Investigation of some physiological, biochemica characteristics and multiple antibiotic resistance of *Staphylococcus aureus* isolated from wound samples

Hasanain Ahmed Enad Obaid

Department of Biology, Graduate School of Natural and Applied Sciences, Çankiri Karatekin University, Cankiri, Turkey

Correspondence Author: Hasanain Ahmed Enad Obaid

Received 21 Mar 2023; Accepted 3 May 2023; Published 11 May 2023

Abstract

In this study, carried out at Baghdad University, it was aimed to determine the optimum antibiotics that will affect *Staphylococcus aureus* infection and live contaminated microorganisms, which cause different disease problems in Iraqi patients who have been exposed to various burns and injuries. For this purpose, about 100 samples were collected from these patients, who were in different age ranges and had various injuries and burn wounds, and were transferred to different culture media. As a result of the growths occurring in the environment, 60% of *S. aureus*bacteria and 40% of other bacterial species were detected. Specimens contaminated with *S. aureus*bacteria showed high resistance to the antibiotic tobramycin (56.7%), and less resistance to Gentamaisin (30%) and Imikacin (10%). Bacterial isolators showed a negative effect on the endol reaction and a positive effect on the oxidis reaction in the filling culture medium. Next, two methods were applied to examine the ability of bacteria to form a thin biofilm. The first is the red congou medium method, which measures the ability of bacteria to form an adhesive layer. In the second method, micro-titer plate method (MTP) were used to measure the bacteria's ability to form the sticky layer. As a result, all insulators showed very low adhesive formation in the method using calibration cups.

Keywords: *Staphylococcus aureus*, antibiotic resistance, biochemical properties, burns and wounds

Introduction

Staphylococcus aureus is a bacterium that can be found on the skin and throat, as well as in the gastrointestinal and urinary systems. It can also be found in the perineum, genitals, and urethra (Den Heijer *et al*. 2013) [12]. It causes a wide variety of diseases in humans and animals (Luzzago *et al*. 2014) [25]. It causes very serious public health problems due to its zoonotic potential as well as its capacity to acquire and lose resistance and virulence genes (Holden *et al*. 2004, Saleha and Zunita 2010, Luzzago *et al*. 2014) [20, 36, 25]. From a clinical point of view, *S. aureus*is the most dangerous member of the Staphylococcus genus. This bacterium is an antibiotic-resistant species that can cause various diseases such as superficial skin abscesses, food poisoning, necrotic pneumonia in infants, and cardiac endocarditis (Shaw *et al*. 2004). It is also responsible for mastitis in cows, botryomycosis in horses, dermatitis in dogs, septicemia and arthritis in chickens (Zunita *et al*. 2008, Luzzago *et al*. 2014) [44, 25]. Methicillin resistant mecA gene, vanA gene highly resistant to vancomycin and teicoplanin, staphylococcal exotoxins and antibiotic resistance genes are the factors responsible for the severity of the disease by causing disease onset, weakening of immunity and host tissue damage. In the mid-1940s, a strain of *S. aureus*was shown to develop resistance to penicillin through the production of a hydrolytic enzyme called penicillinase (Basset *et al*. 2011) [8]. In the United Kingdom and the United States, penicillin-resistant *S. aureus*strains were more common in cases of bacteremia. Hospital-acquired penicillin-resistant bacteria are so named because they were first isolated only from patients and healthcare workers. At the same time, penicillin-resistant strains have been identified among community members without significant risk factors associated with nosocomial infections (Chuang and Huang 2013) [11]. Between the late 1940s and early 1960s, the antibiotic Methicillin, used to treat *S. aureus*infection, showed increasing resistance to penicillin (Jevons 1961) [19].

Methicillin-resistant *S. aureus*is the result of a genomic study containing the mecA resistance marker (MRSA) obtained. Since its discovery in the UK in the early 1960s, methicillin-resistant *S. aureus*has emerged as the leading source of infection in humans and animals worldwide. In the last few years, MRSA has evolved, most likely due to clonal expansion of already existing clones and conversion of MSSA to MRSA, and methicillin resistance genes encoding a penicillin-binding protein that is less sensitive to all classes of beta-lactam antibiotics have been discovered (Noto *et al*. 2008) [30]. Today, although antibiotics are no longer as effective as before, there has been a prolongation of hospitalization and length of stay in humans (Purrello *et al*. 2012) [29].

Methods

Sterilization

Sterilization using autoclave

The prepared media and solutions were sterilized at 121°C and 1 atmosphere pressure for 15 minutes (Tille 2015) [40]. Sterilization with dry hot air (pastor oven), Glassware was sterilized in a pasteur oven at 175°C for one hour (Tille 2014) [39], Sterilization using Millipore filters, The high temperature

solutions were sterilized with a 0.22 µm diameter Millipore Filter (Kumari *et al*. 2008).

Preparation of reagents and solutions

Catalase reagent

To investigate the catalase producing abilities of the reagent isolates, a 3% hydrogen peroxide (H2O2) concentration was prepared by taking 12 mL of hydrogen peroxide at 21% concentration and making the volume up to 177 mL with distilled water and storing it in an opaque reagent vial. (Tadesse and Alem 2006) [38].

Oxidase reagent

To demonstrate the ability of bacterial isolates to produce the enzyme oxidase, the Reagent was prepared immediately by dissolving it in 100 mL of distilled water sterilized with NNNN-tetramethylphenylenediamine dihydrochloride Millipore filter units and stored in a dark bottle refrigerated until use (Macfaddin 2000) [28].

Voges-proskauer reagent

It consists of two solutions:

- The first solution was prepared by dissolving 1g of a-nephthol in 100 mL of absolute ethyl alcohol.
- Second solution: 0.7 g of potassium hydroxide solution was prepared by dissolving 67 mL of distilled water and making its volume up to 100 ml. This test aims to demonstrate the ability of isolates to ferment glucose sugars and produce acetone (Cruickshank *et al*. 1975) [10].

Preparation of crystal violet dye solution

To examine the biofilm forming abilities of bacterial isolates, the solution was prepared by adding 7.1 g of crystalline violet powder to 17 mL of distilled water and sterilized in microfiltration units and then stored at 4 °C (Mathur *et al*. 2006, Babapour *et al*. 2016) [27, 7].

Preparation of culture medium

Ready media

All prepared media were prepared with Peptone water s and MR / VP water according to the manufacturer's instructions and those indicated on the packaging. MacConkey agar, Muller-Hinton agar, brain-heart infusion broth agar, brain-heart infusion agar, nutrient agar, Luria broth, Luria-Berton broth, tryptone soy broth, Simon citrate agar, Kligler iron agar. All were sterilized with an oxidizing agent at 121°C and 1.5 pressure for 11 minutes, then cooled, then placed in sterilized containers or tubes and stored in the refrigerator until use.

Preparation of synthetic media

a) Müller agar

To isolate and diagnose Staphylococcus aureus, Medium was prepared by dissolving 0.9 g of Cetrimide agar in 1000 mL of distilled water containing 17 mL of glycerol, then 11 minutes by osmosis at 121 °C and 1.1 pounds/inch 2 pressure. sterilized and then cooled. It was left to solidify at 45 °C and then poured into sterile containers after 11 amalgam was thoroughly mixed with nalidixic acid (Hashim 2013) [18].

b) Blood agar

To demonstrate the ability of the isolates to produce the hemolysis enzyme, blood agar medium was prepared by dissolving 36.1 g of agar in one liter of distilled water, mixing the medium until homogeneous, and then sterilizing the medium at 121°C and pressure for 11 minutes, according to the manufacturer's instructions. Then, 5-7% blood was added, shaken well, then poured into sterile petri dishes and left to solidify (Vandepitte *et al*. 2003) [42].

c) Urea agar

To examine the ability of bacteria to produce the urease enzyme, this medium was prepared by dissolving 2.0 g of urea agar base in 95 mL of distilled water, then sterilized by osmosis and cooled to 01 °C. 5 mL of urea solution was added to the sterilized medium with a 7,01 micro diameter microfilter at a concentration of 07%. The medium was then poured into sterile tubes, placed at an angle and kept at 0 °C (Forbes *et al*. 2007) [13].

d) Indole test environment

To demonstrate the ability of bacterial isolates to produce indole rings, the solution was prepared by dissolving 27 g peptone and 1 g sodium chloride in 617 mL distilled water, the volume of the solution was completed to 1 liter, sterilized by oxidation, separated into tubes and stored at 4 °C (Collee *et al*. 1996) [9].

e) Skim milk agar

To demonstrate the ability of bacterial isolates to produce the enzyme protease, 10 g of skim milk powder was dissolved in 100 mL of distilled water and sterilized in a clarifier for 5 minutes. Then, the solid culture medium was prepared by dissolving in 900 mL of distilled water and sterilized with clarifier for 15 minutes and allowed to cool to a temperature of 45°C. In addition, the filtered milk solution was added to the nutrient agar, mixed well and allowed to solidify by pouring into sterilized containers (Senior *et al*. 1988) [35].

f) Preparation of brain heart infusion water with 2% glucose

To demonstrate the biofilm forming ability of bacteria, 0.2 g of glucose sugar was dissolved in 100 mL of heart and brain infusion water. Afterwards, the prepared solution was sterilized with a clarifier and filtered (Babapour *et al*. 2016) [7].

g) Use of congo red agar to investigate biofilm formation

This medium, which was prepared to show the phenotypic formation of a thin film on solid surfaces, was obtained by mixing 37 g/liter heart and brain infusion water with 50 gr/liter glucose and 10 gr/liter agar followed by 0.8 gr/liter Congo red dye. Congo red dye powder was prepared as a concentrated aqueous solution and sterilized separately from other components of the medium. Next, the powder was added to the sterilized medium after cooling to a temperature of 45 °C,

mixing well and then pouring into sterilized containers (Freeman *et al*. 1989) [14].

h) Preparation of king-a agar medium for the preparation of piocyanin solid dyes

To demonstrate the ability of the blue-green pigments of Staphylococcus aureus to produce pyocyanin, 46 g of the medium was dissolved in one liter of distilled water and 10 mL of glycerol was added to the medium. The medium was then sterilized and allowed to cool. It was transferred to sterilized containers and stored at 4°C until use (Murray *et al*. 2007) [26].

Collection of samples

A total of 100 samples were collected from various health institutions and ages. The ages of the patients from whom the samples were taken vary between 20-60 years of age. Wound infection (48) and blood (2) samples were obtained from City Hospital, Baghdad Training Hospital, and Gazi Hariri Hospital in Baghdad. The remaining 4 skin samples and 46 burn infection samples were obtained from Iraq Burn Hospital and Training laboratories.

Discussion and conclusion

Isolation and Identification of Staphylococcus aureus

100 samples were collected from the City Hospital, Baghdad Training Hospital, Gazi Hariri Hospital, Burn Specialized Hospital and Training Laboratory in Baghdad.

Table 1: Species, numbers and percentages of *S. aureus*samples and isolates

Example Type	Total number of samples	Number of isolated Staphylococcus aureus	Percentage of isolates %*
Wound Infections	48	30	62.5
Burn Infections	46	26	56.52
Blood	2	2	100
Skin scraping	4	2	50
Total number	100	60	60

* Calculated percentage of total isolates

Because bacterial colonies appear pale in color due to their inability to ferment the lactose sugar, identification of bacteria was made by culturing MacConkey agar on solid media (Forbes *et al*. 2007) [13]. In order to determine the type of β-hemolysis, which is evidence of the ability of bacteria to produce the hemolysis enzyme, solid cetrimide was added to the blood agar medium and incubated (Selim *et al*. 2015) [33]. As a result, it was determined that the pigments that cause greenish discoloration around the colony under UV rays contain Pyocyanin and these pigments are evaluated as water-soluble (Sudhakar *et al*. 2015).

Microscopic examination (Al-Dahmoshi 2013) [5] was performed to detect the presence of non-spore forming gram-positive rods. Additionally, biochemical tests were performed as 60 out of 100 isolates showed positive results for both the oxidase test and the catalase test. Of these tests, the Indole tests, Methyl red test, and Voges Proskauer test showed negative results and the IMViC, which included the Citrate use test, showed positive results. In the urease test, the results were different from each other. The reaction for the iron purifier was found to be basic and the bacteria grew at a temperature of 42°C (Table 2). These results were similar to the results of the studies conducted by Tadess and Alem 2006 [38] and Todar 2011.

Table 2: Phenotypic and biochemical tests for *S. aureus*isolates

No	Test	Conclusion
1	Gram stain	-
2	Cell shape and grouping	Root
3	Growth on MacConkey agar	unfermented to lactose
4	Growth in the middle of blood agar	β- hemolysis
5	Growth on cetrimide agar	+
6	growth at 4°C	-
7	Growth at 42°C	+
8	oxidase enzyme	+
9	catalase enzyme	+
10	urease enzyme	-/+
11	Alandol	-
12	Methyl Red	-
13	Voges Proskauer	-
14	Citrate consumption	+
15	Iron Refiner	C/C(base/base)

+ Test positive, - Test negative, (+/-) heterogeneous, K: base reaction

Determination of MIC value of antibiotics using Vitek2 compact system

This system was implemented according to the procedure in Pincus 2006. According to the procedure, thirteen antibiotics including amikacin, cefepime, ceftazidime, ciprofloxacin, piperacillin, meropenem, imipenem, gentamicin, colistin, ticarcillin/clavulin/clavulanic acid were added to the Vitek2 compact system for labeling in AST analysis. These antibiotics were used to determine the minimum inhibitory concentration values (Table. 3). The results showed that of the minimum inhibitory concentration of anti-amikacin, 3 were 10% resistant, 1 was 3.3% intermediate resistant, and 26 was 87.6% susceptible isolates. As for the antagonist cefapime, the results show that 4 isolates (13.3%) were resistant, 8 (26.7%) moderately resistant and 18 (60%) susceptible. As for the antagonist ceftazidime, 9 isolates (30%) were resistant, 8 (26.7%) moderately resistant and 13 (43.3%) susceptible**.**

Anti-ciprofloxacin results showed that 8 isolates (26.7%) were resistant, 6 isolates (20%) were moderately resistant, and 16 (53.3%) were susceptible. When the anti-colistin results were analyzed, it was observed that 16 isolates (53.3) were resistant and 14 (46.7%) were susceptible to moderately resistant isolates.

As for anti-gentamicin, 9 isolates (30%) are resistant, 3 isolates (10%) are moderately resistant, and 18 isolates (60%) are susceptible. In the imipenem results, 4 (13.3%) isolates were resistant and 26 (86.7%) were susceptible, while no moderately resistant isolates were found. For anti-meropenem, 4 isolates (13.3%) resistant, 26 (86.7%) susceptible and moderately resistant isolates were not found.

In anti-piperacillin, 15 (50%) resistance and 15 (50%) susceptibility were found, while intermediate resistant isolates were not found. As for the piperacillin/tazobactam antagonist, all isolates are susceptible (100%) to this antagonist. Results of the antagonist ticarcillin showed 50% resistance in 15 isolates and 50% susceptibility in 15 isolates, while intermediate resistant samples did not emerge. Antagonist ticarcillin/clavulanic acid results showed that 13 (43.3%) resistant and 17 (56.7%) isolates were susceptible, while moderately resistant isolates did not occur. Finally, the results of the Tobramycin antagonist are ranked as most resistant in 47 (56.7%) isolates, moderately resistant in 1 isolate (3.3%) and susceptible in 12 (40%).

Table 3: Percentages of resistance and susceptibility of minimum inhibitory antibiotic concentration for *S. aureus*bacterial isolates

Antibiotic	Resistance			Medium resistance			Sensitive		
	Number	Percentage %	MIC µg	Number	Percentage %	MIC µg	Number	Percentage %	MIC µg
Tobramycin	47	56,7%	>=16	1	3,3%	8	12	40%	1= >
Piperacillin	15	80	=128	/	/	/	15	50	8
Piperacillin/Tazobactam	/	/	/	/	/	/	30	100	8
Meropenem	4	13,3	>=16	/	/	/	26	86,7	<=0,25
Imipenem	4	13,3	>=16	/	/	/	26	86,7	<=0,25
Gentamicin	9	12	>=16	3	10	8	18	60	>=1
Colistin	16	53,3	>=16	/	/	/	14	46,7	>=0.5
Ciprofloxacin	8	26,7	3	6	20	2	16	54	0,25
Ceftazidime	9	30	63	8	26	16	13	43	4
Cefapim	4	13	>=64	8	25	16	18	60	2
Amikacin	3	10	64	1	3	32	26	86	2
Ticarcillin	15	50	128	/	/	/	15	50	8
Ticarcillin/Clavulanic	13	43	128	/	/	/	17	56	32

In the study by Khan and Faiz (2016) [22], they found that the anti-ticarcillin resistance of *S. aureus*isolates in Saudi Arabia was 55%. The results of this study showed similar results with our study. According to Sekar *et al*. 2014 [32] Another study conducted in Iraq by revealed that clinical isolates of Staphylococcus aureus were 24.4% resistant to ciprofloxacin, cefapime, and tobramycin, and 27% to gentamicin and ceftazidime. Al-Asady *et al*. (2016) reported that 56.6% of Staphylococcus aureus isolates showed resistance to piperacillin. In another study, 20% of root isolates were found to be resistant to piperacillin (Karthi *et al*. 2012) [21]. Likewise, Al-Taee *et al*. (2017) [3] to determine MIC determination on 31 Staphylococcus aureus isolates, resistance to amikacin was 65%, resistance to gentamicin and piperacillin/tazobactam was 80%, resistance to meropenem was 75%, resistance to imipenem was 70%, and lastly, resistance to ciprofloxacin was 65%.

In a study by Helal and Khan (2015), on 136 bacterial isolates, anti-ticarcillin resistance was found to be 61%. Sharma and Dhar (2017), on the other hand, stated in their study that there was a 54% resistance against anti-ticarcillin/clavulanic acid and a 79% resistance rate against anti-tobramycin.

The resistance of the bacterial isolates obtained in our study to anti-colistin with a rate of 53.3% was determined by Mohanty *et al*. It reveals that there are results in line with the study carried out by 2019. When all these results are evaluated, it is understood from our study and previous studies that the reason for the resistance of *S. aureus*bacteria to antibiotics is due to the fact that they have many different mechanisms. These mechanisms are listed as the ability to change the permeability of the outer membrane, having efflux pumps, production of broad-spectrum beta-lactamase enzymes, biofilm formation, and having resistance plasmids carrying resistance genes to various antibiotics (Hong *et al*. 2016) [17].

When Table 4. is examined, it is seen that Staphylococcus aureus isolates (30 isolates) are divided into seven different types according to their antibiotic resistance. While type 1 was 6.6% resistant to eleven antibiotics, type 2 was resistant to nine antibiotics except anti-ciprofloxacin and colistin at the rate of 6.6%, type 3 was resistant to eight antibiotics (6%), type 4 was 3.3% to seven antibiotics, then the 5th type is susceptible to six antibiotics (6.6%), the 6th type is sensitive to five antibiotics with a rate of 26.6%, and finally to the 7th type of three antibiotics (10%).

Table 4: Antibiotic resistance values of *S. aureus*isolates

Number of Species	Number of antibiotics	Number of resistant isolates	Percentage (%)
Type	11	2	6,6
Type	9	2	6,6
Type	8	2	6,6
Type	7	1	3,3
Type	6	2	6,6
Type	5	8	26
Type	3	3	10

Determination of virulence factors

The ability of bacterial isolates to cause infection due to virulence factors plays an important role in triggering infections (LaSarre and Federle 2013) [24]. Some virulence

factors of *S. aureus*bacteria were investigated. In addition, the ability to produce pigment, the enzyme hemolysin, protease production, siderophore production, or the ability to form biofilms that are either directly or under the control of nucleotide-sensing genes may be included (Table. 5) (García-Contreras 2016).

Pigment production

In the study, it was seen that Pyocyanin dye 18/30 enlarged the *S. aureus*isolate by 60% using the drawing method on nitrite agar medium (Figure 1).

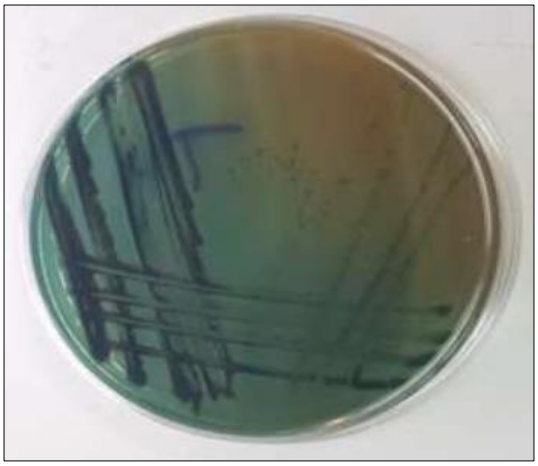

Fig 1: Production of pyocyanin dye for *S. aureus*bacteria on nitrite agar medium

Pigment Pyocyanin is an important virulence factor. This pigment is produced during biofilm formation as it plays an important role in acute and chronic bronchitis. It is also an antimicrobial compound as it acts as an oxidant and allows bacteria to survive under anaerobic conditions (Hotterbeekx *et al*. 2017) [16] Through intracellular signaling, Pyocyanin allows *S. aureus*to compete successfully against other bacteria and even fungi. (Tashiro *et al*. 2013, Stringfellow *et al*. 2009) [37, 31]. Exposure of the respiratory tract to the pigment pyocyanin damages the lungs and causes the development of cystic fibrosis disease. For these reasons, it has become necessary to find a substance that inhibits the production of Pyocyanin pigment.

Production of hemolysis

According to Khalil *et al* 2015. A study conducted bydetermined that all isolates have the ability to produce β-hemolysin enzyme on blood agar. 95.2% of isolates in Turkey produce this enzyme. In the study conducted by Al-Arnaouti 2015, it was found that the production rate of hemolysin was 100% in *S. aureus*isolates isolated from burns and wounds. Production of toxins such as hemolysin causes tissue damage that facilitates the spread of bacteria and the release of nutrients to the host. They also have the ability to alter host signaling, which can affect many processes, including host survival as well as inflammatory responses and cytoskeletal dynamics (Wiles *et al*. 2008) [43] (Figure 2).

Fig 2: Production of ß-hemolysis enzyme in blood medium.

Protease production

The results showed the ability of bacteria to produce protease enzymes in 83.3% of isolates on agar medium (25/30) with an average diameter of 12-20 mm. Al-Tikrity *et al*. 2015 and Al-Tikrity *et al*. A study conducted in Iraq by 2019 revealed that 88.3% and 86.1% of *S. aureus*bacteria from skimmed milk produce proteases, respectively. In our study, it was found that all colistin-resistant isolates produced this enzyme. (Table 5) Some studies have shown that expression of the protease enzyme is required for the ability to internally resist polymyxin antigens in some bacterial species (Velkov *et al*. 2013) [41], suggesting a relationship between the ability to produce proteases and resistance to polymyxin antigens.

Table 5: Production percentage of some virulence factors of *S. aureus*bacteria

Disease Inducing Factor	Total Number of Isolates	Number of Isolates Produced	Percentage (%)
Pigment Production	30	18	60
Production of Hemolysin	30	30	100
Protease Production	30	25	83,3
Biofilm Production	30	30	100

Biofilm forming ability

Detection of adhesion layer formation ability of staphylococcus aureus using the Congo red method

The data obtained as a result of the study showed that all *S. aureus*isolates in which the test was performed had the ability to form adhesion layers at a rate of 100%. The differences between these layers depending on the color are shown in Figure 3.

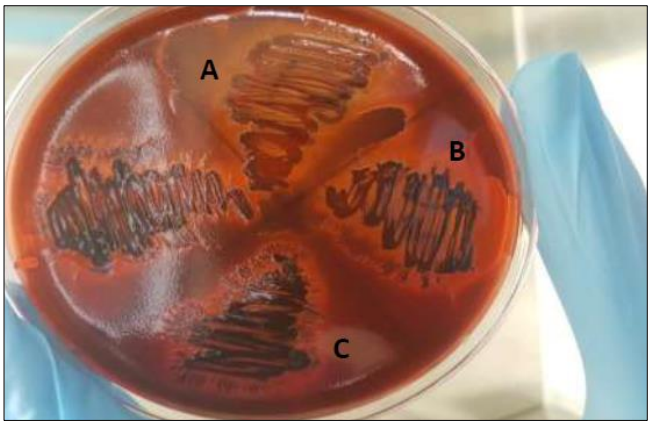

Fig 3: Adhesion layers of *S. aureus*bacteria in the middle of the red congo: A- Weak formation, B- Medium formation, C- Strong formation

The method in which Congo Red CRA medium is used is a descriptive test that reflects the secondary metabolic factors that produce exopolysaccharides, revealing the change in color and colonies of the phenotype depending on the increase in sucrose concentration in the medium. In the later stages of incubation, if black color decreases, roughness, dryness, and crystalline form increase in the production of thin adherent isolate layers, it is considered to belong to the strong forming category, but if the color is pale black, the isolates are of medium composition, with more red colored or pale pink colonies in the weak formation category (Hou *et al*. 2012) [15]. Production of the thin layers according to the Congo red method in this study showed that only three isolates, ie 15%, out of 20 isolates of *S. aureus*bacteria were able to do so. This ratio, Lima *et al*. It is higher than the study conducted in Brazil in 2017 (Table.6).

Table 6: Ability of *S. aureus*bacteria to form a biofilm using the Congo red method

Number of Samples	Strong		Middle		Weak	
	Number of Isolates	Percentage	Number of Isolates	Percentage	Number of Isolates	Percentage
11,41,17,11,13,2 4,41,18,60, 16,14, 11	16	53	/	/	/	/
4,34,25,39,19,35 9,58,59	/	/	12	42	/	/
24,34	/	/	/	/	2	6.6

Detection of susceptibility of Staphylococcus aureus to biofilm formation

Biofilm formation is one of the most common methods used by bacteria to survive in harsh environmental conditions. Bacteria can form biofilms on various abiotic surfaces in water systems as well as in natural aquatic environments (Bronowskil *et al*. 2014) [6]. The biofilm forming abilities of *S. aureus*isolates were investigated using the microtiter plate (MTP) method. While 12 isolates of 40% formed a moderate biofilm, 4 isolates of 13% formed a high biofilm. The results of our study showed that 53% of the bacterial isolates have a high biofilm forming ability. Comparing the results with other studies, Al-Taee *et al*. (2017) [3] and Al-Asady *et al*. (2020) [1] are in line with the results.

Table 7: Biofilm formation ability of *S. aureus*bacteria using microcalibration dishes

Strong Formation		Medium Formation		Weak Formation	
Number of isolates	Percentage (%)	Number of Isolates	Percentage (%)	Number of Isolates	Percentage (%)
4	13	12	40	14	47

The studies conducted serve a common purpose to determine the change of some physical and chemical properties of Staphylococcus aureus bacteria, which is frequently seen in public hospitals, according to multi-antibiotic resistance. When all these obtained data are evaluated and compared with previous studies, it has been determined that chemical reactions and resistance to antibiotics show consistent results. In line with this information, we will continue to examine Staphylococcus aureus bacteria, determine existing or existing resistances to different antibiotics, control bacterial species with these characteristics, and overcome infections caused by bacteria in other studies to be carried out in detail. We recommend taking it.

References

1. Al-Asady FM, Al-Saray DA, Obed AW. Incidence of septicemia. Etiology and antimicrobial susceptibility testing among patients admitted to tertiary care hospital. The Journal of Infection in Developing Countries. 2020;14(12):1387-1394.
2. Aklilu E, Zunita Z, Hassan L, Cheng CH. Molecular epidemiology of methicillin- resistant Staphylococcus aureus (MRSA) among veterinary students and personnel at a veterinary hospital in Malaysia.Veterinary Microbiology. 2013;164(3-4):352-358.
3. Al-Taee EH, Al-Naimi RA, Znad KH, Al-Tamimi AA. Study the Histopathological Changes and Bacteriological Causes of Natural Infection of the Livers in Sheep at Diyala Province. Diyala Journal for Pure Science, 2017, 13(4-part 1).
4. AL-Tikrity TAH, Al-Douri MTA, Abdul-Aziz MM, Al-Jebouri UMA. Diagnostic Values of Complete Blood Count in Patients with Urinary Tract Infection. Indian Journal of Public Health Research & Development, 2019, 10(10).
5. Al-Dahmoshi HOM. Genotypic and phenotypic investigation of alginate biofilm formation among Pseudomonas aeruginosa isolated from burn victims in Babylon, Iraq. Science Journal of Microbiology, 2013.
6. Bronowskil C, James CE, Winstanley C. Role of environmental survival in transmission of Campylobacter jejuni. FEMS Microbiol. Lett. 2014;356(1):8-19.
7. Babapour E, Haddadi A, Mirnejad R, Angaji SA, Amirmozafari N. Biofilm formation in clinical isolates of

nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. Asian Pac. J. Trop. Biomed. 2016;6(6):528-533.
8. Basset P, Feil EJ, Zanetti G, Blanc DS. 25-The Evolution and Dynamics of Methicillin-Resistant Staphylococcus aureus. In M. Tibayrenc (Ed.), Genetics and Evolutionof Infectious Disease, 2011, 669-688.
9. Collee JG, Fraser AG, Marmion BP, Simmons A. Practical medical microbiology. 14th ed. Longman. Singapore Publishers (pet) ltd. Singapore, 1996, 363-373.
10. Cruickshank R, Duguid JP, Marmion BP, Swain RHA. Medical Microbiology. 12th ed Churchill. Livingstone (publ.), London, 1975.
11. Chuang YY, Huang YC. Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Asia. The Lancet Infectious Diseases. 2013;13(8):698-708.
12. Den Heijer CDJ, Van Bijnen EME, Paget WJ, Pringle M, Goossens H, Bruggeman CA, *et al*. Prevalence and resistance of commensal Staphylococcus aureus, including meticillin-resistant S aureus, in nine European countries: a cross-sectionalstudy. The Lancet Infectious Diseases. 2013;13(5):409-415.
13. Forbes BA, Schaberg DR. Transfer of resistance plasmids from Staphylococcus epidermidis to Staphylococcus aureus: evidence for conjugative exchange of resistance. Journal of Bacteriology. 2007;153(2):627-634.
14. Freeman D, Falkiner F, Keane C. New method for detecting slime production by coagulase-negative staphylococci. J. Clin. Pathol. 1989;42:872-874.
15. Hou W, Sun X, Wang Z, Zhang Y. Biofilm-forming capacity of Staphylococcus epidermis's, Staphylococcus aureus, and Pseudomonas aeruginosa from ocular infections. Investigative Ophthalmol. Visual Sci. 2012;53(9):5624-5631.
16. Hotterbeekx A, Kumar-Singh S, Goossens H, Malhotra-Kumar S. *In vivo* and *In vitro* Interactions between *Pseudomonas aeruginosa* and *Staphylococcus* spp. J. Front. Cell. Infect. Microbiol. 2017;7:106.
17. Hong JS, Yoon E-J, Lee H, Jeong SH, Lee K. Clonal Dissemination of Pseudomonas aeruginosa sequence type 235 isolates carrying bla IMP-6 and emergence of bla GES-24 and blaIMP-10 on novel genomic islands PAGI-15 and -16 in South Korea. Antimicrob. Agents Chemother. 2016;60:7216-7223.
18. Hashim I. Microbiology culture media in pharmaceutical industry, 2013.
19. Jevons MP. "Celbenin"-resistant staphylococci. British Medical Journal. 1961;1(5219):124.
20. Holden MTG, Feil EJ, Lindsay JA, Peacock SJ, Day NPJ, Enright MC, *et al*. Complete genomes of two clinical Staphylococcus aureus strains: evidence for the rapid evolution of virulence and drug resistance. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(26):9786-9791.
21. Karthi R, Selvachandra M, Jamuna Devi P. Perspective of insurance agents' about LIC of India-A study at Nagapattinam District. In IEEE-International Conference on Advances in Engineering, Science And Management (ICAESM), 2012, 63-69.
22. Khan MA, Faiz A. Antimicrobial resistance patterns of Pseudomonas aeruginosa in tertiary care hospitals of Makah and Jeddah. Ann. Saudi Med. 2016;36(1):23-28.
23. Giridhar G, Kreger AS, Myrvik QN, Gristina AG. Inhibition of Staphylococcus adherence to biomaterials by extracellular slime of S. epidermidis RP12. Journal of biomedical materials research. 1994;28(11):1289-1294.
24. LaSarre B, Federle MJ. Exploiting quorum sensing to confuse bacterial pathogens. Microbiol. Mol. Biol. Rev. 2013;77:73-111.
25. Luzzago C, Locatelli C, Franco A, Scaccabarozzi L, Gualdi V, Vigano R, *et al*. Clonal diversity, virulence-associated genes and antimicrobial resistance profile of Staphylococcus aureus isolates from nasal cavities and soft tissue infections in wild ruminants in Italian Alps. Veterinary Microbiology. 2014;170(1):157-61.
26. Murray PR, Baron EJ, Sen JH, Landry PM. Manual of Clinical Microbiology. 9th ed (9th ed). American Society for Microbiology, 2007.
27. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian journal of medical microbiology. 2006;24(1):25-29.
28. MacFaddin FJ. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. The Williams and Wilkins Baltimore. U.S.A, 2000.
29. Purrello SM, Daum RS, Edwards GFS, Lina G, Lindsay J, Peters G, *et al*. Meticillin-resistant Staphylococcus aureus (MRSA) update: New insights into bacterial adaptation and therapeutic targets. Journal of Global Antimicrobial Resistance. 2012;2(2):61-69.
30. Noto MJ, Kreiswirth BN, Monk AB, Archer GL. Gene acquisition at the insertion site for SCCmec, the genomic island conferring methicillin resistance in Staphylococcusaureus. Journal of Bacteriology. 2008;190(4):1276-1283.
31. Stringfellow K, Anderson P, Caldwell D, Lee J, Byrd J, McReynolds J. Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions. Poultry science. 2009;88(6):1151-1155.
32. Sekar M, Jaffar FNA, Zahari NH, Mokhtar N, Zulkifli NA, Kamaruzaman RA, *et al*. Comparative evaluation of antimicrobial properties of red and yellow rambutan fruit peel extracts. Annual Research & Review in Biology, 2014, 3869-3874.
33. Selim S, Elkholy I, Hagagy N, ElAlfay S, Abedl Aziz M. Rapid identification of *Pseudomonas aeruginosa* by pulsed - field gel electrophoresis. Biotechnol. Biotechnol. Equipment. 2015;1(29):152-156.
34. Shulman JA, Nahmias AJ. Staphylococcal infections; clinical aspects. In: The Staphylococci. Edited by Cohen, J O, 1972, 457-481.
35. Senior BW, Albrechtsen M, Kerr M. A survey of IgA

protease production among clinical isolates of the proteases. J. Med. Microbiol. 1988;25:27-31.
36. Saleha A, Zunita Z. Methicillin resistant Staphylococcus aureus (MRSA): An emerging veterinary and zoonotic pathogen of public health concern and some studies in Malaysia. Journal Animal Veterinary Advances. 2010;9(7):1094-1098.
37. Tashiro Y, Yawata Y, Toyofuku M, Uchiyama H, Nomura N. Interspecies interaction between *Pseudomonas aeruginosa* and other microorganisms. J. Microbes Environ. 2013;28:13-24.
38. Tadesse A, Alem M. Medical bacteriology. University of Gondar, 2006, 61-63.
39. Tille P. Bailey and S cotts Diagnostic Microbiology –E-Book: Elsevier Health Sciences, 2014.
40. Tille P. Bailey and Scotts Diagnostic Microbiology–E-Book: Elsevier Health Sciences, 2015.
41. Velkov T, Roberts KD, Nation RL, Thompson PE, Li J. Pharmacology of polymyxins: new insights into an 'old' class of antibiotics. Future microbiology. 2013;8(6):711-724.
42. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical Bacteriology. 2nd.World Health Organization Geneva, 2003, 109-120.
43. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic Escherichia coli. Exper. Molec. Pathol. 2008;85(1):11-19.
44. Zunita Z, Bashir A, Hafizal A. Occurrence of Multidrug Resistant Staphylococcus aureus in horses in Malaysia. Veterinary World. 2008;1(6):165-167.