Antifungal activity of staphylococcin produce from MRSA and resistance pseudomonos aeruginosa isolated from clinical specimen

Abstract

This research was designed to study the inhibitory effect of crud bacteriocin (Staphyloccin, Pyocin) production frame MRSA and resistance Pseudomonas aeruginosa which has been isolated from isolated from Baghdad, Iraq samples of different sources (urine and wounds, ear & 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 aeruginosa 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. Some common conditions that Candida overgrowth is responsible for include thrush, vaginal yeast infections and even diaper rash. 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, polynes, 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. 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.

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 animal.

Materials and methods

Isolation and identification of MRSAand 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 & 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. Fifty four isolates of S. aureus were subjected to API Staph System tested and vitek 2 system to confirm the identification of this pathogen. The resistance Pseudomonas aeruginosa collected from different sources such as sources urine and wounds, nesil & eye swab on MacConkey agar plates and Kliger 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 identification 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 (1.5×108 CFU/ml), incubated at 37°C for 18hrs. Supernatant fluid after centrifuged at 5000×g for 10min 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 were discarded and the cell free extract was filtered using a syringe with 0.2µm filter. The cell free extract was gently filtered into sterilized test tubes with 0.2µm acetate cellulose filter.

Determining inhibitory effect of staphyloccin and pyocin on yeast

The antibacterial spectrum of the bacteriocin (pyocin) from P. aeruginosa & (Staphylococcin) frame MRSA was determined using the well diffusion method. The supernatant from a 24-h culture of P. aeruginosa & MRSA was filter sterilized by passage through a 0.45: m pore size membrane filter (PALL Corporation, Mumbai). of the sterile
supernatant were placed in 6mm-diameter wells that had been cut in Sabourad agar plate previously seeded with the indicator yeast. After 12-24h 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.

**Result and discussion**

Bacteriocin Typing of MRSA Among the 25 S. aureus isolates, four bacterial isolates S4, S12, S16, S19, S23 produced an efficient staphylococcin, 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 1h and sub sequently incubated at 30°C for 24h. The antimicrobial activity was quantified by the diameter of the inhibition zone around each sample. Bacteriocin Typing of Producing Staphyloccocin & 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.

![Antimicrobial activity of crud staphyloccin & pyocin Results of the well-diffusion assay against after incubated at 30°C for 24h](image)

**Figure 1** Antimicrobial activity of crud staphyloccin & pyocin Results of the well-diffusion assay against after incubated at 30°C for 24h

| 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 & pyocin) | 22 | 19 | 20 | 30 |

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None.

**Conflict of interest**

Author declares that there is no conflict of interest.

**References**

1. Oscar M, Philippe M, Michel PG, et al. Potent synergism of the combination of fluconazole and cyclosporine in Candida albicans. *Antimicrobial Agents Chemotherapy*. 2000;44(9):2373–2381.
2. Debbie AH, Quentin LS, Sanders RJ, et al. Identification of the diacylable serum inducer of germtube formation in Candida albicans. *Microbiol*. 2000;150:3041–3049.
3. Tserkovniak LS, Roi AO, Kurdysh IK. Synthesis of amino acids of Bacillus subtilis IMV V-7023 in the medium with glycerophosphates. *Mikrobiol Z*. 2009;71(5):18–32.
4. Cheryl G, Maryam GN, Mark M, et al. Candida albicans Int1p interacts with the septin ring in yeast and hyphal cells. *Molecular Biology of the Cell*. 2001;12(11):3538–3549.
5. Boonnaert CJ, Rouxhet PG. Surface of lactic acid bacteria:relationships between chemical composition and physic-chemical properties. *App Environ Microbiol*. 2000;66(6):2548–2554.
6. Ndote-Nembe A, Vu KD, Doucet N, et al. Antimicrobial effects of essential

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oils, nisin, and irradiation treatments against Listeria monocytogenes on Ready-to-Eat carrots. *J Food Sci.* 2015;80(4):M795–M799.

7. Hena JV, Sudha SS. Characterization of staphylococcin by peptide mass fingerprinting, *Intern. J Pharm Bio Sci.* 2011;2:269–274.

8. Sharma V, Aseri GK, Sohal JS, et al. Exploration of Bacteriocins as Potential Food Preservatives. *IJPTB.* 2016;3(1):55–82.

9. Baron EJ, Finegold SM. *Bailey & Scott’s: Diagnostic Microbiology.* 8th ed. USA: Mocby Company; 1990.

10. Majeed HAL. *Identification and immunological study of Candida spp. causing vaginitis.* M.Sc. thesis, College of Science for Women, University of Baghdad; 2004.

11. Papon N, Courdavault V, Clastre M, et al. Emerging and emerged pathogenic Candida species: beyond the Candida albicans paradigm. *PLoS Pathog.* 2013;9(9):e1003550.

12. Pfaller MA. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. *Am J Med.* 2012;125(Suppl 1):S3–S13.

13. Le Lay C, Akerey B, Fliss I, et al. Nisin Z inhibits the growth of Candida albicans and its transition from blastospore to hyphal form. *J Appl Microbiol.* 2008;105(5):1630–1939.