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RESEARCH ARTICLE

In Vitro Effect of MRSAc in Bacteriocins Produced from MRSA on Propionibacterium Acnes Comparing with Antibiotics

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Abstract

Propionibacterium acnes most isolation higher in female than male (14% and 4%) respectively all strain resistance antibiotic and using purification Bacteriocins from MRSA isolation from wound to control acne vulgaris. In this study, Futurity one skin swabs were obtained from patients suffering from acne vulgaris from consultation unit in Baghdad teaching Hospital, from both sexes. Ten isolates (18%) of anaerobic gram positive *Probionibacterium acnes* were identified and diagnosis by culture, Api 20A system, Resistance to antibiotics and Vitek 2 ANC System. The local isolation Methicillin-resistant *Staphylococcus aureus* MRSA strain from wound specimen diagnosis by cultures, Biochemical test and Vitek 2 ANC System. It was found that *P. acne* isolates were higher in female than male (14% and 4%) respectively, and more prevalence in age (16-21) years patients and lower in older age. The effect of some antibiotics on *Probionibacterium acnes* was investigated, and the results showed that all isolates were resistance to antibiotics (Azithromycin, Erythromycin, Metronidazole and Clindamycin) and have been sensitive to (Levofloxacin). There is a need potently replacements to antibiotics to axing the impedance to treat acne infections. Purified bacteriocin (MRSAcin) produce from using against resistance *P. acnes* their wide inhibition zone by the well diffusion assay (WDA) method and Paper disc method reached (15 and 11) mm respectively at concentration 62.5 µg/ml compared with sensitive antibiotic disc of Levofloxacin that diameter reached 9mm. The current in vitro study proved that *P. acne* was resistant to most antibiotics except levofloxacin. Bacteriocins (MRSAcin) had a good antibacterial activity and its effects exceed the effects of levofloxacin antibiotic.

Keywords: *Bacteriocin, MRSA, P. acnes*

Introduction

A commonly (AV) Acne vulgaris is cause inflammatory condition skin that affects (pediatric and adult) patients. Although traditionally as condition adolescent (it 90% develops in almost of patients starting at age 12-13 years), as 8 years patients as young can present with AV and the condition can persist into adulthood at age of 45 [1].

Anaerobe *P. acnes* the Gve+ the skin inhabits and *P. acnes* sebaceous follicles that releases two enzymes. (Lipases and proteases) are responsible for the creation of inflammatory mediators. Aspects of immunity may be the targets for the future development of therapeutic agents [2]. Most types of acne, disease from papules, nodulocystic can cause scarring and adequate treatment early must be started. Acne scars classified can also be as (mild, moderate or severe) depending many factors. Very common skin disease (Acne vulgaris) is an among young people which might be associated with scarring that has a great impact on the emotional, psychological and social life of the patients [3]. The skin first human barrier against attack by organisms foreign, is an ecosystem balanced that harbors a broad range of beneficial microorganism's bacteriocin that act against more 66 pathogens [4]. There is an opportunistic pathogen in implant-associated contagion (joint prosthetic, breast fibrosis, cardiovascular device-related infections or osteomyelitis spinal [5].

Drug today antimicrobial resistance is a growing threat to global public health and the widespread use of antibiotics has been associated with the increase in the occurrence of resistant organisms. Acne is an

important skin quandary amongst the youth, and custom started by androgen and propagated by bacterial flora of hair follicle treatments natural for acne vulgaris have much to offer although clinical studies are lacking [6]. MRSA isolate have gene that acquired a industrialized them nearly antibiotics resistant all beta-lactam, *S. aureus* is an opportunistic human and pathogen widely distributed.

It simple's infections skin and actually life-threatening complication such as (syndrome toxic shock) is the causative agent MRSA is a concern major in environments hospital because a symptomatic represent carriers a highest dangers factor to patients exposed to hospitalization intervals long [7]. Bacteriocins are a family of ribosomally synthesized antimicrobial peptides/proteins secreted by bacteria that inhibit the growth of across genera (broad spectrum) and closely related species (narrow spectrum) or, Bacteriocins are biotechnologically relevant since show toxicity low and can be wield in the food industry as inbred preservatives [8].

Some bacteriocins furthermore, have notable therapeutic possibility systematical bacterial infections, its value as viable alternative to antibiotics highlighting. Several publications on the ability of some type bacteriocins to inhibit *P. acnes*, reducing inflammatory lesions caused by this bacterium. Abound studies Iraqi connotation an epidemiological had showed that acne vulgaris in commonalty was more generic in males than females (74.24%) versus (61.9%). conducted on female patients with acne to investigate the pathogenesis of this disease, however, few involved male acne patients [9].

In fact our results confirm the strong bactericidal activity purification from MRSA strain fight against clinical strains *P. acnes*, even against those strains more the antibiotics to resistant. The results open novel prospects for developing topical treatments with bacteriocin from MRSA in the control of this bacterium.

Materials and Methods

Bacterial Isolates of MRSA

Isolation and Identification of MRSA

Sterile swab was moistened with sterile normal saline and directly inoculated on Mannitol Salt Agar (MSA) and incubated at 37°C for 18-24 hr. All colonies from primary cultures were purified by subculture on Brain-Heart Infusion Agar (BHIA) and then re-inoculated onto (MSA) and incubated at 37°C for 18-24 hrs. [10]. The samples collected from previously from wound specimen from (Al-Yarmouk Teaching Hospital) on media mannitol salt broth were incubated at 37C^{o} for 24 h and Selected isolates with colony morphology, Gram stain reactions and biochemical characteristics (Coagulase, Catalase reagent and Oxidase reagent).

Confirmation of MRSA by VITEK 2 ANC System

A specific number of the bacterial isolates were selected to confirm their identification susceptibility using the vitek 2 ANC system.

Bacterial Isolates of *P. acnes*

Fifty-one samples were collected from acne patients who attended the consultation unit of Dermatology in Baghdad teaching Hospital between the ages of (10-31) years in both sexes during the period of January 2017 till the end of June 2017. Samples were cultured on blood agar media and Brain heart infusion agar (Hi media, India) aseptically add 5% (v/v) horse blood (from horsemanship club, Baghdad University) under anaerobic conditions with anaerobic jar (Oxoid Anaerobic Jar with Anaerogen at 37°C for three days for isolation.

All isolates were obtained directly from the pus formed in the bottom part of the inflamed follicle. The contents were taken by sterile disposable cotton swabs, samples were then inserted into Thioglycolate broth (Oxide, UK) as transport media and carry to the laboratory. Samples were cultured on blood agar media and Brain heart infusion agar aseptically add 5%(v/v) horse blood (from horsemanship club, Baghdad university) under anaerobic conditions with anaerobic jar at 37°C for three days for isolation of *Propionibacterium acnes* [11,12].

Bacteria were identified by colonial morphology, Gram stain, standard biochemical tests for identification of *P. acnes* isolates [13]. All isolates were obtained directly from the pus formed in the bottom part of the inflamed follicle. The contents were taken by sterile disposable cotton swabs, samples were then inserted into

Thioglycolate broth as transport media and carry to the laboratory. Conformation using Vitek 2 ANC System.

Antibiotic sensitivity test *P. acnes*

The sensitivity test of *P. acnes* to antibiotics was designed accordance to the Kirbauy method [14] using 5 type antibiotics (Azithromycin, Clindamycin, Erythromycin, Levofloxacin and Metronidazole).

Purification of Bacteriocin (MRSAcin)

Obtain of Purification Bacteriocin by two steps included (Ion exchange and Gel filtration) from [15] (College of Science / University of Baghdad).

Detection MRSAcin

Agar Well Diffusion Method (WDA)

Mueller-Hinton agar surface was streaked by a sterile (swab cotton) with the assign bacterial strain. Agar plate was punched with a of sterile borer cork (4 mm) size and 100 µL of (MRSAcin) was holed with micropipette in the well. The plates were allowing standing by for 30 min. The plates were incubated at 37°C for 24 hrs. [16].

Paper Disc Method

Exchange (3-5) state and pure pipeline to the central container BHIB and brooded tubes at a temperature of 37 ° C for a time of 18 hrs. After that exchange 0.1 mL of airborne bacterial and send consistently on the focal agar MH, leaving the dish for 10 min. at room temperature to dry, circles of channel paper (Whattman NO.1) intervened by hand knowledge into a width of 5 mm arranged ahead of time by including 1 ml (MRSAcin) of each readied fixation before the cannula compartment 100 clean plate, was introduced drives interceded clean forceps and incubated at 37°C for (18-24) hrs. Recorded comes about by measuring the inhibition zone appeared to determine on every circle by four tablets in every dish [17].

Concentration of MRSAcin

It was delineating according to [18]. The antibacterial activity and concentration of (MRSAcin) bacteriocin were determined in all assays of this examine.

Results

In this study, the distribution of this disease among patient ages was studied. Most cases were in patients ages (16-21) years old in both sexes. Furthermore, the distribution of acne was higher among female than male (14% and 4%) respectively Figure (1).

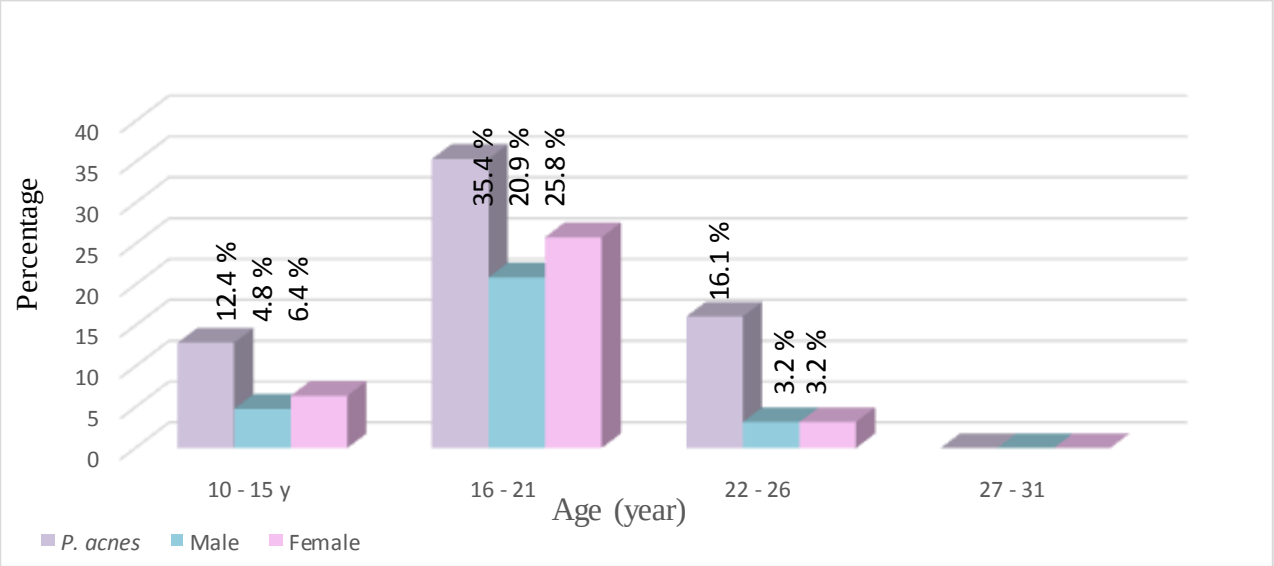

Figure 1: Distribution of *P. acnes* infection according to age and Gender groups

Identification of MRSA and *P. acnes*

The microscopic examination of *S. aureus* was showed that gram positive cocci appeared as single cells pairs and *P. acnes* isolates were giving Gram positive, polymorphism cells that appeared different forms, such as bacillus or spherical, cells were appeared different arrangement: single, in pairs, clustered, short chains, results as shown in Figure (2, A-B).

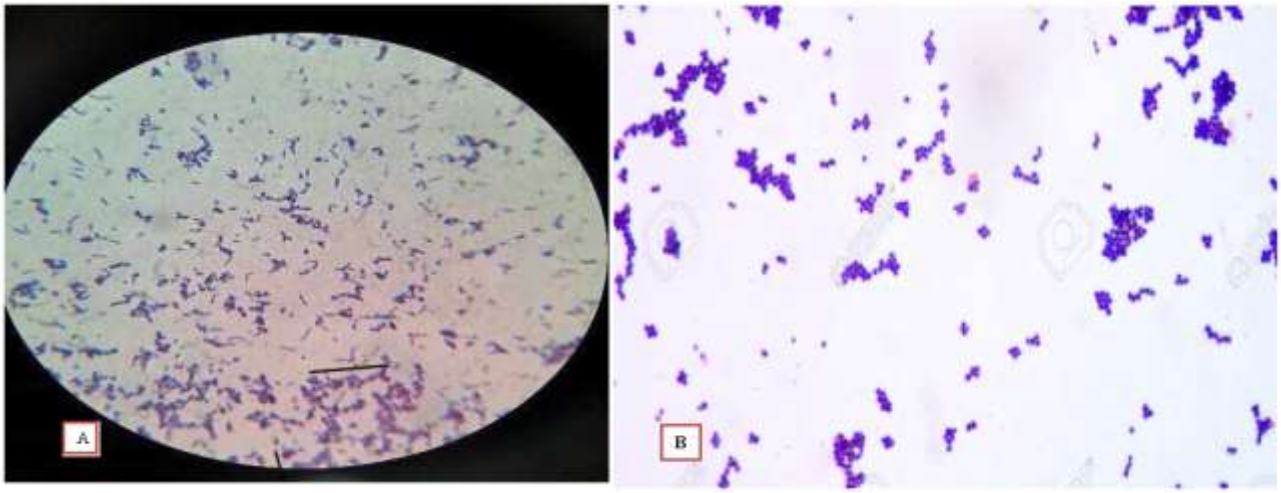

Figure (2, A-B) A: *P. acnes* were Gram positive, with different forms and arrangements (1000x) B: Gram positive, cocci cell shape of *S. aureus* (100x)

The macroscopic examination of isolates on (Mannitol Salt Agar) have an ability to ferment mannitol and turn the color of medium from red to yellow were classified as a presumptive *S. aureus* isolates. Colonies of *P. acnes* appeared glistening, circular and opaque with different colors, including white, yellow or gray, and their colonies grew larger in size over time as their young colonies were 1-2 mm in diameter that smaller than their old colonies. Figure (3,A-B).

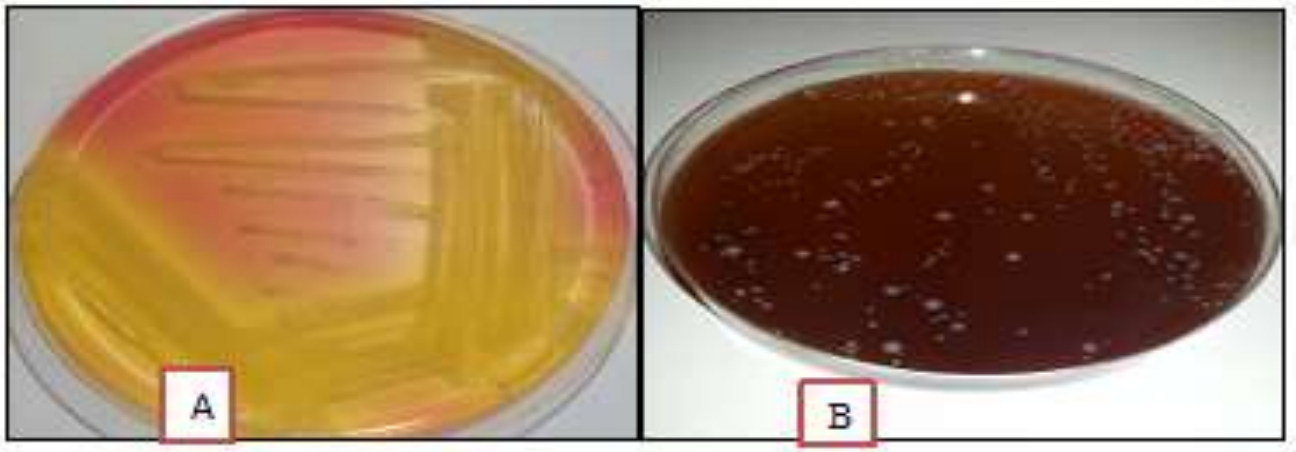

Figure (3-A-B) A: presumptive of *S. aureus* on mannitol salt agar. B: Colonies of *P. acnes* on Blood agar, under anaerobic condition at 37˚C was done between (24-48) hrs

The isolates of *S. aureus* on Blood agar showed yellow-gray colonies are (4-3) mm in diameter on the zones of ß- hemolysis while *P. acnes* colonies on anaerobic blood agar were with weak or no hemolysis. Figure (4, A-B).

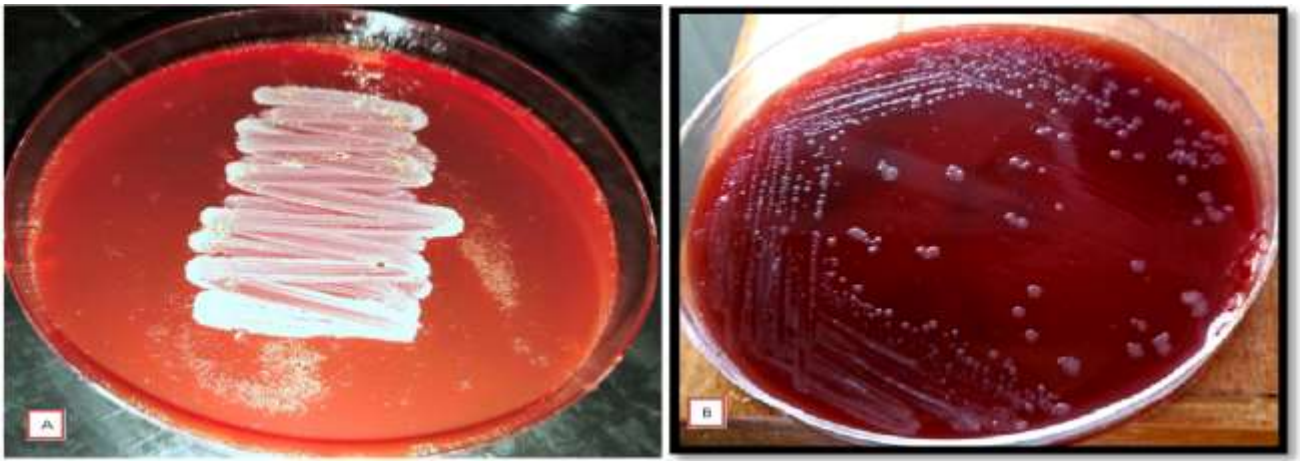

Figure (4, A-B) A: A presumptive of *S. aureus* on a blood agar B: Colonies of *P. acnes* on Blood agar, under anaerobic condition at 37˚C was done between (24-48) hrs

Biochemical Characteristics

The results of biochemical test of *S. aureus* referred that the isolates were positive to coagulase, catalase, citrate and ferment of mannitol, while oxidase and motility test were negative. While *Propionibacterium acnes* isolates shown Catalase (+), gelatin (+), Indole (+), Urease(−).

Vitek 2 ANC System

Vitek 2 ANC system gives confirmation of positive results for MRSA as a selected organism with probability (98-99%).

While the probability of *Propionibacterium acnes* isolates was: five isolates were (98%), two isolates were (94%) and two isolates were (92%).

Antibiotic Sensitivity Test *P. acnes*

Result show in (Figure 5, 6) *P. acnes* sensitive to antibiotic (Levofloxacin) then Clindamycin, and resistance (Azithromycin, Erythromycin and Metronidazole).

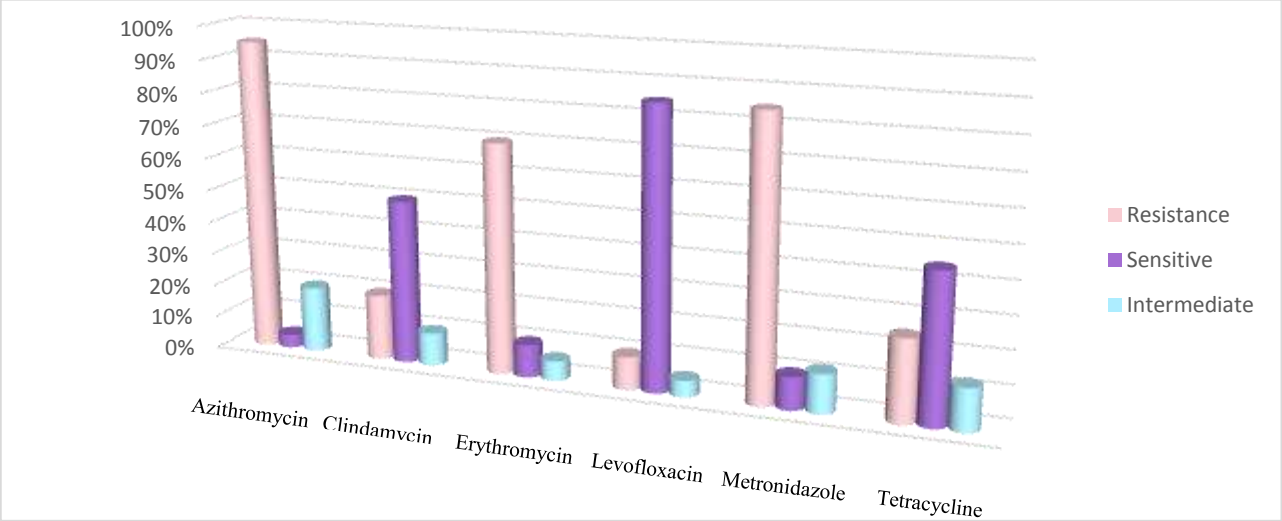

Figure 5: Antibiotic susceptibility test of *Propionibacterium acne*

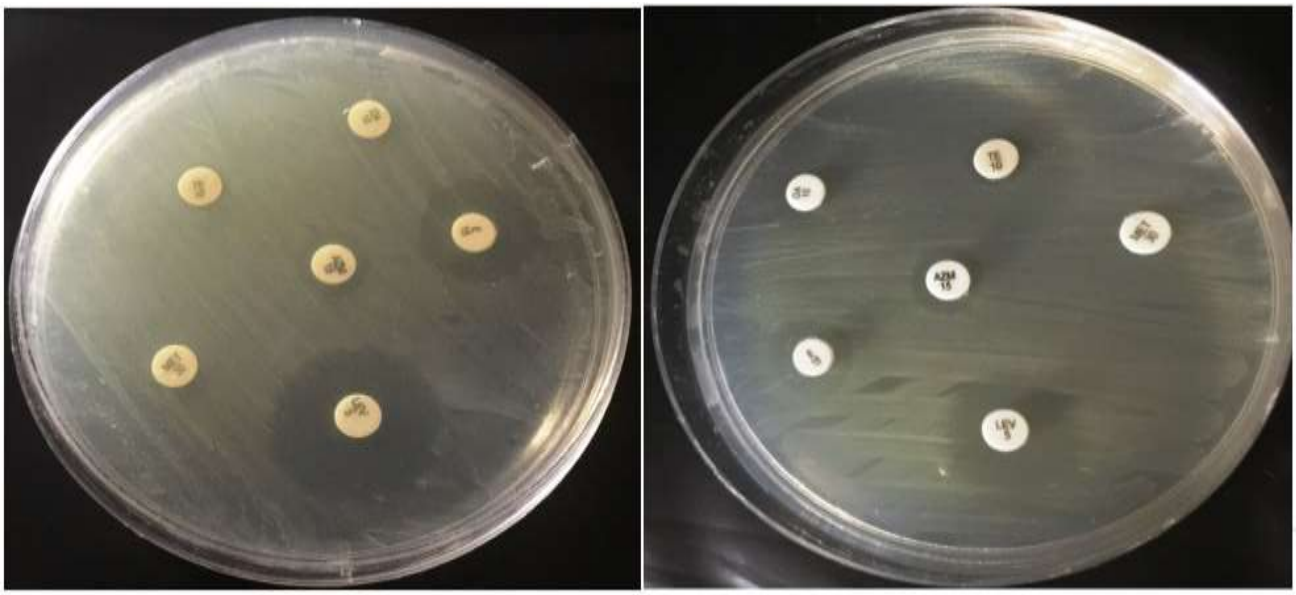

Figure 6: Antibiotic susceptibility test of *Propionibacterium acne* isolates Muller-Hinton agar plates at 37 °C for 24 hours

MRSAcin Activity Assay

The Purified MRSAcin producer by two steps to obtain full purification was from MRSA isolate from wounds infections Get ready to produce application against the indicator organism was *P. acne*. In vitro antibacterial activity of MRSAcin were studied for inhibitory ableness using the (AWD) assay method shows the inhibition zone diameter reached (15) mm in concentration (62.5 µg/ml). While by another method by paper disc method show decrease diameter reach (11) mm (Figure 7).

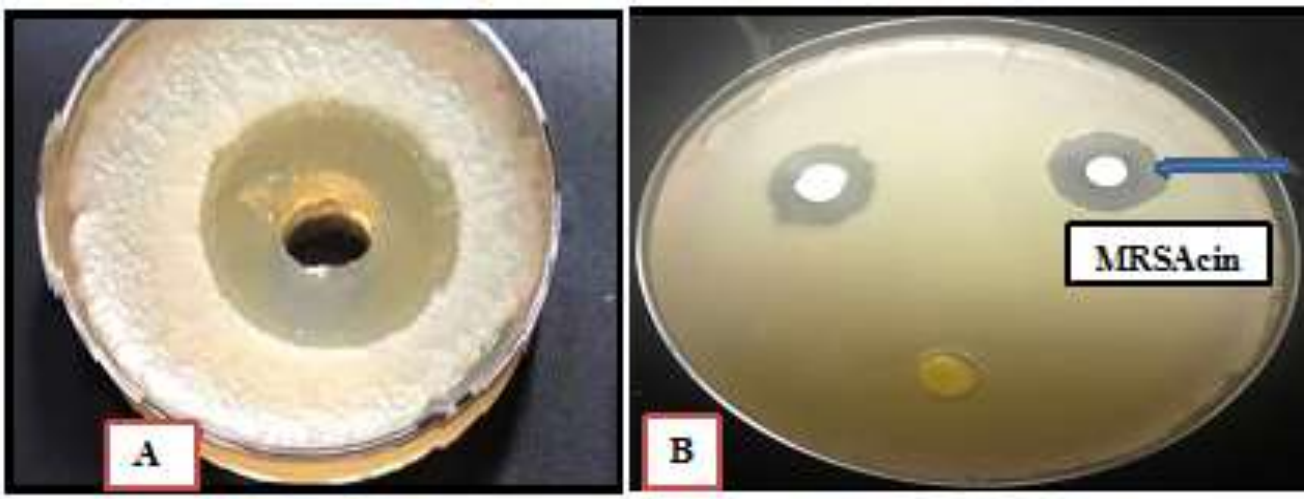

Figure 7: A: Inhibitory effect Pure MRSAcin by WDA B: spot-on-the-lawn method against *Propionibacterium acne* 24-48 hrs. At 37 °C

Comparison between MRSAcin and Antibiotics

Figure (8) shows the effect of Bacteriocin MRSAcin compared with antibiotic Levofloxacin. The local strain of *Propionibacterium acne* has been more sensitive to MRSAcin (15) mm the inhibition zone compared with higher sensitive antibiotic disc (Levofloxacin) that reached (9) mm. Also, when using MRSAcin against resistance *P. acnes*.

Figure 8: Comparison between purified MRSAcin and Antibiotics Levofloxacin

Discussion

These results were coinciding with pervious results in Iraq of *P. acne* in culture [19]. This result agrees with that mentioned by [20] had pointed that acne prevalence was more among female than in male, and most cases were in patient ages (15-20). Also coincides with previous study in Iraq isolated *P. acnes* [21]. This result for identification P. acnes were coincides with [22] Biochemical test and identification for *S. aureus* as mentioned by [23] and Results coincides with for *P. acnes* [24]. Using Vitek-2 ANC System was a rapid, sensitive, and accurate, assay that was developed to distinguish clinical isolates of MRSA from clinical isolates of methicillin-sensitive Staphylococcus aureus (MSSA) [25]. The result agrees with [26] that (erythromycin) were the fewer potently antibiotics for *Propionibacterium acne*. On the base of these results, we propose that Levofloxacin is a favoredly antibiotic for acne patients. [27] who shows the four groups antibiotic erythromycin, azithromycin and clindamycin *P. acnes* resistant and levofloxacin sensitive. Thematic use of antibiotics is presently a widely accepted effective and safe treatment for acne. That topical application of antibiotics such as tetracycline and clindamycin but the growing in *P. acnes* resistance antibiotic should be cause major problem [28].

Conclusion

Antibiotics although play an major role in acne administer, the excess in *P. acnes* resistance should be cause for anxiety and servants as the batch for change in patterns

describing and corrective algorithms. Not only are strains resistant linked to lack or worsening of clinical reacting to treatment, but the pathogenicity of *P. acnes* has increased above recent years, Limiting the frequency and period of antibiotic use and addition the topical antimicrobial agent Bacterocin will curtail the development of resistance while maintaining adequacy in the treatment of inflammatory and non-inflammatory acne wounds.

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