ORIGINAL ARTICLE

Novel determination of *spa* gene diversity and its molecular typing among *Staphylococcus aureus* Iraqi isolates obtained from different clinical samples

S. M. Kareem, S. S. Aljubori and M. R. Ali
Biology Department, College of Science, Mustansiriyah University, P.O. Box :14022 palastien st., Baghdad, Iraq

Abstract

Staphylococcus aureus is the most frequent agent causing nosocomial infections in Baghdad hospitals. This study aimed to determine *S. aureus* methicillin resistance, *spa* gene typing and phylogenic analysis in Iraqi *S. aureus* isolates. Two hundred samples including clinical (n = 100) and environmental (n = 100) specimens were collected. *S. aureus* isolates were identified using multiplex PCR amplification of *fem*A and *mec*A (for methicillin-resistant *S. aureus* (MRSA) strains) genes. The *spa* gene was also amplified. Sequence alignment and identification of *spa* types was then obtained. Of 74 studied *S. aureus* isolates, 61 (82.43%) harboured the *mec*A gene ($p < 0.001$). A *spa* gene variation was detected in 41 (67.2%) of 61 (p 0.0011) MRSA and 6 (46.15%) of 13 methicillin-susceptible *S. aureus* isolates. Amino acid sequence analysis revealed a great change in amino acid pattern among local isolates compared to National Center for Biotechnology Information control. Some of the MRSA isolates had high-level similarity with t10214. No genetic relationship with the infection sources was observed. None of the environmental isolates had *spa* gene variations. Most *S. aureus* isolates were MRSA. The *spa* gene variations was significantly higher among clinical isolates. *spa* sequencing showed different tandem repeats in local MRSA isolates compared to global *spa* types. We conclude that there was no outbreak in hospital settings in the city of Baghdad. However, our data suggest that isolates from the hospital environment are highly clonal.
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Corresponding author: S. M. Kareem.
E-mail: stsq@uomustansiriyah.edu.iq

Introduction

Staphylococcus aureus is a common and versatile human pathogen [1]. Despite continuous progress in the medical and diagnostic fields, it is harboured by 20% to 30% of the population without causing any clinical manifestation. The bacterium has the ability to adapt to numerous conditions, and one successful isolate could become an epidemic or even a pandemic clone with high morbidity and mortality [2]. Methicillin-resistant *S. aureus* (MRSA) causes both hospital-acquired and community-acquired MRSA [1]. *Staphylococcus aureus* may harbour numerous virulence factors, including staphylococcal protein A (*spa*), in addition to its ability to resist a variety of antibiotics [3].

Genotyping methods have the capacity to rapidly and reliably identify the relatedness of clinical isolates. Such methods are crucial for investigating outbreaks as well as to enable epidemiologic studies and surveillance of isolate dissemination [4]. Several molecular techniques have been applied for typing, including pulsed-field gel electrophoresis, PCR restriction fragment length polymorphism, DNA sequencing of *spa* and *coa* genes and screening for toxins [5,6].

The aims of this study were to determine the *spa* gene diversity; and to genotype clinical and environmental *S. aureus* isolates in Iraq.

Materials and methods

Seventy-four dereplicated *S. aureus* isolates were obtained from clinical sources and hospital environments in Baghdad (Table 1). For molecular identification, a multiplex PCR was used to amplify the *fem*A housekeeping gene. The *mec*A mobile genetic

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TABLE 1. Source of isolation of studied *Staphylococcus aureus* isolates

Source of isolation	No. of isolates	%
Skin infection	38	51.35
Urinary tract infection	12	16.21
Ear infection	5	6.75
Nasal infection	3	4.05
Blood	3	4.05
Eye infection	2	2.70
Sputum	1	1.35
Seminal fluid	1	1.35
Hospital environment	9	12.16
Total isolates	74	100

element was amplified to detect MRSA isolates. Primers are listed in Table 2.

DNA extraction

The template DNA was prepared by the boiling method [7]. Briefly, a few bacterial colonies taken from overnight bacterial growth culture were suspended in 1 mL of TE buffer and boiled in a water bath for 5 minutes. After centrifugation, the supernatant was separated and used as a DNA template.

Polymerase chain reaction

The PCR reaction mixture was prepared by adding 12.5 μL 2 × GoTaq Green Master Mix (Promega, Madison, WI, USA), 1.5 μL of each of the forward and reverse primers [8] (10 pmol/μL), 5 μL template DNA and nuclease-free H_2O to a final volume of 25 μL. PCR conditions are listed in Table 3. The PCR products were visualized on a 1% agarose gel for 1 hour at 50 V, stained with ethidium bromide or diamond and visualized by a transilluminator.

Single-locus sequencing typing of *spa* gene

All isolates were subjected to *spa* gene screening using specific primers for a variable region amplifying a PCR product size of 300 bp (Table 2) [9].

Next, all PCR products were sequenced by sending samples to the NICEM Company in the New York, United States. Pairwise sequence alignment was used to identify the amplified DNA fragment of the *spa* gene that might indicate the structural, functional and/or evolutionary relationships among DNA sequences. The sequences were submitted to the National Center for Biotechnology Information (NCBI) under accession number LC038119-LC38142. DNA alignments were analysed by Geneious 8.0.3 software (Geneious, Auckland, New Zealand), and a phylogenic tree was drawn using the Tamura-Nei genetic distance model with cost matrix identity (1.0–0.0) and UPGMA (unweighted pair group method with arithmetic mean). *spa* tandem repeats were calculated by *spa*-Typer online software (http://spatyper.fortinbras.us/). The results were then compared to an online website for world *spa* types (Ridom *spa* Server, https://www.spaserver.ridom.de/).

Data analysis

Data were analysed by SPSS 20 (IBM, Armonk, NY, USA). ANOVA and the Student *t* test were used considering the 95% confidence interval; $p < 0.05$ was considered statistically significant.

Results

All 74 studied isolates contained *fem*A, thus confirming that all were *S. aureus,* among which 61 (82.43%) of 74 contained *mec*A (MRSA) and 13 (17.57%) of 74 were devoid of *mec*A (methicillin-susceptible *S. aureus,* MSSA).

A total of 47 of 74 isolates had *spa*-typing positive results. Of these, 67.2% of MRSA isolates showed positive results vs. 46.15% of MSSA isolates. The amplified fragments ranged from 65 to 300 bp compared to the reference *S. aureus* 01-111 strain as analysed by Geneious software. None of the environmental isolates had *spa* gene variations (Figs. 1 and 2).

The amplified *spa* sequence of MRSA N1 was compared to the control strain using a GenBank (accession no. GI:482576487) MRSA strain isolated in Egypt (Egy 52A). Protein_id AGK23551 and pairwise identity was 262 (97%) of 270, which represents the percentage of identical residues in alignment, including gaps and nongap residues. Notably, there was a difference in base pairs causing a gap in the upper identity green line compared to DNA sequencing alignment. Mutations were

TABLE 2. PCR primers used

Primer name	Primer sequence (5′–3′)	Product size (bp)	Reference
*fem*A F	CGATCCATATTTACCATATCA	450	[8]
*fem*A R	ATCACGCTCTTCGTTTAGTT		
*mec*A F	GTAGAAATGACTGAACGTCCGATAA	314	[8]
*mec*A R	CCAATTCCACATTGTTTCGGTCTAA		
spa F	TCAAGCACCAAAAGAGGAAGA	300	[9]
spa R	ACGACATGTACTCCGTTGCCG		

F, forward; R, reverse.

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TABLE 3. PCR conditions

Amplified gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Elongation	Final extension
*fem*A, *mec*A	95°C/5 min	35	94°C/30 s	53°C/30 s	72°C/1 min	10 min/72°C
spa	95°C/5 min	35	94°C/30 s	52°C/30 s	72°C/30 s	10 min/72°C

evident at 32, 72, 81 and 259 bp, where C, C, C and G were altered to T, T, T and A, respectively.

A high diversity in *spa* genes obtained from different sources was obtained compared to the consensus and compared to the control obtained from NCBI. Results revealed that some changes took place in nucleotide sequences compared to *spa* gene control, and most isolates displayed different genetic variations. The highlighted nucleotides are variable ones and illustrate the diversity between all base pairs. The data divided into four monophyletic groups and one unique pattern group. Group 1 contained MRSA B1, S2, 16 and 32; group 2 contained MRSA U1, S7, 8, E3 and B1 as well as MSSA B1. Group 3 contained MRSA S5 and S19. Group 4 contained MSSA S5 and the NCBI strain control.

Nucleotide sequence variations were observed compared to *spa* gene control, and most isolates displayed genetic

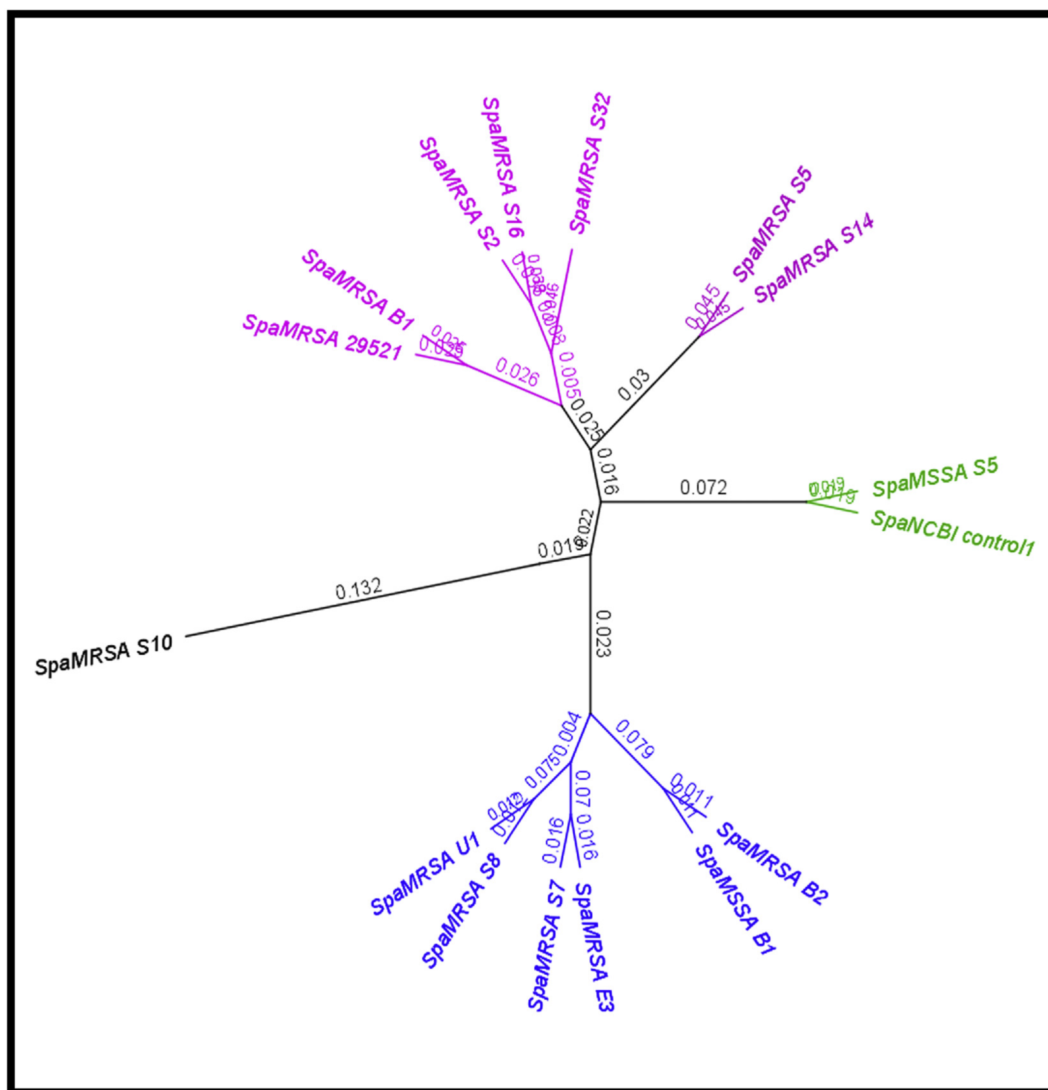

FIG. 1. Dendrogram and phylogenetic tree to long sequence (190–335 bp) of *spa* gene.

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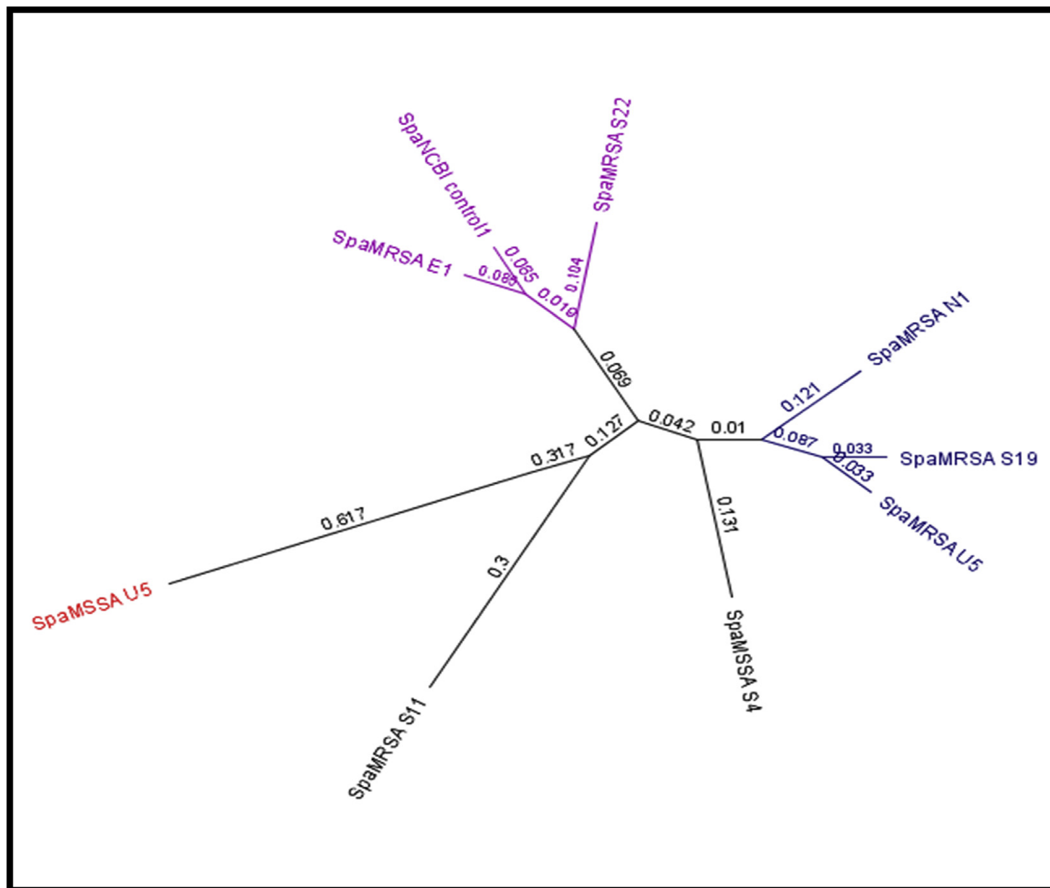

FIG. 2. Dendrogram and phylogenetic tree to short sequence (65–190 bp) of *spa* gene related to methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus*.

diversity. Our isolates divided into three monophyletic groups and one unique pattern group (data not shown). Group 1 contained NCBI standard strain control, MRSA E1 and S22. Group 2 contained MRSA N1, S19 and U5. Group 3 contained MRSA S11 and MSSA U5. The results illustrated that the isolate MSSA S4 was more distant genetically than the species studied, with a 0.131 value. The converged genetic relation for groups 1, 2 (at 0.069 and 0.042) and group 3 *S. aureus* isolates were more distant (0.127) from the other groups. MSSA S5 had a high similarity with the NCBI strain control.

In addition to analysis based on DNA sequence data from single locus *spa* gene, the amino acid profile of the *spa* repeats was performed according to the translation DNA sequencing of the variable region in the *spa* gene. The protein translation profile for each isolate subjected to *spa* gene sequencing, as well as the alignment between them, revealed that some amino acids are predominant in the isolates.

spa tandem repeats were calculated by bioinformatic analysis. Results revealed partial identity, with global *spa* types missing some repeats. They were detected by the starting coordinate of the repeat in the sequence alignment, numbers of repeat units, length of entire variable-number tandem repeat, Kreiswirth nomenclature, Ridom *spa* type name and repeats. Isolates that appear with ellipsis dots refer to missing repeats; an asterisk marks the indicated sequence as a *spa* repeat that was not found in the database we queried.

Single nucleotide polymorphism analysis was performed on the on *spa* gene by creating a DualBrothers (https://msuchard.faculty.biomath.ucla.edu/DualBrothers/index.html) phylogenetic tree and by analysis using the Geneious software; no genetic relation was observed among isolates. There was also no genetic relation regarding infection sources. None of the environmental isolates had *spa* gene variations. The data suggested a genetic relation (clonal distribution) of those isolates from the hospital environment.

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TABLE 4. ***spa* gene tandem repeats and *spa* gene types of local *Staphylococcus aureus* isolates compared to global *spa* gene tandem repeats and types, Seq: sequence, R: repeat**

Isolate	Seq. start	R. unit	Length (bp)	Repeat	Kreiswirth ID	*spa* type in Ridom	Repeat
MSSA S5	34	11	264	… –13–23–31–29–17–25–17–24–25–16–28	EJNF2MOMQOKR	t11434	26–23–23–13–23–31–29–17–25–17–24–25–16–28
MSSA B1	32	9	216	… –21–17–13–13–34–34–34–33–34	FMEEBBBPB	t690	07–12–21–17–13–13–34–34–34–33–34
MRSA S32	107	6	144	… 17–25–17–25–16–28	MOMOKR	t10214	26–31–13–23–05–17–25–17–25–16–28
MRSA S16	33	8	192	… –29–17–25–17–25–16–28	F2MOMOKR	t554, t032 more than one type	26–23–17–31–29–17–25–17–25–16–28
MRSA S14	32	9	216	… –31–13–23–05–17–25–17–25–16–…	NEJCMOMOK	t10214	26–31–13–23–05–17–25–17–25–16–28
MRSA S10	131	2	48	—	—	—	—
MRSA S5	107	7	168	…–29–17–25–17–25–16–28	F2MOMOKR	t554, t032 more than one type	26–23–17–31–29–17–25–17–25–16–28
MRSA S2	33	10	240	94–23–31–29–17–25–17–25–16–28	JNF2MOMOKR	—	—
MRSA S7	84	5	120	16–2–25–17–24	KAOMQ	—	—
MSSA S3	26	7	168	13–12–17–307–23–18–17	EGM[r307]JH2M	—	—
MRSA U1	31	7	168	*–12–17–307–23–18–17	GM[r307]JH2M	—	—
MRSA E1	33	5	120	16–2–16–2–25	KAKAO	—	—
MRSA S11	32	1	24	405	—	—	—
MRSA U5	29	4	96	48–34–34–33	V2BBP	t1590	07–16–48–34–34–33–34
MRSA E3	33	6	144	16–2–16–2–25–17	KAKAOM	—	—
MRSA N1	30	1	24	13	—	—	—
MRSA S19	29	5	120	*–34–34–33–34	BBPB	t2421 more than 100 type	26–34–34–33–34
MSSA S4	31	2	48	12–17	—	More than 100 type	—
MRSA B1	79	7	168	…–05–17–25–17–25–16–28	CMOMOKR	t309	26–23–05–17–25–17–25–16–28
MRSA B2	33	1	24	21	—	—	—
MSSA U2	33	10	240	16–2–16–34–2–25–17–24–24–24	KAKBAOMQQQ	—	—
MSRA S22	32	4	96	25–82–16–17	O[r82]KM	—	—
MRSA S28	30	9	216	94–23–5–17–25–17–25–16–28	[r94]JCMOMOKR	—	—

Ridom refers to Ridom *spa* Server (https://www.spaserver.ridom.de/).
MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-susceptible *S. aureus.*
Ellipses indicate missing repeats.
*Sequence is *spa* repeat but not found in database.

Discussion

The prevalence of *mec*A gene was in agreement with previously published local studies that showed *mec*A prevalence to be 75.5% among *S. aureus* isolates [10,11]. Another local study mentioned the prevalence of *mec*A to be 100% [12]. MRSA isolates have risk factors that increase the prevalence of MRSA isolate colonization in Asia, the result of antibiotic misuse and limited socioeconomic status [13]. Further, patients who are infected with MRSA isolates in the hospital may harbour the microbe for long time [14,15]. In Iraq, a significant problem with MRSA is the result of an increased rate of incidence and hospitalization. Rapid and accurate typing of MRSA isolates is therefore essential for screening, epidemiology, surveillance and infection control.

Indeed, *spa* gene typing is easy and quick [6]. The selected region of the *spa* gene is usually a short sequence repeat with sufficient polymorphism to permit isolate typing [5]. Sakwinska et al. [16] found that 10% of healthy carriers carried the *spa* gene mutation. Another study found that a relation existed between MSSA and a control strain, and that diversity among the MRSA isolates were based on the multiple insertion of the staphylococcal chromosome cassette SCC*mec,* especially the *mec*A gene, into MSSA lineages, shifting them to MRSA isolates [17]. Furthermore, the diversity in amino acids indicates the emergence of a synonymous mutation to protect the amino acid sequence under varying levels of evolutionary pressure; many nonsynonymous mutations have been shown to cause a shift in amino acids [18].

In addition, the variation in tandem repeats of the *spa* gene encoding protein A is critical for analysing host–parasite interactions, as it can help bacteria evade the host immune system [19]. According to Ridom, MSSA S5 differs from t11434 by loss of the three-tandem repeat 26–23–23, found in New Zealand with a frequency of 0, while MSSA B1 differs from t690 by the two-tandem repeat 07–12, with a frequency of 0.09%, as has been found in the Middle East in places like Lebanon and the United Arab Emirates (Table 4). However, MRSA S32 and S14 exhibited higher similarities with t10214; the first lost four repeats at the beginning of the frame, and the second lost one repeat at the beginning and end of the frame. The latter was reported from Sweden, and different genetic cluster groups were shown in dendrogram and phylogenetic trees. Although MRSA S5 and S16 belong to different genetic cluster groups in

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the dendrogram, they exhibited tandem repeats similar to more than one type of *spa,* like t554 and t032. Indeed, the latter is found more frequently worldwide (10.41%), considering common *spa* types in MRSA isolates in Germany [2] in addition to its distribution in the areas surrounding Iraq, such as Lebanon, Kuwait and the United Arab Emirates. This clone has also emerged with resistance to aminoglycoside (known as EMRSA-15 or Barnium epidemic isolates [20]); it is close to our isolates, which showed resistance to gentamicin. MSSA S5 differed from t2421in one repeat, 26, which has been found in the United Kingdom. Humphreys et al. [21] reported availability of the t037 CC-22 isolate in Iraq and the Middle East, and mentioned the possibility of its transmission from Baghdad to Dublin. Koreen et al. [22] reported the common *spa* type t033, which depended on previously finding the predominant *spa* type of *S. aureus* in diverse parts of the world [23]. Other isolates have only two or one repeats (Table 4).

However, this cannot provide a real view in terms of similarities: MSSA U2, MRSA S22 and MRSA S28 display a pattern arrangement of repeats not found in Ridom. Perhaps these isolates have a new *spa* type. Indeed, *spa* types appear evolve by a combination of a faster changes in the number of repeats and a slower nucleotide point mutation [22] by slipped strand mispairing during DNA replication [24]. The *spa* repeat seems more prone to duplication and deletion than point mutation [22]. The maximum repetitive *x*-region in the current samples is 11 times, compared to reports which mentioned the maximum repetitive *x*-region to be 13 and 16 times in India and Iran, respectively [18,23]. The variation in the number of *x*-regions is a result of the deletion process. In other hands, it has been observed that the variation in *spa* types among MSSA isolates is greater than in MRSA isolates. This finding is closely related to the work of Fenner et al. [25], who mentioned that the diversity of the *spa* gene among MSSA isolates is higher than in MRSA isolates. This finding can be considered a novelty in Iraq and comprises the first local data about *spa* repeats. Other researchers have also reported that the discrimination between MRSA isolates is possible by determining the repeat sequence numbers within the *x*-region of the *spa* gene.

The *spa* typing technique represents as excellent tool for national and international surveillance as well as for short-term local epidemiology [23]. The DualBrothers model allows for changes in topology and evolutionary rates across sites in multiple sequence alignments. A huge alteration in the *spa* pattern could thus be recognized, with each *spa* sequence containing a different pattern. There was also no genetic relation regarding infection sources. None of the environmental isolates had *spa* gene variations. These data suggest that the genetic relation (clonal distribution) of these isolates is the result of a hospital environment.

Conclusion

The rate of MRSA was high among clinical isolates. Sequencing results showed different tandem repeats in local MRSA isolates. The pairwise result for the total sequence was 75.8%, with an identity of 15.8%. We found no genetic relation among *S. aureus* isolates or regarding infection sources. None of the environmental isolates had *spa* gene variations. The data suggested that the genetic relation (clonal distribution) of these isolates results from a hospital environment.

Conflict of interest

None declared.

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References

[1] Okwu MU, Mitsan O, Okeke OP. Prevalence and antimicrobial susceptibility profiles of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates among healthy individuals in Okada, south-south, Nigeria. US Open Pharm Biol Chem Sci J 2014;1:1–9.
[2] Holden MT, Hsu L, Kurt K, Weinert LA, Mather AE, Harrism SR, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin resistant *Staphylococcus aureus* pandemic. Genome Res 2013;23:653–64.
[3] Faccone D, Togneri AM, Podesta L, Perez M, Gagetti P, Sanchez S, et al. MRSA pediatric clone expressing *erm*C plus *lnu*A genes causing nosocomial transmission and healthcare workers colonization in a neonatal intensive care unit. Infect Genet Evol 2014;25:78–80.
[4] Mohammadi S, Sekawi Z, Monjezi A, Maleki M, Soroush S, Sadeghifard N, et al. Emergence of SCCmec type III with variable antimicrobial resistance profiles and *spa* types among methicillin-resistant *Staphylococcus aureus* isolated from healthcare- and community-acquired infections in the west of Iran. Int J Infect Dis 2014;25:152–8.
[5] O'Hara FP, Suaya JA, Ray GT, Baxter R, Brown ML, Mera RM, Close NM, et al. *spa* typing and multilocus sequence typing show comparable performance in a macroepidemiologic study of *Staphylococcus aureus* in the United States. Microb Drug Resist 2016;22:88–96.
[6] Shopsin B, Gomez M, Waddington M, Riehman M, Kreiswirth BN. Use of coagulase gene (*coa*) repeat region nucleotide sequences for the typing of methicillin-resistant *Staphylococcus aureus* strains. J Clin Microbiol 2000;38:34536.

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[7] Ruppé E, Hem S, Lath S, Gautier V, Ariey F, Sarthou JL, et al. CTX-M-lactamases in *Escherichia coli* from community acquired urinary tract infections, Cambodia. Emerg Infect Dis 2009;15:741–8.

[8] Kareem SM, Al-Jubori SS, Ali MR. Prevalence of *pvl* gene among methicillin resistance *S. aureus* isolates in Bagdad city. World J Pharm Res 2015;4:455–71.

[9] Walker J, Borrow R, Edwards-Jonesz V, Oppenheim BA, Fox AJ. Epidemiological characterization of methicillin-resistant *Staphylococcus aureus* isolated in the North West of England by protein A (*spa*) and coagulase (*coa*) gene polymorphisms. Epidemiol Infect 1998;121:507–14.

[10] Ibed AN, Hamim SS. Molecular detection of methicillin resistant staphylococcus aureus isolated from burns infection in Al-nasiriyah city. World J Pharm Pharmaceut Sci 2014;2:950–4.

[11] Kareem SM, Al-Jubori SS, Ali MR. Prevalence of *erm* genes among methicillin resistant *Staphylococcus aureus* MRSA Iraqi isolates. Int J Curr Microbiol Appl Sci 2015;4:575–85.

[12] Al-Kadmy IM. A genetic study to differential HC/AC MRSA isolated from clinical cases in Iraq hospitals. Mintage J Pharm Med Sci 2013;2:57–62.

[13] Beam JW, Buckley B. Community-acquired methicillin-resistant *Staphylococcus aureus:* prevalence and risk factors. J Athl Train 2006;41:337–40.

[14] McMullen KM, Warren DK, Woeltje KF. The changing susceptibilities of methicillin-resistant *Staphylococcus aureus* at a Midwestern hospital: the emergence of 'community-associated' MRSA. Am J Infect Control 2009;37:454–7.

[15] Ratnayake L, Olver WJ. Rapid PCR detection of methicillinresistant *Staphylococcus aureus* and methicillin-sensitive *S. aureus* samples from charcoal-containing blood culture bottles. J Clin Microbiol 2011;49:2382.

[16] Sakwinska O, Blanc DS, Lazor-Blanchet C, Moreillon M, Giddey M, Moreillon P. Ecological temporal stability of *Staphylococcus aureus* nasal carriage. J Clin Microbiol 2010;48:2724–8.

[17] Asghar AH. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from tertiary care hospitals. Pak J Med Sci 2014;30:698–702.

[18] Mehndiratta PL, Bhalla P, Ahmed A, Sharma YD. Molecular typing of methicillin-resistant *Staphylococcus aureus* strains by PCR-RFLP of *spa* gene: a reference laboratory perspective. Indian J Med Microbiol 2009;27:116–22.

[19] Benson G. Tandem repeat finder: a program to analyze DNA sequences. Nucleic Acids Res 1999;27:573–80.

[20] Monecke S, Leila Skakni L, Hasan R, Antje Ruppelt A, Ghazal SS, Hakawi A, et al. Characterization of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. BMC Microbiol 2012;12:146.

[21] Humphreys H, Carroll JD, Keane CT, Cafferkey MT, Pomeroy HM, Coleman DC. Importation of methicillin-resistant *Staphylococcus aureus* from Baghdad to Dublin and subsequent nosocomial spread. J Hosp Infect 1990;15:127–35.

[22] Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol 2004;42:792–9.

[23] Shakeri F, Mansourian AZ, Ghaeim EA. *Staphylococcus aureus* protein A gene typing by PCR-RFLP. Afr J Microb Res 2013;7:3448–52.

[24] van Belkum A. The role of short sequence repeats in epidemiological typing. Curr Opin Microbiol 1999;2:306–11.

[25] Fenner L, Anderas WF, Dangel M, Frei R. Distribution of *spa* types among methicillin resistant *Staphylococcus aureus* isolates during a 6 years periods at low-prevalence university hospital. J Med Microbiol 2008;57:612–6.

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