Originally published as:

Shittu, A., Nübel, U., Udo, E., Lin, J., Gaogakwe, S.
Characterization of meticillin-resistant Staphylococcus aureus isolates from hospitals in KwaZulu-Natal province, Republic of South Africa
(2009) Journal of Medical Microbiology, 58 (9), pp. 1219-1226.

DOI: 10.1099/jmm.0.011452-0

Journal of Medical Microbiology Papers in Press. Published June 15, 2009 as doi:10.1099/jmm.0.011452-0

Characterization of methicillin-resistant *Staphylococcus aureus* isolates from hospitals in KwaZulu-Natal (KZN) province, Republic of South Africa

Adebayo Shittu,[1,4*] Ulrich Nübel,[2] Edet Udo,[3] Johnson Lin,[4] and Sedio Gaogakwe Steven[4]

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria

[2]Robert Koch Institute, 38855 Wernigerode, Germany

[3]Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

[4]School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal (Westville Campus), Private Bag X54001, Durban, Republic of South Africa

Corresponding author: Dr Adebayo Shittu

Department of Microbiology

Obafemi Awolowo University

Ile-Ife, Nigeria

Tel: +234-806-244-3286

Email: bayo_shittu@yahoo.com

ABSTRACT

Epidemiological data based on phenotypic and molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) in sub-Saharan Africa are limited. This investigation studied 61 MRSA isolates obtained from 13 health care institutions in KwaZulu-Natal province (KZN), South Africa, from March 2001 to August 2003. More than 80% of the isolates were resistant to at least four classes of antibiotics and six isolates were resistant to the aminoglycoside, macrolide-lincosamide and tetracycline groups of antibiotics, heavy metals and nucleic acid binding compounds. Pulsed field gel electrophoresis (PFGE) of *Sma*I digested genomic DNA revealed seven types, designated A to G. Type A was the main pulsotype (62.3%) and identified in 11 of the 13 health care institutions, suggesting that it represented a major clone in health care institutions in KZN province, South Africa. Analysis of representative members of the three major pulsotypes by *spa*, multilocus sequence typing (MLST) and SCC*mec* typing revealed the types t064-ST1173-SCC*mec* IV and t064-ST1338-SCC*mec* IV (PFGE type A, single-locus and double-locus variants of ST8), t037-ST239-SCC*mec* III (PFGE type F) and t045-ST5-SCC*mec* III (PFGE type G). The combination of various typing methods provided useful information on the geographic dissemination of MRSA clones in health care institutions in KZN, South Africa. The observation of major clones circulating in health care facilities in KZN indicates that adequate infection control measures are urgently needed.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported from the United Kingdom in 1961 (Jevons, 1961) and is currently one of the most important nosocomial pathogens worldwide (Tiemersma *et al*., 2004; Huang *et al*., 2006; Sola *et al*., 2006). Moreover, reports on community-associated MRSA (CA-MRSA), particularly USA300 (ST8-SCC*mec*IV, *pvl*+) outbreaks in hospitals are increasing and CA-MRSA threatens to replace hospital-associated MRSA in health care facilities (Deurenberg *et al*., 2006; Gonzalez *et al*., 2006; Seybold *et al*., 2006; Huang *et al*., 2007; Maree *et al*., 2007; Otter & French, 2008). The high morbidity and mortality rates of MRSA infections, the potential for intra- and inter-hospital dissemination, and spread of epidemic strains (Aires De Sousa *et al*., 1998; Deplano *et al*., 2000; Cosgrove *et al*., 2003; Lodise & McKinnon, 2007) have led to an interest in tracking of strains to gain a better picture of the dynamics of clonal spread.

Phenotypic and genotypic data play an important role in understanding the epidemiology of MRSA and evaluating the effectiveness of infection control measures (Murchan *et al*., 2003). Molecular typing approaches have been used to a great advantage in identifying and monitoring the international spread of some unique MRSA strains (Oliveira *et al*., 2002; Enright, 2003, Aires de Sousa & Lencastre, 2004). Until recently, it was generally believed that the spread of MRSA resulted from the global dissemination of a few highly epidemic clones. However, a recent study by Nübel *et al*. (2008) has provided evidence that the population of MRSA in one of the clones (ST5) is geographically structured and that MRSA could have emerged very frequently in different parts of the world through

independent imports of the methicillin resistance determinant into their genomes (Nübel *et al*., 2008). Similar investigations on other clonal lineages are ongoing. To gain more insights into the global rise and spread of MRSA, data from Africa will be useful in understanding clone distribution. At the moment, however, data on the molecular epidemiology of MRSA in Africa are scarce.

In a recent investigation, we reported the prevalence of MRSA in KwaZulu-Natal (KZN) province of South Africa to be approximately 27% (Shittu & Lin, 2006). However, there is paucity of data on MRSA isolates from this province so that the mechanisms of MRSA emergence and spread are unknown. In this study, we apply a combination of phenotypic and molecular techniques to infer isolate inter-relatedness in order to provide health personnel and policy makers in KZN with baseline information needed to establish appropriate infection control programmes and health intervention strategies.

METHODS

MRSA isolates

The 61 MRSA isolates investigated in this study have been described previously (Shittu & Lin, 2006). A total of 48 isolates (78.7%) were recovered from wound samples, six (9.8%) from sputum, two (3.3%) from otitis media, and one isolate each from a blood and urine sample, an eye-related infection and endotracheal aspirate. No clinical information was available for one isolate (Shittu & Lin, 2006).

Antibiotic susceptibility testing

In addition to the antibiotics mentioned in the previous study (gentamicin, kanamycin, streptomycin, neomycin, erythromycin, clindamycin, tetracycline and minocycline), the isolates were tested against a set of six antibiotics. The antibiotics (Oxoid) included azithromycin (15 µg), fosfomycin (50 µg), linezolid (30 µg), oleandomycin (15 µg), tobramycin (10 µg) and quinipristin/dalfopristin (15 µg). Interpretative zone diameters for resistance not stated in the Clinical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) guidelines (NCCLS, 2003) to the following antibiotics were considered as follows: oleandomycin ≤17 mm and fosfomycin ≤14 mm (Members of the SFM Antibiogram Committee, 2003). Susceptibility to heavy metals (cadmium acetate, mercuric chloride) and nucleic-acid binding compounds (ethidium bromide and propamidine isethionate) was performed on the MRSA isolates as previously described (Emslie *et al.*, 1985). Disks were prepared in the laboratory with the indicated concentrations: cadmium acetate (50 µg), propamidine isethionate (50 µg), mercuric chloride (109 µg) and ethidium bromide (60 µg). Antibiotyping was based on their susceptibility pattern to selected antibiotics, representing various classes of antimicrobial agents as previously reported (Shittu & Lin, 2006).

SCC*mec* typing

SCC*mec* types were determined by the multiplex PCR strategy reported by Oliveira and de Lencastre (2002). The following strains were used as controls: EMRSA-16 (SCC*mec* type II), EMRSA-1 (SCC*mec* type III) and K1814 (SCC*mec* type IV).

Detection of Panton-Valentine Leukocidin (PVL) genes

The PVL genes (*lukS-PV* and *lukF-PV*) were detected by PCR as described by Lina *et al*. (1999). A methicillin-susceptible *S. aureus* (*nuc*+, *pvl*+) isolate from Nigeria served as the positive control.

PFGE Typing

Pulsed field gel electrophoresis (PFGE) of *Sma*I (Fermentas, UK) digested DNA was carried out by a modification of the protocol previously described by Bannerman *et al*. (1995). Electrophoresis was performed in 0.5X TBE buffer (0.045 M Tris, 0.045 M boric acid, EDTA disodium 0.001 M) (pH 8) by the contour-clamped homogenous electric field method using a CHEF MAPPER system (Bio-Rad). The fragments were separated with a linear ramped pulse time of 6.8-63.8 s over a period of 23 h at 14°C. The gels were stained with 1 µg ml^{-1} ethidium bromide (Sigma) solution for 1 h, visualized under UV and photographed using a SynGene Bioimaging System.

The banding patterns were interpreted visually and the relatedness of the strains was determined according to the recommendation of Tenover *et al*. (1995). In addition, the Gel Compar II software version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium) was used to calculate the Dice similarity indices and to construct a dendogram after cluster analysis by unweighted-pair-group-matching-analysis (UPGMA). Band position tolerance was set at 1.5% and two strains belonged to the same cluster if their Dice similarity index was 80% or more. Strains showing the same PFGE pattern were grouped

as a pulsotype and assigned alphabetically (e.g. A, B, C, etc). Numeric sub-codes were used to represent up to three band difference (subtypes, e.g. A1, B1, etc).

spa typing and multilocus sequence typing (MLST)

Isolates representing the major PFGE patterns were characterized by *spa* typing (Strommenger *et al*., 2008) and MLST (Enright *et al*., 2000) as previously described.

RESULTS AND DISCUSSION

All the isolates were susceptible to quinipristin/dalfopristin, fosfomycin and linezolid. However, more than 90% of MRSA were resistant to gentamicin, kanamycin and tobramycin, and more than 80% of MRSA were resistant to antibiotics in the macrolide-lincosamide group. Resistotyping revealed that 46% and 82% of the MRSA isolates were resistant to mercuric chloride and cadmium acetate; however, about 70% were susceptible to propamidine isethionate and ethidium bromide (Table 1). Moreover, 14 MRSA isolates were resistant to the heavy metal/nucleic-binding compounds. Overall, six MRSA isolates obtained from clinical samples in health care institutions in Durban, Pietermartizburg and Empangeni were resistant to the aminoglycoside, macrolide-lincosamide and tetracycline groups of antibiotics, heavy metals and nucleic acid binding compounds.

SCC*mec* typing showed that SCC*mec* IV was identified in 38 strains (62.3%), followed by Type III (11 strains; 18.3%) and Type IIIa (5 strains; 8.2%). Two isolates belonged to Types II, IIIb and one isolate in Type I. Two isolates were non-typable (Table 1).

The PFGE profiles and dendogram of the MRSA strains representing the various pulsotypes and the distribution of pulsotypes in health care institutions within KZN province, South Africa are shown in Figures 1a and 1b. PFGE analysis grouped the 61 isolates into seven pulsotypes consisting of pulsotypes A (38 of 61 strains; 62.3%), F (10 of 61; 16.4%), G (6 of 61; 9.8%) and B (4 of 61; 6.5%). Types C, D and E were each represented by single strains. PFGE types A and F were subdivided into nine and two subtypes respectively.

Four MRSA isolates representing the three major pulsotypes (pulsotype A - 2 isolates, G and F - 1 isolate each) were analysed by *spa* typing and MLST. The two isolates assigned to pulsotype A comprised of two related clones namely *spa* type t064, ST1173 and SCC*mec* IV and t064, ST1338 and SCC*mec* IV. MLST sequence types ST1173 (allelic profile 3-3-1-1-4-131-3) and ST1338 (3-3-1-1-4-131-83) are single-locus and double-locus variants of ST8 (3-3-1-1-4-4-3), a clone that has been observed previously in Europe, Australia, and North America (www.mlst.net). Furthermore, MRSA in pulsotype F had *spa* type t037, ST239 and SCC*mec* III while those of pulsotype G had *spa* type t045, ST5 and SCC*mec* III.

This study has defined the clonal types of MRSA isolates in health-care institutions in KZN province of South Africa. The majority of the isolates studied belonged to pulsotype A with SCC*mec* IV. Generally, SCC*mec* IV strains are not multiresistant and carry only the *mecA* gene (Ito *et al*., 2003; Hanssen & Ericson Sollid, 2006). However, studies have indicated that some SCC*mec* IV strains are resistant to agents other than ß-lactams and

aminoglycosides (Donnio *et al*., 2004; Kim *et al*., 2006; Laplana *et al*., 2007). A similar feature was observed in this study in which all the SCC*mec* IV strains were resistant to tetracycline and minocycline, and more than 95% were resistant to gentamicin, kanamycin and tobramycin. Furthermore, 73.7% of MRSA in SCC*mec* type IV were resistant to the macrolide-lincosamide group of antibiotics. These data suggest that some multiresistant strains with SCC*mec* type IV may have acquired resistance to non- ß-lactam antibiotics in order to be able to survive in the hospital environment (Aires de Sousa and de Lencastre, 2003) or through exposure to the antibiotics (Okuma *et al*., 2002). All the MRSA in this group with SCC*mec* IV element were PVL negative, a feature which has been reported in nosocomial MRSA in previous studies (Cuevas *et al*., 2007; Aires de Sousa *et al*., 2008). The ability of pulsotype A (ST1173, ST1338) to spread over distances within KZN is suggested by our results as shown in Figure 1b. It was observed in three hospitals in Durban, and in health care institutions in Pietermaritzburg, Newcastle, Greytown, Kokstad, Port Shepstone, Empangeni, Scottburgh and Eshowe. These findings suggest that inter-hospital spread of this clone occurs frequently and is a major clone circulating in health institutions in the KZN province of South Africa.

The second major clone t037-ST239-SCC*mec* III (PFGE type F) was identified in three of the four health care institutions in Durban and health care facilities in Pietermaritzburg and Empangeni. Some degree of correlation between antibiotyping and the molecular typing methods were observed in the characterization of this clone. MRSA in antibiotypes 1 and 2 were grouped in this clone and SCC*mec* type III was identified in

strains grouped in the two antibiotypes. Moreover, resistance to ciprofloxacin and susceptibility to rifampicin was a unique feature in the multi-resistant MRSA clone and all the isolates exhibited low-level resistance to mupirocin (Table 2). The t037-ST239-SCC*mec* III (called the Brazilian/Hungarian clone based on PFGE) was reported to be widely disseminated in Brazilian hospitals (Teixeira *et al*., 1995) and is widespread in many countries in South America (Corso *et al*., 1998), Europe (Aires de Sousa *et al*., 1998) and Asia (Feil *et al*., 2008). Moreover, geographic stratification of this clone was recently reported (Smyth *et al*., 2008). Our investigation appears to be the first on the detection of this clone in Africa.

The third major clone t045-ST5-SCC*mec* III (PFGE type G), is similar to the New York/Japan (ST5-SCCmec II) and the pediatric clone (ST5-SCC*mec* IV). In contrast, however, the clone in this study carried SCC*mec* III. It was observed in two hospitals in Durban and Pietermaritzburg and a health care facility in Eshowe and Scottburg. A study by Nübel *et al*. (2008) indicated that the ST5 clone is associated with at least six types of SCC*mec* and investigation of sequence diversity within ST5 revealed that ST5-MRSA clones have emerged many times by multiple independent introductions of SCC*mec* into methicillin-susceptible ST5. The study also observed that MRSA in ST5 from South Africa and Kenya formed a unique sub-lineage (termed 'ST5-D') and were not closely related to MRSA from other continents which shared identical *spa*-types (t045). Furthermore, the closest relatives of MRSA from South Africa were methicillin-susceptible, lukS/F-positive *S. aureus* from Kenya. These observations suggest that long-distance spread of MRSA may not have occurred between different countries in Africa,

but rather the widespread occurrence of MSSA clones which may become resistant through multiple independent imports of SCC*mec*.

MRSA grouped in ST5 were not multiresistant (Table 2) but we observed that they were characteristically resistant to heavy metals/nucleic binding compounds and susceptible to tetracycline and minocycline. Characterization based on antibiotic susceptibility testing has been regarded as a timely and inexpensive tool for MRSA phenotyping and for identifying specific clones (Amorim *et al*., 2007; Nimmo *et al*., 2008). The susceptibility of MRSA to trimethoprim-sulfamethoxazole and spectinomycin was useful in differentiating the Iberian and Brazilian clones (Aires de Sousa *et al*., 1998), and susceptibility of MRSA isolates to ciprofloxacin was an important phenotypic marker of community-associated MRSA in a London teaching hospital (Otter & French, 2008). Although antibiotyping showed that multi-drug resistant MRSA were detected in the different pulsotypes and the dominant antibiotype was not discriminatory for MRSA as it was detected in PFGE types A-E (Table 2), the antibiotic resistance markers identified in MRSA in types F and G could be useful in monitoring the spread of such clones and alert clinical microbiologists on the detection of new clones as and when they arise.

This study examined only a small number of isolates but the combination of various typing tools especially *spa* and MLST provided useful information on the existence and geographic distribution of MRSA clones in KZN, South Africa. The identification of widely disseminated clones in health care institutions in KZN indicate that urgent measures are needed to curtail their emergence, spread and establishment in this

province. More studies are needed to investigate the clonal evolution of MRSA over time and the emergence of community-acquired MRSA in the hospital environment in South Africa.

ACKNOWLEDGMENTS

We would like to thank Professor Sabiha Essack, Faculty of Pharmacy, University of KwaZulu-Natal and the management of the hospitals for their support in the collection of the isolates. The assistance of the Department of Microbiology, Faculty of Medicine, Kuwait, and Mrs. Bindu Matthew during the research visit of AS is appreciated. We gratefully acknowledge the technical assistance of Annette Weller and the staff at the Central Sequencing Unit at the Robert Koch Institute. The National Research Foundation, Republic of South Africa, supported the study.

REFERENCES

Aires de Sousa, M., Santos Sanches, I., Ferro, M.L., Vaz, M.J., Saraiva, Z., Tendeiro, T., Serra, J. & de Lencastre, H. (1998). Intercontinental spread of a multidrug-resistant methicillin-resistant *Staphylococcus aureus* clone. *J Clin Microbiol* **36**, 2590-2596.

Aires de Sousa, M. & de Lencastre, H. (2003). Evolution of sporadic clones of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital and their similarities to isolates of community-acquired MRSA. *J Clin Microbiol* **41**, 3806-3815.

Aires de Sousa, M. & de Lencastre, H. (2004). Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones. *FEMS Immunol Med Microbiol* **40**, 101-111.

Aires de Sousa, M., Correia, B., de Lencastre, H. & the Multilaboratory Project Collaborators (2008). Changing Patterns in Frequency of Recovery of five methicillin-resistant *Staphylococcus aureus* clones in Portuguese Hospitals: Surveillance over a 16-year period. *J Clin Microbiol* **46**, 2912-2917.

Amorim, M.L., Faria, N.A., Oliveira, D.C., Vasconcelos, C., Cabeda, J.C., Mendes, A.C., Calado, E., Castro, A.P., Ramos, M.H., Amorim, J.M., & de Lencastre, H. (2007). Changes in the clonal nature and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* isolates associated with spread of the EMRSA-15 clone in a tertiary care Portuguese Hospital. *J Clin Microbiol* **45**, 2881-2888.

Bannerman, T.L., Hancock, G.A., Tenover, F.C. & Miller, J.M. (1995). Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus. J Clin Microbiol* **33**, 551-555.

Corso, A., Santos Sanches, I., Aires de Sousa, M., Rossi, A. & de Lencastre, H (1998). Spread of a methicillin-resistant and multiresistant epidemic clone of *Staphylococcus aureus* in Argentina. *Microb Drug Resist* **4**, 277-288.

Cosgrove, S.E., Sakoulas, G., Perencevich, E.N., Schwaber, M.J., Karchmer, A.W. & Carmeli, Y. (2003). Comparison of mortality associated with methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* bacteriaemia: a meta analysis. *Clin Infect Dis* **36**, 53-59.

Cuevas, O., Cercenado, E., Bouza, E., Castellares, C., Trincado, P., Cabrera, R., Vindel, A. & the Spanish Group for the Study of *Staphylococcus*. (2007). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Spain: a multicentre prevalence study (2002). *Clin Microbiol Infect* **13**, 250-256.

Deplano, A., Witte, W., van Leeuwen, W., Brun, Y. & Struelens, M.J. (2000). Clonal dissemination of epidemic methicillin-resistant *Staphylococcus aureus* in Belgium and neighbouring countries. *Clin Microbiol Infect* **6**, 239-245.

Deurenberg, R.H., Vink, C., Kalenic, S., Friedrich, A.W., Bruggeman, C.A. & Stobberingh E.E. (2006). The evolution of methicillin-resistant *Staphylococcus aureus.* *Clin Microbiol Infect* **13**, 222-235.

Donnio, P.Y., Preney, L., Gautier-Lerestif, A.L., Avril, J.L. & Lafforgue, N. (2004). Changes in staphylococcal cassette chromosome type and antibiotic resistance profile in methicillin-resistant *Staphylococcus aureus* isolates from a French hospital over an 11 year period. *J Antimicrob Chemother* **53**, 808-813.

Emslie, K.R., Townsend, D.E. & Grubb, W.B. (1985). A resistance determinant to nucleic acid binding compounds in methicillin-resistant *Staphylococcus aureus*. *J Med Microbiol* **20,** 139-145.

Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J. & Spratt, B. G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* **38**, 1008-1015.

Enright, M.C. (2003). The evolution of a resistant pathogen – the case of MRSA. *Curr Opin Pharmacol* **3**, 474-479.

Feil, E.J., Nickerson, E.K., Chantratita, N., Wuthuekanun, V., Srisomang, P., Cousins, R., Pan, W., Zhang, G., Xu, B., Day, N.P. & Peacock, S.J. (2008). Rapid detection of the pandemic methicillin-resistant *Staphylococcus aureus* clone ST239 and its dominance in Asian hospitals. *J Clin Microbiol* **46**, 1520-1522.

Gonzalez, B.E., Rueda, A.M., Shelburne III, S.A., Musher, D.M., Hamill, R.J. & Hulten, K.G. (2006). Community-associated strains of methicillin-resistant *Staphylococcus aureus* as the cause of healthcare-associated infection. *Infect Control Hosp Epidemiol*, **27**, 1051-1056.

Hanssen, A.M. & Ericson Sollid, J.U. (2006). SCC*mec* in staphylococci: genes on the move. *FEMS Immunol Med Microbiol* **46**, 8-20.

Huang, Y.C., Su, L.H., Wu, T.L. & Lin, T.Y. (2006). Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* bloodstream isolates from a teaching hospital in Northern Taiwan. *J Clin Microbiol* **44**, 2268-2270.

Huang, Y.H., Tseng, S.P., Hu, J.M., Tsai, J.C., Hsueh, P.R. & Teng, L.J. (2007). Clonal spread of SCC*mec* type IV methicillin-resistant *Staphylococcus aureus* between community and hospital. *Clin Microbiol Infect* **13**, 717-724.

Ito, T., Okuma, K., Ma, X.X., Yuzama, H. & Hiramatsu, K. (2003). Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist Updat* **6**, 41-52.

Jevons, M.P. (1961). "Celbenin"-resistant staphylococci. *BMJ* **1**, 124-125.

Kim, J.S., Song, W., Kim, H.S., Cho, H.C., Lee K.M. Choi, M.S. & Kim, E.C. (2006). Association between the methicillin resistance of clinical isolates of *Staphylococcus aureus*, their staphylococcal cassette chromosome mec (SCC*mec*) subtype classification, and their toxin gene profiles. *Diagn Microbiol Infect Dis* **56**, 289-295.

Laplana, L.M., Goni Cepero, M.P., Ruiz, J., Zolezzi, P.C., Calvo, M.C.B., Erazo, M.C. & Gomez-Lus, R. (2007). Molecular typing of *Staphylococcus aureus* clinical isolates by pulsed-field gel electrophoresis, staphylococcal cassette chromosome *mec* type determination and dissemination of antibiotic resistance genes. *Int J Antimicrob Agents* **30**, 505-513.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M.O., Gauduchon, V., Vandenesch, F. & Etienne, J. (1999). Involvement of Panton-Valentine Leukocidin-Producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* **29**, 1128-1132.

Lodise, T.P. Jnr. & McKinnon, P.S. (2007). Burden of methicillin-resistant *Staphylococcus aureus*: focus on clinical and economic outcomes. *Pharmacother* **27**, 1001-1012.

Maree, C.L., Daum, R.S., Boyle-Vavra, S., Matayoshi, K. & Miller, L.G. (2007). Community-associated methicillin resistant *Staphylococcus aureus* isolates causing health-care associated infections. *Emerg Infect Dis* **13**, 236-242.

Members of the SFM Antibiogram Committee (2003). Comite de l'Antibiogramme de la Societe Francaise de Microbiologie Report 2003. *Int J Antimicrob Agents* **21**, 364-391.

Murchan, S., Kaufmann, M.E., Deplano, A., de Ryck, R., Struelens, M., Zinn, C.E., Fussing, V., Salmenlinna, S., Vuopio-Varkila, J., El Solh, N., Cuny, C., Witte, W., Tassios, P.T., Legakis, N., van Leeuwen, W., van Belkum, A., Vindel, A., Laconcha, I., Garaizar, J., Coombs, G. & Cookson, B. (2003). Harmonization of pulsed-field gel electrophoresis for epidemiological typing of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European centers and its application for tracing the spread of related strains. *J Clin Microbiol* **41**, 1574-1585.

NCCLS (2003). *Performance standards for antimicrobial disk susceptibility testing*. NCCLS document M2-A8 2003, Wayne, PA: National Committee for Clinical Laboratory Standards Institute.

Nimmo, G.R., Fong, J., Paterson, D.L. & McLaws, M.L. (2008). Changing epidemiology of methicillin-resistant *S. aureus* in Queensland, Australia, 2000-2006: use of passive surveillance of susceptibility phenotypes. *J Hosp Infect* **70**, 305-314.

Nübel, U., Roumagnac, P., Feldkamp, M., Song, J. H., Ko, K. S., Huang, Y. C., Coombs, G., Ip, M., Westh, H., Skov, R., Struelens, M. J., Goering, R. V., Strommenger, B., Weller, A., Witte, W. & Achtman, M. (2008). Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* **105**, 14130-14135.

Okuma, K., Iwakawa, K., Turnidge, J.D., Grubb, W.B., Bell, J.M., O'Brien, F.G., Coombs, G.W., Pearman, J.W., Tenover, F.C., Kapi, M., Tiensasitorn, C., Ito, T. & Hiramatsu K. (2002). Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* **40**, 4289-4294.

Oliveira, D.C. & 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.

Oliveira, D.C., Tomasz, A. & de Lencastre, H. (2002). Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis* **2**, 180-189.

Otter, J.A. & French, G.L. (2008). The emergence of community-associated methicillin-resistant *Staphylococcus aureus* at a London teaching hospital, 2000-2006. *Clin Microbiol Infect* **14**, 670-676.

Seybold, U., Kourbatova, E.V., Johnson, J.G., Halvosa S.J., Wang Y.F., King, M.D., Ray, S.M. & Blumberg, H.M. (2006). Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of healthcare-associated blood stream infections. *Clin Infect Dis* **42**, 647-656.

Shittu, A.O. & Lin J (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. *BMC Infect Dis*, 125.

Smyth, D. S., Song, J.-H., Enright, M., de Lencastre, H., Robinson, A. (2008). Global phylogeny of the ST239-MRSA-III clone. Program of the 13th International Symposium on Staphylococci and Staphylococcal infections, Cairns, Australia, Abstract 409.

Sola, C., Cortes, P., Saka, H., Cordoba MRSA Collaborative Study Group., Vindel, A. & Bocco, J.L. (2006). Evolution and molecular characterization of methicillin-resistant *Staphylococcus aureus* epidemic and sporadic clones in Cordoba, Argentina. *J Clin Microbiol* **44**, 192-200.

Strommenger, B., Braulke, C., Heuck, D., Schmidt, C., Pasemann, B., Nübel, U. & Witte, W. (2008). *Spa*-typing of *Staphylococcus aureus* as frontline tool in epidemiological typing. *J Clin Microbiol* **46**, 574-581.

Teixeira, L.A., Resende, C.A., Ormonde, L.R., Rosenbaum, R., Figueiredo, A.M., de Lencastre, H. & Tomasz, A. (1995). Geographic spread of epidemic multiresistant *Staphylococcus aureus* clone in Brazil. *J Clin Microbiol* **33**, 2400-2404.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233-2239.

Tiemersma, E.W., Bronzwaer, S.L., Lyytikainen,O., Degener, J.E., Schrijnemakers, N., Briuinsma, N., Monen, J., Witte, W. & Grundman, H. (2004). Methicillin-resistant *Staphylococcus aureus* in Europe, 1999-2002. *Emerg Infect Dis* **10**, 1627-1634.

Table 1: Relationship between antimicrobial resistance, PFGE and SCC*mec* typing of MRSA isolates

Antimicrobial agents	Number of isolates resistant (%)	PFGE A (n=38)	PFGE B (n=4)	PFGE C (n=1)	PFGE D (n=1)	PFGE E (n=1)	PFGE F (n=10)	PFGE G (n=6)
Gentamicin*	59 (96.7)	36	4	1	1	1	10	6
Kanamycin*	59 (96.7)	36	4	1	1	1	10	6
Streptomycin*	19 (31.1)	3	0	0	0	0	10	6
Neomycin*	19 (31.1)	3	0	0	0	0	10	6
Tobramycin	58 (95.1)	36	4	0	1	1	10	6
Erythromycin*	50 (82.0)	28	3	1	1	1	10	6
Oleandomycin	50 (82.0)	28	3	1	1	1	10	6
Azithromycin	50 (82.0)	28	3	1	1	1	10	6
Clindamycin*	50 (82.0)	28	3	1	1	1	10	6
Tetracycline*	55 (90.2)	38	4	1	1	1	10	0
Minocycline*	55 (90.2)	38	4	1	1	1	10	0
Ethidium bromide	19 (31.1)	7	0	0	0	0	6	6
Propamidine isethionate	18 (29.5)	5	1	0	0	0	6	6
Mercuric chloride	28 (45.9)	12	0	0	0	0	10	6
Cadmium acetate	50 (82.0)	31	4	1	1	0	7	6
Antimicrobial agents	Number of isolates resistant (%)	SCC*mec* Type I (n=1; 1.6 %)	SCC*mec* Type II (n=2; 3.3 %)	SCC*mec* Type III (n=11; 18.3 %)	SCC*mec* Type IIIa (n=5; 8.2 %)	SCC*mec* Type IIIb (n=2; 3.3 %)	SCC*mec* Type IV (n=38; 62.3 %)	Non-type (n=2; 3.3 %)
Gentamicin*	59 (96.7)	1	1	11	5	2	37	2
Kanamycin*	59 (96.7)	1	1	11	5	2	37	2
Streptomycin*	19 (31.1)	0	0	11	4	1	3	0
Neomycin*	19 (31.1)	0	0	11	4	1	3	0
Tobramycin	58 (95.1)	1	1	11	5	2	36	2
Erythromycin*	50 (82.0)	1	1	11	5	2	28	2
Oleandomycin	50 (82.0)	1	1	11	5	2	28	2
Azithromycin	50 (82.0)	1	1	11	5	2	28	2
Clindamycin*	50 (82.0)	1	1	11	5	2	28	2
Tetracycline*	55 (90.2)	1	2	5	5	2	38	2
Minocycline*	55 (90.2)	1	2	5	5	2	38	2
Ethidium bromide	19 (31.1)	0	0	9	2	1	7	0
Propamidine isethionate	18 (29.5)	0	0	9	2	1	6	0
Mercuric chloride	28 (45.9)	0	0	11	4	1	12	0
Cadmium acetate	50 (82.0)	1	1	9	4	2	31	2

All the MRSA isolates were susceptible to quinipristin/dalfopristin, fosfomycin and linezolid; *Previously reported data (Shittu and Lin, 2006)

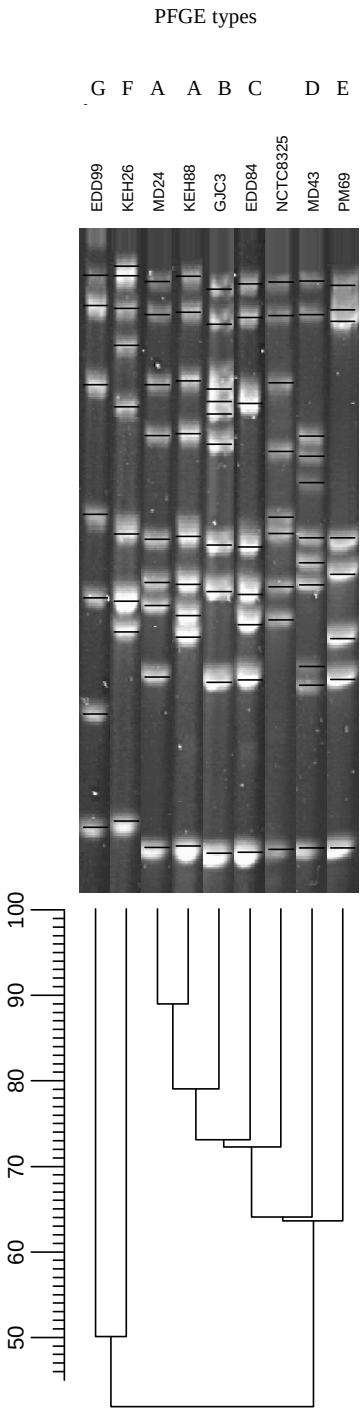
Table 2: Correlation between antibiotyping, PFGE and SCC*mec* typing of 61 MRSA strains from South Africa

Antibiotypes (number of strains)	PFGE types (number of strains)	SCC*mec* types (number of strains)
1. PEN, ERY, GM, TET, MU5, TS, CIP, CHL (4)	F1 (3) F1 (1)	IIIa (3) IIIb (1)
2. PEN, ERY, GM, TET, MU5, TS, CIP (6)	F1 (5) F2 (1)	III (4), IIIa (1) III (1)
3. PEN, ERY, GM, TET, TS, RF, CHL (4)	A1 (3) A6 (1)	IV (2), II (1) IV (1)
4. PEN, ERY, GM, TET, TS, RF, MU (3)	A3 (1) A5 (1) A8 (1)	IV (1) IIIa (1) IV (1)
5. PEN, ERY, GM, TET, TS, RF, CIP (1)	A5 (1)	IV (1)
6. PEN, ERY, GM, TET, TS, RF (25)	A1 (10) A2 (1) A3 (2) A4 (1) A5 (1) A6 (1) A8 (1) A9 (2) B (3) C (1) D (1) E (1)	I (1) IV (7) IIIb (1) NT (1) IV (1) IV (1) NT (1) IV (1) IV (1) IV (1) IV (1) IV (2) IV (3) IV (1) IV (1) IV (1)
7. PEN, GM, TET, TS, RF, CHL (2)	A3 (1) A8 (1)	IV (1) IV (1)
8. PEN, GM, RF, TET, MU5, TS (2)	A8 (2)	IV (2)
9. PEN, GM, RF, TET, TS (5)	A1 (1) A6 (3) B (1)	IV (1) IV (3) IV (1)
10. PEN, ERY, GM, RF, TET (1)	A3 (1)	IV (1)
11. PEN, ERY, GM (6)	G (6)	III (6)
12. PEN, RF, TET (2)	A5 (1) A7 (1)	II (1) IV (1)

KEY

PEN - Penicillin; ERY - Erythromycin; GM - Gentamicin; TET - Tetracycline; MU5 - Low-level mupirocin resistance; TS - Trimethoprim; CIP - Ciprofloxacin; RF - Rifampicin; CHL – Chloramphenicol

Figure 1a: Dendogram of representative MRSA types in KZN, South Africa



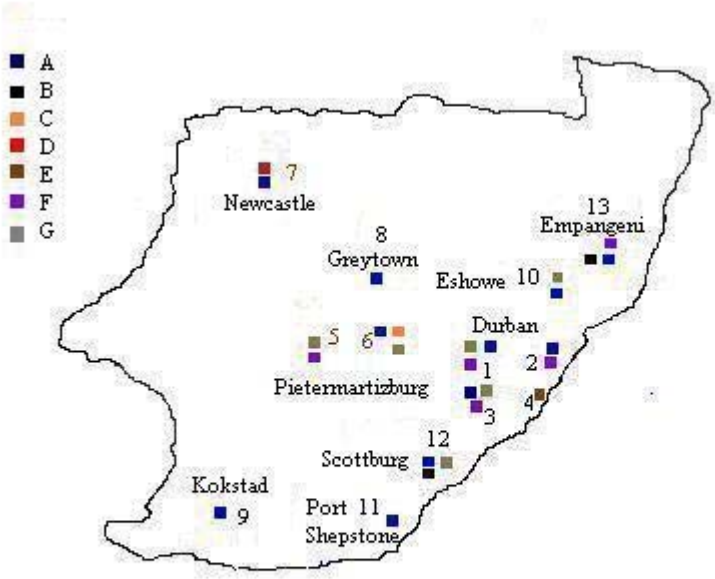


Figure 1b: Map of KwaZulu-Natal province, South Africa and PFGE types in the various locations (illustrated in colours). The numbers indicate the health care institutions.