

A study on the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in clinical samples of Baghdad hospital

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Abstract:

The serious nosocomial illness, Methicillin-resistant *Staphylococcus aureus*, which is spreading across hospitals and the general populace in Iraq, is a major problem. The goal of our research was to figure out how common MRSA was in the province of Baghdad's hospitals and community settings. A total of 150 clinical samples were processed over the course of three months. Two different antibiotic susceptibility tests were done on the MRSA that was found, and the results were analyzed using CLSI 2019 guidelines. β -lactamase production was discovered utilizing phenotypic techniques. The findings showed that 55 of the 150 samples collected from various clinical regions were positive for *Staphylococcus* spp. On the other hand, PCR was used to determine whether *mecA* was present in the 30 isolates. Ten isolates had the 533 bp *mecA*-specific PCR result.

Keywords: Methicillin resistance, *Staphylococcus aureus*, MRSA.

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Introduction

A potentially deadly bacterium called *Staphylococcus aureus* causes a wide range of illnesses and is capable of quickly adapting to the pushing characteristics of anti-infection drugs. MRSA has grown and spread as a result of this [1].

Methicillin-resistant *Staphylococcus aureus*, or MRSA, is a clinical illness that is dangerous for the world's health [2]. This is often because MRSA germs respond unfavorably to pertinent tests and are resistant to a number of antibiotic classes [1]. The *mecA* gene produces the extraordinary penicillin-restriction protein PBP2a, which confers resistance to methicillin and other beta-lactam antibiotics. *Staphylococci* can endure high doses of all-lactam and methicillin antibiotics because PBP2a has a poor affinity for these drugs. This method renders all beta-lactam experts' methicillin-resistant [4]. Because they can be transferred between cells by even or vertical quality exchange from different cells in three different ways—formation, transduction, and transformation—*Staphylococcal* tape chromosome *mec* (SCC*mec*) MGEs play a significant role in the development of antimicrobial obstruction tools and destructiveness. Overuse of antibiotics in healthy local populations leads to the development of community-associated MRSA (CA-MRSA), which encourages bacterial competition. Hospital-acquired MRSA (HA-MRSA) infection may arise in healthcare settings, including clinics and nursing homes [5]. According to epidemiological research, the phenotype and genotype of CA-MRSA are different from those of HA-MRSA [6]. Contrary to HA-MRSA, CA-MRSA often responds better to a range of non-lactam

antibiotics [7]. Furthermore, the majority of CA-MRSA strains exhibit pathogenicity [8]. The boundaries of CA-MRSA are linked to kinds IV, V, and VII, and type IV generally has pvl quality [10]. There have been few studies on the actual incidence of local MRSA epidemiological classes, and despite the fact that both HA- and CA-MRSA cases have previously been reported there, there are no reliable studies on MRSA SCCmec composition and MLST in Iraq, despite the fact that both HA- and CA-MRSA cases have previously been reported there [12],[13]. The purpose of this research was to evaluate the prevalence, geographic distribution, and molecular characterization of MRSA isolates in Baghdad hospitals. Consequently, the following are the goals of the current research:

- 1- Bacteriological testing for MRSA isolation and identification.
- 2- Molecular tests for MRSA isolate identification.

Materials and Methods

Case Study: From patients at the Baghdad hospital, 150 clinical samples were taken between January and March 2021. Swabs from the nares, vagina, burn wounds, sputum, diabetic foot ulcer, and skin were used to gather clinical samples. The sterile cotton swabs were transported to the lab after being submerged in BHI broth tubes. Based on physical characteristics of the culture medium and biochemical tests in accordance with Bergey's manual's classification, isolates were identified [8]. All isolates underwent PCR amplification of the coa gene as a confirming molecular method of detection.

Materials

3.1.1. Instruments and Equipment

Table 1 includes a list of the apparatus and instruments utilised in this study along with their manufacturer.

Table 1: Apparatus and instrument.

No.	Instruments	Company	Origin
1	Camera	Sony	Japan
2	Centrifuge	Tuiup	Italy
3	Distillator	GFL	Germany
4	Security cabinet	Lipshow	USA
5	Sensible Harmony	Sartorius	Germany
6	Aquatic Bath	Memmert	Germany
7	Incubator	Memmert	Germany
9	Multiple Light Microscope	Genex	USA
10	Autoclave	Hirayama	Japan
11	Oven	Memmert	Germany
12	Burner	Shndon	England
13	Vitek 2 system apparatus	Biomerieux	France
14	Micropipettes 5-50,0.5-10,100-1000 µl	Gillson Instruments	France
15	Multichannel micropipette reservoir	Siamed	Germany
16	Shaker	TKA	Italy
17	Soxholet Extractor		
18	Ultraviolet transelumanter	Exc-Ism	UK
19	Horizontal electrophoresis	Shandon scientific CO	UK
20	Vortex	Scientific	Bohemia
21	Cooled centrifuge	Hettich	Germany
22	Epindroff tubes	Sterellin Ltd	UK
23	PCR tubes	Sterellin Ltd	UK
24	Micro centrifuge	Hettich	Germany
25	Water still	Lab tech	Korea
26	Hot plate stirrer	Lab tech	Korea
27	Thermo cycler apparatus	MWG Biotech	Germany

3.1.2. Chemicals

Table 2 lists the compounds used for this investigation.

Table 2: Used Chemical substances

No	Chemical substance	Company	Origin
1	Iodine (10%)	Al Ansar	Syria
2	Gram stain	AL-Hilal	K.S.A

3	McFarland's answer	BDH	UK
4	Hydrogen peroxide	Oxoid	UK
5	Gelatin	Oxoid	UK
6	Ethanol 95 %	BDH	UK
7	Oil Immersion	BDH	UK
8	Catalase Reagent	Himedia	India
9	Physiological normal saline	Alfaiha	Jordan
10	oxidase Reagent	Himedia	India
11	Teramethyl-p-phenylen Diamin Dihydrochloride	Fluka	UK
12	Kovacs reagent	Fluka	UK
13	Urea solution 40%	Himedia	India
14	VP reagent	Himedia	India
15	Methyl red reagent	Himedia	India
16	Ethanol	BDH	UK
17	Isopropanole	Sigma	USA
18	Agarose	Difco	UAS
19	TBE buffer	BioBasic	Canada

Commercial kits

The commercial kits that were used in current study have been illustrated in table 3.

Table 3: The commercial kits.

No.	Type of kit	Company	Origin
1	API20 Staph kit	BioMerieux	France
2	Vitek 2 system kit	Biomerieux	France
3	Genomic DNA Extraction Kit	Norgen	Canada
4	Hot-start master mix	Bioneer	Korea

Culture media have been presented in table 4.

Table 4: Culture media.

No	Type of media	Manufacturing company	Origin
1	Blood agar base	Oxoid	UK
2	Brain heart infusion broth	Oxoid	UK
3	BBL TMCHRO Magar TM MRSA	Becton and Dickinson	France
4	MacConkey agar	Oxoid	UK
5	The Mueller-Hinton agar	Himedia	India
6	Tryptic soya agar	Himedia	India
7	Mannitol salt agar	Himedia	India

Gotten the culture media ready:

The culture media used in this investigation was sterilised by autoclaving it for 15 minutes at 121 °C and 15 bar/in² pressure, as per the manufacturer's recommendations. These media outlets were mentioned:

Blood agar

It was made by combining 40 g of blood agar base (from the Himedia firm) with 1000 ml of distilled water, bringing to a boil, sterilising in an autoclave, and adding 5% fresh human blood after allowing the mixture to cool to 45 °C. It is used to demonstrate bacterial hemolysis of human blood cells.

Heart and brain infusion broth

It was made by dissolving 4 mg of BHI broth in 100 DW, as instructed during manufacture, and sterilising it in an autoclave to activate a range of odious bacteria.

Soy broth with trypticase

The instructions from the manufacturer were followed, and 5 grammes of TS broth were dissolved in 100 millilitres of water before the bacteria were activated in an autoclave [8].

Agar trypticase soy

It has been sterilised in an autoclave after being prepared in accordance with manufacturing instructions by dissolving 5 g of TS agar in 100 DW. It was used to store and maintain bacterial isolates (Short – term and long – term conservation).

Agar Mueller-Hinton

is employed in the disc diffusion method for antimicrobial sensitivity testing.

Using distilled water as a suspension, 35 grammes of powder were boiled and sterilised for 15 minutes in an autoclave at 121.

3.1.6. Solution, dyes and Reagent

Gram's stain remover

Crystal violet, safranin, ethanol, and iodine are the four constituent solutions. This pigment was used to examine the appearance and structure of bacterial cells .

Catalase testing substance

It has a 3% concentration of hydrogen peroxide (H₂O₂) in it. ready immediately before use, as recommended by

Oxidase test substance

This reagent was kept in a container without a label. It was made by mixing together 1 g of (tetramethyl-paraphenylenediamine-dihydrochloride) and 100 ml of D.W.

Usually, saline solution

Using D.W., the solution was created by mixing 0.85g of sodium chloride with 90ml of distilled water.

System diagnosis for Vitek 2

The experiment was done the way the manufacturer told us to, and the results of the biochemical test were checked with the Vitek 2 System:

- ❖ A test tube with three ml of normal saline and a loop full of an isolated colony were combined. It must be a 24-hour-old colony.
- ❖ The colony was standardized to McFarland's standard solution (1.5 10⁸ cells/ml) using a density check machine.
The cassette was filled with standard inoculums; A sample ID number was entered into the computer programmer via a barcode. Thus, a connection exists between the sample ID number and the Vitek 2 card. The cassette was transferred from the filler module to the reader/incubator module after the cards had been filled. All subsequent steps, such as controlling the temperature during incubation and reading optical cards, are handled by the device. Additionally, it sends test findings to a computer for analysis and continuously monitors test outcomes.

3.2.15 Test for Antimicrobial Susceptibility

In this study, the antibiotics oxacillin and methicillin were utilized. The results of the experiment were judged by the standards set by the National Committee for Clinical Laboratory Standards [8] According to , this test was carried out via the Kirby-Bauer disc diffusion method:

- ❖ The inoculums used in this experiment were made by combining 5.0 ml of sterile normal saline with (3-5) isolated colonies that had grown on blood agar plates and (1.5 x 10⁸ cells/ml) McFarland standard tubes. Inoculums were taken out of the bacterial solution using a sterile swab. These inoculums were smeared over blood agar plates and given time to dry.
- ❖ Using flame-up forceps or a disc applicator, the antibiotic discs were introduced to the medium at similar diffusion out intervals. The media was then incubated for 24 hours at 37°C. By measuring the inhibition zone with a ruler or calliper and comparing it to the zones of inhibition set by CLSI, it was possible to tell if an antibiotic could kill an organism or not.

McFarland benchmark	1% BaCl ₂ (ml)	1% H ₂ so ₄ (ml)	Bacterial suspension approximately/ml
0.5	0.05	9.95	1.5x10 ⁸

. Test for chromogenic screens

The materials were streaked for isolation, subculture on MHA, or inoculated onto a CHROMagar plate before antimicrobial susceptibility testing. A colony suspension of the pure cultures from the MHA was created, and its turbidity matched the 0.5 McFarland standard. The plates were turned over and kept at 35°C for 24 hours in a dark incubator since it was expected that light would prevent the isolates from recovering and/or changing color.

The plates were read against a white background. The pink to purple coloration of MRSA colonies on CHROMagar media. (<http://www.chromagar.com>; Flayhart et al., 2005) Other organisms (non-MRSA) were inhibited or displayed colonies that were colorless, white, and blue to green. Each clinical sample was streaked immediately before being tested on HiCrome agar. At 35°C, the plates were incubated aerobically for 48 hours. The HiCrome agar medium had bluish to green MRSA colonies. Colors including metallic blue, pale bluish green, and purple were present in non-MRSA colonies. The incubation period can be expanded to 48 hours to boost sensitivity (<http://www.himedialabs.com>).

. Genotypic identification

. DNA extraction

According to the G-spin DNA Extraction Kit, Cat. No. 01 from Intron Biotechnology, the basic DNA purification procedure was followed. 17045 elements of a set

Label	Contents 50 columns
Buffer CL	25.0 ml
Buffer BL	25.0 ml
Buffer WA	40.0 ml
Buffer CE	10.0 ml
Collection/Spin Column Tube	50.0 ea
A RNase (Lyophilized powder)	3.0 mg* 1 vial
k proteinase (Lyophilized powder)	22.0 * 1 vial

Transfer 1-2 ml of cultivated cells to a tube of 2 ml capacity. The cell should be centrifuged for one minute at 13,000 rpm to form a pellet. The supernatant must be disposed of. To thoroughly resuspend the cell pellet and remaining supernatant, vortex or tap them vigorously. Rapidly vortex the sample tube and add 5 milliliters of RNase A solution, 20 milliliters of proteinase K, and 200 microliters of buffer cl. The 200 l of buffer BL should be added to the top sample tube when the lysis is finished, and it should be well mixed. After that, the lysate must be incubated for 10–30 minutes at 56°C (in a water bath or heated heat block). Centrifuge the sample tube at 13,000 rpm for 5 minutes to remove any remaining tissue that hasn't been thoroughly lysed. The mixture is then incubated for 5 minutes at 70 °C. 350–400 l of the supernatant should be carefully poured into a fresh 1.5 ml tube. Pipette or gently invert the lysate five to six times in 200 ml of 100% ethanol to completely blend it. After a brief period of mixing, centrifuge the 1.5 mL tube to catch any drops from the cap. A tiny quantity of the mixture should be applied to the spin column (in a 2 ml collection tube), the cover should be sealed, and the spin column should be placed in the collection tube after centrifuging at 13,000 rpm for one minute. Spin at 13,000 rpm for one minute while adding 700 l of WA buffer to the spin column without soaking the rim. Use the collecting tube again, but discard the waste flow. Without wetting the rim, add 700 cc of buffer WEB to the spin column. Then, spin at 13,000 rpm for 1 minute. Place the column in a 2.0 mL collecting tube, discard the flow-through, and repeat the centrifugation process one more time for one minute to dry the membrane. The flow-through and collecting tubes must both be discarded. Put the spin column in a clean 1.5 ml tube and the membrane in direct contact with 30 to 100 ml of CE buffer. spin for one minute to elute after one minute of room temperature incubation.

PCR Primers

In order to create a stock solution with a final concentration of 100 pmol/l, lyophilized primers were first dissolved in free ddH₂O. After a stock solution was kept at -20 degrees, a work primer suspension with a concentration of 10 pmol/l was made. IDT researched this (integrated DNA technologies company, Canada).

Table 5: DNA extraction result.

Primers	Sequence	Amplicon	
<i>Coa</i>	F	CGAGACCAAGATTCAACAAG	810
	R	AAAGAAAACCACTCACATCAGT	
<i>mecA</i>	F	AAAATCGATGGTAAAGGTTGGC	533
	R	AGTTCTGCAGTACCGGATTTTGC	

Maxime PCR pre mix kit (20 l prescription) (cat.NO.25025)

The iNtRONs Maxime PCR PreMixkit includes a 2X master mix solution as well as a variety of premix kits designed for various skill levels. The Maxime PCR premix kit (i-taq) contains everything necessary for one PCR reaction in a single tube. Therefore, it is the most practical method for achieving the best results. A template DNA, a set of primers, and D.W. are all that are necessary to do PCR. The second component is the presence of a gel

loading solution for electrophoresis, which permits gel loading without the need for any preparation. Due to its speed and ease of use, it is suited for a range of samples.

Table 6: combination of the precise gene interactions for diagnosis

Components	Concentration
Taq pcr pre Mix	5.0 µl
Forward primer	10 picomols/ µl (1 µl)
Backward primer	10 picomols/ µl (1 µl)
DNA	1.5 µl
Distilled water	16.5 µl
Last volume	25.0 µl

The ideal parameters for initial denaturation and annealing have been identified after a number of trials. Gradient PCR was used to vary the temperature for all samples, and a range of 1.5 to 2.0 l of DNA template concentration was tested to determine the optimal conditions. These two factors are crucial when annealing primers with complement.

Table 7: DNA amplification program for *mec*.

PCR steps	Temperature	Time	Number of cycle
first-order denaturation	94°C	30 sec	1
Denaturation	94°C	30 sec	40
Annealing	52°C	30 sec	
Extension	72°C	1 min	
The last extension	72°C	10 min	

Table 8: combination of the precise gene interactions for diagnosis

Components	Concentration
Taq pcr pre Mix	5.0 µl
Forward primer	10 picomols/ µl (1 µl)
Backward primer	10 picomols/ µl (1 µl)
DNA	1.5 µl
Distilled water	16.5 µl
Last volume	25.0 µl

The ideal parameters for initial denaturation and annealing were established after several attempts. Gradient PCR was used to adjust the temperature for all samples, and a concentration range of 1.5 to 2.0 l was chosen for the DNA template concentration. These two elements play a crucial role in primer annealing with complement.

Table 9: The best circumstances for detecting the COA gene

PCR steps	Temperature	Time	Number of cycle
first-order denaturation	94°C	2 min	1
Denaturation	94°C	30 sec	30
Annealing	55°C	2 sec	
Extension	72°C	4 sec	
final prolongation	72°C	7 min	1

PCR product detection using agarose electrophoresis

3 l of the processor loading buffer (intron/Korea) and 5 l of purportedly to be electrophoresed DNA have been joined (loading dye). after the blending. The perforations in the gel will now be used in the loading process. Before a source of 336 nm UV light looked at the gel, It was placed in a swimming pool with 30 l of water and Red Safe Nucleic Acid Staining Solution. The gel was subjected to a 7 v/c2 electric current for one to two hours until the tincture reached the gel's opposing side.

Results

. Demographics of the Subject and *S. aureus*

This prospective, descriptive, and cross-sectional study was conducted at Baghdad's three largest hospitals. The size of the patient group was determined for practical reasons, and the recruiting approach remained unchanged for three months. The goal of the study was to find out how common MRSA is now, how it reacts to antibiotics, and how to identify it genetically in patients.

Randomly, 150 samples from different clinical departments were chosen for *S. Staphylococcus* spp. made up 55 (36.66%) of the aureus-positive isolates that were collected during the study.

Out of 55 species of *Staphylococcus*, 30 (54.4%) of the isolates were *S. aureus*. 10 of their isolates (33.33%) of *S. aureus* were MRSA.

The samples included three (30%) skin abscesses, two (20%) diabetic foot ulcers, two (20%) burn wounds, and one (10%) wound exudate. This study did not include environmental isolates or additional samples from the same individuals.

the Api 20 Staph system figure and common biochemical tests were used to identify the clinical isolates (1). Gram's staining, catalase, and oxidase tests were performed on all the organisms cultured on primary isolation and selective media, followed by slide and tube coagulase assays. Other biochemical tests were also carried out. All of the samples could be identified as staphylococci because they could all grow on mannitol salt agar (figure 2), oxidase negativity, catalase positivity, resistance to bacitracin, and sensitivity to novobiocin. One hundred ten of these staphylococci isolates had positive slide and tube coagulase findings. Based on categorization, 10 of these isolates exhibited α -hemolysis, which is characterized as a visible ring of hemolysis around the colony, and were capable of fermenting mannitol in mannitol salt agar.

Table 10: S. distribution isolates of uraeus from clinical samples

Patient profile	Age	No.(patient infected)	%
Age group (years)	1-10	2	1.33
	11-20	4	2.66
	21-30	4	2.66
	31-40	5	3.33
	41-50	8	5.33
	51-60	7	4.66
Gender	Male	13	8.66
	Female	17	11.33

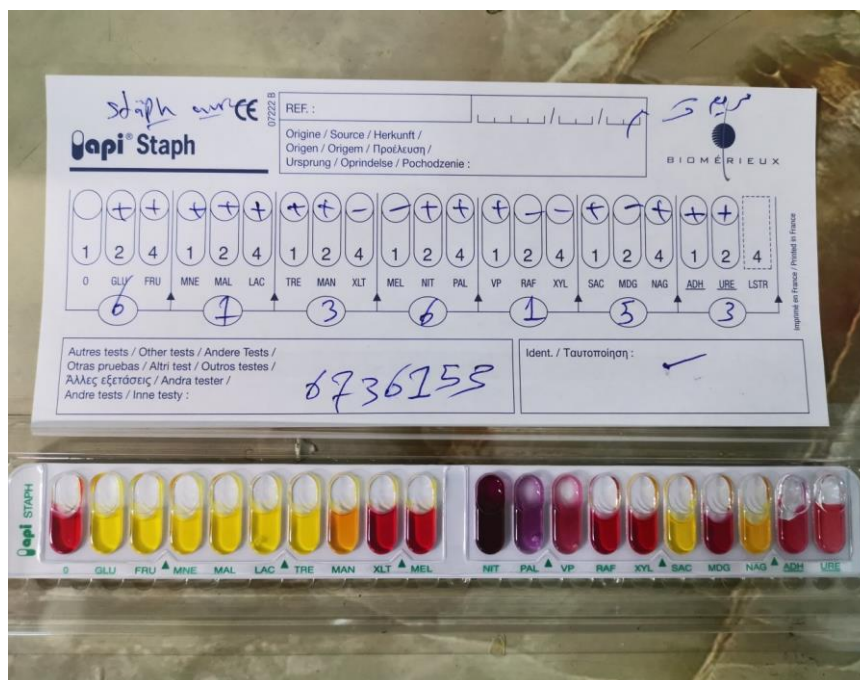


Figure 1: API20 Staph System for *S. aureus* isolates diagnosis (eg. glucose fermentation, lactose fermentation)



Figure 2: colonial morphology of *S. aureus* on mannitol salt agar showing round, smooth, small, yellow colonies and mannitol fermenter.

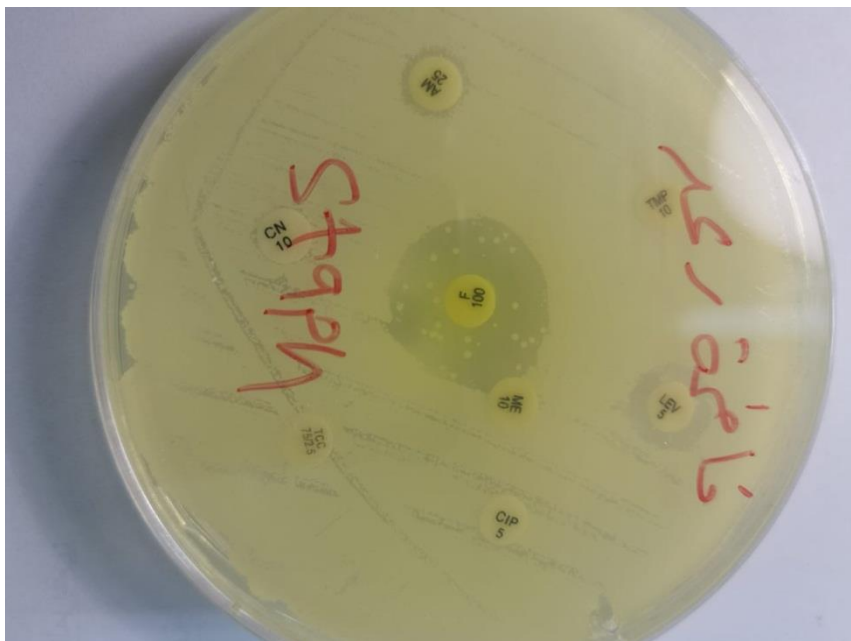


Figure 3: antibiotics sensitivity tests of *S. aureus* on muller hinton agar showing resistant of MRSA for most antibiotics especially for methicillin.

4.2. Vitek system identification: -

Among the biochemical test methods that are used to the identification of bacteria, vitek system for identification of *S. aureus*. The analytical profile index of this system shows probability 99% identification percentage. The test's outcomes are shown in figure 3.

bioMérieux Customer: Wahj AL-DNA
 Microbiology Chart Report
 Printed April 14, 2021 2:43:20 PM CDT

Lab ID: 1 A ast
 Organism Quantity:
 Selected Organism : *Staphylococcus aureus*
 Isolate Number: 1

Comments:

Susceptibility Information Analysis Time: 13.35 hours Status: Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Teicoplanin	1	S
Benzylpenicillin	0.25	R	Vancomycin	1	S
Oxacillin	2*	*R	Tetracycline	<= 1	S
Gentamicin	<= 0.5	S	Tigecycline	<= 0.12	S
Tobramycin	<= 1	S	Fosfomycin		
Levofloxacin	0.25	S	Nitrofurantoin	<= 16	S
Moxifloxacin	<= 0.25	S	Fusidic Acid	<= 0.5	S
Inducible Clindamycin Resistance	NEG	-	Mupirocin		
Erythromycin	<= 0.25	S	Rifampicin	<= 0.5	S
Clindamycin	<= 0.25	S	Trimethoprim/Sulfamethoxazole	<= 10	S
Linezolid	2	S			

*= AES modified **= User modified

AES Findings
 Confidence: Consistent with correction

Figure 4: antibiotics sensitivity tests of *S. aureus* by vitek system showing resistant of MRSA for most antibiotics especially for methicillin .

4.3. Methicillin-resistant *S. aureus* detection with CHROMagar

The possible causes of these isolates' varied expression of methicillin resistance are shown in figure (4). The majority of these isolates displayed resistance just beyond the inhibitory zone, earning them the moniker "moderately resistant *S.* according to Pillai et al. (2012).



Figure 5: The chromogenic media's MRSA colonies' appearance and colour. On CHROMagar, MRSA colonies from the Baghdad isolate are green.

4.4. Methicillin-Resistant *S. aureus* Detection Through molecular assay, aureus

PCR was used to determine whether *mecA* was present in the from (30) sample. Six isolates carried the 533 bp *mecA*-specific PCR product (MRSA). The remaining 24 isolates were *mecA*-negative (MSSA), indicating the prevalence of MRSA. Additionally, PCR amplification of the *coa* gene was carried out on all 30 isolates. 3 isolates have *S* expression. They all produced PCR results with a *S. aureus*-specific band, which supported the theory that they were all *S. aureus* isolates.

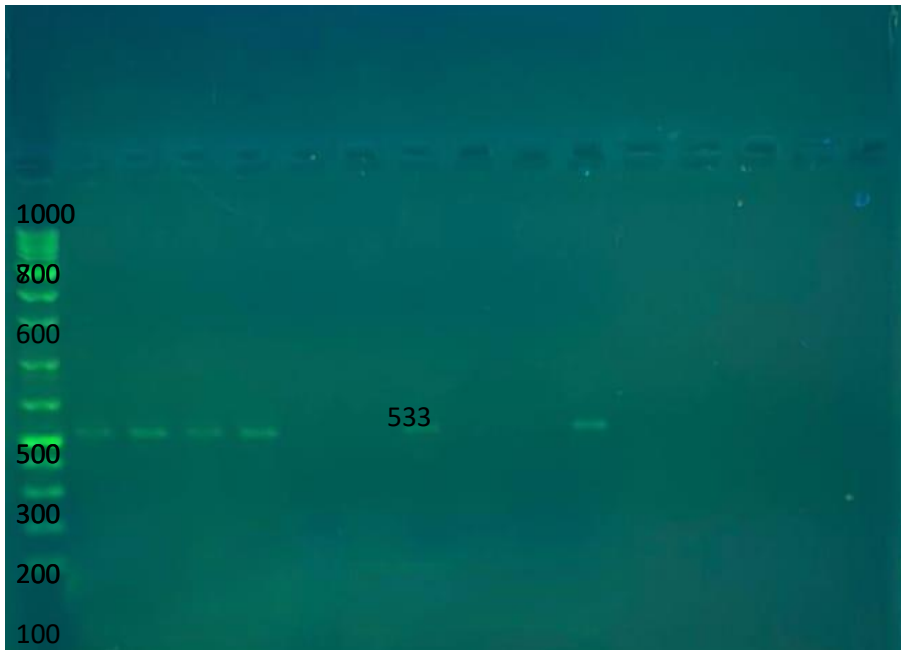


Figure 6: The usual banding patterns seen with a monopole PCR experiment are displayed on a gel. *MecA* gene PCR amplification from *S. aureus* with an amplicon size of 533 bp, is *aureus*. In a 2% agarose gel, DNA amplification products were electrophoretically separated. The electrophoresis was place for 1.5 hours at 70 volts.

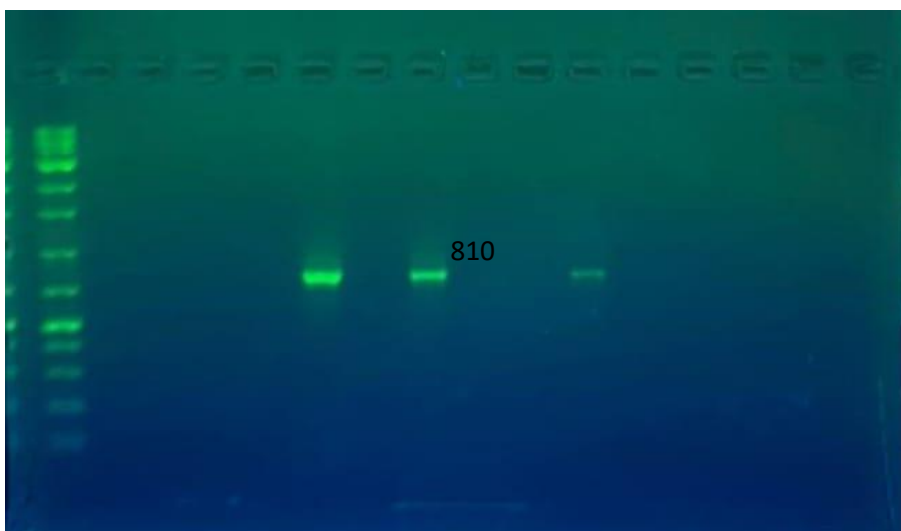


Figure 7: The usual banding patterns seen with a monoplex PCR experiment are displayed on a gel.

Coa gene PCR amplification from *S. aureus* 810 bp amplicon size for *aureus*. In a 2% agarose gel, DNA amplification products were electrophoretically separated. The electrophoresis was place for 1.5 hours at 70 volts.

Discussion

Aureus In addition to being a major infectious agent in boils, carbuncles, infected wounds, deep abscesses, and septicemia, *aureus* is a versatile bacterium that tends to infect most organ systems of the body. *Aureus* was found in 10 percent of the clinical samples in the current investigation. *aureus*, followed by samples of wound exudate, diabetic feet, burn wound infections, and wounds. Skin abscesses were frequently used to isolate *aureus*.

Numerous proteins that specifically interact with elements of the human cellular matrix are encoded by aureus. One of the most frequent microorganisms discovered to colonize burn wounds is aureus. Compared to other samples, aureus. 34% of burn wound cases were caused by aureus, the most isolated agent, although a study from Turkey has revealed that *Pseudomonas* spp.

One percent of the species isolated from wound infections were aureus. The existence of these microorganisms on human skin may help to partially explain aureus infection. Common surface bacteria called aureus are often able to enter wounds. aureus was the most frequently isolated bacterial pathogen from post-operative wound infections, Aureus is incredibly virulent and capable of invasion.

The presence of aureus was significant. aureus, which is thought to be the main pathogen linked to diabetic foot ulcers, has increased. Isolation of aureus was 2%. nasal colonization Despite being widespread, aureus plays a crucial role in the pathogenesis and spread of *S. aureus* infections serve as a reservoir for infections at other locations, such as surgical sites and bloodstream infections.

« aureus » in the nasal swab of its study subjects. « These can be challenging to identify from resistant isolates that possess the organism *Staphylococcus aureus*.

Conclusion

It may be deduced that the vitek system is a useful bacteriological test to confirm the diagnosis of MRSA since bacteriological technique settled the debate over the false negative and or false positive outcomes of some biochemical tests.

Molecular (PCR) assessments by *Meca* gene have an efficient diagnostic performance which can be included for confirmation of MRSA.

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