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Bacteriocin Typing of *Staphylococcus Aureus* Isolated from Clinical Specimens in Nagpur City

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Abstract: *Bacteriocin synthesis is a valuable character of some staphylococcal strains. Staphylococcal bacteriocins are lethal to strains belonging to the same or related species as well as have a broad activity spectrum against many Gram-positive and Gram-negative bacteria. On the other hand, 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 in typing of S. aureus. A total of 22 S. aureus were isolated from 50 clinical specimens of wound infections. Four staphylococci isolates (S. aureus 5, S. aureus 8, S. aureus 13 and S. aureus 20) were selected on the basis of sensitivity to most antibiotics which were used as basic indicator strains to determine the most producing staphylococcin isolates. Five staphylococcal isolates (S. aureus 1, S. aureus 4, S. aureus 12, S. aureus 17 and S. aureus 19) were chosen as good Staphylococcin producers according to their widest inhibition zone on the basic indicator isolates. Then the five isolates (producers) were tested against 22 S. aureus (Indicator) by well diffusion method. Staphylococcin of S. aureus 1, S. aureus 4, S. aureus 12, S. aureus 17 and S. aureus 19 strains inhibited 17 (77.27%), 20 (90.90%), 18 (81.81%), 16 (72.72%) and 20 (90.90%) of the tested isolates respectively. Depending on the sensitivity to the staphylococcin used, the isolates of S. aureus were classified into 4 groups. The most numerous group was characterized by the susceptibility to all five staphylococcin and comprised 13 (59.09%) isolates of S. aureus, followed by four isolates (18.18%) susceptible to three staphylococcin, three isolates (13.63%) susceptible to four staphylococcin while the lowest numerous were found in 2 isolates (9.09%) susceptible to only one staphylococcin.*

Keywords: *Antibiotics, Bacteriocin, Resistance, Staphylococcin, Staphylococcus aureus*

1. INTRODUCTION

Staphylococcus aureus is one of the pathogen, that can cause minor skin infections (pimples, boils, cellulites, toxic shock syndrome, impetigo and abscesses) as well as life threatening diseases (pneumonia, meningitis, endocarditis and septicemia); it is also responsible for severe morbidity and mortality worldwide [1] [2]. Staphylococcal infections are frequently treated with antibiotics and consequently acquire resistances to antibiotics [3]. The resistance to antimicrobial agents is an increasing problem worldwide [4]. 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*.

In *Staphylococcus aureus,* bacteriocins, also called Staphylococcins have been reported to play an important role in the control of infections [5]. Bacteriocin synthesis is a valuable character of some staphylococcal strains [6]. Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria [7]. 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 [8]. 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. 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 [9]. *S*tudies 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 in typing of *S. aureus*.

2. METHODS

2.1. Sample Collection

A total of 50 clinical specimens were collected from different sources such as wound infections were collected from the pathology laboratory in Nagpur. The specimens were immediately transferred to the microbiology laboratory for further isolation of bacterial pathogens.

2.2. Isolation and Identification of Bacterial Pathogens

Each specimen was inoculated on Mannitol Salt Agar plates. The plates were incubated at 37^0C 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, 9th edition. Out of 50 specimens 22 bacterial pathogens were identified as *Staphylococcus aureus*.

2.3. Antimicrobial Susceptibility Test

The antibiotic susceptibility pattern of all isolated *S. aureus* (22) 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 37^0C, 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 37^0C for 18 hours [11]. The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded [12], and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines [13].

Table1. *Antibiotic Discs Used in the Study*

Antibiotics	Concentration	Antibiotics	Concentration
Azithromycin	30mcg	Imipenem	10mcg
Cefixime	5mcg	Linezolid	30mcg
Erythromycin	5mcg	Oxacillin	5mcg
Gentamycin	50mcg	Vancomycin	30mcg

2.4. Bacteriocin Typing of S. Aureus

2.4.1. Investigation of the Efficient Strains Producing Staphylococcin

Four staphylococci isolates were selected from which were sensitive to most antibiotics (*S. aureus* 5, *S. aureus* 8, *S. aureus* 13 and *S. aureus* 20) were used as basic indicator strains to determine the most producing staphylococcin isolates, by well diffusion method [14]. 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 (1.5×108 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 37^0C for 24 hrs. The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells.

2.4.2. Typing of S. aureus Strains

Five staphylococcal isolates (*S. aureus* 1, *S. aureus* 4, *S. aureus* 12, *S. aureus* 17 and *S. aureus* 19) were chosen as good Staphylococcin producers according to their widest inhibition zone on the basic indicator isolates. Then the five isolates (producers) were tested against 22 *S. aureus* (Indicator) by well diffusion method, as described earlier [14].

3. RESULTS AND DISCUSSION

From the 50 clinical specimens i.e. samples from wound infections were collected from pathology laboratory in Nagpur, only 22 isolates were found to be *Staphylococcus aureus*. All the *S. aureus* were tested for antibiotic susceptibility pattern against eight different antibiotic discs selected.

Table2. *Antibiotic Susceptibility Test of S. aureus*

S. aureus	**Azithromycin**	**Cefixime**	**Erythromycin**	**Gentamycin**	**Imipenem**	**Linezolid**	**Oxacillin**	**Vancomycin**	**Resistance**	**Sensitive**
S1	19	NZ	17	21	NZ	NZ	NZ	18	4	4
S2	20	NZ	20	NZ	NZ	NZ	NZ	19	5	3
S3	22	NZ	25	22	NZ	NZ	NZ	19	4	4
S4	25	NZ	17	24	NZ	NZ	NZ	20	4	4
S5	**31**	**NZ**	**27**	**27**	**NZ**	**32**	**13**	**16**	**2**	**6**
S6	20	NZ	20	24	NZ	NZ	NZ	17	4	4
S7	19	NZ	21	24	NZ	NZ	NZ	18	4	4
S8	**17**	**NZ**	**14**	**20**	**NZ**	**25**	**11**	**16**	**2**	**6**
S9	21	NZ	17	NZ	NZ	NZ	NZ	14	5	3
S10	21	NZ	18	23	NZ	NZ	NZ	16	4	4
S11	20	NZ	17	23	NZ	NZ	NZ	19	4	4
S12	19	NZ	21	18	NZ	NZ	NZ	16	4	4
S13	**18**	**NZ**	**28**	**32**	**NZ**	**40**	**25**	**13**	**2**	**6**
S14	19	NZ	26	26	NZ	NZ	NZ	16	4	4
S15	19	NZ	17	22	NZ	NZ	NZ	16	4	4
S16	21	NZ	15	23	NZ	NZ	NZ	16	4	4
S17	20	NZ	22	18	NZ	NZ	NZ	18	4	4
S18	23	NZ	21	21	NZ	NZ	NZ	16	4	4
S19	21	NZ	19	20	NZ	NZ	NZ	16	4	4
S20	**22**	**NZ**	**19**	**16**	**NZ**	**24**	**13**	**11**	**2**	**6**
S21	20	NZ	NZ	NZ	NZ	NZ	NZ	16	6	2
S22	20	NZ	17	19	NZ	NZ	NZ	16	4	4
Resistant	**0**	**22**	**1**	**3**	**22**	**18**	**18**	**0**	**No. of Isolates**	
Sensitive	**22**	**0**	**21**	**19**	**0**	**4**	**4**	**22**		

Where, NZ = No Zone

On the basis of antibiotic susceptibility test it was found that four *S. aureus* isolates (S5, S8, S13 and S20) were sensitive to most of the antibiotic discs tested. All the isolates (22) were resistant to Imipenem and Cefixime (100% each), followed by Linezolid and oxacillin (81.81% each). Gentamycin had moderate effect on isolates (13.63%). However most isolates were highly susceptible to Azithromycin (100%), Erythromycin (95.45%) and Vancomycin (100%) (Table 2). These findings were agreed with that of the previous study [15]. The widespread use of antibiotics has been responsible for the development of numerous problems, including the emergence of multi drug resistance bacteria, an increased number of acquired infections from community and hospitals, and increased health care costs [16] [17].

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 [18]. Among the eight antibiotics used in the present study, Azithromycin, Erythromycin, Gentamycin and Vancomycin are the best choices for treating *S. aureus* infection. *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 [16].

Among the 22 *S. aureus* isolates, five bacterial isolates (*S.aureus* 1, *S.aureus* 4, *S.aureus* 12, *S. aureus*17, *S.aureus* 19) 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.

Five staphylococcal isolates (*S. aureus* 1, *S. aureus* 4, *S. aureus* 12, *S. aureus* 17 and *S. aureus* 19) were chosen as good Staphylococcin producers according to their widest inhibition zone on the basic indicator isolates. Then the five isolates (producers) were tested against 22 *S. aureus* (Indicator) by

well diffusion method, as described earlier. Staphylococcin of *S. aureus* 1, *S. aureus* 4, *S. aureus* 12, *S. aureus* 17 and *S. aureus* 19 strains were inhibited 17 (77.27%), 20 (90.90%), 18 (81.81%), 16 (72.72%) and 20 (90.90%) of the tested isolates respectively (Table 3). 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 eubacterial taxonomic branches; they are lethal to bacteria closely related to the producing bacteria, the latter being protected by an immunity phenomenon. Bacteriocins may serve as anti-competitor compounds enabling an invasion of a strain or species in an established microbial community [19] [20]. Determining staphylococcin producing strains depends upon the susceptibility of the indicator strain [21]. Previous studies reported that merely twelve out of 300 *S. aureus* strains were convenient indicators. The importance of the indicators was also emphasized by many authors [22-25].

Table3. *Susceptibility patterns of bacteriocin typing of Staphylococcus aureus*

Typing *S. aureus* isolates (22)	***S. aureus* 1**	***S. aureus* 4**	***S. aureus*12**	***S. aureus*17**	***S. aureus*19**
13	+	+	+	+	+
4	+	+	-	-	+
3	-	+	+	+	+
2	-	-	+	-	-
Total Inhibited Isolates	17	20	18	16	20

Where, + = Inhibition Zone - = Without Effect

Depending on the sensitivity to the staphylococcin used, the isolates of *S. aureus* were classified into 4 groups. The most numerous group was characterized by the susceptibility to all five staphylococcin and comprised 13 (59.09%) isolates of *S. aureus*, followed by four isolates (18.18%) susceptible to three staphylococcin, three isolates (13.63%) susceptible to four staphylococcin while the lowest numerous were found in 2 isolates (9.09%) susceptible to only one staphylococcin (Table 3). The results obtained with the typing set strains on tested isolates show that the producers isolates having a wide spectrum and a high intensity of activity against indicator strains. However, the producer's isolates contributed to the achievement of greater differentiation of typed staphylococci. Furthermore, since bacteriocins, produced by bacteria, are thought to have an important role in establishing the ecosystem [26], the bacteriocin presented here may be responsible for a part of the control mechanism of microbial ecology. Bacteriocins are found in almost every bacterial species examined to date [27]. Bacteriocins are part of widespread applications in epidemiological studies as specific markers for bacteria. Various typing schemes have been based on either the production of, or sensitivity to a range of different bacteriocins [9].

4. CONCLUSION

On the basis of present findings, it was concluded that out of 22 isolates of *S.aureus* only four *S.aureus* strains were sensitive to most of the antibiotics tested and therefore considered as basic indicator strains. By agar well diffusion method the five isolates of *S.aureus* that are *S.aureus* 1, S.a*ureus* 12, *S.aureus* 4, *S.aureus* 17, *S.aureus* 19 were chosen as good staphylococcin producers according to their widest zone of inhibition on basic indicator strains.

*S.aureus*4 and *S.aureus*19 inhibited 20 isolates of *S.aureus* followed by *S.aureus*12 inhibited 18 isolate while *S.aureus*1 and *S.aureus*17 inhibited 17 and 16 *S.aureus* isolates respectively. Bacteriocin produced by bacteria plays an important role in establishing the ecosystem. Bacteriocin produced in this study may be responsible for part of control mechanism of microbial ecology.

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