.1% of 104 of *P. aeruginosa* isolates formed biofilms. [33].

Since all of our isolates were MDR, this could account for the high rate of biofilm development in this research. Previous results revealed that the MDR *P. aeruginosa* isolates experienced a greater amount of biofilm

development compared to the non-MDR isolates. [34, 35].

In our investigation, CFS of *L. fermentum* and *L. acidophilus* was able to decrease the production of biofilms in both MDR *Klebsiella* and *Pseudomonas* isolates. *L. fermentum and L. acidophilus revealed reductions of* 46.01% and 47.21%, respectively, in biofilm production *in Klebsiella.* While *L. acidophilus* was more effective (61.06%) in inhibiting the formation of biofilm *in Pseudomonas* than *L. fermentum* (46.5%).

Similarly**,** El-Mokhtar et al. reported that when challenging 24-h biofilms with CFS, disruption and removal of biofilm formation of *K. pneumoniae* and *P. aeruginosa* were 52% ± 12 and 41% ± 15, respectively [22].

Also, Asadzadegan et al., evaluated the inhibitory effect of 7 *Lactobacillus* strains derived from infant feces and reported that the CFS of four isolates showed a 100% inhibition against biofilm formation for all *P. aeruginosa* strains [36]. Additionally, Shokri et al. studed the antibiofilm activity of different lactobacilli strains isolated from local dairy sources against *P. aeruginosa* strains, the strongest antagonistic impact against all strains of *P. aeruginosa* was demonstrated by *L. fermentum*. [18]. Similarly, Varma et al. examined the antibiofilm properties of *lactobacilli* against pathogenic microorganisms such as *P. aeruginosa* in vitro and demonstrated that chemicals released by *L. fermentum* suppressed the development and biofilm formation of *P. aeruginosa* strains. [37].

Singh et al. found that *L. brevis* CFS may prevent *P. aeruginosa* and *K. pneumoniae* from forming biofilms. For *P. aeruginosa*, the greatest biofilm inhibition was 52.63%, while for *K. pneumoniae*, the inhibition activity was 22.2%. [38].

The anti-biofilm properties of CFS of new *Lactobacilli* isolated from domestic goats' guts against the "ESKAPE" group of pathogens were assessed in another investigation by Saini et al. and reported a notable reduction of biofilm formation by *E. faecalis, S. aureus, K. pneumoniae, A. baumannii,* and *P. aeruginosa,* however there was a small degree of variance in their efficiency of inhibition. [2].

Given that the antibacterial characteristics of probiotic *Lactobacillus* depend on strain specificity, full characterization and assessment of the probiotic qualities of new Lactobacillus isolates from natural sources is important before their selection for prospective use. [39,40].

Conclusion

Probiotics may offer an alternative preventive or therapeutic strategy against MDR bacteria. The CFS of both *L. fermentum* and *L. acidophilus*, showed strong antimicrobial and antibiofilm formation activity against MDR *Klebsiella* species and *Pseudomonas* species. However, further studies on larger sample sizes and wider scales are required. Moreover, studies of different species of *Lactobacilli* to test their antibacterial effect are recommended.

Conflict of interest

All authors affirm no conflict of interest in the work.

Funding

This research did not take any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

All data generated or analyzed during this study are included in this puplished article.

Authors' contributions

All authors have substantially contributed to the conception and design, acquisition of data, data analysis, and interpretation. All authors have agreed on the content of the manuscript.

References

1- Boucher HW, Talbot GH , Bradley JS , Edwards JE , Gilbert D , Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin. Infect. Dis. 2009; 48: 1-12.

2- Saini P, Ayyanna R, Kumar R, Bhowmick SK, Bhaskar V and Dey B. Restriction of growth and biofilm formation of ESKAPE pathogens by caprine gut-derived probiotic bacteria. Frontiers in Microbiology 2024; 15:1428808.

3- Andersson, DI, Balaban, NQ, Baquero F, Courvalin P, Glaser P, Gophna U, et al. Antibiotic resistance: turning evolutionary principles into clinical reality. FEMS Microbiol. Rev. 2020; 44: 171-188.

4- Mulani MS, Kamble EE, Kumkar SN, Tawre MS, and Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Frontiers in Microbiology 2019; 10:539.
5- da Rosa TF, Coelho SS, Foletto VS., Bottega A, Serafin MB, Machado CS, et al. Alternatives for the treatment of infections caused by ESKAPE pathogens. J. Clin. Pharm. Ther. 2020; 45, 863-873.
6- Shams Eldeen MA, Elnahal AS, Elsawaf MI, Samy SM. Effect of Lactobacillus Plantarum on virulence factors of Pseudomonas aeruginosa isolated from wound infection. Microbes and Infectious Diseases 2021; 2(4): 790-796
7- Ibrahim AM, Ayoub MI, Awad AR. Antibiofilm effects of cell free supernatant of lactobacilli, Egyptian Journal of Medical Microbiology, 29 (3) ,163-170.
8- Pitt T and Simpson A. Identification of Enterobacteriacea In: Hawkey PM, Gillesoie SH, editors: Principles and Practice of Clinical Bacteriology. Chichester: John Wiley and sons 2006; 341-342.
9- Winn W and Koneman E. Enterobacteriaceae, in Koneman's color atlas and textbook of diagnostic microbiology. 6th ed.. Philadelphia, Lippincott Williams & Wilkins; 2006; P.212-289.
10- Cheesbrough M. District laboratory practice in tropical countries. Part 2. 2nd ed. Cambridge, UK: Cambridge University press; 2007.
11- Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resist ant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3):268–81.
12- Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Tests. CLSI 2024, 34th ed, M100, USA
13- Prabhurajeshwar C, Chandrakanth RK. Probiotic potential of Lactobacilli with antagonistic activity against pathogenic strains: an in vitro validation for the production of inhibitory substances. Biom J. 2017; 40(5):270–83.
14- Abdelhalim, MM., Saafan, GS, El-Sayed, HS, & Ghaith, DM. In vitro antibacterial effect of probiotics against Carbapenamase-producing multidrug-resistant Klebsiella pneumoniae clinical isolates, Cairo, Egypt. Journal of the Egyptian Public Health Association 2022;97(1), 19
15- Christensen GD, Simpson WA, Younger JA et al. Adherence of coagulase negative staphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices. Journal of Clinical Microbiology 1985; 22: 996- 1006
16- Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I, et.al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis, 2007; 115(8), 891-899.
17- Harika K, Shenoy VP, Narasimhaswamy N, Chawla K. Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. Journal of global infectious diseases, 2020; 12(3), 129-134.
18- Shokri D , Khorasgani MR., Mohkam M, FatemiSM, Ghasemi Y, Taheri-Kafrani A. The inhibition effect of lactobacilli against growth and biofilm formation of Pseudomonas

aeruginosa. Probiotics and antimicrobial proteins, 2018; 10(1), 34-42.

19- Rushdy AA, Gomaa EZ. Antimicrobial compounds produced by probiotic Lactobacillus brevis isolated from dairy products. Ann Microbiol. 2013; 63(1):81-90.

20- Inglin RC, Stevens MJ, Meile L, Lacroix C, Meile L. High throughput screening assays for antibacterial and antifungal activities of Lactobacillus species. The Journal of Microbiological Methods, 2015; 114:26-29.

21- Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M , Gil A. Mechanisms of action of probiotics. Advances in Nutrition, 2019; 10(1):S49- S66.

22- El-Mokhtar MA , Hassanein KM, Ahmed AS, Gad GF, Amin MM , Hassanein, OF . Antagonistic activities of cell-free supernatants of lactobacilli against extended-spectrum β-lactamase producing Klebsiella pneumoniae and Pseudomonas aeruginosa. Infection and Drug Resistance, 2020; 543-552.

23- Al-Malkey, MK, Ismeeal MC, Abo Al-Hur FJ, Mohammed SW , Nayyef HJ Antimicrobial effect of probiotic Lactobacillus spp. on Pseudomonas aeruginosa. Journal of Contemporary Medical Sciences. 2017; 3(10), 218–223.

24- Hossain, TJ, Mozumder HA , Ferdausi A , Khadiza A. Inhibition of pathogenic microbes by the lactic acid bacteria Limosilactobacillus fermentum strain LAB-1 and Levilactobacillus brevis strain LAB-5 isolated from the dairy beverage borhani Current Research in Nutrition and Food Science.2022 ;10(3): 928-939;

25- Abdelhalim MM, Saafan GS, El-Sayed HS, Ghaith DM. In vitro antibacterial effect of probiotics against Carbapenamase-producing multidrug-resistant Klebsiella pneumoniae clinical isolates, Cairo, Egypt. Journal of the Egyptian Public Health Association 2022; 97(1), 19.

26- Yin W , Xu S , Wang Y , Zhang Y , Chou SH , Galperin MY et al. Ways to control harmful biofilms: prevention, inhibition, and eradication. Critical reviews in microbiology 2021; 47(1), 57-78.

27- Verderosa AD, Totsika M, Fairfull-Smith KE. Bacterial biofilm eradication agents: a current review. Frontiers in chemistry, 2019; 7

28- Penesyan A, Paulsen IT, Kjelleberg S, Gillings MR. Three faces of biofilms: a microbial lifestyle, a nascent multicellular organism, and an incubator for diversity. NPJ Biofilms and Microbiomes 2021; 7(1).

29- Fajingbesi AO, Sheikh-Lemu MM, Tella MO , Ganiyu OT, Biyaosi, SY. Inhibitory effects of Lactobacillus fermentii and Lactococcus lactis against Pseudomonas aeruginosa biofilms. Fountain Journal of Natural and Applied Sciences 2023; 12(1).

30- Zamani H , Rahbar S , Garakoui SR , Afsah Sahebi A , Jafari H. Antibiofilm potential of Lactobacillus plantarum spp. cell free supernatant (CFS) against multidrug resistant bacterial pathogens. Pharmaceutical and Biomedical Research 2017 3(2), 39-44.

31- Nasirmoghadas P, Yadegari S, Moghim S, Esfahani BN, Fazeli H, Poursina F, et al. Evaluation of Biofilm Formation and Frequency of Multidrug-resistant and Extended Drug-resistant Strain in Pseudomonas aeruginosa Isolated from Burn Patients in Isfahan. Adv Biomed Res 2018; 7:61.

32- Jabalameli F, Mirsalehian A, Khoramian B, Aligholi M, Khoramrooz SS, Asadollahi P, et al. Evaluation of biofilm production and characterization of genes encoding type III

secretion system among Pseudomonas aeruginosa isolated from burn patients. Burns 2012;38:1192 7

33- Ghadaksaz A, Fooladi AA, Hosseini HM, Amin M. The prevalence of some Pseudomonas virulence genes related to biofilm formation and alginate production among clinical isolates. J Appl Biomed 2015; 13:61 8.

34- Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, Kazmi SU. Drug resistance profile and biofilm forming potential of Pseudomonas aeruginosa isolated from contact lenses in Karachi Pakistan. BMC Ophthalmol 2013; 13:57.

35- Ghanbarzadeh Corehtash Z, Khorshidi A, Firoozeh F, Akbari H, Mahmoudi Aznaveh A. Biofilm formation and virulence factors among Pseudomonas aeruginosa isolated from burn patients. Jundishapur J Microbiol. 2015;8(10):e22345

36- Asadzadegan R, Haratian N, Sadeghi M, et al. Antibiofilm and antimicrobial activity of Lactobacillus cell free supernatant against Pseudomonas aeruginosa isolated from burn wounds. Int Wound J. 2023; 20(10): 4112-4121.

37- Varma P, Nisha N, Dinesh KR, Kumar AV, Biswas R Anti-infective properties of Lactobacillus fermentum against Staphylococcus aureus and Pseudomonas aeruginosa. J Mol Microbiol Biotechnol 2011; 20(3):137–143

38- Singh V, Ganger S, Patil S. Characterization of Lactobacillus brevis with potential probiotic properties and biofilm inhibition against Pseudomonas aeruginosa. In Proceedings 2020; 66(1), 14 MDPI.

39- Ramos CL, Thorsen L, Schwan RF, Jespersen L. Strain-specific probiotics properties of Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis isolates from Brazilian food products. Food Microbiol. 2013; 36, 22-29.

40- Campana R, van Hemert S, Baffone W. Strain-specific probiotic properties of lactic acid bacteria and their interference with human intestinal pathogens invasion. Gut pathogens 2017; 9, 1-12.