JJBS

Volume 7, Number 1, March .2014
ISSN 1995-6673
Pages 25 - 29

Jordan Journal of Biological Sciences

Bacteriocin Typing of *Staphylococcus aureus* Isolated from Different Sources in Ibb City, Yemen

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Received: October 10,2013 Revised: November 1, 2013 Accepted: November 4, 2013

Abstract

A total of 207 specimens were collected from different sources including patients, health care staff and hospital environment in Ibb city, Yemen. The study used the bacteriocin produced from active producer strains in typing of *Staphylococcus aureus*. Depending on the morphological, cultural and biochemical characteristics, 54 (26.09%) isolates of *Staphylococcus aureus* were identified. An antibiotic sensitivity test was done for the bacterial isolates, and the results showed that there were multiple resistant antibiotics. The Staphylococcin production of these isolates has been detected by using wells assay. Fifty one isolates were Staphylococcin producer. Four isolates (staph19, staph25, staph28 and staph43) were chosen as good Staphylococcin producers, and used locally as indicators in bacteriocin typing. Depending on *S. aureus* typing, the isolates fell into (9) groups. The most numerous group was characterized by susceptibility to all four staphylococcin and comprised 61.11% isolates of *S. aureus*, while the lowest numerous were found in three groups with a ratio of 1.85%; the remaining groups had little percentages ranging from 3.70% to 11.11%. We observed that about (94.44%) of the isolates were bacteriocin producers, and among them, four isolates had a strong bacteriocin production. Based on typing, most isolates had one pattern.

Keywords: Bacteriocin, Staphylococcin, Typing, *Staphylococcus aureus*, Infection,Antibiotics, Resistance.

1. Introduction

Staphylococcus aureus is one of among Staphylococci belonging to the *Micrococaceae* family, that can cause under appropriate conditions, 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 (Singh and Prakash, 2010) and (Noskin *et al.*, 2005). Staphylococcal infections are frequently treated with antibiotics and, consequently, acquire resistances to antibiotics that evolve (Sabra and Farag, 2012). The resistance to antimicrobial agents is an increasing global problem worldwide (Duran *et al.*, 2012). Controlling and understanding *S. aureus* in both hospital and community settings is a significant public health concern that is underscored by the continuous evolution and development of antibiotic-resistant *S. aureus*.

Recent advances in biotechnology allow molecular epidemiology to play an increasing role in the control of *S. aureus* (Arbeit, 1997). Within the genus *Staphylococcus*, bacteriocins, also called Staphylococcins, have been reported from several species, and already been described and characterized in details (Hena and Sudha, 2011). Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria (Cleveland *et al.*, 2001). 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 (Saeed *et al.*, 2004). Production of bacteriocin is a highly important factor in microbial ecology. Bacteriocins have found widespread application in epidemiological studies as specific markers for bacteria. Various typing schemes have been based upon either the production of, or sensitivity to a range of different bacteriocins (Guven, 2000). Staphylococcal bacteriocins are lethal to strains belonging to the same or related species and act by binding to surface receptors followed interaction with an intracellular target. It has a broad activity spectrum against many Gram-positive (e.g. corynebacteria, listeriae, streptococci and bacilli) and Gram-negative bacteria (e.g. *Neisseria gonorrhoeae* and *Escherichia coli*) (Rogolsky and Wiley, 1977; Kader *et al.*, 1984). It is apparent from the reviews and from the recent studies about staphylococcins that the main effort is directed towards the discovery of as many bacteriocin producing strains as possible, which can be used in

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characterization of staphylococcal strain. Bacteriocin synthesis is a valuable character of some staphylococcal strains (Skalka, 1986). 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 is to use the production of bacteriocin from active strains in typing of *S. aureus*.

2. Material and Methods

2.1. Bacteriological Study

A total of 207 specimens were collected from different sources including patients, health care staff and hospital environment in Ibb city, Yemen, from April to September, 2012. For the isolation and identification of *S. aureus*, each specimen was identified, depending on the morphology, cultural characteristics and biochemical reaction (Baron and Feingeld, 1990). Fifty four isolates of *S. aureus* were subjected to API Staph System tested to confirm the identification of this pathogen.

2.2. Antimicrobial Susceptibility Test

The antibiotic susceptibility pattern of all isolated *S. aureus* was tested by 9 antibiotics, determined by the modified Kirby-Bauer disc diffusion technique. In brief, *S. aureus* isolates were grown overnight on nutrient agar at 37°C, 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 Muller Hinton plates, and the plates were incubated at 37°C for 18 hrs. (Ehinmidu, 2003). The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded, and the isolates were classified as “resistant” or “sensitive” based on the standard interpretative according to CLSI (formerly NCCLS) guidelines.

2.3. Bacteriocin typing of S.aureus

2.3.1. Investigation of the Efficient Strains Producing Staphylococcin

Five staphylococci isolates (three isolates were selected from our study which are sensitive to most antibiotics (staph4, staph11 and staph31), and two standard strains (obtained from the Central Laboratories: ATCC 12345 and ATCC 98765)) were used as basic indicator strains to determine the most producing staphylococcin isolates, by well diffusion method (Rasool *et al.*, 1996). 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 °C for 24 hrs. The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the wells.

2.3.2. Typing of S. aureus Strains

Four staphylococcal isolates (staph19, staph25, staph28 and staph43) were chosen as good Staphylococcin producers according to their widest inhibition zone on the basic indicator isolates. Then the four isolates (producers) were tested against fifty four *S. aureus* (Indicator) by well diffusion method, as described earlier (Rasool *et al*., 1996).

3. Results

3.1. Isolation and Identification

From the 207 specimens collected from different sources, only 54 isolates were found to be *S. aureus*, with the ratio 26.09% of the total isolates (Table1). Data analysis, based on the sites of infection (Table2), shows that 16 (29.63%) samples were recovered from nose infection, 11 (20.37%) samples from wound infection, 8 (14.81%) and 5(9.26%) from urine infection and ear swabs, respectively. While samples taken from health care staff showed that 7 (12.96%) were nasal isolates and 3 (5.56%) were skin swabs. But samples taken from the hospital environment showed the lowest percentages: 1 (1.86%) were operation room swabs, and 3 (5.56%) were surgery room swabs.

Table 1. Represents the prevalence of *Staphylococcus aureus* isolated from different sources of infection

Sources of sampling	No. samples	No. isolates	(%)
Patients	125	40	32
Health care staff	40	10	25
Hospital environment	42	4	9.52
Total	207	54	26.09

Table 2. Distribution of *Staphylococcus aureus* isolated from different sources patients based on sites of sampling

Sources of sampling	Site of Sampling	Number	(%)
Patients	Wound	11	20.37
	Ear	5	9.26
	Urine	8	14.81
	Nasal	16	29.30
Health care staff	Skin	3	5.56
	Nasal	7	12.96
Hospital environment	Surgery room	3	5.56
	Operation room	1	1.86
Total		54	100

3.2. Antibiotic Susceptibility Test

All isolates (54) of *S. aureus* exposed to nine antibiotics are used in this study. The resistance phenotype obtained was as follows: all isolates were resistant to ampicillin with ratio (100%), penicillin G (94.44%), gentamycin (77.78%), erythromycin

(68.52%) and cephalexin (57.41%). Vancomycin and amikacin had moderate effects on the isolates (37.04%) and (35.19), respectively. However, most isolates were highly susceptible to ciprofloxacin (75.93%) and ofloxacin (81.48%), as shown in (Figure 1).

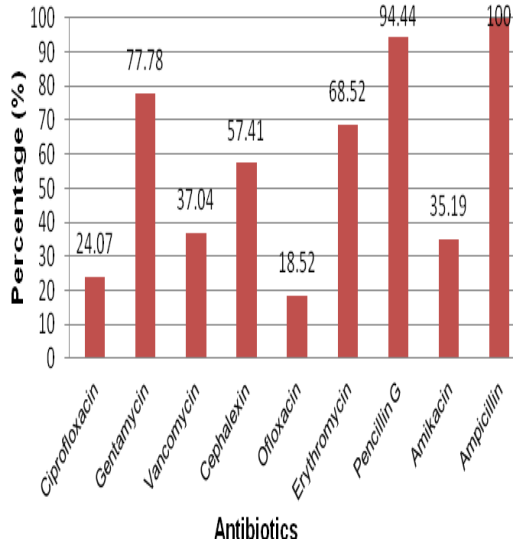

Figure 1. Percentage of Antibiotic-resistance of *S. aureus* isolates from different sites.

3.3. Bacteriocin Typing of S. Aureus

Among the 54 *S. aureus* isolates, four bacterial isolates ($staph_{19}$, $staph_{25}$, $staph_{28}$ and $staph_{43}$) 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. These isolates were used as indicator local in bacteriocin typing, since they have characters compatible with the bacterial strains producing staphylococcin to follow the epidemic and the spread of *S. aureus* isolates in Ibb city, Yemen.

Four staphylococcal producer strains, previously selected and used as indicator in bacteriocin typing, were tested against fifty four isolates of *S. aureus.* Most of these isolates were susceptible to the staphylococcin of the producer isolates, the results showed that 51 isolates with a ratio (94.44%) were sensitive dissimilar to staphylococcin (Table 3), (Figure 2).

Stapylococcin of $staph_{19}$, $staph_{25}$, $staph_{28}$ and $staph_{43}$ were inhibited 46, 46, 41 and 45 with ratios of 85.19%, 79.63%, 75.93% and 83.33% of the tested isolates, respectively (Table 3). Depending on the sensitivity to the staphylococcin used, the isolates of *S. aureus* were classified into nine groups. The most numerous group was characterized by the susceptibility to all four staphylococcin and comprised 61.11% isolates of *S. aureus*, while the lowest numerous were found in three groups with ratio 1.85% ; the remaining groups had little percentages ranging from 3.70% to 11.11%.

Table 3. Susceptibility patterns of bacteriocin typing of *Staphylococcus aureus*

Typing	Producer isolates				Total	No. of groups
	$Staph_{19}$	$Staph_{25}$	$Staph_{28}$	$Staph_{43}$		
results	+	+	+	+	33	1
	+	+	+	-	4	2
	+	+	-	+	6	3
	+	-	-	+	1	4
	+	-	-	-	2	5
	-	+	+	+	3	6
	-	-	+	+	1	7
	-	-	-	+	1	8
	-	-	-	-	3	9
Total	46	46	41	45	54	9

+ = inhibition of growth - = without effect

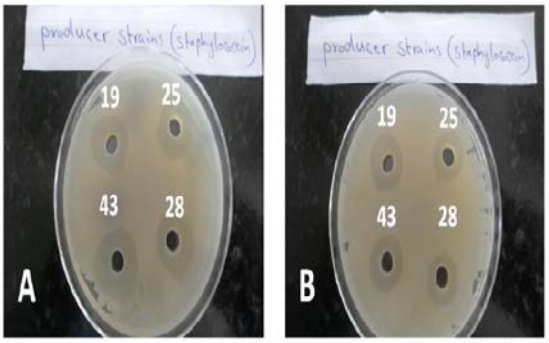

Figure 2. Effect of staphylococcin producer isolates (staph19, staph25, staph28 and staph43) on A) staph16 B) staph38

4. Discussion

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 (Snyder *et al.*, 2000). In this study, all isolates of *S. aureus* were resistant to ampicillin while most of these isolates were resistant to penicillin-G, which agrees with the study by Syed *et al.* (2011). The indiscriminate use of antibiotics may be a cause of this multidrug resistance. (Aucken *et al.*, 2002). High resistance of these isolates against gentamycin, erythromycin and cephalexin approximately agrees with other previous studies (Ounungа and Awhowho, 2012; Shazia and Jyothsna, 2011). This resistance against a particular antibiotic may be due to its frequent and long-term use (Sabour *et al.*, 2004). Among the nine antibiotics used in the present study, ofloxacin and ciprofloxacin 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 (Aucken *et al.*, 2002).

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 (Ahmad *et al.*, 2003; Desriac *et al.*, 2010).

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 producers 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 (Nakamura *et al.*, 1983), 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 (Riley and Wertz, 2002). 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 (Guven, 2000).

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