Molecular Characterisation of Methicillin-Resistant *Staphylococcus aureus* Surveillance Isolates in South Africa

Ashika Singh-Moodley[1,2] and Olga Perovic[1,2]

National Institute for Communicable Diseases, a Division of the National Health Laboratory Service Johannesburg, South Africa[1]
University of Witwatersrand, South Africa[2]

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) infections are a significant cause of morbidity and mortality and is increasing in some public hospitals in South Africa. It accounts for more than 50% of bacteraemic staphylococcal infections [1].

In this study we aimed to molecularly characterise MRSA isolates using staphylococcal cassette chromosome *mec* (SCC*mec*)- and *spa*- typing to provide information on strain interrelationship.

Materials and Methods

Methicillin susceptible *Staphylococcus aureus* (MSSA) and MRSA isolates were submitted to the laboratory on Dorset transport media. Each isolate was plated onto a blood agar plate followed by organism identification and antimicrobial susceptibility testing (AST) using automated systems (VITEK II for identification and MicroScan® MIC dried Gram Positive panel for AST). All MRSA isolates were screened for methicillin resistance using real time PCR (LightCyler 480 II) and then typed using conventional typing methods.

Results

A total number of 2560 *Staphylococcus aureus* isolates were processed using the conventional phenotypic methods. Of these, 1457 MRSA isolates from various provinces in South Africa were molecularly tested to detect the presence of the *mecA* and *nuc* genes and typed to identify the current prevalent *mec* element types. Phenotypic and genotypic testing methods correlated for majority (99%) of the isolates tested with the exception of a few which accounted for the remaining 1%. A subset of the MRSA isolates was further molecularly characterised using *spa*-typing.

Two isolates phenotypically identified as MRSA were characterised as MSSA molecularly. The *nuc* gene was detected but the *mecA* gene was not amplified. SCC*mec* typing confirmed this result. The possibility that resistance may have been conferred by *mecC* does exist. This will be further investigated.

A further three isolates identified as MRSA were characterised as methicillin resistant as they harboured the *mecA* gene but were not genotypically classified as a *S. aureus* as the species-specific *nuc* gene could not be amplified. Deoxyribonuclease (DNase) testing for these isolates was negative indicating that these isolates belong to another staphylococcal species thereby demonstrating that the phenotypic automated systems (VITEK II and confirmed with Microflex MALDI-ToF (Bruker Daltonik, GmbH) employed were not accurate. The most prevalent SCC*mec* type identified was SCC*mec* type III (n=679) followed by types IV (n=490), II (n=88), VI (n=6) and V (n=2). No type I SCCmec elements was observed but unknown typing patterns were identified (n=188).

Three isolates produced no SCC*mec* type although positive for the *mecA* gene and classified as MRSA. These isolates may express a SCC*mec* type not detected in this study or the SCC*mec* element may have been excised altogether.

Interestingly of the 113 MSSA isolates subjected to genotypic testing, one isolate possessed a type VI SCC*mec* type element although *mecA* was not present.

Results

Spa-typing of a subset of the isolates revealed 24 different *spa*-types, 4 of which were novel and have not as yet been classified. The five most common *spa*-types were t037 (n=173), t1257 (n=45), t045 (n=32), t064 (n=18) and t012 (n=14) which accounted for 88% of the isolates tested. The *spa*-types clustered into 4 *spa* clonal complexes (*spa*-CC) using the Based Upon Repeat Pattern (BURP) algorithm at a cost setting of ≤4 and excluding *spa*-types with 5 or fewer repeats. *Spa*-CC-1257 (24% of all spa-types) was the largest clonal complex followed by *Spa*-CC-021 (18% of all spa-types) and *Spa*-CC-045 (11% of all spa types). *Spa*-CC-1257 contained isolates displaying predominantly the SCC*mec* type IV element. *Spa*-CC-021 contained isolates that displayed SCC*mec* types II, III, IV and unknown typing patterns (Table 1). These clonal complexes were widespread in South Africa. *Spa*-CC-021 was identified in Gauteng, Western Cape and the North West Province and *spa*-CC-1257 was identified in these provinces as well as in KwaZulu Natal.

Table 1: Genotpyes of 320 MRSA Isolates

Spa-CC	*Spa*-Types	SCC*mec* Type
***spa*-CC1257 (n=78)**	t064	IV, V
	t951	IV
	t1257	IV
	t1443	IV
	t1476	Unknown
	t1555	IV
	t1971	IV
	t2293	IV
	t4833	IV
***spa*-CC012 (n=193)**	t012	II, III
	t018	Unknown
	t021	II
	t037	II, III, IV, Unknown
	t238	II
***spa*-CC045 (n=35)**	t045	Unknown
	t1107	Unknown
	t13165	Unknown
No founder identified (n=5)	t022	IV
	t032	IV
	t148	Unknown

Conclusion

Phenotypic and genotypic methods have correlated for majority of isolates tested. Further, by molecular characterisation we have gained insight in pathogen distribution and relatedness of MRSA isolates from South African sentinel sites. SCC*mec* type III was most common and the most common *spa*-type was t037.

References

Communique, *National Institute for Communicable Diseases a division of the National Health Laboratory Service.* Communicable Diseases Communique - MRSA alerts, May 2010. 9(5): p. 1-2.