Research Article

# Antibiofilm Activity of Dextran Purified from *Saccharomyces boulardii* against MDR-*Pseudomonas aeruginosa* Isolated from Wounds and Burns

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**Abstract** | The current study aimed to evaluate the patterns of antibiotic resistance of *Pseudomonas aeruginosa* isolates obtained from burns and wounds with a focus on multidrug-resistant (MDR) isolates and their biofilm formation, and investigate the effect of dextran purified from *Saccharomyces boulardii* on the growth and biofilm formation by these MDR isolates. Seventy- nine isolates of *P. aeruginosa* were collected from Hospitals in Baghdad, re-identified, tested for their susceptibility to 11 different antibiotic discs using Kirby-Bauer method to detect the MDR isolates, and assessed the ability of the MDR isolates to form biofilm. In this study, 34/79 isolates were identified as MDR. The highest ratio of resistance was to Amikacin (55.7%). In comparison, Piperacillin showed the lowest ratio of resistance recording 32.9%, and 33 out of 34 MDR isolates of *P. aeruginosa* were variable in their ability for biofilm formation, recorded as strong, moderate, and weak. Fourier Transform Infrared Spectroscopy (FTIR), solubility test, and melting point were used for dextran characterization. The tests proved that pure polysaccharide contained each of α (1-6) glycosidic bond and (1-3) α-D-glucan, highly soluble, and melted at 254°C. Its antibacterial effect was determined through detecting its Minimum Inhibitory Concentration (MIC) against MDR isolates at concentrations ranging from (100-0.19 mg/mL). The recorded MIC values were 6.25 and 12.5mg/mL for wounds isolates, and was >100 mg/mL for burns isolates. The antibiofilm potential of purified dextran (at sub-MIC 100 mg/ml) displayed the highest inhibition ratio of 35.85% for biofilm formation after 24 h and the lowest inhibition ratio of 5.57%.

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## Introduction

*Saccharomyces boulardii* (*S. boulardii*) is a probiotic and a non-pathogenic yeast, which is beneficial in double-blinded trials as a preventive and curative treatment for diarrhea and other gastrointestinal issues, especially those brought on by the use of antibiotics (Kaźmierczak-Siedlecka *et al.*, 2020). Certain physiological characteristics and phenotypic traits, such as tolerance of high temperature, resistance to low pH, besides its tolerance to bile salt, make *S. boulardii* a successful probiotic. These are in addition

to its unique mechanisms of action as attributed to a combination of several pathways, including immune modulation, trophic effects, production of antimicrobial peptides, pathogen competitive exclusion, and enhancement of gut barrier function (Pais *et al*., 2020).

Dextran is a linear homopolysaccharide composed of at least 50% D-glucopyranosyl units connected by α-(1–6) links, with different proportions of α-(1–2), or α-(1–3), or α-(1–4) branches (Santra and Banerjee, 2021). Exopolysaccharides (EPS) like dextran are very important because of their hydrophilic, neutral, biodegradable, and biocompatible qualities, and due to its solubility, viscosity, thermal and rheological properties, in addition to lack of hazardous side effects (Mouro *et al*., 2024). Dextran finds widespread use in different applicable fields such as pharmacy, industry, and medicine as a carrier, emulsifier, stabilizer, adjuvant, and medicine delivery system (Díaz-Montes, 2021). Dextran is produced by lactic acid bacteria (LAB), and *S. cerevisiae* that is a yeast has also been proved to produce dextran (Abdulhameed *et al*., 2020). The natural metabolites like dextran could inhibit biofilm formation in several ways, including preventing the production of extracellular matrix, suppressing cell adhesion and attachment, dismantling the structure of microbial membranes, and reducing the synthesis of virulence factors. When this happens, biofilm and quorum-sensing QS network formation will halt (Shariati *et al*., 2024).

*Pseudomonas aeruginosa* is an aerobic, heterotrophic, motile, Gram-negative bacterium. This bacterium is an opportunistic microorganism that poses a serious risk to hospitalized patients, particularly those who have burns, wounds, acquired immunodeficiency, and/or immunosuppression, because of its environmental prevalence and the variety of virulence factors it possesses that compromise the immune system (Diggle and Whiteley, 2020). The pathophysiology of this bacterium involves several virulence factors that make it more capable of causing dangerous infections (Reynolds and Kollef, 2021). This bacterium is resistant to many antibiotics, including aminoglycosides, β-lactams, fluoroquinolones, and several disinfectants, attributed to both acquired and intrinsic mechanisms, which include reduced membrane permeability, overexpression of efflux pumps, and synthesis of enzymes that degrade antibiotics, such as aminoglycoside-modifying enzymes and AmpC β-lactamases (Belay *et al*., 2024). Additionally, in *P. aeruginosa*, the formation of biofilms is believed to be the primary resistance mechanism that this bacterium produces, which makes it up to one thousand times more resistant than when it is found as planktonic cells (Salman *et al*., 2024). Additionally, biofilms are crucial for protecting this bacterium from the host immune responses. Microorganisms form biofilms when they attach themselves to a surface and then enclose themselves in a polymeric matrix, which can include polysaccharides, proteins, lipids, and even e-DNA (Singh *et al*., 2021). The objective of the current study was to determine the antibacterial and antibiofilm properties of purified dextran from *S. boulardii* against MDR-*P. aeruginosa* isolates with strong biofilm formation ability, which were isolated from wounds and burns.

## Materials and Methods

*Microorganisms*

*Saccharomyces boulardii CNCM I-3799* strain was obtained from ADM Protexin Limited, United Kingdom. Seventy-nine isolates of *Pseudomonas aeruginosa* were collected from burns and wounds between October 2024 to January 2025 from five different hospital laboratories, including Al-Kindi General Teaching Hospital, Specialized Burns Hospital in Medical City, Al-Shaheed ghazi Al-Hariri Hospital in Medical city, Ibn Al-baladi Hospital, and Al-Kadhimiya Teaching Hospital in Baghdad, Iraq. After collection, all these bacterial isolates were re-identified through testing their cultural characteristics using streak plate method on Nutrient agar (Hi-media, India), macConkey agar (TM-media, India), Blood agar (Oxoid, UK), Certrimide agar (Liofilchem, Italy), and kings A base media (Hi-media, India) (Murray *et al*., 2020), microscopical characteristics using Gram stain, and biochemical tests such as catalase and oxidase assays (Tille, 2022), in addition to VITEK 2 COMPACT system (BioMerieux, France) (Mahon and Lehman, 2023).

*Antibiotic susceptibility assay*

The Kirby-Bauer disc diffusion method reported by Bauer *et al*. (1966) was employed to conduct the antibiotic susceptibility assay of the tested bacterial isolates using 11 antibiotic discs, mainly Ciprofloxacin (5μg), Norfloxacin (10μg), Ofloxacin (5μg), Imipenem (10μg), Ceftazidime (30μg), Gentamicin (10μg),

Amikacin (30µg), Piperacillin (100µg), Cefepime (30µg), Tobramycin (10µg), and Aztreonam (30µg) (Bioanalyse, Turkey). The bacterial isolates were grown in Mueller-Hinton broth (MH broth) (Hi-media, India) for 24h at 37˚C, then the turbidity was adjusted to (1.5 ×$10^8$ CFU/mL) equivalent to 0.5 McFarland. Sterile cotton swabs were used to individually disseminate 0.1 ml of each bacterial culture suspension on the surface of MH agar (Hi-media, India) petri plates. The antibiotic disks were aseptically placed on the surface of the plates (4 discs/ plate) and incubated for 24 h at 37˚C. After incubation, the diameter of the developed inhibition zones were measured using a calibrated ruler (mm), and the results were interpreted as sensitive, resistant, and intermediate according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2024). Isolates that expressed resistance to three or more classes of antibiotics were recorded as multidrug-resistant (MDR) (Magiorakos *et al*., 2012).

*Biofilm formation assay*

The biofilm quantification was performed using Micro-titer plate (MTP) assay as described by (Mathur *et al*., 2006). Approximately, 180 µl of Brain Heart Infusion Broth (BHI broth) (Hi-media, India) supplemented with 2% (g/ml) of sucrose (Loba Chemie, India) were added to 96 flat bottom well microtiter plates (Sigma-Aldrich, USA). Afterward, 20 µL of an overnight bacterial culture equivalent to 0.5 McFarland standards were then added as an inoculant in duplicate. The control wells contained BHI broth without a bacterial culture, and all plates were covered and incubated at 37˚C. After 24 h of incubation, the bacterial suspensions were removed, and each well was washed three times with Phosphate Buffered Saline (PBS) (pH 7.2) (Syrbio, Syria), and left to dry at room temperature for 15 min. Subsequently, the plates were stained with 200 µL of 0.1% crystal violet solution (Vion Biosciences, USA) for 20 min. The plates were washed three times with PBS, and then left to dry at room temperature for 15 min. Afterward, 200 µL of cold ethanol (95%) were added and absorbance readings were taken using an ELISA reader (HumaReader HS, Germany) at a wavelength of 630 nm. The formed biofilms were categorized into the following groups based on the obtained absorbance values (Babapour *et al*., 2016): OD ≤ ODc (None), ODc < OD ≤ 2 ODc (Weak), 2 ODc < OD ≤ 4 ODc (Moderate), and 4 ODc < OD (Strong). *Optical density of control is represented by the letters ODc.

*Production and precipitation of dextran*

The experiments of production and precipitation of dextran were carried out according to the steps outlined by Salman and Salim (2016). Briefly 100 mL of dextran production medium (DPM), composed of 150 g sucrose, (Loba Chemie, India), 5 g peptone (Difco, USA), 0.01 g NaCl ((BDH, UK), 15 g $K_2HPO_4$ (Merck, Germany), 0.01 g $MnCl_2.4H_2O$ (Merck, Germany), 5 g yeast extract (Hi-media, India), and 0.05 g $CaCl_2$ (BDH, UK) fused into one liter of dist. water, were inoculated with 2% (3 × $10^7$ CFU/ml ) of *S. boulardii* suspension with an absorbance measured at (600 nm) using a spectrophotometer (Optima, Japan), and incubated for 24 h at 37ºC. After incubation, the *S. boulardii* culture was precipitated with an equal volume (1:1 v/v) of cold ethanol (99%) (Fisher Chemical, France). Afterward, the culture was agitated rapidly, and centrifuged (Faithful, China) at 4,000 g for 30min. The supernatant was decanted and the precipitate was dissolved in dist. water (1:1 v/v). To eliminate the impurities, the dextran slurry of *S. boulardii* was precipitated again using the same volume of cold ethanol (1:1 v/v) to remove cell debris, and then re-dissolving, precipitation, and washing process were performed three times. After 50 min in an oven (JRAD, Syria) set at 50ºC, dextran was dried and the dry weight was calculated (Abedin *et al*., 2013).

*Purification of dextran*

The crude dextran produced from *S. boulardii* was dissolved in dist water (1:1 v/v). Thereafter, the dextran slurry was precipitated with an equivalent volume (1:1 v/v) of cold ethanol (99%) and then centrifuged for 30 min. at 4,000g (Abedin *et al*., 2013). Re-dissolving, precipitation, and washing steps were carried out five times to eliminated any yeast cell debris. The final precipitate of dextran was re-dissolved in dist. water (1:1 v/v), the pH of the solution was adjusted to pH 7.5, mixed with Protease K (Geneaid, Taiwan) (70 U/100 mL dextran solution), and the mixture was allowed to react at 40˚C for 1 h. Enzyme inactivation was conducted by heating, followed by cooling the solution, dialyzed [7000 Dalton-Molecular weight cut-off (MWCO)] (Schuchardt München, Germany) with dist. water for 24h, and lyophilized (Alpha 2-4 LSC basic freeze dryer, Martin Christ, Germany) for 20h (Lee *et al*., 2017). Thereafter, the dextran purity was confirmed using UV-visible spectrophotometry

(Optima, Japan). The UV absorption spectra were recorded the wavelength range of 200-800 nm.

*Characterization of the purified dextran*

*Fourier-transform infrared spectroscopy (FTIR)*

This analysis was performed using infrared spectroscopy (FTIR) (PerkinElmer TOW with ATR unit, USA). The device worked by measuring the amount of infrared (IR) radiation which was reflected or passed through the tested sample and analyzed across the wave number spectrum of 400–4000 $cm^{-1}$. A graphical chart was made to express the obtained results, where the X-axis on chart signified the number of waves and the Y-axis signified the ratio of transmittance (Faustino *et al.*, 2023).

*Melting point*

Melting Point of purified dextran was determined by using melting point apparatus (HMPD-100, HFH, China). The capillary tube was filled to a depth of 2-3 mm with dextran at the open ending and then heated in the melting point apparatus. There were two recorded temperatures, the initial temperature at which the dextran began to melt and the final temperature at which it completely melt, which was considered as the melting point of purified dextran (Al-Helli and Salman, 2023).

*Solubility test*

The dextran solubility test was conducted in accordance with the procedures specified in the European Pharmacopoeia (Zimmer *et al.*, 2022). Solubility classification was determined by mixing 1 g of dextran with increasing volumes of dist. water. The amount of dist. water required for complete solubilization of dextran dictated the classification. The dextran samples were dissolved in 10-30 mL, 30-100 mL, 100-1000 mL, and 1000-10000 mL of dist. water, which were categorized as soluble; sparingly soluble, slightly soluble, and very slightly soluble, respectively.

*Antibacterial potential of the purified dextran*

To determine the antibacterial efficacy of the purified dextran against the MDR isolates, the Micro-dilution method was employed using 96 flat-bottom wells micro-titer plates to evaluate the minimum inhibitory concentration (MIC) values. The procedures outlined by Salman and Kareem (2021) were followed during the experiment. A stock solution (200 mg/mL) of dextran purified from *S. boulardii* was diluted to concentrations ranging from 100 to 0.19 mg/mL in sterile dist. water. At the beginning, 100 µL of MH broth were added in the first column of the plate and then 100 µL of dextran stock solution were added. Then serially, 100 µL of the mixture were transferred to following wells and discarded from the last column. The wells containing 100 µL of MH broth only without dextran were referred to as the controls. A bacterial suspension of MDR–*P. aeruginosa* that was incubated overnight was then diluted to 1:100 in MH broth equivalent to ($1.5\times10^8$ CFU/mL) equal to 0.5 McFarlan, and 50 µL of the diluted bacterial suspension were added to all wells. Thereafter, covering and incubating the microplates at 37°C were performed. After incubation for 24 h, 30 µL of resazurin dye (0.015%) (Sigma-Aldrich) were added to all wells and incubated for 2–4 h at 37°C to observe any color changes. When incubation completed, the MIC of dextran was determined (Elshikh *et al.*, 2016).

*Antibiofilm effect of the purified dextran*

The antibiofilm influence of dextran purified from probiotic *S. boulardii* against MDR–*P. aeruginosa* isolates, including Pb61, Pb64, and Pb65 was detected by using 96-flat bottom well micro-titer plates in accordance with the protocol outlined by Salman and Kareem (2021). MDR isolates were cultured at 37°C with and without dextran at sub-MIC concentration (100 mg/ml) for 24h. Each well contained 80 µl of BHI broth medium supplemented with 2% sucrose, 20 µL of MDR isolates suspension ($1.5\times10^8$ CFU/mL) were added, and 100 µL of dextran were mixed in each well. The control group contained 180 µL of BHI medium and 20 µL of *P. aeruginosa* suspension. Following incubation, the plates were freed from the excess medium and washed three times with sterile PBS to get rid of any unattached MDR–*P. aeruginosa* cells. The wells were dried for 15 min. at room temperature, filled with 200 µL of 0.1% crystal violet stain, and left for 20 min. at room temperature. After three PBS (pH 7.2) successive rinses, the stained wells were allowed to dry at room temperature for 15 min. Afterward, 200 µL of 95% ethanol were added to each well and an ELISA Reader (HumaReader HS, Germany) was used to measure the OD at 630 nm. The biofilm formation inhibition percentage was determined using the formula given by Namasivayam *et al.* (2012):

$$\text{The biofilm formation inhibition percentage} = \frac{(O.D\ control - O.D\ treatment)}{((O.D\ control))} \times 100$$

Where; O.D control: Optical density of biofilm

formation in absence of dextran, O.D treatment: Optical density of biofilm formation in presence of dextran.

*Examination of biofilm inhibition using field emission scanning electron microscopy (FE-SEM)*

To examine the antibiofilm potential of dextran purified from *S. boulardii* by field emission scanning electron microscopy (FE-SEM), developed biofilm of each MDR- isolate was placed individually on cover slips, followed by addition of cover slips with purified dextran as a treatment for the isolate and without dextran as a control to the same isolate, and incubated at 37°C for 24h (Chopra *et al*., 2015). Afterward the slides were washed for three time with PBS (pH 7.2), and the cells were fixed with 2.5% glutaraldehyde (Hi-media, India). Slides were dehydrated using a graded ethanol series (30–100%) to remove water without causing shrinkage. Subsequently, the samples were dried using a critical point dryer. The dried slides were sputter-coated with a thin layer of gold. Finally, the samples were examined using FE-SEM, images were obtained at an accelerating voltage of 30 kV, with a working distance of 6.9 mm and a magnification of 23,000X. Scale bars representing 4 µm are included in all images (Wang *et al*., 2022).

*Statistical analysis*

To analyze the significance level, or *p*-value, among the various factors included in the study, the percentage and chi-square were computed. A t-test was employed to identify any significant differences between the groups. *P* values greater than 0.05 were regarded as statistically non-significant, whereas *p* values less than or equal to 0.05 were deemed statistically significant. The statistical analysis was carried out by SPSS (v 23).

## Results and Discussion

*Collection and identification of the bacterial isolates*

The colonies of *P. aeruginosa* isolates produced a clear zone with gunmetal or gray color on Blood Agar (Oxoid, UK) as a result of β-hemolysis generation, and they smelled like grapes or tortillas. The developing colonies appeared flat, round, and colorless on MacConkey Agar (TM Media, India), due to their lactose non-fermenting nature. On Cetrimide Agar (Liofilchem, Italy) and Nutrient Agar (Hi-Media, India), the colonies exhibited a greenish-yellow to blue coloration. When grown on King A Base medium (Hi-Media, India), *P. aeruginosa* isolates produced a characteristic greenish-blue pigment (pyocyanin). Additionally, after being stained with Gram-stain, the bacteria were identified microscopically as Gram-negative bacilli, single or pair, and were identified as oxidase and catalase positive, as result of conducted biochemical assays. All of 79 isolates were confirmed as *P. aeruginosa* by using Vitek2 compact system supply with ID kit.

*Antibiotic susceptibility test*

Antibiotic susceptibility of every tested isolate of *P. aeruginosa* was determined. The obtained results indicated that the highest detected resistance ratio was against Amikacin (55.7%), followed by Ofloxacin, Tobramycin, Gentamicin, Ciprofloxacin, Norfloxacin, and Imipenem, which recorded resistances range of 48%-40.5%. On other hand, the resistance ratio of Ceftazidime, Aztreonam and Cefepime were 39.24%, 37.97%, and 35.55%, respectively. The lowest resistance ratio was detected against Piperacillin (32.9%). Moreover, the susceptibility ratio was in the range of 37.97%-59.5% for all the tested antibiotics. The antibiotic susceptibility patterns varied significantly, according to the statistical analysis ($\chi^2$ = 14.5762, p = 0.0001), indicating a high level of statistical significance as shown in (Table 1). In the current study, 34(43.04%) of the tested isolates were categorized as multidrug-resistant (MDR), 32(40.5%) as drug resistant (DR), while the remaining 13(16.46%) of isolates were observed to be susceptible. Statistical analysis revealed significant differences in resistance patterns of *P. aeruginosa* isolated from burn and wound sources ($\chi^2$= 9.3657, *p*= 0.009253) (Table 2).

**Table 1:** *Antibiotic resistance percentages among Pseudomonas aeruginosa isolates.*

| Antibiotics | Antibiotic susceptibility profile | | |
|---|---|---|---|
| | Sensitive | Intermediate | Resistant |
| Amikacin | 30(37.97%) | 5(6.33%) | 44(55.7%) |
| Ofloxacin | 40(50.6%) | 1(1.3%) | 38(48.1%) |
| Tobramycin | 42(53.2%) | 0(0%) | 37(46.8%) |
| Gentamicin | 37(45.6%) | 6(7.6%) | 37(46.84%) |
| Ciprofloxacin | 42(53.2%) | 2(2.5%) | 35(44.30%) |
| Norfloxacin | 45(55.7%) | 1(1.3%) | 34(43.04%) |
| Imipenem | 42(53.2%) | 5(6.33%) | 32(40.5%) |
| Ceftazidime | 47(59.5%) | 1(1.3%) | 31(39.24%) |
| Aztreonam | 43(54.4%) | 6(7.6%) | 30(37.97%) |
| Cefepime | 46(58.23%) | 5(6.33%) | 28(35.44%) |
| Piperacillin | 47(59.5%) | 6(7.6%) | 26(32.91%) |

*Where; The chi-square statistic is 14.5762, while the p-value is 0.0001. The result was considered significant at p < 0.05.*

**Table 2:** *Antibiotic resistant profile of Pseudomonas aeruginosa isolates.*

| Source of sample | Number of isolates | DR isolates | MDR isolates | Susceptible isolates |
|---|---|---|---|---|
| Wounds | 47(59.5%) | 25(31.6%) | 14(17.72%) | 8(10.13%) |
| Burns | 32(40.5%) | 7(8.9%) | 20(25.32%) | 5(6.33%) |
| Total number | 79 (100%) | 32(40.5%) | 34(43.04%) | 13(16.46%) |

*Where; DR refers to drug resistant isolates (i.e., resistant to one or two classes of antibiotics), MDR refers to multidrug resistant isolates (i.e., resistant to three or more classes of antibiotics). The chi-square statistic is 9.3657. The p-value is 0.009253. The result was considered significant at $p < 0.05$.*

The findings of the present study are consistent with a previous study conducted in Iran by Ghasemian *et al.* (2023) on 40 *P. aeruginosa* isolates obtained from burn and wound infections, where the recorded resistance rates toward Imipenem, Ciprofloxacin, Ceftazidim, Piperacillin, and Gentamicin were reported to be 45%, 32.4%, 40%, 40% and 35%, respectively, with a susceptibility rate ranging between 55-60%. Similarly, the previous studies conducted in Iraq by Humady and Hadi (2024); Younus *et al.* (2021) on *P. aeruginosa* isolated from burns and wounds patients, revealed that the isolates exhibited resistance ratios as following: Ofloxacin (45.9%), Norfloxacin (45.9%), Amikacin, Tobromycin, Gentamicin (45%), and Aztreonam (32.5%), which were comparable with our results. While in the same study reported by Younus *et al.* (2021), the susceptibility ratio was ranging between 67.5-100%, in consistence with results of this study, which recorded high rate of intermediate susceptibility compared to the reported study that was 0%. According to the results of a study conducted by Singh *et al.* (2024) in India, the resistance ratios for Cefepime (15%), Ceftazidime (45%), Gentmacin (45%), Ciprofloxacin (60%), Amikacin (80%), Imipenem (100%), and Tobromycin (60%) which disagree with results of the current study; except for Gentmacin, where some antibiotics expressed lower resistance ratios and others displayed higher resistance ratios.

In the present investigation, 34(43.04%) was the ratio of MDR isolates which was compatible with the findings reported by Ghasemian *et al.* (2023); Singh *et al.* (2024), who found that ratio of MDR isolates was recorded as 42.5% and 45%, while disagreeing with Humady and Hadi (2024), which found that MDR was represented 78.6%. It is widely recognized that *P. aeruginosa* is a major source of infections contracted in healthcare facilities, which are difficult-to-treat infections. Moreover, this bacterium can be deadly because of its inherent MDR and capacity to develop resistance to most of the effective antimicrobial therapies (Reynolds and Kollef, 2021).

The results of our investigation revealed that *P. aeruginosa* isolates were more resistant to aminoglycoside group followed by fluoroquinolones group, due to the presence of several enzymes such as Phosphotransferase and N-acetyl transferase, and resistance genes encoded on chromosomes or plasmids, contributing to the *P. aeruginosa* resistance to aminoglycosides. Meanwhile, the *P. aeruginosa* resistance to quinolone antibiotics was attributed to the fact that these drugs impede DNA synthesis through mutating DNA gyrase and topoisomerase enzymes (Bharadwaj *et al.*, 2022). Amikacin showed high resistance towards the same isolates compared to the other types of aminoglycosides. Resistance of clinical isolates to aminoglycoside antibiotics was found to vary with specific drugs, chemical structure of drugs, and presence of a specific modifying enzyme within certain isolates, which had an effect on specific drugs within the same class. These are in addition to several mechanisms of resistance within the same isolate that may have an effect on certain antibiotics but not others within the same class, e.g. resistance of isolate to Gentamicin but susceptibility to Amikacin. The microorganism, its mechanism of resistance, geographic area, and many other factors affect not only antibiotic resistance within the same class, but also resistance to the of antibiotics in general (Mohamed and Abdelhamid, 2020).

*Biofilm formation by MDR isolates*

Micro-titer plate was used to investigate the biofilm-forming capacity of MDR *P. aeruginosa* isolates. In total, 33/34(97.06%) of MDR- *P. aeruginosa* isolates were biofilm forming. The obtained results were divided into four types depending on biofilm analysis, 10/34(29.41%) that were weak biofilm-forming, 17/34(50%) moderate, 6/34(17.65%) strong, and 1/34(2.9%) isolate was unable to generate biofilm. Based on the chi-square test, a statistically significant difference was observed in the biofilm formation ability among the isolates particularly in the moderate biofilm-forming group ($\chi^2$= 12.55, $p$= 0.0003), indicating a high level of significant association as shown in (Figure 1).

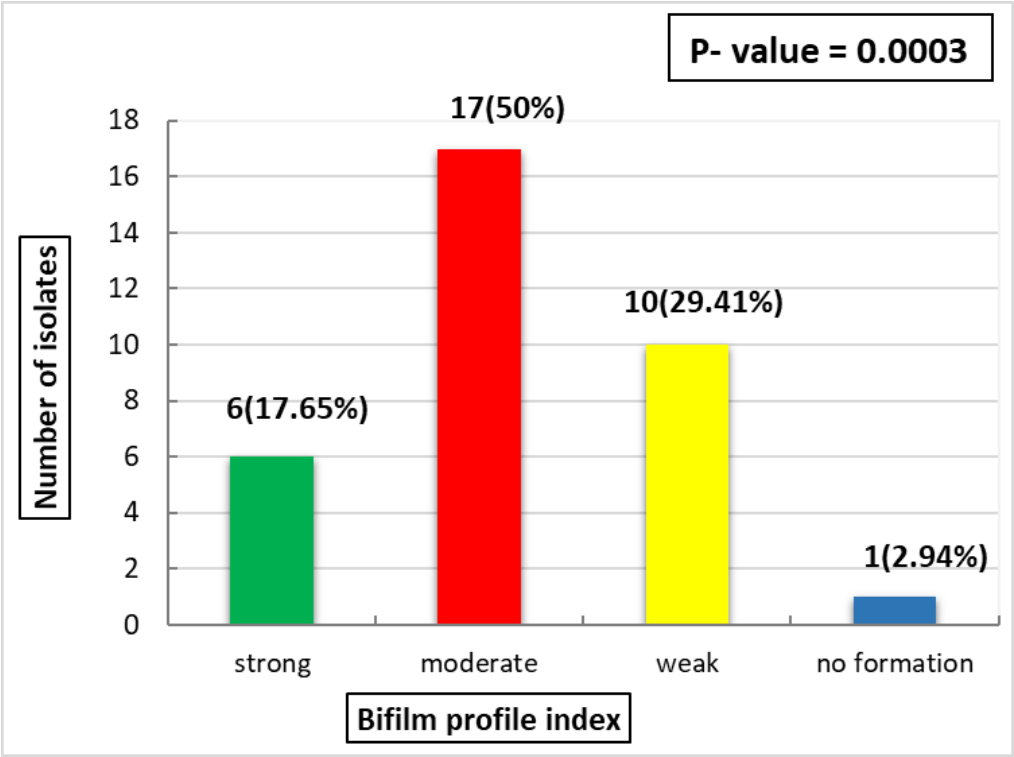


**Figure 1:** *Biofilm formation by MDR-Pseudomonas aeruginosa isolates.*
*Where; The chi-square statistic is 12.5503. The p-value is 0.000396. The result is regarded significant at p < 0.05.*

Biofilms are complex structures composed of a tightly organized bacterial population interacting with each other to create a diverse and protective extracellular matrix. These structures are the main sources of wounds and burns infections, due to their resistance to physical and chemical eradicators, along with the wound and burn ability to facilitate biofilm formation, making therapy more challenging. Wound and burns infections caused by biofilms can also spread throughout the body, potentially leading to fatal systemic infections (Zhao *et al.*, 2023).

In the present study, 33 (97.06%) of the MDR isolates were recorded as biofilm formers. The results of biofilm formation agreed with the previous studies conducted by Ghasemian *et al.* (2023); Khadam and Salman (2024), which recorded that 100% of tested *P. aeruginosa* isolates had biofilm forming capability. In their study, Singh *et al.* (2024) reported that a lower percentage up to 80 % of *P. aeruginosa* strain were biofilm producers. According to Khadam and Salman (2024), *P. aeruginosa* was the dominant biofilm producer among the other tested microorganisms in burn and wound isolates, and the best biofilm producer. However, these results disagree with Kunwar *et al.* (2021) study that reported lower rates of biofilms production 25%. The hospital hygiene and cleanliness protocols could be the reason for generating variants with higher capacity to form biofilms in some isolates than others.

The ability of certain pathogens to colonize both living and nonliving environments is greatly enhanced by their ability to produce biofilms (Zhao *et al.*, 2023). Biofilm production makes it more difficult to completely eradicate diseases by increasing antibiotic resistance (Belay *et al.*, 2024). Quorum sensing works to enhance and extend the overall pathogenicity by regulating biofilm formation and coordinating the extra-virulence agents production (Salman *et al.*, 2024). Biofilms are incredibly resistant to the defenses of the host, disinfectants, and antibiotics which is a well-known characteristic of *P. aeruginosa*. This hinders elimination of the bacteria from the host and may lead to the emergence of persistent infections that are incredibly resistant, becoming problematic to the medicine (Reynolds and Kollef, 2021).

*Precipitation and purification of dextran*

Purification of dextran was carried out after the production process. The total dry weight of dextran purified from *S. boulardii* was 1.25 g/L, which denoted the dextran yield. White, and being granular and chalky-like, were the characterizations of the purified dextran. The dextran purity was verified by utilizing of UV-Vis spectrophotometry analysis (Optima, Japan),

demonstrating that *S. boulardii* dextran was devoid of any nucleic acids and proteins, proved by the lack of any peaks at 260 and 280 nm as shown in Figure 2.

According to Kareem and Salman (2019), the whole dry weight of dextran obtained from a probiotic bacterium *L. gasseri* isolated from feces and vaginal fluid after purification was 1.12 g/ L, while in another study conducted on 10 strains of lactic acid bacteria (LAB), the highest potent isolate produced dextran of 0.515 g/L (Mıdık *et al*., 2020).

According to Du *et al*. (2023), dextran purity can be proved by UV-Vis method by observing the absence of any peaks at 260-280 nm, because the absence of both peaks revealed that the sample was a pure carbohydrate only which agreed with our results. Direct measurement of the protein by UV-Vis provided a faster and easier alternative to colorimetric methods that had been previously used for protein quantification (Reinmuth-Selzle *et al*., 2022). UV-Vis method could potentially interfere with the formation of other compounds such as DNA, which absorbs light at a wavelength of 260 nm, which is the most important matter that should be noticed (Minhas-Khan *et al*., 2021). This method is used for assessment of the contamination of a protein sample with nucleic acids (Oliveira *et al*., 2022). The protein spectrum displays a very sensitive area at approximately 280 nm. This area includes *S. cerevisiae* mannan, which has residual glucose coupled with proteins in the periplasmic space and the yeast cell wall (Liu *et al*., 2018).

*Characterization of dextran purified from S. boulardii*

*Dextran analysis by FTIR*: The functional groups of standard dextran and that purified from *S. boulardii* were detected by FTIR-spectra analysis. The bands in the region of 3420.39 and 3429.51 $cm^{-1}$ were attributed to V (OH) stretching vibration of the polysaccharide in both samples, while the band in the region of 2925.79, 2925.12, and 2956.80 $cm^{-1}$ corresponded to a C-H stretching vibration. A sharp band found in the region of 1642.99 and 1647.53 $cm^{-1}$ was assigned to a Carboxylic group in both types of dextran. In addition, the peaks at the region of 1460.14, 1457.01, and 1429.24 $cm^{-1}$ were attributed to C-H and O-H stretching, while the band in the region of 1239.88, 1014.74, 1246.02, and 1082.55 $cm^{-1}$ confirmed the existence of both C-O and C-C stretching vibrations in both samples. Furthermore, the band region of 1359.60, 1385.09 $cm^{-1}$ was attributed to (1-3) α-D-glucan,

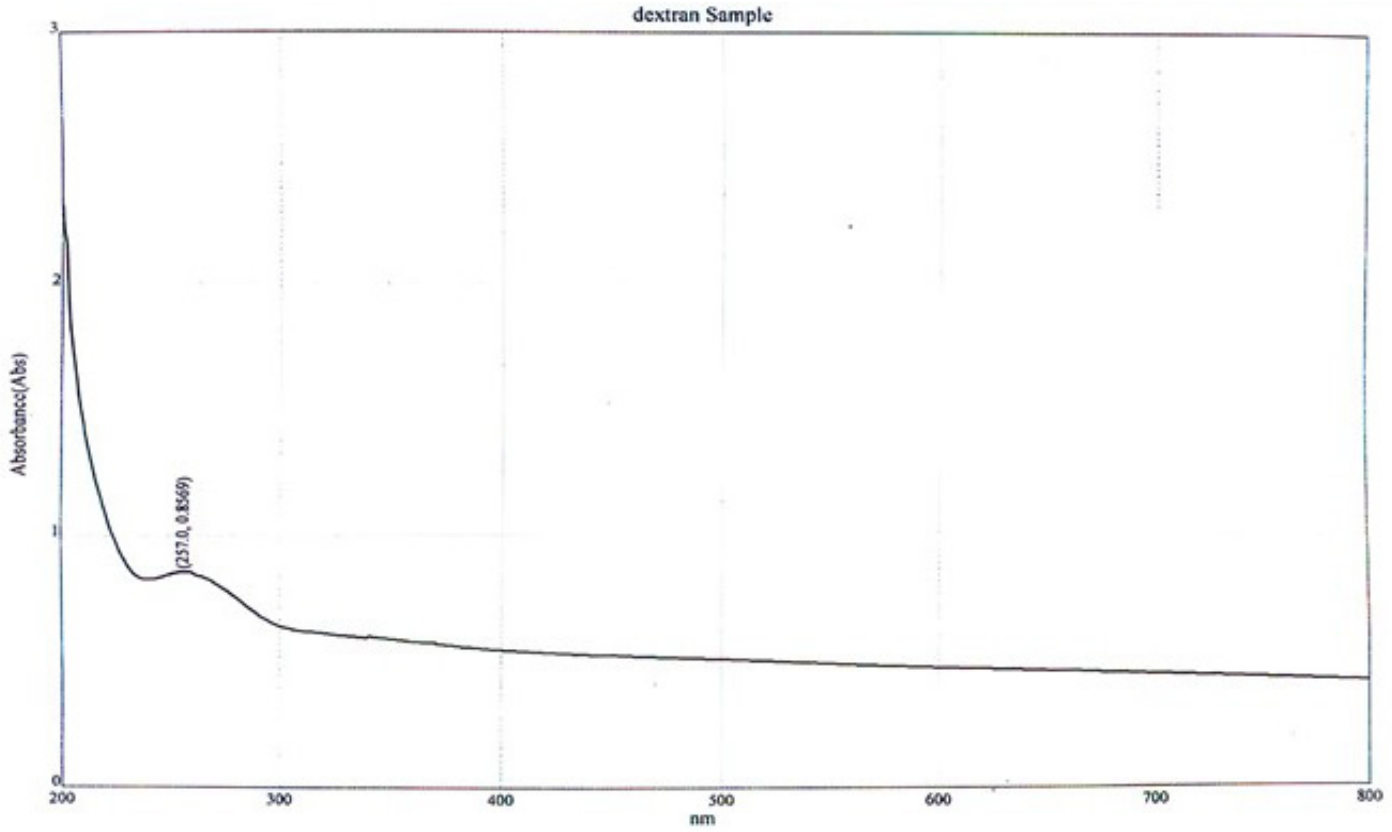


**Figure 2:** *UV-Vis absorbance spectrum of purified dextran produced by S. boulardii showing absorbance (Y-axis) versus wavelength (nm) (X-axis).*


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which confirmed the polysaccharide nature of dextran. Additionally, the distinct band detected at 1014.74, 1082.55 $cm^{-1}$ was assigned to α-(1-6) glycosidic bond, indicating a glycosidic bond in dextran. Along with a band region in the 548.67 and 557.03 $cm^{-1}$ for each sample of dextran was attributed to the presence of (1-3) alpha-D-glucan. The broad peak at the region 1457.01 and 1385.09 $cm^{-1}$ proved the polysaccharide nature for both of tested samples. The presence of alpha-(1-6) and (1-3) links verified that the polysaccharide investigated in the current study was dextran, as shown in (Table 3).

**Table 3:** *Functional groups of standard and purified dextran obtained from a probiotic yeast S. boulardii by FTIR analysis.*

| Purified dextran | Standard dextran | Group | Comments |
|---|---|---|---|
| Position | Position | | |
| 3429.51 | 3420.39 | V(OH) | -Stretching vibration of the polysaccharide |
| 2925.12 2956.8 | 2925.79 | C-H | -CH stretching of $CH_2$ and $CH_3$ groups |
| 1642.99 | 1647.53 | Carboxylic group | |
| 1457.01 | 1460.14 1429.24 | C-H O-H | -Is for confirming the polysaccharide nature of the dextran compound |
| 1246.02 1082.55 | 1239.88 1014.74 | C-O and C-C | |
| 1385.09 | 1359.6 | (1-3)α-D-glucan | |
| 1082.55 | 1014.74 | α-(1,6) glycosidic bond | -Chain flexibility in dextran around the glycosidic bond. |
| 557.03 | 548.67 | (1-3)α-D-glucan | -Is for confirming the polysaccharide nature of the dextran compound. |

Abdulhameed *et al.* (2020) found that purified dextran from *S. cerevisiae* showed several bands in the same regions, including 3404.47 $cm^{-1}$ due to (O-H) stretching vibration of the polysaccharide, 2920.32 $cm^{-1}$ due to (C-H) stretching vibration, 1635 $cm^{-1}$ for carboxyl group, 1024,24 $cm^{-1}$ due to α-(1→6) glycosidic bond, and 821.70 $cm^{-1}$ due to (1→3) α-D-glucan.

Du *et al.* (2023) showed that the strong absorptions at the region of 3422.10 $cm^{-1}$ and 2925.53 $cm^{-1}$ were attributed to the existence of O-H and C-H groups, while the absorptions observed at the region of 1639.05 and 1456.67 $cm^{-1}$ were assigned to C=O and C-O stretching vibration of the carboxyl group of dextran produced from *Leuconostoc pseudomesenteroides*. According to Salman and Kareem (2021), the dextran exhibited an extensive and board peaks at region of 1219.10$cm^{-1}$ and 1043.52 $cm^{-1}$ that assigned to stretching of (C-O) and (C-C).

*Melting point*

To find the temperature at which the dextran compound becomes completely melted, a melting point analysis was employed. This analysis provided information about purity of the sample. According to the finding of the current study the purified dextran has been melting at 254 ˚C.

According to Zhou *et al.* (2018), the EPS exhibited excellent thermal stability with a melting point of 274.14˚C and a degradation temperature of 313.80˚C, as demonstrated by thermal gravimetric analysis and differential scanning calorimetric, while in another study, the recorded melting point of dextran purified from *Leuconostoc pseudomesenteroides* was 325.62˚C (Du *et al.*, 2023). In contrast to our result, in a previous study, the observed melting point of *Lactobacillus fermentum* dextran was 228˚C (Al-Dabbagh *et al.*, 2023).

According to Du *et al.* (2023), the molecular mass, molecular structure, and monosaccharide composition are the reasons for the observed differences in the melting point of dextran and a polysaccharide from one microorganism to another.

*Solubility test*

In the solubility test, 1 g of the finely ground dextran powder was dissolved entirely in 10 ml of water within 1 min., leaving no agglomerates or leftover particles. This demonstrated that the purified dextran was perfectly soluble has and had excellent water solubility.

According to Letinski *et al.* (2021), as dextran plays a major role in water-based settings, its solubility in water is crucial, because dextran and other water-soluble polymers offer enormous potentials and uses in various fields. They might be used to make oral dissolvable tablets or thin films, enabling quick and effective drug absorption into the body (Benalaya *et al.*, 2024). Furthermore, the food and beverage sector uses water-soluble polymers as stabilizers and

dispersants in a variety of ways to improve the stability, consistency, and texture of food products (Al-Helli and Salman, 2023). Size of the dextran particles may have an impact on how quickly it dissolves since smaller dextran particles expose a greater surface area to water, which speeds up the dissolution process. Additionally, vehicles with a larger amorphous components should have higher solubility (Faustino *et al.*, 2023).

*Antibacterial activity of purified dextran*

Purified dextran from *S. boulardii* was used to determine the MIC at concentrations ranging between 100-0.19 mg/mL against MDR isolates of *P. aeruginosa*. Compared to the control wells, the obtained results denoted that the MIC of dextran was >100 mg/mL for isolates Pb61, Pb64, and Pb65 (isolated from burns), while the MIC of dextran was 12.5 mg/mL for Pw29 and 6.25 mg/mL for Pw14 and Pw36 (wound isolates). Among them, the isolates with high MIC were selected for subsequent experiments, according to their high resistance to antibiotics and virulence factors (Table 4).

**Table 4:** *Minimum inhibitory concentration (MIC) of dextran purified from S. boulardii against MDR isolates.*

| ***Pseudomonas* isolates** | **MIC dextran concentration (mg/mL)** |
|---|---|
| *P. aeruginosa* (Pw14) | 6.25 |
| *P. aeruginosa* (Pw29) | 12.5 |
| *P. aeruginosa* (Pw36) | 6.25 |
| *P. aeruginosa* (Pb61) | >100 |
| *P. aeruginosa* (Pb64) | >100 |
| *P. aeruginosa* (Pb65) | >100 |

*Where; MIC: Minimum inhibitory concentration. Pw: indicates isolates obtained from wounds, while Pb: indicates isolates obtained from burns.*

The findings of this study differed from those reported by Salman and Kareem (2021), who found that the MIC was 50 mg/mL for purified dextran obtained from *Lactobacillus gasseri* against all the *P. aeruginosa* isolates, and the MIC of EPS was 200 mg/mL against *P. aeruginosa* in another study conductd by Zainulabdeen *et al.* (2021). Abdulhameed *et al.* (2020) reported that the MIC of purified dextran from *S. cerevisiae* for *Escherichia coli*, *Salmonella sonnei*, and *S. flexneri* was 50 mg/ml, while for *S. typhi* and *S. enteritidis* was 25 mg/ml. The variations observed among these studies might be attributed to the differences in bacterial isolates and environmental conditions.

The EPS have the potential to be widely used as innovative antibacterial agents in food and medicine, because they are easily accessible and non-toxic (Sun *et al.*, 2021). The main mechanisms by which polysaccharides work against bacteria are through weakening the cell wall and membrane, preventing the formation of biofilms, altering bacterial metabolism, preventing the production of proteins, and preventing the absorption of nutrients (Zhou *et al.*, 2022). In addition to their effect on DNA of the bacterial cell (Lu *et al.*, 2024). Although antibiotics are commonly used as antibacterial agents, their abuse damages human health by upsetting the delicate balance of gut microbiota and leads to the emergence of drug-resistant genes (Sun *et al.*, 2021). On the other hand, polysaccharides are thought to be non-toxic, harmless, and effective for the intestinal microorganisms (Zhou *et al.*, 2022). Dextran is one of the molecules that has been used to improve the antibacterial activity of many medicinal products, such as hydrogel (Flynn *et al.*, 2023).

*Antibiofilm effect of purified dextran*

The inhibitory assay used for biofilm formation by purified dextran was performed on the previously identified MDR *P. aeruginosa* isolates that exhibited high biofilm formation. Three isolates were selected from burns infection because they showed strong resistance to all the tested antibiotics, compared to the wounds isolates. The results demonstrated that purified dextran effectively inhibited biofilm formation across all the tested MDR-*P. aeruginosa* isolates after 24 h of incubation, compared to the control group. The highest percentage of inhibition of biofilm formation was observed in MDR-*P. aeruginosa* isolate Pb65 recording 35.85%, followed by isolate Pb61 (26.02%). Conversely, the lowest percentage of inhibition reaching 5.57% was recorded for isolate Pb64. The statistical analysis revealed that *P. aeruginosa* isolates formed substantially less biofilms when treated with dextran, as observed by lower O.D values in detected in treated samples compared to the controls. According to Table 5, there were significant differences for isolates Pb65 ($p$= 0.002) and Pb61 ($p$= 0.05), but no effect was recorded for isolate Pb64 ($p$= 0.91).

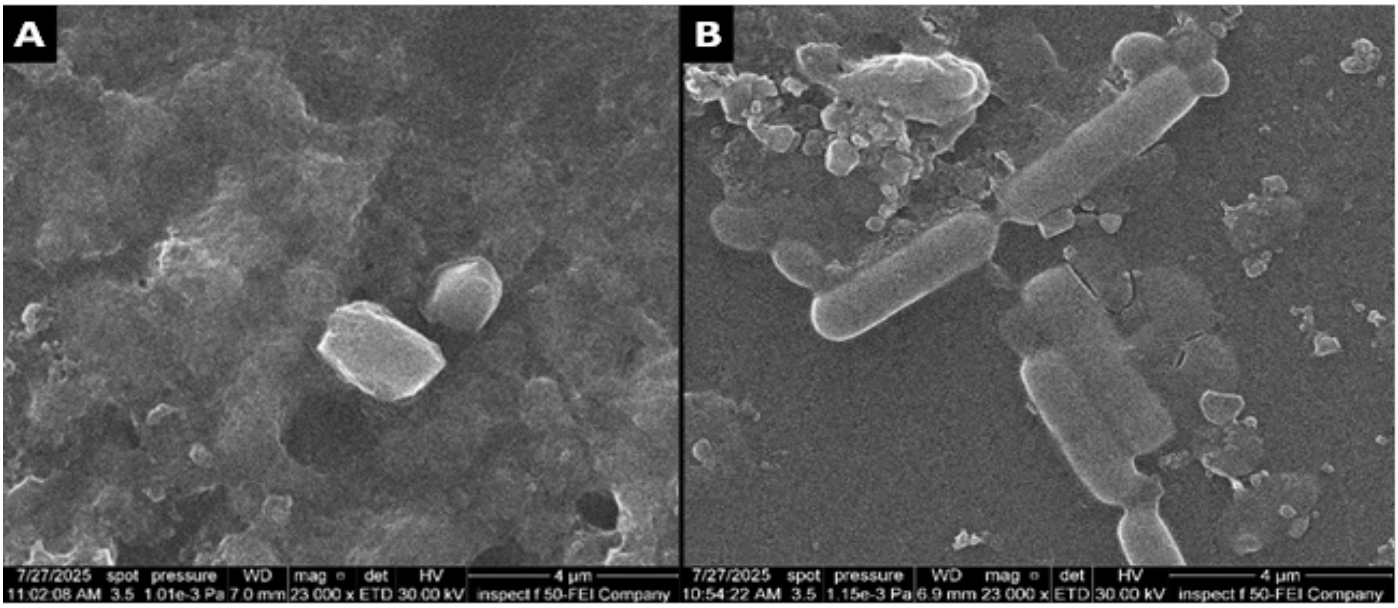


**Figure 3:** *The SEM image of the antibiofilm effect of purified dextran against MDR-P. aeruginosa (Pb61) isolate fixed with 2.5% glutaraldehyde. Where; A control (without purified dextran), B treated with purified dextran at 23,000x*

**Table 5:** *Percentages of biofilm formation inhibition by dextran purified from S. boulardii.*

| ***Pseudomonas* isolates** | **$OD_{(630nm)}$** | | ***p* value** | **Inhibition of biofilm formation (%) at 24h** |
|---|---|---|---|---|
| | **Control** | **Treatment** | | |
| *P. aeruginosa* (Pb61) | 1.026±0.05 | 0.759±0.01 | 0.05 | 26.02% |
| *P. aeruginosa* (Pb64) | 0.574±0.07 | 0.542±0.02 | 0.91 | 5.57% |
| *P. aeruginosa* (Pb65) | 0.756±0.04 | 0.485±0.1 | 0.002 | 35.85% |
| p ≤ 0.05 was considered statistically significant | | | | |

*Where; Pb: isolates obtained from burns, OD: Optical Density, Control: (in absence of dextran), Treatment: (in presence of dextran).*

The FE-SEM image of MDR-*P. aeruginosa* (Pb61) isolate treated with purified dextran of *S. boulardii* displayed high reductions in biofilm formation, while the image of the control (without purified dextran) displayed a formed biofilm, composed of multiple layers of attached bacterial cells as shown in Figure 3. The measured dimensions of the observed structures ranged from approximately 1.027 µm to 4.075 µm. This result supports the findings of Salman and Kareem (2021), who reported that purified dextran produced from *Lactobacillus gasseri* could inhibit biofilm formation by *P. aeruginosa* isolated from the catheters; with a recorded inhibition ratio that ranged between 37%-47% after 24h of incubation. This result agrees with our result except that we investigated the effect of dextran on isolates that showed strong ability to form biofilm; however, the previous study focused on moderate and weak biofilm formers. Abdulhameed *et al.* (2020) showed that dextran purified from *S. cerevisiae* displayed a strong inhibition against the formation of biofilm by diarrheal causative bacteria, which proved that yeasts can produce dextran with high inhibitory activity against the pathogenic bacteria, in accordance with our study, especially since all previous studies focused on EPS produced from the bacteria only.

Zainulabdeen *et al.* (2021) revealed that EPS purified from a probiotic *L. gasseri* can act as an inhibitory agent against biofilm formation by *P. aeruginosa* isolates with a range of inhibition of 27%-51% at 24h. EPS has been shown to lowers cell adhesion and drug tolerance in biofilms (Deokar and Kadam, 2020). A study conducted by Song *et al.* (2020) examined the impact of purified EPS from *L. plantarum* 12 on biofilm of *S. flexneri*, where the obtained results showed that EPS in addition to its effect on growth and development of biofilm, it also effected the primary active components of biofilms such as polysaccharides. According to Almuhanna (2020), *P. aeruginosa* has lectin dependent proteins which are carbohydrate binding proteins found on bacterial membrane that have special functions in formation and adhesion of biofilms, host interactions, contribute in antibiotic resistance, and had a major rule in virulence of the bacteria. EPS inhibited biofilm formation by competing for lectin-binding

sites, preventing the adhesion of pili and fimbriae of pathogenic bacteria, and thereby prevented biofilm formation. In addition to acting as a signal molecule, EPS decreased the expression of many genes involved in biofilm formation and its virulence, further contributing to the inhibition of biofilm development (Zammuto *et al*., 2023).

According to Mouro *et al*. (2024), polysaccharides have many antibacterial mechanisms and focus on damaging the cellular structure and inhibiting bioenergetics metabolism, which includes biofilm formation, because of their structural diversity and uniqueness. These sugar scaffolds are important entities for designing antibiofilm agents. The cell walls of yeasts, fungi, seaweed, and cereals can all provide dextran; a readily available water-soluble polysaccharide, which exhibits significant application potentials in the biomedical field, due to its superior uniformity and long-cycling properties (Chen *et al*., 2025).

## Conclusions and Recommendations

In the current study, the majority of *P. aeruginosa* exhibited multidrug resistance to the proposed antibiotics, with varying rates of resistance in each isolate. Almost all of the isolates that were recorded as MDR had the ability to form biofilm with a ratio of 97.06%. We concluded that *S. boulardii* has the ability to produce dextran with anti-bacterial and antibiofilm activity against MDR-*P. aeruginosa* isolates. The wounds isolates were more susceptible to dextran than burn isolates. The purified dextran provided a promising platform to enhance the efficacy of antibiotics and develop innovative strategies against bacterial infections, particularly in the context of the growing challenge of antimicrobial resistance. We recommend studying the synergistic impact of antibiotics and purified dextran, to increase the effectiveness of antibiotics and solve the problem of resistance to these antibiotics.

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## Novelty Statement

The novelty of the study lies in inhibiting the growth and biofilm formation by MDR *Pseudomonas aeruginosa* isolates obtained from local hospitals using dextran purified from a probiotic yeast strain *Saccharomyces boulardii.*

## Author's Contribution

MAH: Conceptualization, methodology, investigations, and writing of the original draft.
JAS: Supervision, review, and editing.
KKJ: Supervision, and editing.
The final version of the manuscript was approved by all of the authors.

*Ethical approval*
This study was approved by the ethical committee of Mustansiriyah University, Baghdad, Iraq, and the ethical approval code is BCSMU/1221/00045M, Date 1/12/2024. Written consents of the participants were provided.

*Funding source*
None to declare.

*Generative Al or Al-assisted technology statement*
The authors declare that no Al or Al-technology was employed in conducting or writing of this work.

*Conflict of interests*
The authors have declared no conflicts of interest.

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