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Isolation, prevalence, and drug susceptibility patterns of Methicillin resistant *Staphylococcus aureus* in Mansoura city.

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Abstract: *Staphylococcus aureus*, particularly MRSA strains, are common multidrug resistant pathogens worldwide. Treatment for nosocomial infections has been complicated by MRSA because it has a potent resistance strategy that enables it to be less susceptible to all β-lactam antibiotics. The principal objective of this investigation was to isolate methicillin resistant *S. aureus* from different clinical specimens and determine both the antibiotic susceptibility pattern and the prevalence rate of MRSA. Various clinical specimens were collected and cultivated on mannitol salt agar and blood agar, and biochemical tests using catalase and coagulase tests revealed that the isolates were *S. aureus*. The disc diffusion method was used to screen MRSA isolates using 30 µg cefoxitin and confirmed by the detection of the *mec A* gene. The prevalence of MRSA, the percentage of Multidrug Resistant (MDR) isolates, and the value of Multiple Antibiotic Resistance Indices (MARI) for isolates were determined. Of the 150 clinical samples evaluated, *S. aureus* was detected in 45 (30 %) samples. The highest rate of *S. aureus* isolation was 31.1% from wound swab samples. 60% of *S. aureus* isolates were MRSA isolates, and 55.5% were found to be MDR. 70% of MRSA isolates have a MARI value greater than 0.2. This investigation exhibited a high MRSA prevalence rate and also multidrug resistance to several tested antibiotics in a hospital setting. Thus, restricted antibiotic use and infection control became necessary to reduce MRSA infections.

keywords: Staphylococcus aureus, MRSA, Cefoxitin, Prevalence

1.Introduction

Staphylococcus aureus is a highly serious pathogen for humans, causing both nosocomial and community-acquired infections. A variety of infectious diseases, from simple illnesses like skin and soft tissue infections to serious, potentially fatal infections, can be caused by *S. aureus* [1,2]. Before the discovery of antibiotics, *S. aureus*-caused invasive infections were frequently fatal [3]. Methicillin-resistant *S. aureus* (MRSA) strains were first identified in the early 1960s, not long after the drug was introduced into hospitals. MRSA spreads easily within hospitals. In certain countries, 75 % of all hospitalized *S. aureus* isolates were recovered by MRSA [4]**.** It can be transmitted mainly from patients or staff who are colonized or infected to other patients or staff, or vice versa [5]. As a result, the prevalence rate varies noticeably between hospitals in the same country and between countries [6]. Methicillin resistance is managed by the presence of penicillin-binding protein 2a (PBP-2a), encoded by the mecA gene, which has a poor affinity for β-lactam antibiotics [7]**.** In addition, MRSA showed resistance to Fluoroquinolones and aminoglycosides [8]**.** Nowadays, vancomycin is recognized globally as the final option for treating MRSA infections [9]. The rise of *S. aureus* isolates resistant to vancomycin and other broad spectrum antibiotics has turned MRSA into a multidrug-resistant superbug,

making it a greater risk in both hospital and community settings [10,11].

In the current investigation, MRSA strains were isolated from various clinical samples and identified, and both the antibiotic susceptibility pattern and the prevalence rate were determined.

2. Materials and methods

2.1. Collection and processing of Clinical bacterial specimens

The following chemicals were provided for this study: Mannitol salt agar medium (Himedia, India), Muller-Hinton agar medium (Oxoid, UK), hydrogen peroxide (Oxford, India), commercial antibiotic disks (Oxoid, UK), green Master Mix 2× (Genetix Biotech Asia, India), and DNA Marker GeneRuler 1 kb Plus DNA Ladder ranged between 75 to 20000 bp (ThermoFisher Scientific, SM 1333).

One hundred and fifty different clinical specimens, including urine, blood, nasal swaps, and wound swaps, were collected from Mansoura University Hospital in Egypt. The samples were taken under aseptic conditions and transmitted right away in ice packs to the laboratory of bacteriology for bacterial isolation [12]. Blood samples were cultivated in Brain heart infusion broth medium, and then the samples were incubated at 37 °C for 24 h. Next, these samples were cultivated on blood agar by the streaking method [13]. While swabs and urine samples were directly streaked on blood agar. The inoculated plates were placed in an incubator at 35-37 °C for 18-24 h [14].

2.2. Isolation of Staphylococcus aureus

The bacterial colonies that showed beta hemolytic activity on blood agar were picked up and streaked on mannitol salt agar, and then the plates were incubated at 35-37 °C for 18-24 h. Bacterial colonies exhibiting the characteristic golden-yellow pigmentation on Mannitol salt agar were picked up and cultivated on nutrient agar slant. Slants were incubated for 24 h at 37 °C and then stored at 4 °C for preservation until further characterization [14].

2.3. Identification of Staphylococcus aureus

The *S. aureus* isolates were biochemically tested with a catalase test by adding three drops of 3% hydrogen peroxide to 5 ml of bacterial suspension, and the evolution of oxygen bubbles immediately indicated a positive result. The coagulase test was also carried out by mixing 200 μl of plasma with 800 μl of tested bacteria culture, which was then incubated at 37 °C for 1 h. Clotting formation implies coagulase positive *S. aureus* [15].

2.4. Antibiotics susceptibility test

The Kirby-Bauer disk diffusion method was used to test the susceptibility of *S. aureus* isolates to different antibiotics, as described by Dilnessa and Bitew [14]. Fourteen standard commercial antibiotic disks were used, involving Cefoxitin (30 µg), Gentamicin (10µg), Azithromycin (15 µg), Clarithromycin (15 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Lomefloxacin (10 µg), Norfloxacin (10 µg), Trimethoprim/ sulfamethoxazole (1.25/23.75 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Doxycycline (30 µg), Nitrofurantoins (300 µg) and Clindamycin (2 µg). Briefly, the mid log phase of each bacterial isolate grown in Muller Hinton Broth medium (MHB) was suspended in saline to obtain bacterial turbidity matched with 0.5 McFarland standards ~ 1.5×10^8 colony forming units (CFU) per ml. Next, a sterile cotton swab was used by dipping into the diluted bacterial suspension, applied to Muller Hinton agar (MHA) plates, and spread on the medium. After the inoculum was dried, antibiotic disks were carefully set on the agar plates using sterile forceps. The plates were inverted and incubated at 37±2 °C for 16-18 h. The diameter of the inhibition zone produced by each antibiotic disk was measured and represented in millimeters (mm). The size of the inhibition zone surrounding each antibiotic disc was interpreted as resistant (R), intermediate (I), or sensitive (S) according to the guidelines of Clinical and Laboratory Standards Institute [16].

2.5. Determination of Multi Drug Resistant (MDR) and Multiple Antibiotic Resistance Indices (MARI)

The proportion of isolates with MDRs was calculated [17]**,** as it is the resistance of bacterial isolates to three or more groups of tested antibiotics. Also, the MARI calculations were done [18]**.** It is done by dividing the

number of antibiotics to which an isolate has demonstrated resistance by the total number of antibiotics to which the isolate has been exposed.

2.6. Detection of mec A gene

PCR amplification of *mec A* gene was carried out to confirm the identity of the isolates as MRSA. Specific primers (*mec A*-F: AAAATCGATGGTAAAGGTTGGC and *mec A*-R: AGTTCTGGAGTACCGGATTTGC) were used [19]. Briefly, bacterial DNA was extracted by taking a single colony from an overnight culture plate and dissolved in 100 µl of lysis solution (TritonX-100, 10 mM Tris-HCL, 100 mM NaCL, 1 mM EDTA). The mixture was heated to 100°C for ten minutes, then centrifuged at 14000 rpm for 1 min and the supernatant was used as a template DNA**.** A 25 µL PCR mixture was the final volume to which 1 µL of the extracted DNA was added. The PCR mixture contains 10 µL of green Master Mix 2× GeneTaq^{TM}, 0.7 µL of 0.8 µmol/L of each primer, and 12.6 µL of nuclease-free water. The PCR thermal cycling protocol was carried out with an initial denaturation temperature of 95 °C for 3 min, followed by 33 cycles of 94 °C for 1 min, 53 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 6 min. The PCR products were loaded on a 2% agarose gel containing ethidium bromide and electrophoresed at 100 volts for 30-45 min. Bands were observed by a UV transilluminator and documented by a gel analyzer machine [19, 20].

3. Results and Discussion

3.1. Isolation and Identification of *S. aureus*

The prevalence of *S. aureus* and MRSA varies from one geographic region to another and between different institutions in a given area [6]. In this investigation, 45 *S. aureus* isolates were recovered out of 150 collected clinical specimens with a prevalence of about 30%, which is similar to the prevalence of *S. aureus* conducted by a previous study [21], which was 32.8%, while other study [22] showed a low prevalence rate of *S. aureus* from clinical specimens, which recorded 10% prevalence rate. *S. aureus* isolates showed a β-hemolysis pattern on blood agar, golden yellow colonies on mannitol salt agar, a coagulase test positive, and a catalase test positive, as shown in Table 1.

Table 1. Phenotypic characterization of S. aureus isolated from clinical specimens

Sample	No. of Isolates	β - haemolysis on Blood agar	Golden yellow color on Mannitol salt agar	Positive Coagulase test
Blood	30	11	7	7
Urine	40	16	11	11
Wound swabs	40	22	18	14
Nasal swabs	40	20	16	13

3.2. Classification of of *S. aureus* according to clinical source.

According to the *S. aureus* isolates' distribution analysis, the majority of isolates (31.1%) were found in wounds, which was slightly higher than the value (23.3%) conducted in another study [23]**,** and this majority might be because *S. aureus* is a prevalent skin flora that may penetrate the body through cracks, cuts, scratches, burns, and surgical wounds. It can also cause pyogenic infections. The following part of *S. aureus* isolates was recovered from nasal swabs (28.8%), then urine samples (24.4%), and the lowest percentage (15.5%) was isolated from blood (Figure 1).

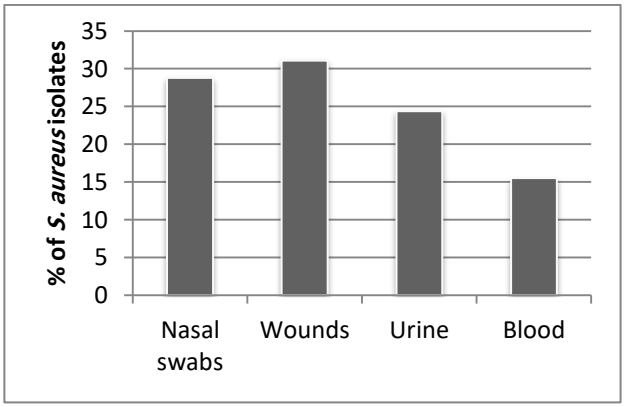

Figure 1. Distribution of *S. aureus* isolates in different clinical specimens

3.3. Antibiotic resistance profiles

The antimicrobial susceptibility test conducted using the agar disk diffusion method among *S. aureus* isolates showed that the percentage of resistance to Cefoxitin, Gentamicin, Azithromycin, Clarithromycin,

Ciprofloxacin, Lomefloxacin, Norfloxacin, Levofloxacin, Doxycycline, Tetracycline, Clindamycin, Trimethoprim/ sulfamethoxazole, and Chloramphenicol were 60%, 46.6%, 28.8%, 28.8%, 17.7%, 20%, 17.7%, 11.1%, 17.7%, 53.3%, 11.1%, 6.6%, and 24.4%, respectively. On the other hand, 15.5% (7/45) of *S. aureus* isolates were sensitive to all tested antibiotics (Figure 2). 55.5% (25/45) of *S. aureus* isolates were multidrug resistant (MDR), while 70.3% (19/27) of MRSA isolates were MDR, as they were resistant to at least three classes of antibiotics [17]. The Multiple Antibiotic Resistance Indices (MARI) (Table 2) showed that 70.3% (19/27) of MRSA isolates gave MARI higher than 0.20 and this implies that the site of bacterial collection overused or misused several antibiotics. Thus, a large proportion of the bacterial isolates have been subjected to multiple antibiotics and developed antibiotic resistance [24]. Fortunately, Nitrofurantoin was the only antibiotic that was effective against every isolate in this research. So, it is the recommended treatment for *S. aureus* which is resistant to multiple drugs.

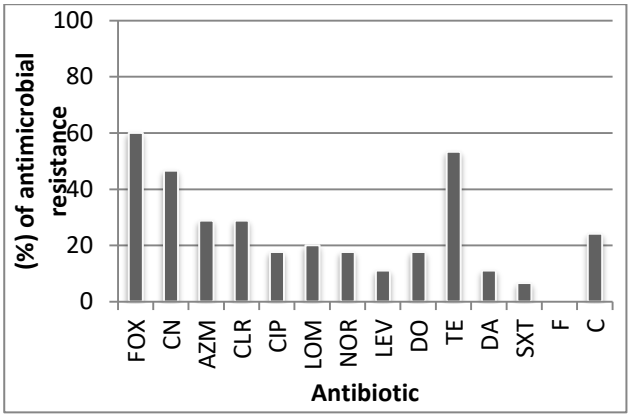

Figure 2. The antibiogram of *S. aureus* isolates including Cefoxitin (FOX), Gentamycin (CN), Azithromycin (AZM), Clarithromycin (CLR), Ciprofloxacin (CIP), Levofloxacin (LEV), Lomefloxacin (LOM), Norfloxacin (NOR), Doxycycline (DO), Tetracycline (TE), Clindamycin (DA), Trimethoprim/ sulfamethoxazole (SXT), Nitrofurantoins (F), and Chloramphenicol (C).

3.4. Prevalence of MRSA among clinical specimens

The prevalence of MRSA was detected by testing the susceptibility of isolates to cefoxitin. (27/45) of *S. aureus* isolates were resistant to cefoxitin. In addition, PCR amplification of the *mec A* gene of MRSA isolates showed a single band of 533 bp, as revealed in Figure 3. The prevalence rate of MRSA was 60%, and this agreed with a previous study carried out by Abouelfetouh, A. [25] which revealed that 40% to 70% of *S. aureus* infections were caused by methicillin resistant strains in Egypt. In comparison to values previously reported in other settings in the region, the value was also higher. In sub-Saharan Africa (SSA), the prevalence of MRSA is either less than 25% or between 25% and 50%, according to multiple reviews [26]. A high prevalence rate of MRSA has also been reported in other global locations, such as Peru (80%) [27].The highest rate of MRSA was recovered from wound samples, with a value of 78.5%. This value is in line with the study [28] performed which showed a prevalence rate of 75% MRSA in wound samples. The following part of MRSA was recovered from nasal swabs at a rate of 69.2%. In conclusion, this study demonstrated high prevalence rate of MRSA isolates from Mansoura University Hospital which are resistant to multiple commercial antibiotics making a greater risk in both hospital and community settings. Thus, it is recommended to conduct routine surveillance of hospital acquired infections, which included monitoring drug susceptibility patterns and enforcing strict drug policies regarding the use of antibiotics both inside and outside of hospitals. It may be possible to lower the frequency of MRSA infections by implementing good infection control measures like hand washing, identifying MRSA carriers and treating them.

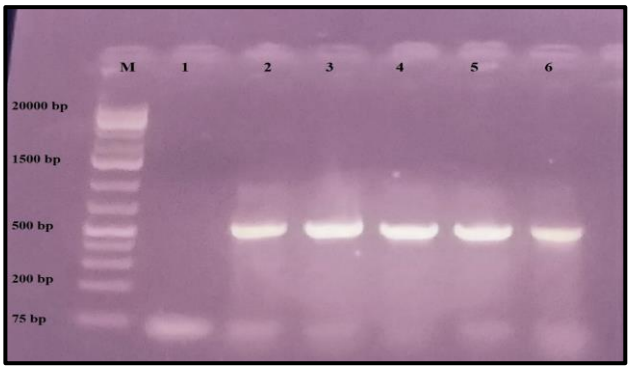

Figure 3. Electrophoretic graph of PCR products of the *mec A* gene on a 2 % agarose gel stained with ethidium bromide. MRSA isolates showed a single band of 533 bp (lane 2-6), MSSA isolate was negative control (lane 1), DNA ladder ranged between 75 to 20000 bp**.**

Table 2. Multiple Antibiotic Resistance of the MRSA isolates

MRSA Isolate code	MDR	MARI	Antibiotics to which the isolate is resistant
15	+	0.50	FOX, AZM, CLR, CIP, LOM, NOR, LEV
26	-	0.14	FOX, CN
35	+	0.42	FOX, CN, AZM, CLR, TE, DA
36	+	0.64	FOX, CN, CIP, LOM, NOR, LEV, DO, TE, C
62	-	0.07	FOX
63	+	0.78	FOX, AZM, CLR, CIP, LOM, NOR, LEV, TE, DA, SXT, C
68	-	0.07	FOX
72	+	0.28	FOX, CN, TE, C
78	+	0.42	FOX, CN, AZM, CLR, TE, DA
80	+	0.21	FOX, TE, C
81	-	0.14	FOX, CN
84	+	0.21	FOX, CN, TE
85	+	0.35	FOX, LOM, DO, TE, C
87	+	0.21	FOX, CN, TE
94	+	0.42	FOX, CN, AZM, CLR, DO, TE
100	-	0.14	FOX, CN
101	-	0.07	FOX
104	-	0.14	FOX, C
112	+	0.21	FOX, CN, TE
113	+	0.21	FOX, CN, TE
117	+	0.21	FOX, CN, TE
138	+	0.21	FOX, TE, C
139	+	0.42	FOX, CN, AZM, CLR, DO, TE
140	-	0.07	FOX
144	+	0.21	FOX, CN, TE
145	+	0.35	FOX, CIP, LOM, NOR, LEV
147	+	0.50	FOX, AZM, CLR, CIP, LOM, NOR, LEV

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