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Genotyping of Methicillin Resistant *Staphylococcus aureus* Clinical Isolates in Sulaimani City using Pulsed -Field Gel Electrophoresis

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ABSTRACT
Methicillin-resistant *Staphylococcus aureus* (MRSA) strains cause serious nosocomial infections. The objective of this study was to detect and find the epidemiological relatedness among MRSA isolates which was collected in Emergency burn-and-plastic surgery hospital in Sulaimani city using Pulsed-filed gel electrophoresis (PFGE). Routine methods were used for detection of *Staphylococcus aureus*, further molecular confirmation was done by PCR assays targeting *16S rRNA* gene. The cefoxitin-disc diffusion assay was used for detection MRSA, which is subsequently confirmed by the presence of the *mecA* gene. PFGE was chosen to observe the genetic relatedness of 10 MRSA strains using *Sma*I restriction endonuclease. All the isolates (100%, 50/50) carried *16S rRNA* gene specific for *S.aureus* and (42%, 21/50) exhibited resistance to cefoxitin and harboured the *mecA* gene, thereby being classified as MRSA. Among 10 MRSA isolates, the PFGE pattern were grouped into four clusters. Three isolates belonging to cluster (C3) were indistinguishable, 4 isolates belonging to cluster (C4) were closely related, 2 isolates of the second cluster (C2) were possibly related and the first cluster (C1) with 1 MRSA isolate was unrelated. This finding shows 30% of the samples had indistinguishable fingerprints with no changes in banding profiles, while 40% of the samples had closely related patterns. PFGE is a good discriminatory tool for typing MRSA strains.

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التنميط الجيني للعزلات السريرية للمكورات العنقودية الذهبية المقاومة للميثيسيلين في مدينة السليمانية باستخدام تقنية الترحيل الكهربائي الهلامي النبضي

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الملخص

تسبب سلالات المكورات العنقودية الذهبية المقاومة للميثيسيلين عدوى المستشفيات الخطيرة داخل المستشفيات. .الهدف من هذه الدراسة هو الكشف عن العلاقة الوبائية بين عزلات MRSA التي تم جمعها من حالات الحروق الطارئة و الجراحة التجميلية في مدينة السليمانية باستخدام الرحلان الكهربائي للهلام النبضي(PFGE). تم استخدام الطرق الروتينية للكشف عن *Staphylococcus aureus* ، وتم التأكيد بواسطة فحص PCR التي استهدفت جين *16S rRNA* ,كما استخدم فحص انتشار قرص سيفوكسيتين للكشف عن MRSA وتم تأكيد الفحص من خلال الكشف عن جين *mecA*.تم اختيار PFGE لتحديد الارتباط الوراثي لـ ١٠ سلالات MRSA باستخدام انزيم القطع *Sma*I. جميع العزلات ١٠٠٪ (٥٠/٥٠) حملت جين *16S rRNA*الخاص بالبكتريا العنقودية الذهبية. وأظهرت ٥٠/٢١ (٤٢٪) من العزلات مقاومة للسيفوكسيتين واحتوائها على جين *mecA* وتم اعتبارها ضمن مجموعة MRSA. من خلال نتائج PFGE تم تقسيم ١٠ عزلات الى أربع مجاميع؛ المجموعه (C3) شملت ٣ عزلات غير مميزه ، وكانت ٤ عزلات تنتمي إلى المجموعة (C4) وهي مرتبطة ارتباطًا وثيقًا ، المجموعة الثانية (C2) و التي احتوت على عزلتين اعطت احتماليه الارتباط, اما المجموعة الأولى (C1) والتي شملت عزله واحدة فقط فقد كانت غير مرتبطة. تُظهر هذه النتائج أن ٣٠٪ من العينات لا يمكن تمييزها مع عدم وجود تغييرات في نمط قطعDNA ، في حين أن ٤٠٪ من العينات لديها أنماط مرتبطة ارتباطًا وثيقًا. لذلك، نستنتج أن تفشي المرض حدث خلال فترة أخذ العينات.

الكلمات المفتاحية: المكورات العنقودية الذهبية، المكورات العنقودية الذهبية المقاومة للميثيسيلين، جين mecA, *16S rRNA*,. PFGE

Introduction

Nowadays, drug-resistant *S. aureus* isolates have become a serious issue in most hospitals. MRSA isolates cause infections that are severe and difficult to treat. In order to track the spread of MRSA, it is important to type isolates from more than one source[1], as a result, a number of Various molecular genotyping approaches are involved. Methods like *SCCmec* typing, *spa* typing, restriction fragment length polymorphism (RFLP), ribotyping, multi locus sequencing typing (MLST),multi locus variable tandem repeat (MLVA), fluorescence amplified-fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE) are all included [2]. Due to its great discriminative power, the molecular typing technique of pulsed-field gel electrophoresis (PFGE) is widely used for epidemiological research in a wide range of bacterial infections across the world. It's crucial for identifying outbreak sources and preventing the spread of them[3,4].The PFGE method is considered the "gold standard" of molecular-based typing methods for infectious agents and nosocomial infections. This technique enables precise clustering to be made during epidemics, and it simply differentiates epidemiologically connected cases from spontaneous cases[5].However, it has some drawbacks, in spite of time-consuming and pricey, this technique necessitates the use of specialized instruments and a high level of technical expertise, in addition to low comparability between different laboratories [6]. Because of this, the National Center for Disease Control and Prevention's Division of Healthcare Quality Promotion has created a PFGE protocol to unify and improve the molecular strain typing of different clinically important pathogens that are under routine surveillance or investigation by the Division[7]. In this study, for the first time in Sulaimani city, PFGE molecular typing techniques were employed to investigate MRSA clones among patients in Emergency burn-and-plastic surgery.

Materials &Methods

Samples collection

The authors had access to 50 *S. aureus* strains isolated from different clinical samples (burn, blood and urine) taken from patients at Emergency burn-and-plastic surgery hospital in Sulaimani city between January and December 2020.The acquired samples were transported to the advanced research microbiology laboratory and advanced research molecular laboratory in the biology department at Sulaimani University for further investigations.

Bacterial identification

The samples were cultured on blood agar and incubated overnight at 37 °C. Subsequently, *S. aureus* was finally verified using conventional tests (Gram's stain, catalase, coagulase, and growth on mannitol salt agar)[8].

Molecular confirmation of *S.aureus*

Bacterial DNA was extracted using a DNA extraction kit according to the manufacturer's instructions (Geneaid Bioteck Ltd- Taiwan). A set of primers (Sinaclone, Iran) was used to target the*16S rRNA* gene by PCR (Applied-Biosystem, USA). PCR reactions were carried out in a final volume of 20 µL including; 5µLThe OnePCRTM Ultra master mix (Gene Direx-Taiwan), 1µl of each primer (10 pmol), 11µl of nuclease-free water, and 2µl of purified DNA template were used. Thermal cycler was used for the amplification (Applied-Biosystem, USA). The primer sequences and conditions of *16S rRNA* are summarized in Table 1. The evaluation of the quantity and quality of the extracted DNA samples was conducted using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

Phenotypic & Genotypic detection of MRSA

All *Staphylococcus aureus* isolates were tested using 30 µg cefoxitin discs (Hi-Media). A bacterial suspension (McFarland 0.5) inoculated on Mueller–Hinton agar. After incubation for 16–18 hr at 37°C, the CLSI guidelines were applied to determine the growth inhibition zones, and methicillin-resistant isolates were identified accordingly [9]. Further confirmation was done through genotypic detection by targeting *mecA* gene. 5µL The OnePCRTM Ultra master mix (Gene Direx-Taiwan), 1µL of each primer (10 pmol), 11µL of nuclease-free water, and 2µL of purified DNA template were used in PCR reactions in a total volume of 20µL (Table 1).

Table 1: Primer sequence and conditions of *16S rRNA* and *mecA* genes

Genes Name	Name of primers	Primer sequence	Primer conditions	Length (bp)	References
16S rRNA	*16S* F	AACTCTGTTATTAGGGAAGAACA	Denaturation 94℃ 5min, Annealing 55℃ 45 s, No. of cycles 35, Final extension 72℃ 7 min	756	[10]
	16S R	CCACCTTCCTCCGGTTTGTCACC			
Methicillin Resistant	*mecA* F	CCTAGTAAAGCTCCGGAA	Denaturation 94℃ 5min, Annealing 50℃ 45 s, No. of cycles 35, Final extension 72℃ 7 min	310	[11]
	mecA R	CTAGTCCATTCGGTCCA			

Gel Electrophoresis

The PCR amplified products were stained with ethidium bromide and analyzed by 1% agarose gel electrophoresis. 2 µL of the product was run through the gel using electrophoresis at 90 volts for one hour. The length of the amplified PCR product was validated using a 100 bp DNA ladder. Images were acquired using a Bio-Rad Gel Doc XR+ imaging system [12].

DNA sequence analysis

Twenty-one MRSA isolates carrying the *16S rRNA* and *mecA* genes were chosen for DNA sequencing, 20µl of PCR products, 2µl of forward and 2µl of reverse primers were sent to (Sinaclon, Iran) for sequencing, the sequenced genes examined in this study were recorded in the NCBI database under the following GenBank accession numbers: ON17148 to ON171501.

Pulsed-Filed Gel Electrophoresis

PFGE was carried out in accordance with the Centres for Disease Control and Prevention (CDC) protocol (https://www.cdc.gov/pulsenet/pathogens/pfge.html). using CHEF Mapper XA Pulsed Field Electrophoresis System (Bio-Rad, USA) and CHEF Genomic DNA Plug Kits for Bacteria (Bio-Rad, USA) with few modifications [13].

Preparing Cell Suspension

The *S. aureus* isolates were grown at 37 °C in 5 ml of Trypticase soy broth (QUELAB ,UK) for 24 hrs, 1 ml of the overnight bacterial culture was harvested and re-suspended 1 ml Trypticase soy broth media and put in shaker incubator for about 4 hours. The cell suspension concentrations were adjusted by saline using a spectrophotometer to an absorbance of 0.8 to 1.0 at the wavelength of 600 nm using an eppendorf tube a 0.5 ml of adjusted cell suspension was centrifuged at 14,000 rpm for 5 min (Eppendorf, Germany), then the supernatant was aspirated. The cells were re-suspended in 200µl cell suspension buffer with 3 µl of lysostaphin stock solution (1 mg/ml in 20 mM sodium acetate (pH 4.5) was added and vortexed gently.

Plug formation

After that, an equal volume of bacterial-lysostaphin suspension was mixed with preheated 2% CleanCut agarose (Bio-Rad, USA) at 56 °C then quickly100 µl dispensed in a disposable mold. The plugs were allowed to solidify at room temperature for 30 minutes.

Cell lysis

After solidification, placing the plug in an eppendorf tube containing 300 µl of lysis buffer (1M tris, 0.5 M EDTA, 0.5% Brij 58, 0.2% sodium deoxycholate,0.5% sodium laureyl sarcosine) at 37 °C for 1hr without shaking. Subsequently, lysis buffer was removed; the plugs were incubated overnight at 55°C with 2.5ml Proteinase K buffer. The plugs were then washed, 4 to 5 times in a shaking incubator for 1hr in 1 × Wash at 50°C, during the third wash 100 µl of 1mM PMSF (Sigma; St. Louis, MO USA) was used. The plug was then moved to 1 ml of fresh 1x wash-buffer for storage at 4°C until used.

Restriction-endonuclease digestion

The plug slices of 3 × 5 mm-wide were placed in a 1.5 ml microcentrifuge tube containing 200 µl of 1X restriction buffer with 10 U of *Sma*I (Sigma; St. Louis, MO USA), and incubated overnight at room temperature.

Gel casting

The plug slices were loaded directly on the end of the comb tooth before placing the comb into the comb holder, and the equilibrated 1% Megabase agarose (Bio-rad, USA) and poured carefully into a gel tray. The wells containing the plug slices were sealed with 1% Megabase agarose gel. Positioned in a contour-clamped homogeneous electric field (CHEF) (Biorad) tank and submerged in 2000 ml of 0.5 × Tris-Borate-EDTA (TBE agarose was carefully poured into the gel casting platform.

Running conditions

The following were the running parameters: initial pulse, 5.3 s; final pulse, 34.9 s; voltage, 6 V/cm; time, 15 hours; and temperature, 14°C. Following the completion of the electrophoresis run, the gel was stained with ethidium bromide (1 g/ml, Invitrogen) for 20 minutes in a covered container, de-stained in fresh distilled water for 45 minutes, and viewed using The Gel Doc XR+ imaging system (Bio-rad, USA) [7]. The criteria for interpretation of PFGE banding pattern is summarized in Table 2[14].

Table 2: Criteria for interpreting PFGE patterns [14].

Category	No. of genetic differences compared with outbreak strain	Typical no. of fragment differences compared with outbreak pattern	Epidemiologic interpretation
Indistinguishable	0	0	Isolate is part of the outbreak
Closely related	1	2-3	Isolate is probably part of the outbreak
Possible related	2	4-6	Isolate is possibly part of the outbreak
Different	≥3	≥7	Isolate is not part of the outbreak

Results

In the current investigation, all *S. aureus* isolates (50/50) were at first identified by the examination of their morphological characteristics, such as growth on manitol-salt agar and gram stain, as well as performing two biochemical assays, namely catalase and coagulase tests. Figure 1 shows the morphological and biochemical characterization of *S. aureus*. Additional validation was conducted utilizing molecular methodology targeting two genes, particularly the *16S rRNA* and *mecA* genes.

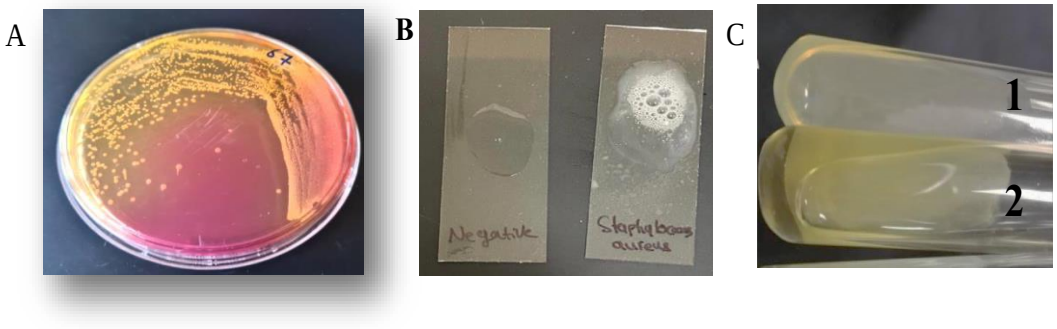

Fig 1: Identification of *S.aureus*

A:*S. aureus* colonies on Manitol-salt agar media. **B**: Catalase activity of *S.aureus* . **C**: Tube coagulase test: 2+ve test, 1-ve test.

Cefoxitin disc diffusion test

A total of 50 samples were analyzed, and 21 of them (42%) demonstrated resistance to cefoxitin according to CLSI results. The results of the study revealed that those isolates of *S. aureus* were MRSA, whereas a majority of the strains, specifically 58%, were recognized as methicillin-susceptible *Staphylococcus aureus* (MSSA), as depicted in Figure 2.

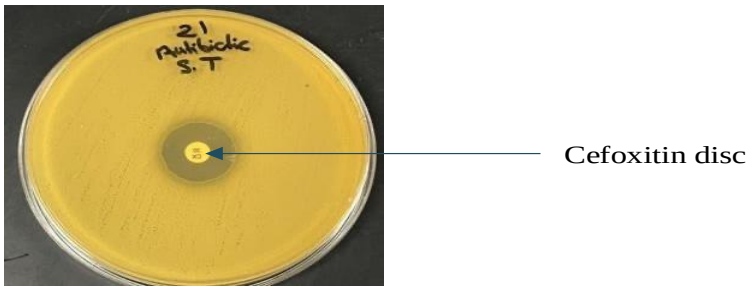

Fig 2: Cefoxitin disk susceptibility pattern of MRSA isolate on Muller-Hinton agar.

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DNA purification and quantitation

The purity and quantity of extracted DNA was evaluated as illustrated in table 3.

Table 3: Extracted DNA purity and quantity

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
Blank	ND2000	2/6/2022 9:54:32 AM	5	ng/µl	0.1	0.052	1.95	0.81	DNA	50
1	ND2000	2/6/2022 9:54:48 AM	5	ng/µl	0.1	0.058	1.71	0.79	DNA	50
2	ND2000	2/6/2022 9:57:34 AM	9	ng/µl	0.18	0.107	1.68	0.8	DNA	50
3	ND2000	2/6/2022 10:00:08 AM	3.8	ng/µl	0.076	0.036	2.09	0.66	DNA	50
4	ND2000	2/6/2022 10:01:57 AM	13.9	ng/µl	0.278	0.138	2.01	1.31	DNA	50
5	ND2000	2/6/2022 10:03:01 AM	15.8	ng/µl	0.315	0.164	1.92	1.11	DNA	50
6	ND2000	2/6/2022 10:05:42 AM	36.8	ng/µl	0.735	0.414	1.78	0.88	DNA	50
blank	ND2000	2/6/2022 10:06:41 AM	-0.7	ng/µl	-0.014	-0.017	0.79	0.5	DNA	50
7	ND2000	2/6/2022 10:07:53 AM	9.1	ng/µl	0.182	0.089	2.04	1.13	DNA	50
8	ND2000	2/6/2022 10:10:18 AM	83.8	ng/µl	1.677	1.167	1.44	0.79	DNA	50
9	ND2000	2/6/2022 10:12:57 AM	16.5	ng/µl	0.33	0.225	1.46	0.57	DNA	50
10	ND2000	2/6/2022 10:14:27 AM	7.8	ng/µl	0.155	0.088	1.76	0.82	DNA	50
11	ND2000	2/6/2022 10:23:15 AM	8.5	ng/µl	0.17	0.081	2.09	0.62	DNA	50
18	ND2000	2/6/2022 10:24:20 AM	32	ng/µl	0.64	0.33	1.94	1.59	DNA	50
54	ND2000	2/6/2022 10:25:41 AM	37	ng/µl	0.741	0.401	1.84	1.39	DNA	50
blank	ND2000	2/6/2022 10:27:15 AM	0.3	ng/µl	0.006	-0.007	-0.94	1.69	DNA	50
20	ND2000	2/6/2022 10:29:40 AM	35.6	ng/µl	0.712	0.451	1.58	0.77	DNA	50
33	ND2000	2/6/2022 10:31:18 AM	7.8	ng/µl	0.155	0.079	1.96	0.87	DNA	50
75	ND2000	2/6/2022 10:32:41 AM	57.3	ng/µl	1.145	0.796	1.44	0.64	DNA	50
66	ND2000	2/6/2022 10:33:55 AM	25.6	ng/µl	0.513	0.239	2.14	1.66	DNA	50
blank	ND2000	2/6/2022 10:36:35 AM	0	ng/µl	-0.001	-0.009	0.08	0.14	DNA	50
21	ND2000	2/6/2022 10:41:31 AM	24.7	ng/µl	0.495	0.251	1.97	1.38	DNA	50
blank	ND2000	2/6/2022 10:44:58 AM	-0.2	ng/µl	-0.004	-0.01	0.42	0.33	DNA	50
65	ND2000	2/6/2022 10:46:53 AM	29.2	ng/µl	0.583	0.32	1.82	1.3	DNA	50
52	ND2000	2/6/2022 10:49:51 AM	21.8	ng/µl	0.435	0.282	1.55	2.4	DNA	50
blank	ND2000	2/6/2022 10:52:34 AM	-0.9	ng/µl	-0.019	-0.024	0.8	0.71	DNA	50
17	ND2000	2/6/2022 10:56:49 AM	12.5	ng/µl	0.249	0.16	1.56	0.74	DNA	50
27	ND2000	2/6/2022 10:58:36 AM	21.1	ng/µl	0.421	0.202	2.09	1.51	DNA	50
67	ND2000	2/6/2022 10:59:55 AM	22.2	ng/µl	0.444	0.293	1.51	0.67	DNA	50
31	ND2000	2/6/2022 11:01:05 AM	21	ng/µl	0.421	0.187	2.25	1.32	DNA	50
32	ND2000	2/6/2022 11:02:43 AM	5	ng/µl	0.1	0.042	2.38	1.02	DNA	50
blank	ND2000	2/6/2022 11:04:01 AM	-0.4	ng/µl	-0.008	-0.019	0.41	0.42	DNA	50
55	ND2000	2/6/2022 11:05:06 AM	4.9	ng/µl	0.098	0.051	1.91	0.6	DNA	50
50	ND2000	2/6/2022 11:06:20 AM	146.2	ng/µl	2.925	2.238	1.31	0.65	DNA	50

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Genotypic confirmation of *S.aureus* and MRSA

Both *S.aureus* and MRSA have been confirmed using PCR to verify the presence of specific *16S rRNA* and *mecA* genes, respectively. All of the *S.aureus* isolates, including MRSA and MSSA (50/50), were positive for the presence of *16S rRNA*. Twenty-one out of fifty cefoxitin-resistant MRSA isolates also tested positive for the *mecA* gene shown in Figure 3 and Table 4.

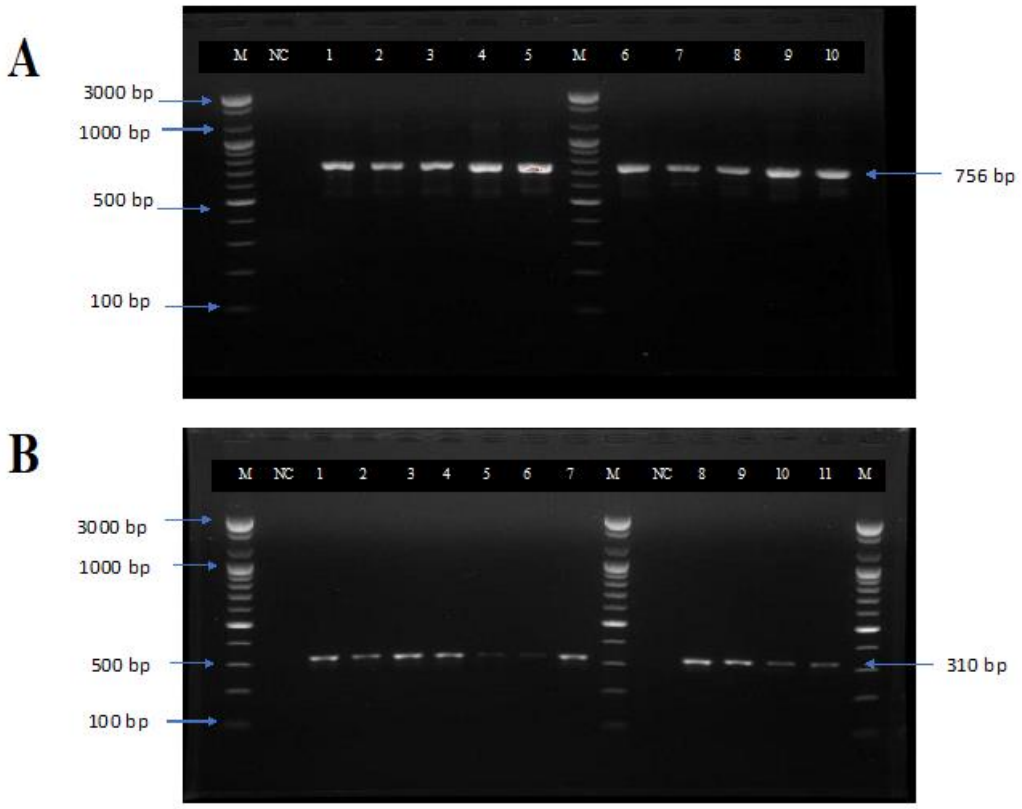

Fig 3: Gel electrophoresis image of PCR amplification of *16S rRNA* and *mecA* genes.

A: Amplified fragments of *16S rRNA* gene (756 bp) in *S.aureus* (1-10), Negative control (NC), Lane M: DNA ladder 100bp. **B**: PCR amplification of *mecA* gene (310 bp) of *S.aureus* (1-11), Negative control (NC), Lane M: DNA ladder 100bp.

Table 4: Bacterial source and the rate of cefoxitin resistance and *mecA* gene

Isolates	Source	Hospital	MRSA	*MecA*
Suli1	Burn wound swab	Emergency hospital	+	+
Suli2	Burn wound swab	Emergency hospital	+	+
Suli3	Burn wound swab	Emergency hospital	+	+
Suli4	Burn wound swab	Emergency hospital	+	+
Suli5	Burn wound swab	Emergency hospital	+	+
Suli6	Burn wound swab	Emergency hospital	+	+
Suli7	Burn wound swab	Emergency hospital	+	+
Suli8	Burn wound swab	Emergency hospital	+	+
Suli9	Blood	Emergency hospital	+	+
Suli10	Blood	Emergency hospital	+	+
Suli11	Blood	Emergency hospital	+	+
Suli12	Burn wound swab	Emergency hospital		
Suli13	Blood	Emergency hospital		
Suli14	Burn wound swab	Emergency hospital		
Suli15	Burn wound swab	Emergency hospital		
Suli16	Burn wound swab	Emergency hospital		
Suli17	Blood	Emergency hospital	+	+
Suli18	Burn wound swab	Emergency hospital	+	+
Suli19	Blood	Emergency hospital		
Suli20	Burn wound swab	Emergency hospital	+	+
Suli21	Burn wound swab	Emergency hospital	+	+
Suli22	Blood	Emergency hospital		
Suli23	Blood	Emergency hospital		
Suli24	Blood	Emergency hospital		
Suli25	Blood	Emergency hospital		
Suli26	Blood	Emergency hospital		
Suli27	Blood	Emergency hospital		
Suli28	Blood	Emergency hospital	+	+
Suli29	Burn wound swab	Emergency hospital		
Suli30	Burn wound swab	Emergency hospital		
Suli31	Burn wound swab	Emergency hospital	+	+
Suli32	Burn wound swab	Emergency hospital	+	+
Suli33	Burn wound swab	Emergency hospital		
Suli34	Burn wound swab	Emergency hospital		
Suli35	Burn wound swab	Emergency hospital		
Suli36	Burn wound swab	Emergency hospital		
Suli37	Burn wound swab	Emergency hospital		
Suli38	Burn wound swab	Emergency hospital		
Suli50	Blood	Emergency hospital		
Suli51	Urine	Emergency hospital		
Suli52	Urine	Emergency hospital		
Suli53	Urine	Emergency hospital		
Suli54	Urine	Emergency hospital		
Suli55	Blood	Emergency hospital		
Suli56	Urine	Emergency hospital		
Suli57	Urine	Emergency hospital		
Suli65	Blood	Emergency hospital	+	+
Suli66	Blood	Emergency hospital	+	+
Suli67	Blood	Emergency hospital	+	+
Suli75	Blood	Emergency hospital		

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Sequence analysis

Phylogenetic analysis using *16S rRNA* gene sequences from Emergency hospital in Sulaimani-Iraq involved 33 nucleotide sequences (21 of our MRSA isolates named suli and 12 reference MRSA strains from NCBI data bases). 75 % (6/8) among MRSA isolates isolated in the present research were corelated as shown in Figure 4.

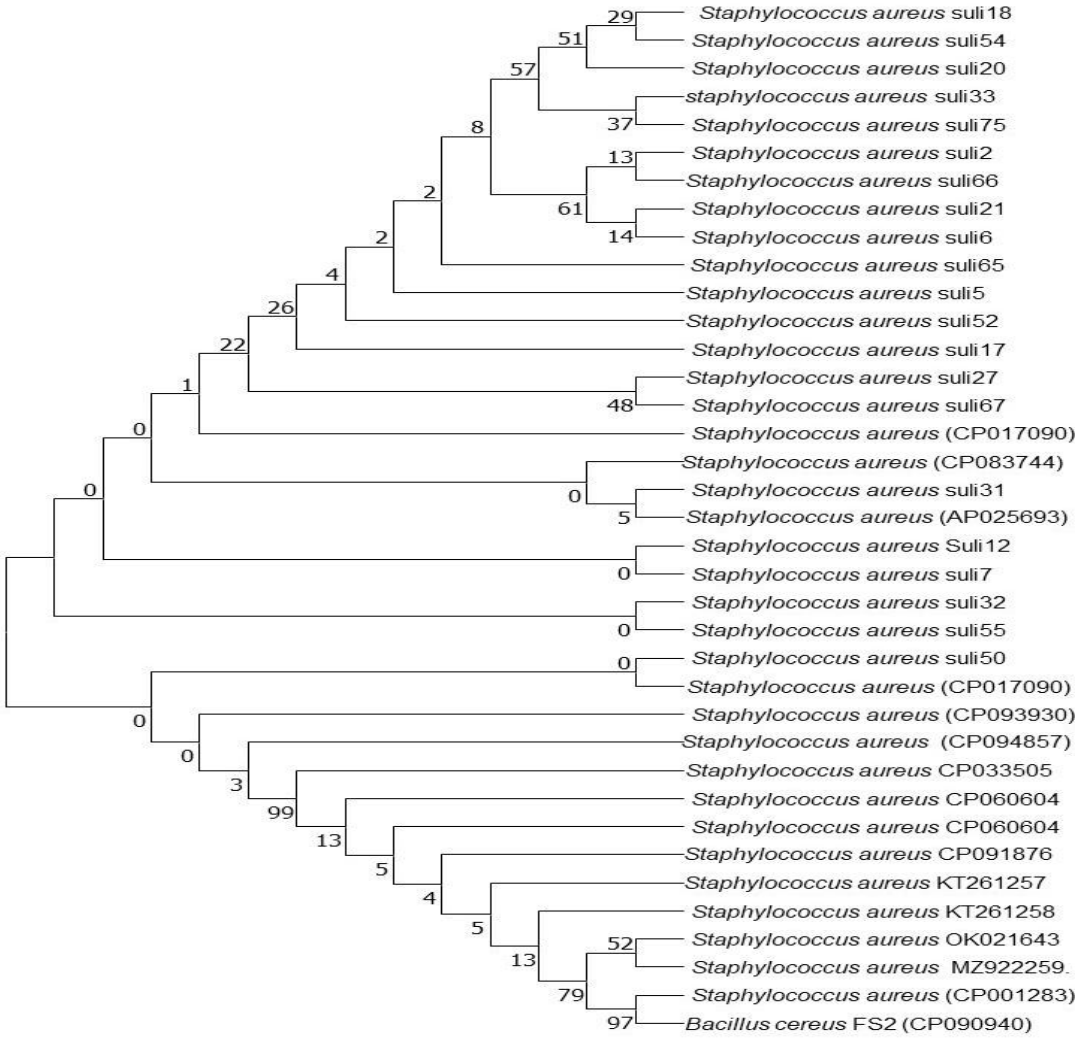

Fig 4: **Phylogenetic tree and evolutionary relationships of *Staphylococcus aureus* taxa**.
The optimal tree, with a branch length sum = 5.44226012, is displayed. The tree was drawn to scale, with branch lengths represented by the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analyses were conducted with MEGA X software.

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PFGE analysis

The studied MRSA's chromosomal DNA was fragmented into 10 to 13 fragments by *SmaI*. A dendogram percentage of similarities calculated with dice coefficient from the examination of the PFGE data through a cutoff. 75% revealed the presence of 4 clusters. The first cluster(C1) contained 1 MRSA strain (S3) recovered from burn-wound swab was unrelated with more than 7 fragment differences. The second cluster(C2) contained 2 MRSA strains (S4, S2) both recovered from burn-wound swabs were possibly related with 5 fragment differences. The third cluster(C3) contained 3 MRSA strains, the isolates S5 and S6 recovered from burn-wound swabs, the isolate S10 recovered from blood were indistinguishable with no fragment difference while the fourth cluster(C4) contained 4 MRSA strains in which isolates S1, S7 and S8 recovered from burn-wound swab in contrast to isolate S9 recovered from blood were closely related with three fragment differences as shown in figure 5.

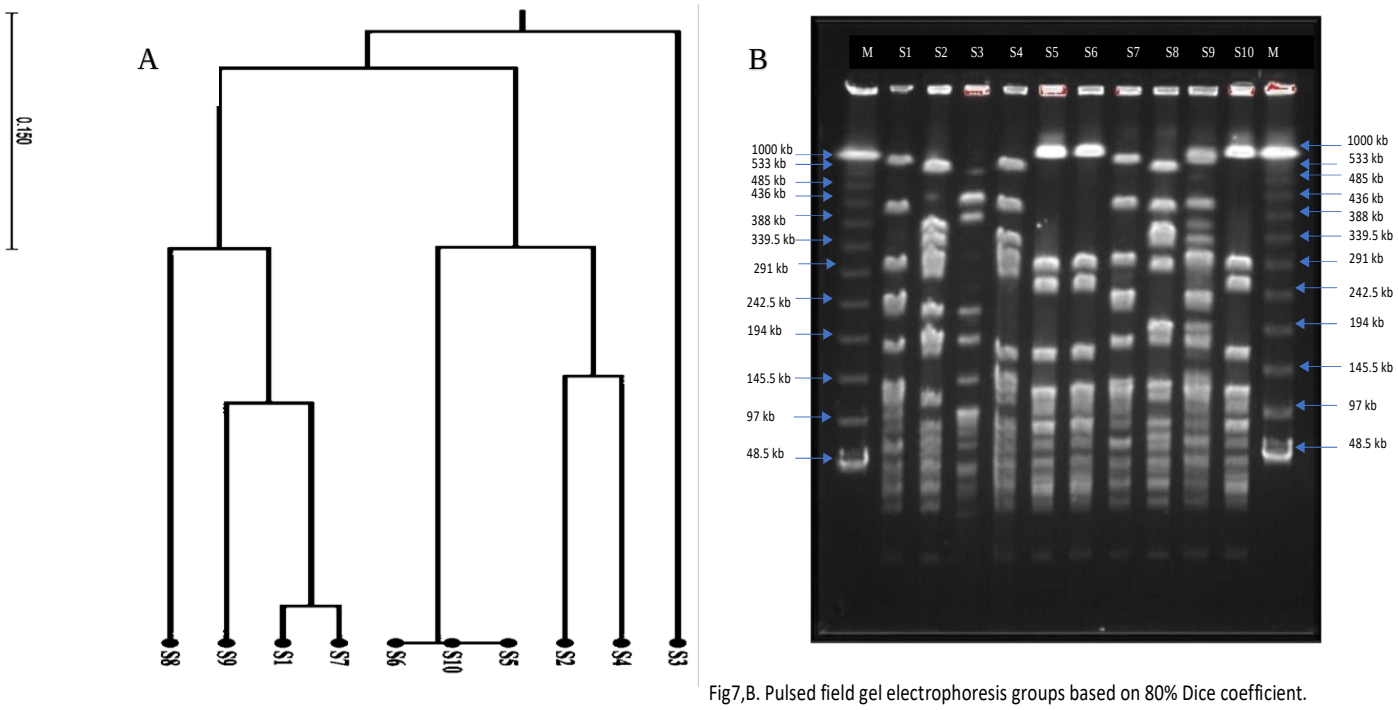

Fig7,B. Pulsed field gel electrophoresis groups based on 80% Dice coefficient.

Fig 5 : PFGE results of 10 MRSA isolates

A : Dendrogram of percent similarity, revealed four major clusters .**B :**PFGE separation of restriction fragments of the MRSA genome digested with SmaI of 10 MRSA isolates.

Discussion

The MRSA strains have emerged as a significant multi-drug resistant bacterium causing nosocomial infections in Iraq and around the world, as reported by various studies [15, 16]. The present investigation revealed that 42% (21 out of 50) of the isolates exhibited resistance to cefoxitin and were found to possess the *mecA* gene. A study was carried out among hospital and the community in Duhok Governorate /KRG-Iraq, found that 50.4% of isolates were MRSA [17]. Researchers in Kirkuk Governorate, conducted a study during 2021 indicated the prevalence of MRSA was found to be 54.2% in 25 clinical samples that were collected from burn patients [18]. In another study

done in Al-Diwaniya City, 22.2% of *S. aureus* isolates from burn-wounds were MRSA [19]. In Al-Nasiriyah city from 90 isolates from burn units, 75.5% revealed MRSA in 2014[20]. The prevalence of methicillin-resistant *S. aureus* (MRSA) among burn patients in Asia has been reported to be 33.3% in Bangladesh [21] and 57.14% in India [22].

The utilization of *16S rRNA* analysis has proven to be a proficient approach to discriminating among non-related isolates [23]. The findings of this investigation indicated that MRSA isolates exhibited a high degree of similarity based on the *16S rRNA* partial sequencing. According to a study, MRSA strains found in Syrian refugees in Duhok Governorate, KRG- Iraq, were subjected to amplification using *16S rRNA* and were observed to exhibit genetic relatedness in sequence, indicating a shared ancestry from which they have evolved. The proximity of neighboring countries may have contributed to the observed similarity in MRSA strains between the two research groups, as it may have facilitated the transmission of MRSA strains across borders [24].

MRSA infections are rapidly increasing in hospitals and communities, making them a public health concern. A variety of epidemiological markers were used for typing MRSA. PFGE is a gold standard technique for genotyping MRSA, it has been used for its reliability, discriminatory and reproducibility in several studies [25,26,27]. This is the first study in Sulaimani City to investigate the clonal relationship of MRSA strains using the PFGE technique. The dendrogram exhibited the presence of four primary clusters, denoted as C. The most abundant PFGE clusters were cluster (C4) and cluster (C3), which contained 4/10 and 3/10 MRSA isolates, respectively, while clusters (C1) and (C2) contained 1/10 and 2/10 MRSA isolates, respectively. According to Tenover PFGE pattern interpretation guidelines [14], the isolates belonging to cluster C3, S5 (from burn-wound swab), S10 (from blood), and S6 (from burn-wound swab) were indistinguishable, which means the isolates are part of the outbreak. Thus, the findings indicate that these three MRSA isolates have a common origin and belong to the same clone. The isolates obtained from Cluster C4, S7 (burn-wound swab), S1 (burn-wound swab), S9 (blood), and S8 (burn-wound swab) exhibit a close genetic relationship, with only three band differences. This suggests that the isolates are likely part of the outbreak. A single recognition site loss or addition can result in up to three band changes. Spontaneous genetic events, including single mutations, DNA insertions, and DNA deletions, are more likely to affect PFGE patterns during an outbreak [14]. Isolates that belonged to cluster C2, S4 and S2 (burn-wound swabs), were possibly related (5 band differences), which indicates that the isolates are possibly part of the outbreak. Hussein reported that 114 isolates of S. aureus were categorized into eight primary PFGE pulsotypes, denoted as types A through H. Type A (43, 37.7%) was found to be the most commonly occurring PFGE type, which exhibited a total of seven subtypes. Type B was found to exhibit three distinct subtypes. The prevalence of pulsotypes C-H was comparatively lower, with a reduced number of subtypes. Other pulsotypes, E - H, were found to lack any subtypes, which indicated a significant genotypic variety within the MRSA population [28]. A study from Japan [29] reported that MRSA isolates belonging to cluster 3 from the same hospital were genetically identical, similarly, a study done by Alp et al [30], observed that 22 PFGE patterns out of 23 were closely related and that 98% were clonally related, another study by Ho et al in Malaysia [31], 41 MRSA strains were identified three strains belonging to cluster A were closely related. The PFGE patterns of isolates reflecting the epidemic strain involved in the outbreak would not be distinguishable from one another and would be clearly distinguished from those of epidemiologically irrelevant strains [14]. The findings suggest that 30% of the samples (C3) had indistinguishable fingerprints with no variations in banding profiles, whereas 40% of the samples (C4) displayed

closely related patterns. Based on the data collected, there is evidence to suggest a potential outbreak may have occurred during the time of sample collection. The outbreak of MRSA may be attributed to the colonization of patients or the hospital environment, as well as healthcare workers. The prevalence of MRSA was found to be 10.1% among healthcare workers hailing from Jordan [32], 22.5% among those from Iraq [33], and 73% among healthcare workers from Saudi Arabia [34]. However, the current study did not incorporate healthcare workers and environmental samples. Further investigations are required to explore the origin of the outbreak from both patients or the environment, with a larger sample size being preferable.

Conclusions

According to the results, it can be concluded that 30% of the samples (C3) exhibited identical fingerprints with no discernible differences in banding profiles. However further studies are needed for epidemiological detection. Evidence suggests dissemination of bacterial infection may have occurred around the time samples were collected.

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