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Antifungal Activity of Staphyloccin Produce from MRSA and Resistance *Pseudomonus aeruginosa* Isolated from Clinical Specimen

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ABSTRACT

This research was designed to study the inhibitory effect of crud bacteriocin (Staphyloccin, Pyocin) production from MRSA and resistance *Pseudomonas aeruginosa* which has been isolated from isolated from Baghdad, Iraq samples of different sources (urine and wounds, ear and eye swab) according to biochemical test and vitek 2 system. *In vitro* assay with the antagonists and their cell-free culture supernatants crud bacteriocin from MRSA and resistance *Pseudomonas aeruginos*a strains on agar plates showed that the effectively inhibited growth of the yeast (*Candida albicans*, *Candida tropicalis*, *Candida kefyer*).

The results showed that the Minimum Fungicidal Concentrations were 60% and 70% respectively, also the inhibition zone of reached (26 to 24) mm in solid medium.

Keywords: MRSA, Antibacterial activity, Pyocin, Candida

INTRODUCTION

Candida albicans is a virulent strain of yeast which naturally present in every body, often within areas of mucous membrane such as the inside of the mouth, on moist skin, vagina, intestines, lungs, on or under the fingers and toenails [1]. Some common conditions that Candida overgrowth is responsible for include thrush, vaginal yeast infections and even diaper rash [2]. Although harmless, under various circumstances such as immune-compromised conditions, cancer, diabetics, increased estrogen levels in the body and long term antibiotic usage, Candida can cause infection. Candidiasis is a common yeast infection caused by Candid Common mode of treatment for candidiasis is the application of azole derivatives, polyenes, fluoropyrimidines and echinocandins. Azole derivatives are the major drugs used in candidiasis, they act by interfering with biosynthesis of ergosterol in the fungal cell membrane [3]. When the immune system is suppressed, the yeast can multiply rapidly, penetrate the intestinal lining and move into the bloodstream, Yeast population is controlled by probiotic or bacteriocin from bacteria [4,5]. Bacteriocins are antimicrobial peptides or proteins ribosomally synthesized by bacteria. The antimicrobial resistance has been linked mostly to the use of antimicrobial drugs in food-producing animals [6]. Staphylococcin Bac188 also showed very potent activity against many clinical isolates is active against many gram positive but not gram-negative bacteria and anti-candida [7]. Protease resistant pyocin *P. aeruginosa* of its kind, further investigations elaborated that it adhered to the cell surface of sensitive bacteria that led to their ultimate killing [8]. This research was designed to study the inhibitory effect of staphyloccin and pyocin which isolated from MRSA and resistance *Pseudomonas aeruginosa* in reduction of (*Candida albicans*, *Candida tropicalis*, *Candida kefyer*) growth *in vitro*.

MATERIALS AND METHODS

Isolation and identification of MRSA and resistance *Pseudomonas aeruginosa*

Sample Collection A total of 25 clinical specimens of MRSA and resistance *Pseudomonas aeruginosa* were collected from different sources such as sources urine and wounds, nesil and eye swab were collected from the pathology Hospital in Iraq. For the isolation and identification of MRSA, each specimen was identified, depending on the morphology, cultural characteristics and biochemical reaction [9]. Fifty four isolates of *S. aureus* were subjected to API Staph System tested and vitek 2

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systems to confirm the identification of this pathogen. The resistance *Pseudomonas aeruginosa* collected from different sources such as sources urine and wounds, nesil and eye swab on MacConkey agar plates and Kligler Iron agar that we used, were purchased from Sigma Company, both media were recommended for differentiation of Gram-negative bacilli from clinical specimens. Additional chemicals; Indole, Simmen Citrate and Urea test and identifications by vitek 2 system .the strain of *Candida albicans*, *Candida tropicalis*, *Candida kefyer* obtain Isolation and identification from (College of Science for Women, University of Baghdad).

Production crud staphyloccin and pyocin

After growing MRSA and *Pseudomonas aeruginosa* in a Brain-Heart infusion broth and diluting appropriately to a 0.5 McFarland standard (1.5×10^8 CFU/ml), incubated at 37°C for 18 h. Supernatant fluid after centrifuged at 5000x g for 10 min of the isolates were placed into the antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells. Preparation of cell free extract, the cells was discarded and the cell free extract was filtered using a syringe with 0.2 µm filter. The cell free extract was gently filtered into sterilized test tubes with 0.2 µm acetate cellulose filter [10].

Determining inhibitory effect of staphyloccin and pyocin on Yeast

The antibacterial spectrum of the bacteriocin (pyocin) from *P. aeruginosa* and (Staphyloccin) from MRSA was determined using the well diffusion method. The supernatant from a 24 h culture of *P. aeruginosa* and MRSA was filter sterilized by passage through a 0.45 µm pore size membrane filter (PALL Corporation, Mumbai). of the sterile supernatant were placed in 6-mm-diameter wells that had been cut in Sabourad agar plate previously seeded with the indicator yeast, After 12-24 h of incubation, the diameters of the zones of growth inhibition were measured. Antimicrobial activity was expressed in arbitrary units (AU/ml). One AU was defined as the reciprocal of the highest level of dilute on resulting in a clear zone of growth inhibition [10].

RESULTS AND DISCUSSION

Bacteriocin Typing of MRSA among the 25S. *S. aureus* isolates, four bacterial isolates S4, S12, S16, S19, S23) produced an efficient staphyloccin, identified by wells diffusion method, depending on the widest inhibition zone and the highest sensitive number of the basic indicator isolates S12. These isolates were used as indicator local in bacteriocin typing. Most of these isolates were susceptible to the staphylococcin of the producer isolates, while pyocin production from only five isolates (P1, P7, P9, P21, P26) in the study identified by wells diffusion method, depending on the widest inhibition zone and the highest sensitive number of the basic indicator isolates P26.

Determination of the inhibitory spectrum: Inhibitory activity was detected by techniques

In the agar well diffusion assay, the sample of crud staphyloccin and pyocin was put on well in Sabourad agar plate and the plates were kept at room temperature for 1 h and sub sequently incubated at 30°C for 24 h. The antimicrobial activity was quantified by the diameter of the inhibition zone around each sample. Bacteriocin typing of producing Staphylococcin and pyocin, were selected from were used as basic indicator strains *Candida albicans*, *Candida tropicalis*, *Candida kefyer* to determine the most producing staphylococcin isolates, by well diffusion method show **Figure 1**. The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells result show in **Table 1** similar result of Papon et al. [11], Pfaller [12] and Le Lay et al. [13].

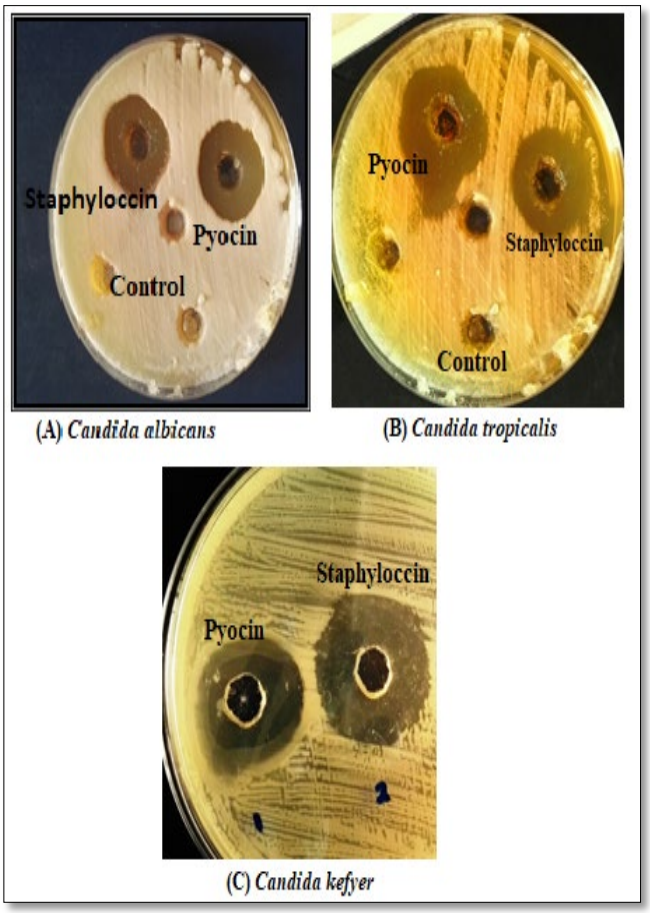

Figure 1. Antimicrobial activity of crud staphyloccin and pyocin Results of the well-diffusion assay against after incubated at 30°C for 24 h, **A**:*Candida albicans* B: *Candida tropicalis* (C) *Candida kefyer*.

Table 1. Antibacterial activity of staphyloccin and pyocin against indicator bacteria.

Type of Bacteriocin	Indicator strain *Candida albicans*	Indicator strain *Candida tropicalis*	Indicator strain *Candida kefyer*	Average Zone of inhibition (mm) diameter
MRSA (Staphyloccin)	20	18	27	27
Pseudomonas aeruginosa (Pyocin)	4	9	7	18
Synergistic (Staphylocci and Pyocin)	22	19	20	30

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