DOI: https://doi.org/10.53350/pjmhs22164920

ORIGINAL ARTICLE

Molecular Detection of Some Virulence Genes of Methicillin Resistant Staphylococcus Aureus Clinical Isolates in Diyala Province

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ABSTRACT

In this study a total of (264) clinical samples were collected from patients with different infections from Baquba and Al Batool Teaching Hospitals during the period from September 2021 to April 2022. The results demonstrated that 50(26.4%) of the isolates were S.aureus distributed according to the sources as follows: wound13 (26%), urine11 (22%), vaginal swab10 (20%), blood8 (16%), and burn8 (16%) by using blood agar and mannitol salt agar, microscopic characteristics, biochemical tests, Vitek-2 system. All the (50) isolates showed catalase positive and oxidase negative. On baired parker agar showed black, convex, and shiny colonies which approve the presence of S. aureus with a clear zone 34(68%) confirm coagulase-positive and 16(32%) of the isolates without a clear zone that confirms coagulase-negative. The detection of some virulence factors as ESBL 2%, Dnase 62%, gelatinase 42%, Beta hemolysin 82%, and 2% MBL. Biofilm formation was detected by Microtiter plate method: strong14%, moderate42%, and 44% weak. The antibiotic susceptibility tests against 16 different types of antibiotics by the Kirby Bauer disc diffusion method reported that the isolates were resistant to Oxacillin (98%), Vancomycin and Nitrofurantoin (100%) Netilimycin (14%) Amikacin and tetracycline (52%), Azithromycin (78%), Ciprofloxacin(62%), Levofloxacin(42%), Clindamycin(80%), Chloramphenicol(32%), Ofloxacin(40%), Gentamycin(56%), Teicoplanin(90%), Clarithromycin(74%), Imipenem(44%). The result showed 41(82%) multidrug resistance (MDR), 7 (14%) as extensive drug resistance (XDR), and the remaining 2(4%) pan drug resistance (PDR).

The minimum inhibitory concentration (MIC) was determined against vancomycin and azithromycin for seven isolates (strong biofilm formation) recorded that (16-1024) µg/ml for Vancomycin while the bacteria showed highly resist to Azithromycin. A polymerase Chain Reaction was performed for the detection of mecA and icaADB were detected in all seven isolates (100%), while coa and spa genes appeared (71.4%) in five isolates.

Keywords: MRSA, mecA, ica ADB, spa, coa gene

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a common source of community- and hospital-acquired infections. MRSA is currently responsible for ten times the number of infections caused by all MDR Gram-negative bacteria combined. The World Health Organization (WHO) recently designated MRSA as one of twelve priority infections that pose a hazard to human health. (Craft et al., 2019).

MRSA causes a variety of hard-to-treat illnesses in people, including skin and soft tissue infections, endocarditis, septicemia, osteomyelitis, pneumonia, enteritis, meningitis, and toxic shock syndrome (Li et al., 2018). There are multiple virulence factors in S. aureus. They are divided into components of bacterial cells involved in disease and chemicals excreted into the environment by bacteria. Structures of bacterial cells involved in pathogenesis are capsule, protein A, and teichoic acid. Also, almost all strains of S. aureus produce a group of extracellular products such as exoproteins: nucleases, hyaluronidase, lipases, proteases, and collagenase, moreover exotoxins: α-hemolysin, βhemolysin, γ-hemolysin, leukocidin, and PantonValentine leukocidin (PVL) (Bien et al., 2011). S. aureus infections and multidrug-resistant strains are becoming increasingly common, making clinical anti-infective treatment more challenging. In bacteria, resistance was developed through the integration of a complex system that included the efflux pump, drug target site change, enzyme inactivation, drug target site mutation, and horizontal gene transfer to acquire resistance determinants (Bitrus et al .,2018).

Detecting mecA gene by polymerase chain reaction is now considered the gold standard for finding methicillin resistance in S. aureus (Pillai et al., 2012), mecA gene is found in Staphylococcus chromosomes and impacts S. aureus pathogenicity by producing methicillin resistance in the Staphylococcus cassette chromosome genomic area (Gittens-St et al., 2020). Penicillin-binding protein-2a (PBP2a), a mobile extrinsic genetic element transported on a genomic island, is encoded by the mecA gene (SCCmec). (Alkharsah et al., 2018). As it blocks the active site from binding B-lactams, PBP-2a has a lower affinity for B-lactams than the usual penicillin-binding protein-2 (PBP2) generated by methicillin-susceptible S. aureus (MSSA) (Hussain, et al., 2019).

For DNA-based S. aureus diagnosis, the coa gene, which codes for the synthesis of coagulase enzyme, since the varied sequences at its 3' terminus, this gene is extremely polymorphic. Variations in amino acid sequences and the number of tandem repeats at the 3' end of the coagulase-producing gene have been discovered in a range of Staphylococcal species. This region, which contains 81 bp tandem repeats expressing repeated 27-amino-acid sequences in the C-terminal region, is heterogeneous (Reza Talebi et al., 2012).

ica operon is encoding biofilm formation, mediated by a polysaccharide intercellular adhesion (PIA), which is considered an important virulence factor in both S. epidermidis and S. aureus. However, the clinical effect of the ica locus and PIA production is less well described in S. aureus. (Eftekhar and Dadaei, 2011).

The gene encodes virulence factor protein A (SpA), which contains a variable polymorphic X region (Afrough et al., 2013). The molecular characterization of the X region of spa gene is documented as an exact method for typing S. aureus strains (Ruppitsch et al., 2006). So this study aimed to determine the prevalence of the clinical isolates of Methicillin Resistance S. aureus (MRSA) and Genotyping detection of some virulence and resistance gene of MRSA isolates in Diyala province.

MATERIALS AND METHODS

Collection of specimens: A total of 264 samples were collected from patients aged 7 days – to 50 years suffering from varied infections, including wound, burn, vaginal swabs, urine, and blood; these samples were collected in the proper way to avoid any possible contamination and exposure to laboratory tests such as catalase, oxidase, and culturing in mannitol salt agar as well as on Baird barker, this media also used to detect coagulase activity by adding fibrinogen plasma(BUCUR et al., 2017).

Phenotyping Detection of some virulence factors: The isolated and diagnosed bacteria were represented for the detection phenotypically of some virulence factors such as; hemolysin, gelatinase, and DNase production was done according to (Benson, 2001; Cappuccino and Welsh, 2020; Harley and Prescott, 2002).

Detection of β-Lactamase production

Detection of Metallo β-Lactamase (MBLs.): Suspensions of S. aureus isolates were made by transferring a single colony to 5 ml

of normal saline until it was comparable to 0.5 McFarland (after 24 hours of incubation), bacterial suspension seeded onto Muller Hinton agar plates, two Imipenem (10 µg) discs were placed 20 mm apart on the Mueller Hinton agar plate, and 5 µl of EDTA was added to one of the Imipenem discs, which was then incubated overnight at 37° C. (Anwar et al., 2016).

Detection of extended-spectrum β-Lactamase production: The ability of the isolates to create ESBLs was determined using the combined disk or double-disk diffusion test method (Patel et al., 2017). The inoculated isolates (comparable to 0.5 McFarland) are streaked on the surface of Muller–Hinton agar plates, in the center of the inoculation plate, an antibiotic disk with a combination of Amoxicillin/Clavulanic acid (30µg/disk) is inserted. Then, at a distance of 3 cm from the disk in the center, an antibiotic disk of Aztreonam and third-generation cephalosporin's Cefotaxime and Ceftazidime. The plates were incubated at 37°C for 24 hours. Zone inhibition of 5 mm or more in the presence of Augmentin is suggested as a positive result for the production of ESBL enzyme.

Quantitative Assay for Biofilm Production: According to the protocol, biofilm development was detected using a microtiter plate assay (Almeida et al., 2013). At 37°C for 24 hours, the bacteria were inoculated on a nutrient broth medium. Following that, using the same medium as the diluent, 200µl of isolate suspension was placed in each of the three wells of a 96-well flat-bottom polystyrene plate and incubated for 24 hours at 37°C. Following that, each well was washed three times with distilled water and vigorously shaken before being fully dried. A total of 200 µl of absolute ethanol was used to fix the adherent bacterial cells. After that, each well was dyed for 15 minutes with 200µl of 0.5% crystal violet. According to Tang et al., (2011), the amount of crystal violet eliminated by 95% ethanol in each well was quantified by measuring the OD 630 nm using an ELISA reader.

Antimicrobial susceptibility test: The disk diffusion method on Mueller-Hinton agar was used to assess the susceptibility of all isolates to different types of antibiotics, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2021).

Genotyping detection of some virulence genes of S. aureus by PCR Technique

Primers and their sequencing: The sequencing of primers used for the detection appeared in Table 1

Table 1: Primers used for gene detection (microgen, Korea)

Primer target gene	Sequence (Macrogen, Korea)	Annealing Temp.(°C)	Product size(bp)	Reference
mecA-F mecA-R	5 -TGG CTA TCG TGT CAC AAT CG-3 5 -CTG GAA CTT GTT GAG CAG AG-3	56	310	(Omar et al.,2014)
coa-F coa-R	5 -CGA GAC CAA GAT TCA ACA AG-3 5 -AAA GAA AAC CAC TCA CAT CA-3	55	650-730	(Omar et al.,2014)
Spa-F Spa-R	5 -ATC TGG TGG CGT AAC ACC TG-3 5 -CGC TGC ACC TAA CGC TAA TG-3	56	1100-1440	(Omar et al.,2014)
IcaADB-F IcaADB-R	5-AAA CTT GGT GCG GTT ACA GG-3 5-TCT GGG CCT GAC CAT GTT G-3	55	750	(Eftekhar and Dadaei, 2011).

The primers were supplied by the Macrogen Company in a lyophilized form. As a stock solution, lyophilized primers were dissolved in nuclease-free water to a final concentration of 100pmol/µl. To make a working primer solution, combine 10 µl of primer stock solution (stored at -20 C) with 90 µl of nuclease-free water to make a 10 pmol/µl working primer solution.

DNA Extraction: DNA of the isolated bacteria was extracted using the ABIO, pure extraction protocol as follows

100 µl of Nuclease-free water and 100 µl of Lysozyme solution were added to the pellet of S. aureus isolates and vortexed, then Incubated for 30 minutes at 37°C in a water bath. After incubation, samples were centrifuged at 13000 rpm for 2 minutes. After that, the supernatant was discarded. Then for protein digestion and cell lysis, 20µl of Proteinase K solution (20 mg/ml) and 200µl of Buffer BL was added to the sample, then the tube was vortexed rapidly and incubated at 56°C for 30 min. 200µl of 100% ethanol was added to the sample and pulse-vortexed. Carefully transfer all of the mixtures to the mini-column, centrifuge for 1 minute at 6,000 x g above (>8,000 rpm), 600µl of Buffer BW was Added to the mini-column, then centrifuged for 1 min at 6,000 x g above (>8,000 rpm). After that 700µl of Buffer TW was applied. Centrifuged for 1 min at 6,000 x g above (>8,000 rpm. The mini-column was Centrifuged at maximum speed (>13,000 x g) for 1 min to remove residual wash buffer, then the mini-column was transferred into a fresh 1.5 ml tube.

From Buffer AE 100µl was added and incubated for 1 min at room temperature, then centrifuged at 5,000 rpm for 5min.

Quantitation of DNA: In order to detect the quality of extraction for downstream applications, a Quantus Fluorimeter was utilized to detect the concentration of extracted DNA.

Agarose Gel Electrophoresis and DNA Loading: Preparation of agarose was carried out as 1.5 gm of the agarose was added to a tube filled with 100 ml of 1X TAE. The solution was heated in the microwave to boiling until all of the gel particles were dissolved, and 1 µl of Ethidium Bromide (10 mg/ml) was added (avoided stirring it), agarose solution was poured into the gel tray and allowed to set at room temperature for 30 minutes. After carefully removing the comb, the gel was deposited in the gel tray. 1X TAE-electrophoresis buffer was poured into the tray until it reached 3-5 mm above the gel's surface.

Each 5µl DNA sample received two microliters of loading dye, carefully placed in individual wells. The PCR products were directly loaded. 5µl of PCR product was directly put into each well. For 60 minutes, electrical power was turned on at 100v/mAmp.

Statistical analyses: Statistical analysis Fisher's test (GraphPad Prism version 6 software) turned into used for the assessment between samples of the contemporary examination. P-values less than 0.05 degrees have been considered statistically significant. P-value is as follows: P-value < 0.01.

RESULTS AND DISCUSSION

A total of 50 isolates of Staphylococcus aureus primary identified from different clinical sources, distrusted as follows: Wounds 13(26%) burn 8(16%) blood 8(16%), vaginal swab 10 (20%), urine 11(22%), all isolates were grown on Blood agar and Mannitol salt agar. Some isolates create clear β -hemolysis around their colonies in blood agar, whereas others exhibit gamma hemolysis. The result showed that catalase was positive while oxidase was negative. The identification of the isolated bacteria was confirmed by Vitek 2 System.

Table 2: Distribution of virulence factors among S.aureus isolates

Virulence factor	% of Positive result	% of Negative result
Gelatinase	42%	58%
Dnase	62%	38%
MBL	2%	98%
Coagulase	68%	32%
ESBL	2%	98%
Hemolysin		
Beta hemolysin	82%	-
Gamma hemolysin	18%	-
Biofilm	56%	44%
P value	*0.001	
Chi-square	289.6	

*P-value < 0.05 is significant

All isolates examined on Baird Parker agar had black, convex, and shiny colonies, indicating the presence of S. aureus with a clear zone 34(68%), confirming coagulase-positive, and some isolates without a clear zone 16(32%), confirming coagulase-negative.

Detection of some virulence factors: The results as shown in figure (2) some virulence factors were investigated and the production of hemolysin, gelatinase, DNase, biofilm, and β-lactamase were detected in all fifty isolates of S. aureus in this study, table (2).

Haemolysin production: The results shown in table (4-3) showed that 41 isolates (82%) of S. aureus produced hemolysin enzyme which was ß-hemolysis, and 9(18%) gamma hemolysis. our result is compatible with a local study in Baghdad by Al Ani and Al Meani (2018), Beta hemolysis was detected in (65.6 %) of isolates, and 34.4% of isolates did not give any zone (gamma hemolysis), while no alpha hemolysis has been detected.

Gelatinase production: All the isolates tested for the production of gelatinase found that only 21 (42%) of the isolates produced gelatinase by hydrolysis of gelatin. This result disagreed with Salman and Ali (2017) in the Diyala governorate who reported a high ability of staph aureus (100%) to produce this enzyme. A connective tissue protein is important in pathogenesis because it allows bacteria to hydrolyze gelatin and metabolize tiny peptides that result from that hydrolysis for energy. Gelatinized activity can increase their virulence, making them more pathogenic, especially in immunocompromised patients (Iseppi et al., 2020).

Dnase production: In all 50 isolates detected for the production of DNase was found that only 31(62%) of the isolate was Dnase producer. our result agreed with the study of Khwen et al (2021) showed that (66%) of isolates producer Dnase.

Biofilm production: The microtiter plate method was used to detect the biofilm formation for all isolates, and the results of the current study showed that 7(14%) isolates of Staphylococcus aureus was strong biofilm and 21(42%) was moderate biofilm while the isolate which weak 22(44%) as appeared figure (1). The prior study by Samadi et al (2018) results showed strong biofilm (1%) isolates, moderate (8.2%) isolate, and weak (54.1%) isolates, while (36.7%) of them had no attachment ability.

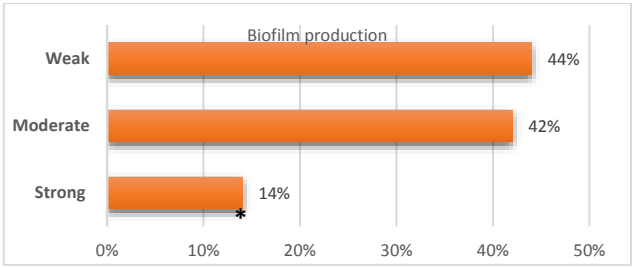

Figure 1: Biofilm production by S. aureus isolates. *Significant difference between percentages using Chi-square test (χ 2 -test) at 0.05 level.

Extended-spectrum β-lactamase enzyme production: It was used to detect the isolate's ability to produce ESBLs enzyme. The results showed that 1(2%) isolates ESBLs enzyme producer. The result of a local study done by Aziz (2020) showed that 34.8% ESBLs enzyme producer. According to their relative size and the genes they contain, S. aureus plasmids have been divided into three classes: class I, II, and III. These plasmids include a diverse set of genes that code for antibiotic resistance. The majority of Class III plasmids are conjugative plasmids (Foster & Geoghegan, 2015). The blaZ gene, which causes penicillin resistance, is carried by a transposable element that can be found either in the core chromosome or as part of a plasmid (Vestergaard et al., 2019).

Metallo β- Lactamase enzyme production: It was used to detect the isolate's ability to produce MBLs enzyme. The results showed that 1(2%) isolates MBLs enzyme producer. This result disagreed with the study of Chikkala (2012) which showed that 50% of isolates were produced. The use of B-lactams has resulted in the development of four major resistance mechanisms: (1) reduced membrane permeability or efflux via multidrug efflux pumps; (2) expression of PBPs with reduced affinity for -lactams or acquisition of "less sensitive variants"; (3) bypassing the crosslinking step with L, D transpeptidases; and (4) degradation of the antibiotic by -lactamases. The mechanisms involve enzymes that are related to peptidoglycan biosynthesis (Nikolaidis et al., 2014).

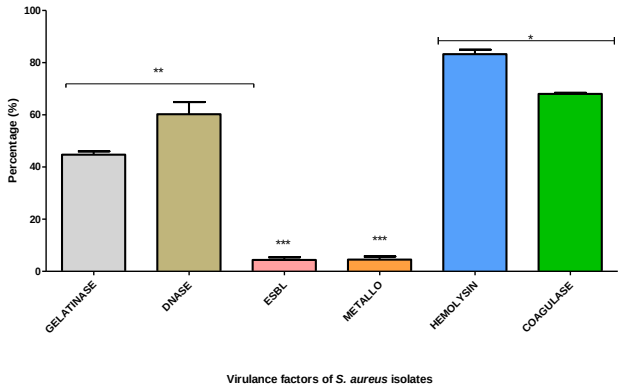

Figure 2: Virulence factors of s.aureus isolates

Bacterial Resistance to Antibiotics: Fifty S. aureus isolates were tested for 16 antibiotic discs from different kinds of antibiotics using the disc diffusion method, and it was found that isolates had varying levels of resistance to different antibiotics (Figure 4.4).

The results of this study 49 (98%) isolate appeared as MRSA and 1 (2%) isolates appeared as MSSA according to complete resistance toward the Oxacillin class of cell wall synthesis inhibitors. Our result agreed with the data published by Haneen and Al-Hamadany (2019) in the Al-Muthanna governorate who recorded that 100% of S. aureus isolates are resistant to oxacillin.S. aureus develops resistance to beta-lactam antibiotics through two mechanisms: beta-lactam penicillinase and the mecA gene. The first mechanism necessitates the development of penicillinase or beta-lactamase enzymes, both of which are found on plasmids and are encoded by the blaZ gene. This enzyme degrades the beta-lactam ring in beta-lactam antibiotic structures, rendering them inactive (Gnanamani et al., 2017). The second defense mechanism necessitates the acquisition of the mecA gene, which encodes the PBP2a protein that aids in bacterial cell wall construction even when beta-lactam antibiotics are present. (Harris et al., 2002). As well as the current study revealed that the resistance was 100% to Nitrofurantoin

The study showed that the isolates' resistance to quinolones antibiotics, which include ofloxacin, ciprofloxacin, and levofloxacin was at 40%, 42%, and 62%, respectively. Aminoglycosides which include gentamycin, amikacin, and Netlimicin, the isolates have appeared level of resistance to gentamicin (56%) Amikacin (52%), while the resistance rate of Netlimicin14% this result agrees with Al-Mayahi and Srhan (2021) showed Resistant to netilmicin (11.1%). Macrolides which include azithromycin whose results indicated the percentage of S. aureus isolates (78%). Lincosamides which include clindamycin results showed that 80% of S. aureus isolates were resistant to clindamycin. Phenicol includes chloramphenicol classes of protein synthesis inhibitors antibiotics which S. aureus isolates were resisted to chloramphenicol by 32%. Tetracyclines include tetracycline the result of our study detected that 52% of the isolates resisted. Glycopeptides include vancomycin and teicoplanin which is cell wall inhibitors, study showed that the resistance rate of vancomycin and teicoplanin was at 100%, 90% this result agrees with the local result study by Albadri (2021) was mentioned to the resistance rate of vancomycin (92.3%) as well the study in Al-Diwaniya by Al-Mayahi and Srhan (2021) found the percentage

resistant 61.2% to teicoplanin. Carbapenems include imipenem the results showed 44% the resistance rate.

Table 3: Resistance percentage and sensitivity and the number of isolates that were resistant to each antibiotic tested.

No. of antibiotic	Antibiotics symbol and its concentration µg	Number and percentage of resistant isolates	Number and percentage of sensitive isolates
1	Netilimycin (30)	7(14%)	43(86%)
2	Chloramphenicol (30)	16(32%)	34(68%)
3	Ofloxacin (5)	20(40%)	30(60%)
4	Levofloxacin (5)	21(42%)	29(58%)
5	Impenem (10)	22(44%)	28(56%)
6	Amikacin (30)	26(52%)	24(48%)
7	Tetracycline (30)	26(52%)	24(48%)
8	Gentamycin (10)	28(56%)	22(44%)
9	Ciprofloxacin (5)	31(62%)	19(38%)
10	Clarithromycin (15)	37(74%)	13(26%)
11	Azithromycin (15)	39(78%)	11(22%)
12	Clindamycin (2)	40(80%)	10(20%)
13	Teicoplanin (30)	45(90%)	5(10%)
14	Oxacillin (1)	49(98%)	1(2%)
15	Nitrofurantoin (300)	50(100%)	0(0%)
16	Vancomycin (30)	501(00%)	0(0%)

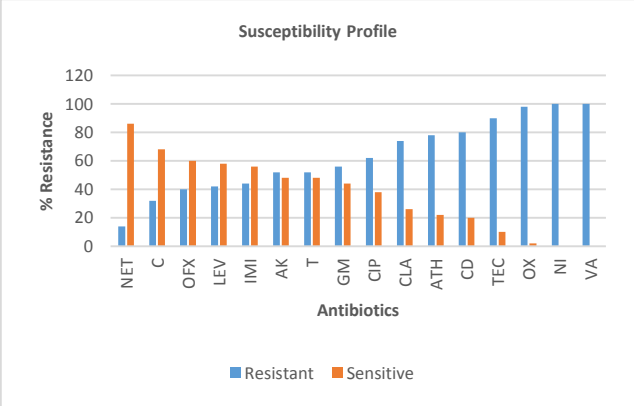

Figure 3: Antibiogram profile of S. aureus isolates using disk diffusion method: In general there is a Significant difference between percentages using the Chi-square test (χ 2 -test) at 0.05 level.

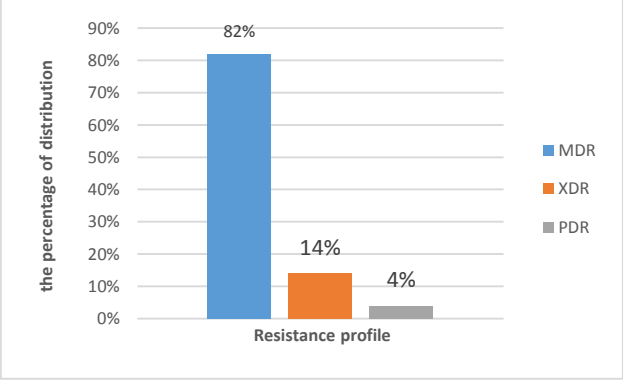

Figure 4: Bar graph of the percentage distribution of multidrug-resistant S. aureus (MDR), extended-drug-resistant S. aureus (XDR), and pan-drug-resistant S. aureus (PDR). P value *0.001 Chi-square 279.1

Multidrug Resistance Patterns in Isolates: All fifty isolates in the study showed a multi-drug resistance (MDR, XDR, and PDR) level of resistance to the tested antibiotics, with a rate of 100%. The incidence of Multi-drug resistance (MDR), extensive drug resistance (XDR), and pan-drug resistance (PDR) was investigated among isolates of 50 S. aureus. MDR isolates were defined as isolates demonstrating resistance to antimicrobials from at least three of all antimicrobial categories tested in this study. While the definition of extensive drug resistance (XDR) is an isolate that is resistant to all but one or two classes. PDR isolates are non-susceptible to all seven antimicrobial categories tested (Magiorakos, 2012). Our result showed that 41(82%) were Confirmed as MDR, 7 (14%) of them were confirmed as XDR, and the remaining 2(4%) were PDR isolates. Antibiotic resistance has evolved as a major problem as a result of the extensive and indiscriminate use of these medications in treatment, particularly among S. aureus bacteria. This could be due to the creation of biofilms, which increases the pathogenic ability (Gomes et al., 2019).

Genotyping detection of S. aureus

Extraction of Genomic DNA and Detection of mecA, coa, spa, icaADB genes: Seven isolates were chosen that formed strong biofilm. The DNA extraction kit approach yielded successful and efficient results, with all extracted DNA being intact and pure. Electrophoresis on a 1% Agarose gel was used to identify the extracted DNA, which was stained with ethidium bromide, electrophoresed at 70 volts for 1 hour, and photographed using an ultraviolet (UV) transilluminator. The concentration and purity of DNA were assessed using a Quantus Fluorometer after DNA extraction, with a range of (1.5-1.8) depending on the measured absorbance of the DNA using a spectrophotometer. Among 7 isolates that detected virulence factors genes and resistance, the results achieved by using PCR to (mecA, coa, spa, icaADB) revealed that 7(100%) isolates carried mecA gene which showed bands of 310 bp as a product size (figure5) Another result converged with what was revealed by Angel et al (2019) and Dhungel et al (2021) in Nepal showed (93.8%), (82.1%) of isolates had mecA gene. The results of coa gene revealed that was identified in 5 isolates (71.4%), which showed bands of (650-821) bp as a product size. (figure6). A study in AL-Diwaniyah City reported by Hezam (2019) mentioned that the presence of coa gene was 86.6%. The variability in the size of coa bands may be due to the presence of different allelic forms of coa gene in MRSA, allowing one strain to produce multiple amplicons (Goh et al., 1992).

The results revealed that spa gene was identified in 5 isolates (71.4%), which showed bands of (1100-1440) bp as a product size as showed figure (7). In a local study by Mohammed et al (2021) was (83.1%) showed the spa gene PCR products with different sizes, The results IcaADB gene revealed that was identified in 7 isolates (100%), which showed bands of (750) bp as a product size as in figure (8) .local study in Al-Najaf by Almayali et al . (2018) for IcaA and IcaD revealed that (97.7%) of Staphylococcus aureus isolate gave positive for IcaA (93.3%) from specimens gave a positive result of IcaD.

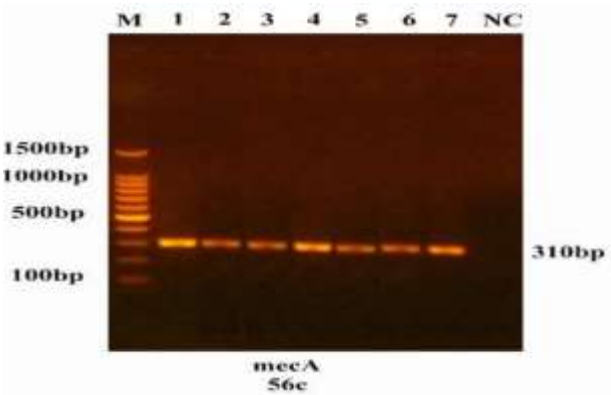

Figure 5: Results of the amplification of mecA gene of S. aureus were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker.

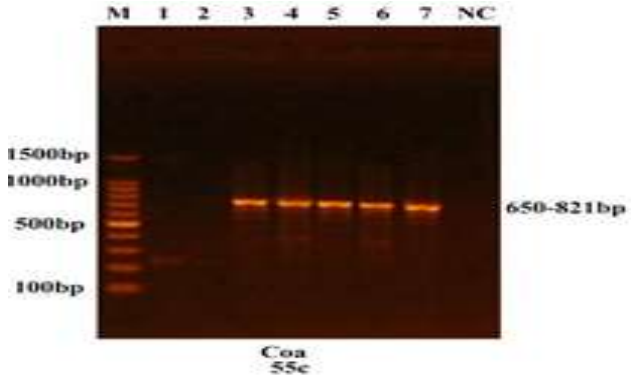

Figure 6: Results of the amplification of coa gene of S. aureus were fractionated on 1% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker.

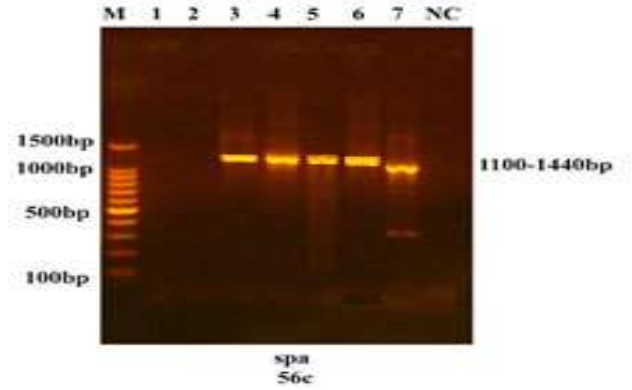

Figure 7: Results of the amplification of spa gene of S. aureus were fractionated on 1% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker.

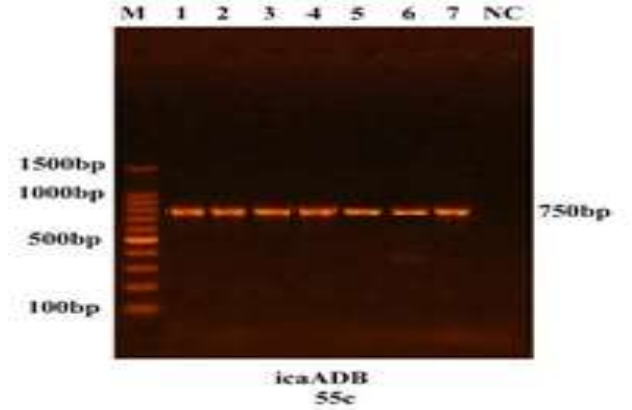

Figure 8: Results of the amplification of icaADB gene of S. aureus were fractionated on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker.

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