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RESEARCH ARTICLE

BACTERIOCIN TYPING OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL SPECIMEN

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ABSTRACT

The antibacterial activity of local isolates frome isolated from Baghdad, Iraq samples of different sources urine and wounds, ear and eye swab(25)strain of *Staphylococcus aureus*. From the collected clinical samples that gave positive result in coagulase of it was (MRSA methicillinresistance *S. aureus* 1 according to sensitivity test and vitek 2 system. Bacteriocin synthesis is a valuable character of some *staphylococcal* strains. Staphylococcal bacteriocinsa broad activity spectrum against many Gram-positive and Gram-negative bacteria are lethal to strains belonging to the same or related species as well as have. On the other hand, studies on the possibility of typing *S. aureus* MRSA, on the basis of their sensitivity to bacteriocins, are rarely published. Therefore, the purpose of this study was to use the production of bacteriocin from active strains in typing of *S. aureus*. A total of 10*S. aureus* were isolated from wound infections. Four staphylococci isolates *(S. aureus*1) were selected on the basis of sensitivity to most antibiotics which were used as basic indicator strains to determine the most producing staphylococcin isolates. (*S. aureus* 1) were chosen as good Staphylococcin producers according to their widest inhibition zone on the basic indicator isolates. Then the 10 isolates (producers) were tested against (Indicator) by well diffusion method. Staphylococcin of *S. aureus* 1, 5,7,8,11,16,18,22,23,25 strains inhibited of the tested isolates respectively (from wound infaction) multiple resistant strains that produced largest inhibition zone against the indicator strain was chosen for further study,

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INTRODUCTION

Staphylococcus aureus is one of the pathogen, as well as life threatening diseases (pneumonia, meningitis, endocarditis and septicemia)that can cause minor skin infections (pimples, boils, cellulites, toxic shock syndrome, impetigo and abscesses); it is also responsible for severe morbidity and mortality worldwide (Noskin *et al*., 2005; Sabra and Farag, 2012). Staphylococcal infections are frequently treated with antibiotics and consequently acquire resistances to antibiotics (Skalka, 1986). The resistance to antimicrobial agents is an increasing problem worldwide (Saeed *et al*., 2004). Controlling and understanding *S. aureus* is a significant public health concern that is underscored by the continuous evolution and development of antibiotic-resistant *S. aureus*. also called Staphylococcinsin *Staphylococcus aureus*. Bacteriocin synthesis is a valuable character of some staphylococcal strains (Syed *et al*., 2011) bacteriocins, have been reported to play an important role in the control of infections (CLSI, 2007).

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Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria (Desriac *et al*., 2010). Production of bacteriocin is very important. Various typing schemes have been based upon either the production of, or sensitivity to a range of different bacteriocins. Bacteriocin-like inhibitory substances (BLIS) are generally described as antagonistic bacterial agents with an active protein moiety; immunity of the producer strain to its own substance is genetically determined (Saranya and Hemashenpagam, 2011) Staphylococcal bacteriocins are lethal to strains belonging to the same or related species. It has a broad activity spectrum against many Gram-positive and Gram-negative bacteria (Montalb´an-L´opez *et al*., 2011). Studies on the possibility of typing *S. aureus*, on the basis of their sensitivity to bacteriocins, are rarely published. Therefore, the purpose of this study was to use the production of bacteriocin from active strains against testing bacteria.

MATERIALS AND METHODS

Sample Collection a total of 25 clinical specimens were collected from different sources such as sources urine and

wounds, nesil and eye swab were collected from the pathology Hospital in Iraq. The specimens were immediately transferred to the microbiology laboratory for further isolation of bacterial pathogens. Isolation and Identification of Bacterial Pathogens Each specimen was inoculated on Mannitol Salt Agar plates. The plates were incubated at 370C for 24 hours. After incubation the isolated colonies were identified on the basis of morphological, cultural and biochemical characteristics (10) and results were compared with Bergey's Manual of Determinative Bacteriology, From the collected clinical samples that gave positive result in coagulase 85(78.7%) of it was (MRSA) methicillinresistance *S. aureus* according to sensitivity test and vitek 2 system..pathogens were identified as *Staphylococcus aureus*.

Fig. 1. *Staphylococcus aureus*in mannitol salt agar

Antimicrobial Susceptibility

Test The antibiotic susceptibility pattern of all isolated *S. aureus* (11) was tested by 8 antibiotic discs obtained from Hi-media Laboratories Pvt. Ltd. Mumbai (Table 1). In brief, *S. aureus* isolates were grown overnight on nutrient agar at 370C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Mueller-Hinton agar. Then, the antibiotic disc was transferred aseptically on to the surface of the inoculated Mueller Hinton agar plates, and the plates were incubated at 370C for 18 hours (Reid *et al*., 2010). The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded (Montalb´an-L´opez *et al*., 2011), and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Lab oratory Standards Institute (CLSI) guidelines (Desriac *et al*., 2010)

Estimation of protein by Lowry's method

The samples were analyzed for protein using Lowry's method. 5 tubes which serve as standard and one tube for the supernatant and one tube for the pellet were taken and 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml of protein solution were added to the standard tubes marked as 0.2 ml of supernatant and 0.8 ml of pellet were also added to the respective tubes and each of the tubes were made up to 4ml by adding water. Then 5.5 ml of reagent C was added to all the tubes and kept at room temperature for 10-15 mins. Then 0.5ml of reagent D was added to all the tubes and kept in dark for 30 mins. (CLSI, 2007)

RESULTS

Antibiotic Resistanc

With other previous studies. This resistance against a particular antibiotic may be due to its frequent and long-term use (Joo *et al*., 2012). Among the eight antibiotics used in the present study, Azithromycin, Erythromycin, Gentamycin and Vancomycin are the best choices for treating *S. aureus* infection (Table 1). *S. aureus* is capable of causing a variety of human infections, including fatal invasive and toxic conditions and also possesses a differential ability to spread and cause hospital associated outbreaks of infections (Benmechernene *et al*., 2013).

Table 1. Antibiotic Discs Used in the Study

Antibiotics	Concentration	Antibiotics	Concentration
Azithromycin	30mcg	Imipenem	10mcg
Cefixime	5mcg	Linezolid	5mcg
Erythromycin	5mcg	Oxacillin	5mcg
Gentamycin	5mcg	Vancomycin	30mcg

High resistance of these isolates against Imipenem and Cefixime (100% each) approximately agrees with other previous studies. This resistance against a particular antibiotic may be due to its frequent and long-term use. Among the eight antibiotics used in the present study, Azithromycin, Erythromycin, Gentamycin and Vancomycin are the best choices for treating *S. aureus* infection. produced an efficient staphylococcin, identified by agar well diffusion method, depending on the widest inhibition zone and the highest sensitive number of the basic indicator isolates. These isolates were used as indicator local in bacteriocin typing. one staphylococcal isolates were chosen as good Staphylococcin producers according to their widest inhibition zone on the basic indicator isolates.

Determination of the inhibitory spectrum

Inhibitory activity was detected by techniques: In the agar diffusion assay, the sample of puri ed StT was put on 6-mm-diameter lter-paper discs, and these were placed on a surface of solid Mueller- Hinton medium inoculated with the tested strain. The plates were kept at room temperature (30°C) for 1h and sub sequently incubated at 37°C for 24h. The antimicrobial activity was quanti ed by the diameter of the inhibition zone around each sample. Bacteriocin Typing of *S. aureus*. Investigation of the Efficient Strains Producing Staphylococcin, were selected from which were sensitive to most antibiotics were used as basic indicator strains to determine the most producing staphylococcin isolates, by well diffusion method (Kopit *et al*., 2014). Nutrient agar plates were inoculated with 100 µL of each basic indicator strains after growing them in a Brain-Heart infusion broth and diluting appropriately to a 0.5 McFarland standard (0.5×108 CFU/ml), then left to dry at room temperature for a period (10-15 minutes). Wells (6 mm) were cut into the plates and 100 µL of supernatant fluid after centrifuged at 5000 × g for 10 min of the isolates were placed into each well. Plates were incubated at 370C for 24 hrs.

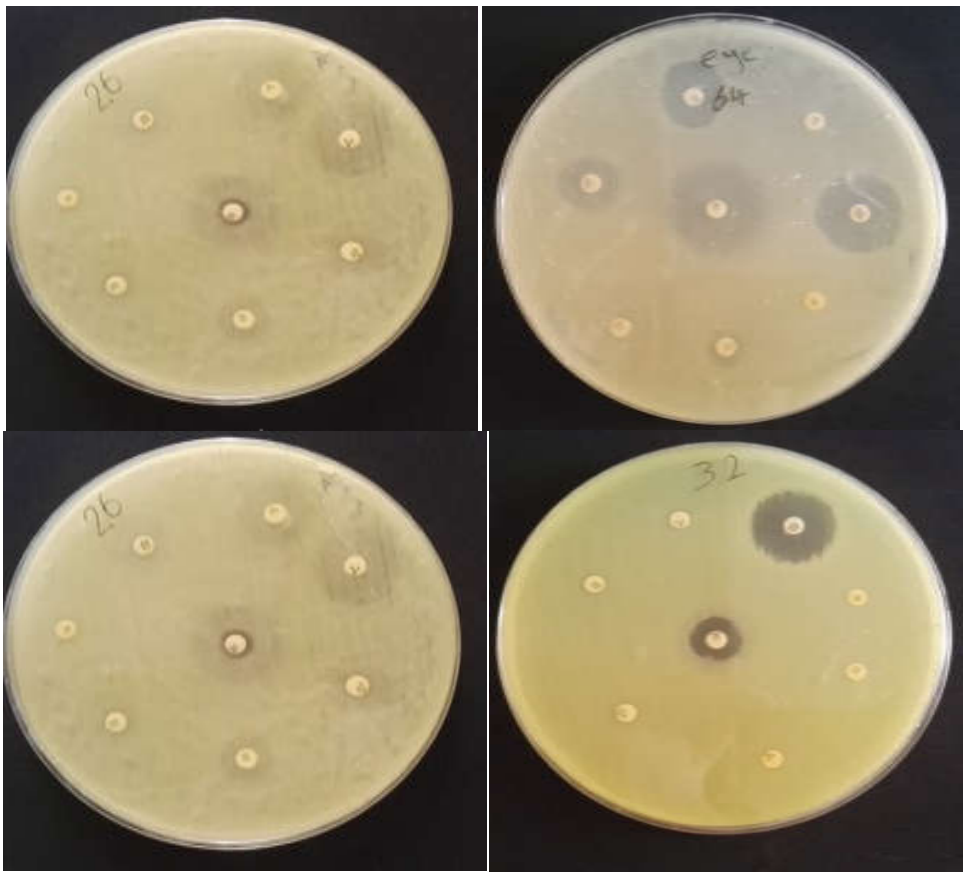

Fig. 2. Antibiotic resistance tests: Bacteria are streaked on the dish on which antibiotic impregnated white disks are placed. Bacteria in the culture on the left are susceptible to the antibiotic in each disk, as show clear rings where bacteria have not grown. Those on the right are fully susceptible to only three of the seven antibiotics tested (Noskin *et al*., 2005)

Table 1. Production

Bacteriocin of *Staphylococcus aureus*1	Indicator /sensitive strain	Average Zone of inhibition (mm)diameter
	*Proteus*sp	27
	Streptococcus pyogens	18
	Klebsiellapneumonia	30
	Salmonella typhia	17
	Listeria monocytogenes	26

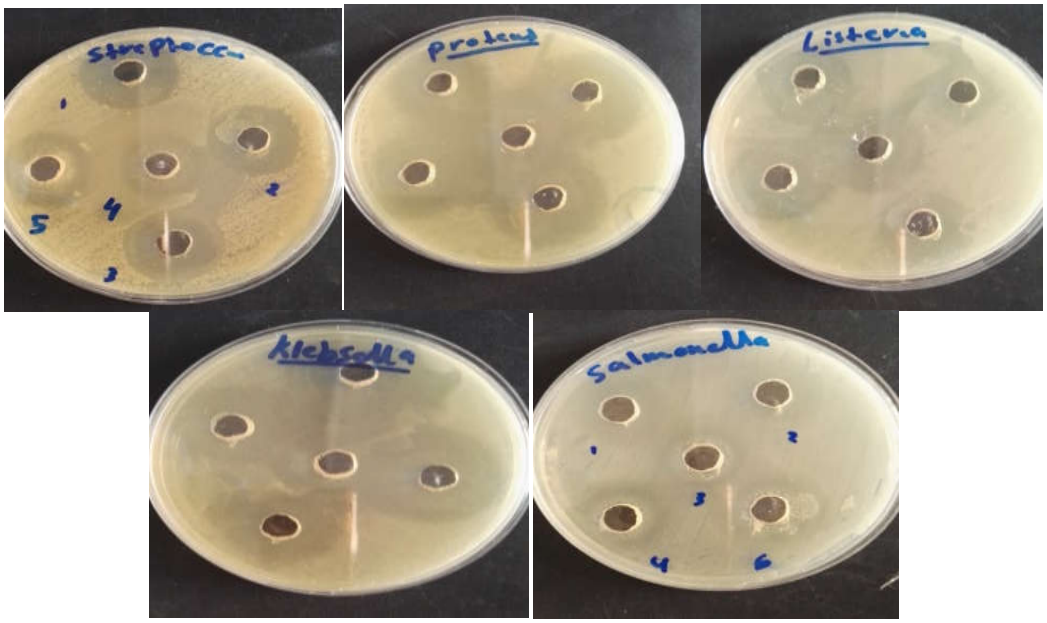

Figure 3. Antimicrobial activity CFCS *S. aureus* 1, Results of the well-diffusion assay of five bacterial strains (a) *Streptococcus pyogens* (b) *Proteus*sp (c) *Listeria monocytogenes* (d) *Klebsilla*(e) *salmonella typha* (c) *Klebsilla*

The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells well diffusion method as described earlier. Staphylococcin of *S. aureus* 1, 5, 7, 8, 11, 16, 18, 22, 23, 25were inhibited of the tested isolates respectively Bacteriocin and bacteriocin- like inhibitory substances (BLIS) are natural antimicrobial agents produced by Gram positive bacteria. BLIS have potential applications against a wide range of human and animal diseases. They are ribosomally synthesized antimicrobial peptides produced by microorganism belonging to different Bacteriocins may serve as anti-competitor compounds enabling an invasion of a strain or species in an established microbial community (Kopit *et al*., 2014; Kamarajan *et al*., 2015). eubacterial taxonomic branches; they are lethal to bacteria closely related to the producing bacteria, the latter being protected by an immunity phenomenon. Determining staphylococcin producing strains depends upon the susceptibility of the indicator strain (Rehaiem *et al*., 2014). Spectrum of activity of *S. aureus* 1 inhibited all the *S. aureus* strains as well as many streptococcal strains in the deferred antagonism test. Its spectrum of activity was not restricted to Gram-positive organisms, but included strains of *Proteus* sp., *Streptococcus pyogens* and *salmonella typha, Klebsilla, Listeria monocytogenes*

Production of staphylococcin

*S. aureus*1was found to produce antibacterial, antifungal and antimycobacterial substance(s). Culture supernatant of *S. aureus* was found to contain a maximal amount of Bac after 7 h of incubation at 37C corresponding to mid exponential phase. For convenience, the *S. aureus*1 culture was harvested after 24 h. The bacteriocin activity was present in the cell-free culture supernatant, indicating that bacteriocins produced by *S. aureus* may not be associated with the cell membrane. The antimicrobial activities of *S. aureus*, were evaluated by agar-well dicusion methods. The activity unit of Bac (AU/ml) against a) *Streptococcuspyogens*, *Proteus*sp, *Listeria monocytogenes*, *Klebsilla*, *salmonella typha*was found (Figure 3). Interestingly, a similar result of Bac has also been observed against Gram-negative bacteria such as *E. coli* and *S. typhi* (data shown) suggesting a similar mechanism of killing on both Gram-negative as well as Gram-positive organisms (e ective protein concen- tration=2.2 lg/ml). On the other hand, when in the agar well di usion method *S. aureus*1 (i.e. the producer) was challenged with staphylococcin, no zone of inhibition was observed showing that it possesses immunity genes which protect it from the lethal e ects

DISCUSSION

The present study demonstrates the activity spectrum, production of bacteriocin and (termed as Staphylococcin Bac1) produced by *S. aureus*1. Staphylococcin1 (>10 kDa fraction) which are characteristic features of class I and II bacteriocins. Bac1 showed a broad spectrum of activities against di erent Gram-positive and Gram-negative bacteria as well as many dermatophytes which is not surprising considering the structural and functional diversity that exist within the bacteriocins of Gram-positive bacteria, particularly staphylococcins (17,11). Furthermore, staphylococci produce many inhibitory substances that are either bactericidal and/or bacteriostatic. In case of *S. aureus* 1, the e ect of staphylococcin Bac1 on log and stationary phase cells of sensitive *S. aureus* 1 was bactericidal. The antagonism between closely related. However, recent studies suggest that they may be as e ective as some currently used therapeutic agents for the treatment of staphylococcal infections in mice as well as humans (14). avirulent strain of *Staphylococcus aureus* in the prevention of serious staphylococcal disease in neonates and in the treatment of furunculosis has also been demonstrated (1). Studies on the antidermatophytic property of staphylococcin Bac1 determined against various common mouldsMycelial plugs taken from the zones of inhibition were found to revive their growth after rein- oculation into fresh media,. Thus it is quite pre- mature to speculate whether these antimicrobial and antifungal activities are related to the bacteriocins. The increased prevalence of fungal infections and non-availability of e ective and safer drugs has prompted a vigorous search for antifungal antibiotics from di erent sources (6). The antidermatophytic e ects of Bac1 may provide an incentive for use of these bacteriocins as chemotherapeutic agents. Indeed, bacteriocin treatment in general has already been proposed for controlling these diseases in view of the low cost, e ectiveness, non- toxic nature and non-immunogenic character (3).

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