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# evaluation of antibiotic resistance in staphylococcus aureus strains isolated from mansoura university hospitals

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**Abstract:** The introduction of antibiotics revolutionized medicine in the 20th-century permitting the treatment of incurable infections. Widespread use of antibiotics has led to the development of resistant microorganisms. Lately, there is increase in community and hospital-acquired infections accompanied by Multi-drug-resistant (MDR) Staphylococcus aureus. The goal of this study is evaluating the features of antibiotic resistance of S. aureus isolates through two years in different clinical samples from Mansoura University Hospitals. Fourty five clinical Staphylococcus aureus isolates were collected from patients from Mansoura University Hospitals; Emergency, Mansoura University, Pediatric, Specialized medical and Ophthalmic. Multidrug resistant S.aureus (MDR) isolates showed varible resistances to Clindamycin (84.45%), Ciprofloxacin (75.55%), Norfloxacin (75.55%), Ofloxacin (73.33%), Erythromycin (66.68%), Gatifloxacin(60%), Levofloxacin (57.79%), Gentamycin (53.34%), Rifampin (46.66%), Linezolid (06.66%), Vancomycin (04.45%) antibiotics. In conclusion, the MDR S. aureus isolated from Mansoura University Hospitals showed high resistance to nine antibiotics and low resistance to Vancomycin and Linezolid. Reducing the average of antibiotic resistance contributes in decreasing therapy cost. Through the years any variation of antibiotic resistance must be determined, and for guiding empirical therapies every hospital must define its own antibiotic resistance profile.

**keywords**: Staphylococcus aureus, clinical isolates, Antibiotic resistance

## 1.Introduction

*Staphylococcus aureus* is one of the most common human pathogens causing different infections in both genders and all age groups. Two obvious shifts appeared in the past two decades in the *Staphylococcus aureus* infections epidemiology: first, increasing hospital acquired infections especially in prosthetic device infections and in infective endocarditis, and second, skin and soft tissue infections caused by strains own specific virulence factors and associated with β-lactam antibiotics resistance [1].

Staphylococcus aureus exists in human microflora and most environments of human as skin, axillae, vagina, throat, perineum and gastrointestinal tract but the main ecological niche of *S. aureus* is the nares. [2].

Although most bacteria don't have the ability to grow in the presence of concentrations of salt (up to 15% NaCl), *S. aureus* has it. Also it is capable of growth over a wide range of temperature 7– 48.5 °C, with an optimum of 30–37 °C [3]. *S. aureus* is distinguished from other species on the basis of positive results of catalase, coagulase, mannitol-fermentation and deoxyribonuclease tests [4].

*S. aureus* can be distinguished from other staphylococcal species on the basis of gold colony pigmentation that is called staphyloxanthin on nutrient agar. The characteristic golden color protects the bacteria from polymorph nuclear cells and phagocytes [5].

Staphylococcus aureus has appeared as life-threatening healthcare- and community-associated infection reason. The antibiotic resistance trouble in MRSA isolates emerged in healthcare infections is associated with elevated

illness rate and death rate. The prevalence of Methicillin resistant Staphylococcus aureus (MRSA) infections can vary from country to country and between hospitals, and it also varies between different units of the same hospital [6, 7, 8]. So the goal is evaluating the features of antibiotic resistance of *S. aureus* isolates through two years in different clinical samples from Mansoura University Hospitals.

## 2. Materials and methods

### 1- Samples collection and transport :

This study was performed on 70 bacterial isolates that randomly selected from cultured plates of different specimens (corneal swab, blood, skin swab, nose swab, wound swab, axilla, pustule, diabetic foot, endotracheal tube, sputum and urine) for patients who attended to inpatients and outpatients clinics in Mansoura University Hospitals from January 2014 to December 2016.The bacterial isolates were identified as *Staphylococci* in Clinical Microbiology laboratories at Faculty of Medicine in Mansoura University. The bacterial isolates were transported to Bacteriology laboratory, Botany department at Faculty of Science in Mansoura University for further identification.

### 2- Identification of isolates:

Samples were cultured on blood agar, nutrient agar and mannitol salt agar (selective medium for *S. aureus*) aerobically for 24 hours at 37 °C. Strains that exhibited a delayed fermentation of mannitol were re-incubated overnight before excluding *S. aureus*. Yellow pigmented β-hemolytic colonies grown on blood agar and yellow colonies on mannitol salt agar and nutrient agar were subjected to the following tests to identify *S. aureus* [9, 10, 11].

### Gram stain:

The Gram staining aimed to identify and characterize the bacteria [9,12].

### Catalase test:

Colony was picked and placed on a clean glass slide. Then, a drop of 3% $H_2O_2$ was added to cover the organism on the slide. Immediate bubbling was observed in a catalase positive test [9].

### DNAse test:

DNAse agar plates were inoculated with the tested colonies. The plates were incubated aerobically for 24h at 37°C. The plates were flooded with 1 N HCl that precipitated DNA and turned the medium cloudy. The presence of clear zone around the area of growth indicates DNAse production by *S. aureus* [9,13].

### Slide Coagulase test

Agglutination (positive result) occurred by converted fibrinogen to fibrin. Dense suspension of isolate is placed on glass slide and blended with drop of EDTA human plasma. Tube coagulase test should be done to negative results for confirmation [9].

### Tube coagulase test:

One ml of plasma was added to sterile test tube containing 9 ml distilled water. The content was divided into 2 tubes then one inoculating loopful of the organism being tested was added to each tube. The tested tubes were incubated at 37 °C and examined for clot formation after 1 hour. If no clotting occurred, the tubes were re-examined after 3 hours. If the tested organism was still negative, the tube was left at room temperature overnight

and examined again. Clotting of the tube contents denoted *S. aureus* [9].

### Automated identification system:

Verification of *S.aureus* isolates were conducted by VITEK2 at Specialized Medical Hospital of Mansoura University. [31].

### Antimicrobial susceptibility test:

The antibiotic susceptibility testing was performed by Kirby Baure disc diffusion method against these antibiotics: Ciprofloxacin (5µg), Norfloxacin (10µg), Ofloxacin (5µg), Gatifloxacin(5µg), Levofloxacin (5µg), Erythromycin (15µg), Gentamycin (10µg), Rifampin (5µg), Linezolid (30µg), Vancomycin (30µg) and Clindamycin (2µg) antibiotics. The results interpreted according to CLSI guidelines [14, 30].

## 3. Results and Discussion

### 1. Collection of samples and isolation of *Staphylococcus aureus* isolates:

Different samples were collected from patients of different ages and sexes for the isolation of *S.aureus* as a possible cause for the isolation.

**Table (1):** Distribution and clinical diagnosis of *S.aureus* strains isolated from Human among different Mansoura University hospitals.

| Number of *S.aureus* isolates | | | | | |
|---|---|---|---|---|---|
| **HospitalSample** | **Emergency** | **MUH** | **Pediatric** | **Specializedmedical** | **Ophthalmic** |
| Corneal swab | 0 | 0 | 0 | 0 | 1 |
| Blood | 4 | 2 | 1 | 0 | 0 |
| Skin swab | 1 | 2 | 0 | 0 | 0 |
| Nose swab | 1 | 6 | 0 | 1 | 0 |
| Wound swab | 0 | 11 | 0 | 0 | 0 |
| Axilla | 0 | 0 | 1 | 0 | 0 |
| Pustule | 0 | 1 | 0 | 0 | 0 |
| Diabetic foot | 0 | 0 | 0 | 4 | 0 |
| Tube | 1 | 1 | 0 | 0 | 0 |
| Endotracheal tube | 0 | 2 | 0 | 0 | 0 |
| Sputum | 2 | 2 | 0 | 0 | 0 |
| Urine | 0 | 1 | 0 | 0 | 0 |
| Total No (%) | 9 (20.00) | 28 (62.23) | 2 (04.44) | 5 (11.11) | 1 (02.22) |

The results in table (1) showed that *S.aureus* exposed the maximum occurance in wound swabs samples. On the other hand, declaring the occurance of *S.aureus* was in corneal swab, axilla, pustule and urine samples. The distribution of *S.aureus* among Mansoura University hospitals showed that *S.aureus* were prevalent in Mansoura University Hospital (MUH) and less distributed in Ophthalmic.

**2. Identification of *S.aureus* isolates**

**Cultural identification:**

On blood agar medium, 38 from 70 bacterial samples showed positive β-hemolysis after 24 hours cultivation at 37°C **(Fig.1A)**.

On mannitol salt agar medium, 60 from seventy bacterial samples showed mannitol fermentation whereas 10 bacterial samples were negative. In positive result, organism can ferment mannitol producing acid turning phenol red in the agar into yellow colour **(Fig.1B)**.

On nutrient agar medium, 45 from 70 bacterial samples exposed smooth surface, yellow colour, circular colonies with 2–3 mm diameter after 24 hours cultivation at 37°C **(Fig.1C)**.

**Morphological and biochemical identification:**

After Gram staining and microscopic examination, all samples were Gram-positive coccoid in cluster shaped like *Staphylococcus* species **(Fig.1D)**.

From catalase test, all bacterial samples grown on nutrient agar media gave positive results **(Fig.2A)**. On DNAse agar medium, 46 from 70 *Staphylococcal* samples exposed a clear zone (+ve result) around the bacterial growth after 24 hours cultivation at 37°C by adding HCl **(Fig.2B)**. Bound coagulase, otherwise known as clumping factor, could be detected by carrying out a slide coagulase test **(Fig.2C)** whereas free coagulase could be detected via tube coagulase test **(Fig.2D)**. From 70 *Staphylococcal* samples, 35 give positive coagulase on slides and 39 positive coagulase in tubes whearas 29 *Staphylococcal* samples were positive for both slide and tube coagulase tests.

Fourty five isolates were considered to be *S.aureus* according to their biochemical characters especially the positive results of catalase and coagulase.

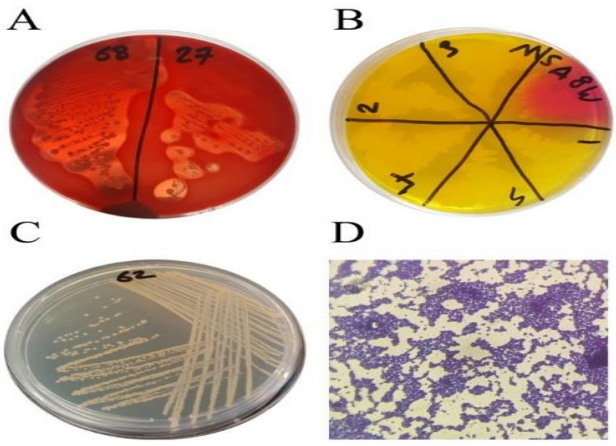


**Fig.(1)**: Cultures of *S. aureus* on (A) blood agar, (B) mannitol salt agar, (C) nutrient agar, (D) microscopic examination of *S.aureus* showed a typical character of Gram positive

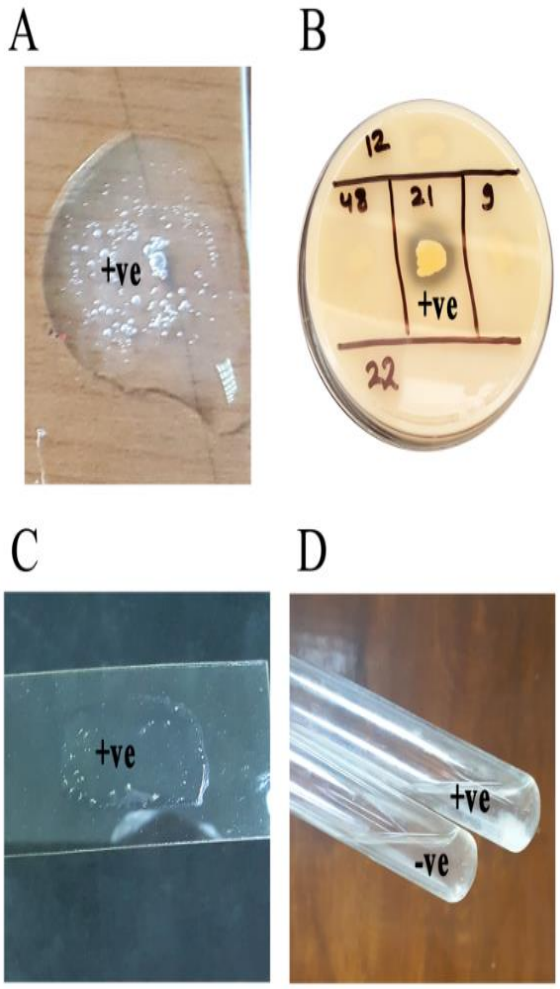


**Fig.(2)**:(A) Culture of *S. aureus* on DNAse agar, (B) Catalase test, (C) Coagulase slide test , (D) Coagulase tube test.+ve =positive result, -ve =negative result.

**3. Antimicrobial susceptibility test:**

Antimicrobial susceptibility pattern of different *S.aureus* isolates to various antibiotic agents were determined by Kirby Baure disc diffusion method.

Fourty five *S.aureus* isolates, recorded resistance to eleven tested antibiotics. Multidrug resistant *S.aureus* isolates showed varible resistances to Clindamycin (84.45%), Ciprofloxacin (75.55%), Norfloxacin (75.55%), Ofloxacin(73.33%), Erythromycin (66.68%), Gatifloxacin(60%), Levofloxacin (57.79%), Gentamycin (53.34%), Rifampin (46.66%), Linezolid (06.66%), Vancomycin (04.45%) antibiotics **(Table.2)**.

The Multidrug resistant *S.aureus* isolated from Mansoura University Hospitals showed high resistance to nine antibiotics and low resistance to Vancomycin and Linezolid.

**Table(2):** Susceptibility of *Staphylococcus aureus* (45 isolates) to eleven antimicrobial agents.

| Antimicrobial agent | Susceptibility groups | | | | | |
|---|---|---|---|---|---|---|
| | Sensitive | | Intermediate | | Resistant | |
| | No. | % | No. | % | No. | % |
| Gatifloxacin | 13 | 28.88 | 05 | 11.12 | 27 | 60.00 |
| Levofloxacin | 16 | 35.55 | 03 | 06.66 | 26 | 57.79 |
| Ofloxacin | 11 | 24.45 | 01 | 02.00 | 33 | 73.33 |
| Norfloxacin | 11 | 24.45 | 00 | 00.00 | 34 | 75.55 |
| Ciprofloxacin | 09 | 20.00 | 02 | 04.45 | 34 | 75.55 |
| Linezolid | 42 | 93.34 | 00 | 00.00 | 03 | 06.66 |
| Vancomycin | 27 | 60.00 | 16 | 35.55 | 02 | 04.45 |
| Clindamycin | 01 | 02.22 | 06 | 13.33 | 38 | 84.45 |
| Gentamycin | 21 | 46.66 | 00 | 00.00 | 24 | 53.34 |
| Erythromycin | 12 | 26.66 | 03 | 06.66 | 30 | 66.68 |
| Rifampin | 20 | 44.46 | 04 | 08.88 | 21 | 46.66 |

## Discussion

The main common bacterial human pathogen is *Staphylococcus aureus* also it is the leading reason of septic arthritis, sepsis, pneumonia and osteomyelitis [15].

The extensive use of antibiotics in the clinical environment has led to the appearance of a wide variety of drug resistance mechanisms among all bacterial pathogens, including *S. aureus* [16].

The mechanism of antibiotic actions are as follows: Inhibition of cell wall synthesis, breakdown of cell membrane structure or function, inhibition of the structure and function of nucleic acids, inhibition of protein synthesis and disturb of key metabolic pathways [17].

Vancomycin is a glycopeptide antibiotic that is widely used to treat serious infections caused by MRSA strains in hospital patients [18]. It inhibits bacterial growth by inhibiting the synthesis of peptidoglycan [19]. In Europe and Turkey no reports about Vancomycin resistance in many studies made there [7, 20]. In India, 0.33% Vancomycin resistance was reported [21]. In USA only 13 cases of VRSA were reported up to 2013 [22]. After the emergence of MRSA infections and prevalence use of Vancomycin in many countries has led to decline in Vancomycin sensitivity. For Japan, the first appearance of Vancomycin Intermediate - resistant *S. aureus* (VISA) strains was in 1997 [23].

Linezolid is an oxazolidinone agent that inhibits bacterial protein synthesis by binding

to the 50S ribosomal subunit, blocking the formation of the initiation complex [24]. Linezolid is a leading antimicrobial for use against *S. aureus*. In Turkey many in vitro antibiotic sensetivity studies made to MRSA isolates and no Linezolid resistance found in the results [25].

Resistance to Linezolid has been reported to be growing steadily since the first report [26]. In USA, the Linezolid resistance level reported less than 2% [27]. In Spain, only 256 Linezolid resistant *S. aureus* (2.8 %) were isolated between 2005 and 2009 [28], whereas in India the occurrence has been reported to be between 2–20% [29].

In a study at Yuzuncu Yil University, Dursun Odabas Medical Center, Microbiology Laboratory *S. aureus* strains from 2009 to 2014 were isolated from various clinical samples. Depending on the results of susceptibility tests, they found that all isolates of *S. aureus* sensitive to Linezolid, Vancomycin and Levofloxacin. The resistance rates to Erythromycin, Rifampicin, Gentamicin, and Clindamycin were 18%, 14%, 14%, and 11%, respectively [20] whereas in our study we found higher resistance rates to Clindamycin, Erythromycin, Levofloxacin, Gentamicin and Rifampicin were 84.45%, 66.68%, 57.79%, 53.34% and 46.66% respectively and low resistance rate in Vancomycin, Linezolid, 04.45%, 06.66% , respectively.

## Conclusion

In conclusion, the MDR *S. aureus* isolated from Mansoura University Hospitals showed high resistance to nine antibiotics and Vancomycin and Linezolid. Improper use of antibiotics in empirical treatment prevents the treatment of multi-drug resistant bacterial infections. Through the years any variation of resistance must be determined and every hospital must define its own antibiotic resistance profile. Reducing antibiotic resistance average contributes in decreasing therapy cost.

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