Borneo Journal of Resource Science and Technology (2025), 15(1): 55-69
DOI: https:doi.org/10.33736/bjrst.7828.2025

Antifungal, Anti-Biofilm, and Anti-Phospholipase Effects of *Pseudomonas aeruginosa* Bacteriocins on Clinical Yeast Pathogens

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Received: 13 September 2024 Accepted: 21 February 2025 Published: 30 June 2025

ABSTRACT

Yeast infections pose a significant challenge around the world, especially with the rising of antifungal drug resistance. This study investigates the antifungal, anti-biofilm and anti-phospholipase activity of bacteriocins produced by the bacterium *Pseudomonas aeruginosa* against 12 yeast isolates which were selected from 57 according to their high resistance to commonly used antifungal drugs, high biofilm production and phospholipase production. Additionally, this study tested the viability of the yeast cells tested after exposure to the bacteriocin. Forty *P. aeruginosa* isolates were tested and the most potent bacteriocin producing isolate was selected. The partially purified pyocins had high antifungal activity with a range of 40.57 µg ml^{-1} to 81.15 µg ml^{-1} minimum inhibitory concentration (MIC) against multiple clinical and drug resistant *Candida* and *Cryptococcus* isolates and surpassed the conventionally used antifungal drugs. It also possessed strong anti-biofilm activity, though its anti-phospholipase activity is varied and isolate dependent, and the viability of the yeast cells was significantly reduced. The high antimicrobial activity of the bacteriocin shows its potential as a therapeutic agent against yeast infections, especially those with high antifungal resistance and biofilm production. These findings can be beneficial to improve patients' outcome as more novel antifungal therapeutic drugs are needed.

Keywords: Bacteriocin, Biofilm, *Candida*, *Cryptococcus*, Phospholipase

INTRODUCTION

The increased resistance of microbes to antimicrobial drugs is rising and with it the deaths related to antimicrobial resistance (Oneill, 2014). Fungal infections represent a public health concern worldwide especially those that are caused by yeasts which result in substantial increase in morbidity and mortality rates (Van de Veerdonk *et al*., 2015; Pal, 2018; Lima *et al*., 2022; Kakar *et al*., 2022), these yeasts can be transported through different door handles, elevators and restaurant tables and staff due to poor personal hygiene, especially *Candida* (Abid *et al*., 2023). Studies have shown that co-infections with pathogenic yeasts can lead to worst outcomes in COVID-19 patients by causing COVID-19 related Candidiasis (Garcia-Vidal *et al*., 2021; Arastehfar *et al*., 2020).

Candida albicans has also been shown to be a causative agent of urinary tract infections (Haghighipour *et al*., 2019; Al-Baqer *et al*., 2021), vaginal infections (Perinelli *et al*., 2018; Hussein *et al*., 2019), oral thrushes (Beute *et al*., 2022; Karajacob *et al*., 2023) and accidental implantation of the yeast during surgery and injections (Wang *et al*., 2019).

Cryptococcus as well as causes a variety of infections especially in immunocompromised patients, by affecting the skin, bones and central nervous system and causing meningitis (Vechi *et al*., 2019; Hayashida *et al*., 2017). The increased use and misuse of antifungal drugs has been related to increased antifungal resistance especially of *C. albicans* and the emergence of resistance in non albicans species as well (Gow *et al*., 2022; M El-Ganiny *et al*., 2021).

One major factor that contributes to the pathogenicity of yeasts is their ability to form biofilms, it serves as a barrier that protects them from antifungal drugs which increases their persistence and makes them hard to manage, the ability to form these biofilms is associated with increased mortality rates especially in patients with candidemia (Vitális *et al*., 2020; Hamady & Marei, 2021; Zhu *et al*., 2022). Another major virulence factor is the extracellular

phospholipases which cause the degradation of phospholipids of the cell membrane of target cells, which disrupts cellular activities and could lead to cell death or changes to the cell membrane that facilitate adherence. *C. albicans* isolates with high phospholipase production were shown to have higher rate of deaths *in vivo* in mice and higher rates of adherence to oral epithelial cells compared to *C. albicans* isolates with lower phospholipase production (Ellepola *et al*., 2014). Phospholipase is an enzyme produced by *Cryptococcus* that hydrolyses phospholipids in the lungs and cellular membranes by breaking down the ester bonds. Phospholipases have shown to enhance the adhesion to lung epithelial cells, it is also required for interstitial tissue growth and efficient transfer from the lung to the brain (Montoya *et al*., 2021).

The requirement for alternative and novel drugs is increasing as the antifungal resistance has been on the rise. Bacteriocins are antimicrobial peptides that have gotten the attention for antimicrobial therapy against bacteria and fungi as seen in the bacteriocin of *Pediococcus pentosaceus* (Taher, 2017). Especially those bacteriocins produced by *P. aeruginosa* show great antibacterial activity (Ling *et al*., 2010; Oluyombo *et al*., 2019; Behrens *et al*., 2020; Paškevičius *et al*., 2022). On the other hand, the antimicrobial activity of these bacteriocins against yeasts, especially those that have high resistance to conventional antifungals is still relatively unexplored.

This study investigated the antimicrobial activity of the cell free supernatant (CFS) of *P. aeruginosa* that contains the pyocins against 4 selected test microbial isolates (two *Staphylococcus aureus* isolates, one *P. aeruginosa*, and one *C. albicans*) which were selected for screening purposes according to previous studies and displayed susceptibility to the pyocins (Mohamed *et al*., 2021; Alqahtani *et al*., 2022). The antifungal drugs resistance, biofilm and phospholipase producing capability of the yeast isolates were investigated and the strongest ones overall were selected. This study also determines the antifungal, anti-biofilm and anti-phospholipase activity of the partially purified bacteriocin of the selected *P. aeruginosa* against multiple species of yeast isolates of *Candida* and *Cryptococcus*, in addition the viability of these yeast isolates was tested after exposure to the bacteriocins. This study may fill a gap in the literature by finding the potential use of *P. aeruginosa* bacteriocins as antifungal, anti-biofilm and anti-phospholipase agents especially against antifungal resistant yeasts, which would improve patients' well-being.

MATERIALS AND METHODS

Source and Identification of Bacterial and Yeast Isolates

A total of 40 *P. aeruginosa* isolates were specifically selected and collected from clinical samples (oral, vaginal and skin) from Al-Baladi Teaching Hospital and Medical City hospitals. The initial identification of the isolates involved microscopic examination and biochemical testing. Microscopic examination involved observation of colony morphology, pigmentation, size and shape; Gram staining to identify characteristic features of *P. aeruginosa*. Biochemical tests involved the use of oxidase and catalase tests, differential media like MacConkey agar (HiMedia Laboratories, Mumbai) and Triple Sugar iron (TSI) slants (HiMedia Laboratories, Mumbai). Pseudomonas Cetrimide Agar (Oxoid, Basingstoke) specialized media was subsequently used to confirm the identity of isolates.

In addition, three different bacterial test isolates (two *S. aureus* isolates, and one *P. aeruginosa*) were employed for screening purposes. They were selected based on previous studies that investigated the antimicrobial effect of *P. aeruginosa* pyocins (Mohamed *et al*., 2021; Alqahtani *et al*., 2022). These isolates were obtained from Microbiology laboratory, at Mustansiriyah University, College of Science, Department of Biology.

A total of 57 yeast isolates were specifically selected from different clinical samples (Oral, Vaginal, sputum, and urine) from Al-Baladi Teaching Hospital and Medical City hospitals. They included different species of *Candida* and *Cryptococcus*. All yeast isolates were identified using microscopic and macroscopic techniques and the specialized media Candida Chromogenic Differential Agar (HiMedia Laboratories, Mumbai) was used for confirmation.

Moreover, all isolates, both bacterial and yeast were further identified using the VITEK 2 Compact System using the manufacturer's procedures (bioMérieux inc, Durhan, NC 27112, USA).

Preparation of Isolates

After identification, all isolates were prepared by culturing them on brain heart infusion (BHI) broth (Neogen, USA) at 37 °C for 24 hours to ensure they were in optimal physiological conditions for the subsequent experimental procedures. BHI broth provides a nutrient-rich environment that promotes a healthy growth and ensures reliable results in the following experimental procedures.

Selection of Yeast Isolates

Of the 57 yeast isolates, 12 of them were selected based on their high resistance to commonly used antifungal drugs and their significant biofilm and phospholipase production. The following techniques were followed to select the 12 yeast isolates:

Antifungal Susceptibility Test of Yeast Isolates

The antifungal susceptibility tests were performed using commercially available discs (HiMedia, Mumbai) for 5 antifungal agents: Amphotericin B (100 µg/Disc), Clotrimazole (10 µg/Disc), Fluconazole (25 µg/Disc), Ketoconazole (10 µg/Disc), and Nystatin (100 µg/Disc). The disc diffusion method was employed, and the results were recorded based on the zone diameters provided in the HiMedia product data sheet. Interpretation of results was conducted in accordance with available breakpoints from HiMedia, EUCAST, and CLSI where applicable. However, it is important to note that CLSI and EUCAST guidelines primarily provide breakpoints for Amphotericin B and Fluconazole, while breakpoints for Clotrimazole, Ketoconazole, and Nystatin were not available for disk diffusion testing. Consequently, the interpretation of results for these agents relied on HiMedia product data.

As a result, the absolute interpretation of susceptibility was less critical in this case than identifying isolates with the strongest resistance profiles. The primary goal of this method was to identify and select the yeast isolates exhibiting the highest resistance to these antifungal agents. These isolates, exhibiting the strongest resistance profiles, were then selected for further testing with *P. aeruginosa* pyocins.

All yeast isolates were diluted in normal saline to a 0.5 McFarland standard (approximately 1×10^6 cell ml^{-1}) and inoculated on Mueller-Hinton Agar supplemented with 2% glucose, in accordance with the Clinical and Laboratory Standards Institute (CLSI), as it enhances fungal growth and ensures consistency. Then the 5 antifungal discs were added to the cultured Petri dishes using sterile forceps and were gently pressed. Then the Petri dishes were cultured for 24 - 48 hours at 35 °C. Finally, the results were measured and recorded. The identification of the resistance of these isolates to the commercially used antifungal drugs also helps to explore the potential inhibitory effects of pyocins compared to the conventionally used antifungal drugs.

Biofilm Production

Biofilm production was assessed using the described technique (Ball *et al*., 2022) with slight alteration. Each yeast isolate was inoculated into 2 mL of BHI broth with 0.25% Glucose then incubated at 37°C for 24 hours. Then all cultures were adjusted to 0.5 McFarland standard and inoculated in a freshly made BHI Broth. After that sterile 96-well microtiter plates were inoculated with 200 µL of the adjusted culture and incubated at 37°C for 24 hours. Next wells were rinsed with PBS 3 times then the microplates were flipped to dry. Then 200 µL of 0.1% Crystal Violet Stain (BDH, England) was added to each well and incubated for 15 minutes. After incubation the wells were rinsed again 3 times with PBS. Then 200 µL of ethanol:acetone of a ratio of 80:20 were added to each well and read at 450 nm using an ELISA Reader. The optical density was recorded for each well.

Each isolate was inoculated on 3 wells and the mean of each of them was calculated. Three wells of sterile BHIB without any microorganisms were also used as a negative control. The threshold for biofilm detection was determined by calculation the mean OD of the negative control and adding two times the standard deviation. This ensures that any OD value above the cutoff accounts for background

noise. Despite the calculated cutoff this study followed commonly used thresholds for classification: Isolates with (OD > 0.320) were considered strong biofilm producers, isolates with (OD value between 0.120 - 0.320) were considered moderate biofilm producers, and isolates with (OD value < 0.120) were considered non/weak biofilm producers. The classifications for biofilm production were selected based on a previously established method (Al-Dabbagh Ali *et al*., 2023). The identification of these strong biofilm-producing isolates lays the groundwork for exploring the potential inhibitory effects of pyocins on biofilm formation.

Phospholipase production

Phospholipase activity was assessed using Egg Yolk Agar (EYA) following the method described by (Fule *et al*., 2015). The EYA was inoculated with 10 µL of the McFarland (1×10^6 CFU mL^{-1}) adjusted yeast isolates and incubated at 37°C for 72 hours. Then the results were determined by measuring the diameter of the colonies and the precipitation zones around them and calculating the PZ (Phospholipase Activity) Value using the following formula, Eq.(1):

$$PZ\ value = \frac{Colony\ Diameter\ (mm)}{Colony\ Diameter\ (mm) + Precipitation\ Diameter\ (mm)} \quad \text{Eq. (1)}$$

Phospholipase activity was categorised into 5 categories (Al-Dabbagh Ali *et al*., 2023) based on the PZ values as follows: 1.00 (Negative), 0.90-0.99 (Weak), 0.80-0.89 (Poor), 0.70-0.79 (Moderate), and <0.70 (Intense). The identification of these strong phospholipase-producing isolates lays the groundwork for exploring the potential effects of pyocins on phospholipase formation.

Screening for Strongest Pyocinogenic *P. aeruginosa* Isolates

The goal of this stage was to screen for the capability of *P. aeruginosa* isolates to produce bacteriocins, and select the strongest one. All 40 isolates of *P. aeruginosa* were screened for their bacteriocins production using a slightly modified version of the original method described by (Morse *et al*., 1976). First the isolates were cultured overnight on nutrient broth. The following day, the isolates were diluted at a 1:50 ratio in fresh nutrient broth medium and incubated for 3 hours in shaker incubator at 80 rpm. Then 2 µg ml^{-1} Mitomycin C was introduced on nutrient broth to induce bacteriocin production. Then it was incubated in shaker incubator at 80 rpm. After about 3 hours the culture was centrifuged at 6700 × g for 20 minutes and the cell free supernatant (CFS) was acquired.

Then CFS was tested against three bacterial and one yeast isolates for screening purposes (two *S. aureus* isolates, one *P. aeruginosa*, and one *C. albicans*) due to lack or no access to standard strains. These three bacterial isolates were selected according to previous studies of antimicrobial activity of *P. aeruginosa* pyocins against these species therefore they were chosen as positive control (Mohamed *et al*., 2021; Alqahtani *et al*., 2022), on the other hand *C. albicans* was used to determine the effectiveness of pyocin against yeast isolates. Agar well diffusion method was used, wells were made and filled with 200 µL of the crude CFS to ensure adequate diffusion and a measurable inhibition zone on Mueller Hinton Agar after it was inoculated with 0.5 McFarland compared yeast suspension. The petri dishes were then incubated at 35 °C for 24 hours (35 °C ± 2 °C as per EUCAST and CLSI guidelines). The inhibition zones were recorded in mm. The isolate of *P. aeruginosa* with the strongest inhibition rate against all test isolates was selected and determined as the strongest bacteriocin producer isolate.

Production of the Partially Purified Bacteriocin

After the screening was done and the strongest bacteriocin producer isolate was selected, this method aims to partially purify the R and S type pyocins. The same method described by (Morse *et al*., 1976) was used for bacteriocin production; however, in this step, the bacteriocin containing CFS was subjected to partial purification. Two successive rounds of ammonium sulphate precipitation were conducted. The first round aimed for 60% saturation, at which point the precipitate was discarded, as R and S pyocins do not precipitated at this level; the second round increased the saturation from 60% to 90%, resulting in the precipitation of R and S pyocins, which are retained at this range. The pyocin precipitate was then collected and dissolved in 25 mL phosphate buffer (PH 7.4). Then 20 ml of the suspension was dialysed overnight against

phosphate buffer using dialysis membranes with a molecular weight cutoff (MWCO) ranging from 8-14 kDa which ensures that S and R type bacteriocins are retained.

Then Bradford technique (Kielkopf *et al*., 2020) was utilised to determine the bacteriocins suspensions protein concentrations. Where a standard curve was plotted using different known concentrations of bovine serum albumin (BSA). BSA was diluted with phosphate buffer, each BSA dilution was then added into cuvettes, Coomassie brilliant blue G-250 (CBB) dye (Cepham Life Sciences, USA) was then added, then the mixture was incubated for 5 minutes before measuring the optical density at 595 nm using a spectrophotometer, then a standard curve was formed.

After that the same procedure was used to measure the bacteriocin concentration, where CBB dye was added to the pyocin suspensions, incubated for 5 minutes, then measuring the optical density at 595 nm using the spectrophotometer where the OD was used to determine the protein concentration of the bacteriocin suspensions by comparing it with the BSA curve.

Determination of Partially Purified Bacteriocins MIC and sub-MIC

The antifungal activity of the partially purified bacteriocins against 10 *Candida* and 2 *Cryptococcus* isolates was measured using the resazurin microtiter plate assay method in a 96 wells microplate (Sarker *et al*., 2007). Each column contained a different isolate. Rows 1-7 contained 50 µL sabouraud dextrose broth (SDB), 100 µL of the serially two fold diluted partially purified bacteriocin, and 50 µL of 0.5 McFarland standard diluted yeast suspension in SDB at a ratio of 1:100. Row 8 served as the control, containing 150 µL of SDB and 50 µL of a 0.5 McFarland standard suspension diluted in SDB at a 1:100 ratios. The Microtiter plate was then incubated at 37°C. After incubation 30 µL of 0.02% resazurin dye (Solarbio, China) was added to each well. The wells would turn red if there is any microbial activity in said wells, otherwise the wells remain blue. The lowest concentration of partially purified bacteriocin added to wells in each column with a blue color was determined as the minimum inhibitory concentration. The sub-MIC values were determined as the concentrations of pyocins in the wells immediately following the MIC wells in the dilution series.

Anti-biofilm and Anti-phospholipase Activity of Pyocin

The effect of pyocin on biofilm formation was studied using a 96 wells microtiter plate, all experiments were done in triplets and 3 times for 24, 48 and 72 hours at 37 °C, for the control and pyocin treatment. Yeast isolates were first incubated overnight on SDB. A turbidity similar to that of McFarland solution (10^6) was then prepared from the yeast culture in normal saline. A volume of 100 µL of the sub-MIC (final concentration) for each isolate was added into the wells. Followed by 80 µL of freshly made SDB and 20 µL of the prepared yeast suspension was then added. The control contained 180 µL SDB and 20 µL of the prepared yeast suspension. After incubation, the medium was discarded from the microtitper plate and was washed two times using PBS and left to dry for 15 minutes, then 200 µL of crystal violet was added to the wells and incubated for 20 minutes. The plate was washed 3 times with PBS and left to dry at room temperature. Finally 200 µL of a mixture of ethanol:acetone (80:20 v/v) was added to the wells and incubated for 10 minutes before reading the optical density at 450 nm using an ELISA Reader (Adeyemo *et al*., 2022). The results were obtained 3 times for 3 consecutive days and the effect of pyocin on biofilm formation was calculated using the following formula, Eq. (2):

$$\%\ \text{Inhibition of Biofilm formation} = \frac{\text{OD of Control} - \text{OD of treatment}}{\text{OD of Control}} \times 100\% \quad \text{Eq. (2)}$$

As for the effect of pyocin on phospholipase production, all experiments were done in triplets for both treatment and control. Yeast isolates were first incubated overnight on SDB. A turbidity similar to that of McFarland solution (10^6) was then prepared from the yeast culture in normal saline. A volume of 20 µL of the prepared yeast suspension was then added to 80 µL of freshly prepared SDB and mixed with 100 µL of sub- MIC (final concentration) pyocin and was incubated at 37 °C for 24 hours. Control contained 180 µL of SDB and 20 µL of the prepared yeast suspension. Then a needle was used to inoculate the yeasts onto the surface of EYA, and incubated at 37 °C for 24 hours. Finally, the colony size and precipitation zone

for control and treatment were measured and the PZ value was calculated. The phospholipase activity was divided into 5 categories according to the PZ values as follows: 1.00 PZ value is negative phospholipase activity, 0.90-0.99 PZ value is weak phospholipase activity, 0.80-0.89 PZ value is poor phospholipase activity, 0.70-0.79 PZ value moderate phospholipase activity, and <0.70 PZ value is intense phospholipase activity (Al-Dabbagh *et al*., 2023).

Effect of the Pyocin on the Viability of Yeast Cells

The effect of pyocin on the yeast isolates viability was tested using (Bahuguna *et al*., 2017) with slight alterations. All experiments were done in triplets. Yeast isolates were first incubated overnight on SDB. A turbidity similar to that of McFarland solution (10^6 CFU mL^{-1}) was then prepared from the yeast culture in normal saline. A volume of 200 µL of the prepared yeast suspension was then added to 800 µL of freshly made SDB in plain tubes, after that 1 mL of sub-MIC (final concentration of the pyocin) was added to the plain tube. Control contained 1.8 mL SDB and 200 µL of the prepared yeast suspension. Isolates were then incubated at 37 °C for 24 hours to test cell viability at various times. Ninety-six-well microtiter plates were used, wells were filled with 50 µL of the treatment and control separately, and 50 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye (Solarbio, China) was added to reach a 0.5 mg mL^{-1} final concentration, then they were incubated for 3 hours. After that the solution was discarded and wells were filled with 150 µL DMSO solvent and was measured at 590 nm using an ELISA reader. Finally, viability inhibition percentages were then calculated using the following formula, Eq.(3):

$$\text{Viability inhibition } \% = \frac{\text{OD Control} - \text{OD Treatment}}{\text{OD Control}} \times 100\% \quad \text{Eq. (3)}$$

RESULTS AND DISCUSSION

Identification of Isolates

A total of 40 *P. aeruginosa* isolates were successfully identified, they appeared with flat and colorless colonies on MacConkey agar and fluorescent green to yellow green on Pseudomonas Cetrimide Agar. They were identified using VITEK 2 compact system (bioMérieux inc, Durhan, NC 27112, USA).

Candida isolates exhibited different colors on *Candida* Chromogenic Differential Agar indicating each different species. *C. albicans* appeared light green, *C. glabrata* appeared cream to white, *C. dubliniensis* appeared pale green, *C. krusei* appeared purple, *C. tropicalis* appeared blue to purple, *C. kefyr* appeared cream to white, *C. utilis* appeared pink, and *C. parapsilosis* appeared white to cream. *Cryptococcus* isolates were also confirmed microscopically through their distinctive capsule formation. All isolates were identified by VITEK 2 compact system (bioMérieux inc, Durhan, NC 27112, USA).

Selection of the Yeast Isolates

Antifungal Susceptibility Test

The antifungal susceptibility test revealed that most the 57 had high antifungal resistance, though the 12 with the highest resistance were selected for further studies. The yeast isolates exhibited a varying susceptibility to Clotrimazole. For instance, *C. kefyr* (isolate 5) demonstrated high resistance to it, while the other isolates exhibited either full or intermediate susceptibility to it. All 12 isolates were resistant to the other antifungal agents tested. Figure 1 shows the results of the antifungal susceptibility test for the selected yeast isolates.

Biofilm and Phospholipase Production

The negative control had a mean OD of 0.042 with a standard deviation of 0.001, resulting in a cutoff value of 0.0446. All isolates exhibited OD values above the cutoff which confirms biofilm production. Since Isolates with (OD > 0.320) were considered strong biofilm producers, isolates with (OD value between 0.120 - 0.320) were considered moderate biofilm producers, and isolates with (OD value < 0.120) were considered non/weak biofilm producers. Table 1 summarises the biofilm production level of the 12 selected isolates

The screening measurements of ODs at 450 nm revealed a diverse spectrum of biofilm production among the tested isolates. Notably, 12 isolates emerged as the strongest biofilm

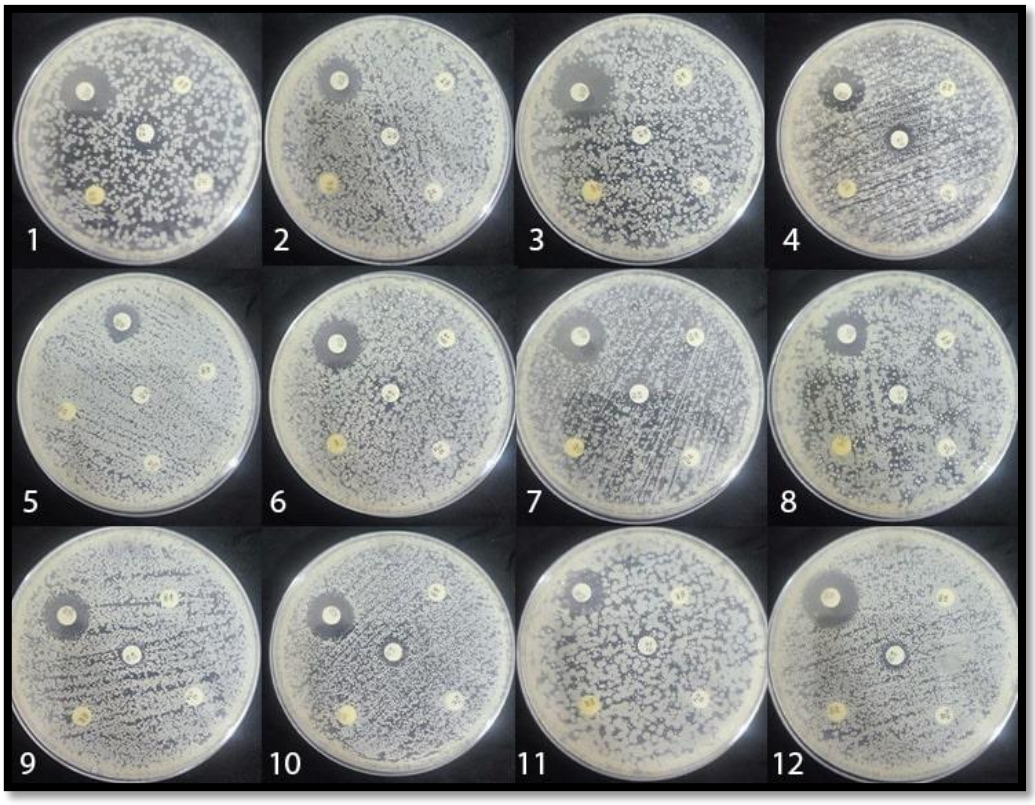

Figure 1. Antifungal susceptibility test against the 12 selected yeast isolates. As shown in the figure all 12 selected isolates showed high resistance to the 4 used antifungal drugs, except for Clotrimazole. The numbers represent the isolates as follows: 1) *C. krusei*, 2) *C. tropicalis*, 3) *C. dubliniensis*, 4) *C. guilliermondii*, 5) *C. kefyr*, 6) *C. parapsilosis*, 7) *C. albicans*, 8) *C. albicans*, 9) *C. glabrata*, 10) *C. utilis*, 11) *C. neoformans*, 12) *C. laurentii*

Table 1. Biofilm producing capability indicated by optical density (OD) of the selected yeast isolates over the course of 24 hours at 37 °C

	Isolate Species	OD mean	Production Level
n.		Cutoff value: 0.0446	
1	*C. krusei*	0.277	Moderate
2	*C. tropicalis*	0.583	Strong
3	*C. dubliniensis*	0.249	Moderate
4	*C. guilliermondii*	0.302	Moderate
5	*C. kefyr*	0.131	Moderate
6	*C. parapsilosis*	0.148	Moderate
7	*C. albicans*	0.400	Strong
8	*C. albicans*	0.342	Strong
9	*C. glabrata*	0.270	Moderate
10	*C. utilis*	0.326	Strong
11	*C. neoformans*	0.178	Moderate
12	*C. laurentii*	0.810	Strong

producers, representing various species within the *Candida* and *Cryptococcus* genera.

Among the identified isolates, *C. tropicalis*, *C. albicans*, *C. utilis*, and *C. laurentii* exhibited particularly strong biofilm production, as evidenced by their elevated optical density readings. While isolates such as *C. krusei*, *C. dubliniensis*, and *C. parapsilosis* demonstrated moderate biofilm production, indicating a lower propensity for biofilm formation. While their biofilm-forming abilities may be less pronounced, these isolates still represent valuable subjects for further investigation, due to their high antifungal resistance and intense phospholipase activity.

Table 2. Phospholipase activity of the yeast isolates according to the measured PZ values

n	Isolate Species	PZ mean	Phospholipase activity
1	*Candida krusei*	0.375	Intense
2	*C. tropicalis*	0.250	Intense
3	*C. dubliniensis*	0.230	Intense
4	*C. guilliermondii*	0.444	Intense
5	*C. kefyr*	0.500	Intense
6	*C. parapsilosis*	0.192	Intense
7	*C. albicans*	0.538	Intense
8	*C. albicans*	0.375	Intense
9	*C. glabrata*	0.187	Intense
10	*C. utilis*	0.238	Intense
11	*C. neoformans*	0.227	Intense
12	*C. laurentii*	0.214	Intense

Utilising PZ values as a measure for phospholipase activity, 12 isolates with high degrees of enzymatic activity, spanning multiple species within the *Candida* and *Cryptococcus* genera were selected. All the 12 isolates selected exhibited intense phospholipase activity as shown in Table 2.

Among the identified isolates, *C. albicans*, *C. glabrata*, and *C. kefyr* demonstrated particularly high PZ values, which is indicative of their high phospholipase production. Isolates such as *C. parapsilosis*, *C. dubliniensis*, and *C. neoformans* exhibited slightly lower PZ values, though still within the range of intense phospholipase activity.

Screening for Strongest Pyocinogenic *P. aeruginosa*

The agar well diffusion method results revealed that (10) 25% out of the 40 *P. aeruginosa* isolates were pyocinogenic and had antimicrobial activity against the test isolates. Among these, isolates 2 and 5 of *P. aeruginosa* demonstrated the strongest antimicrobial activity. However, isolate 5 was preferred due to its superior antimicrobial activity against the test yeast isolate *C. albicans*, which aligned with the study's primary objective. Table 3 presents the antimicrobial activity against the four test isolates.

Production of the Partially Purified Pyocin

Determination of bacteriocin suspension concentration is important to find its MIC. The crude extract which was obtained after centrifugation (6700 × g) of the Mitomycin C induced *P. aeruginosa* culture had a concentration of 1.75 mg mL^{-1}. After the precipitate was dissolved in 25 mL phosphate buffer the concentration was 2.96 mg mL^{-1} and 2.59 mg mL^{-1} after being dialysed. Table 4 presents the Purification profile of Bacteriocin from *P. aeruginosa*. These concentrations were measured using Bradford technique (Kielkopf *et al*., 2020).

Determination of Partially Purified Bacteriocins MIC and sub-MIC by Microtiter Plate Assay Method

The MIC values of the bacteriocin are presented in Table 5 against all tested the yeast isolates. The degree of susceptibility is varied among the yeast isolates, ranging from 40.57 µg mL^{-1} to 81.15 µg mL^{-1}.

The results shed light on the potential of *P. aeruginosa* bacteriocin as an alternative for antifungal drugs against yeasts, especially against clinical antifungal resistant ones. Varying levels of yeast susceptibility and MIC of the partially purified bacteriocin have been revealed indicating its effectiveness against the yeast isolates. Potent antifungal activity has been observed against the yeast isolates with MIC ranging from 40.57 µg mL^{-1} to 81.15 µg mL^{-1} across different isolates. With 50% of the isolates of the *Candida* isolates showed susceptibility to 40.57 µg ml^{-1} MIC and the other 50% of *Candida* isolates showed susceptibility 81.15 µg mL^{-1} MIC. Both the *Cryptococcus* isolates on the other hand showed 40.57 µg mL^{-1} MIC indicating the possibility of having higher susceptibility to the partially purified bacteriocin. Notably the two *C. albicans* isolates had different MICs which could be explained if

the two isolates are of different strains and utilizing a type of resistance against the bacteriocins. The ability of the bacteriocin to inhibit the growth of the drug resistance yeast holds great value especially due to its ability to surpass the antifungal activity of the drugs used in this study. It is important to point out that further studies should be made to test its effectiveness *in vivo*, its possible clinical applications and its safety.

Table 3. Antimicrobial activity of *P. aeruginosa* isolates 2 and 5 which exhibited the highest antimicrobial potential against the selected test isolates (two *S. aureus* isolates, one *P. aeruginosa*, and one *C. albicans*). All zones were measured and noted in mm units

	Test isolate species			
P. aeruginosa Isolate number	*S. aureus 1*	*S. aureus 2*	*P. aeruginosa*	*C. albicans*
2	19	13	19	18
5	13	19	0	25

Table 4. Purification profile of Bacteriocin from *P. aeruginosa*

Purification Step	Volume (mL)	Activity (AU/mL)	Protein concentration (mg/mL)	Specific Activity (AU/mg)	Total Activity (AU)	Purification (folds)	Yield (%)
Crude extract	200	125	1.75	71.4	25000	1.00	100%
Ammonium sulphate precipitation	25	130	2.96	43.9	3250	0.61	13%
Dialysis	20	135	2.59	52.1	2700	0.72	10.8%

Table 5. MIC (μg ml^{-1}) of the *P. aeruginosa* bacteriocin against the 12 pathogenic yeast isolates

n.	Species	MIC (μg ml^{-1})
1	*C. krusei*	81.15
2	*C. tropicalis*	81.15
3	*C. dubliniensis*	40.57
4	*C. guilliermondii*	81.15
5	*C. kefyr*	81.15
6	*C. parapsilosis*	40.57
7	*C. albicans*	81.15
8	*C. albicans*	40.57
9	*C. glabrata*	40.57
10	*C. utilis*	40.57
11	*C. neoformans*	40.57
12	*C. laurentii*	40.57

Anti-biofilm and Anti-phospholipase Activity of Pyocin

Results for the biofilm inhibition percentage were calculated. The means, standard error and inhibition percentage (24, 48 and 72 hours) of the control and pyocin treated ODs were calculated and presented in Table 6, P values were calculated for the 24, 48 and 72 hours. They were all significantly lower than 0.05. Treatment with the pyocin showed high inhibition of biofilm for all isolates, reaching a maximum of 82% at 72 hours for isolate number 1 which is *C. krusei*, and a minimum of 14% at 24 hours also for the same *C. krusei* isolate. At sub-MIC concentrations, pyocin does not kill the yeast cells but induces significant stress that impairs their ability to form biofilms. The stress response triggered by pyocin likely disrupts the early stages of biofilm formation, including cell adhesion and the production of the extracellular matrix. This leads to a reduction in biofilm formation without completely inhibiting cell growth. The progressive inhibition observed over time suggests that pyocin acts as a stress factor that prevents the initiation of biofilm

formation. The experiment conducted aimed to assess the impact of a treatment compared to a control group on optical density at 450 nm and inhibition percentage over three time intervals: 24, 48, and 72 hours. Each row represents a distinct condition, presented as Control and Treatment, while each column corresponds to a specific time point.

Analyzing the data, it's evident that the control group exhibited increasing optical densities overtime and treatment group exhibited consistent optical densities indicating high inhibition rates over time. For instance, in the first row, the control group demonstrated optical densities of 0.282 ± 0.011, 0.511 ± 0.027, and Treatment group demonstrated optical densities of 1.343 ± 0.292 at 24, 48, and 72 hours, respectively. The inhibition percentages for these time points were 14.87%, 57.86%, and 82.22%, indicating a progressive increase in inhibition over time.

Comparatively, the treatment group displayed lower optical densities at all time points (0.240 ± 0.005, 0.215 ± 0.033, and 0.238 ± 0.010, respectively), suggesting the potential inhibitory effect induced by the treatment. These differences between the control and treatment groups were statistically significant, as indicated by the p values ($p<0.05$) for all time points.

Furthermore, considering the trends across all rows, it's notable that the Treatment consistently led to decreased optical densities and inhibition percentages compared to the control group, emphasizing its inhibitory effect. The results of the phospholipase experiment suggest a nuanced response among different isolates to the treatment, with an overall non-significant difference observed between the control and treatment groups ($p = 0.899$). This indicates that the treatment did not have a uniform effect across all isolates collectively. However, individual isolate responses highlight a diverse range of reactions to the treatment, suggesting an isolate-specific effect. P values for each isolate have been calculated and presented in Table **7** to show the significance of the effect.

For instance, while isolate 2 (*C. tropicalis*) exhibited an increase in phospholipase activity under treatment conditions compared to the control group, isolates 3, 6, 7, 8, 10, 11 and 12 displayed a notable decrease in phospholipase activity. In contrast, Isolates 1, 4, 5 and 9 showed no significant change. Isolate 7 and 8 which are both *C. albicans* in particular, demonstrated complete inhibition of phospholipase activity under treatment conditions. These observations indicate that the treatment possesses an isolate-specific effect, such as genetic makeup or stress response mechanisms which influences phospholipase production. Isolates with minimal changes in phospholipase activity between control and treatment groups may indicate a lack of response to the treatment or negligible impact on phospholipase production.

Overall, the varied responses among isolates underscore the importance of considering isolate-specific effects when assessing the efficacy of treatments targeting phospholipase activity. Further investigation into the underlying mechanisms driving these differential responses is important to explain the treatment's isolate-specific effects comprehensively.

Effect of the Pyocin on the Viability of Yeast Cells

The MTT assay was utilized to assess cell viability as an indicator with optical density (OD) measured at 630 nm. The extremely low P value obtained (<0.05) indicates a statistically significant difference between the control and treatment groups, suggesting a notable impact of the treatment on cell viability.

Examining the individual results presented in Table 8, it's evident that the treatment exerted a substantial effect on cell viability compared to the control condition. Across all isolates, the treatment led to a decrease in optical density which is indicative of reduced cell viability. For example, in isolate 1 (*C. krusei*), the control group exhibited an OD of 0.624 ± 0.144, while the treatment group showed a significantly lower OD of 0.303 ± 0.020, indicating a substantial decrease in cell viability corresponding to an inhibition percentage of 51.44%.

This trend is consistent across all isolates, with the treatment group consistently demonstrating lower OD values compared to the control group. Notably, isolate 10 (*C. utilis*) displayed a particularly high inhibition percentage (83.98%), indicating a potent effect of the treatment on reducing cell viability.

Table 6. The effect of pyocin on the biofilm activity of the yeast isolates recorded 3 times over the course of 3 consecutive days (24, 48 and 72 hours)

Isolate			Optical Density at 450 nm			Inhibition %		
			24 hours ± SE	48 hours ± SE	72 hours ± SE	24 H	48 H	72 H
1	*C. krusei*	Control	0.282 ± 0.011	0.511 ± 0.027	1.343 ± 0.292	14.87	57.86	82.22
		Treatment	0.240 ± 0.005	0.215 ± 0.033	0.238 ± 0.010			
2	*C. tropicalis*	Control	0.262 ± 0.005	0.452 ± 0.024	1.085 ± 0.090	15.12	68.95	81.39
		Treatment	0.222 ± 0.006	0.140 ± 0.019	0.202 ± 0.031			
3	*C. dubliniensis*	Control	0.345 ± 0.081	0.492 ± 0.019	0.780 ± 0.172	29.12	57.24	70.31
		Treatment	0.245 ± 0.012	0.210 ± 0.022	0.231 ± 0.018			
4	*C. guilliermondii*	Control	0.339 ± 0.034	0.395 ± 0.019	0.912 ± 0.166	52.40	63.32	61.23
		Treatment	0.161 ± 0.002	0.145 ± 0.008	0.353 ± 0.026			
5	*C. kefyr*	Control	0.236 ± 0.022	0.412 ± 0.034	0.740 ± 0.116	39.49	51.09	67.47
		Treatment	0.143 ± 0.002	0.201 ± 0.034	0.240 ± 0.003			
6	*C. parapsilosis*	Control	0.253 ± 0.002	0.494 ± 0.035	0.728 ± 0.056	16.84	43.93	63.56
		Treatment	0.210 ± 0.009	0.277 ± 0.003	0.265 ± 0.047			
7	*C. albicans*	Control	0.317 ± 0.077	0.470 ± 0.011	0.719 ± 0.085	53.04	60.19	68.33
		Treatment	0.149 ± 0.011	0.187 ± 0.022	0.227 ± 0.042			
8	*C. albicans*	Control	0.349 ± 0.056	0.485 ± 0.033	0.894 ± 0.131	49.85	69.82	79.72
		Treatment	0.175 ± 0.013	0.146 ± 0.012	0.181 ± 0.017			
9	*C. glabrata*	Control	0.228 ± 0.032	0.487 ± 0.028	0.748 ± 0.070	25.36	61.01	69.16
		Treatment	0.170 ± 0.023	0.190 ± 0.032	0.230 ± 0.007			
10	*C. utilis*	Control	0.437 ± 0.043	0.447 ± 0.030	0.730 ± 0.024	60.33	56.03	57.75
		Treatment	0.173 ± 0.014	0.196 ± 0.028	0.308 ± 0.027			
11	*Cryptococcus neoformans*	Control	0.343 ± 0.050	0.435 ± 0.060	0.654 ± 0.107	42.09	58.03	62.02
		Treatment	0.199 ± 0.019	0.182 ± 0.021	0.248 ± 0.036			
12	*C. laurentii*	Control	0.491 ± 0.007	0.435 ± 0.026	0.968 ± 0.133	55.32	53.98	54.87
		Treatment	0.219 ± 0.019	0.200 ± 0.013	0.437 ± 0.025			
		p Value	p<0.05	p<0.05	p<0.05			

Table 7. The effect of pyocin on the phospholipase activity of the yeast isolates. SE stands for Standard Error

Isolate			Pz value ± SE	P value
1	*C. krusei*	Control	0.430 ± 0.009	0.188
		Treatment	0.480 ± 0.035	
2	*C. tropicalis*	Control	0.571 ± 0.051	0.012
		Treatment	0.385 ± 0.036	
3	*C. dubliniensis*	Control	0.282 ± 0.085	0.017
		Treatment	0.475 ± 0.070	
4	*C. guilliermondii*	Control	0.392 ± 0.032	0.578
		Treatment	0.437 ± 0.036	
5	*C. kefyr*	Control	0.386 ± 0.017	0.651
		Treatment	0.433 ± 0.097	
6	*C. parapsilosis*	Control	0.320 ± 0.064	0.141
		Treatment	0.434 ± 0.019	
7	*C. albicans*	Control	0.504 ± 0.119	0.051
		Treatment	1.000 ± 0.000	
8	*C. albicans*	Control	0.431 ± 0.056	0.016
		Treatment	1.000 ± 0.000	
9	*C. glabrata*	Control	0.397 ± 0.028	0.696
		Treatment	0.408 ± 0.018	
10	*C. utilis*	Control	0.238 ± 2E-17	0.021
		Treatment	0.392 ± 0.022	
11	*Cryptococcus neoformans*	Control	0.310 ± 0.082	0.0002
		Treatment	0.641 ± 0.088	
12	*C. laurentii*	Control	0.214 ± 0.023	0.0007
		Treatment	0.507 ± 0.007	

Overall, these findings underscore the efficacy of the treatment in reducing cell viability, as evidenced by the significant differences observed between the control and treatment groups across all isolates. This reduction is consistent across all isolates, with varying levels of inhibition. Notably, in some cases, the decrease in viability is linked to the stress induced by pyocin at sub-MIC concentrations, which likely interferes with metabolic processes and cell wall integrity, ultimately affecting the cell's ability to proliferate and perform other metabolic processes.

Table 8: Effect of pyocins on the optical density (O. D) the viability of the yeast isolates over 24 hours. SE stands for Standard Error

Isolate			O. D ± SE	Inhibition %
1	*C. krusei*	Control	0.624 ± 0.144	51.44
		Treatment	0.303 ± 0.020	
2	*C. tropicalis*	Control	0.863 ± 0.208	69.78
		Treatment	0.261 ± 0.020	
3	*C. dubliniensis*	Control	0.643 ± 0.034	57.40
		Treatment	0.274 ± 0.010	
4	*C. guilliermondii*	Control	0.937 ± 0.012	70.43
		Treatment	0.277 ± 0.009	
5	*C. kefyr*	Control	0.832 ± 0.130	68.49
		Treatment	0.262 ± 0.019	
6	*C. parapsilosis*	Control	0.708 ± 0.022	65.88
		Treatment	0.241 ± 0.018	
7	*C. albicans*	Control	0.848 ± 0.054	74.76
		Treatment	0.214 ± 0.016	
8	*C. albicans*	Control	0.624 ± 0.003	76.61
		Treatment	0.146 ± 0.015	
9	*C. glabrata*	Control	0.802 ± 0.025	80.68
		Treatment	0.155 ± 0.020	
10	*C. utilis*	Control	0.899 ± 0.081	83.98
		Treatment	0.144 ± 0.007	
11	*Cryptococcus neoformans*	Control	1.155 ± 0.072	77.06
		Treatment	0.265 ± 0.024	
12	*C. laurentii*	Control	0.991 ± 0.075	79.05
		Treatment	0.207 ± 0.020	
		p Value	$p<0.05$	

CONCLUSION

This study highlights the potential of *P. aeruginosa* bacteriocins as an alternative for treating clinical yeast infections. The high antifungal activity of the bacteriocin and inhibiting the growth of yeasts especially those that are resistant to conventional antifungal drugs shows its value as being possibly an alternative therapeutic option where other antifungals are ineffective with MICs ranging between 40.57 - 81.15 µg ml^{-1} against *Candida* and *Cryptococcus* isolates. This observed efficacy of the pyocins against a diverse range of *Candida* and *Cryptococcus* species underscores their potential as a valuable therapeutic option. This may represent a significant step forward in the field of antimicrobial therapy against yeasts especially in the light of the rising challenge of antifungal resistance.

Additionally, the pyocins were highly effective in inhibiting biofilm formation and treatment with the pyocin showed high inhibition of biofilm for all isolates, reaching a maximum of 82% at 72 hours against *C. krusei*.

Finally, the effect of pyocin on phospholipase production showed varying results which can indicate specie-specific response indicated by the two *C. albicans* isolates having a complete cessation of phospholipase production. It also indicates a stress induced virulence which led some of the isolates to display an increase in phospholipase production. A significant reduction in the viability of yeast across all isolates was observed, confirming the efficacy of

pyocins as a valuable treatment option. These findings highlight the promise of pyocins as a broad spectrum antimicrobial agent and their potential role in addressing the rising issue of antifungal resistance.

ACKNOWLEDGMENTS

The authors would like to thank Mustansiriyah University in Iraq (www.uomustansiriyah.edu.iq) for their support in the current work.

REFERENCES

Abid, S.A., Aziz, S.N., Saeed, N.A.-H.A.H., Mizil, S.N., Al-Kadmy, I.M.S., Hussein, N.H., Al-Saryi, N., Ibrahim, S.A. & Hussein, J.D. (2023). Investigation of virulence factors in microbial organisms that associated with public health risk isolates from different environmental regions. *Al-Mustansiriyah Journal of Science*, 33: 5. DOI: 10.23851/mjs.v33i5.1303

Adeyemo, R. O., Famuyide, I. M., Dzoyem, J. P., & Lyndy Joy, M. (2022). Anti-biofilm, antibacterial, and anti-quorum sensing activities of selected South African plants traditionally used to treat diarrhoea. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2022, 1307801. DOI:10.1155/2022/1307801

Al-Baqer, T. M., Al-Gharrawi, S. A. R., & Saeed, N. A. A. (2021). Causative microorganisms and antibiotics susceptibilities in children with urinary tract infection. *Al-Mustansiriyah Journal of Science*, 32(1): 5–9. DOI:10.23851/mjs.v32i1.948

Ali Hameed Al-Dabbagh, A., Ali Ajah, H. & Abdul Sattar Salman, J. (2023). Detection of virulence factors from *Candida* spp. Isolated from oral and vaginal candidiasis in Iraqi patients. *Archives of Razi Institute*, 78(1): 465–474. DOI:10.22092/ARI.2022.359464.2420

Alqahtani, A., Kopel, J. & Hamood, A. (2022). The in vivo and in vitro assessment of pyocins in treating *Pseudomonas aeruginosa* infections. *Antibiotics (Basel, Switzerland)*, 11(10): 1366. DOI:10.3390/antibiotics11101366

Arastehfar, A., Carvalho, A., Nguyen, M.H., Hedayati, M.T., Netea, M.G., Perlin, D.S. & Hoenigl, M. (2020). COVID-19-Associated Candidiasis (CAC): An underestimated complication in the absence of immunological predispositions? *Journal of Fungi*, 6: 211. DOI: 10.3390/jof6040211

Bahuguna, A., Khan, I., Bajpai, V.K. & Kang, S.C. (2017). MTT assay to evaluate the cytotoxic potential of a drug. *Bangladesh Journal of Pharmacology*, 12(2): 8. DOI:10.3329/bjp.v12i2.30892

Ball, A.L., Augenstein, E.D., Wienclaw, T.M., Richmond, B.C., Freestone, C.A., Lewis, J.M., Thompson, J.S., Pickett, B.E. & Berges, B.K. (2022). Characterization of *Staphylococcus aureus* biofilms via crystal violet binding and biochemical composition assays of isolates from hospitals, raw meat, and biofilm-associated gene mutants. *Microbial Pathogenesis*, 167: 105554. DOI: 10.1016/j.micpath.2022.105554

Behrens, H.M., Lowe, E.D., Gault, J., Housden, N.G., Kaminska, R., Weber, T.M., Thompson, C.M. A., Mislin, G.L.A., Schalk, I.J., Walker, D., Robinson, C.V. & Kleanthous, C. (2020). Pyocin S5 import into *Pseudomonas aeruginosa* reveals a generic mode of bacteriocin transport. *mBio*, 11(2): 10-1128. DOI: 10.1128/mBio.03230-19

Beute, J.E., Kim, A.Y., Park, J.J., Yang, A., Torres-Shafer, K., Mullins, D.W. & Sundstrom, P. (2022). The IL-20RB receptor and the IL-20 signaling pathway in regulating host defense in oral mucosal candidiasis. *Frontiers in Cellular and Infection Microbiology*, 12: 979701. DOI: 10.3389/fcimb.2022.979701

Ellepola, A.N.B., Joseph, B.K. & Khan, Z.U. (2014). The postantifungal effect and phospholipase production of oral *Candida albicans* from smokers, diabetics, asthmatics, denture wearers and healthy individuals following brief exposure to subtherapeutic concentrations of chlorhexidine gluconate. *Mycoses*, 57(9): 553–559. DOI:10.1111/myc.12194

Fule, S.R., Das, D. & Fule, R.P. (2015). Detection of phospholipase activity of *Candida albicans* and non albicans isolated from women of reproductive age with vulvovaginal candidiasis in rural area. *Indian Journal of Medical Microbiology*, 33(1): 92–95. DOI:10.4103/0255-0857.148392

Garcia-Vidal, C., Sanjuan, G., Moreno-García, E., Puerta-Alcalde, P., Garcia-Pouton, N., Chumbita, M., Fernandez-Pittol, M., Pitart, C., Inciarte, A., Bodro, M., Morata, L., Ambrosioni, J., Grafia, I., Meira, F., Macaya, I., Cardozo, C., Casals, C., Tellez, A., Castro, P., Marco, F., García, F., Mensa, J., Martínez, J.A., Soriano, A. & COVID-19 Researchers Group. (2021). Incidence of co-infections and superinfections in hospitalized patients with COVID-19: A retrospective cohort study. Clinical Microbiology and Infection: The Official Publication of the European Society of

Clinical Microbiology and Infectious Diseases, 27: 83–88. DOI: 10.1016/j.cmi.2020.07.041

Gow, N.A.R., Johnson, C., Berman, J., Coste, A.T., Cuomo, C.A., Perlin, D.S., Bicanic, T., Harrison, T.S., Wiederhold, N., Bromley, M., Chiller, T. & Edgar, K. (2022). The importance of antimicrobial resistance in medical mycology. *Nature Communications*, 13: 5352. DOI: 10.1038/s41467-022-32249-5

Haghighipour, S., Pourahmad, M., Noorbakhsh, M. & Mohammadi, R. (2019). *Candida* urinary tract infection among ICU patients in Isfahan, Iran. *Archives of Clinical Infectious Diseases*, 14. DOI: 10.5812/archcid.86472

Hamady, A. & Marei, Y. (2021). Detection of als1 and hwp1 genes involved in biofilm formation in *Candida albicans* isolated from catheter-associated candiduria. *Microbes and Infectious Diseases*, 2(3): 558–566. DOI: 10.21608/mid.2021.76052.1153

Hayashida, M.Z., Seque, C.A., Pasin, V.P., Enokihara, M.M.S.E. & Porro, A.M. (2017). Disseminated cryptococcosis with skin lesions: Report of a case series. *Anais Brasileiros de Dermatologia*, 92: 69–72. DOI: 10.1590/abd1806-4841.20176343

Hussein, M., Yassin, A. & El-Gelany, F. (2019). Characterization, virulence factors, and antifungal susceptibility of vulvovaginal *Candida* isolated from women at Qena, Egypt. *Egyptian Journal of Microbiology*, 54: 13–24. DOI: 10.21608/ejm.2019.10543.1091

Kakar, A., Sastré-Velásquez, L.E., Hess, M., Galgóczy, L., Papp, C., Holzknecht, J., Romanelli, A., Váradi, G., Malanovic, N. & Marx, F. (2022). The membrane activity of the amphibian temporin B peptide analog TB_KKG6K sheds light on the mechanism that kills *Candida albicans*. *mSphere*, 7: e0029022. DOI: 10.1128/msphere.00290-22

Karajacob, A.S., Azizan, N.B., Al-Maleki, A.R.M., Goh, J.P.E., Loke, M.F., Khor, H.M., Ho, G.F., Ponnampalavanar, S. & Tay, S.T. (2023). *Candida* species and oral mycobiota of patients clinically diagnosed with oral thrush. *PloS One*, 18: e0284043. DOI: 10.1371/journal.pone.0284043

Kielkopf, C.L., Bauer, W. & Urbatsch, I.L. (2020). Bradford assay for determining protein concentration. *Cold Spring Harbor Protocols*, 2020: 102269. DOI: 10.1101/pdb.prot102269

Lima, T., Gunnarsson, S.B., Coelho, E., Evtuguin, D. V., Correia, A., Coimbra, M.A., Cedervall, T. & Vilanova, M. (2022). β-glucan-functionalized nanoparticles down-modulate the proinflammatory response of mononuclear phagocytes challenged with *Candida albicans*. *Nanomaterials (Basel, Switzerland)*, 12: 2475. DOI: 10.3390/nano12142475

Ling, H., Saeidi, N., Rasouliha, B.H. & Chang, M.W. (2010). A predicted S-type pyocin shows a bactericidal activity against clinical *Pseudomonas aeruginosa* isolates through membrane damage. *FEBS Letters*, 584: 3354–3358. DOI: 10.1016/j.febslet.2010.06.021

El-Ganiny, M.A., Yossef, E.N. & Kamel, A.H. (2021). Prevalence and antifungal drug resistance of nosocomial *Candida* species isolated from two university hospitals in Egypt. *Current Medical Mycology*, 7(1): 31–37. DOI: 10.18502/cmm.7.1.6181

Mohamed, A.A., Elshawadfy, A.M., Amin, G. & Askora, A. (2021). Characterization of R-pyocin activity against Gram-positive pathogens for the first time with special focus on *Staphylococcus aureus. Journal of Applied Microbiology*, 131(6): 2780–2792. DOI:10.1111/jam.15134

Montoya, M.C., Magwene, P.M. & Perfect, J.R. (2021). Associations between Cryptococcus genotypes, phenotypes, and clinical parameters of human disease: A review. *Journal of Fungi (Basel, Switzerland)*, 7(4): 260. DOI:10.3390/jof7040260

Morse, S.A., Vaughan, P., Johnson, D. & Iglewski, B. H. (1976). Inhibition of *Neisseria gonorrhoeae* by a bacteriocin from *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*, 10(2): 354–362. DOI: 10.1128/AAC.10.2.354

Oluyombo, O., Penfold, C.N. & Diggle, S.P. (2019). Competition in biofilms between cystic fibrosis isolates of *Pseudomonas aeruginosa* is shaped by R-pyocins. *mBio*, 10(1): e01828-18. DOI: 10.1128/mBio.01828-18

O'Neill, J. (2014). Antimicrobial resistance: Tackling a crisis for the health and wealth of nations (The Review on Antimicrobial Resistance). United Kingdom: The Review on Antimicrobial Resistance. Available at: https://amr-review.org/

Pal, M. (2018). Morbidity and mortality due to fungal infections. *Journal of Applied Microbiology and Biochemistry*, 1(1): 1-3. DOI: 10.21767/2576-1412.100002.

Paškevičius, Š., Dapkutė, V., Misiūnas, A., Balzaris, M., Thommes, P., Sattar, A., Gleba, Y. & Ražanskienė, A. (2022). Chimeric bacteriocin S5-PmnH engineered by domain swapping efficiently controls *Pseudomonas aeruginosa* infection in murine keratitis and lung models. *Scientific Reports*, 12: 5865. DOI: 10.1038/s41598-022-09865-8

Perinelli, D., Campana, R., Skouras, A., Bonacucina, G., Cespi, M., Mastrotto, F., Baffone, W. & Casettari, L. (2018). Chitosan loaded into a hydrogel delivery system as a strategy to treat vaginal co-infection. *Pharmaceutics*, 10(1): 23. DOI: 10.3390/pharmaceutics10010023.

Sarker, S.D., Nahar, L. & Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42(4): 321-324. DOI: 10.1016/j.ymeth.2007.01.006

Taher, N.A. (2017). Antimicrobial effect of bacteriocin produced by *Pediococcus pentosaceus* on some clinical isolates. *Al-Mustansiriyah Journal of Science*, 27: 26–30. DOI: 10.23851/mjs.v27i5.163

Van de Veerdonk, F.L., Joosten, L.A.B. & Netea, M.G. (2015). The interplay between inflammasome activation and antifungal host defense. *Immunological Reviews*, 265: 172–180. DOI: 10.1111/imr.12280

Vechi, H.T., Theodoro, R.C., de Oliveira, A.L., da Silva Gomes, R.M., de Almeida Soares, R.D., Freire, M.G. & Baumgardt Bay, M. (2019). Invasive fungal infection by *Cryptococcus neoformans var. grubii* with bone marrow and meningeal involvement in a HIV-infected patient: A case report. *BMC Infectious Diseases*, 19: 220. DOI: 10.1186/s12879-019-3831-8

Vitális, E., Nagy, F., Tóth, Z., Forgács, L., Bozó, A., Kardos, G., Majoros, L. & Kovács, R. (2020). *Candida* biofilm production is associated with higher mortality in patients with candidaemia. *Mycoses*, 63(4): 352-360. DOI: 10.1111/myc.13049

Wang, J., Zhang, Z., Zhang, M., Yang, B., Wang, T., Sun, X., Chen, X., Zhang, M.Y., Guo, Z.Y. & Jiang, X. (2019). A rare primary *Candida parapsilosis* infection of the knee joint in a patient without predisposing factors: A case report. *Medicine*, 98(6): e14327. DOI: 10.1097/MD.0000000000014327

Zhu, B., Li, Z., Yin, H., Hu, J., Xue, Y., Zhang, G., Zheng, X., Chen, W., & Hu, X. (2022). Synergistic antibiofilm effects of pseudolaric acid A combined with fluconazole against *Candida albicans* via inhibition of adhesion and yeast-to-hypha transition. *Microbiology Spectrum*, 10(2): e01478-21. DOI: 10.1128/spectrum.01478-21.