Review Article

Methicillin-Resistant *Staphylococcus Aureus* in Saudi Arabia: Genotypes Distribution Review

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections in hospital have obviously imposed a significant burden of morbidity and mortality, and strain on healthcare resources. Here, we review the genotype distribution of these pathogens in the Kingdom of Saudi Arabia (KSA). A PubMed literature search (until May 2014) specified 12 articles that characterized MRSA clones in KSA. Only two regions (Riyadh and Damamm) were represented in ten articles. Data from these articles showed that the pandemic Vienna/Hungarian/Brazilian clone (CC8/ST239-III) is the most frequent in Saudi regions (Riyadh and Damamm). Several other clones such as Barnim/UK-EMRSA-15 (CC22-IV), Southwest Pacific clone (ST30-IV) and European community-associated-MRSA clone (CC80-IV) have been detected in the Riyadh region. A variety of MRSA clones is beginning to circulate in Saudi hospitals. Continued collection and molecular characterization of MRSA is crucial for the effective prevention and treatment.

Key words: Genotypes, methicillin-resistant *Staphylococcus aureus*, Saudi

ملخص البحث :

العدوى الناشئة عن البكتيريا العنقودية المقاومة للميثيسيلين في المستشفيات شكلت عبئاً كبيراً على موارد الرعاية الصحية بالإضافة إلى زيادة معدلات المرض والوفيات. يستعرض الباحثان توزيع الأنماط الجينية بين هذه الجراثيم في المملكة العربية السعودية. أجري البحث في قاعدة البيانات (PupMed) واستعرض الباحثان عشرة ابحاث منشورة لمنطقتي الرياض والدمام. وأظهرت المعلومات انتشار نسيله فيينا / المجر/ البرازيل بصورة وبائية في المملكة. وكذلك انتشار أعداد أخرى من النسائل في الرياض وهي بارنيم/ المملكة المتحدة وجنوب غرب المحيط الهادي والنسيلة الأوربية المكتسبة من المجتمع. يتضح من ذلك ان هناك العديد من النسائل لهذه البكتيريا العنقودية والتي بدأت في الانتشار في المستشفيات السعودية. الاستمرار الدائم في جمع والتوصيف الجزيئي لهذه البكتيريا العنقودية أمر بالغ الأهمية للوقاية والعلاج الفعال.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections represent a major challenge to hospital microbiologists because of the emergence and spread of clones that have decreased susceptibility to many antibiotic classes.[1,2] Methicillin-resistance in staphylococci is due to the acquisition of one of the several staphylococcal cassette chromosomes (SCC*mec*), that carries a *mec* A gene that encodes a penicillin-binding protein conferring resistance to methicillin.[3] Molecular typing approaches have been used to great advantage in establishing clonal relationships between strains besides identifying and monitoring the international spread of some unique MRSA strains.[4,5] A variety of molecular techniques has now been introduced for the study of the epidemiology of MRSA.[6] These include SCC*mec* typing,[7-9] pulse field gel electrophoresis (PFGE),[10] multilocus sequence typing (MLST),[11] and Staphylococcus protein A gene (*spa*) typing.[12-14] PFGE, MLST, and *spa* typing are the most widely used typing techniques because of their high discriminatory power and reproducibility.[15]

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In Saudi studies, the prevalence of MRSA among *S. aureus* and temporal increases have been shown to vary widely among the regions.[16,17] Recently, the overall estimation of the prevalence of MRSA in Saudi Arabia (KSA) was 35.6% from pooled estimation of 22,793 *S. aureus* strains from 2002 to 2012.[18]

The aim of the present review was to provide an overview of the genotypes present throughout Saudi Arabia to support the use of effective treatments and to guide strategies for the control of the spread of MRSA.

GEOGRAPHY OF KSA AND DATA ACQUISITION

KSA is one of the most populous countries and the largest in the Arabian Peninsula (2×10^6 Km^2). The population of KSA is estimated around 28 million, about 20% of whom are expatriates mainly from the Indian subcontinent and Southeast Asia. Moreover, KSA hosts more than 4 million Muslim pilgrims from across the globe during the Hajj and Umra seasons.[16,19] Mass gathering of millions of Muslims for the Hajj from all over the world in the same region increases the possibility of infectious pathogens, especially MRSA strains. The above-mentioned conditions make KSA a hot spot for the collection of MRSA and its global spread.

For this review, we performed a literature search to evaluate the history and distribution of MRSA clones within KSA. We searched the PubMed/MEDLINE database of all published articles related to genotyping MRSA in Saudi Arabia until May 2014. Search terms (and combinations thereof) were: "MRSA clones," "'Methicillin resistant *Staphylococcus aureus* clones," "MRSA," "genetic," "molecular characterization," "typing," "genotypes," and "Saudi". Hand search of references listed in relevant articles was also carried out.

Most of the pertinent published data on genotyping MRSA came from only three regions out of a total of 13 regions in KSA. Only 12 relevant published articles reporting data collected were included: Nine from Riyadh (1998-2011), one from Dammam (2001-2003), one from Jeddah (2009-2011), and one from different cities in KSA (1992-1995). A variety of genotyping methods had been used to identify MRSA clones. Some studies applied only one technique (PFGE three articles and SCC*mec* three articles). Three other articles used two techniques (SCC*mec* and MLST). One article used PFGE and SCC*mec*; another study applied PFGE and MLST techniques; and one used three techniques (SCC*mec*, MLST, and *spa*) [Table 1].

DISTRIBUTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS GENOTYPES IN KSA

The data collected in Table 1 show that the earliest publication from KSA described the inter-hospital spread of a single MRSA clone between 1992 and 1995. Ninety-four strains of MRSA, originating from inpatients across geographically diverse regions of KSA were genetically typed by randomly amplified polymorphic DNA and a representative subset of the strains was analyzed by PFGE as well. However, 93% (87/94) of the isolates belonged to a single clonally related lineage of MRSA and seven other isolates differed only slightly from those determined for the clonal type.[20] Eight years later, in contrast to the Romania strains, MRSA strains collected from institutions in Dammam (n = 60) and Dhahran (n = 8) between April 2001 and May 2003 shared a common PFGE pattern and MLST (ST239). This indicated that a single epidemic clone was spreading in Saudi Arabia. The MRSA strains were differentiated using PFGE of *sma* I DNA macrorestriction fragments. A comparison of PFGE fingerprints identified clusters of strains with clear segregation branches according to the hospital they had originated from. However, MLST revealed that all strains except two (ST5 and ST254) shared the ST239 genotype. Even the relatively closed PFGE-based dendrogram indicated a diversity of ST239.[21]

Data based on evolutionary patterns and genotypic characteristics again indicated that a single epidemic clone of MRSA was widespread in Saudi Arabia compared with other Asian countries. In this review, five MRSA strains (from King Khalid University Hospital, Riyadh during 1998-2003 were analyzed by MLST and SCC*mec* typing. Data indicated that all five MRSA strains belonged to a single, epidemic clone ST241 (a single-locus variant of ST239) and CC239 with SCC*mec* IIIA.[22] A description of SCC*mec* elements carried by 19 MRSA strains isolated in King Khalid University Hospital, Riyadh, from 1998 to 1999 was reported. Eighteen MRSA strains were classified as SCC*mec* 3A (III) type, and the frequency of type SCC*mec* 2B (IV) was very low (1/19). Subsequently, the genotypes of representative five isolates were investigated by MLST. The dominant four MRSA strains belonged to CC8 (ST239), and one minor strain of CC5 (ST5) were reported.[23]

Table 1: Distribution of MRSA isolates from Saudi regions by their genotypic characteristics until May 2014

Location		Study period	Isolates number	PFGE (isolates number)	*Mec* type	SCC*mec* type (isolates number)	MLST			*spa* type (isolates number)	PVL	References
Region	Hospital						CC	ST	Allelic profile			
Cities in KSA	KFSH RCTC	1992-1995	94	Clone 1 (87) Clone 2 (7)	—*	—	—	—	—	—	—	[20]
Dammam	DCH KAAH DGH	2001-2003	68	PFGE patterns clustered strains to hospitals origin with clear segregation branches	—	—	—	239 (66) 254 (1) 5 (1)	—	—	—	[21]
Riyadh	KSUH	1998-2003	5	—	A	III	239	241	2-3-1-1-4-4-30	—	—	[22]
Riyadh	KSUH	1998-1999	19	—	A	3A—III (18)	8	239	2-3-1-1-4-4-3	—	—	[23]
					B	2B—IV (1)	5	5	1-4-1-4-12-1-10			
Riyadh	RMC SFH MH KFSH KAUH KFMC KKUH	2004-2005	512	M1 (187) M2 (105) M3 (27) M4 (73) M5 (29) M6 (7) Unique (84)	—	—	—	—	—	—	—	[24]
Riyadh	KKUH	2007	37	—	A	IVa	—	—	—	—	3	[26]
Riyadh	MHL PHC	2008-2009	135	—	A	IVa	—	—	—	—	18	[27]
Riyadh	MHL PHC	2009	30	Cluster-1 (17) Cluster-2 (12) Cluster-3 (1)	A	—	—	—	—	—	—	[25]
Riyadh	KKUH	2009	10	Pattern-1 (9) Pattern-2 (1)	—	IV (10)	—	—	—	—	—	[28]
Jeddah	MHL PHC	2009-2011	101	—	A	III (39) V (43) IVa (16) IVc (3)	—	—	—	—	38	[29]

Table 1: (Continud)

Location		Study period	Isolates number	PFGE (isolates number)	*Mec* type	SCC*mec* type (isolates number)	MLST			*spa* type (isolates number)	PVL	References
Region	Hospital						CC	ST	Allelic profile			
Riyadh	KFMC	2010-2011	107	—	A	IV (1)	1	—	—	—	58	[30]
						V (1)		ST772				
						IV (8)	5	—				
						V (1)						
						IV (3)	6					
						III (22)	8	239				
						Atypical (1)	9	834				
						IV (30)	22					
						IV (13)	30					
						IV (1)	45					
						1V (21)	80					
						IV (3)	88					
						V (2)	97					
Riyadh	AFH	2010	120	—	—	III (120)		ST239 (60)	2-3-1-1-4-4-3	*spa* CC037 (66) t019-t030-t037-t138-t363-t388-t459-t631-t748-t932-t1070	0	[31]
								ST08 (7)	3-3-1-1-4-4-3			
								ST241 (3)	2-3-1-1-4-4-30	*spa* CC790 (9) t032-t223-t790-t4573-t7604-t8506-t8855-		
								ST22 (10)	7-6-1-5-8-8-6	*spa* CC376 (25) t044-t376-t8731-		
								ST217 (2)	7-6-1-5-8-5-6			
								ST80 (25)	3-3-1-14-11-4-10			
								ST71 (3)	18-1-1-1-1-5-3	*spa* CC690 (5) t690-t729-t8507-		
								ST30 (3)	2-2-2-2-26-3-2			
								ST88 (6)	22-1-14-23-12-4-3	No founder (6) t304-t701-		
								ST82 (1)	18-18-6-2-13-15-18	Singletons (9) t002-t364-t2235-t3059		

— – No data; KFSH – King faisal specialist hospital; RCTC – Research centre for tertiary care; DCH – Dammam central hospital; KAAH – King abdulaziz airbase hospital; DGH – Dhahran general hospital; KSUH – King saud university hospital; RMC – Riyadh medical compound; SFH – Security force hospital; KAUH – King abdulaziz university hospital; KFMC – King fahad medical city; KKUH – King khalid university Hospital; MHL – Major hospital laboratories; PHC – Public health centers; AFH – Armed force hospital; MRSA – Methicillin—resistant *Staphylococcus aureus*; PFGE – Pulse field gel electrophoresis; SCC*mec* – Staphylococcal cassette chromosomes; MLST – Multilocus sequence typing; PVL – Panton—Valentine leukocidin

Although one MRSA clone is widespread in KSA, other clones are also beginning to spread. Baddour *et al.* performed comparative chromosomal DNA analysis of 512 MRSA strains from seven hospitals in Riyadh between 2004 and 2005 using PFGE. They were categorized into six major PFGE patterns (M1–M6), and M1 was numerically predominant and widespread (187 strains, 36.5%) followed by an M2 type (105 strains, 20.5%). Unique PFGE type was designated and reported as a prevalent pattern (84 strains, 16.4%).[24] In the same way, three major clusters were revealed from the 30 MRSA strains collected from major hospital laboratories and public health centers in Riyadh in 2009 and typed by using PFGE. The first, which included 17 strains were subdivided into four groups, and the second consisted of 12 strains, while the third cluster contained only one strain.[25]

Other MRSA strains have been described with genes encoding Panton-Valentine leukocidin (PVL), harboring SCC*mec* type IV-traits that are usually associated with community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). MRSA genotyping data showed a much lower PVL prevalence of only 8% (three out of 37) in SCC*mec* IV strains from outpatient clinics in King Khalid University Hospital, Riyadh, in 2007.[26] In another report, Moussa and Hessan found that only 18 strains (13.33%) recovered from skin and soft tissue infections were positive for PVL and (SCC*mec*) type IV.[27] Furthermore, in an investigation of a CA-MRSA outbreak among healthy neonates at a tertiary care teaching hospital in Riyadh between October and November 2009, 10 MRSA isolates (nine infants and one mother) were characterized using PFGE and SCC*mec* typing. Although all 10 MRSA isolates harbored SCC*mec* IV type, nine MRSA were of the same PFGE pattern but the remaining one was different.[28] Recently, 101 clinical isolates of MRSA, taken from a Jeddah Hospital and Health Centers from August 2009 to May 2011, were investigated genetically by SCC*mec* typing and genes encoding PVL. SCC*mec* III (39 strains) were the most predominant type. Only 38 strains (37.6%) harbored PVL gene while SCC*mec* V (43 strains). Some minor strains belonged to SCC*mec* IVa and IVc (16 and 3 strains, respectively), but no SCC*mec* types I, II, IVb or IVd were detected.[29]

The first MRSA typing data from Saudi Arabia were obtained by Monecke *et al*. When 107 MRSA isolates from King Fahad Medical City in Riyadh between summer 2010 and spring 2011 were characterized by DNA microarrays, the most predominant five MRSA strains from four clonal complexes found were CC8/ST239-III (Vienna/Hungary/Brazil Epidemic strain-20.75%) PVL-positive, and negative CC22-IV (UK-EMRSA-15/Barnim Epidemic strain-18.87% and 9.43%, respectively), PVL-positive CC30-IV (Southwest Pacific clone-12.26%) and PVL-positive CC80-IV (European CA-MRSA clone-17.92%). Other strains which accounted for <3% each included PVL-negative CC5-IV, CC5-IV/SCCfus, CC6-IV (West Australian, WA, MRSA-51/66) and PVL-positive CC88-IV, PVL-positive CC5-IV, PVL-positive CC80-IV, CC97-V as well as CC1-IV/SCCfus (WA MRSA-1/45), PVL-positive CC1/ST772-V (Bengal Bay clone/WA MRSA-60), PVL-negative CC5-V, CC45-IV (WA MRSA-23), and a CC9/ST834-MRSA strain with an identified SCC*mec* element. Surprisingly, the prevalence of PVL genes was significantly higher (54.21%).[30] Alreshidi *et al*. provided the first data on MRSA genotypes and virulence gene profiles in cancer patients. A total of 120 MRSA isolates from cancer and noncancer patients in the Armed Forces Hospital in Riyadh in February and August 2010, were investigated using SCC*mec*, MLST, *spa*, and virulence genes detection. SCC*mec* type III was detected in all MRSA isolates, but no PVL gene was detected. According to *spa* typing, MRSA strains were clustered into six groups including cluster-1 *spa* CC 037 (66 strains), cluster 3 *spa* CC 376 (25 strains), cluster 2 *spa* CC 790 (9 strain) as well as some minor clusters. Four novel *spa* types (local *spa* types) were detected and identified as t7604, t8506, t8507, and t8855. MRSA strains were classified into two different clonal clusters and three singletons. Group-1 including ST239 (70 strains), ST08 (seven strains), and ST241 (three strains) was the most prevalent followed by group-2 (ST22 and ST217) and five minor singleton groups (ST182, ST71, ST88, ST30, and ST80,).[31]

PREDOMINANT METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CLONES IN KSA

Although few published data about MRSA genotyping in KSA (12 published papers) are available, what are presented above give an overview of the genotyping of MRSA currently circulating in KSA and allow a comparison to be made with other countries. In this review, we found that the pandemic Vienna/Hungarian/Brazilian clone (CC8/ST239-III) and its variants continue to circulate in Dammam and Riyadh. ST239-III, mainly hospital-associated was the largest group detected in most studies from 2001 to 2013. Another clone, the pediatric clone (CC5), has been found in KSA hospitals though cases are rare. In recent years (2012-2013), many diverse strains of MRSA have been identified in the

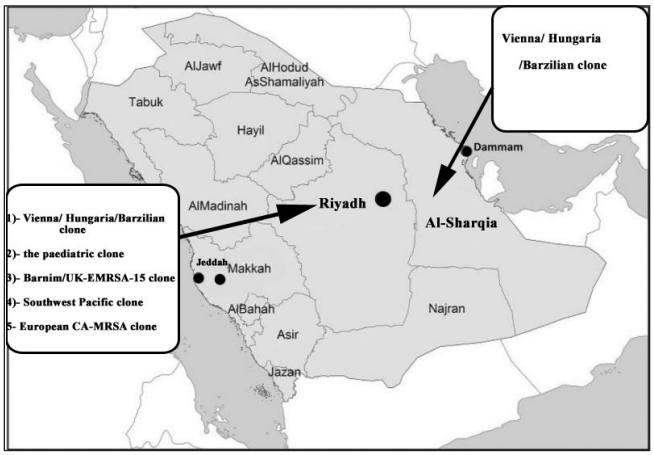

Figure 1: Distribution of most predominant Methicillin-resistant *Staphylococcus aureus* clones in Saudi regions

Riyadh region. Besides the Vienna/Hungarian/Brazilian and pediatric clones disseminated in KSA, several other common clones have also been identified. Barnim/UK-EMRSA-15 (CC22-IV), Southwest Pacific clone (ST30-IV), and European CA-MRSA clone (CC80-IV) are the most common clones in Riyadh hospitals [Figure 1].

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