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Purification Of Bacteriocin Produced By *Staphylococcus Aureus* And Its Effect On Some *Candida* Spp.

Sara Salim Maryoush[1], Raghad A. Abdulrazaq[2] , Hamzia AliAjah[3]
***Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq**
Email: sarahsalim670@gmail.com
Email: raghadmustafa65@gmail.com
Email: Hamzia@uomostansiriyah.edu.iq

Abstract:
Candida is the most implicated fungal pathogen in the clinical setting. Virulence factors, such as biofilms play important roles in its pathogenesis. Hence, this study aimed to investigate role of staphylococcal bacteriocin in inhibit biofilm and viability of *Candida* spp. Seventy-two isolates were collected from abscess, wounds and burns . All isolates were reidentified using different examinations. In addition, eight species of previously isolated clinical *Candida* species, from oral and vaginal cavities of patients with candidiasis, including *C. albicans*, C. *glabrata*, *C. parasilosis*, *C. krusei*, *C. lusitaniae*, *C. kefyr*, *C. tropicalis* and *C. ciferrii*, were utilized to check the antimicrobial activity of *S. aureus* on *Candida* spp. Agar well diffusion method were used to detect bacteriocin production by *S. aureus*, whereas only 5 isolates were selected. The antibacterial effect of selected *S. aureus* isolates were tested against indicators (*Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*). Isolate of *S. aureus* no.2 was showed the best bacteriocin production. Bacteriocin was purified using ammonium sulfate and dialysis. Different concentrations was utilized to determine sub-minimal inhibitory concentration (sub-MIC) of bacteriocin. Sub-MIC of bacteriocin was utilized to estimate its inhibitory effect on viability of *Candida* spp. Bacteriocin showed the maximal inhibition percentage against *C. parapsilosis* CV26 with 85.42%, followed by *C. tropicalis* CO49 with 80.49%. The viability of *C. kefyr* CO45, *C. krusei* CO41, *C. glabrata* CO38, *C. albicans* CV5 and *C. lusitaniae* was inhibited by 77.78, 75.41, 74.67, 71.43 and 70.18%, respectively. In addition, 69.84 and 64.62% of *C. albicans* CV1 and *C. ciferrii*. Using microtiter plate, sub-MIC of bacteriocin showed maximal inhibitory effect against *C. ciferrii* with 82.26%, followed by *C. lusitaniae* with 80.4%, the biofilm formation of *C. parapsilosis* CV26 and *C. tropicalis* CO49 was inhibited by 80.49% and 79.82%, respectively.

Keywords: *Candida*, Bacteriocin, Antifungal , Biofilm.

Introduction:

Candida species are found on the skin, in the gastrointestinal tract, and in the vaginal tract. Because they are commensals, they are a typical component of human flora. Nevertheless, in susceptible individuals, such as the elderly, hospitalized, or immunocompromised, *Candida* can also result in other illnesses. Globally, one of the most common fungal illnesses is invasive Candida infection (1,2).

Proteinaceous substances called bacteriocins are produced by practically all bacteria and are specifically lethal to closely related species . Bacteriocins are group of peptides or proteins which have several biochemical properties, mechanism of action, molecular weight, sequence of amino acids, location and spectrum of activity (3).

Both Gram-positive (including *Lactococcus, Lactobacillus, Streptococcus, Enterococcus, Leuconostoc, Propionibacterium* and *Pediococcus*) (4) and Gram-negative (including *Serratia, E. coli, Shigella, Klebsiella* and *Pseudomonas*) can produce bacteriocins (5).

Staphylococcus aureus can produce bacteriocin, whereas staphylococcal-produced bacteriocin can inhibit some *Candida* species (6) and even suppressing growth of *S. aureus* (7). This staphylococcal-produced bacteriocin called staphylococcins (8). Also, different studies were reported the roles of staphylococcal-produced bacteriocins, such as their immunomodulation activities, anti-inflammatory effects, anti-fungi activities, anti-viral potentials, anti-tubercular, anti-cancer and biopreservatives (9). Biotechnological strategies such as bioengineering or chemical synthesis of bacteriocins may be useful tools for improving antimicrobial action, changing physicochemical features, or lowering manufacturing costs (10).

Because of the high potential of bacteriocins, particularly those generated by commensal isolates, examining their antimicrobial activity on *Candida* spp. was the top aim of this work.

Materials and Methods

Collection of specimens

Seventy-two isolates of specimens were collected during the period of November 2022 and May 2023 from abscess, wounds and burns for producing bacteriocin. Also, two ready isolates of *E. coli* and *P. aeruginosa* were utilized as indicators. Both these isolates were identified using different examinations, including VITEK2 system (11).

In addition to eight species of previously isolates of clinical *Candida* species, including *C. albicans*, C. *glabrata*, *C. parasilosis*, *C. krusei*, *C. lusitaniae*, *C. kefyr*, *C. tropicalis* and *C. ciferrii*, that were isolated from oral and vaginal cavities of patients with candidiasis, were utilized to check the antimicrobial activity of *S. aureus* on *Candida* spp.

Identification of *Candida* species

all specimens were cultured on chloramphenicol (250mg/L) enriched-sabouraud dextrose agar (SDA) (Himedia/India) and incubated for 48 hours at 37°C under aerobic conditions. The size, texture, color, form, opacity, and margin of colonies were among the cultural traits that were examined using (SDA). Chromatin Candida agar (Himedia/India), a selective and differential medium, was next used to reculture the Candida spp. isolates for 48 hours at 37°C. Yeast was identified after incubation based on the color of the colonies. Colony color and form are used to differentiate Candida krusei, Candida tropicalis, and Candida albicans in CHROMagar Candida, a chromogenic medium that is ready to use. Colonies of Candida albicans range in color from light green to medium green; colonies of Candida tropicalis range in color from dark blue to metallic blue; and colonies of Candida krusei and Candida lusitaniae are pink with a white border. On isolation media, other yeasts like Candida glabrata can produce cream or light to dark mauve colors. Colonies of Candida ciferrii grow rapidly, with a blue core and a white periphery, and eventually get rough with ridges and bumps that resemble gyruses (12).

Identification of *S. aureus* isolates

All specimens were subjected into different examination, including microscopic examination (using gram staining), cultural characteristics (on mannitol salt agar) and vitek system identification (13,14).

Extraction of bacteriocin

Staphylococcal-produced bacteriocin was purified using method described by (15), whereas the procedure was performed as follows: five isolates of *S. aureus* were inoculated in test tubes, whereas all these tubes were containing 10 milliliters of sterile brain heart infusion broth (BHIB). All tubes were placed in incubator for 16 hours at 37^{o}C. Then, 50 milliliters-containing BHIB tube were added into the inoculated BHIB, then placed in shaker incubator for 180 rpm/ minute at 37°C. After 60 minutes, 0.5 microliters/milliliters of mitomycin C was placed into the inoculated BHIB and placed in shaker incubator for 180 rpm/ minute at 37°C for 8 hours. The

inoculated BHIB was placed in centrifuge (temperature at 4°C, 10 minutes and 10,000 rpm). The supernatant was obtained and utilized as bacteriocin crude.

Antimicrobial activity of Bacteriocin

Well-diffusion assay was utilized to estimate the antimicrobial activity of bacteriocin, as method described by (16). After preparation and sterilization of Mueller Hinton Agar (MHA) using autoclave, this medium was poured into plates. Each selected indicator (*E. coli* and *P. aeruginosa*) was streaked on MHA plates with properly labeling. Using cork borer, wells were formed on plates. The crude of bacteriocin was placed into each well. Each plate was placed into incubator for 24 hours at 37°C (17). The diameter (mm) of clear zone of inhibition was estimated.

Purification of bacteriocin

Using ammonium sulfate precipitation and dialysis, purification of bacteriocin was performed. After centrifugation (10,000 x g; 4^{o}C), 50 milliliters of bacteriocin crude was placed into small beakers with labeling. All these beakers were putted in box of ice. Each beaker was filled with ammonium sulfate (with different concentration, including 60, 70 and 80%). For fully dissolving of ammonium sulfate, continuous stirring should be performed. Then, centrifugation (10 minutes; 10,000 x g) was performed. In supernatant, the content of protein was determined after precipitation using three concentrations. Using deionized water, each precipitate was re-suspended. The dialysis bag (3.5 kDa) was utilized to purified bacteriocin for 24 hours. After dialysis, 35 milliliters of crude bacteriocin was gained. The activity (18) and concentration (19) of protein were determined for each step.

Determination of minimal inhibitory concentration of bacteriocin

Different concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 micrograms pr milliliters) were prepared from 10 milligrams per milliliters of stock bacteriocin in microtiter plate using Muller Hinton broth (MHB) as diluent. 20 microliters of 1.5 x 10^{8} CFU/ml bacterial suspension were putted in each well except the wells of control (only MHB). After incubation of microplate for 18-20 hours at 37^{o}C, 20 microlites of resazurin dye were putted into each well and the microplate was placed in incubator for two hours until changing in color. When the color was changed from blue to pink, the sub-MIC was determined (20).

Biofilm

Polystyrene 96-well microplate method was used to estimate the ability of *Candida* spp. according to Tartor *et al.* (21), whereas the results were represented as strong, moderate and weak biofilm producers.

Extracellular Phospholipase Production

The approach of using egg-yolk agar plates to measure extracellular phospholipase production was employed, as described by Fule *et al.*, (22). The represented as negative, weak, moderate and strong phospholipase producers.

Effect of bacteriocin on the viability of *Candida* spp.

The sub-MIC of bacteriocin was utilized to estimate the viability of each *Candida* sp. using MTT (3- [4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Yellow color of tetrazolium MTT was changed into purple when it converted into insoluble formazan with presence of oxidoreductase and dissolved in dimethyl sulfoxide (DMSO). At 590 nm, the number of cells was estimated using ELIZA reader based on the intensity of purple absorbance. This experiment was done with triplicate. Before 35^{o}C of incubation for 24 hours, one milliliter of bacteriocin was placed into one milliliter of 1.5 x 10^{8} CFU/ml *Candida* suspension in treatment test tubes. The tubes of control were containing only *Candida* suspension (without treatment). Then, 50 microliters of each tube was placed into each well of microplate. MTT reagent was putted in each well and the microplate was placed in 5% CO_2 incubator for two to three hours then the solution was eliminated. 150 microliters was placed to each well in microplate leading to change color into purple. At 590 nm, the number of cells was estimated using ELIZA reader based on the intensity of purple absorbance. The percentage of inhibition (%) was estimated using the following equation (23):

$$Inhibition\ of\ viability = \frac{OD_{control} - OD_{Treatment}}{OD_{control}} \times 100\%$$

Effect of bacteriocin on the biofilm formation of *Candida* spp.

The effect of Bacteriocin on *Candida* spp. biofilm formation was investigated using a 96-well flat bottom microtiter plate according to the procedure described by Paniágua *et al.* (24). The experiments were conducted in triplicate. *Candida* spp. isolates were allowed to grow fully at 37°C for 24, 48, and 72 hours in the presence and absence of bacteriocin at sub-MIC concentrations. To each well, 20 microlites of suspension of *Candida* spp., equivalent to 0.5 McFarland standard, was placed. The wells contained 80

microlites of YPD broth mixed with 100 microlites of dextran. The control wells contained only 180 microlites of YPD broth and 20 microlites of *Candida* suspension. After incubation, the medium was removed from the wells and washed two times with sterile phosphate buffer solution (PBS) to eliminate any unbound cells of *Candida* spp. The wells were then left to dry for 15 minutes at room temperature. Subsequently, 200 microlites of crystal violet was placed into each well and allowed to sit for 20 minutes. The crystal violet solution was then eliminated, and PBS (pH 7.2) was utilized for washing of wells for three times with to eliminate any unattached stain. Once more, room temperature was used to allow the wells to dry. Finally, 200 microlites of a mixture of acetone and ethanol (20:80 v/v) was placed into wells and left for approximately 10 minutes. The optical density at 450 nm was measured using an ELISA reader. The percentage of biofilm formation inhibition was calculated using the equation described by Chevalier *et al.* (25).

$$\text{Inhibition of biofilm formation\%} = \frac{OD_{control} - OD_{Treatment}}{OD_{control}} \times 100\%$$

Results and discussion

Identification bacterial isolates

72 isolates of *S. aureus* were obtained. According to results of different examinations, were mentioned in Table 1, only five isolates of *S. aureus*, from total of 72 isolates, were selected. In addition to the diagnosis of the ready-selected indicators. All bacterial samples were aerobically incubated on mannitol salt agar at 37C° for 24 hrs. Microscopic Examination was indicated that both *E. coli* and *P. aeruginosa* were negative rod bacteria, while *S. aureus* was positive cocci bacterium. On mannitol salt agar (MSA), *S. aureus* was appeared as golden colonies. On other hand, *E. coli* and *P. aeruginosa* were appeared as pink and colorless colonies on MacConkey agar plates, respectively.

Based on biochemical tests, *S. aureus* was coagulase, catalase and citrate positive, but oxidase and indole negative. In addition, *E. coli* was catalase and indole positive, but oxidase and citrate negative. *P. aeruginosa* was catalase, oxidase and citrate positive, but indole negative.

Staphylococcus aureus is a common human pathogen that is present in both hospitals and the general population. It is an opportunistic pathogen that can cause a variety of illnesses in people, some of which are both self-limiting and potentially fatal [24]. The common source of these bacteria are wounds,

as reported by [25], [26]. *S. aureus* may colonize tissue, multiply in the respiratory system, and cause several illnesses. *Pseudomonas aeruginosa* is a leading cause of illness and death in hospitalized patients owing to its high level of antibiotic resistance [28]. Emerging strains of *E. coli* that are resistant to drugs pose a greater treatment challenge and increase the risk of bacteremia and mortality [29].

Bacteriocin Production

In order to estimate the ability of *S. aureus* to produce bacteriocin, agar well diffusion method was employed. One benefit of the well diffusion experiment is that it permits the bacteriocin to permeate into the agar prior to the indicator strains starting to proliferate [30]. Only five isolates of *S. aureus* were showed inhibition zones as an effect of bacteriocin production against *E. coli* and *P. aeruginosa*, as represented in figure (1) and table (1). Based on the results, the antibacterial effect of *S. aureus* (S1, S2, S3, S4 and S5) isolates were higher against *E. coli* (with inhibition zones of 21, 28, 20, 19 and 23 mm) than *P. aeruginosa* (with inhibition zones of 19, 26, 18, 17 and 21 mm), respectively.

In addition, the isolate of *S. aureus* No.2 was showed the best bacteriocin production against *E. coli* and *P. aeruginosa*. Also, effect of bacteriocin against *C. albicans* was estimated with inhibition zone (29 mm), as shown in figure (1).

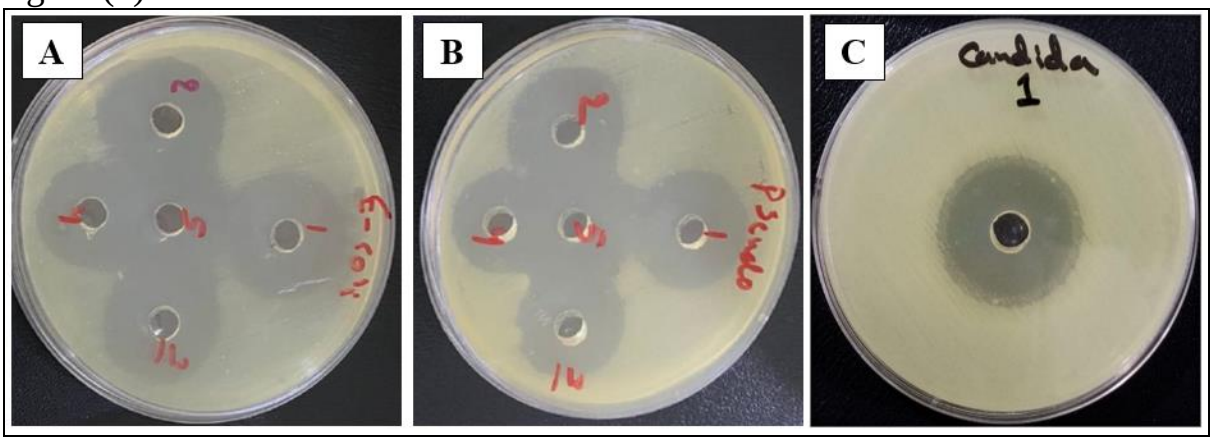

Figure 1: Antibacterial activity of bacteriocin on nutrient agar plates (A: *E. coli*; B: *P. aeruginosa*; C: *Candida albicans*) overnight at 37˚C.

Table 1: Antibacterial activity of bacteriocin against *E. coli*, *P. aeruginosa* and *C. albicans*.

No.	Inhibition zone (mm) for *E. coli*	Inhibition zone (mm) for *P. aeruginosa*	Inhibition zone (mm) for *C. albicans*
***S. aureus* 2**	**28**	**26**	**29**
***S. aureus* 5**	23	21	-
***S. aureus* 1**	21	19	-
***S. aureus* 3**	20	18	-
***S. aureus* 4**	19	17	-

Bacteriocin is an antimicrobial peptide or protein formed by bacterial species, naturally to prevent other bacterial species from growing. This protein is thought to be a type of antimicrobial defense mechanism that bacteria naturally utilize to compete with other microbes in their surroundings. Bacteriocins can be effective against a wide range of bacterial species, or they might have a restricted or broad spectrum of action, which means they can only target certain closely related bacteria [31].

In study conducted by [32], none of the Gram-negative species assayed, including *P. aeruginosa* and *E. coli*, were susceptible to BacCH91, which produced by *S. aureus*, even at the highest concentration tested (100 µM). Indeed, different strains of *S. aureus* were reported as bacteriocin producers, whereas each bacteriocin has shown a specific spectrum of antagonistic effect, hence, effecting certain microorganisms. In study conducted by [33] and [34], Aureocin A70 and Aureocin 4181 were bacteriocin produced by *S. aureus*, which isolated from commercial milk and bovine mastitis, whereas exhibited their antagonistic effects against *L. monocytogenes* and *S. aureus*, respectively. Also, *S. aureus* 4185, isolated from bovine mastitis, which reported as an Aureocyclicin 4185 (AclA) producer, act as antimicrobial agent against *S. aureus* and coagulase-negative Staphylococci (CoNS), including multi-drug resistant bacteria (MDR) strains [35]. Staphylococcins, other examples of bacteriocins produced by *S. aureus* that isolated from different sources, such as human skin, milk, human saliva, human vagina, wound, poultry, dog skin and bovine mastitis, were exhibited wide range of gram-positive bacteria (*S. aureus*, streptococci, pneumococci, *Corynebacterium*, *Enterococcus* and *Bacillus*) and gram-negative bacteria (*Neisseria*, *Haemophilus*, *Moraxella*, *Bordetella*, *Pasteurella*), respectively .

Purification of bacteriocin

Crude extracted bacteriocin was obtained from isolate of *S. aureus* 2 by growing of this isolate in trypticase broth with presence of mitomycin C and incubation at 37ºC for 24 hours. After incubation, bacterial cells were removed from the growth medium by centrifugation (10,000×g for 15 min), and the cell-free supernatant used as crude bacteriocin. This stage show that protein concentration of crude was (0.421) mg/ml, specific activity (59.83) unit/mg with purification fold was (1) and yield (100) % .

Different saturation rates of ammonium sulfate used (60, 70 and 80 %), to determine the best range for bacteriocin precipitation, the results showed that the best range of enzyme precipitation was in the saturation range 80%, This technique is useful to quickly remove large amounts of contaminant proteins were commonly used in precipitation, it was highly solubility, very purity, low cost and no effect on protein [36], [37]. The concentration of ammonium sulfate appears to be based on the concept of the charger neutralization on the protein surface and attraction to the water layer around, it due to the effect of precipitation depending on what is regarded as salting out [38].

Table (2) show that specific activity of 67.26U/mg indicated that there was an increase in the specific activity compared with that of the crude extract 59.83 U/mg and the protein concentration was 0.223 mg/ml, with a fold of purification 1.1327 and enzymic yield 73.61 %.

In order to extract tiny molecules from a protein solution, including salts, dialysis is a frequently employed approach in protein purification. It is founded on the idea of diffusion, which describes how solutes flow from a region of greater concentration to a region of lower concentration across a semipermeable barrier [39]. In this study, yield was reached into 48.7 % with purification fold 1.3917, with using dialysis.

Table 2: Purification steps of bacteriocin.

Step	Volume	Protein Concentration mg/ml	Total Protein mg	Activity U/ML	Specific activity U/mg	Total Activity U	Yield %	Fold
crude	100	0.421	42.1	0.183	59.38	5938	100	1
Ammonium sulfate 80%	65	0.223	14.495	0.217	67.26	4371	73.61	1.1327
Dialysis	35	0.121	4.235	0.433	82.64	2892	48.7	1.3917

Determination of minimal inhibitory concentration of bacteriocin

According to antifungal susceptibility test, phospholipase activity and biofilm formation, nine isolates of *Candida* spp. isolated from oral cavity and vaginal infection, selected for further steps. Different concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml) was utilized to determine MIC and sub-MIC of Staphylococcal bacteriocin using microtiter plate method. The results were illustrated in table (3) and figure (2), whereas the MIC of bacteriocin against *C. albicans* CV3 was 32 µg/ml, while the MIC of bacteriocin against *C. parapsilosis* was 64 µg/ml, also *C. albicans* CV5, *C. tropicalis*, *C. kefyr were* 512 µg/ml, moreover *C. ciferrii*, C. *glabrata*, *C. krusei* were 1024 µg/ml.

Table 3: Determination of MIC and Sub-MIC of Staphylococcal bacteriocin against *Candida* spp.

Isolate	MIC (µg/ml)	Sub-MIC (µg/ml)
***C. albicans* CV1**	32	16
***C. albicans* CV5**	512	256
***C. ciferrii* CO50**	1024	512
***C. tropicalis* CO49**	512	256
***C. parapsilosis* CV26**	64	32
***C. glabrata* CO38**	1024	512
***C. krusei* CO41**	1024	512
***C. lusitaniae* CO27**	512	256
***C. kefyr* CO45**	512	256

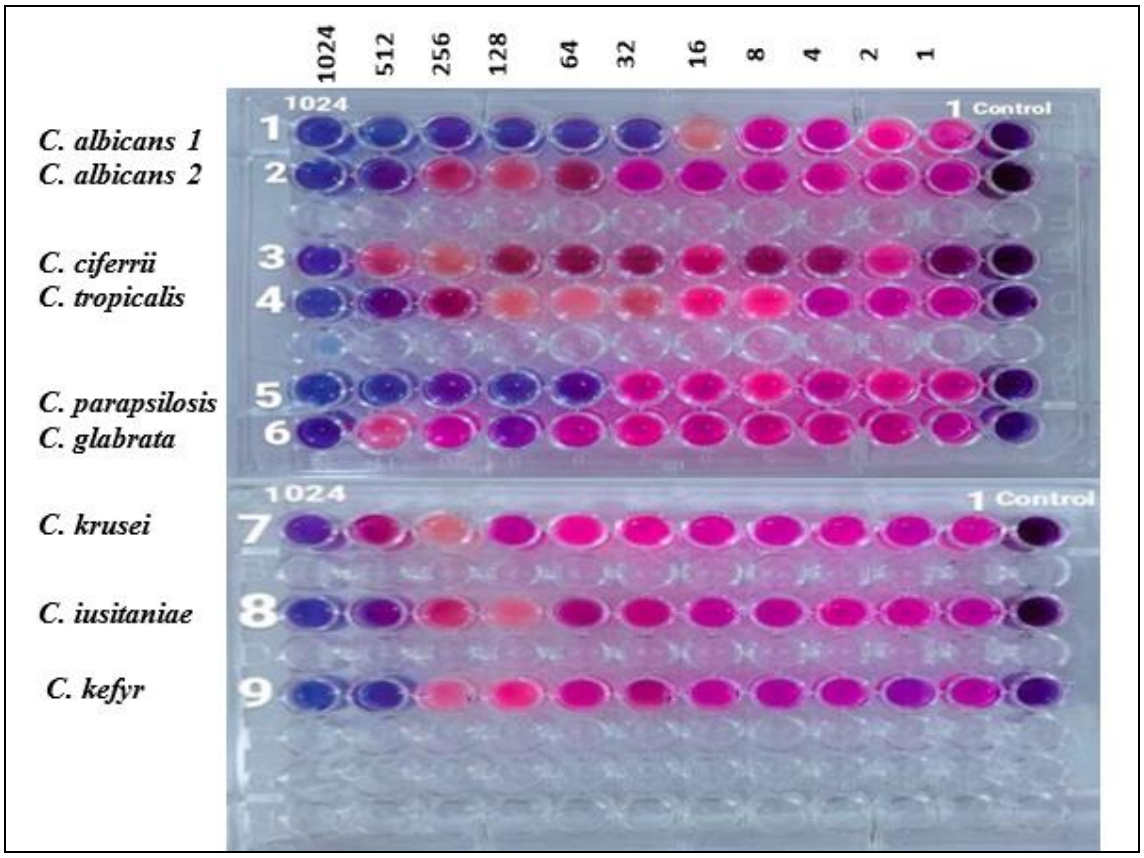

Figure 2: Determination of MIC and Sub-MIC of Staphylococcal bacteriocin against *Candida* spp.

Biofilm and phospholipase production

Polystyrene 96-well microplate method and egg-yolk agar plate method were used to estimate biofilm and phospholipase production. The results were indicated that all isolates of *Candida* species were strong biofilm and phospholipase producers.

Effect of bacteriocin on the viability of *Candida* spp.

The sub-MIC of bacteriocin for each isolate of *Candida* was utilized to estimate the inhibition percentage of bacteriocin on viability of *Candida* spp. and the results were illustrated in Figure 2. According to results, bacteriocin showed the maximal inhibition percentage against *C. parapsilosis* CV26 with 85.42%, followed by *C. tropicalis* CO49 with 80.49%. The viability of *C. kefyr* CO45, *C. krusei* CO41, *C. glabrata* CO38, *C. albicans* CV5 and *C. lusitaniae* was inhibited by 77.78, 75.41, 74.67, 71.43 and 70.18%, respectively. In addition, 69.84 and 64.62% of *C. albicans* CV1 and *C. ciferrii*, respectively, as shown in figure (3) and table (4). In addition, there was non-significant variations in viability of bacteriocin-treated and non-

treated isolates, but there were significantly variations in viability of *Candida* spp. before and after treatment with $P \leq 0.001$.

Table 4: Effect of Bacteriocin on the viability of *Candida* Spp.

Isolate	Viability before treatment Mean ± SD	Viability after treatment Mean ± SD	*p*-value
***C. albicans* CV1**	0.65±0.05	0.23±0.03	0.001
***C. albicans* CV5**	0.70±0.09	0.20±0.04	0.001
***C. ciferrii* CO50**	0.63±0.06	0.19±0.01	0.001
***C. tropicalis* CO49**	0.82±0.12	0.16±0.03	0.001
***C. parapsilosis* CV26**	0.96±0.03	0.14±0.04	0.001
***C. glabrata* CO38**	0.75±0.04	0.19±0.03	0.001
***C. krusei* CO41**	0.61±0.05	0.15±0.05	0.001
***C. lusitaniae* CO27**	0.57±0.12	0.17±0.04	0.001
***C. kefyr* CO45**	0.72±0.15	0.16±0.04	0.001
***p*-value**	NS	NS	

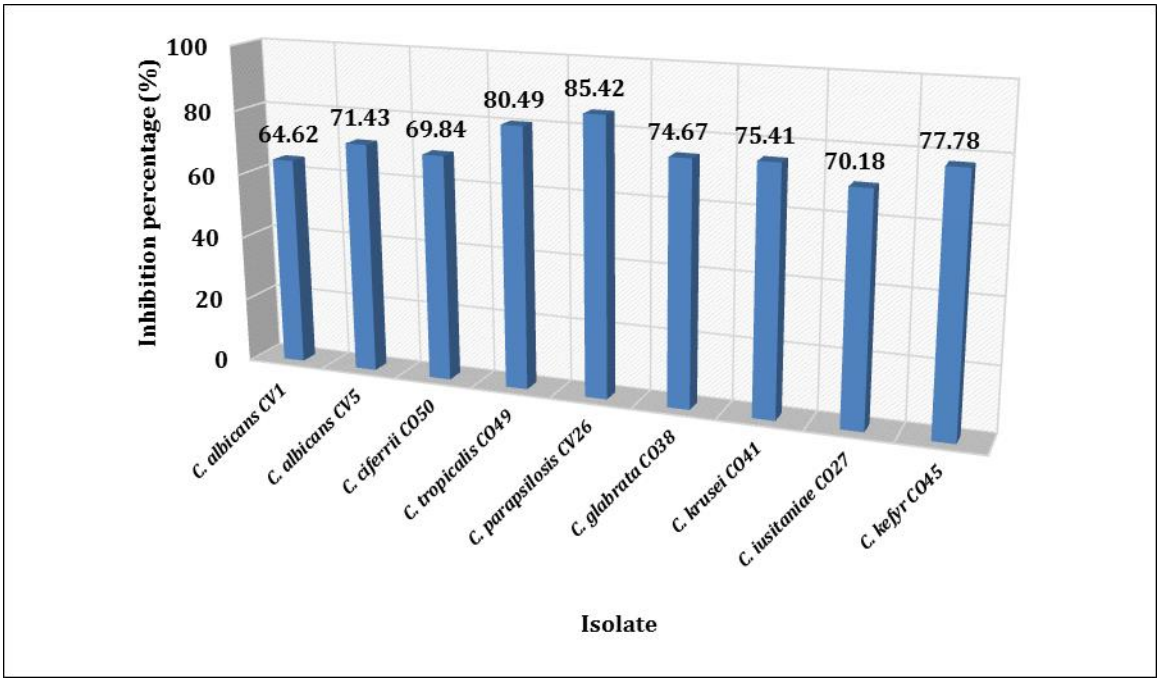

Figure 3: Inhibition percentage of bacteriocin of viability of *Candida* spp.

Neither the gram-negative species nor the fungi were susceptible to the Staphylococcal bacteriocin BacSp222, even at the highest concentration tested (100 µM) [40]. However, *E. faecalis* bacteriocin, EntV has no effect of viability of *C. albicans* [41]. Several studies indicated the antifungal potential of bacteriocin, which produced from different bacterial species, against

Candida pp. For example, Mohsin, (2021) reported that *L. plantarum* WZD3 bacteriocin could be a good antifungal agent to inhibit *C. albicans*. The weak or no effect of bacteriocin produced by *S. aureus* on *C. albicans* may attribute to their effect on closely related species [43]. The mechanisms of cell free supernatant (CFS) *Lactobacillus* strains' antimicrobial activity include (i) competitive exclusion of bacteria, which forces them to adhere and compete for adhesion receptors and nutrients; (ii) co-aggregation, which is the assembly of microbial communities into distinct, interconnected structures; and (iii) intense production of antimicrobial compounds, such as lactic acid, which lowers the pH in the reaction environment and eventually inhibits the growth of bacteria, hydrogen peroxide (H2O2), biosurfactants, and substances similar to bacteriocins [44]. Small antimicrobial peptides known as "bacteriocins" can kill or inhibit certain kinds of bacteria. Bacteria are killed as a result of their adsorption to specific receptors on their surface, which results in critical and phenotypic metabolic changes [45], [46].

Effect of Bacteriocin on Biofilm Formation of *Candida* spp.

The effect of Bacteriocin on Biofilm Formation of *Candida* spp. isolated from oral and vaginal candidiasis were studied by using microtiter plate. In this study, bacteriocin from *Staphylococcus* aureus effect on the Biofilm formation of all *Candida* spp. isolates. The biofilm formation was decreased in all *Candida* spp. The sub-MIC of bacteriocin for each isolate of *Candida* was utilized to estimate the inhibition percentage of bacteriocin on biofilm formation of *Candida* spp. and the results were illustrated in table 5 and figure 4. According to results, bacteriocin showed the maximal inhibition percentage against *C. ciferrii* with 82.26%, followed by *C. lusitaniae* with 80.4%, the biofilm formation of *C. parapsilosis* CV26 and *C. tropicalis* CO49 was inhibited by 80.49% and 79.82%, respectively. In addition, 78.08, 72.36, 69.49, 54.4% and 51.7% of *C. kefyr* CO45, *C. glabrata* CO38, *C. krusei* CO41, *C. albicans* CV5 and *C. albicans* CV1, respectively. Also, there was non-significant variations in biofilm formation of bacteriocin-treated and non-treated isolates, but there were significantly variations in biofilm formation before and after treatment with $p \geq 0.001$.

Table 5: Effect of Bacteriocin on the Biofilm Formation of *Candida* Spp.

Isolate	Biofilm before treatment Mean ± SD	Biofilm after treatment Mean ± SD	*p*-value
***C. albicans* CV1**	1.14 ± 0.05	0.71 ± 0.10	0.001
***C. albicans* CV5**	1.15 ± 0.02	0.70 ± 0.04	0.001
***C. ciferrii* CO50**	1.24 ± 0.12	0.22 ± 0.07	0.001
***C. tropicalis* CO49**	1.14 ± 0.05	0.23 ± 0.02	0.001
***C. parapsilosis* CV26**	1.13 ± 0.04	0.37 ± 0.01	0.001
***C. glabrata* CO38**	1.23 ± 0.09	0.34 ± 0.02	0.001
***C. krusei* CO41**	1.18 ± 0.04	0.36 ± 0.04	0.001
***C. lusitaniae* CO27**	0.82 ± 0.02	0.16 ± 0.02	0.001
***C. kefyr* CO45**	0.73 ± 0.02	0.16 ± 0.02	0.001
***p*-value**	**	**	

** High significant

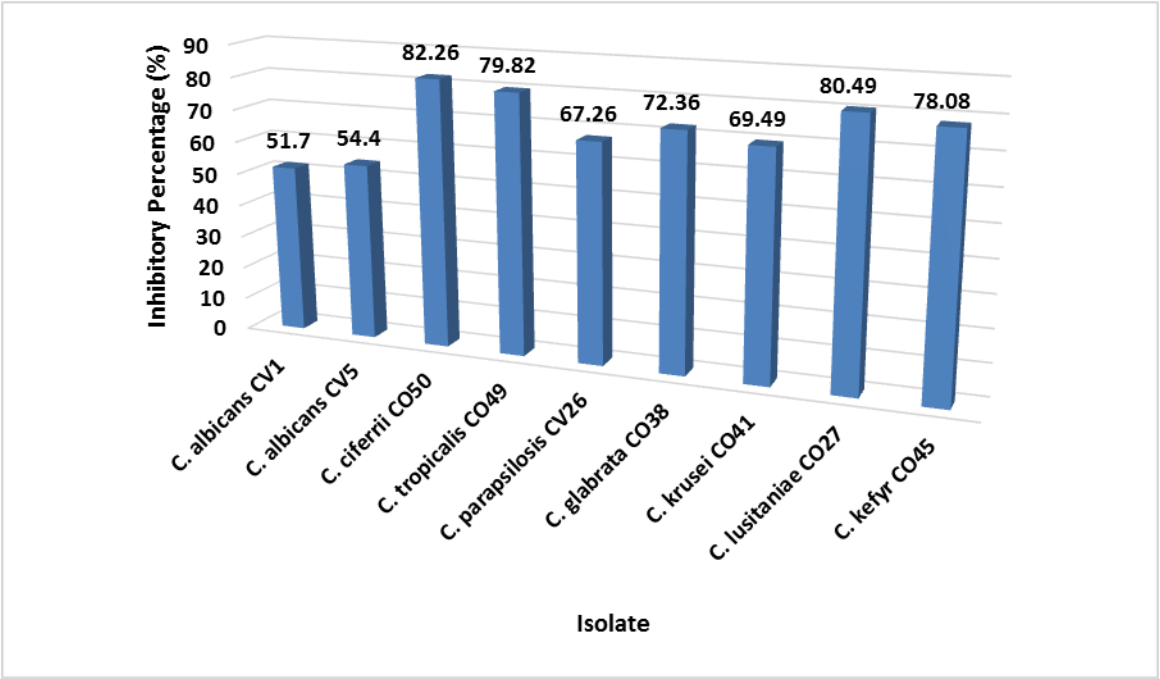

Figure 4: Effect of Bacteriocin on the Biofilm Formation of *Candida* Spp.

They investigated the impact of the cell-free bacterial supernatant (CFBS) on *C. glabrata* biofilms in an effort to show if the antagonistic connection between C. glabrata and S. aureus was affected by the release of secreted bacterial extracellular compounds. Following exposure to CFBS, *C. glabrata*'s ability to build a biofilm was impacted in a dose-dependent way. This experiment demonstrated an unexpected behaviour: the *C. glabrata* biofilm was greatly reduced (83.24%, $p < 0.050$) when the yeast was

immediately exposed to CFBS. In study reported by (48), Staphyloccin produced by multi-drug resistant *S. aureus* (MRSA) showed that the effectively inhibited growth of the yeast (*C. albicans*, *C. tropicalis* and *C. kefyr*).

The precise mechanisms of action of bacteriocins and *Candida* biofilms might differ based on the type of *Candida* and the bacteriocin in question. Bacteriocins have the ability to break down or interfere with the extracellular polymeric materials that make up *Candida* biofilms' matrix. As a result, the biofilm structure may become more brittle and vulnerable to further antimicrobial therapies. Bacteriocins can also stop *Candida* cells from adhering to surfaces in the first place, which stops biofilm development. Bacteriocins have the ability to impede the development of mature and resistant biofilms by disrupting the first phases of biofilm formation. Bacteriocins have the ability to either directly kill or stop the proliferation of *Candida* cells within the biofilm. Bacteriocins can decrease the viability of *Candida* biofilms and increase the effectiveness of other antifungal therapies by going after the fungal cells directly (49,50).

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تنقية البكتريوسين المنتج من بكتريا *Staphylococcus aureus* وتأثيره على بعض العزلات لخميرة *Candida* spp.

مستخلص البحث:

كانديدا هو أكثر الكائنات الفطرية التي يُشتبه فيها في البيئة السريرية. تلعب عوامل الضراوة مثل الأغشية الحيوية أدوارًا مهمة في طريقة تكون المرض. لذلك، هدفت الدراسة إلى التحقيق في دور بيكتيريوسين العنقوديات في تثبيط الأغشية الحيوية وفعالية أنواع الكانديدا. تم جمع 72 عزلة من الخراجات والجروح والحروح. تم اعادة فحص العزلات باستخدام فحوصات مختلفة. بالإضافة إلى ذلك، استُخدِمت ثمانية أنواع من سلالات الكانديدا السريرية المعزولة سابقًا، من تجاويف الفم والمهبل للمرضى الذين يعانون من عدوى الكانديدا، بما في ذلك *C. albicans* و *C. glabrata* و *C. parasilosis* و *C. krusei* و *C. lusitaniae* و *C. kefyr* و *C. tropicalis* و *C. ciferrii*، لفحص النشاط المضاد للميكروبات لبكتيريا المكورات العنقودية الذهبية على انواع الكانديدا. تم استخدام طريقة (well diffusion method) في الزرع على السطح الصلب لاكتشاف إنتاج بيكتيريوسين بواسطة المكورات العنقودية الذهبية، حيث تم اختيار 5 عزلات فقط. تم اختبار التأثير المضاد للبكتيريوسين المحدد من بكتيريا المكورات العنقودية الذهبية ضد المؤشرات (*E. coli*) و(*P. aeruginosa*) و(*Candida albicans*). أظهرت عزلة المكورات العنقودية الذهبية رقم 2 أعلى إنتاج للبكتيريوسين. تم تنقية البكتيريوسين باستخدام كبريتات الأمونيوم والتصفية الناعمة. تم استخدام تراكيز مختلفة لتحديد اقل تركيز مثبط ثاني (sub-MIC) للبكتيريوسين. استخدمت تراكيز البكتيريوسين لتقدير تأثيرها المثبط على قابلية بقاء أنواع الكانديدا. أظهر البكتيريوسين النسبة القصوى للتثبيط ضد *C. parapsilosis* CV26 بنسبة 85.42٪، تليها *C. tropicalis* CO49 بنسبة 80.49٪. تم تثبيط قابلية بقاء *C. kefyr* CO45 و *C. krusei* CO41 و *C. glabrata* CO38 و *C. albicans* CV5 و *C. lusitaniae* بنسب بلغت 77.78٪ و75.41٪ و74.67٪ و71.43٪ و70.18٪ على التوالي. بالإضافة، تم تثبيط 69.84٪ و64.62٪ من *C. albicans* CV1 و*C. ciferrii*. باستخدام لوحة الميكروتيتر، أظهرت تراكيز البكتيريوسين اعلى تأثير مثبط ضد *C. ciferrii* بنسبة 82.26٪، تليتها *C. lusitaniae* بنسبة 80.4٪، وتم تثبيط تكون الأغشية الحيوية لـ *C. parapsilosis* CV26 و *C. tropicalis* CO49 بنسبة 80.49٪ و79.82٪ على التوالي.

الكلمات المفتاحية: المبيضات، البكتريوسين، مضادات الفطريات، الأغشية الحيوية.