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العدد 23

# مجلة التربوي

## مجلة علمية محكمة تصدر عن كلية التربية

# جامعة المرقب

**العدد الثالث والعشرون**
**يوليو 2023م**



- المجلة ترحب بما يرد عليها من أبحاث وعلى استعداد لنشرها بعد التحكيم .
- المجلة تحترم كل الاحترام آراء المحكمين وتعمل بمقتضاها .
- كافة الآراء والأفكار المنشورة تعبر عن آراء أصحابها ولا تتحمل المجلة تبعاتها .
- يتحمل الباحث مسؤولية الأمانة العلمية وهو المسؤول عما ينشر له .
- البحوث المقدمة للنشر لا ترد لأصحابها نشرت أو لم تنشر .

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**ضوابط النشر:**

يشترط في البحوث العلمية المقدمة للنشر أن يراعى فيها ما يأتي :

- أصول البحث العلمي وقواعده .
- ألا تكون المادة العلمية قد سبق نشرها أو كانت جزءا من رسالة علمية .
- يرفق بالبحث تزكية لغوية وفق أنموذج معد .
- تعدل البحوث المقبولة وتصحح وفق ما يراه المحكمون .
- التزام الباحث بالضوابط التي وضعتها المجلة من عدد الصفحات ، ونوع الخط ورقمه ، والفترات الزمنية الممنوحة للتعديل ، وما يستجد من ضوابط تضعها المجلة مستقبلا .

**تنبيهات** :

- للمجلة الحق في تعديل البحث أو طلب تعديله أو رفضه .
- يخضع البحث في النشر لأولويات المجلة وسياستها .
- البحوث المنشورة تعبر عن وجهة نظر أصحابها ، ولا تعبر عن وجهة نظر المجلة .

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# Isolation of *Staphylococcus Aureus* From Different Clinical Samples And Detects on Its Antibiotic Resistance

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**Abstract:** The current study was designed to isolate and identify of forty-three samples were collected from Mosallata Central Hospital, which included different samples. Including urine, ear, sputum, and nose and wound swabs. The resistance strains increased the challenge in treating the infections caused by *S. aureus*, which are resistant to antibiotics. The present work aims to Isolate and identifying of *S. aureus* and detects its resistance and sensitive to common use antibiotics. While the number of isolates from the hospital environmental was five isolates, at a rate of (20.8%), followed by nasal samples, with four isolates with a rate of (16.6%). After that, the wound sample by three isolates at a rate of (12.5%), and at least the ear samples, by two isolates at a rate of (8.3%). Susceptibility test was determined for 43 isolates of *S. aureus* against 06 different antibiotics penicillin G (10 units), gentamicin (10µg), vancomycin (30µg), cefoxitin (30µg), ampicillin (10µg) and methicillin (5µg), using the disk diffusion method using the Müeller-Hinton agar medium. The growth inhibition zone was measured in millimeters using an accurate ruler in order to read the results and compared to the standard table of the Clinical and Laboratory Standard Institute.

The efficacy of antibiotics against *S. aureus* which was as follow was 23.2% in penicillin, 13.9% in methicillin, 9.3% in vancomycin, 6.9% in ampicillin, 4.6 in cefoxitin, 2.3% in gentamicin. In the conflicting of that, the other isolates of *S. aureus* which were resistant to antibiotics appears efficacy against antibiotics as follow was 16.2% in penicillin, 6.9% in both methicillin and vancomycin, 4.6% in both ampicillin and cefoxitin, but no resistant appear against gentamicin.

**Key words:** *Staphylococcus aureus*, Antibiotic Resistance, Nosocomial Infection.

## Introduction

*Staphylococcus aureus* (*S. aureus* ) is a gram positive considered one of the most important species in the coccus family from a medical point of view, and it is characterized by its wide spread in nature, as it is found in the air, soil, mucous membranes, skin, upper respiratory tract, and alimentary canal in humans (Herbert *et al*., 2001). *S. aureus* is one of the most common types of cocci. *S. aureus* is pathogenic, although it is a part of the normal flora, but it is distinguished by its ability to cause a variety of infections that vary from simple skin infections to life-threatening systemic diseases, and because it possesses many virulence factors such as surface factors, coagulase, and the beta-lactamase enzyme, as well as having toxins that have a major role in infections such as hemolysin, enterotoxin, and others (Ferry *et al*., 2005).

Further, the virulence of *S. aureus* has risen with existence of antibiotics resistance strains such as, Methicillin resistant *S. aureus* (MRSA) and Vancomycin resistance *S. aureus* (VRSA) (Oliveira *et al*., 2018; Turner *et al*., 2019) so it has become one of the main causes of nosocomial infections with high rates all over the world (Koziol–mantewka *et al*., 2006), many Studies have shown that approximately 70% of hospital isolates of *S. aureus* are resistant to antibiotics, especially those of the beta-lactam species, which were the first line of defense in medical treatment until recently (Mccarthy *et al*., 2004)**.**

The antibiotic penicillin was used as the first antibiotic to treat infections caused by *S. aureus*, and it was considered a successful treatment in the early forties, and because of its misuse, resistant isolates appeared because *S. aureus* is notorious for its ability to become resistant (Chambers and Deleo, 2009), and as a result of that resistance, new generations of these antibiotics were discovered. It works against these bacteria, which is an anti-Methicillin, as well as other semi-synthetic antibiotics such as Oxacilln were used (Klevens *et al*., 2007), the reason for the indiscriminate use of these antibiotics was the emergence of the first case of resistance to anti-Methicillin in 1961 (Jarvis *et al*., 2007), the emergence of the resistant semi-synthetic and natural penicillin antibiotics have made it difficult to treat infections caused by *S. aureus*, particularly *S. aureus*, and posed a challenge to health professionals (Kim *et al*., 2006)**.**

Treatment with antibiotics not only expensive but the risk of bacteria resistance to antimicrobial agents and the side effects such as acidity burning sensation and damage to natural fauna of intestine are also involved (Attiya Mohamedin *et al*., 2018). The resistance strains increased the challenge in treating the infections caused by them. The circulation of these strains in health care settings and community changed the epidemiology of their spread. Using preventive control measures are critical in controlling *S. aureus* infections (Oliveira *et al*., 2018). Natural alternative of antibiotics are require because of bacterial antibiotic resistant (Attiya Mohamedin *et al*., 2018).

**The present work aims to**

Isolate and identifying of *S. aureus* isolated bacteria from Massalata Central Hospital/ Libya and its resistance and sensitive to common use antibiotics.

## Material and Methods

### Sample collection

Forty three clinical specimens including urine, ear, sputum, nose and wound swabs, collected from patients attending Massalata Central Hospital/ Libya.

### Location

Massalata city/ Libya

### Period

The period from April to June 2022.

### Ages

6-40 years

**Gender**

Both Genders male and female

**Isolation of *S. aureus***

Isolation of *S. aureus* from different clinical samples collected from different localities of Massalata Central Hospital/ Libya by specific way depending on routine laboratory techniques, all samples were streaked on mannitol salt agar for detecting ability of bacterial isolates to grow on this media and incubated aerobically for 24 h at 37°C. The media used for isolation, cultivation and stock maintenance of isolated strains was nutrient agar. The culture was continued every period to activate the bacteria and prevent incidence of contamination and persistence of isolates (Mendez-Vilas, 2012).

**Identification of staphylococci**

The isolates were identified depending on the morphological features on culture media, gram staining and biochemical tests [catalase, coagulase (slide and tube) and oxidase] according to Bergey's Manual (Holt *et al*., 1994).

**Microscopic examination**

Microscopic Character it is an initial routine test for diagnosis, as the microscopic examination of the slides stained with a gram stain showed spherical cells positive for the gram stain arranged in pairs or clusters, and this is consistent with the characteristics of the genus Staphylococcus (Matar, 2014). The isolates bacteria were stained by Gram stain to detect their response to stain, cocci shapes and their arrangement.

**Colonial morphology on blood agar and mannitol salt agar**

The colonies grown on blood agar plate were tested for their shape, size, color and blood haemolysis pattern, while those grown on mannitol salt agar plate, were tested for their ability to ferment mannitol sugar.

**Implant for diagnosis**

Bacterial isolates belonging to the genus were initially diagnosed based on their morphological characteristics on blood agar media at a temperature of 37 °C for 24 hours. Their medium to large colonies appear regular and smooth, yellow to golden. These colonies are surrounded by a transparent zone, a narrow zone of lysis as a result of its complete lysis of the blood (Benson, 2001).

**Biochemical tests**

Biochemical tests were performed to confirm *S. aureus* using and mannitol fermentation test (Gillespie & Hawkey; 2006), catalase test, coagulase test and oxidase test (Macfaddin, 2000).

**Antibiotics susceptibility test of *S. aureus***

Susceptibility test was determined for 43 isolates of *S. aureus* against 06 different antibiotics penicillin G (10 units), gentamicin (10µg), vancomycin (30µg), cefoxitin (30µg), ampicillin (10µg) and methicillin (5µg), Oxoid CMO337, UK) using the disk diffusion method using the Müeller-Hinton agar medium. The growth inhibition zone was measured in millimeters using an accurate ruler in order to read the results and compared to the standard table of the Clinical and Laboratory Standard Institute (CLSI, 2018).

Initially, a bacterial suspension was prepared with a turbidity equivalent to 0.5 McFarland tube, and a lawn culture was conducted three times using a sterile swab on the Müeller-Hinton agar medium plate. The discs were removed from the freezer one hour before, placed on the culture media with pliers, and stabilized with the tip of a pair of pliers, and the plates were incubated at the temperature of 37°C for 24 hours. The diameter of the inhibition zone was measured in millimeters using an accurate ruler in order to read the results. The findings were classified as sensitivity (S1) and resistance (R2). This study was conducted in accordance with ethical principles, and the confidentiality of the information was preserved (Fernades, *et al*. 2013).

**Results and Discussion**

**Biochemical tests for isolating and identifications *S. aureus* bacteria**

The current study was designed to isolate and identify of forty-three samples were collected from Mosallata Central Hospital, which included different samples. All samples were diagnosed by conventional methods with *S. aureus* bacteria, based on the morphological, microscopic and biochemical tests as reported, (Macfaddin, 2000), (Benson`s Microbiological Applications Laboratory). All the isolates were screened for drug resistance profile by disc diffusion method with commercially available disc of six antibiotics, that is, Penicillin, Methicillin, Vancomycin, Cefoxitin, Gentamicin, and Ampicillin.

**Table (1): Biochemical tests for isolating *S. aureus* bacteria**

| Test type | Result |
|---|---|
| Mannitol fermentation test | Positive |
| Oxidase test | Negative |
| Catalase test | Positive |
| Coagulase test | Positive |
| Agglomeration factor test (Plasma coagulant bound test). | Positive |

Table (1), showed the biochemical tests for isolating *S. aureus* bacteria. Which were, the results of all tests of samples under study were positive except oxidase test was negative.

**Distribution of bacterial isolates according to isolation sources**

The samples of urine, wound infection, nasal infection, ear infection and sample from hospital environment were distributed according to free from bacterial growth and number of isolates of *S. aureus*.

**Table (2). Distribution of bacterial isolates according to isolation sources**

| Type of Sample | Numbers of samples free from *S. aureus* growth No (%) | Number of isolates of *S. aureus* No (%) | Total number of samples |
|---|---|---|---|
| Urine | 3 (6.97%) | 10 (41.6%) | 13 |
| Hospital environment | 10 (23.2%) | 5 (20.8%) | 15 |
| Nasal infection | 2 (4.65%) | 4 (16.6%) | 6 |
| Wound infection | 1 (2.32%) | 3 (12.5%) | 4 |
| Ear infection | 3 (6.9%) | 2 (8.3%) | 5 |
| Total summation | 19 (44.1%) | 24 (100%) | 43 |

Table (2), showed the distribution of *S. aureus* bacteria isolates among the number and the percentage of isolates of *S. aureus*. Which were as fallow, the urine samples were the most samples from which *S. aureus* was isolated, with ten isolates at a rate of (41.6%), that explain the reason of urine tract infections (UTI) are often chronic, approximately one in four people with a previous history of.UTI continue to develop to chronic infections (Chieng, et al., 2023).

While the number of isolates from the hospital environmental was five isolates, at a rate of (20.8%), followed by nasal samples, with four isolates with a rate of (16.6%). After that, the wound sample by three isolates at a rate of (12.5%), and at least the ear samples, by two isolates at a rate of (8.3%). These results showed that urine more infected with *S. aureus* than nasal, wound and ear specimens respectively. While Debnath and Chikkaswamy in 2015 arrange the more infected as fallow wound swabs, urine samples, ear swabs this is may be due to the different between the more common types antibiotics used in Bengaluru of

**Bacteria sensitivity tests for antibiotics**

The antibiotic Susceptibility test was done to investigate the sensitivity of twenty *S. aureus* isolates under study to six antibiotics. Which were tested using the disc diffusion method according to (CLSI; 2018). The results were counted and calculated the percentage of sensitive of bacteria to antibiotics which appears efficacy of antibiotics. On the other hand counted the resistance of bacteria to antibiotic which appears the efficacy of bacteria against antibiotics, table (3).

**Table (3) Total Percentages efficacy of different antibiotics among bacterial isolates Total number of isolates (43)**

| Antibiotics | Sensitive | Efficacy % | Resistant | Usefulness % |
|---|---|---|---|---|
| Penicillin | 10 | 23.2 | 7 | 16.2 |
| Methicillin | 6 | 13.9 | 3 | 6.9 |
| Vancomycin | 4 | 9.3 | 3 | 6.9 |
| Ampicillin | 3 | 6.9 | 2 | 4.6 |
| Cefoxitin | 2 | 4.6 | 2 | 4,6 |
| Gentamicin | 1 | 2.3 | 0 | 0.0 |

Table (3), showed the sensitivity of bacteria to common use antibiotics and the resistant of bacteria to common use antibiotics. Also, the efficacy of antibiotics against *S. aureus* which was as follow was 23.2% in penicillin, 13.9% in methicillin, 9.3% in Vancomycin, 6.9% in ampicillin, 4.6 in cefoxitin, 2.3% in gentamicin. In the conflicting of that, the other isolates of *S. aureus* which were resistant to antibiotics appears efficacy against antibiotics as follow was 16.2% in penicillin, 6.9% in both methicillin and vancomycin, 4.6% in both ampicillin and cefoxitin, but no resistant appear against gentamicin.

These results of this study were very similar to the findings of a study (Bastidas *et al*., 2019), if the bacteria were resistant to penicillin at a rate of (93.5%). But the results of this study were different of Debnath and Chikkaswamy in 2015 that found that *S. aureus* are more resistant to vancomycin from to penicillin, gentamycin, and ampicillin respectively. This increased resistance may be due to its irregular and continuous use, which enhances the resistance of the inducing bacteria. In addition to the production of *S. aureus* bacteria, the enzyme hydrolyzed by *S. aureus*. While a small percentage appeared towards the anti-gentamicin.

The results showed that the second highest rate of resistance, after penicillin, was for the antibiotic methicillin, which belongs to the cephalosporin, as the rate of bacteria resistance to it was (13.9%), and this was the conclusion of a study (Turner *et al*., 2019).

As for the anti-cefoxtin, the resistance rate was (4.6%), and as for the anti-vancomycin, the resistance rate was (4.6%). With regard to Ampicillin, the resistance rate was (25%). The rise of gentamycin resistance from 0% before 1996 to 80% after 1996 has been described but only 8% of MRSA were resistance to gentamycin. However, in the current study of twenty one century found 0% resistance of *S. aureus* against gentamycin.

## Conclusions

The bacterium *S. aureus* is more commonly found in various infections (urinary fluid, samples from the hospital environment, nose, ear, wounds). The urine sample ranked first in terms of the percentage of positive isolation, followed by samples from the hospital environment, nose, ear, and wounds. *S. aureus* showed high resistance to antibiotics, as it was more resistant to Penicillin, followed by Methicillin and less resistant to Gentamicin.

## Recommendations

In hospital, people infected with or carries of resistant *S. aureus* must be kept in isolation as measure to prevent its spread. Visitors and healthcare workers caring for people in isolation may be required to wear protective clothing. Careful hand washing remain the best germ prevention method. So, rub hands carefully for at least 20 seconds. Looking for an alternative to the antibiotics that have been shown to be resistant by these bacteria. Detection of genetic mutations responsible for antibiotic resistance of bacteria using modern technology. It is necessary to conduct susceptibility tests before prescript antibiotics, to limit the indiscriminate use of antibiotics and thus limit the spread of bacterial resistance.

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