

Evaluation of the Infection Environment of *Staphylococcus aureus* in Patients and Workers in Kirkuk Governorate Hospitals

Nadya Esmaeel Mustafa Al-Joboury, Ph. D¹, Riedh Abass Abdul Jabbar, Ph. D², Mahmood K. Saleh, Ph. D³

¹ Department of Biology, College of Science, Tikrit University

² Professor, Department of Biology, College of Science, Tikrit University

³ Assistant Professor, Department of Biology, College of Education For pure Science, Tikrit University

ABSTRACT

This study included collecting 300 samples from various clinical sources (operating room, emergency rooms, surgical instruments, laboratory equipment, the hospital floor) and cases of patients of different ages and works, from Kirkuk Teaching Hospital and Children's Hospital, with 150 samples for each hospital in Kirkuk Governorate for the period from April 2022 to December 2022, where the presence of *Staphylococcus aureus* was verified. There were 40% positive isolates and 60% negative isolates. The bacteria were diagnosed by the characteristics of the colony, the microscopic characteristics of the bacteria, and the biochemical tests IMViC. It was found that the contamination in Kirkuk Teaching Hospital was more than the Children's Hospital and in females more than males. A susceptibility test was conducted using the disk-diffusion method and the percentages of bacteria sensitive to antibiotics were as follows: Cefuroxime 79% • Ceftriaxone 79% • Cephalothin 80% • Cefepime 80% • Ertapenem 91% • Imipenem 99% • Meropenem 99% • Amikacin 87% • and Nitrofurantoin 79%. While there are many antibiotics to which bacteria have shown high resistance, these antibiotics are: Amoxicillin 83% • piperacillin 97% • Fosfomycin 98% • Tobramycin 93% • Norfloxacin 83% • Tetracycline 83% • Aztreonam 90% • Azithromycin 98% • Erythromycin 73% • Novobiocin 96%, and Clindamycin 84%.

Keywords: *Staphylococcus* spp., IMViC

INTRODUCTION

Staphylococcus aureus: It is a gram-positive bacterium whose cells divide at more than one level and remain connected to each other in an irregular manner, resembling grape clusters [1]. The name *Staphylococcus* comes from the Greek word *Staphylococcus*, which means clusters of grapes, while the name *aureus* comes from the Greek word *Staphylococcus aureus* [2]. The bacteria are transmitted in several ways between patients by the hands of healthcare providers after they have been in contact with an infected patient, and this is a reason for the spread of bacteria in some healthcare hospitals for chronic conditions as well [3]. Those who carry antibiotic-resistant bacteria have a fundamental role in the spread of infection through the air, as transmission of infection through the air is not considered important except in certain cases, including patients in the burn units. As for transmission of infection through the inanimate environment, it is very important for some patients, including burn patients and intensive care unit patients, as there is a possibility of comprehensive pollution in the environment [4].

The period between infection with *Staphylococcus* bacteria and recovery requires increased health care, with the probability of increasing the risk of infection [5]. The environment is considered an external source of infection and includes water, air, food, surfaces surrounding the patient, and all accessories and medical devices. The patient's environment is contaminated with pathogens of infectious diseases in hospitals and is considered a reservoir for the transmission of pathogens, directly through the patient's contact with the environment or indirectly through contamination or infection of the hands of healthcare workers and their gloves. These surfaces are a source of the spread of *Staphylococcus aureus* bacteria [6].

Methods

Geographical Area

A number of 300 clinical samples were collected from various clinical sources (operating room, emergency rooms, surgical instruments, laboratory equipment, the hospital floor) and cases of patients of different ages and works from Kirkuk Teaching Hospital and Children's Hospital, 150 samples for each hospital, in Kirkuk Governorate for the period from April 2022 to December 2022.

Collection of Samples

Swabs were taken from the operating room, emergency rooms, surgical instruments, laboratory equipment, the hospital floor, and patients and works. Single-use sterile medical swabs from the Indian company "Hi Media" were used to examine for *Staphylococcus aureus*, to evaluate the above-mentioned hospital environments, which grows on 7.5% mannitol Salt agar medium for *Staphylococcus aureus* that inhibits all other bacterial species. A questionnaire was used to collect information, and swabs were taken under the supervision of doctors and specialized employees at the hospital, in accordance with the approval of the Iraqi Ministry of Health and the Kirkuk Health Directorate. After taking the swabs, they were brought to the bacteriology laboratory for the purpose of culturing them within an hour or less, and the *Staphylococcus aureus* were diagnosed. They were under investigation to study its impact on environmental pollution and transmission of infection between people. This bacterium was diagnosed based on morphological and cultural tests, and the diagnosis was confirmed using Vitek-2 Compact System.

Culture Characterizations and Morphology

A pure colony was taken from every growth on the culture plates of the primary cultures and were diagnosed initially based on morphological characteristics which include the size, color, edges, and height of the colony, then the bacterial cells characteristics were studied under the microscope after gram staining those including the shape and the grouping of those cells, after that these cells were diagnosed according to MacFaddin [7].

Biochemical Tests

Many of the biochemical tests were performed and these tests are IMViC which were performed according to Brown [8]. and several diagnostic tests for *S. aureus*, such as Coagulase, Catalase, and Oxidase.

Antibiotic Susceptibility Testing

Susceptibility testing was conducted using disk diffusion method for many types of antibiotics on the isolates under study using the modified Kirby-Bauer disk method and approved by [9]. Moreover, a

drug sensitivity test to antibiotics at different concentrations was conducted to find the minimum inhibitory concentration using the Vitec-2 Compact System. This is done by testing 26 antibiotics at different concentrations.

Results

Isolation and Identification

This study aimed to isolate *Staphylococcus* bacteria from different sources, as swabs were taken from operating and emergency rooms, lying-in rooms, laboratories, patients, and workers. The total number of samples reached 300 samples collected from Kirkuk Teaching Hospital and Children's Hospital in Kirkuk Governorate for the period from 1/4/2022 until 1/12/2022, for both genders and different ages.

The results showed that 120 positive isolates, 40% of the total number of swabs, and 180 negative isolates, 60% of the total number of swabs, as shown in Table (1).

Table 1: Number of Positive and Negative Samples Taken from Different Sources with their Percentages.

Implant Results	Number of Samples	Percentage
Positive Cases	120	40%
Negative Cases	180	60%
Total	300	100%

The isolated colonies on the blood agar appeared as gold colonies surrounded by a clear halo indicating beta hemolysis as it's shown in

Figure (1) and after culturing it on Manitol Salt Agar the red color of the agar will change to yellow as it's shown in Figure (2) [10].



Figure 1: Red Agar

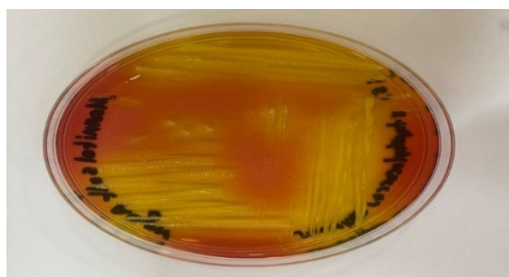


Figure 2: Yellow Agar

Table 2: The Biochemical Tests to Diagnose Bacterial Isolates

Test	Catalase	Oxidase	Coagulase	Urease	Indole	Methyl Red	Voges Proskauer	Citrate	Kligler
<i>S. aureus</i>	+	—	+	+	—	+	+	+	+

The current study showed that the percentage of people carrying the *S. aureus* bacteria among females in general, from medical staff and those hospitalized in Kirkuk Teaching Hospital and Children's Hospital was 33.3%, while its percentage among males was 12.5%. It indicates that the isolation rate in females is higher than its percentage

in males as shown in Table (3). The prevalence of *S. aureus* bacteria in general in the hospital environment of Kirkuk Governorate was studied, where the isolation rate was 41.6% from Kirkuk Teaching Hospital, which is much higher than the bacterial isolation rate 12.5% from the Children's Hospital, as summarized in Table (4).

Table 3: Number of Positive Samples According to Gender with their Percentages.

Implant results	Males	Percentage	Females	Percentage
Positive Cases	15	12.5%	40	33.3%
Total	120		120	

Table 4: Number of Positive Samples Collected from Kirkuk Hospital Environment with their Percentages.

Implant results	Kirkuk General Hospital		Children's Hospital	
	Number	Percentage	Number	Percentage
Positive Cases	50	41.6%	15	12.5%
Total	120		120	

The drug susceptibility test for these isolated bacteria was conducted using the disk diffusion method, where the drug susceptibility to antibiotics for 120 isolates was 100%, which showed high sensitivity to the antibiotics as described by Cefuroxime 79%, Ceftriaxone 79%, Cephalotin 80%, Cefepime 80%, Ertapenem 91%, Imipenem 99%, Meropenem 99%, Amikacin 87%, and Nitrofurantoin

79%, while there are many antibiotics to which bacteria have shown high resistance, and among these antibiotics are Amoxicillin 83%, Piperacillin 97%, Fosfomycin 98%, Tobramycin 93%, Norfloxacin 83%, Tetracycline 83%, Aztreonam 90%, Azithromycin 98%, Erythromycin 73%, Novobiocin 96%, and Clindamycin 84%. The details are shown in Table (5).

Table 5(a): Susceptibility and Resistance of *Staphylococcus aureus* isolates to Antibiotics.

<i>Staphylococcus Aureus</i> , N = 120 (100%)					
Antimicrobial	Resistance		Sensitive		
	N	%	N	%	
Amoxicillin / C. Acid	100	83	20	17	
piperacillin/Tazobactam	117	97	3	3	
Cefazolin	75	63	45	37	
Cefuroxime	25	21	95	79	
Cefuroxime Axetil	90	75	30	25	
Ceftazidime	60	50	60	50	
Cephalothin	24	20	96	80	
Ceftriaxone	25	21	95	79	
Cefoxilin	118	98	2	2	
Cefepime	24	20	96	80	
Ertapenem	11	9	109	91	
Imipenem	1	1	119	99	
Meropenem	1	1	119	99	
Amikacin	15	13	105	87	
Gentamicin	80	67	40	33	

Table 5(b): Susceptibility and Resistance of *Staphylococcus aureus* isolates to Antibiotics.

<i>Staphylococcus Aureus</i> , N = 120 (100%)					
Antimicrobial	Resistance		Sensitive		
	N	%	N	%	
Ciprofloxacin	75	63	45	37	
Fosfomycin	117	98	3	2	
Nitrofurantoin	25	21	95	79	
Tobramycin	112	93	8	7	
Norfloxacin	100	83	20	17	
Tetracycline	100	83	20	17	
Aztreonam	117	98	3	2	
Azithromycin	118	98	2	2	
Erythromycin	88	73	32	37	
Novobiocin	115	96	5	4	
Clindamycin	101	84	19	16	

Discussion

The laboratory culture results, as shown in Table (1), showed that 120 isolates from the total number of samples showed bacterial growth 40%, which is close to the results of [11], which was 34%, and on the other hand, much less than the results of [12] of which 79% were infected with *Staphylococcus aureus*. The reason may be due to the number of studied samples, the social level of the people, or the age of the people from whom the samples were collected. As for the samples that were collected and did not show bacterial growth, they amounted to 180 isolates, or 60%. This may be due to the presence of pathogens other than bacteria, such as viruses, parasites, or chlamydia, which cannot be detected in this study, or due to the efficiency of antibiotics used in treating diseases caused by bacteria and the ability of antibiotics to kill bacteria and disappear them [13]. In Table (2) the results of the biochemical tests performed on the *S.aureus* isolates show that all isolates were positive for the catalase test, where the

bacteria was able to breakdown hydrogen peroxide (H₂O₂) to water and oxygen [14], coagulase test were also positive, hemolytic clear zones around the colonies on a blood agar indicating a positive result for hemolysin test shown in picture (1) the isolates were all negative for cytochrome oxidase test [15]. Other biochemical tests were performed for diagnosis including methyl red, urea test, Indole test, voges-proskauer test, and citrate test [16]. In Table (3), it was shown that the isolation rate in females is higher than its rate in males, and these results are close to the results of Al-Juboori [11], while they were contrary to the results of Soe et al. and Shibabaw et al. [12, 17].

The reason for the noticeable increase in the percentage of *S.aureus* isolated bacteria compared to males may be attributed to the factors and circumstances surrounding them, the nature of the work they perform, their level of health awareness, the random use of antibiotics, and other physiological aspects that may be related to the childbirth [18]. Another reason for the high rates of *S.aureus* among females working in the health field is the exchange and use of personal

makeup and cosmetics among female staff in those institutions, as it may be considered a second source of bacterial transmission. The spread of bacteria has also been observed among young age groups ranging in their twenties. This is due to their large number among the staff, the large arrival of patients and hospitalized patients, lack of experience and absence of information on some matters regarding policies for controlling disease infections, and their loss of the required experience in dealing with and caring for patients [17].

Table (4) showed that the rate of contamination with *S. aureus* bacteria in Kirkuk Teaching Hospital is higher than the Children's Hospital, and this result is close to what was found by Al-Janabi in Baghdad and Al-Duri in Tikrit [19, 20], and higher than what was found [21] in Germany. The most important reason for the high rate of contamination with *S. aureus* bacteria in Kirkuk Teaching Hospital is the increase in the number of patients arriving and staying at the hospital and the increase in the number of healthcare workers, employees, visitors, and companions of patients. Other reasons are not following the correct methods of cleaning, and sterilization and the fact that many workers dilute sterilizers, which are not among the lethal concentrations of bacteria, and thus are considered a major cause of contamination of the hospital environment [22]. In addition, bacteria can survive from several weeks to several months on various surfaces, which increases the possibility of medical staff coming into contact with them and transmitting them in an unauthorized manner to inpatient patients and to the hospital environment [23]. The cause of hospital contamination may be due to poor ventilation, humidity, and the lack of sunlight entering the lobbies [24], in addition to the arrival of a large number of visitors and patients, entering the lobbies and other sections of the hospital, and transferring bacteria from the surfaces without following any method to prevent the transfer of bacteria to another place from the hospital [25, 26]. Bacteria can be transmitted between hospital staff, especially in the laboratory, because the laboratory staff works with samples that may be contaminated and transmitted to it easily. It is possible that there is another cause of pollution, which is eating meals inside the laboratories on a daily and routine basis that leads to a high percentage of pollution among the staff, in addition to the fact that the cleaning process that occurs in the laboratory is repeated once a day and at sometimes simple detergents are used that do not rise to the level of disinfection and sterilization required due to the lack of health awareness of the staff. As for the nursing staff, due to contact and direct contact with inpatients and visitors, and their long stay in the hospital lobby and their contact with surfaces around the patient, they are more susceptible to infection with bacteria [26] than doctors and pharmacists because they are more committed to the subject of sterilization and do not have a deal with patient lobbies or surrounding surfaces [17].

Table (5) shows that *Staphylococcus aureus* has the ability to resist many agents that fight bacterial growth, especially antibiotics. Susceptibility testing for these organisms is very important to evaluate their resistance to different types of antibiotics, in addition to medical applications related to the effect of antibiotics in treating bacterial diseases. Continuous exposure to antibiotics has a significant impact on the emergence of resistance among bacterial isolates [27]. As some have shown sensitivity to many antibiotics, the reason why all isolates appeared sensitive to the group of Carbapenems is that they are β -lactam antibiotics, as they have the ability to kill negative bacteria and anaerobic bacteria, so they are useful in treating a wide range of

moderate to severe infections [28] and that the high sensitivity to it is due to the limited use of these antibiotics, whose prices are relatively high and are often not available in hospitals. These results are close to the results of Al-Ani *et al.*, Al-Juboori [11, 29] and opposite to the results of Yehouenou *et al.* [30] as they showed resistance to the same antibiotics. As for Cephalosporin antibiotics, the result of this study is similar to a study conducted by Abdulla [31] and opposite of the results of Hamada; Al-Douri [18, 20]. It also does not agree with the findings of Al-Tamimi, [32], who appeared to have high resistance to these antibiotics. On the other hand, the findings of Salman, [33] showed a similarity to what we have reached in terms of the sensitivity of bacteria to aminoglycoside antibiotics.

Bacteria have shown high resistance against many available antibiotics. The reason for bacterial resistance to penicillin group antibiotics is due to the bacteria secreting broad-spectrum beta-lactamase enzymes that work to nullify the effectiveness of beta-lactam antibiotics by breaking the β -lactam ring. The reason for resistance may also be due to the lack of affinity for the binding of antibiotics to penicillin-binding proteins. These antibiotics may inhibit the process of manufacturing the bacterial empty wall through interference with the process of manufacturing the peptidoglycan layer [34]. These results are similar to the results of Salman and Al-Juboori [11, 33]. The high resistance to the aminoglycoside group has been increasing significantly in recent times, and this resistance results from the secretion of enzymes by the resistant bacteria, which mutate the antibiotic and then it lose its effectiveness [35]. As for the Macrolides group, it faced resistance from bacteria because the antibiotic has the ability to inhibit bacterial protein synthesis, as it targets the cell envelope on one hand and ribosomes and nucleic acids on the other hand [36]. These results are similar to Kim *et al.* and Salman [33, 37], while the bacteria showed high sensitivity to this group of antibiotics, and it was contrary to what we found, as in the results of the researchers Abd Zaid and Kandala; Al-Douri [20, 38].

As for the Tetracycline antibiotic, the bacteria showed high resistance as the result was close enough [11, 33]. The main reason for the increase in antibiotic resistance is due to many reasons, including the incorrect use of doses, the use of medications without a prescription, or the use of medications for non-bacterial diseases, and that the continuous exposure of bacteria to antibiotics leads to the selection of resistant breeds [39]. As for the rest of the antibiotics, they showed resistance with moderate sensitivity.

Conclusions

The occurrence of *Staphylococcus aureus* bacteria in Kirkuk Teaching Hospital is at a higher rate compared to the Children's Hospital. The proportions of isolated bacteria were greater in females than in males. The emergence of high resistance of *Staphylococcus aureus* to some antibiotics used in the current study is a result of the indiscriminate use of antibiotics and their widespread spread.

Emphasis on the health departments in the governorates in general and in Kirkuk in particular to conduct an examination of medical staff and the environment of hospital lobbies to determine the rates of spread of resistant bacteria isolates periodically and Emphasizing the controls of dispensing antibiotics in pharmacies and educating patients about the danger of using antibiotics according to personal mood, randomly and intermittently.

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