Hadhramout University Journal of Natural & Applied Sciences

Volume 19 | Issue 1 Article 2

2022

Identification and Antibiotic-Susceptibility Profiling of Non-Hospital Wound Infecting Staphylococcus aureus Isolated from Ghail-Bawazeer Patients, Yemen

Maged Salem Bin-Masalam
Hadhramout University, dr_maged@hu.edu.ye

A. Ba-Swaqi
Department of Medical Laboratories, Athar Institute of Medical Sciences, Ghail Bawazeer, Yemen.

A. Hwydi
Department of Medical Laboratories, Athar Institute of Medical Sciences, Ghail Bawazeer, Yemen

A. Mhedy
Department of Medical Laboratories, Athar Institute of Medical Sciences, Ghail Bawazeer, Yemen

E. Alamudi
Department of Medical Laboratories, Athar Institute of Medical Sciences, Ghail Bawazeer, Yemen

Identification and Antibiotic-Susceptibility Profiling of Non-Hospital Wound Infecting Staphylococcus aureus Isolated from Ghail-Bawazeer Patients, Yemen

Authors

Maged Salem Bin-Masalam, A. Ba-Swaqi, A. Hwydi, A. Mhedy, E. Alamudi, H. Bin-Nashe, M. Abozid, S. Aljomhy, and S. Badaood

Contents lists available at https://digitalcommons.aaru.edu.jo/huj_nas/.

Hadhramout University Journal of Natural & Applied Science

Article

Digital Object Identifier:
Received 14 November 2021,
Accepted 9 May 2022,
Available online 13 September 2022

Identification and Antibiotic-Susceptibility Profiling of Non-Hospital Wound Infecting *Staphylococcus aureus* Isolated from Ghail-Bawazeer Patients, Yemen

Maged S. Bin-Masalam[1*], A. Ba-Swaqi[2], A. Hwydi[2], A. Mhedy[2], E. Alamudi[2], H. Bin-Nashe[2], M. Abozid[2], S. Aljomhy[2], S. Badaood[2]

[1]Department of Food Science and Technology, Faculty of Environmental Science and Marine Biology, Hadhramout University, Yemen.
[2]Department of Medical Laboratories, Athar Institute of Medical Sciences, Ghail Bawazeer, Yemen.

*Corresponding author: dr_maged@hu.edu.ye

Abstract: The continuous increase in the resistance of pathogenic *Staphylococcus aureus* isolated from non-hospital infected wounds to the novel chemical classes of antibiotics is of great public health importance. This problem prompted the researchers to study the antibiotic susceptibility of such strains. Twenty wound pus samples were collected and selected randomly from 20 non-hospital wound infected patients of different ages who attended different medical laboratories in Ghail-Bawazeer, Yemen, from October to December 2020, and 24 bacterial isolates were analyzed using morphological, cultural, and biochemical characterization for the isolation and identification of *S. aureus*. Based on the morphological and cultural characteristics, only 17 isolates appeared in the form of golden yellow colonies, non-motile, gram-positive clustered cocci, from which 11 isolates were distinguished biochemically as *S. aureus*. The antibiotic susceptibility of the identified *S. aureus* to ten antibiotics of different classes was determined using the modified Kirby-Bauer disk diffusion technique. The major risk was the emergence of methicillin-resistant *S. aureus* isolates (MRSA) (n = 11, 100%), and (n = 9, 81.8%) of isolates were multidrug-resistant. There was a feeling of reassurance represented by the presence of other antibiotics that inhibited the growth of all isolates, starting with Tetracycline, Gentamicin, and Levofloxacin and ending with Novobiocin and Norfloxacin. The inhibitory effects of the tested antibiotics against each expected *S. aureus* were significantly different at the 0.05 level, except Novobiocin, Gentamycin, Methicillin, and Metronidazole. There were no significant differences. Continuity of work to identify the remaining wound strains associated with patients residing in Ghail-Bawazeer and their antibiotic sensitivity is required.

Key words: Antibiotic sensitivity; Multi-drug; *S. aureus*; Wound; biochemical.

1. Introduction

Staphylococcus aureus is a major opportunistic pathogen causing sever morbidity and mortality [1]. *S. aureus* is a common bacterial pathogen causing both hospital and community-acquired infections, and it is considered as commensal human normal flora. *S. aureus* can infect the body through different environments with exposure to the health care and without exposure. Soil is an important reservoir of pathogen and plays a significant role in the infection and invasion of pathogen [2, 3]. Alexander Ogston was the first surgeon, from Scotland who identified Staphylococcus from pus of a knee joint abscess in 1880 [4-6]. *S. aureus* are frequently present in the skin, nose and respiratory tract, and is classified as a Gram-positive, aerobic or microaerophilic bacterium. *S. aureus* may infect the soft tissues, wounds, skin, bones, lungs and blood stream leading to nosocomial infections, bacteremia and pneumonia [7, 8, 9].

The term conventional antibiotics refers to the natural substances produced by microorganisms and act against the growth of other microorganisms [10]. The antibiotics have been modified chemically to improve potency and to avoid their resistance mechanisms by increasing of

pharmacokinetics and their solubility [11]. *S. aureus* frequently acquires resistance to several antibiotic classes [7, 12]. Emergence of antibiotic resistance may be acquired due to horizontal transfer of mobile genes leading to mutation of the targets and alteration of their drug binding sites. The envelope, nucleic acids, ribosome of staphylococci are the main targets for modified antibiotics in addition to the ClpP protease and FtsZ from their division machinery. [11, 13, 14].

S. aureus is a pathogen causing infectious diseases and the increasing of its drug-resistance causes high risk to human health [15]. The emergence of antibiotic resistance is caused by the continuous and incorrect use of antibiotics [16, 17]. Leading to huge treatment problem for the bacterial infectious diseases [18]. Superficial skin infections caused by Methicillin-resistant *S. aureus* (MRSA) can be complicated, causing health-threatening conditions such as pneumonia, endocarditis, bacteremia or toxic shock syndrome [19]. The first known MRSA was identified in the UK in 1961, then its knowledge spread throughout the world [20]. Recently, the occurrence of MRSA has been high due to the continuous use of new generations of cephalosporins [21].

Characterization of bacteria and their antibiotic resistance are two keys for alleviation of the most challenging threat in public health [22]. Therefore, the study has a great importance to determine the classes of antibiotics, which the isolated *S. aureus* resist. The emerged study was aimed to assess the antibiotic susceptibility of *S. aureus* strains isolated from non-hospital infected wounds of patients living in district of Ghail Bawazeer, Hadhramout, Yemen due to several reasons. The most important reasons are the exposure of patients, especially those with wounds, to airborne soil and dust, and the poor sanitary conditions in most medical facilities, and the emergence of drug resistant *S. aureus*. Ten antibiotics were tested in this study; these antibiotics represent different novel chemical classes introduced in the past 30 years [23].

2.Materials and methods

2.1.Media used

Media used in this study were made in India by Himedia company, including Peptone Water (PW), Blood Agar (BA), Mannitol Salt Agar (MSA), Glucose Phosphate Broth, Simmon's Citrate Agar, Muller Hinton Agar (MHA) and Sulfur, Indole, Motility (SIM) Agar.

2.2. Area of Study

The area of study was located in Ghail-Bawazeer district, Hadhramout, Yemen. A total of 20 samples was selected randomly from 20 non-hospital wound infecting patients attending the medical laboratories in Ghail-Bawazeer for routine clinical and laboratory monitoring in a period from October to December 2020.

2.3.Sample collection

Twenty wound swab specimens were collected randomly from infected patients of age ranged between 9-50 years from different body parts (knee, foot, hand, arm, finger, face and ass) in the scheduled period from October to December 2020 (Table 1). All available patients were males, because the nature of their work in Ghail_Bawazeer exposes them to this type of wounds. Therefore, female samples were not available at the time of collection. The samples were taken randomly from different places in the patient's body, more samples were taken from the foot and from the arm, while only one sample was taken from knee and one from ass, this indicates that the foot and arm organs are more susceptible to injury. The specimens were transported to the bacteriology laboratory of the Athar Institute for Medical Sciences within one hour under aseptic conditions, depending on the international guidelines for Word Health Organization regarding handling of specimens from human subjects [24].

Table 1. General information about the collection of pus specimens from wounds of infected patients

No.	Age of patients years	date	Site of woun
1	20	11/10	Knee
2	26	11/10	Ass
3	22	11/10	Finger
4	35	11/10	Face
5	17	28/11	Hand
6	26	28/11	Foot
7	25	28/11	Hand
8	40	28/11	Arm
9	12	30/11	Foot finger
10	25	30/11	Arm
11	34	2/12	Foot
12	33	2/12	Arm
13	21	2/12	Foot
14	50	2/12	Arm
15	12	2/12	Finger
16	23	13/12	Face
17	30	13/12	Finger
18	9	13/12	Hand
19	26	13/12	Finger
20	33	13/12	Foot

2.4. Methods

2.4.1. Isolation and identification *of S. aureus*

The wound swab specimens were inoculated in PW at 37°C for 18-24 h under aseptic condition and the bacteria was primarily isolated by incubation of cultured MSA and BA plates at 37°C for 18-24 h [25, 26].

2.4.1.1. Morphological characterization of colonies

For isolation, purification and identification of *S. aureus*, the culture plates having significant growth (>10 CFU/ml) were subcultured onto fresh MSA plates. The growing colonies were identified depending on the morphology, microscopy and biochemical tests of isolated strains [4, 27, 28].

2.4.1.2. Microscopic examination

The isolated bacteria were examined microscopically after Gram staining (Himedia, India) to detect their Gram reactions, cell morphology (shape and arrangement of cells) using a light microscope.

2.4.1.3. Motility test

An overnight colony (24 h) was picked up using a straight needle and stabbed once in the center of the SIM Agar tube, then incubated at 37°C; the result was examined daily for up to 7 days [29].

2.4.1.4. Biochemical characterization

2.4.1.4.1. Mannitol fermentation and blood haemolysis

The non-motile, Gram positive cocci (17 isolates) were selected and inoculated on MSA and BA plates separately, then incubated to examine their ability to ferment minnitol and to haemolize blood, respectively, at 37°C for 24 h.

2.4.1.4.2. Catalase test

The ability of the isolated strains to produce catalase and to decompose hydrogen peroxide into water and free oxygen, slide (drop) method was employed. A sterile loop was used to collect a small amount of overnight growth (only non-motile, Gram positive cocci were studied), and placed onto the microscope slide. One drop of 3% H_2O_2 was dropped onto the organism. Occurrence of immediate bubble formation (effervescence) indicates the positive result and presumptive presence of *S. aureus*. Tube method was used also by the addition of 5 drops of 3% H_2O_2 to the test tube, using a dropper; small amount of organism was taken from the overnight growing colony and placed into the test tube. Immediate effervescence (bubble formation) indicates the positive reaction [30].

2.4.1.4.3. Coagulase or Thrombin clotting time (TCT) test

Coagulase test was used to identify *S. aureus*, which secrete free coagulase. In plasma, coagulase reacting factor reacts with the secreted coagulase enzyme forming thrombin complex, which converts fibrinogen to fibrin causing plasma clotting. One loop of fresh colonies (only the non-motile, Gram positive cocci) was mixed with 1 ml plasma and incubated at 37°C for 4, 6 and 24 h followed by horizontal shaking. The clotting of plasma indicates the positive coagulase test [30].

2.4.1.4.4. Indole test

The suspected bacterial isolate was inoculated overnight at 37°C in SIM broth containing amino acid tryptophan. After incubation, 15 drops of Kovac's reagent were added to the culture broth. Kovac's reagent is composed from para-dimethyl aminobenzaldehyde, isoamyl alcohol and conc. HCl. Occurrence of red or pink coloured ring at the top of the solution indicates the positive result [31].

2.4.1.4.5. Methyl-Red (MR) Test

The suspected bacterial isolate was inoculated at 37°C for 48 h in Glucose Phosphate Broth containing phosphate buffer and glucose. After two days, the organism may produce sufficient amount of acid to overcome the phosphate buffer. The pH of the medium was tested by addition of five drops of MR reagent. The positive result is indicated by the appearance of red color [32].

2.4.1.4.6. Voges-Proskauer (VP) Test

Glucose Phosphate Broth was used for inoculation of suspected bacterial isolate and incubated at 37°C for at least 48 h. The culture broth was mixed with 0.6 ml of alpha-naphthol and shaken, followed by the addition of 0.2 ml of 40% KOH and shaken. After 15 minutes, occurrence of red color means a positive result, and the negative tubes must be held for one hour [32].

2.4.1.4.7. Citrate Utilization Test

The suspected colonies of isolated strains were picked up and inoculated separately onto Simmon's Citrate Agar and incubated at 37°Cfor 24 hr. The color of medium changes from green to blue due to utilization of citrate by *S. aureus* [33].

2.4.2. Antibiotics susceptibility test

The modified Kirby-Bauer disk diffusion technique was used to test the antimicrobial susceptibility of the isolated strains using Mueller-Hinton (MH) Agar plates depending on the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [4, 26, 34-36]. The antimicrobial susceptibility was tested using ten different types of antibiotic discs (Norfloxacin, 10 µg [fluoroquinolone], Erythromycin, 15 µg [macrolide], Levofloxacin, 5 µg [quinolone], Gentamicin, 10 µg, [aminoglycoside], Metronidazole, 5 µg [nitroimidazole], Vancomycin, 30 µg [glycopeptide], Azithromycin, 30 µg [macrolide], Tetracycline, 30 µg [tetracycline], Novobiocin, 30 µg [aminocoumarin] and Methicillin, 5 µg [β-lactam] [Himedia, India]). All inoculated plates were incubated at 37°C for 18-24 h; and Inhibition Zone Diameters (IZDs) were measured employing the standard breakpoints recommended by the CLSI [35]. The degrees of susceptibility of Staphylococci to the used antibiotics were recorded as susceptible (S), intermediate (I) or resistant (R) to a particular antibiotic comparing to the standard IZDs.

Statistical analysis

The resulted data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 23.0 (SPSS, Chicago, IL, USA) using Post Hoc tests (Tukey HSD and LSD) and homogeneity to analyze the multiple comparisons between the IZDs of the different used antibiotics and the susceptibility of the homogenous subsets of the isolates. The differences in the resulting data were considered significant if the p-value was less than 0.05.

3.Results

3.1. Isolation, colony characterization, microscopic examination and motility

Twenty four bacterial strains were isolated on MSA and BA plates from twenty samples of wound pus, and the colonies occurring throughout the medium with a distinct color and hazy diffuse growth were selected, picked up and purified separately on freshly prepared MSA plates and labeled by serial numbers ranging from 1 to 24 (Table 2). The application of Gram staining technique distinguished the 24 bacterial isolates into Gram positive and Gram negative bacteria (17 and 7 isolates, respectively). The growth of all Gram positive isolates had sharp margins in the stab line without spreading, representing that they were non-motile. The Gram negative isolates were excluded from the next work.

Table 2. Characteristic information about the bacteria isolated from wound pus samples

Sample No.	Number of colonies on a plate	Isolate number	Colonies color	Gram Reaction	Motility
1	>300	1	Golden yellow	Gram positive grape like aggregated cocci	-ve
		2	Golden yellow	Gram positive grape like aggregated cocci	-ve
2	>300	3	Creamy	Gram positive grape like aggregated cocci	-ve
		4	Golden yellow	Gram positive grape like aggregated cocci	-ve
3	2	5	Golden yellow	Gram positive grape like aggregated cocci	-ve
4	NG	-	-	-	-
5	> 300	6	Yellow	Gram negative bacilli	ND
6	> 300	7	Golden yellow	Gram positive grape like aggregated cocci	-ve
		8	Golden yellow	Gram positive grape like aggregated cocci	-ve
7	NG	-	-	-	-
8	20	9	Golden yellow	Gram positive grape like aggregated cocci	-ve
		10	White	Gram positive grape like aggregated cocci	-ve
9	> 300	11	White	Gram positive grape like aggregated cocci	-ve
10	NG	-	-	-	-
11	> 300	12	Golden yellow	Gram positive grape like aggregated cocci	-ve
		13	Golden yellow	Gram positive grape like aggregated cocci	-ve
12	NG	-	-	-	-
13	> 300	14	Golden yellow	Gram positive grape like aggregated cocci	-ve
14	NG	-	-	-	-
15	5	15	White	Gram positive grape like aggregated cocci	-ve
16	95	16	Creamy	Gram positive grape like aggregated cocci	-ve
		17	White	Gram negative bacilli	ND
		18	Golden yellow	Gram positive grape like aggregated cocci	-ve
17	50	19	White	Gram positive grape like aggregated cocci	-ve
		20	White	Gram negative bacilli	ND
18	> 300	21	Yellow	Gram negative bacilli	ND
		22	Gray	Gram negative bacilli	ND
19	NG	-	-	-	-
20	100	23	White	Gram negative bacilli	ND
		24	Gray	Gram negative bacilli	ND

NG: No growth, ND: not done.

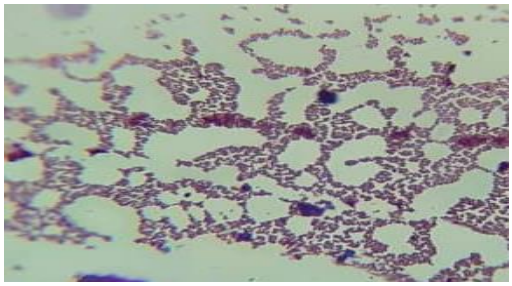

Figure 1. Microscopic field of Gram Reaction showing cluster Gram positive cocci bacteria.

3.2. Biochemical characterization

3.2.1. Mannitol fermentation and blood hemolysis activity:

The current study showed that all isolated bacteria were able to ferment mannitol sugar except the isolates no. 10, 11, 15 and 19 were unable to ferment mannitol (Tab. 3), (Fig. 2). The isolates differed in their ability to hemolyze blood, nine isolates (isolates no. 1, 2, 5, 7, 8, 12, 13, 14 and 18) exhibited β-hemolysis, while two isolates (no. 4 and 9) were partial hemolytic (α-hemolysis), while the rest (no. 3, 10, 11, 15, 16 and 19) were non-hemolytic (γ-hemolysis).

3.2.2. Catalase and coagulase activity

The catalase and coagulase tests were carried out using the slide and tube methods, it was found that the whole selected Gram positive cocci isolates were positive in their catalase activity, and only eleven isolates were positive in their coagulase activity, but isolates no. 3, 10, 11, 15, 16 and 19 were negative [24] (Tab. 3) (Fig. 3 and 4).

Table 3. Description of blood hemolysis and mannitol fermentation activities for non-motile, Gram-positive isolated cocci

Isolate number	Mannitol fermentation	Blood haemoly	Coagulase activity
1	+ve	B	+ve
2	+ve	B	+ve
3	+ve	Γ	- ve
4	+ve	α	+ ve
5	+ve	B	+ve
7	+ve	B	+ve
8	+ve	B	+ve
9	+ ve	α	+ve
10	- ve	Γ	- ve
11	- ve	Γ	- ve
12	+ve	B	+ve
13	+ve	B	+ve
14	+ve	B	+ve
15	- ve	Γ	- ve
16	+ ve	Γ	- ve
18	+ve	B	+ve
19	- ve	Γ	- ve

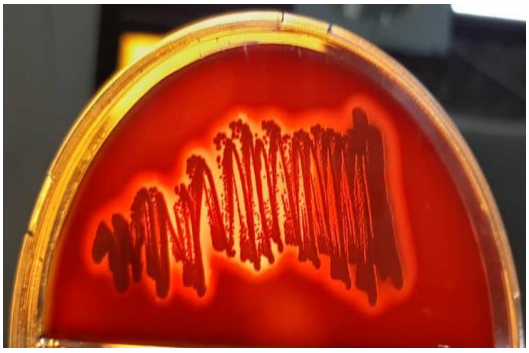

Figure 2. Description of complete hemolysis (β hemolysis) produced by the isolated bacteria no. 14 inoculating on blood agar medium

Figure 3. Description of a positive result of catalase activity test for the selected Gram positive coccal isolate no. 14 represented by immediate effervescence (bubble formation).

Figure 4. Description of the positive coagulase activity test for the selected Gram positive cocca isolate no. 14, represented by the plasma clotting appearance using the slide method.

2.3. Indole, Methyl-Red, Vogues-Proskauer and Citrate utilization activity

Eleven isolates of suspected *S. aureus* isolates were analyzed by IMVIC technique to characterize their properties to produce indole and Methyl-Red, Vogues-Proskauer and Citrate utilization. All isolates were positive for all tests and negative for indole test (Table 4), (Figure 5 and 6).

Table 4. Characterization of the suspected *S. aureus* isolates using IMVIC technique.

Isolate no.	Identification tests (IMVIC)				Urease
	Indole	Methyl-Red	Vogues-Proskauer	Citrate	
1	- ve	+ ve	+ ve	+ ve	+ ve
2	- ve	+ ve	+ ve	+ ve	+ ve
4	- ve	+ ve	+ ve	+ ve	+ ve weak
5	- ve	+ ve	+ ve	+ ve	+ ve weak
7	- ve	+ ve	+ ve	+ ve	+ ve
8	- ve	+ ve	+ ve	+ ve	+ ve
9	- ve	+ ve	+ ve	+ ve	+ ve weak
12	- ve	+ ve	+ ve	+ ve	+ ve
13	- ve	+ ve	+ ve	+ ve	+ ve
14	- ve	+ ve	+ ve	+ ve	+ ve weak
18	- ve	+ ve	+ ve	+ ve	+ ve weak

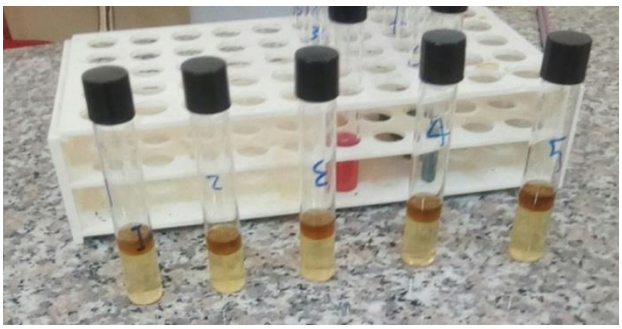

Figure 5. Description of negative indole production activity of some suspected S. aureus isolates.

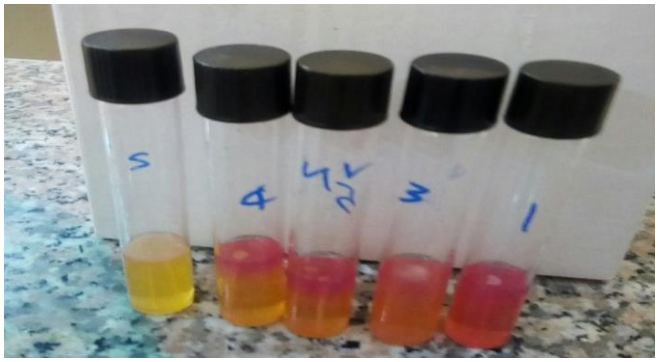

Figure 6. Description of urease activity of some suspected *S. aureus* isolates.

3.3. Antibiotics susceptibility

The antibiotic susceptibility of the identified *S. aureus* (11 isolate) was detected against ten different classes of antibiotics mentioned previously, using the disk diffusion method. The antibiotic susceptibilities of *S. aureus* strains were measured depending on the means of inhibition zone diameters IZDs, the IZDs were descriptive statistically analyzed at the 0.05 level. The values of IZDs varied from <10 to 39 mm IZDs, depending on the concentration and the type of the used antibiotics and on the type of the bacterial strains. Novobyocin inhibited the growth of *S. aureus* in the range from 21 to 30 mm IZD, while the Methicillin, Erythromycin and Metronidazole reduced the growth of *S. aureus* with values less than 10 mm IZDs, except species no. 8, 9, 13 and 14, they were inhibited by Erythromycin more than 10 mm IZDs. Tetracycline, Gentamicin, Levofloxacin, Vancomycin, Norfloxacin and Azithromycin inhibited *S. aureus* in the range of 23–39 mm, 19-35 mm, 27-32 mm, 15-30 mm, 13-29 mm and <10-34 mm IZDs, respectively (Table 5) (Figures 7 and 8). Statistical analysis was performed using Post Hoc tests (Tukey HSD and LSD) and homogeneity to analyze the multiple comparisons between the IZDs of the used antibiotics and the types of isolates.

Table 5. Characterization of the antibiotic susceptibility of *S. aureus* strains to ten different classes of antibiotics, depending on the Inhibition Zone Diameter (IZD) in mm

	Inhibition zone diameter (IZD) in mm									
No.	**NV 30 µg**	**MET 5 µg**	**TE 30 µg**	**GEN 10 µg**	**LE 5 µg**	**VA 30 µg**	**NX 10 µg**	**E 15 µg**	**AZM 30 µg**	**MT 5 µg**
1	S	R	S	S	S	I	S	R	R	R
2	S	R	S	S	S	S	S	R	R	R
4	S	R	S	S	S	I	S	R	R	R
5	S	R	S	S	S	S	S	R	I	R
7	S	R	S	S	S	S	S	R	R	R
8	I	R	S	S	S	R	S	S	S	R
9	S	R	S	S	S	S	S	I	R	R
12	S	R	S	S	S	S	S	R	R	R
13	S	R	S	S	S	S	I	I	S	R
14	S	R	S	S	S	S	S	I	S	R
18	S	R	S	S	S	I	S	R	R	R

NV: Novobiocin (30 µg); MET: Methicillin (5 µg); TE: Tetracycline (30 µg); GEN: Gentamicin (10 µg); LE: Levofloxacin (5 µg); VA: Vancomycin (30 µg).
NX: Norfloxacin (10 µg); E: Erythromycin (15 µg); AZM: Azithromycin (30 µg); MT: Metronidazole (5 µg); S: sensitive; R: resistant.

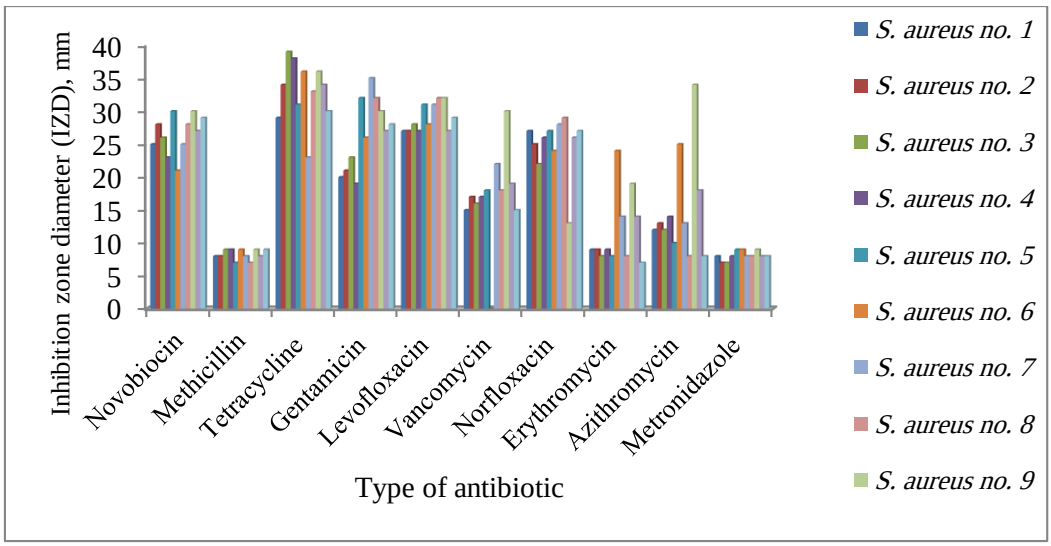

Figure 7. Histogramatic comparison between the antibiotic susceptibility of the isolated *S. aureus* strains to ten different classes of antibiotics, using the Inhibition Zone Diameter (IZD), mm.

Figure 8. Description of the inhibition zones induced by the used antibiotics to measure the sensitivity of some *S. aureus* isolates using the IZDs, mm

4. Discussion

The study of the antibiotic susceptibility of infectious bacteria helps in the selection of the suitable drug and the successful beneficial chemotherapy. Twenty four bacterial isolates were grown from twenty skin wound pus swabs, only 11 strains were identified as *Staphylococcus aureus* depending on the morphology of cells and colonies in addition to the Gram reaction (Golden yellow colonies on mannitol salt agar, non-motile, Gram positive grape like cocci) and on the biochemical characteristics (Mannitole fermenters, Blood hemolytics, Catalase, Coagulase and Urease positive, Indole producers, Methyl-Red and Voges-Proskauer test positive organisms). The current study revealed that the most common etiological agent of the local wound infections was *S. aureus* by a ratio of (n=11, 45.83%) of the total sample isolates.

The Inhibition Zone Diameter "IZD", mm was used as the dependent variable in the statistical analysis of tow way ANOVA in which the isolated strain and the antibiotic were used as independent variables. To calculate the multiple comparisons among the inhibition effects of antibiotics and among the susceptibility of S. aureus isolates and between the antibiotics and isolates, Post Hoc tests (Tukey HSD and LSD) were analyzed, and significant mean differences were detected among the inhibition effects of the ten used antibiotics on each expected *S. aureus* isolate at the 0.05 level, except the cases between the inhibition effects induced by Novobiocin and Gentamycin on all isolates, in addition to that of Methicillin and Metronidazole, there were no significant differences. Likewise, the inhibition effects of each used antibiotic were different significantly at significance level (α=0.0) among all suspected *S. aureus* isolates, but it becomes clear that these differences are lower than what's mentioned previously, because some isolates did not exhibit significant differences in the inhibition affected by the used antibiotics, such as the isolates 2, 4, 5 and 18, in addition to 7, 8, 9, 12 and 14. The homogeneity analysis divided the suspected isolated *S. aureus* into four subsets at $\alpha \leq 0.05$, the first subset includes isolate no. 1, the second subset: 2, 4, 5 and 18, the third subset: 7, 8, 9, 12 and 14, the fourth subset: 13.

The emergence of infecting multidrug-resistant and methicillin-resistant *S. aureus* (MRSA) limits the possibility of treatment and impedes the clinical follow-up [5]. So, the susceptibility of the identified *S. aureus* toward ten different classes of antibiotics was important to be studied. All staph isolates were resistant to Methacillin (5 µg) (MRSA), and among the total of 11 MRSA isolates, (n=9, 81.8%) were multidrug-resistant (MDR) relying on the definition of Magiorakos et al. [37] approved by CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST). One of the most important results was that all strains (100% total isolates) were resistant to Metronidazol (5 µg) and were very sensitive to Gentamicin (10 µg), Tetracycline (30 µg), Levofloxacin (5 µg), Novobiocin (30 µg), Norfloxacin (10 µg) and Vancomycin (30 µg), confirming what was stated in the study of Shi et al. [16] who reported that the minimum inhibitory concentration (MIC) of Gentamycin was ≤4.0 µg mL−1, and confirming what was recorded by Abie et al. [38] who found that the MRSA isolates were very sensitive to Tetracycline (n=14, 66.7%), Erythromycin (n=16, 76.2%), Gentamicin (n=16, 76.2%). Some results of this study did not agree with what previously mentioned, as the isolate no. 8 (n=1; 9.09%) showed complete resistance against Vancomycin, and (n=7; 63.64%) of *S. aureus* isolates were sensitive, and (n=3; 27.27%) were intermediate. In the study conducted by Shibabaw et al. [39], the MRSA isolates were resistant to common antibiotics, and 86.7% of the isolates were sensitive to Vancomycin, in agreement with the results of this study. In respect to Novobiocin and Norfloxacin, *S. aureus* isolates differed in their antibiotic susceptibility between sensitive (n=10; 90.9%) and intermediate (n=1; 9.1%), resistant strains were absent. In respect to Erythromycin (15 µg), (n=1; 9.09%) of strains were sensitive, (n=3; 27.27%) were intermediate and the highest percentage of isolates (n=7; 63.64%) was resistant to Erythromycin, contrary to what was stated in the study of Abie et al. [38]. Azithromycin (30 µg) inhibited the growth of *S. aureus* isolates at different levels, five isolates (n=7; 63.64%) were resistant, (n=3; 27.27%) were sensitive and (n=1; 9.09%) were intermediate.

The study of Swathirajan et al. [5] showed that the *S. aureus* strains of high susceptibility (63.1%) against Tetracycline (Tetracyclines class), followed by (53.4%) against Rifampicin (Ansamycins), (50.3%) against Nitrofurantoin (Nitrofurans), (48.7%) against Doxycycline (Tetracyclines), (45.5%) against Gentamycin (Aminoglycosides) and (44.7%) against Clindamycin (Lincosamides). Al-Quraishi [4] reported that the wound isolated Methecillin resistant *S. aureus* (MRSA) strains were completely resistant against Methecillin (5 µg) and completely susceptible to Azithromycin (AZM) (15 µg). Okonkwo et al. [26] recorded that the *S. aureus* isolates were resistant to Levofloxacin (59.0%) contrary to what was stated in this study, and sensitive to Gentamicin (77.2%) and Erythromycin (50.0%), in agreement with the current results. It is very clear that the *S. aureus* isolated in this study have acquired resistance against several classes of antibiotics, especially Methicillin (MRSA), followed by Metronidazole, Erythromycin and Azithromycin (Multidrug resistant *S. aureus*). The results of this study reflected a dangerous indication of health risk and gave a painful truth that the treatment of infection may be difficult in the future.

5. Conclusion

The current study relied on the characterization of morphological, cultural and biochemical properties for identification of the isolated *S. aureus* from 20 wound pus samples which were selected randomly and collected from 20 different aged patients suffering from non-hospital wound infections. The study found that only 11 of the studied 17 isolates were identified as *S. aureus*. The results indicated that there is a major health risk, represented in the fact that *all S. aureus* isolates (n=11, 100%) were Methicillin-resistant (MRSA), and (n=9, 81.8%) of isolates were multidrug-resistant, this makes the matter more seriously. The bright side of this study is the presence of some inhibitory antibiotics for all isolates, especially Tetracycline, Gentamicin and Levofloxacin followed by Novobiocin and Norfloxacin. The growth inhibiting effects of all tested antibiotics against the same *S. aureus* isolate were significantly different at the 0.05 level, except the effects of Novobiocin and Gentamycin

against the isolates. Regarding to Methicillin and Metronidazole, there were no significant differences. The current study highlighted the need to continue research and study to find out the most important antibiotics with inhibitory efficacy and to treat any inflection.

Acknowledgments

The authors would like to acknowledge Athar Institute for Medical Sciences (AIMS), Ghail Bawazeer, Hadhramout, Yemen, for their technical and financial support.

Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

References

[1] Bae, H. Jin, J. Kim, M. Park, J. Lee, and S. Kim, "Molecular Characteristics and Exotoxins of Methicillin-Resistant Staphylococcus aureus," *Biomedical Science Letters*, vol. 27, no. 4, pp. 195–207, Dec. 2021, doi: 10.15616/bsl.2021.27.4.195.

[2] Z. W. Jaradat, Q. O. Ababneh, S. T. Sha'aban, A. A. Alkofahi, D. Assaleh, and A. Al Shara, "Methicillin Resistant Staphylococcus aureus and public fomites: a review," *Pathogens and Global Health*, vol. 114, no. 8, pp. 426–450, Oct. 2020, doi: 10.1080/20477724.2020.1824112.

[3] V. S. Sonola, G. Misinzo, and M. I. Matee, "Occurrence of Multidrug-Resistant Staphylococcus aureus among Humans, Rodents, Chickens, and Household Soils in Karatu, Northern Tanzania," *International Journal of Environmental Research and Public Health*, vol. 18, no. 16, p. 8496, Aug. 2021, doi: 10.3390/ijerph18168496.

[4] G. M. Al-Quraishi, "The relationship between biofilm production and antibiotic sensitivity of (MRSA) *Staphylococcus aureus*" *Int. J. Pharm. Biol. Sci.* 8 (1) 220-226. 2018.

[5] R. S. Chinnambedu, R. R. Marimuthu, S. S. Sunil, P. Amrose, V. Ramachandran, and B. Pachamuthu, "Changing antibiotic resistance profile of Staphylococcus aureus isolated from HIV patients (2012–2017) in Southern India," *Journal of Infection and Public Health*, vol. 13, no. 1, pp. 75–79, Jan. 2020, doi: 10.1016/j.jiph.2019.06.015.

[6] Z. W. Jaradat, M. Khwaileh, W. Al Mousa, Q. O. Ababneh, and A. Al Nabulsi, "Occurrence, distribution and pattern analysis of methicillin resistant (MRSA) and methicillin sensitive (MSSA) Staphylococcus aureus on fomites in public facilities," *Pathogens and Global Health*, vol. 115, no. 6, pp. 377–391, Aug. 2021, doi: 10.1080/20477724.2021.1906563.

[7] G. Mancuso, A. Midiri, E. Gerace, and C. Biondo, "Bacterial Antibiotic Resistance: The Most Critical Pathogens," *Pathogens*, vol. 10, no. 10, p. 1310, Oct. 2021, doi: 10.3390/pathogens10101310.

[8] E. J. M. Raineri, D. Altulea, and J. M. van Dijl, "Staphylococcal trafficking and infection—from 'nose to gut' and back," *FEMS Microbiology Reviews*, vol. 46, no. 1, Jul. 2021, doi: 10.1093/femsre/fuab041.

[9] L. Abraham, D. M. Bamberger, "*Staphylococcus aureus* Bacteremia: Contemporary Management" *Missouri Medicine*, 117 (4) 341-345. 2020.

[10] C. Walsh, T. A. Wencewicz, "Antibiotics: Challenges, Mechanisms, Opportunities" ASM Press, Washington, DC, 2016.

[11] T. J. Foster, "Antibiotic resistance in Staphylococcus aureus. Current status and future prospects," *FEMS Microbiology Reviews*, vol. 41, no. 3, pp. 430–449, Apr. 2017, doi: 10.1093/femsre/fux007.

[12] A. Senthilkumar, S. Kumar, and J. N. Sheagren, "Increased Incidence of Staphylococcus aureus Bacteremia in Hospitalized Patients with Acquired Immunodeficiency Syndrome," *Clinical Infectious Diseases*, vol. 33, no. 8, pp. 1412–1416, Oct. 2001, doi: 10.1086/322656.

[13] S. O. Jensen and B. R. Lyon, "Genetics of antimicrobial resistance in Staphylococcus aureus," *Future Microbiology*, vol. 4, no. 5, pp. 565–582, Jun. 2009, doi: 10.2217/fmb.09.30.

[14] N. Silber, S. Pan, S. Schäkermann, C. Mayer, H. Brötz-Oesterhelt, and P. Sass, "Cell Division Protein FtsZ Is Unfolded for N-Terminal Degradation by Antibiotic-Activated ClpP," *mBio*, vol. 11, no. 3, Jun. 2020, doi: 10.1128/mbio.01006-20

[15] D. Raoult, N. Mouffok, I. Bitam, R. Piarroux, and M. Drancourt, "Plague: History and contemporary analysis," *Journal of Infection*, vol. 66, no. 1, pp. 18–26, Jan. 2013, doi: 10.1016/j.jinf.2012.09.010.

[16] Y. Shi, H. Yang, Y. He, and Z. Fu, "Antibiotic-affinity chromatographic test strip for quantitative analysis and antibiotic resistance testing of Staphylococcus aureus," *Talanta*, vol. 205, p. 120130, Dec. 2019, doi: 10.1016/j.talanta.2019.120130.

[17] R. M. Humphries *et al.*, "Carbapenem-Resistant Enterobacteriaceae Detection Practices in California: What Are We Missing?," *Clinical Infectious Diseases*, vol. 66, no. 7, pp. 1061–1067, Nov. 2017, doi: 10.1093/cid/cix942.

[18] R. Laxminarayan *et al.*, "Antibiotic resistance—the need for global solutions," *The Lancet Infectious Diseases*, vol. 13, no. 12, pp. 1057–1098, Dec. 2013, doi: 10.1016/s1473-3099(13)70318-9.

[19] N. A. Turner *et al.*, "Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research," *Nature Reviews Microbiology*, vol. 17, no. 4, pp. 203–218, Feb. 2019, doi: 10.1038/s41579-018-0147-4.

[20] Y. Rhee, A. Aroutcheva, B. Hota, R. A. Weinstein, and K. J. Popovich, "Evolving Epidemiology ofStaphylococcus aureusBacteremia," *Infection Control & Hospital Epidemiology*, vol. 36, no. 12, pp. 1417–1422, Sep. 2015, doi: 10.1017/ice.2015.213.

[21] C. Li *et al.*, "Two optimized antimicrobial peptides with therapeutic potential for clinical antibiotic-resistant Staphylococcus aureus," *European Journal of Medicinal Chemistry*, vol. 183, p. 111686, Dec. 2019, doi: 10.1016/j.ejmech.2019.111686.

[22] G. D. Kaprou, I. Bergšpica, E. A. Alexa, A. Alvarez-Ordóñez, and M. Prieto, "Rapid Methods for Antimicrobial Resistance Diagnostics," *Antibiotics*, vol. 10, no. 2, p. 209, Feb. 2021, doi: 10.3390/antibiotics10020209.

[23]L. L. Silver, "Challenges of Antibacterial Discovery," *Clinical Microbiology Reviews*, vol. 24, no. 1, pp. 71–109, Jan. 2011, doi: 10.1128/cmr.00030-10.

[24] Engbaek K, El Nageh M M and Groen J 1995 Specimen collection and transport for microbiological investigation, Word Health organization Regional Publications, Eastern Mediterranean Series No 8, WHO Regional Office for the

Eastern Mediterranean, Alexandria, WHO Library Cataloguing in Publication Data, 88-92.
[25] M. Cheesbrough, "District laboratory practice in tropical countries" part 2 Cambridge University press. New York 12 248-266. 2006.
[26]O. EC *et al.*, "Prevalence and Antibiotic Sensitivity Pattern of Staphylococcus aureus Isolates of Non-Hospital Origin," *Archives of Clinical Microbiology*, vol. 09, no. 01, 2018, doi: 10.4172/1989-8436.100076.
[27] J. G. Collee, A. G. Fransser, G. P. Marmion, Simmons A Mackie and MoCantrey Practical Medical Microbiology 14th ed. Churchill Livingstone, New York. 1996.
[28] J. P. Harley, C. M. Prescst, Laboratory Exercises microbiology 5th ed. The McGraw – Hill. 2002.
[29] P. M. Tille, Forbes B A Bailey and Scott's diagnostic microbiology 13th edition, St. Louis, Missouri: Elsevier. 2014.
[30]N. Pumipuntu *et al.*, "Screening method for Staphylococcus aureus identification in subclinical bovine mastitis from dairy farms," *Veterinary World*, vol. 10, no. 7, pp. 721–726, Jul. 2017, doi: 10.14202/vetworld.2017.721-726.
[31] M. P. MacWilliams, Indole Test Protocol American Society for Microbiology 1-9. 2009.
[32] McDevitt S Methyl Red and Voges-Proskauer Test Protocols American Society for Microbiology 1-9. 2009.
[33] M. P. MacWilliams, Citrate Test Protocol American Society for Microbiology, pp. 1-9. 2009.
[34] A. W. Bauer, W. M. Kirby, J. C. Sherris, M. Turck, "Antibiotic susceptibility testing by standarized single disk method" *Am. J. Clin. pathol*. 45 493-496. 1966.
[35] CLSI (Clinical Laboratory Standard Institute) Performance standards for antimicrobial disk susceptibility test, Fifteenth informational supplement CLSI document M100-S15, Wayne, USA 112. 2011.
[36] E. Chika *et al.*, "Multiple Antibiotic Resistance, Antibiogram and Phenotypic Detection of Metallo-Beta-Lactamase (MBL) from Escherichia coli of Poultry Origin," *Journal of Applied Microbiology and Biochemistry*, vol. 01, no. 04, 2017, doi: 10.21767/2576-1412.100015.
[37] A. P. Magiorakos *et al.*, "Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance," *Clinical Microbiology and Infection*, vol. 18, no. 3, pp. 268–281, Mar. 2012, doi: 10.1111/j.1469-0691.2011.03570.x.
[38] S. Abie, M. Tiruneh, and W. Abebe, "Methicillin-resistant Staphylococcus aureus nasal carriage among janitors working in hospital and non-hospital areas: a comparative cross-sectional study," *Annals of Clinical Microbiology and Antimicrobials*, vol. 19, no. 1, Oct. 2020, doi: 10.1186/s12941-020-00391-x.
[39] A. Shibabaw, T. Abebe, and A. Mihret, "Antimicrobial susceptibility pattern of nasal Staphylococcus aureus among Dessie Referral Hospital health care workers, Dessie, Northeast Ethiopia," International Journal of Infectious Diseases, vol. 25, pp. 22–25, Aug. 2014, doi: 10.1016/j.ijid.2014.03.1386.

توصيف ودراسة حساسية المضادات الحيوية لبكتيريا المكورات العنقودية الذهبية المعزولة من الجروح المصابة خارج المستشفى لمرضى من غيل باوزير، اليمن

ماجد سالم بن مسلم[1,*], عبدالله محمد باسواقي[2], عادل هويدي[2], عوض مهيدي[2], اسلام العمودي[2], هاجر بن ناشي[2], محمد ابو زيد[2], صالح الجمحي[2], سمية باداؤود[2]

الملخص: إن الزيادة المستمرة في مقاومة بكتيريا المكورات العنقودية الذهبية المعزولة من الجروح المصابة خارج المستشفى لفئات المضادات الحيوية الكيميائية الجديدة لها أهميتها الكبيرة على الصحة العامة. هذه المشكلة دفعت الباحثين إلى دراسة قابلية عزلات هذه البكتيريا لهذه المضادات الحيوية، حيث جمع الباحثون عشرين عينة من صديد الجروح بطريقة عشوائية من عشرين مريضاً يعاني من جروح مصابة خارج المستشفى من أعمار مختلفة يفحصون في مختبرات طبية في مدينة غيل باوزير، حضرموت، اليمن، في المدة من أكتوبر حتى ديسمبر 2020م. تم فحص 24 عزلة بكتيرية باستخدام الخصائص الزرعية والظاهرية والكيموحيوية لعزل وتوصيف بكتيريا المكورات العنقودية الذهبية. بالاعتماد على الخصائص الزرعية والظاهرية ظهرت 17 عزلة في شكل مستعمرات صفراء ذهبية، مكورات عنقودية غير متحركة موجبة الجرام، تميزت منها كيموحيوياً 11 عزلة من بكتيريا المكورات العنقودية الذهبية. باستخدام تقنية انتشار قرص كربي باور المعدلة، تم معرفة حساسية عزلات المكورات العنقودية الذهبية لعشرة أنواع من فئات مختلفة من المضادات الحيوية. وكان الخطر الرئيسي هو مقاومة جميع العزلات (11 عزلة، 100%) للمضاد الحيوي ميثيسيلين (MRSA)، وتميز 9 منها (81.8%) بمقاومتها للأدوية المتعددة .(MDR) ورغم ذلك إلا أنه كان هناك ما يدعو للطمانينة وهو وجود مضادات حيوية أخرى تثبط نمو جميع العزلات بدءاً من التتراسايكلين والجنتامايسين والليفوفلوكساسين وانتهاءً بالنوفوبايوسين والنورفلوكساسين. اختلفت التأثيرات التثبيطية للمضادات الحيوية المختبرة ضد كل المكورات العنقودية الذهبية اختلافاً معنوياً عند مستوى 0.05 ماعدا نوفوبايوسين وجنتامايسين وميثيسيلين وميترونيدازول لم تكن هناك فروق معنوية. من الضرورة بمكان استمرارية العمل البحثي للتعرف على سلالات أخرى معزولة من الجروح المصابة خارج المستشفيات للمرضى الساكنين في مديرية غيل باوزير ودراسة حساسيتها للمضادات الحيوية.

الكلمات المفتاحية: جرح، كيموحيوي، حساسية المضادات الحيوية، مقاوم للأدوية المتعددة، بكتيريا المكورة العنقودية الذهبية.