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Impact of mass migrations on the clonal variation of clinical *Staphylococcus aureus* strains isolated from the Western region of Saudi Arabia

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ABSTRACT

Objectives: A rapid molecular typing system was used to determine the impact of mass migration on the clonal variation of *Staphylococcus aureus* isolates recovered from King Abdulaziz University Hospital (KAUH) Jeddah, in the western region of Saudi Arabia. This region experiences an annual influx of millions of pilgrims.
*Methods: Sma*I-multiplex PCR typing (SMT) was used for the initial analysis of strains and the resulting data subsequently supported by Multi-Locus Sequence Typing (MLST).
Results: A total of 89 *S. aureus* isolates were SMT typed and revealed a high degree of genetic variation, with 40 SMT profiles detected among the isolates. Representatives of all forty SMT types were subsequently analysed by MLST, identifying 26 sequence types. A novel sequence type (ST), named ST3303, was identified in two methicillin-sensitive *S. aureus* (MSSA) isolates. MSSA strains exhibited more diversity than methicillin-resistant *S. aureus* (MRSA) strains, with community acquired MSSA and MRSA strains reaching alarmingly high levels.
Conclusion: The relatively high degree of genetic diversity found among *S. aureus* isolates of single hospital was attributed to the fact that Jeddah is the principal gateway to Mecca, visited each year by millions of pilgrims from many countries. The observed diversity clearly reflects the impact of such mass migrations in the rapid dissemination of strains world-wide. Our findings suggest the importance of surveillance programmes in locations affected by mass migrations, both to monitor their impact on endemic strains and for the detection of pandemic strains. SMT provides a cost-effective and sensitive typing method for achieving this objective.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is still considered among the most important nosocomial pathogens. Although, there is no national surveillance program for MRSA in Saudi Arabia, studies conducted in the seven hospitals in Riyadh reported prevalences of methicillin resistance among isolates of *S. aureus* ranging from 12% to 49% [1]. In addition, a study conducted in King Abdul-Aziz University Hospital (KAUH) in Jeddah has pointed to a rapid rise in the prevalence of MRSA [2], while the prevalence of MRSA nasal carriage among medical students was 18.7% [3]. Moreover, community acquired (CA)-MRSA infections have reached alarmingly high levels in some Saudi hospitals [4]. Therefore, an understanding of the epidemiological characteristics of *S. aureus* is essential in the management and control of such infections in both hospital and community settings. *S. aureus* typing is an effective means of aiding infection control since it allows epidemic and sporadic strains to be distinguished and potential sources of infection to be identified [5]. Molecular epidemiological techniques are now the methods of choice for strain typing because

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they can be performed with a high level of accuracy. Pulsed-field gel electrophoresis (PFGE), *Staphylococcus* protein A gene (*spa*) typing and Multi-Locus Sequence Typing (MLST) are currently the most widely used molecular typing techniques for *S. aureus* with the former being regarded as the gold standard due to its discriminatory power and reproducibility [6,7]. However, their routine application in many hospital laboratories is restricted by technical limitations: they are time-consuming, expensive, and require specific expertise and specialist equipment [8]. Ultimately, whole genome sequencing (WGS) is likely to replace current molecular typing methods since, in additional to the epidemiological data it provides, it can also provide data useful for treatment, such as the presence of genes encoding antibiotic resistance and virulence factors. Nevertheless, WGS is currently relatively expensive, not rapid enough for routine use and requires an automated data processing pipeline to interpret and extract the required clinically useful information [9,10]. These limitations are global issues, but especially applicable to developing countries. In contrast, the *Sma*I-multiplex PCR typing (SMT) technique, based on single nucleotide polymorphisms (SNP) in and around *Sma*I-restriction sites (CCCGGG), is inexpensive and can be performed rapidly. SMT has been evaluated against a variety of clinical *S. aureus* isolates and shown to provide reliable epidemiological information for the investigation of on-going staphylococcal outbreaks [11,12]. SMT is more discriminatory than MLST and SCC*mec* typing but less discriminatory than PFGE [12].

Saudi Arabia receives more than six million Muslims for the Hajj and Umrah pilgrimages each year. This huge number of visitors can contribute to the spread of many bacterial species and their clones. The primary aim of this study was to identify the impact of mass migration on the clonal variation of clinical *S. aureus* isolated from King Abdulaziz University Hospital (KAUH), Jeddah, in the Western region of Saudi Arabia, in the light of the mass movements of peoples in this particular geographical region.

Materials and methods

Sources of clinical S. aureus *isolates*

A cross-sectional study was carried out over five months in KAUH. KAUH is one of the largest hospitals in the Western region of Saudi Arabia with a capacity of 1000 beds. In addition to its role as a teaching hospital, KAUH offers many specialist medical services to large population in the Western region of Saudi Arabia.

Bacterial strains and growth conditions

Eighty-nine *S. aureus* (MSSA/MRSA) isolates were collected from the clinical microbiology laboratory of KAUH. Clinical information about the isolates was recorded and patients were coded to facilitate cross-reference between samples. All *S. aureus* isolates were identified by the Vitek 2 system (bioMérieux Inc., Durham, North Carolina/USA) using GP ID cards. Confirmed MSSA and MRSA strains were grown on fresh blood agar and isolates were transferred to Microbank porous carrier beads (Pro-Lab Diagnostics) for long-term storage and maintained at −80 °C. For molecular typing, bacterial isolates stored at −80° C were thawed and subcultured on brain-heart infusion (BHI) agar overnight at 37° C.

Molecular typing

Sma*I-multiplex PCR typing (SMT)*

SMT was carried out for all isolates according to the method of Al-Zahrani et al. [12]. Primers were obtained from MWG Biotech (Germany) and the resulting PCR products were separated on 4% low melting point (LMP) agarose (Cleaver Scientific LTD., Warwickshire, UK) in 1 × TBE at 110 V for 3 h. The gels were stained for 15 min in one litre of staining buffer (0.5 μg/ml ethidium bromide in 1 × TBE), destained in ddH_2O for 15 min and then visualized with a gel documentation system.

MLST

For MLST, chromosomal DNA was extracted using the method of Al-Zahrani et al. [12] and the Qiagen DNeasy extraction kit, with minor modifications to the manufacturer's instructions. A suspension of cells and glass beads was shaken in a TissueLyser II (Qiagen) for 5 min at 30 Hz. MLST primers were obtained from Macrogen (Seoul, Korea) and MLST was performed using the protocol of Enright and colleagues [6]. The PCR products were purified with the Qiagen PCR purification kit according to the manufacturer's instructions. The purified PCR products were sequenced by Macrogen (Seoul, Korea).

*SCC*mec

SCC*mec* typing was performed using a multiplex PCR as previously published by Ghaznavi-Rad et al. [13].

Data analysis

Gel images were saved as TIFF files and analyzed using the BioNumerics software (7.5; Applied Maths, Belgium). The analysis of multiplex PCR gels was based on band differences and a clustering dendrogram was generated by the unweighted-pair group method with arithmetic mean (UPGMA) using a Dice coefficient of similarity of 1.5%. For MLST, nucleotide sequences were compared using the MLST database (http://www.mlst.net) for assignment of allelic profiles and sequence type (ST). One novel ST identified in this study has been deposited in MLST database, and assigned number ST3303. The MLST data was analysed and the neighbour-joining tree was constructed using the START2 software (http://pubmlst.org/software/analysis/start2/).

Results

During the examination period, 89 non-duplicate *S. aureus* isolates were recovered from various clinical samples. The vast majority of clinical isolates (45%, n = 40) were isolated from wounds while only 10% (n = 9) were from blood. More than 61% (n = 55) of the patients were non-Saudi citizens. There were slightly more male patients (57.3%) than female patients (42.7%). Twenty-four (27%) patients were under 10 years old while 22 (24%) were 60 years or older. The frequency of MRSA strains among the isolates was relatively high at 48% (n = 43).

Molecular typing of S. aureus *isolates*

The eighty-nine *S. aureus* isolates were analysed using SMT and all isolates were typeable. The isolates showed relatively high genetic diversity and forty SMT types (A to V2) were reported among the isolates (Table 1 & Fig. 1). The most common SMT types were Types J and K, which, with their subtypes, were found in 21 isolates (J1: n = 3; J2: n = 5; J3: n = 4; K1: n = 6 and K2: n = 3). Other SMT types detected contained between 1 and 5 representatives (Table 1). SMT Types J, K and their subtypes have similar SMT profiles to community-acquired strains, MSSA476 and MRSA-MW2 with the exception of a fragment generated from the *mec*A gene in MSSA isolates [12]. Most of these isolates were recovered from non-Saudi citizens (Yemeni, Sudanese, Syrian, Egypt, Chad, Somalia and Pakistani), but at least one isolate of each type, except J1, was recovered from Saudi patients. The K2 subtype isolates (n = 3) were confined to Saudi patients (Table 1). Type C isolates MRSA-KAUHSA-12, 50, 64 and 76, and MSSA-KAUHSA-66 have the same basic profile as vancomycin-intermediate *S. aureus* (VISA) strains

Table 1
Typing data obtained by *Sma*I-multiplex PCR, MLST and SCC*mec* typing.

SMT type	No. of isolates (names)	PN	Representative isolate	MLST			SCC*mec*
				ST	Allelic profile	CC	
A	2 (MSSA-KAUH-55 and 32)	MM, SO	MSSA-KAUH-55	1675	10-14-8-6-14-185-2	–	NA
B	1 (MSSA-KAUH-82)	SY	MSSA-KAUH-82	152	46-75-49-44-13-68-60	152	NA
C1	4 (MRSA-KAUH-12, 50, 64 and 76)	EG, EG, SA, BD	MRSA-KAUH-50	5	1-4-1-4-12-1-10	5	V
C2	1 (MSSA-KAUH-66)	YE	MSSA-KAUH-66	5	1-4-1-4-12-1-10	5	NA
D1	1 (MSSA-KAUH-7)	SA	MSSA-KAUH-7	1292	3-38-1-1-1-40	9	NA
D2	1 (MSSA-KAUH-52)	SA	MSSA-KAUH-52	96	12-1-1-15-11-1-40	–	NA
E	2 (MSSA-KAUH-8 and 20)	SA, ET	MSSA-KAUH-8	25	4-1-4-1-5-5-4	25	NA
F1	1 (MRSA-KAUH-58)	PS	MRSA-KAUH-58	361	4-3-1-1-11-72-64	361	V
F2	1 (MSSA-KAUH-59)	SA	MSSA-KAUH-59	672	4-3-1-1-11-72-11	361	NA
F3	1 (MRSA-KAUH-31)	SO	MRSA-KAUH-31	88	22-1-14-23-12-4-31	88	IVa
G1	1 (MRSA-KAUH-11)	PK	MRSA-KAUH-11	217	7-6-1-5-8-5-6	22	IVa
G2	1 (MRSA-KAUH-45)	TD	MRSA-KAUH-45	217	7-6-1-5-8-5-6	22	IVa
G3	2 (MRSA-KAUH-5 and 24)	SA, PS	MRSA-KAUH-5	36	2-2-2-2-3-3-2	30	II
G4	2 (MSSA-KAUH-89 and 33)	YE, IN	MSSA-KAUH-89	1478	2-2-2-2-6-3-163	30	NA
H	3 (MSSA-KAUH-10, 42 and 65)	ET, SO, JO	MSSA-KAUH-10	1478	2-2-2-2-6-3-163	30	NA
I1	1 (MRSA-KAUH-68)	SD	MRSA-KAUH-68	728	1-3-1-14-11-27-10	80	IVe
I2	1 (MSSA-KAUH-81)	SD	MSSA-KAUH-81	188	3-1-1-8-1-1-1-1	1	NA
I3	1 (MRSA- KAUH-67)	SO	MRSA- KAUH-67	6	12-4-1-4-12-1-3	6	IVa
I4	2 (MSSA-KAUH-70 and 85)	SA, AF	MSSA-KAUH-85	6	12-4-1-4-12-1-3	6	NA
J1	3 (MRSA-KAUH-14, 78 and 79)	SD, SY, EG	MRSA-KAUH-14	2592	1-1-1-1-1-1-295	1	Non-typable
J2	5 (MSSA-KAUH-25, 26, 72, 83 and 87)	PK, SA, PS, PK, ER	MSSA-KAUH-87	1	1-1-1-1-1-1-1	1	NA
J3	4 (MRSA-KAUH-16, 21, 56 and 75)	YE, SD, PK, SA	MRSA-KAUH-16	728	1-3-1-14-11-27-10	80	IVe
K1	6 (MRSA-KAUH-4, 19, 23, 29, 35 and 36)	YE, YE, SO, SA, TD	MRSA-KAUH-23	728	1-3-1-14-11-27-10	80	IVe
K2	3 (MSSA-KAUH-17, 41 and 84)	SA, SA, SA	MSSA-KAUH-84	728	1-3-1-14-11-27-10	80	NA
L1	3 (MRSA-KAUH-9, 13 and 30)	SA, PK, PS	MRSA-KAUH-9	5	1-4-1-4-12-1-10	5	IVa
L2	2 (MSSA-KAUH-18 and 86)	SA, YE	MSSA-KAUH-18	15	13-13-1-1-12-11-13	15	NA
M	1 (MSSA-KAUH-71)	MM	MSSA-KAUH-71	121	6-5-6-2-7-14-5	121	NA
N	1 (MSSA-KAUH-88)	YE	MSSA-KAUH-88	15	13-13-1-1-12-11-13	15	NA
O	2 (MRSA-KAUH-43 and 54)	SA, SA	MRSA-KAUH-54	30	2-2-2-2-6-3-2	30	IVe
P	1 (MSSA-KAUH-44)	YE	MSSA-KAUH-44	2816	1-68-359-15-310-5-2	–	NA
Q1	5 (MRSA-KAUH-3, 39, 57, 62 and 77)	SA, SA, PK, PK, YE	MRSA-KAUH-39	97	3-1-1-1-1-5-3	97	V
Q2	2 (MSSA-KAUH-61 and 74)	SY, YE	MSSA-KAUH-74	97	3-1-1-1-1-5-3	97	NA
R	2 (MSSA-KAUH-27 and 34)	SA, YE	MSSA-KAUH-27	88	22-1-14-23-12-4-31	88	NA
S	2 (MSSA-KAUH-37 and 47)	SA, SA	MSSA-KAUH-47	15	13-13-1-1-12-11-13	15	NA
T1	4 (MSSA-KAUH-1, 6, 15 and 48)	SA, SA, SA, YE	MSSA-KAUH-1	789	1-3-1-76-11-5-13	152	NA
T2	1 (MRSA-KAUH-38)	SA	MRSA-KAUH-38	217	7-6-1-5-8-5-6	22	IVa
T3	5 (MRSA-KAUH-40, 46, 63, 69 and 80)	SA, SA, SA, NA, MM	MRSA-KAUH-46	241	2-3-1-1-4-4-30	–	III
U	4 (MSSA-KAUH-2, 28, 53 and 73)	SA, SA, SO, MM	MSSA-KAUH-2	791	10-14-8-6-10-3-32	–	NA
V1	3 (MSSA-KAUH-22, 49 and 51)	PH, SY, SA	MSSA-KAUH-22	3303	151-5-215-34-175-180-169	–	NA
V2	1 (MSSA-KAUH-60)	MM	MSSA-KAUH-60	3303	151-5-215-34-175-180-169	–	NA

PN: patient nationality; MM: Myanmar; SY: Syrian; EG: Egypt; YE: Yemen; SA: Saudi Arabia; PS: Palestine; SO: Somalia; PK: Pakistan; TD: Chad; ET: Ethiopia; SD: Sudan; NA: Nigeria; PH: Philippines BD: Bangladesh; JO: Jordan; AF: Afghanistan; ER: Eritrea; TD: Chad. CC: Clonal complex. NA: Not applicable.

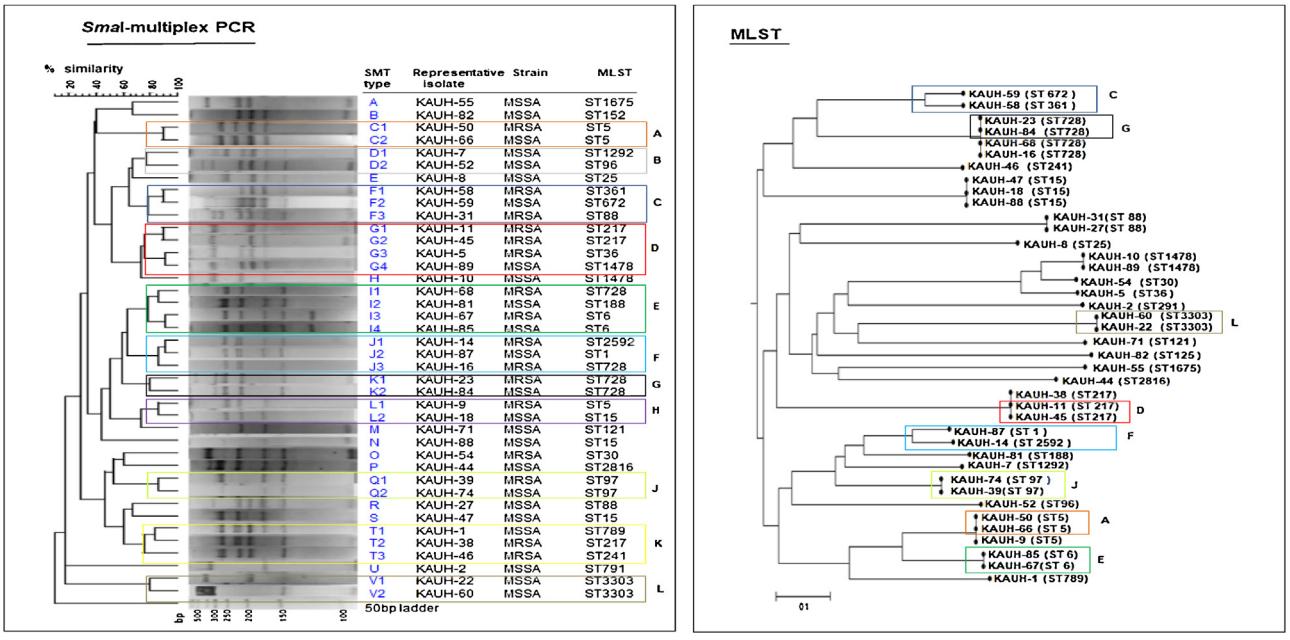

Fig. 1. The UPGMA dendrogram of banding patterns obtained by SMT based on Dice similarity coefficient, and the neighbor-joining tree of MLST illustrating the genetic relationship of 40 *S. aureus* isolates. The rectangles with different colours show the groups of clusters.

(Mu50, Mu3 and N315) [12]. These isolates were recovered from Egyptian, Bangladeshi, Saudi and Yemeni patients, respectively. The subtypes G1 and G2, isolates MRSA-KAUHSA-11 and MSSA-KAUHSA-45, were isolated respectively from Pakistani and Chadi patients and showed profiles that were identical to the UK epidemic clone EMRSA-15. Subtypes G3 and G4, strains MRSA-KAUHSA-5, 24, MSSA-KAUHSA-89, 33, and the type H isolate, strain MSSA-KAUHSA-10, 42 and 65, have profiles that are closely related to the UK epidemic clone EMRSA-16 [12]. Most of those isolates were recovered from intensive care unit (ICU) patients of seven nationalities (Saudi Arabia, Palestine, Yemen, India, Ethiopia, Somalia and Jordan).

In order to better understand the clonality of the SMT types, representatives of all 40 SMT profiles were typed by MLST to provide a linkage to the MLST database. Twenty-six sequence types (STs) were identified among the 40 representatives SMT types. One sequence type, identified in MSSA isolates KAUHSA-22 and KAUHSA-60, was not present in the MLST database and was subsequently assigned as ST3303 (Table 1). The eBURST analysis showed that the SMT types belonged to two clonal complexes (CC30: ST30, ST36 and ST1478, and CC1: ST1, ST188 and 2592), two doubletons (ST5 and ST6) and (ST361 and 672), and 16 singletons (ST1, ST152, ST188, ST672, ST361, ST1675, ST30, ST96, ST241, 2816, ST25, ST1292, ST36, ST291, ST789, ST3303). The most commonly identified sequence type was ST728 (n = 4), with ST5, ST15 and ST217 the next most common (n = 3).

Among the 40 *S. aureus* SMT types, 15 were methicillin-resistant and these were tested using multiplex PCR to identify the SCC*mec* type. Ten of these MRSA strains were found to harbour SCC*mec*-IV and its subtypes (six were SCC*mec*-IVa and four SCC*mec*-IVe). Three MRSA isolates (MRSA-KAUHSA-39, 50 and 58) belonged to SCC*mec* type V while isolates MRSA-KAUHSA-5 and 46 belonged to SCC*mec* types II and III, respectively. The remaining single MRSA strain, MRSA-KAUHSA-14, was non-typeable (Table 1).

Comparative analysis of typing data

One aim of this study was to establish a surveillance programme to monitor the impact of mass migration on the clonality of *S. aureus* strains isolated in Saudi Arabian hospitals. Consequently, epidemiological data obtained in the current study was subjected to a comparative analysis to determine whether the more cost-effectivity SMT typing system provided sufficient data for utilisation in a large-scale surveillance programme. The banding patterns obtained with SMT were analysed using the fingerprinting analysis software BioNumerics v7.5 (Applied Maths), while neighbour-joining tree software was used to construct the allelic profiles and STs of all 40 representative *S. aureus* isolates. Analysis of the correlation between the SMT and MLST types, using UGPMA, showed a good correlation between the two methods (Fig. 1). Cluster analysis showed that SMT grouped most of the *S. aureus* isolates into the same clusters as MLST, although there were differences in the relationships between other isolates. SMT type I isolates were grouped in very tight clusters by SMT, but not by MLST that grouped them into three unrelated clusters (Fig. 1, cluster E). Two isolates of SMT type I strains MRSA-KAUHSA-67 and MSSA-KAUHSA-85, have the same MLST allelic profile (ST6) while SMT type I strains MRSA-KAUHSA-68 and MSSA-KAUHSA-81 were identified as ST728, and ST188, respectively. Consistent with this result, the two MRSA isolates in SMT type I harboured subtypes SCC*mec* IV, namely IVe and IVa (Table 1). Similarly, MRSA-KAUHSA-14, 16 and MSSA-KAUHSA-87 were tightly clustered by SMT (Fig. 1, cluster F) and were identified as sequence types ST2592, ST728 and ST1, respectively. MRSA-KAUHSA-16 was shown to harbour SCC*mec*-IVe, while that of MRSA-KAUHSA-14 encoded a non-typeable by SCC*mec* typing.

Other clusters (A, C, F, J, and L) were consistently grouped by the two methods. For example, ST217-SCC*mec*-IVa isolates MRSA-KAUHSA-45 and MRSA-KAUHSA-11, were clustered with 85% similarity) (Fig. 1, cluster D), while ST5 isolates MRSA-KAUHSA-50 and MSSA-KAUHSA-66, clustered with 90% similarity (Fig. 1, cluster A). Isolates MRSA-KAUHSA-50 and MSSA-KAUHSA-66 have the same SMT profile to that of VISA strains Mu50, Mu3 and N315 and this was confirmed by MLST since both isolates have the same sequence type (ST5) of the VISA strains. Two isolates (KAUHSA-89 and MRSA-KAUHSA-5) were closely related to the UK nosocomial epidemic strain EMRSA-16 and were tightly clustered by SMT and MLST; they were identified as sequence types ST1478 and ST36 respectively, and belong to clonal complex CC30 (Fig. 1, cluster D) (Table 1). MSSA-KAUHSA-74 and MRSA-KAUHSA-39 had identical SMT profile with the exception of a fragment generated from the *mec*A gene in KAUHSA-39 and both isolates were identified as ST97 (CC97). Three MSSA isolates (KAUHSA-18, 47and 88) were identified as ST15 but were clustered differently by SMT (Fig. 1).

Discussion

Although the frequency of MRSA has decreased in some countries [14], it continues to be a predominant cause of nosocomial and community-acquired infections. Consequently, understanding the epidemiology of *S. aureus* strains (whether MSSA or MRSA) will continue to be essential for monitoring and controlling *S. aureus* infections. Human migration undoubtedly plays an important role in the spread and diversity bacterial infections, and genotyping provides a powerful means to explore the resulting clonality. Recently, DNA sequencing-based technologies such as MLST and WGS have been used to study the impact of human migrations on diversity of other bacterial pathogens such as *Helicobacter pylori* and *Mycobacterium tuberculosis* [15,16].

Mass population movements (e.g. air travel, migration and pilgrimages) are responsible for the world-wide dissemination of newly emerging strains and infections. Muslims from over one hundred countries visit the Saudi Arabian holy places annually. This mass movement undoubtedly contributes to the spread bacterial species and their clones throughout Saudi Arabia and subsequently globally. In particular, Jeddah city, the main gateway to these holy places, is likely to encounter a broader range of bacterial species and clones. However, the absence of a national surveillance programme means that there is a lack of information regarding the impact of visitors on the epidemiology and clonality of clinical *S. aureus* isolates. The aim of this study was to identify the impact on the clonal variation of clinical *S. aureus* isolates recovered from Jeddah city and to relate this to the likely geographical source. Isolates (n = 89) of *S. aureus* (MSSA and MRSA) were collected from clinical patient samples, primarily of non-Saudi Arabian origin. All isolates were typeable by SMT and there was a remarkably high degree of diversity among the forty distinct SMT profiles. A subsequent MLST analysis of representative of the forty SMT types confirmed a high level of concordance between the two typing methods (Fig. 1), albeit with SMT typing being more discriminatory. Seven sequence types (MSSA-ST16**75**, MRSA-ST361, MSSA-ST14**78**, MSSA-ST188, MSSA-ST121, MSSA-**ST152** and MSSA-ST2816) were only reported in non-Saudi patients and MRSA-ST30 only in Saudi patients. However, the remaining STs types were shared by both Saudi and non-Saudi citizens (Table 1). A small number of studies have been conducted to determine the most common genotypes of *S. aureus* and their clonal distribution in Saudi Arabia [17]. These studies were carried out in Riyadh and the Eastern provinces and it is unclear how representative they are of the Western provinces. However, these studies provide information about the most common *S. aureus* STs, with the so-called Hungarian clone (ST239-III) being the most prevalent clone in these hospitals, followed by ST80.

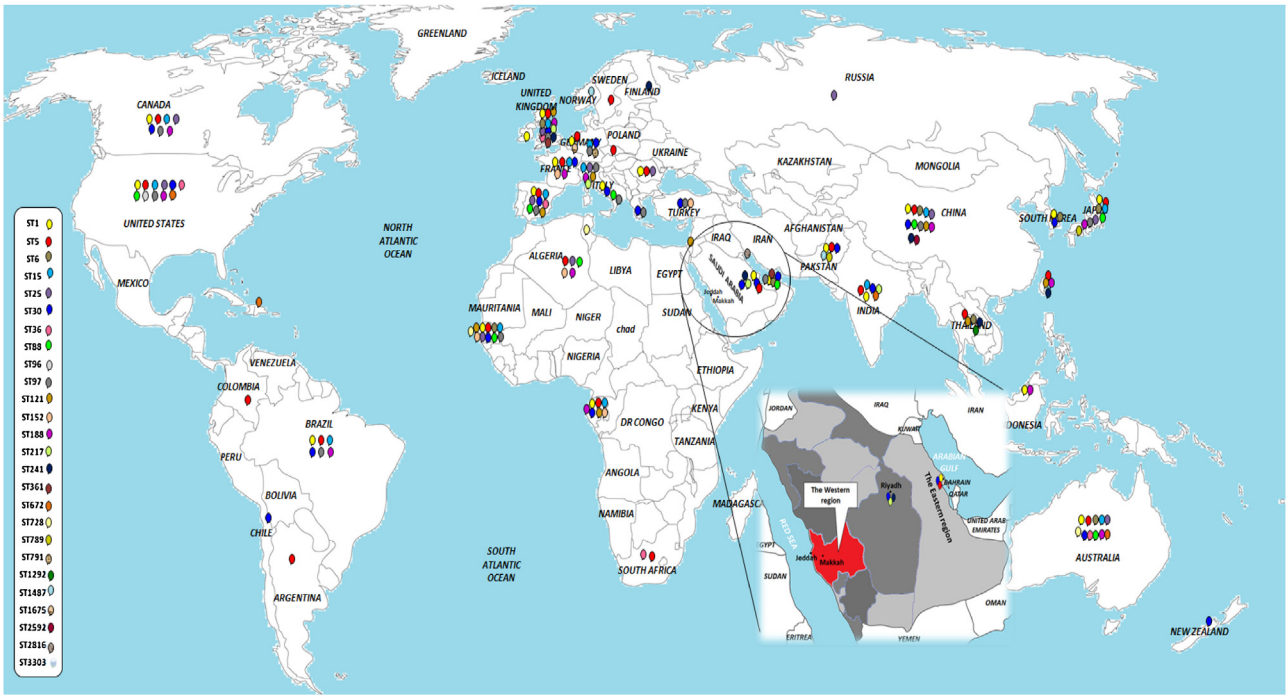

Fig. 2. Worldwide distribution of MRSA and MSSA clones (26 STs) reported in this study.

Moreover, the two hospital clones (the UK epidemic clone EMRSA-15, ST22-IV and the Southwest Pacific clone ST30-IV), and sequence types ST88, ST241, ST5 were also reported sporadically [17–19].

In the current study, SMT types J, K and their subtypes are closely related to community-acquired strains, MSSA476 and MW2 (both are members of ST1) [12,20]. Among the SMT types J and K, ST1 and ST728 encoded SCC*mec*-IVe while ST2592 encoded a SCC*mec* that was non-typeable. ST2592 is a single locus variant of ST1 and both types belong to the same clonal complex, namely CC1. ST1 has been recently reported in six isolates in the Eastern province hospitals, Saudi Arabia [19]. Clonal complex 1 strains include some of the most commonly isolated world-wide and have been reported to be isolated in more than 22 countries. In contrast there are only rare reports of ST728 (CC80), from Australia, Tunisia and the Gambia (Fig. 2). Clonal complexes CC1 and CC80 are associated with CA-MRSA clones [21–23]. In the current study, isolates of ST2592 were associated with wound infections in three non-Saudi patients. This clonal type has only recently been identified from a hospital infection in South China [24], and encodes a similarly untypeable SCC*mec* cassette. All of the CC1 and CC80 isolates were recovered from patients of different nationalities including Yemen, Sudan, Palestine, Somalia, Pakistan, Chad and Saudi Arabia (Table 1). Other community-acquired clones, ST361 (MSSA) and ST672 (MRSA), belonging to CC361, were also identified in this study in Palestinian and Saudi patients respectively. ST672 was reported in USA, Australia, Haiti and India while ST361 was identified only in UAE and the UK (Fig. 2). These results may be explained by the fact that community acquired MRSA strains have only recently become prevalent and their infections are no longer confined to the community but have started to replace HA-MRSA in some health care settings [25,26].

The SMT profiles of the closely related hospital-associated VISA strains Mu3, Mu50 and N315 [12] were reported in two isolates recovered from Egyptian and Yemeni patients. Both isolates had the same sequence type (ST5) as VISA strains. ST5 belongs to CC5 which is the most common clonal type, having been reported in more than 30 countries including Saudi Arabia (Fig. 2) and includes many HA-MRSA and CA-MRSA isolates [22,17]. The SMT profile of the UK epidemic clone EMRSA-15 was observed in two isolates. Both isolates shared the same SCC*mec* cassette (IVa) and sequence type (ST217), the latter a single locus variant of ST22, the sequence type of EMRSA-15. ST217 has only previously been reported in the UK, Switzerland, Italy and India (Fig. 2) [27,28]. ST217 has also identified in two isolates from hospitals in Riyadh [18]. Similarly, we detected a SMT profile similar to that of UK epidemic variant EMRSA-16 (also known as USA200) in one MRSA and two MSSA isolates. The MRSA representative isolate was identified as ST36 and carried SCC*mec*-II while the MSSA strains were of ST1478, a double-locus variant of epidemic ST36 and a member of clonal complex CC30. All ST1478 strains were recovered from non-Saudi patients and have not been described previously in Saudi Arabia. ST1487 strains have been reported in Norway and Pakistan, while ST36-MRSA-II is a more widespread clone from this lineage, found in USA, Australia, the UK, Spain and South Africa [22] (Fig. 2). MRSA-KAUHSA-54, identified as ST30-MRSA-IVe, also belongs to clonal complex (CC30) but was clustered separately by SMT. ST30-MRSA-IVe is known as a Southwest pacific clone or, in the UK, as epidemic variant EMRSA-16. This clone was also identified in three MRSA isolates from Riyadh hospitals [18]. The Southwest pacific clone is also described as the most widely distributed clone of the CC30 lineage [22,29,30] (Fig. 2). CC15 is commonly associated with MSSA, but MRSA clones are occasionally observed among healthy carriers [22]. In the current study, the ST15 (CC15) MSSA isolates were recovered from blood, urine and wound infections with many Saudi patients involved. A novel sequence type, named ST3303, was identified in two MSSA isolates in this study, both isolates were recovered from non-Saudi citizens. ST3303 is single locus variant of ST3387, ST2483, ST2250, ST2196 and ST1667 that differ only within the *aroE* locus.

In conclusion, a high genotypic diversity was found among *S. aureus* isolates and this was attributed to the fact that Jeddah is the principal gateway to Mecca and other pilgrimage centres, MSSA strains exhibited greater diversity than MRSA strains and a high proportion of these strains are likely to have been community-acquired. Such migrations are ideal events during which to study the impacts of large one-of meetings on the spread of bacterial

clonal types. Our data strongly suggests that there would be a value in extending such studies to include large numbers of isolates from the other hospitals of the Western region. A limitation of the current study is the lack of any previous epidemiological data in this particular region of Saudi Arabia. However, the relatively high genetic diversity reported among *S. aureus* isolates in a single hospital could reflect the impact of migration and form the basis for a more extensive survey. Our findings suggest that in locations affected by mass migrations, surveillance programmes should be established to monitor their impact on endemic strains and SMT has been shown to be a cost-effective and sensitive typing method for achieving this objective.

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Competing interests

None declared.

Ethical approval

I have read the ethical responsibilities of authors and I hereby declare that the content of this article complies to the Ethical Rules applicable for this journal.

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