academicJournals

Vol. 10(22), pp. 775-782, 14 June, 2016
DOI: 10.5897/AJMR2016.7996
Article Number: 894341F58932
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Diversity of MRSA SCC*mec* elements in Pretoria region of South Africa: The problem of variation in the assigned SCC*mec* types by different multiplex-polymerase chain reaction (PCR) methods and a call for an African consensus

John F. Antiabong[1], Marleen M. Kock[1,2], Tsidiso G Maphanga[1], Adeola M. Salawu[1] and Marthie M. Ehlers[1,2]*

[1]Department of Medical Microbiology, University of Pretoria, Gauteng, South Africa.
[2]South Africa National Health Laboratory Service, South Africa.

Received 7 March 2016, Accepted 5 May, 2016.

The SCC*mec* element is one of the recommended targets for MRSA characterization and several multiplex-PCR SCC*mec* typing methods have been developed over the past years. However, there are no data on the consistency of the SCC*mec* types in clinical isolates as detected by these methods. Using different previously published, commonly used M-PCR methods, this report describes the diversity of SCC*mec* elements in MRSA isolates in the Pretoria region of South Africa and the discrepancies observed in the assigned SCC*mec* types. Different SCC*mec* types were assigned to the same clinical MRSA isolates. The discrepancies included the assignment of composite SCC*mec* types [(SCC*mec* II and SCC*mecury*) 20.7% (40/193)] and [(SCC*mec* type II+IVc) 22.3% (43/193)] to some of the clinical MRSA isolates. Summarily, the combination of the result of the M-PCR methods showed that the MRSA genotypes circulating in the healthcare facility studied potentially carried SCC*mec* types I, II, IV (subtypes IVa, IVb and IVd) and V. No SCC*mec* types III or VIII was detected among the isolates. At least 25.91% of SCC*mec* type IV was detected in this study, thus corroborating previous findings of the global encroachment of MRSA strains into the hospital settings. The associated epidemiological significance of these observations is discussed and we also call for an African consensus SCC*mec* typing method in order to allow effective epidemiological data comparison across the countries.

Key words: MRSA genotype, SCC*mec* elements, multiplex-polymerase chain reaction (PCR), variation.

INTRODUCTION

Staphylococcus aureus is a virulent bacterial pathogen which is responsible for infections seen in healthcare and

*Corresponding author. E-mail: marthie.ehlers@up.ac.za.

community settings (Kim, 2009). Infections caused by MRSA were previously associated with healthcare settings [Healthcare-associated MRSA (HA-MRSA)] but the emergence of community-associated MRSA (CA-MRSA) worsened the health challenges associated with MRSA (Moussa et al., 2012). Epidemiological history shows that, the CA-MRSA differed from the HA-MRSA in various ways: (i) the lack of traditional risk factors associated with MRSA among patients, (ii) a susceptibility pattern with resistance to few antimicrobial agents and (iii) the inclusion of specific virulence factors such as the Panton Valentine leucocidin (PVL) genes (Weber, 2005; Lo et al., 2011). In addition, a previous study have shown that CA-MRSA and HA-MRSA are demographically, clinically, and microbiologically different (Naimi et al., 2003). However, recent reports now show that the clinical definition of CA-MRSA and HA-MRSA (based on disease on-set, risk-factors and possession of PVL gene) are becoming blurred (David et al., 2010; Prosperi et al., 2013). A study by Peterson et al. (2012) showed that demographics including the disease on-set and the associated risk-factors are not consistent with the genotypic classification of CA-MRSA and HA-MRSA.

The *S. aureus* genome includes a mobile genetic element [staphylococcal cassette chromosome *mec* elements (SCC*mec*)] that carries the determinant for beta-lactam resistance encoded by *mec*A (IWG-SCC, 2009) and *mec*C (Paterson et al., 2013). Earlier reports indicated that the HA-MRSA strains harbor primarily SCC*mec* type I, II, III or VI (Naimi et al., 2003), while CA-MRSA carries the SCC*mec* type IV, V, VII, or VIII and are resistant to only β-lactam antibiotics and sensitive to non-β-lactam antibiotics (Daum et al., 2002). The possibility of transfer of the antimicrobial resistance determinant (the SCC*mec*) between CA-MRSA and HA-MRSA isolates in healthcare and community settings necessitates accurate and reliable methods for the detection and identification of these strains (Song et al., 2011). Moreover, the lack of healthcare associated risk factors for the definition of CA-MRSA as prescribed by the Centers for Disease Control and Prevention (CDC) (Morrison et al., 2006) was not sufficient in defining the emergence of CA-MRSA- and HA-MRSA-associated infection in the community and the association of CA-MRSA strains with healthcare-associated infections (O'Brien et al., 1999; Saiman et al., 2003). This led to the use of molecular typing tools (based on SCC*mec* element) for the classification of MRSA (Daum et al., 2002; Naimi et al., 2003). Several methods for SCC*mec* typing have been developed and have been previously validated and characterized using MRSA strains with known SCC*mec* elements. These methods were designed in response to new epidemiological and genomic information. For an extensive review of structure of the SCC*mec* element in *S. aureus,* refer to the work of Shore et al. (2013). An in-depth description of the molecular basis for the SCC*mec* typing and other typing methods have been previously reviewed (Stefani et al., 2012). Consequently, a brief description of the regions targeted by the primer sets/SCCmec typing methods investigated in this report and their limitations in detecting SCC*mec* types are thus presented: the primer sets of Oliveira and de Lencastre (2002) targets the upstream and downstream of *mec*A complex incorporating the cassette chromosome recombinase (*ccr*) allele AB. The Oliviera and de Lencastre (2002) method described SCC*mec* type V as type III and did not consider the differentiation between type IV subtypes. An updated version of the Oliviera and de Lencastre (2002) method focused on the detection of SCC*mec* type IV (Milheiriço et al., 2007) by amplifying regions within the *ccr*AB allotypes, five polymorphic J1 regions and a new J1 region that was detected in EMRSA-15 clone. The multiplex PCR (M-PCR) primer sets designed by Zhang et al. (2005) focused on the identification of types 1-4 using the *mec* and *ccr* elements and the subtypes designation are based on the junkyard region. Five isolates were not typable by this method however, Oliveira and de Lencastre (2002) method designated those isolates as SCC*mec* type III while four isolates identified as types I or II were designated as type II by the Oliveira and de Lencastre (2002) method.

It is noteworthy that these two methods showed a 100% agreement in typing control strains. An updated version (Zhang et al., 2012) of the previous Zhang and colleagues' (2005) method was later reported. This improvement addressed the following: (a) detection of SCC*mec* type II strains that lack the *kdp*E gene; (b) SCC*mec* type III lacking the J1 region; (c) detection of subtype IVc by targeting the J1 region and subtype IVe by targeting the J3 region; (d) differentiation of the SCC*mec* VIII and II. Despite this updated method, 4.5% (24/533) of the isolates were not typable.

Boye et al. (2007) developed a method to differentiate between HAMRSA from CA-MRSA carrying SCC*mec* types IV and V thereby, preventing the mistyping of SCC*mec* type V as type III. Six (1.92%) of the isolates tested by this method were not typable. However, four of the six isolates were typable using the Oliveira and de Lancaster (2002) method. The McClure et al. (2010) method focused on the detection of SCC*mec* type VIII by amplifying regions within the class A *mec* gene and type IV *ccr* gene complexes using five PCR targets.

In an effort to incorporate more variable regions within the *S. aureus* genomic make up, Kondo et al. (2007) described a method that included the *ccr* genes, *mec* class A-C, open reading frame of J1 region, transposons *Tn554* and Ψ*Tn554* in the J2 regions and plasmids *PT181* and *Pub110* in the J3 regions. Despite the extensive coverage of the variable regions 93/99 MRSA control strains could be assigned by this method while the *ccr* genes of six *mec*A positive strains could not be defined by this method. The authors reported that the M-PCR reported did not conflict with previous methods by Oliveira and de Lancaster (2002) and Zhang et al. (2005).

While the complete review of the various SCC*mec* typing methods is not the primary scope of this report, a snap shot of the adoption frequency of the methods (discussed in this report) by different laboratories indicates that there is no uniform method or criteria for the use of a particular method (Table 1).

To circumvent the inherent limitations of individual SCC*mec* typing methods, five published M-PCR based SCC*mec* typing were combined in order to determine the diversity of SCC*mec* elements in the Pretoria region of South Africa and to observe the differences in the SCC*mec* types assigned by methods that detect the same range of known SCC*mec* types.

MATERIALS AND METHODS

MRSA sample source and total bacterial DNA purification

One hundred and ninety three (193) MRSA isolates were obtained from the Diagnostic Laboratory, Department of Medical Microbiology, University of Pretoria Tshwane Academic Division, National Health Laboratory Service. The MRSA isolates were sub-cultured on Blood agar plates (Oxoid, England) at 37°C for 18 to 24 h to obtain single colonies for Gram-staining in order to confirm the purity. Genomic DNA was purified from the 193 MRSA isolates using the ZR Fungal/Bacterial DNA MiniPrep (Zymo Research, Thermo Scientific, USA), according to the manufacturer's instructions. Ethical approval for this study was obtained from the Research Ethics Committee of the University of Pretoria (protocol number S189/2010 and S175/2011).

Multiplex-PCR assays for the designation SCCmec types

Five commonly described SCC*mec* typing methods in the literature were investigated. Multiplex PCR (M-PCR) reactions using specific primers were employed as previously described for each of the methods tested (Table 1) and the genomic DNA from a CA-MRSA strain (ATCC CA05) served as a positive control in the M-PCR assays. The samples were reconfirmed using the *S. aureus* specific primers (McClure et al., 2006). The M-PCR amplicons were electrophoretically separated at 100 V/cm in a 1% MetaPhor^{TM} agarose gel (Lonza, Rockland, USA) containing 5 µl of ethidium bromide (10 mg/ml) (Promega, Madison, USA) and visualized using an Ultra Violet light box (DigiDoc, UVP product, Upland, California). The assignments of SCC*mec* types were performed as previously described for individual methods (Table 2).

RESULTS AND DISCUSSION

All the 193 previously determined MRSA samples were reconfirmed using the *S. aureus* specific primers (McClure et al., 2006). The 16S rRNA and the *mec*A gene were detected in all the samples tested. However, variations were observed in the proportion of samples designated as a specific SCC*mec* type or untypable by each SCC*mec* typing method assessed (Table 3). The electrophoretic pattern of the M-PCR amplicons used for the assignment of the SCC*mec* types is shown in supplementary material (Figure S1-S7). Table 3 shows that methods1 and 3 were able to designate equal number and same set of MRSA isolates as SCC*mec* I (3.1%) and SCC*mec* II (9.33%). The number of isolates assigned as SCC*mercury* by methods 1, 2 and 3 were different, with method 3 designating 61.14% (118/193) of the isolates as SCC*mercury* followed by method 1 {(41.5% (80/193)} and method 2 {16.1% (31/193)}. The number of MRSA isolates designated as SCC*mec* I, II and SCC*mercury* by method 2 did not correspond to any of the other methods tested. Method 4, an updated version of method 2 designated 10 additional MRSA isolates as SCC*mec* II, giving a total of 35.75% (69/193) SCC*mec* type II MRSA isolates as compared to method 2 which assigned SCC*mec* type II to 30.7% (59/193) to the isolates. Method 2 was able to subtype the same set of isolates [SCC*mec* type IVa, 1.03% (2/193); SCC*mec* type IVb, 0.52% (1/193); SCC*mec* type IVd, 24.4% (47/193)] designated as SCC*mec* type IV [25.91% (50/193)] by Method 3. Moreover, one isolate was designated as SCC*mec* type V by Method 3.

The rest of the MRSA isolates were designated as composite SCC*mec* types. These included SCC*mec* type II+SCC*mercury,* 20.7% (40/193) assigned by method 1; SCC*mec* type II+IVc, 22.3% (43/193) and 26.42% (51/193) assigned by method 2 and 3 respectively. As SCC*mercury* was detected by methods 1, 2 and 3 and also in composite SCC*mec* type detected by method 1, it is possible that the SCC*mercury* is carried in separate plasmid within the bacterial cell. No SCC*mec* type III or type VIII was detected by the methods 4 and 5, respectively. The proportion of untypable MRSA isolates was 4.7% [(9/193); (Method 2)] and 37.82% {(73/193); (Method 4)}.

Table 4 shows the number of clinical MRSA isolates that were designated the same SCC*mec* type by different M-PCR methods. Methods 2 and 4 assigned SCC*mec* type II to 30.57% (59/193) of the same set of MRSA isolates. This was the highest number of isolates designated the same SCC*mec* type by the different methods investigated. Moreover, about 26% (50/193) of the same isolates were assigned SCC*mec* type IV by methods 2 and 3, while 15.54% (30/193) of the MRSA isolates were designated as SCC*mecury* by methods 1, 2 and 3.

These observations indicated that the assessed M-PCR methods were able to assign a specific SCC*mec* type to the same MRSA isolates, most of the remaining isolates were designated different SCC*mec* types by the methods investigated. In a separate experiment, an attempt to categorize the SCC*mec* types defined by each SCC*mec* typing method in this study revealed that there was no specific distribution pattern of SCC*mec* type(s) among the pulsed field gel electrophoresis derived pulsotypes (data not shown) suggesting that there was no specific association between the chromosomal DNA content of the MRSA isolates and the SCC*mec* type assigned by the methods evaluated.

Table 1. List of primers for six SCC*mec* typing methods including *S. aureus* confirmation.

Primer	Oligonucleotide sequence (5'- 3')	Target gene	Amplicon Size (bp)	Reference
Staph 756F	-AACTCTGTTATTAGGGAAGAACA-			
Staph 756R	-CCACCTTCCTCCGGTTTGTCACC-	16S rRNA	756	Primers for *Staphylococcus aureus* confirmation
MecA1-F	-GTAGAAATGACTGAACGTCCGATAA-			McClure et al. (2006)
MecA2-R	-CCAATTCCACATTGTTTCGGTCTAA-	*mec*A	310	
CIF2-F2	-TTCGAGTTGCTGATGAAGAAGG-			
CIF2-R2	-ATTTACCACAAGGACTACCAGC-	SCC*mec* I	495	
KDP-F1	-AATCATCTGCCATTGGTGATGC-			
KDP-R1	-CGAATGAAGTGAAAGAAAGTGG-	SCC*mec* II	284	(Method 1)
RIF5-F10	-TTCTTAAGTACACGCTGAATCG-	SCC*mec*		Oliveira and De Lencastre (2002)
RIF5-F13	-GTCACAGTAATTCCATCAATGC-	III	414	
MECA P4	-TCCAGATTACAACTTCACCAGG-			
MECA P7	-CCACTTCATATCTTGTAACG-	*mec*A	162	
Type I-F	-GCTTTAAAGAGTGTCGTTACAGG-			
Type I-R	-GTTCTCTCATAGTATGACGTCC-	SCC*mec* I	613	
Type II-F	-CGTTGAAGATGATGAAGCG-			
Type II-R	-CGAAATCAATGGTTAATGGACC-	SCC*mec* II	398	
Type III-F	-CCATATTGTGTACGATGCG-			(Method 2)
Type III-R	-CCTTAGTTGTCGTAACAGATCG-	SCC*mec* III	280	Zhang et al. (2005)
Type IVa-F	-GCCTTATTCGAAGAAACCG-			
Type IVa-R	-CTACTCTTCTGAAAAGCGTCG-	SCC*mec*IV a	776	
Type IVb-F	-TCTGGAATTACTTCAGCTGC-			
Type IVb-R	-AAACAATATTGCTCTCCCTC-	SCC*mec*IV b	493	
Type IVc-F	-ACAATATTTGTATTATCGGAGAGC-			
Type IVc-R	-TTGGTATGAGGTATTGCTGG-	SCC*mec*IV c	200	
Type IVd-F	-CTCAAAATACGGACCCCAATACA-			
Type IVd-R	-TGCTCCAGTAATTGCTAAAG-	SCC*mec*IV d	881	
Type V-F	-GAACATTGTTACTTAAATGAGCG-			
TypeV-R	-TGAAAGTTGTACCCTTGACACC-	SCC*mec* V	325	
MecA147-F	-GTGAAGATATACCAAGTGATT-			
MecA147-R	-ATGCGCTATAGATTGAAAGGAT-	*mec*A	147	
B-F	-ATTGCCTTGATAATAGCCYTCT-			
α3-R	-TAAAGGCATCAATGCACAAACACT-	*ccr*A2-B	937	
*ccr*CF-F	-CGTCTATTACAAGATGTTAAGGATAAT-			(Method 3)
*ccr*CR-R	-CCTTTATAGACTGGATTATTCAAAATA-	*ccr*C	518	Boye et al. (2007)
1272-F1	-GCCACTCATAACATATGGAA-			
1272-R1	-CATCCGAGTGAAACCCAAA-	IS1272	415	

Table 1. Contd.

5R*mec*A-F	-TATACCAAACCCGACAACTAC-	*mec*A-IS431	359	Boye et al. (2007)
5R431-R	-CGGCTACAGTGATAACATCC-			
Type II-F2	-TAGCTTATGGTGCTTATGCG-	SCC*mec* II,VIII	128	(Method 4) Zhang et al. (2012)
Type II-R2	-GTGCATGATTTCATTTGTGGC-			
Type III-F5	-TTCTCATTGATGCTGAAGCC-	SCC*mec* III, IIIA	257	
Type III-R6	-GTGTAATTTCTTTTGAAAGATATGG-			
mecI-F	-CCCTTTTTATACAATCTCGTT-	*mec*I	147	
mecI-R	-ATATCATCTGCAGAATGGG-			
ccr4-Fd	-ATCGCTCATTATGGATACYGC-	ccr4	428	(Method 5) McClure et al. (2010)
ccr4-R2	-CAAAACAACCTTTTCTATAACG-			
SCCRP62A	-CAATATTGATTTCCTTCATCGTTTACCTCC-	SCC*mec*VIII	1957	
SCC-CI	-GAGCATCATAAGAAGCAATTTTATGTTACGC-			
nuc1	-GCGATTGATGGTGATACGGTT-	nuc	279	
nuc2	-AGCCAAGCCTTGACGAACTAAAGC-			
mecA147-F	-GTGAAGATATACCAAGTGATT-	*mec*A	112	
mecA112-R	-ATCAGTATTTCACCTTGTCCG-			

Table 2. Number of citations of some SCC*mec* typing methods as observed in Google scholar database.

Method	Number of citations in peer reviewed articles	Number of citation since 2014 till date*
Oliveira and de Lencastre (2002)	1223	91
Zhang et al. (2005)	635	84
Boye et al. (2007)	132	28
Milheirico et al. (2007)	310	58
McClure et al. (2010)	212	38
Zhang et al. (2012)	6	5

*Google Scholar access date: April 25th 2015.

A spectacular instance of misassigned ST398-SCC*mec* III MRSA isolates that took about two years to be reassigned as SCC*mec* type V has been previously reported (van Loo et al., 2007; Jansen et al., 2009) Such incidence would include a redesignation of the isolates from SCC*mec* III to SCC*mec* V based on the molecular typing criteria (Ito et al., 2001, 2004). This report showed that at least 25.91% of the MRSA isolates was of SCC*mec* type VI and correlates with a number of recent reports which have indicated an increase in the number of infections associated with SCC*mec* type IV, V, VII or VIII in the hospital setting (Magilner et al., 2008; David et al., 2010) including the presence of the different genotypes in specific environments (Marchese et al., 2009). Although, the overall epidemiological picture presented in these reports may still be biologically relevant based on the general pattern observed across the different countries involved, the estimated statistics may be misrepresented due to the lack of a unified standard method for SCC*mec* classification.

The need for standardization of SCC*mec* typing and genotype designation is evident by a number of reports including: (i) the continuous blurring of the clinical and genetic distinctions between CA-MRSA and HA-MRSA (David et al., 2010; Prosperi et al., 2013) (ii) the probability that CA-MRSA isolates might displace HA-MRSA in future and become the most prevalent strains in clinical settings (Popovich et al., 2008) and (iii) the likelihood for the eventual co-existence of the two MRSA genotypes based on epidemiological modeling (Kouyos et al., 2013). Therefore, the lack of a consensus typing method will make it difficult to predict the actual genetic changes and evolution of the SCC*mec* elements in *S. aureus*. A standard and consensus typing method will

Table 3. Summary of the proportion of SCC*mec* types detected among the 193 clinical MRSA isolate tested by the M-PCR typing methods assessed in this study.

*SCC*mec* types and subtypes	Oliveira and De Lencastre (2002) {types I to III} Method 1	Zhang et al. (2005) {types I to V & subtype IVa to IVd} Method 2	Boye et al. (2007) {types I to V} Method 3	Zhang et al. (2012) {types II & III} Method 4
SCC*mec* type I	3.1% (6/193)	0.52% (1/193)	3.1% (6/193)	-
SCC*mec* type II	9.33% (18/193)	30.7% (59/193)	9.33% (18/193)	35.75% (69/193)
SCC*mercury*	41.5% (80/193)	16.1% (31/193)	61.14% (118/143)	-
SCC*mec*type IV	-	-	25.91% (50/193)	-
SCC*mec*type IVa	-	1.03% (2/193)	-	-
SCC*mec*type IVb	-	0.52% (1/193)	-	-
SCC*mec*type IVc	-	-	-	-
SCC*mec*type IVd	-	24.4% (47/193)	-	-
SCC*mec* type V	-	-	0.52% (1/193)	-
SCC*mec* type II+SCC*mercury*	20.7% (40/193)	-	-	-
SCC*mec* type II+IVc	-	22.3% (43/193)	26.42% (51/193)±	-
Not typeable	-	4.7% (9/193)	-	37.82% (73/193)#

*****Not all the primer sets described in the original methods were tested in this study. Therefore, data shown are those of the primers tested for each method. See Table 2 for details of the primers tested in each method. Methods 4 and 5 shown in Table 2 did not detect any SCC*mec* type III and VIII in this study. ND: Not detected. This indicates the non-detection of expected SCC*mec* types detectable by the primers used in this study. # The number of isolates that were not typeaple by the Zhang et al. (2012) method was derived by deducting the number of isolates that were already assigned other SCC*mec* types not covered by this method. ±Result obtained using a single-target PCR method by Boye et al. (2007) assigned a composite SCC*mec* type (SCC*mec* type II+IVc) to 26.42% (51/193).

ensure accurate epidemiological assessments within and across different countries and effective management and control of MRSA infections.

Currently, the classification of SCC*mec* elements in *S. aureus* is based on the combination of *mec* and *ccr* genes which have variations upon which the different classes of SCC*mec* elements are inferred (IWG-SCC, 2009).

The multiplex PCR method described by Kondo et al. (2007) attempts to improve the accuracy of detection by an initial PCR identification of the *mec* and cassette chromosome recombinases (*ccr*) types followed by identifying the genes in the "joining regions" (J-regions). Accordingly, sequence variations in the joining regions are then used to classify SCC*mec* I-V. There is an ongoing effort to test the performance of this method on clinical MRSA isolates from a number of African countries, in our laboratory. Despite this continuous improvement, consensus criteria for choosing a typing method for SCC*mec* typing is required.

Based on the variations observed in the designation of SCC*mec* types by various methods targeting different sites and genes within the SCC*mec* elements, it is obvious that the designation of SCC*mec* types across different laboratories around the world may not be in synchrony. This is epitomized in the fact that laboratories across the globe adopt different SCC*mec* typing methods (Table 1).

A recent review indicated that SCC*mec* typing was recommended as one of the methods for the monitoring of the molecular epidemiology of MRSA at national and international levels (IWG-SCC, 2009). The current study presents one of the challenges in the practicality of such endeavor. A more detailed study primarily designed to compare all published SCC*mec* typing methods on MRSA strains with known SCC*mec* sequence information would be required to make informed decision on a consensus M-PCR characterization of the SCC*mec* element.

While the SCC*mec* elements described to date include types I-XI, this study focused on the SCC*mec* types I-V and VIII based on our laboratory dataset on the prevalence of the SCC*mec* types in Pretoria, South Africa. This work attempts to paint a practical picture of the difficulties encountered in low income laboratories that are still using M-PCR for MRSA genotyping and hence focuses on the mostly reported M-PCR methods as presented in Table 2. Therefore, not all reported M-PCR methods could be covered for an in-depth comparative study.

In conclusion, this report shows the differences in the assigned SCC*mec* types by the different M-PCR methods as observed in our laboratory. The fact that in spite of the extensive coverage of the variable regions as observed for each method, some clinical isolates could not be SCC*mec*-typed in the original reports by the authors of

these methods was also highlighted.

The M-PCR detection of composite SCC*mec* types in clinical MRSA isolates (SCC*mec* II + SCC*mecury* and SCC*mec* type II+IVc)) was also reported. A plan is underway to investigate the whole genome sequence of these isolates in order to confirm this finding. From the above discussion, a number of questions thus arise: Is the inability to type clinical strains by SCC*mec* method attributed to different structural types or rearrangement and/or recombination of known SCC*mec* elements? Is there still a clinical-epidemiological relevance of HA-MRSA and CA-MRSA differentiation using SCC*mec* element, considering the reported blurring of the distinction (Peterson et al., 2012) between these two categories? If yes, do we have a consensus algorithm for making this distinction? Is SCC*mec* element still a reliable tool for typing MRSA isolates as previously suggested (IWG-SCC, 2009) taking into consideration the variations in the nucleic acid content of this element and the associated discrepancies in identification? While it is obvious that diagnostic microarray, sequencing of SCC*mec* elements and whole genome sequencing are among the modern methods of choice that may resolve this problem, majority of the laboratories in low income countries of Africa are still not able to afford the routine use of these methods. As a way forward, the adoption of a consensus method in South Africa and Africa in general is recommended, in order to allow effective epidemiological data comparison.

Limitation of the study

This report was based on empirical observations of real-world scenarios in the laboratory and therefore was not designed to effectively compare and contrast the individual methods mentioned. Such experiments will include the use of well characterized ATCC strains of MRSA and all published SCC*mec* typing algorithms. However, the results are useful as a basis for an agreement on a consensus SCC*mec* typing method in Africa.

Conflict of interests

The authors declare that there is no conflict of interest in relation to the content of this report.

ACKNOWLEDGEMENT

This work was supported by the Medical Research Council (MRC) and the National Research Foundation (NRF) of South Africa.

REFERENCES

Boye K, Bartels MD, Andersen IS, Moller JA, Westh H (2007). A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I-V. Clin. Microbiol. Infect. 13:725-727.

Daum RS, Ito T, Hiramatsu K, Hussain F, Mongkolrattanothai K, Jamklang M, Boyle-Vavra S (2002). A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds. J. Infect. Dis. 186:1344-1347.

David MZ, Daum RS (2010). Community-associated methicillin-resistant *Staphylococcus aureus:* epidemiology and clinical consequences of an emerging epidemic. Clin. Microbiol. Rev. 23:616-87.

International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (2009). Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. Antimicrob. Agents Chemother. 53:4961-4967.

Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, Hiramatsu K (2001). Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 45:3677.

Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K (2004). Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob. Agents Chemother. 48: 2637-2651.

Jansen MD, Box ATA, Fluit AC (2009). SCC*mec* typing in methicillin-resistant *Staphylococcus aureus* strains of animal origin. Emerg. Infect. Dis. 15:136.

Kim J (2009). Understanding the evolution of methicillin resistant *Staphylococcus aureus*. Clin. Microbiol. Newsletter. 31:17-23.

Kondo Y, Ito T, Ma X X, Watanabe S, Kreiswirth B N, Etienne J, Hiramatsu K (2007). Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec, ccr*, and major differences in junkyard regions. Antimicrob. Agents Chemother. 51:264-274.

Kouyos R, Klein E, Grenfell B (2013). Hospital-community interactions foster coexistence between methicillin-resistant strains of *Staphylococcus aureus*. PLOS Pathog. 9:e1003134.

Lo WT, Wang CC (2011). Panton-Valentine leukocidin in the pathogenesis of community-associated methicillin resistant *Staphylococcus aureus* infection. Pediatr. Neonatol. 52:59-65.

Magilner D, Byerly MM, Cline DM (2008). The prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in skin abscesses presenting to the pediatric emergency department. NC Med. J. 69:351-354.

Marchese A, Gualco L, Maioli E, Debbia E (2009). Molecular analysis and susceptibility patterns of meticillin-resistant *Staphylococcus aureus* (MRSA) strains circulating in the community in the Ligurian area, a northern region of Italy: emergence of USA300 and EMRSA-15 clones. Int. J. Antimicrob. Ag. 34:424-428.

McClure JA, Conly JM, Elsayed S, Zhang K (2010). Multiplex PCR assay to facilitate identification of the recently described Staphylococcal cassette chromosome *mec* type VIII. Mol. Cell. Probe 24:229-232.

McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, Zhang K (2006). Novel multiplex-PCR assay for detection of the staphylococcal virulence marker Panton- Valentine leukocidin genes and simultaneous discrimination of methicillin susceptible from resistant staphylococci. J. Clin. Microbiol. 44: 1141-1144.

Milheiriço C, Oliveira DC, de Lencastre H (2007). Multiplex PCR strategy for subtyping thestaphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: 'SCCmec IV multiplex'. J. Antimicrob. Chemother. 60:42-48.

Morrison MA, Hageman JC, Klevens RM (2006). Case definition for community-associated methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. 62:241.

Moussa IMI, Kabli SA, Hemeg HA, Al-Garni SM, Shibl AM (2012). A novel multiplex PCR for molecular characterization of methicillin resistant *Staphylococcus aureus* recovered from Jeddah, Kingdom of Saudi Arabia. Indian J. Med. Microbiol. 30:296-301.

Naimi TS, LeDell K.H, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Susan K, Lynfield R (2003). Comparison of community-

and health care–associated methicillin-16 resistant *Staphylococcus aureus* infection. JAMA. 290:2976-2984.
O'Brien FG, Pearman JW, Gracey M, Riley TV, Grubb WB (1999). Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J. Clin. Microbiol. 37:2858-2862.
Oliveira DC, de Lencastre H (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 46:2155-2161.
Paterson GK, Harrison EM, Holmes MA (2013). The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. 22:42-47.
Peterson AE, Davis MF, Julian KG, Awantang G, Greene WH, Price LB, Andrew W, Whitener CJ (2012). Molecular and phenotypic characteristics of healthcare-and community associated methicillin-resistant *Staphylococcus aureus* at a rural hospital. PloS one. 7:e38354.
Popovich KJ, Weinstein RA, Hota B (2008). Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains. Clin. Infect. Dis. 46:787-794.
Prosperi M, Veras N, Azarian T, Rathore M, Nolan D, Rand K, Cook RL, Johnson J, Morris JG, Jr., .Salemi M (2013). Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in the genomic era: a cross-sectional study. Sci Rep. 3:1902.
Saiman L, O'Keefe M, Graham PL, Wu F, Said-Salim B, Kreiswirth B, LaSala A, Schlievert PM, Della-Latta P (2003). Hospital transmission of community-acquired methicillin-resistant *Staphylococcus aureus* among postpartum women. Clin. Infect. Dis. 37:1313-1319.
Shore AC, Coleman DC (2013). Staphylococcal cassette chromosome *mec*: Recent advances and new insights. Int. J. Med. Microbiol. 303:350-359.
Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, Yeom JS., Kim SW,Chang HH, Kim YS, Jung SI, Son JS, So TMK, Lalitha MK, Yang YH, Huang SG,Wang H, Lu QA, Carlos CC, Perera JA, Chiu CH, Liu JW, Chongthaleong A,Thamlikitkul V, Van PH, Grp AS (2011). Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. J. Antimicrob. Chemother. 66:1061-1069.
Stefani S, Chung, DR, Lindsay J A, Friedrich AW, Kearns AM, Westh H, MacKenzie FM (2012). Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int. J. Antimicrob. Agents 39:273-282.
van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J (2007). Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg. Infect. Dis. 13:1834-1839.
Weber JD (2005). Community-associated methicillin resistant *Staphylococcus aureus*. Clin. Infect. Dis. 41:269-72.
Zhang KY, McClure JA, Conly JM (2012). Enhanced multiplex PCR assay for typing of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. Mol. Cell Probe. 26:218-221.
Zhang KY, McClure JA, Elsayed S, Louie T, Conly JM (2005). Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 43:5026-5033.