Nasopharyngeal carriage with *Staphylococcus aureus* in healthy children during the first year of life - The Drakenstein Child Health study

By

Shima Mohammed Ahmed Algalaa Abdulgader

Submitted to the University of Cape Town for the degree of Doctor of Philosophy in Medical Microbiology

September 2016
The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.
Declaration

I, Shima Mohammed Ahmed Algalaa Abdulgader, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

Signature

Date: 2nd September 2016
**Supervisor**

Professor Mark Patrick Nicol (MBBCh, MMed (Med Microbiol), SA FCPath (Microbiol), PhD)\(^1, 2, 3\)

\(^1\)Division of Medical Microbiology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.

\(^2\)Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa.

\(^3\)National Health Laboratory Service of South Africa, Groote Schuur Hospital, Cape Town, South Africa

**Co-supervisor**

Dr. Lourens Robberts PhD, D (ABMM), FCCM\(^1\)

\(^1\)Clinical and public Microbiologist, American Society of Microbiology
Acknowledgements

Thanks to Allah Almighty for granting me the opportunity to fulfil this research.

This dissertation would not have been possible without the endless encouragement and support from a number of people. First, I would like to express my sincere appreciation to my principal supervisor Prof. Mark Nicol, thank you for giving me the opportunity to be part of the medie micro family and for always supporting me. Secondly, a special thanks to my co-supervisor Dr. Lourens Robberts, you taught me how to be confident with myself and my work which helped me through a very critical time during my PhD journey.

A great thanks goes to Prof. Heather Zar - the PI of the Drakenstein Study - and the Drakenstein Study Child Health Study clinical team and of course to all the study participants (mothers and infants).

Thanks also goes out to those who provided me with statistical advice at times of critical need: A/Prof. Sugnet Lubbe (I really cannot thank you enough for always willing to help despite your busy schedule), Prof. Rohmatul Fajryiah, Mr. Polite Nduru, and Mr. Jordache Smith. I am so grateful for Dr. Adebayo Shittu for his invaluable critique of this dissertation. Thanks to Dr. Mamadou Kaba and Dr. Eliya Madikane (who was my MSc project supervisor) for their valuable input and for passing on their experience which was an absolute assistance during my studies.

I would like thank all the senior scientists, staff, and members of the Division of Medical Microbiology for always making me feel like I am home. To all my colleagues in the Division of Medical Microbiology for their moral support and assistance I really appreciate it. Thank you Ms Shantelle Claassen for always being there for me.

I would also like to thank my father, mother, brothers and sisters for the support they provided me through my entire life. A special thanks to my beloved husband and best friend Mohammed Fadul and my daughter Joud for being the joy of my life. Mohammed: I wouldn’t have gotten this far without your support, encouragement, motivation, and unconditional love- Thank you for being you!
In conclusion, I recognize that this research would not have been possible without the financial assistance of the Organisation for Women in Science (OWSD), and Bill and Melinda Gates Foundation through the Division of Medical Microbiology. I was fortunate to receive a financial support from the Wellcome Trust to attend the Working with Pathogen Genomes course in January 2016, Cambridge, United Kingdom. I am equally thankful for receiving the Elsevier travel award to attend the 13th International Molecular Epidemiology Evolutionary Genetic of Infectious Diseases in May 2016 in Antwerp, Belgium.
# Table of Contents

Declaration .......................................................................................................................... i
Acknowledgements ........................................................................................................... iii
Table of Contents .............................................................................................................. v
List of Figures .................................................................................................................. ix
List of Tables ................................................................................................................... xi
List of abbreviations and acronyms ................................................................................ xii
Abstract ........................................................................................................................... xv

Chapter One ................................................................................................................... 0
General Introduction and Thesis Outline ........................................................................ 0
  1.1 General Introduction .................................................................................................. 1
  1.2 Aims of the study ..................................................................................................... 2
    1.2.1 General aim ....................................................................................................... 2
  1.3 The Drakenstein Child Health study design and population .................................. 3
  1.4 S. aureus nasopharyngeal carriage: study design and population ......................... 4
  1.5 Ethics consideration ................................................................................................. 5
  1.6 Overview of the laboratory work flow .................................................................... 5
  1.7 Outline of the thesis ............................................................................................... 6
References .......................................................................................................................... 8

Chapter Two .................................................................................................................. 10

*Staphylococcus aureus Nasal Carriage: A Review of the Literature* ......................... 10
  2. *Staphylococcus aureus* ............................................................................................ 11
  2.1 *S. aureus*: competence as a coloniser strain ........................................................... 12
    2.1.1 *S. aureus* carriage niches ................................................................................ 12
    2.1.2 *S. aureus* carriage patterns and dynamics ....................................................... 16
  2.2 Clinical relevance of *S. aureus* nasal carriage ...................................................... 19
  2.3 Determinants for *S. aureus* nasal carriage ............................................................ 21
    2.3.1 Bacterial determinants ..................................................................................... 21
    2.3.2 Host determinants of carriage by *S. aureus* .................................................... 26
  2.4 Molecular basis for methicillin resistance in *S. aureus* ......................................... 29
    2.4.1 The *mec* complex ......................................................................................... 29
    2.4.2 The *ccr* complex and Junction regions .......................................................... 30
  2.5 *S. aureus* epidemiology ......................................................................................... 32
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5.1</td>
<td>Staphylococcal cassette chromosome mec (SCCmec) typing</td>
<td>32</td>
</tr>
<tr>
<td>2.5.2</td>
<td>Staphylococcal protein A (spa) typing</td>
<td>32</td>
</tr>
<tr>
<td>2.5.3</td>
<td>Multilocus sequence typing (MLST)</td>
<td>34</td>
</tr>
<tr>
<td>2.6</td>
<td>The global population structure of <em>S. aureus</em></td>
<td>35</td>
</tr>
<tr>
<td>2.6.1</td>
<td>Molecular epidemiology of hospital-associated (HA) and community-associated (CA) MRSA</td>
<td>36</td>
</tr>
<tr>
<td>2.7</td>
<td>Decolonisation, eradication and control of <em>S. aureus</em> carriage</td>
<td>37</td>
</tr>
<tr>
<td>2.8</td>
<td><em>S. aureus</em> vaccination</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>44</td>
</tr>
</tbody>
</table>

**Chapter Three**

**Molecular epidemiology of Methicillin-resistant *S. aureus* in Africa: a systematic review**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Summary</td>
<td>59</td>
</tr>
<tr>
<td>3.2</td>
<td>Background</td>
<td>60</td>
</tr>
<tr>
<td>3.3</td>
<td>Method</td>
<td>62</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Literature search strategy</td>
<td>62</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Eligible articles identification</td>
<td>64</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Data extraction and synthesis</td>
<td>64</td>
</tr>
<tr>
<td>3.3.4</td>
<td>eBURST analysis</td>
<td>64</td>
</tr>
<tr>
<td>3.4</td>
<td>Results</td>
<td>65</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Literature search</td>
<td>65</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Characteristics of the studies included in the systematic review</td>
<td>65</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Community- and hospital-associated MRSA</td>
<td>72</td>
</tr>
<tr>
<td>3.4.4</td>
<td>Detection of Panton-Valentine leukocidin (PVL) genes</td>
<td>72</td>
</tr>
<tr>
<td>3.4.5</td>
<td>MRSA clones reported in Africa using the current eBURST scheme</td>
<td>73</td>
</tr>
<tr>
<td>3.5</td>
<td>Discussion</td>
<td>82</td>
</tr>
<tr>
<td>3.6</td>
<td>Conclusion</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>86</td>
</tr>
</tbody>
</table>

**Chapter Four**

**Prevalence, incidence and determinants of *S. aureus* nasopharyngeal carriage during infancy**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Summary</td>
<td>95</td>
</tr>
<tr>
<td>4.2</td>
<td>Background</td>
<td>96</td>
</tr>
<tr>
<td>4.3</td>
<td>Methods</td>
<td>97</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Study design and population</td>
<td>97</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Important definitions used in this study</td>
<td>98</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Nasopharyngeal (NP) swab collection</td>
<td>98</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Characterisation of <em>S. aureus</em> isolates</td>
<td>98</td>
</tr>
</tbody>
</table>
6.3.3 Confirmation of Tigecycline non-susceptible strains using E-test..............................152
6.3.4 Calculation of the antibiotics resistance rate.................................................................152
6.4 Results..............................................................................................................................152
6.4.1 Characteristics of participants ......................................................................................152
6.4.2 Antibiotic susceptibility patterns for S. aureus isolates..................................................153
6.4.3 Antibiotic susceptibility patterns of methicillin-resistant S. aureus strains..............154
6.4.4 Antibiotypes of multi drug resistant of S. aureus isolates............................................155
6.4.5 Antibiotic resistance trends over time ..........................................................................156
6.4.6 Relationship between antibiotic resistance and spa-clonal complexes.................158
6.4.7 Changes in antibiotic susceptibility patterns over time within the same infant159
6.5 Discussion.........................................................................................................................161
6.6 Conclusion........................................................................................................................164
References .............................................................................................................................165

Chapter Seven.........................................................................................................................168

Longitudinal assessment of the dynamics of S. aureus nasopharyngeal carriage in healthy infants during the first year of life .................................................................................................168

7.1 Summary.............................................................................................................................169
7.2 Background........................................................................................................................170
7.3 Methods .............................................................................................................................172
7.4 Results...............................................................................................................................174
7.4.1 S. aureus NP carriage dynamics......................................................................................174
7.4.2 S. aureus nasopharyngeal carriage patterns among infants during the first year of life 181
7.5 Discussion...........................................................................................................................184
7.6 Conclusion........................................................................................................................188
References .............................................................................................................................189

Chapter Eight.........................................................................................................................192

General discussion.................................................................................................................192

Future outlook........................................................................................................................196

Appendices..............................................................................................................................199
List of Figures

Figure 1.1: The main study schedule of procedures and sample collection. Adopted from Zar et al. 2014 ..................................................................................................................................................... 4
Figure 1.2: Schedule of procedures and sample collection for S. aureus NP carriage study......... 5
Figure 1.3: Overview of the laboratory work flow for the S. aureus NP carriage study.................. 6
Figure 2.1: The different types of epithelia prone to S. aureus colonisation from selected body sites. .............................................................................................................................................. 14
Figure 2.2: Increasing number of studies on S. aureus nasal carriage as indexed in PubMed database between 1946 and 2016. .............................................................................................................................................. 15
Figure 2.3: Schematic diagram describing steps of S. aureus colonisation of the anterior........ 24 nares as an example. .................................................................................................................................................. 24
Figure 2.4: Schematic diagram of the 11 staphylococcal cassette chromosome mec (SCCmec) types. ..................................................................................................................................................... 30
Figure 2.5: Schematic diagram for the S. aureus protein A (spa) locus........................................ 32
Figure 3.1: Preferred reporting item for systematic reviews. ......................................................... 65
Figure 3.2: Methicillin-resistant S. aureus clones reported in Africa............................................. 66
Figure 3.3: A minimum spanning showing MRSA clones circulating in Africa clustered with 223 randomly selected MLST ST circulating world-wide ................................................................................. 76
Figure 4.1: The point prevalence of S. aureus NP carriage during the first year of life.............. 102
Figure 4.2: Incidence of acquisition of S. aureus NP carriage during the first year of life........ 103
Figure 4.3: Changes in the detection rates of S. aureus over different seasons.......................... 104
Figure 4.4: Multivariate analysis of variables associated with S. aureus NP carriage during the first year of life ................................................................................................................................. 105
Figure 4.5: The proportion of S. aureus NP carriage by HIV-exposure during four age intervals.
............................................................................................................................................................................. 106
Figure 4.6 estimated odds ratio of S. aureus carriage for both HIV-exposure and S. pneumoniae carriage overtime. ............................................................................................................................................. 107
Figure 4.7: Multivariate analysis for S. aureus NP carriage stratified by study site..................... 109
Figure 5.1: Amplification of mecA gene (162bp) from methicillin-resistant S. aureus (MRSA) strains........................................................................................................................................................ 131
Figure 5.2: Characterisation of the SCCmec-types for methicillin-resistant S. aureus (MRSA) control strains and the 12 MRSA strains identified from infants......................................................... 132
Figure 5.3: ‘Based Upon Repeat Pattern’ clustering analysis on identified spa types. ............... 134
Figure 5.4: Genotype distribution of the 11 spa-clonal complexes and the singleton isolates identified from the 137 infants at the 27 time points. ...................................................................................... 135
Figure 5.5: The molecular epidemiology of *S. aureus* among MRSA carriers analysed by time. 136

Figure 5.6: The repeat sequence for the *spa* untypeable isolates showing the atypical sequence for the “GAP” repeat. ................................................................. 137

Figure 5.7: Schematic diagram of the rearrangements identified in the *spa* hypervariable region in eight isolates compared to prototyped control strains. ......................................................... 138

Figure 6.2: The longitudinal resistance patterns in the 725 *S. aureus* isolates identified from the 137 mother-infant pairs. ............................................................................................ 157

Figure 6.3: The relationship between antibiotic resistance and *spa*-clonal complexes (*spa*-CC). .......................................................................................................................... 158

Figure 6.4: Longitudinal *S. aureus* carriage profiles showing the shifts in resistance profiles for antibiotics which occurred in six infants during the first year of life.............................................. 160

Figure 7.1: Survival time (days) to *S. aureus* acquisition events in 137 healthy infants during the first year of life........................................................................................................... 175

Figure 7.2: Incidence of acquisition of different genotypes during the first year of life. .......... 176

Figure 7.3: Transmission of *S. aureus* (*spa* types) within mother-infant pairs.................... 178

Figure 7.4: The carriage duration of the different genotypes (*spa*-clonal complexes) carried by the 137 infants distributed over different time intervals.............................................. 179

Figure 7.5: The longitudinal *S. aureus* carriage profiles by different genotypes (*spa*-clonal complexes) for the 137 mother-infant pairs during the first year of life.............................................. 180

Figure 7.6: The carrier indices for 137 infants followed up longitudinally every two weeks for the first year of life........................................................................................................... 182

Figure 7.7: A scatter plot for the carrier index and the diversity measures............................ 183

Appendix 1: The number of missing NP swabs over the 27 time points.............................. 199

Appendix 4: The relative proportions of each *spa*-CC among the range of isolates at each time point .......................................................................................................................... 201

Appendix 5: Histograms for the carriage duration for each of the *spa*-clonal complexes........ 202
List of Tables

Table 2.1: Protocols used to define persistent nasal carriage of Staphylococcus aureus.............17
Table 2.2: Summary of Staphylococcus aureus active vaccine trials reported by Botelho-Nevers et al. ........................................................................................................................................42
Table 3.1: Keywords used to identify eligible studies available in four biomedical databases.... 63
Table 3.2: Characteristics of eligible articles that studied Methicillin-resistant S. aureus......... 67
Table 3.3: Panton-Valentine Leukocidin prevalence as reported by the eligible studies with sample size of 30 or above.............................................................................................................73
Table 3.4: Methicillin-resistant Staphylococcus aureus clones reported in 34 eligible studies... 79
Table 4.1: Participant characteristics..........................................................................................101
Table 4.2: S. aureus carriage determinants between carriers and non-carriers......................110
Table 5.1: The prevalence of PVL in each spa-clonal complex (CC) and its distribution among spa-types ........................................................................................................................................139
Table 6.1: Clinical characteristics of MRSA carriers................................................................155
Table 6.2: Multi-drug resistance profiles of Staphylococcus aureus strains..............................156
Table 7.1: Factors independently associated with S. aureus first acquisition and loss............178
Table 7.2: Summary of the median (carriage duration) permutation for the 11 spa-CCs........... 181
Appendix 2: The incidence rate and the confidence intervals for the incidence of S. aureus acquisition at the species and the genotype levels.................................................................................................................................200
## List of abbreviations and acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ºC</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>µl</td>
<td>Microliter</td>
</tr>
<tr>
<td>AD</td>
<td>Atopic dermatitis</td>
</tr>
<tr>
<td>AFLP</td>
<td>Amplified Fragment Length polymorphism</td>
</tr>
<tr>
<td>agr</td>
<td>Accessory gene regulator</td>
</tr>
<tr>
<td>AST</td>
<td>Antibiotic Susceptibility testing</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>BA</td>
<td>Blood agar</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>BURP</td>
<td>Based Upon Repeat Pattern</td>
</tr>
<tr>
<td>BURST</td>
<td>Based Upon Related Sequence Types</td>
</tr>
<tr>
<td>CA-MRSA</td>
<td>Community-associated methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>CC</td>
<td>Clonal Complex</td>
</tr>
<tr>
<td>ccr</td>
<td>Cassette Chromosome Recombinases</td>
</tr>
<tr>
<td>CHIPS</td>
<td>Chemotaxis inhibitory protein of staphylococci</td>
</tr>
<tr>
<td>CHL</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>CIP</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>clfA, B</td>
<td>Clumping factor A, and B genes</td>
</tr>
<tr>
<td>CLIN</td>
<td>Clindamycin</td>
</tr>
<tr>
<td>DLV</td>
<td>Double Locus variant</td>
</tr>
<tr>
<td>Dnase</td>
<td>Deoxyribonucleic acid digesting enzyme</td>
</tr>
<tr>
<td>dNTPs</td>
<td>Deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>ERY</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>FOX</td>
<td>Cefoxitin</td>
</tr>
<tr>
<td>FUS</td>
<td>Fusidic acid</td>
</tr>
<tr>
<td>GEN</td>
<td>Gentamicin</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td>Hospital-associated methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>HCL</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immune Deficiency Virus</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>IEC</td>
<td>Immune Evasion Cluster</td>
</tr>
<tr>
<td>IsdA</td>
<td>Iron-regulated surface determinant protein A</td>
</tr>
<tr>
<td>Kb</td>
<td>Kilobase/s</td>
</tr>
<tr>
<td>LZD</td>
<td>Linezolid</td>
</tr>
<tr>
<td>mecA</td>
<td>Methicillin resistance gene A</td>
</tr>
</tbody>
</table>
MGI Mobile genetic island
ml Millilitre
MLST Multi-locus sequence typing
mM MilliMolar
MRSA Methicillin-resistant *Staphylococcus aureus*
MSA Mannitol Salt agar
MSCRAMMs Microbial-surface components recognising adhesive matrix molecules
MSSA Methicillin-susceptible *Staphylococcus aureus*
MUP Mupirocin
NHLS National Health Laboratory Services
NICD National Institute for Communicable Diseases
NP Nasopharyngeal
OR Odds ration
OXA Oxacillin
PBP Penicillin-binding protein
PCR Polymerase Chain reaction
PCV Pneumococcal conjugate vaccine
PEN Penicillin
PFGE Pulsed Field Gel Electrophoresis
PMNPs polymorphonuclear phagocytes
PVL Pantone-Valentine Leuckocidin
PYR Pyrrolidonyl Arylamidase
RIF Rifampicin
sae *S. aureus* enterotoxin
SAK Staphylokinase
SasG *S. aureus* surface protein G
SCC*mec* Staphylococcal Cassette Chromosome *mec*
SCIN staphylococcal complement inhibitor
SdrC Serine-aspartic acid repeat protein
SLV Single Locus variant
*Spa* Staphylococcal protein A
*Spa-CC* *spa*-Clonal Complex
SSTI Skin and soft tissue infections
ST Sequence Type
SXT Cotrimoxazole
TAE Tris-acetate EDTA
TCY Tetracycline
TGC Tigecycline
VAN Vancomycin
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGS</td>
<td>Whole genome sequencing</td>
</tr>
<tr>
<td>WTA</td>
<td>Wall teichoic acid</td>
</tr>
</tbody>
</table>
Abstract

Background

*Staphylococcus aureus* carriage is a risk factor for subsequent infections. Data on the prevalence, determinants, population structure and molecular epidemiology of *S. aureus* nasal carriage among healthy African populations are scarce, especially during infancy. The different *S. aureus* nasal carriage patterns (intermittent and persistent) were defined in the adult population, however, were ill defined during infancy. A consensus definition for these patterns is still lacking since different approaches were used to define carriage patterns. In addition, few studies used strain genotype to support the intermittent and persistent carriage patterns. This thesis describes the prevalence, determinants, population structure and carriage patterns of *S. aureus* nasopharyngeal carriage among healthy South African infants and their mothers participating in an intensively sampled cohort.

Methods

Nasopharyngeal swabs (NP) were collected on the day of birth from 137 mother-infant pairs and two-weekly thereafter from infants during the first year of life. *S. aureus* isolates were characterized by antibiotic susceptibility testing, PCR detection of the *mecA* and Panton-Valentine Leuckocidin (PVL) genes, and typed by targeting the Staphylococcal protein A locus. All genetically related *spa* types were clustered into *spa*-clonal complexes using the 'based upon repeat pattern clustering analysis. The Logistic regression model for binomial outcomes, Cox proportional hazards model, and Pearson's Chi-square and correlation coefficient tests were used to determine *S. aureus* NP carriage dynamics and determinants. Median permutation test was performed to determine the difference in the median carriage duration for each genotype. The NP carriage dynamics i.e. incidence, acquisition, and carriage patterns were analysed at the species and the genotype levels. Genotype diversity (number of *spa*-clonal complexes carried by
the infant and the alpha diversity) were incorporated with the carrier index to define the carriage patterns.

**Results**

*S. aureus* was identified in 21% (725/3292) of the NP swabs; 704 isolates from infants and 21 from mothers. *S. aureus* NP carriage occurred from birth, peaked by four weeks of age and declined over the following 11 months. Male gender, higher socioeconomic status, maternal carriage, large family size and hospitalization were risk factors for *S. aureus* NP carriage. The prevalence of methicillin-resistant *S. aureus* (MRSA) was 1.7% among infants and none of the mothers carried MRSA at birth. Genotyping of 725 *S. aureus* isolates by targeting the spa gene resulted in 85 spa types. BURP analysis clustered 71 spa types (n=578) into 11 spa-clonal complexes (spa-CCs). Eleven spa types (n=116) were singletons and three spa types (n=27) were excluded from the cluster analysis due to the small number of repeats. The PVL prevalence was 21% (155/725) consisted mainly on MSSA. Eighty three percent of *S. aureus* strains were resistant to penicillin, 9.5% to gentamicin, 4% to tetracycline and co-trimoxazole, and 2.4% to rifampicin. Constitutive erythromycin resistance was identified in 1.3% (n=4), whereas the inducible MLS\textsubscript{B} phenotype (ICR) was observed in 3% (n=8) of isolates. All isolates were susceptible to tigecycline, linezolid and mupirocin. A strong relationship between the spa-clonal complexes and antimicrobial resistance phenotypes was noted. We also documented shifts in the resistance patterns over time within the same genotype carried by the same infant. This study demonstrates the importance of strain genotyping to fully describe carriage dynamics; the incidence of acquisition was higher (0.65 episodes per 100 child days) at the genotype level compared to (0.24 episodes) the species level. During the first year of life, the acquisition rate was 1.8 acquisitions per 137 child-year at the species level compared to 2.4 acquisitions per 137 child-year. At the level of the individual child, a positive correlation (r=0.6; 95% CI 0.46 – 0.70, p < 0.0001) was observed between genotype diversity and the proportion of samples testing positive for *S. aureus*. Using the genotype diversity measure we found that true persistent carriage with a single strain is rare (2%) during infancy.
**Conclusion**

This study provide baseline data on the prevalence, determinants, population structure and dynamics of *S. aureus* NP carriage among South African infants. A low prevalence of MRSA was observed in this cohort. A diverse MSSA population with relatively high PVL prevalence was observed. Persistent carriage with a single strain was uncommon during the first year of life. The detailed phenotypic and genotypic analysis of *S. aureus* in this intensively sampled birth cohort has extended our knowledge of the nature and determinants of NP carriage during infancy.
Chapter One

General Introduction and Thesis Outline
1.1 General Introduction

*Staphylococcus aureus* is not only a human commensal but also a major cause of serious invasive diseases in community and hospital settings. It colonizes different body sites including the nasal cavity. Carriage in the nasopharynx has been frequently reported in cohort and cross-sectional studies in children. *S. aureus* nasal carriage is a risk factor for infections ranging from superficial skin infections to life-threatening infections such as bacteraemia and endocarditis.

Three main carriage patterns have been defined in healthy adult populations; 20% of adults are persistent carriers and 80% are intermittent or non-carriers. However, a consensus definition or a clear distinction between persistent and intermittent carriage is still lacking, due to inconsistency in the approaches used to define these patterns. Although several studies suggest that persistent carriage is observed more frequently among children, the carriage dynamics in healthy paediatric populations have not been thoroughly described, either in longitudinal cohorts or at the strain genotype level. Risk factors such as maternal *S. aureus* carriage, male gender, number of siblings, day care attendance and antibiotic use are associated with *S. aureus* nasal carriage in infancy, however, the risk factors for different carriage patterns still need to be investigated. Further, although few reports describe the prevalence of *S. aureus* carriage in African children, there is paucity of data on *S. aureus* carriage dynamics and population structure in healthy African children.

Methicillin-resistant *S. aureus* (MRSA) infections are a serious global problem with considerable impact on morbidity and mortality, and huge health care costs. MRSA have an acquired mobile genetic element - Staphylococcal Cassette Chromosome *mec* (SCCmec) - integrated into their genome that harbours the methicillin resistance gene (*mecA* gene) and other antimicrobial resistance determinants. The *mecA* gene is responsible for the synthesis of an altered penicillin binding protein (PBP) 2a which in turn confers cross-resistance to beta-lactam antibiotics. Recently a *mecA* homologue (*mecC*) which also contributes to methicillin resistance has been described and its association with human and animal infection has attracted considerable attention. In Africa, data on the prevalence and molecular epidemiology of MRSA is limited.
to certain countries, and mainly focusing on healthcare settings \cite{21}. Therefore, there is a need for community-based surveillance studies to better understand the population structure of the MRSA clones which are circulating among the healthy community.

Panton-Valentine Leukocidin (PVL) is a virulence factor which is commonly associated with skin and soft tissue infections and \textit{S. aureus} related pneumonia \cite{22,23}, and is one of the most important virulence factors associated with community acquired \textit{S. aureus} infections independent of methicillin resistance \cite{24}. Moreover, since almost 80\% of \textit{S. aureus} invasive diseases in humans are linked to the host's colonising strain \cite{25}, individuals who harbour PVL-positive \textit{S. aureus} strains could be predisposed to these infections. A number of studies \cite{26–29} have reported high prevalence of PVL-positive \textit{S. aureus} in the African continent, however, many of these investigations were conducted in hospital settings and data on the prevalence of PVL in \textit{S. aureus} carried by the healthy population in Africa are scanty.

In view of the clinical significance of \textit{S. aureus} nasal carriage, the spread of community-associated methicillin-resistant \textit{S. aureus} (CA-MRSA) and the lack of data on the population structure and molecular epidemiology of \textit{S. aureus} among healthy individuals particularly during infancy, we conducted a comprehensive longitudinal investigation of nasopharyngeal colonisation in healthy children in order to investigate the different carriage patterns of \textit{S. aureus} and associated risk factors.

1.2 Aims of the study

1.2.1 General aim

This study aims to describe the prevalence and dynamics of \textit{S. aureus} nasopharyngeal carriage in healthy African children during the first year life and associated risk factors.

1.2.2 Specific aims

(1) To study the prevalence and determinants of \textit{S. aureus} nasopharyngeal carriage in infants during the first year of life.
To describe the antibiotic resistance profiles for all identified *S. aureus* isolates during the study period and determine the proportion of infants and mothers colonized with methicillin-resistant *S. aureus* (MRSA).

To determine the proportion of *S. aureus* isolates harbouring the Panton Valentine Leucocidin (PVL) genes.

To describe, at the strain genotype level, the different *S. aureus* carriage dynamics in children under the age of one year and identify children who are intermittently and persistently colonized during the study period.

To compare the genetic background of *S. aureus* isolated from children with intermittent and persistent *S. aureus* carriage.

### 1.3 The Drakenstein Child Health study design and population

The Drakenstein Child Lung Health study (DCHS) is a large population-based birth cohort study. The Drakenstein region is a semi-urban, stable, but low socio-economic status community with a high burden of HIV, tuberculosis and childhood pneumonia. It is situated about 60 kilometres outside Cape Town. This region has an estimated population of 200,000 and limited immigration and emigration. The main aim of the broader cohort study is to elucidate the epidemiology, progression, risk factors and long-term impact of lower respiratory tract infections on child health in low or middle income countries including South Africa. DCHS study has enrolled 1000 pregnant women during their second trimester and their infants are followed up from birth to five years of life. To be enrolled in the study, pregnant women should be 18 years or older, attend one of the study clinics for antenatal care, and should remain in the study area for at least one year. Figure 1.1 shows the schedule of procedures and sample collection of the main study.
1.4 *S. aureus* nasopharyngeal carriage: study design and population

The studies described in this PhD dissertation are nested within the DCHS. A sub-cohort of 137 mother-infant pairs was randomly selected based on the following criterion: Infants should have a minimum number of 24 nasopharyngeal swabs (NP) collected at birth and every two weeks over the first 12 months of life. The selected participants were enrolled during June 2012-May 2013. Data on *Streptococcus pneumoniae* NP carriage for this sub-cohort was extracted from the main study database to investigate its association with *S. aureus* NP carriage during infancy. Demographic data and data on risk factors such as delivery mode, feeding method and HIV exposure were collected longitudinally through case report forms (CRFs) at seven time points (Figure 1.2).
Introduction and Thesis Outline

Figure 1.2: Schedule of procedures and sample collection for *S. aureus* NP carriage study.

### 1.5 Ethics consideration

Written informed consent was obtained from parents and was renewed annually. The DCHS was approved by the University of Cape Town Human Research Ethics Committee (HREC REF Number 401/2009) and the Provincial Government of the Western Cape, South Africa (RP 45/2011). Additional specific approval for the studies described in this thesis was given (reference number HREC REF 741/2013).

### 1.6 Overview of the laboratory work flow

Figure 1.3 shows a flow diagram of the laboratory work flow; a detailed description of all laboratory techniques used in this study will be given in the relevant chapters. A total of 3417 NP swabs were collected from the 137 mother-infant pairs and placed in the transport and storage medium, skimmed milk tryptone glucose and glycerine medium (STGG). *S. aureus* isolation and identification was performed following standard procedures using protocols from the National Institute for Communicable Diseases (NICD). *S. aureus* isolates were stored at -80 °C for further investigations. All *S. aureus* isolates were typed by targeting the staphylococcal protein A locus (*spa*). The genes encoding for the virulence factor Panton-Valentine Leukocidin (PVL) were screened for using real-time PCR. Antibiotic susceptibility testing was performed on all *S. aureus* isolates using the Kirby-Baur disc diffusion method and breakpoints were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. Methicillin resistance (phenotypic resistance to oxacillin) was further confirmed by amplifying the gene *mecA* (Figure 1.3).
1.7 Outline of the thesis

Chapter 2 is a narrative review of the published literature on *S. aureus* nasal carriage, its clinical relevance, and the different bacterial, host and environmental determinants. In addition, this chapter also discusses *S. aureus* global population, emergence of MRSA clones, in addition to, the strategies to control the dissemination of *S. aureus*; decolonisation and vaccines. To understand the molecular epidemiology of MRSA strains circulating in the African continent, we conducted a systematic review (Chapter 3) to highlight the gap in knowledge on MRSA population structure within Africa. In Chapter 4, we describe the prevalence and incidence of acquisition of nasopharyngeal carriage with *S. aureus* during the first year of life. In addition, in this chapter we also identify determinants of *S. aureus* nasopharyngeal carriage over time. Together Chapters 5
and 6 describe the population structure of *S. aureus* in the Drakenstein cohort; **Chapter 5** gives a detailed description of the molecular epidemiology of *S. aureus* within the Drakenstein cohort; the different *spa* types circulating in the cohort, the proportion of MRSA and the prevalence of PVL among both MSSA and MRSA, and **Chapter 6**, focuses on the antimicrobial resistance patterns of all identified *S. aureus* isolates. A longitudinal assessment of *S. aureus* carriage dynamics over the first year of life is described in **Chapter 7**, this includes description of acquisition and loss, carriage duration of the different genotypes, transmission between mother-infant pairs and defining the carriage patterns during the first year of life. Finally, the last chapter (**Chapter 8**) provides a general discussion and concluding remarks on the study and proposes future perspectives.
References


Chapter Two

*Staphylococcus aureus Nasal Carriage: A Review of the Literature*
2. **Staphylococcus aureus**

The grape-like (*Staphylē*) and coci (*kókkoc in Greek*) forms of this genus were first described in the late nineteenth century by the surgeon Ogston \(^1\). In 1884, Rosenbach, succeeded in isolating and growing the bacteria and subsequently named it "*Staphylococcus aureus*" (*aureus* means gold in Greek), referring to the golden-yellow pigmentation produced when grown on solid media. Historically, staphylococci were classified together with the genus *Micrococcus* in a single group \(^1\), however, these two genera differ in a number of characteristics such as the low DNA content of guanidine-cytosine (G+C), and the presence of surface bound teichoic acid in staphylococci \(^2\). The genus *Staphylococcus* is gram positive, and appears as single form, short chains or irregular grape-like clusters. It belongs to the phylum *Firmicutes*, class *Bacilli*, order *Bacillales*, and family *Staphylococcaceae*. Members of this genus are facultatively anaerobic, non-motile, non-spore forming, and catalase positive cocci. At least 40 Staphylococcal species are known, 16 of these represent human commensals. Based on expression of the clotting factor coagulase (binds to prothrombin to form fibrin, i.e. clots), staphylococci are divided into coagulase positive and coagulase negative. *Staphylococcus aureus* which is coagulase positive and *Staphylococcus epidermidis* a coagulase negative are the most common commensals of the nose and the skin, respectively. The most virulent species is *S. aureus* both in human and animals, while coagulase negative staphylococci like *S. epidermidis, S. saprophyticus* and *S. haemolyticus* are generally less associated with severe diseases compared to *S. aureus* – they are more commonly associated with urinary tract and device related infections \(^2\text{-}^4\).

*Staphylococcus aureus* is a well-established coloniser that can be carried asymptomatically for short periods of time on epithelial surfaces, and for relatively longer periods on mucous membranes \(^5\). Extended carriage suggests the development of a joint tolerance between the host and the bacterium \(^6\). Infections, however, occur as a result of the disturbance of this balance \(^6\). *S. aureus* causes a variety of infections, ranging from mild skin infections such as boils, cellulitis, and folliculitis to invasive life-threatening infections like pneumonia, infective endocarditis and deep
Literature review

abscesses. In addition, *S. aureus* is also associated with carriage and infections in companion and live-stock animals (e.g. cats, dogs, sheep and cows).

2.1 *S. aureus*: competence as a coloniser strain

*Staphylococcus aureus* is a global health concern, however, it remains a normal commensal bacterium found in different body sites. Approximately one third of the human adult population carries this bacterium persistently yet asymptomatically, while approximately 60% carry the bacterium intermittently. In contrast, some individuals remain un-colonised after frequent exposure to *S. aureus* and they account for approximately 20% of the adult population. *S. aureus* can be transmitted between individuals easily via skin contact or contact with a contaminated environment.

2.1.1 *S. aureus* carriage niches

*Staphylococcus aureus* preferably colonises the mucosal and epithelial surfaces of several body sites such as the nose, throat, gut, axilla, groin, vagina and perineum. The epithelium is the common tissue shared among the skin, gut and upper respiratory tract along with more specialised structures relevant to each site (Figure 2.1). The anterior part of the nose, also known as “vestibulum nasi”, is coated with keratinized, non-ciliated, stratified squamous epithelial cells. In the anterior nares, *S. aureus* attaches to certain proteins found on this keratinized non-ciliated squamous layer such as irocin and cytokeratin through surface bound proteins. In addition to the nares, *S. aureus* can multiply independently in the skin, axilla and the groin since the keratinized layer, sebaceous glands as well as the apocrine sweat glands are shared features between these anatomic sites and the anterior nares. In contrast to the anterior nares, the posterior part of the nose including the nasopharynx is layered with non-keratinized, ciliated, pseudostratified columnar epithelia. In addition, this epithelium also contains goblet cells that produce mucus – as does the oropharynx and the throat. *S. aureus* is better able to adhere to mucus coated surfaces than to non-mucin coated epithelial cells as shown by *in-vitro* studies. (Figure 2.1).
2.1.1.1 Carriage on the skin

Skin *S. aureus* carriage rates vary depending on the targeted population, age and health status. Only 27% of the non-nasal carrier population harbour *S. aureus* on their hands compared to 90% among all nasal carriers. It was suggested that nasal carriage is a source of contamination of other body sites especially the skin. In support of this, eradication of *S. aureus* nasal carriage using either ampicillin or mupirocin as topical antibiotic ointments successfully eliminated *S. aureus* from the skin. *S. aureus* skin carriage is mainly associated with skin and soft tissue infections, stye, impetigo and skin sycosis barbae with an average of almost 90% of infected individuals being nasal carriers. In addition, skin carriage is associated with secondary bacterial infections in children with atopic dermatitis (AD). 

2.1.1.2 Carriage in the intestine

It is proposed that *S. aureus* intestinal carriage occurs after nasal carriage, however, exclusive intestinal carriage occurs in one out of three intestinal carriers. In their review, Acton and colleagues estimated that the average detection rate of intestinal carriage in the adult population (including both healthy and at risk patients) was 20% for *S. aureus* (including MRSA), which represented half of that of the nasal carriage. However, for hospitalised adult patients at risk, the frequency of methicillin-resistant *S. aureus* (MRSA) intestinal carriage was almost 10% of all individuals compared to 12% MRSA nasal carriage in the same population. Rectal swabs showed more efficacy in identifying intestinal carriage compared to passed stool samples, and sampling the perineum or perianal areas can also be used to identify intestinal carriage. Higher rates of intestinal carriage (60%) were reported in children during infancy compared to adults. Two European longitudinal studies involved mother-infant pairs during the first year of life, showed that both breast-feeding and maternal skin carriage contribute to early *S. aureus* intestinal colonisation in neonates. Moreover, hospitalised patients who carry *S. aureus* in their gut have a high risk of developing surgical wound-site infections due to the spread of *S. aureus* to other skin areas (e.g. groin and thighs). This might also contaminate the hospital environment resulting in outbreaks.
keeping with this, the asymptomatic enteric MRSA carriage maintained a hospital outbreak for almost four years in a tertiary care hospital in Switzerland, suggesting the important role of unrecognised intestinal carriers in spreading and maintaining outbreaks.  

![Figure 2.1](image.jpg)

**Figure 2.1**: The different types of epithelia prone to *S. aureus* colonisation from selected body sites. (a) Keratinized non-ciliated stratified squamous epithelium of the skin and the anterior nares. (b) Non-keratinized ciliated pseudostratified columnar epithelium featuring the goblet glands lining the upper respiratory tract of the nasopharynx. (c) Simple columnar epithelium lining the intestinal tract of the small intestine.

### 2.1.1.3 Carriage in the upper respiratory tract

*Staphylococcus aureus* nasal carriage has been more carefully studied (Figure 2.2) whereas extra-nasal body sites were overlooked until recently. Recent studies investigated *S. aureus* carriage in the oropharynx as well as the nasopharynx. *S. aureus* was isolated from the throat of orthopaedic patients at a significantly higher percentage (41%) compared to the nose (31%). However, among healthy medical students *S. aureus* carriage in the throat was less frequent (24%) than carriage in the nares (34%). In addition, *S. aureus* was one of the most frequently isolated bacteria from the tonsils of children younger than two years and from the oropharynx in school-aged children. Throat carriage may occur independently of *S. aureus* nasal carriage.
with evidence of genetically distinct strains in both sites from the same individual. Typically the nasal swab is collected by rotating the swab on the tip of the nares, while the nasopharyngeal swab is passed through the nasal cavity to the reach the nasopharynx carefully avoiding contamination with secretions or surfaces from the nares. A study by Petersen and colleagues which was conducted in an adult population, suggested a very strong correlation between carriage of *S. aureus* in the nasopharynx and the oropharynx especially in individuals negative for nasal carriage. However, in this study, the anterior nares were still the most colonised site. Among children younger than five years, *S. aureus* was frequently reported to colonise the nasopharynx. Nasopharyngeal carriage occurs early in life starting from birth at high rates up to 50% within the first 4 weeks of life both in developed and developing countries, and declines with increasing age. *S. aureus* oro-nasopharyngeal carriage is a risk factor for lung infections in individuals with cystic fibrosis, ventilator-associated pneumonia, necrotizing pneumonia and tonsillitis.

![Figure 2.2](image-url) Figure 2.2: Increasing number of studies on *S. aureus* nasal carriage as indexed in PubMed database between 1946 and 2016.
2.1.2 *S. aureus* carriage patterns and dynamics

Three main patterns of *S. aureus* nasal carriage are described: persistent, intermittent and non-carriage. Persistent carriers account for 10 to 35% of the adult population, whereas 20 to 80% are intermittent carriers and 5 to 50% have never carried *S. aureus*. However, different approaches have been used to distinguish between *S. aureus* intermittent and persistent carriage and it must be noted that study designs were each unique and may therefore have limited comparability (Table 2.1). One method used to define carriage status is the carriage index which is defined by the number of samples positive for *S. aureus* in a subject, divided by the total number of samples collected for the same subject during the study period. Individuals with a zero index represent non-carriers, and those with index of 0.8 or higher (in some studies index must be 1.0) are considered persistent carriers, with values between these indices being the intermittent carriers. Even though carriage index provides a quantitative cut-off between the different patterns, the distinction between intermittent and persistent carriage is still not entirely clear. Furthermore, persistent carriers usually harbour higher bacterial loads in their nasal cavity compared to intermittent carriers. This high load leads to inoculation or contamination of other body sites, the surrounding environment as well as to transmission to other individuals.

The median survival (duration of carriage) also differs between these two patterns; 22 weeks vs. two weeks for persistent and intermittent carriers, respectively. In addition, serum samples from *S. aureus* persistent, intermittent and non-carriers were tested for anti-staphylococcal antibodies against 17 *S. aureus* proteins. The median serum level for immune globulin G directed against *S. aureus* surface protein G and toxic shock syndrome toxin-1 were significantly higher among persistent carriers than intermittent or non-carriers. In addition, the median serum level of immune globulin A elicited by clumping factor A, staphylococcal enterotoxin A and chemotaxis inhibitory protein of *S. aureus* was higher in persistent carriers as oppose to intermittent or non-carriers. No significant difference were observed in the median fluorescence intensities for these antibodies between intermittent and non-carriers. Therefore, based on the difference in carriage duration, bacterial load and the antibody (Ab) response to *S. aureus* antigens (Ags), van Belkum
et al. reclassified nasal carriage patterns to persistent carriers and “others” 54. To differentiate between *S. aureus* persistent and intermittent carriers, a “culture rule” which assesses the quantitative and qualitative results of two consecutive nasal swabs, collected one week apart was proposed. Based on this rule, a persistent carrier should have *S. aureus* on both swabs (qualitative) with high bacterial load (quantitative). When tested on a cohort with predefined carriage status using the carrier index, the “culture rule” accurately predicted persistent carriage with 93% reliability 55. Another method which was used to differentiate *S. aureus* persistent from intermittent carriers was an algorithm based on one to three quantitative nasal samples. A persistent carrier should have ≥10^3 colony forming units (CFUs) from the first swab; this algorithm had a 83% sensitivity and 96% specificity to predict persistent carriage among healthy volunteers as defined by a carrier index (% *S. aureus* positive over total number of samples) of ≥80%53.

Genotyping of carriage strains allows a better understanding of the dynamics of *S. aureus* nasal carriage 56. When considering the genotype of the carriage strain, persistent carriage has been defined as harbouring the same or closely related genotype in all positive samples 51,56,57. Studies that analysed the carriage strain at the genotype level revealed a low exchange rate of strains among persistent carriers (i.e. carriers retained the same genotype or carried less diverse genotypes) in repeated cultures compared to intermittent carriers 54,58,59. In support of this, a trial was conducted on healthy persistent carriers who were decolonised and re-challenged again with a mixture of *S. aureus* strains. Those persistent carriers retained their autologous strain from the mixture 54. However, a recent longitudinal study on healthy adults described a similar strain exchange rate between persistent and intermittent carriers 51. Another benefit of using the genotype rather than the species when describing *S. aureus* nasal carriage dynamics, is the ability to successfully detect simultaneous carriage of multiple genotypes in the same sample 60,61.
**Table 2.1: Protocols used to define persistent nasal carriage of *Staphylococcus aureus*.**

<table>
<thead>
<tr>
<th>Definition of persistent carriage</th>
<th>No. of swabs</th>
<th>Interval between swabs</th>
<th>Study duration</th>
<th>Population health status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 80% positive</td>
<td>10</td>
<td>ND</td>
<td>19 months</td>
<td>Healthy</td>
<td>62</td>
</tr>
<tr>
<td>≥ 80% positive</td>
<td>10-12</td>
<td>1 week</td>
<td>10-12 weeks, then 8 years</td>
<td>Healthy</td>
<td>50</td>
</tr>
<tr>
<td>Carriage of a single strain</td>
<td>9</td>
<td>2 weeks for 3 months, 1-month for 3 months</td>
<td>6 month</td>
<td>Healthy infants</td>
<td>63</td>
</tr>
<tr>
<td>≥ 80% positive and two semi quantitative swabs</td>
<td>3-12</td>
<td>1 week</td>
<td>12 weeks</td>
<td>Healthy</td>
<td>55</td>
</tr>
<tr>
<td>≥ 80% positive plus identical genotype</td>
<td>6</td>
<td>1 week</td>
<td>6 weeks</td>
<td>CPD patients</td>
<td>64</td>
</tr>
<tr>
<td>100% positive plus identical genotype</td>
<td>3</td>
<td>At 1.5, 6, and 14 months of age</td>
<td>14 months</td>
<td>Healthy infants</td>
<td>44</td>
</tr>
<tr>
<td>≥ 80% positive</td>
<td>5-10</td>
<td>3-4 weeks</td>
<td>6 months</td>
<td>Healthy</td>
<td>54</td>
</tr>
<tr>
<td>100% positive</td>
<td>6</td>
<td>1 year</td>
<td>6 years</td>
<td>Healthy</td>
<td>58</td>
</tr>
<tr>
<td>≥ 80% positive compared to two semi quantitative swab</td>
<td>9</td>
<td>1 week</td>
<td>9 weeks</td>
<td>Healthy</td>
<td>65</td>
</tr>
<tr>
<td>100% positive</td>
<td>2</td>
<td>28 days (mean)</td>
<td>ND</td>
<td>Healthy</td>
<td>66</td>
</tr>
<tr>
<td>2 consecutive with ≥10³ CFUs</td>
<td>7</td>
<td>1 week</td>
<td>5 weeks</td>
<td>Healthy</td>
<td>53</td>
</tr>
<tr>
<td>2 consecutive positive</td>
<td>3</td>
<td>Season</td>
<td>1 year</td>
<td>Healthy</td>
<td>57</td>
</tr>
<tr>
<td>100% positive</td>
<td>2-18</td>
<td>Variable</td>
<td>Up to 3 years</td>
<td>Healthy</td>
<td>51</td>
</tr>
<tr>
<td>Two consecutive positive swabs</td>
<td>4</td>
<td>At birth, 1, 9, and 12 months of age</td>
<td>12 months</td>
<td>Healthy infants</td>
<td>67</td>
</tr>
<tr>
<td>≥ 80% positive of the first 12 swabs</td>
<td>72</td>
<td>2 weeks</td>
<td>3 years</td>
<td>Adults attending GP</td>
<td>56</td>
</tr>
</tbody>
</table>

Note: This table was modified from Verhoeven et al. 52. CPD: continuous peritoneal dialysis; GP, General practitioner; ND, not determined
2.2 Clinical relevance of *S. aureus* nasal carriage

*S. aureus* is considered to be a global public health concern in both community and clinical settings with substantial morbidity and mortality, particularly with the emergence of hospital-and community-associated MRSA \(^9,68,69\). *S. aureus* can be carried asymptptomatically for extended periods without causing any infection or disease. Entrance into the blood circulation might be a “dead end” for the bacterium, or, can serve as the “escape route” to other anatomical sites, causing invasive infections and chronic diseases \(^70\). *S. aureus* nasal carriage is associated with subsequent infections, mostly in hospital \(^9,71\). It is involved in a range of minor, self-limiting infections such as skin and soft tissue infections (SSTI), as well as life-threatening diseases such as necrotizing pneumonia \(^48,72,73\). Von Eiff *et al.* reported that approximately 85% of *S. aureus* bacteraemia patients were *S. aureus* nasal carriers, with a high proportion of these episodes caused by the nasal carriage strain \(^71\). In addition, a genetic analysis on *S. aureus* strains showed minimal differences in the genomes between carriage and invasive strains \(^74\). Another study determined the genetic evolutionary relationships between clinical strains and nasal carriage strains from healthy population in the USA \(^75\). The study focused on evolution events within the MLST housekeeping genes and the hypervariable regions within virulence genes such as clumping factors A and B and indicated that both carriage and clinical strains belonged to the same lineages and had 100% sequence similarity within the virulence genes \(^75\).

In the community, *S. aureus* nasal carriage is frequently associated with recurrent skin and soft tissue infections such as furunculosis, stye, and impetigo among individuals with no previous exposure to hospitals. In children under five years, impetigo (i.e. skin blistering) occurs in 1% of all children. In moderate climates, exfoliative toxin-producing *S. aureus* strains are the most common pathogen, while *Streptococcus pyogenes* mainly causes tropical impetigo *S. aureus* \(^76\). Also, Staphylococcal Scalded Skin Syndrome - a skin exfoliation following an erythematous cellulitis which affects the skin tissues but not the mucous membranes caused by skin-splitting
toxins of *S. aureus* - mainly occurs in children, and to lesser extent among adults; specially immune compromised individuals like HIV-positive patients. Moreover, *S. aureus* is the main bacterial cause of secondary skin infections in AD patients, particularly during childhood. *S. aureus*-carrier AD patients are at a four-fold increased risk for developing secondary infections compared to non-carrier patients. Atopic dermatitis complications are typically caused by enterotoxin producing *S. aureus* strains (e.g. *S. aureus* enterotoxin (SE) A, SEB, SEC and toxic shock syndrome toxin-1 (TSST-1)). Staphylococcal Enterotoxins SEA, SEB, SEC and TSST-1 act as super-antigens that elicit Immunoglobulin E antibodies which increase the disease severity. A population-based birth cohort study reported the involvement of *S. aureus* enterotoxins in atopic disorders early in life. This was evident when the serum level of the immune globulin E antibodies specific to staphylococcal enterotoxins were measured in 510 children followed from birth to five years of age and correlated with atopic diseases. Children with atopic disorders (eczema and wheeze) were more frequently sensitized (high antibody levels against *S. aureus* enterotoxins) than the children without. In addition, the sensitization rate significantly increased with increasing eczema severity.

A strong epidemiological association is evident between the bi-component toxin Panton-Valentine Leuckocidin (PVL) and the emergence of community associated skin and soft tissue infections and to lesser degree with invasive diseases among individuals with no previous contact with hospitals. In a collection of 172 *S. aureus* isolates collected from different infected body sites, Lina et al. identified PVL genes in 93% of isolates associated with furunculosis and in 85% of isolates associated with severe necrotic haemorrhagic pneumonia. Necrotizing pneumonia - also known as cavitary pneumonia- which is caused by PVL-positive *S. aureus* is usually severe and often fatal, mainly affecting young children and healthy individuals with mortality rate up to 75%.* S. aureus* necrotizing pneumonia is considered rare compared to SSTIs, however, the spread of PVL-positive community-associated MRSA (CA-MRSA), carrying the small in size Staphylococcal Cassette Chromosome *mec* element (SCCmec) type-IV, contributed to the spread
of this severe form of community-acquired pneumonia \(^{82,84}\). SCCmec type IV [2B] is transmitted easily and is found in twice as many clones as any other SCCmec types \(^{85}\). *S. aureus*-related severe pneumonia has received increasing attention, particularly since pneumococcal vaccination has resulted in a reduction in the rate of paediatric hospitalisations due to pneumococcal community-acquired pneumonia \(^{86,87}\).

In hospital settings, *S. aureus* nasal carriage is associated with development of nosocomial infections among health care workers and inpatients \(^{52}\). *S. aureus* is the second most common cause of intravascular device-related bacteraemia/septicaemia in intensive care units, following only coagulase negative *Staphylococci* \(^{9,88}\). In addition, higher rates (approximately 40\%) of *S. aureus* infections were reported among carriers admitted to intensive care units compared to 15\% among non-carriers; endogenous MRSA was the predominant cause of these infections \(^{88}\). Orthopaedic patients carrying *S. aureus* are at a 16-fold increased risk of *S. aureus* surgical site infections (SSIs) while cardiac patients are at a nine-fold increased risk \(^{89,90}\). Moreover, peritonitis and exit site infections are common in *S. aureus* carrier patients undergoing haemodialysis and continuous peritoneal dialysis \(^{64,91}\). These infections are mostly due to endogenous (i.e. carriage) strains, and often lead to loss of the catheter \(^{12,92,93}\).

### 2.3 Determinants for *S. aureus* nasal carriage

*Staphylococcus aureus* nasal carriage is common and may be influenced by bacterial, host and environmental factors \(^{63,94}\). These same factors may also contribute to infection/disease caused by *S. aureus* \(^{15}\).

#### 2.3.1 Bacterial determinants

The colonisation process involves a sequence of events listed below with their mechanisms

- i) bacterial adhesion to the epithelial cells surface with subsequent multiplication;
- ii) evasion of the host mucosal immune system and competition with other microorganisms in the nasal cavity; and
iii) invasion and survival inside the host’s cells which may further lead to intracellular persistence.  

i) **Adhesion.** Adhesion is mediated by extra-cellular surface bound Microbial-Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs). MSCRAMMs mainly adhere to the extracellular matrix of the nasal epithelial cells, including fibronectin, cytokeratin, lactoferrin and fibrinogen. MSCRAMMs include the cell wall-teichoic acids (WTA), iron-regulated surface determinant protein A (IsdA), and clumping factor B (ClfB). WTA is essential for initializing the colonisation process *in-vitro*, as well as adhesion to epithelial and endothelial cells. WTA biosynthesis involves the genes tagO and tarK which have relatively higher transcriptional levels during the early stages of colonisation, as shown in an *ex-vivo* study. In an animal model, disruption of WTA biosynthesis resulted in failure of *S. aureus* strains to colonise the nasal cavity.

IsdA is a surface-protein essential for binding to the host proteins associated with desquamated nasal epithelial cells, specifically irocin, involucin and cytokeratin. The gene IsdA is down-regulated in the presence of iron. An *ex-vivo* transcriptomics study reported elevated expression levels of IsdA during nasal colonisation, consistent with the nasal cavity being an iron-restricted environment. In *vivo*, IsdA mutants were defective in colonizing cotton rat nares compared to wild type isolates. Furthermore, healthy human non-*S. aureus* carriers had higher anti-IsdA antibody titres than carriers, suggesting the involvement of anti-IsdA antibodies in reducing *S. aureus* carriage. ClfB is a surface protein that specifically binds to the keratinized squamous cells through the surface component cytokeratin-10, and cytokeratin 8 which are expressed on epithelial cell surfaces. Similar to IsdA, clfB mutants are unable to colonise the anterior nares of cotton rats. Immunisation with IsdA and clfB prevents *S. aureus* colonisation in animal models and may represent future vaccine targets. Evidence suggests that Serine-aspartic acid repeat proteins C and D (SdrC and SdrD), and *S. aureus* surface protein G (SasG) play a role in adhesion, although their cognate ligands remain unknown.

*S. aureus*...
strains deficient in clfB, IsdA, SdrC and SdrD are completely defective in adherence. This suggests that adhesion to the nasal epithelial cells is multifactorial.

During nasal colonisation, *S. aureus* is found in conditions that favour adhesion and proliferation rather than spreading into other sites or causing infections. High replication and growth levels are necessary to compensate for clearance through shedding of the nasal epithelial cells and mucus. The elevated expression levels of the genes involved in cell wall biosynthesis during colonisation, namely *sak* and *sceD*, suggest active, exponential growth of *S. aureus*. In addition, the regulatory systems involved in expression of virulence toxin genes (quorum-sensing accessory gene regulator-agr and enterotoxin genes regulator Sae) are down-regulated.

**ii) Evasion.** In addition to resisting host mechanical clearance, colonisation requires evasion of the host immune system. A low level of humoral immunity is elicited against *S. aureus* proteins upon establishing colonisation in the nasal cavity, however, this immunity does not provide adequate protection against *S. aureus* infections. *S. aureus* evades the immune system through interfering with opsonisation, chemotaxis, and induction of immune modulatory components.

Interestingly, the same virulence factors that facilitate bacterial adhesion are involved in resistance to antibacterial mechanisms (Figure 2.3). For example, IsdA and WTA bind to lactoferrin which reduces the net negative charge of the cell wall and increases its hydrophillicity to combat antibacterial fatty acids produced by sebum glands. Moreover, the chemotaxis inhibitory protein of staphylococci (CHIPS) and staphylococcal complement inhibitor (SCIN) interfere with chemotaxis, the complement system and phagocytosis. The genes encoding for CHIP and SCIN are located in the immune evasion cluster (IEC) which is carried on the β-haemolysin converting bacteriophage. This bacteriophage is present in 90% of carriage strains and encodes the anti-opsonisation factors staphylokinase (SAK) and staphylococcal enterotoxin A (SEA). PVL is a leukotoxin that induces pore formation in polymorphonuclear phagocytes (PMNPs) and macrophages.

---

23
The ability of *S. aureus* to colonise the nasal cavity might be influenced by the surrounding microbiome composition, competition over nutrients or space, or interference with either similar or different species \(^{19,120,121}\). The existence of two different *S. aureus* strains in one niche is considered a rare phenomenon \(^15\). Furthermore, colonisation with a susceptible *S. aureus* strain protects against acquisition of MRSA \(^{122}\). Polymorphism in the autoinducing peptide and its corresponding receptors within the regulatory *agr* system may explain the intra-strain interference in *S. aureus*; "cross-activation" of *agr*-controlled gene expression between similar strains, and “cross-inhibition” of signalling between strains from different groups \(^{101,123,124}\). The “cross-inhibition” in this case occurs in the form of blocking the expression of virulence genes rather than mutual growth \(^{15,123}\).
Figure 2.3: Schematic diagram describing steps of *S. aureus* colonisation of the anterior nares as an example.

1. **Initiation** of the colonisation process mediated by the wall teichoic acid (WTA) which binds to human nasal epithelial cells as suggested by (Weidenmaier *et al.* 2004), followed by (2) **Adhesion and attachment** of *S. aureus* to the epithelium cells via Microbial-Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), including iron-regulated surface determinant protein A (IsdA), clumping factors A/B (ClfA/B), serine-aspartic acid repeat proteins C and D (SdrC, and SdrD) and staphylococcal surface protein G (SasG). (3) **S. aureus multiplication** and interaction/competition with other commensals in the nasal cavity. (4) **Immune evasion** by blocking opsonisation through the complement system via staphylococcal protein A (SPA) which breaks the immunoglobulin G-C3b (IgG-C3b) complex, and staphylococcal complement inhibitor (SCIN). Impairment of neutrophil chemotaxis via chemotaxis inhibitory protein of staphylococci (CHIPs). Secreted molecules such as staphylokinase (SAK), staphylococcal enterotoxin A (SEA) and pantone-valentine leucocidin (PVL) prevent opsonisation and phagocytosis (neutrophil cell is used as an example). WTA and IsdA resist antimicrobial fatty acids by changing *S. aureus* cell wall polarity to hydrophilic. (5) The **cellular invasion** step is initiated by the extra-cellular adhesion protein (Eap) which facilitate adhesion of fibrinogen binding protein (FnBP) to fibrinogen (Fn), the latter serving as a bridge between FnBP and the integrin receptors on the host cell. This complex modulates the host's cell-wall structure to allow *S. aureus* invasion to either immune cells or normal tissue cells.
iii) Invasion and survival. In-vitro studies showed that *S. aureus* has the potential to invade the host’s phagocytic (such as neutrophils and macrophages) or non-phagocytic cells (such as keratinocytes, fibroblasts and epithelial cells) and persist for extended periods of time. For example, in a mouse model, polymorphonuclear phagocytes isolated from the experimentally infected site contained viable intracellular bacterial cells which were sufficient to cause an infection in a naïve animal. The fibronectin binding proteins (FnBPs) play a key role in the internalisation process (Table 2.2). In a knock-out mouse model, elimination of FnBP genes delayed *S. aureus* invasion into the nasal epithelial cells but did not completely stop it suggesting that the cell invasion process is multifactorial. In addition, persistence of *S. aureus* colonisation, be it intracellular or extracellular, requires specific modulation of gene expression toward biofilm formation; where after, it becomes resistant to the mechanical forces such as removal by mucus or saliva. Continuous expression of the genes involved in the colonisation process during persistent carriage (e.g. adhesins, evasins and invasins) provides a selective pressure for maintenance of these genes. This is probably why *S. aureus* is able to cause a wide range of infections since the very variables involved in colonisation have also been showed to be involved in invasive infections. During colonisation, the expression level of toxin genes is low due to down-regulation of the *agr* system. However, recurrent superficial skin infections by *S. aureus* might activate the *agr* system and subsequently trigger toxin production. This may indirectly facilitate the spread of *S. aureus* and transmission, resulting in maintenance and thereafter colonisation.

### 2.3.2 Host determinants of carriage by *S. aureus*

Host factors that influence *S. aureus* nasal carriage can be divided into immune factors, host genetic factors, and demographic and behavioural factors that impact the carrier status. *Staphylococcus aureus* nasal carriers are at a higher risk of infections compared to non-carriers, however, they appear to have less severe disease outcomes upon hospitalisation. This can be explained by the production of antibodies to staphylococcal antigens during colonisation.
which provides natural immunisation \(^{94,110}\). Host innate immunity is largely involved in defence against \(S.\ aureus\) nasal carriage through antimicrobial molecules and nasal epithelium cellular components (Figure 2.3). In a human experimental colonisation study Holfreret et al. compared the anti- \(S.\ aureus\) antibody profiles before and 28 days after colonisation in 16 healthy volunteers using a super antigen-negative (for safety purposes) \(S.\ aureus\) strain 8325-4. In this study, a core immune proteome was observed in 75\% of healthy adult volunteers. This core proteome was represented by common antibodies elicited against conserved virulence factors within the species \(S.\ aureus\) including alpha-hemolysin, beta-haemolysin, phospholipase C, staphylococcal serine protease, and cysteine protease. This experimental colonisation did not boost the humoral response; therefore, it is unlikely that the high antibody prevalence in human is due to short-term nasal colonisation, rather, minor infections are required to elicit anti-\(S.\ aureus\) antibody responses \(^{94,110,130}\).

There are a number of host factors that possibly have a role in determining carriage by \(S.\ aureus\). Pattern-recognition receptors such as toll-like receptor 2 and nucleotide-binding oligomerization domain 2 recognise cell wall components of gram positive bacteria and activate defensins and cytokines against the bacterium \(^{131}\). In keeping with this, \(S.\ aureus\) nasopharyngeal carriage rate was higher among children with polymorphism in toll-like receptors 2 and 4 compared to those without \(^{132,133}\). In the healthy normal skin, the fatty acid sphingosine is known to have an antimicrobial activity against \(S.\ aureus\) at physiological levels. The possible alteration of the sphingosine metabolism in patients with atopic dermatitis may explain their high vulnerability to colonisation/secondary infections by \(S.\ aureus\) \(^{94,134}\). The antimicrobial peptides found in human skin \(\alpha\)- and \(\beta\)-defensins, Cathelicidin and ribonuclease 7 may also influence the carriage status by \(S.\ aureus\). For example, when comparing the expression level of human \(\beta\)-defensin-3 among healthy adults with predefined carriage status, Zanger et al reported significantly low expression levels of \(\beta\)-defensin-3 in the skin of persistent carriers, suggesting that individuals with deficiency in expression of human \(\beta\)-defensin-3 are more likely to carry \(S.\ aureus\) persistently \(^{135}\). In addition, RNAse 7 which is highly expressed in healthy skin, showed high
killing activity when incubated with *S. aureus* in-vitro. The complement system also plays a critical role in the innate defence against microbes. Polymorphism in complement regulators such as mannose-binding lectin (activates the alternative pathway), C-reactive protein (activates opsonisation), and serine protease C1 inhibitor (prevents spontaneous activation of complement system) influence nasal carriage status. The presence of haemoglobin in the human nasal secretions promotes *S. aureus* surface colonisation ex-vivo. Haemoglobin prevents the expression of the *agr* system and favours expression of adhesion genes necessary for colonisation. Furthermore, vitamin D binds to the vitamin D receptor on the surface of immune cells resulting in expression of antimicrobial peptides and T-cell dependent responses in-vitro. A large population study of 3789 participants investigated the association between serum vitamin D and *S. aureus* nasal colonisation. This study showed that reduced levels of serum vitamin D was associated with high risk of *S. aureus* nasal carriage especially among type 1 diabetes mellitus patients. According to the factors described above, it is clear that allelic variation in the genes that encode for *S. aureus* antigen recognition, processing and/or clearance play a critical role in determining carriage status.

Additionally demographical factors such as age and gender are key determinants for *S. aureus* carriage. Also, there are a number of behavioural and environmental factors that may influence *S. aureus* nasal carriage either positively or negatively. These factors will be discussed in detail in Chapter 4.
2.4 Molecular basis for methicillin resistance in *S. aureus*

Penicillin-binding proteins (PBPs) are cell-wall bound enzymes necessary for peptidoglycan synthesis. In methicillin-susceptible *S. aureus* (MSSA) strains β-lactam antibiotics bind to PBPs and disrupt formation of the peptidoglycan. However, methicillin-resistant strains (MRSA), produce an alternative penicillin-binding protein (PBP2a) encoded by *mecA* gene. This protein (78-kDa) has low affinity for methicillin and all other β-lactam antibiotics, therefore, peptidoglycan synthesis is not disrupted in the resistant strains resulting in continuous growth. The methicillin-resistance gene (*mecA*) is located on a mobile genetic island (MGI) called Staphylococcal cassette chromosome mec (*SCCmec*). Even though the origin of the SCCmec element is still under investigation, it was hypothesised that this element was transferred on several occasions from the coagulase negative strain *S. sciuri* to different MSSA strains. *S. sciuri* PBP2a has an 88% amino acid similarity to that of *S. aureus*. The SCCmec element consists of three main components; *mec* complex, cassette chromosome recombinases (ccr) complex, and the joining regions (J-region).

2.4.1 The *mec* complex

The *mec* complex contains the *mecA* gene and its regulator genes *mecRI* and *mecI*. Specifically, the proteins mecR and the mecl function as a trans-membrane β-lactam-sensing signal-transducer and a suppresser for *mecA* and *mecR-mecI* in the absence of β-lactam antibiotic, respectively. Currently, five main *mec* classes (A-E) are known, all classes were identified in *S. aureus* isolates except for class D which was described in the species *S. caprae*. Class A is the only SCCmec that contains the complete *mec* region (*IS341-meca-mecRI-mecI*), whereas in classes B, C, D and E these genes are disrupted by insertion sequences (*IS1272-Δmeca-mecRI-IS431* in class B, or *IS431- mecA-ΔmecRI-IS431* in class C). Both classes D and E share similar structures (*mecA-ΔmecRI- IS431*) with a 976bp deletion in the mecRI region of class E compared to class D (Figure 2.4).

Until recently, *mecA* was the only described gene encoding for methicillin resistance, however, in 2011, a new *mecA* homologue gene called *mecC* was described. *mecC* shares almost 70%
sequence similarity with mecA gene and 62% amino acid identity to PBP2a \(^8\). Therefore, this homologue will be overlooked using the conventional polymerase chain reaction (PCR) and commercial phenotypic methods designed to detect mec/PBP2a \(^8\).

### 2.4.2 The ccr complex and Junction regions

The ccr complex mediates integration/excision of the SCCmec element at a specific attachment site (attBscC) at the 3’ end of the open-reading frame X (orfX). Thus far, eight ccr gene allotypes have been identified, designated A1B1, A2B2, A3B3, A4B4, C, A5B3, A1B6, and A1B3 ([http://www.sccmec.org/Pages/SCC_TypesEN.html](http://www.sccmec.org/Pages/SCC_TypesEN.html)). Moreover, the SCCmec element contains Junction regions (J) which flank the ccr and mec complexes on each side (Figure 2.4a). Structural differences in the J-regions result in SCCmec subtypes (Figure 2.4) \(^146,148,151,154\). The J-regions carry additional MGIs which confer resistance genes to other classes of antibiotics such as the transposon Tn554 and pT181 (Figure 2.4) \(^151\). Based on the combination and the arrangement of the mec and ccr complexes, eleven SCCmec allotypes designated I-XI have been described ([http://www.sccmec.org/Pages/SCC_TypesEN.html](http://www.sccmec.org/Pages/SCC_TypesEN.html)). SCCmec-types I-VIII are mainly identified from MRSA associated with humans. SCCmec types IX-XI were recently identified in MRSA strains from livestock animals and SCCmec-XI is the only type that carries the mecC gene \(^153\). These livestock-associated MRSA strains (types IX-XI) have caused sporadic zoonotic infections \(^153,155-158\).
Figure 2.4: Schematic diagram of the 11 staphylococcal cassette chromosome mec (SCCmec) types. 
(a) SCCmec-types I-VIII associated with humans. (b) SCCmec-types IX, X and XI mainly associated with animals. The mec and ccr complexes in all types are highlighted in grey. SCCmec types II, III and VIII carry the complete mec complex-class A (IS341-mecA-mecR1-mecI), types I, IV and VI carry mec class B (IS1272-\Delta mecA-mecR1-IS431), class C1 and C2 differ in the orientation of the IS341, and carried by types VII and X and V-IX respectively. Class E is carried by type XI which contains the β-lactamase gene (blaZ). J1, 2 and 3 are junction regions. Tn554 encodes resistance to erythromycin (erm) and aminoglycosides (spc or aad9); ΨTn554 encodes cadmium resistance; Tn4001 encodes aminoglycosides resistance operon (aacA-aphD); plasmid pUB110 encodes bleomycin resistance; pT 181 resistance to tetracycline; copA,B encode copper-transport ATPase; hdsR, M and S a partial restriction modification system; ars arsenic resistance operon; ISSha1 insertion sequence typically found in S. haemolyticus. This figure was adapted from Malachowa et al 2010, Shore et al 2011 and Li et al 2013.
2.5  **S. aureus epidemiology**

To better understand the genetic evolution and dissemination of *S. aureus* in a setting, an appropriate genotyping tool should be applied \(^{159}\). Genotyping tools can be grouped into phenotypic and genotypic techniques. Phenotypic techniques focus on detection of biological substances expressed by bacteria under certain conditions \(^{160}\). For decades, *S. aureus* was mainly typed using phage typing, antibiotic susceptibility testing (AST) and multilocus enzyme electrophoresis. However, most of these methods lack discriminatory power and reproducibility due to differences in protein expression level in different conditions \(^{6,161}\). Genotyping techniques measure the variation at the genomic DNA level and can be classified into DNA band-based amplification and sequencing based techniques. Pulse Field Gel Electrophoresis (PFGE) is an example for the band-based techniques and staphylococcal protein A (*spa*) typing exemplifies DNA sequencing-based techniques, in addition to hybridisation-based techniques such as DNA based microarrays \(^{151,161}\).

Selection of a specific typing tool is largely reliant on the study design and setting (e.g., diagnostics or research), the question to be addressed, cost and time to results \(^{161}\). The molecular genotyping tools used to describe *S. aureus* epidemiology and population structure in this study include: SCC*mec* typing, and single locus sequence typing (*spa*), which will be described in greater details in Chapter 5.

### 2.5.1  Staphylococcal cassette chromosome mec (SCCmec) typing

SCC*mec* typing is based on amplification of both *mec* and *ccr* complexes using a multiplex PCR, and identification of the different subtypes by targeting the J-regions \(^{152}\). SCC*mec* types and subtypes are identified by distinct amplification patterns on an agarose gel \(^{151,162–164}\).

### 2.5.2  Staphylococcal protein A (*spa*) typing

*Spa typing* is a single-locus sequence-based typing method, which determines the sequence variation in the polymorphic X-region of the *spa* locus. This polymorphic region consists of many 24bp repeats flanked by the IgG binding domains and the signalling sequence (Figure 2.5) \(^{165}\).
Genetic arrangements, such as point mutations, insertions, deletions, and duplication of these repeats, generate unique spa types. The identified spa types are submitted to a curated central network based spa server (http://spaserver.ridom.de). Clustering analysis can be done using the ‘based upon repeat pattern’ (BURP) algorithm which is implemented in the commercial software Ridom StaphType (Ridom, Münster, Germany). The BURP algorithm clusters the spa types into spa-clonal complexes (spa-CCs) based on their genetic relatedness. Two commercial software packages are currently available for spa typing.

While spa typing is based on the sequencing of a single locus, it has high discriminatory power, reproducibility and typeability, while also being inexpensive, easy to perform and fast. Spa typing showed usefulness in short- and long-term epidemiological investigations in local and global settings because it is able to characterise micro and macro genetic variations. However, since this locus is highly susceptible to genetic rearrangements resulting in different types, this could result in misleading interpretations, especially in investigations related to hospital outbreak source tracing.

**Figure 2.5: Schematic diagram for the S. aureus protein A (spa) locus.**

(a) S is the signal-sequence, A-E are the IgG binding domains, the X-region is the hypervariable region which contains a constant part Xc (codes for cell-wall binding protein) and the variable part Xs (the region for spa typing). (b) An example for the repeats arrangement within the Xs. (c) The typical structure for the 24bp repeat sequence. This figure was adapted from Hallin et al.
2.5.3 Multilocus sequence typing (MLST)

MLST is a multilocus sequence-based method which assesses the genetic variation occurring within seven housekeeping genes. In this method, 400-500bp internal fragments of the seven housekeeping genes are amplified, sequenced, and submitted to the online S. aureus MLST database ([http://saureus.mlst.net/](http://saureus.mlst.net/)). Thereafter, an allelic identification number is assigned to each fragment, and the combination of the seven allelic numbers result in the sequence type (ST). STs that differ by one or two alleles are called single locus variants (SLV) or double locus variants (DLV), respectively. Like spa typing, clustering analysis for STs can be done using the eBURST (based upon related sequence types) algorithm which clusters the different SLVs into MLST-clonal complexes (CC). Furthermore, STs with the most number of SLVs could be assigned as the founder ST to that CC. MLST is a very useful tool in studying S. aureus population structure and long-term genetic evolution rather than short-term epidemiological investigations because it targets slowly evolving housekeeping genes. The combination of ST and SCCmec types has helped to define the major MRSA clones spread world-wide. Even though MLST data is comparable between laboratories locally and globally, it has only a moderate discriminatory power, is time consuming, laborious and relatively expensive.

Other typing tools that have added value to staphylococcal population structure investigations include single locus typing tools targeting the coagulase gene and mec-associated direct repeat units (dru) mainly for MRSA. Amplified Fragment Length polymorphism (AFLP) and multilocus variable number of tandem repeat analysis (MLVA) are PCR based methods which have been used in place of PFGE in hospital outbreak investigations. Whole genome sequencing (WGS) is currently the most advanced and most accurate molecular technique used to-date. It provides high resolution to detect micro-evolutionary changes unlike other techniques, however, it is still expensive and not feasible for studies with large numbers of isolates. WGS showed success in a number of applications such as evolution of MRSA during hospital transmission and intercontinental spread, and source-tracking of hospital outbreaks caused by MRSA strains. S. aureus genome comparisons which can be done at different levels (at the core genome, variable
Literature review

genome or at the mobile genetic elements level) may be a key tool to extend our understanding of genome polymorphism and mutation rate within *S. aureus*\(^{100}\).

### 2.6 The global population structure of *S. aureus*

Using the different typing tools described above has contributed to the understanding of *S. aureus* population structure, outbreak source tracing and infection control. In the past decade, there has been a substantial increase in understanding of the population structure of *S. aureus*, especially after the application of the WGS\(^{6}\). Currently, MLST is one of the most widely used tools to describe global *S. aureus* lineages\(^{181}\). According to the MLST database, there are 82 *S. aureus* CCs and 384 singletons (http://saureus.mlst.net/). Of these, CC5, CC9, CC12, CC22, CC25, CC30, CC45, and CC51 are considered to be the major *S. aureus* lineages associated with humans\(^{182}\). CC5 is the largest *S. aureus* cluster which contains five of previously described major CCs (CC1, 5, 8, 15 and 97)\(^{183}\).

In the current literature, little attention was drawn to study the population structure of MSSA. However, it is established that the population structure of MSSA is more diverse than that of MRSA\(^{184}\). The methicillin-susceptible clonal complexes CC5, CC8, CC30, and CC45 have worldwide dissemination, which explains the successful pandemic spread of their methicillin-resistant counterparts following acquisition of SCC\(^\text{mec}\) element\(^{185}\). On the other hand, some MSSA lineages have limited geographical dispersal such as ST152, which is found mainly in West Africa and Balkan region (http://saureus.mlst.net/). In Africa, ST5 and ST15 are predominantly found in West Africa, while, ST8 is present in Maghreb countries and Nigeria. In Central and Western Africa, ST30, ST121, and ST152 are the common MSSA lineages\(^{186}\). Data on molecular epidemiology in the other parts of Africa is scarce and more baseline surveillance studies are much needed\(^{187}\).

It is of importance to understand the genetic nature of the methicillin susceptible strains found in a setting, as this may help predict resistant strains that may emerge, since acquisition of a SCC\(^\text{mec}\) by endemic MSSA strains is one important mechanism for the emergence of new MRSA strains\(^{85,188,189}\). Initially, the hypothesis was that MRSA epidemics emerged from a single clone,
however, a number of studies have since supported that the acquisition of SCCmec occurred multiple times in genetically diverse MSSA strains. Moreover, the occurrence of new SCCmec acquisitions was four times more common than the replacement of one SCCmec type with another \cite{85,188,189}, supporting the multi-clone theory; "MRSA has initially emerged at least 20 times upon acquisition of SCCmec by different susceptible clones or re-acquisition by putative MRSA clones"\cite{85}. Not all MSSA lineages provide a stable background for SCCmec recombination due to the lack of a specific integration site in some lineages, as suggested by Noto et al. \cite{190}.

### 2.6.1 Molecular epidemiology of hospital-associated (HA) and community-associated (CA) MRSA

Hospital-associated MRSA (HA-MRSA) have considerable impact on patients and healthcare costs. HA-MRSA strains mainly cause invasive infections in hospitalised, compromised and elderly patients \cite{191,192}. The proportions of \textit{S. aureus} isolates which are HA-MRSA isolates within hospital settings as reviewed by Grundmann et al. and Stefani et al. range from 5-25\% in Australia, China and some European countries. In Africa, MRSA prevalence mostly ranges between 25-50\% and is showing trends of decreasing in South Africa \cite{68}. The highest prevalence (>50\%) is reported in North and South America, South Korea, and Japan \cite{181,193}.

MRSA strains belonging to CC5, CC22, CC30 and CC45 are frequently associated with MRSA infections in hospitals. They typically harbour SCCmec types I [1B], II [2A], or III [3A] which confer resistance to a wide range of antibiotics. MRSA clones belonging to CC5, such as ST5-II [2A] (New York/Japan) as well as CC8, such as ST239-III [3A] (Brazil/Hungary) have world-wide distribution \cite{151,181}. ST239-III [3A] (CC8) for example, which was originally described in Latin America, is now more frequent in Asia and is showing successful pandemic spread \cite{194}. Other clones such as the epidemic MRSA (EMRSA) ST22-II [2A] (CC22) and ST36-II [2A] (CC30) also known as UK-EMRSA-15 and 16, respectively, are endemic in the United Kingdom \cite{195}.

Community-associated MRSA (CA-MRSA) emerged in the late 1990s when it was described in individuals with no previous exposure to health care settings such as athletes, prisoners and military personnel \cite{196,197}. CA-MRSA evolved independently of HA-MRSA; strong evidence of this
are the differences in the epidemiological, microbiological characteristics and clinical manifestations. These include carriage of the small-size SCCmec types IV [2B], V [5C2] and VII [5C1], the PVL encoding bacteriophage and resistance genes to only β-lactam antibiotics. CA-MRSA reports are associated with a few distinct lineages with the following PVL-positive clones being the most predominant: ST1-IV [2B] (USA400), ST8-IV [2B] (USA300), ST30-IV [2B] (South West Pacific clone), and ST80-IV [2B] (European clone). USA300 is the most prevalent CA-MRSA in the United States of America and is considered a global health threat since it has been described on every continent. The European clone however, is the most prevalent clone in Europe, North Africa and the Middle East. ST59-IV/V [2B/C2] is frequently reported in Asia and Australia and ST772-I [1B] (Bengal clone) is the main endemic clone in India and Bangladesh. In Africa, ST8-IV [2B] and ST88-IV [2B] were the most predominant CA-MRSA clones in community settings.

Over the years, CA-MRSA has been increasing in prevalence and is frequently seen in healthcare settings. Therefore, it has become difficult to dichotomously define CA- and HA-MRSA; previously known CA-MRSA clones are now circulating in hospital settings and vice versa. Although the Centres for Disease Control and Prevention have set criteria to define CA-MRSA, these are wholly reliant on epidemiological criteria. Therefore, a combination of molecular typing tools, phenotypic characteristics as well as epidemiological data for colonising/infecting strains may help to distinguish CA-MRSA and HA-MRSA, although this distinction is increasingly artificial.

2.7 Decolonisation, eradication and control of \( S. \) aureus carriage

In the relevant literature, “eradication” and “decolonisation” are used interchangeably. Decolonisation is defined as attempts to eradicate \( S. \) aureus or MRSA nasal carriage. Most decolonisation studies focus on applying \( S. \) aureus carriage eradication protocols only without assessing the effectiveness of this eradication. Generally, there are three main approaches used for decolonisation: i) applying topical treatments such as chlorhexidine, mupirocin and Tea Tree oil; ii) using systemic treatments such as fusidic acid, Rifampicin and sulfathiazole; and iii) other strategies such as bacterial interference, \( S. \) aureus phage therapy and vaccines.
SSTIs, decolonisation using mupirocin successfully prevented recurrent furunculosis caused by MSSA (PVL-positive) strains \(^{204}\), but, failed to reduce SSTIs due to MRSA strains especially in football players \(^{205}\). However, these studies did not include controls in their trials. Moreover, an old randomized control trial investigated monthly intranasal mupirocin for a year compared to placebo in immune competent patients with skin infections. This study reported reduced rates of nasal carriage, and 50% reduction of SSTIs among the mupirocin group \(^{206}\). Other randomized controlled trials studied mupirocin alone in soldiers carrying MRSA \(^{207}\), HIV-infected patients with known MRSA carriage \(^{208}\), or applied combined strategies like mupirocin plus chlorhexidine, or mupirocin plus daily dilute bleach baths vs hygiene education on healthy carriers as well as patients with community-onset SSTIs \(^{209}\). All these studies managed to eradicate staphylococcal carriage, but failed to reduce subsequent SSTIs in treated groups. Furthermore, decolonisation targeting index cases as well as their household members showed significant reduction in the incidence of CA-SSTIs compared to decolonising the individual index cases alone \(^{210}\).

For dialysis patients (haemodialysis and peritoneal dialysis) on the other hand, a substantial reduction of staphylococcal carriage and infections including peritonitis following mupirocin treatment was noted by Tacconelli and co-workers \(^{211}\). In addition, the application of topical mupirocin to the vascular catheter cannulation site rather than to the nose, significantly reduced the occurrence of *S. aureus* catheter associated bacteraemia \(^{212,213}\). For efficient decolonisation in this particular group of patients (dialysis), repeated treatment is recommended to prevent reinfection, however, the use of nasal mupirocin for extended periods (especially with peritoneal dialysis patients) leads to increased resistance to this antibiotic and subsequent complicated infections as a result of this resistance \(^{202}\).

At least in some surgery patients such as orthopaedic and cardiothoracic patients, applying decolonisation procedure at the time of surgery reduced the risk of surgical site infections \(^{202,214}\). For example, application of chlorhexidine gluconate to the nasopharynx and the oropharynx of cardiac surgery patients significantly reduced *S. aureus* carriage compared to patients who received placebo. This reduction was associated with decrease incidence of nosocomial infections.
and hospital stay. In a randomized control trial, surgical patients (general, gastrointestinal, cardiothoracic, orthopaedic and vascular) were screened for *S. aureus* carriage by RT-PCR. Patients who were colonised, were randomly assigned to either mupirocin ointment and bathing with chlorhexidine gluconate soap or placebo ointment and soap. The infection rate was 3.4% among the mupirocin group compared to 7.7% in the control group.

The success of *S. aureus*/MRSA decolonisation using mupirocin alone or mupirocin with skin antiseptic washes among non-surgical patients was debatable. Clinical trials on medical patients either showed temporal reduction in the risk of carrying *S. aureus*/MRSA post treatment, but did not reduce the risk of infection, or showed no significant difference in the rate of staphylococcal infections between the treatment and the placebo groups. However, a significant decrease in the rate of *S. aureus* infections was reported among ICU patients after performing a nasal carriage screening followed by mupirocin treatment and chlorhexidine washes.

Among children (median age 2 years), who were candidates for elective ambulatory surgery, it was suggested that screening for and eradication of *S. aureus* carriage is not essential in this population, since no correlation was observed between positive nasal swabs and incidence of wound infections.

Even though decolonisation using mupirocin alone or in combination with systemic antibiotic or aseptic washes could be successful for short-term carriage eradication, a number of factors has been attributed to treatment failure. Host-related factors are more likely to be linked to this, such as underlying conditions and skin lesions. The use of systemic antibiotics with sub-optimal doses, plus development of resistance to mupirocin especially with long-term use, could be main reasons for decolonisation failure. In addition to the possibility of re-colonisation or re-infection after the successful treatment. New alternatives to mupirocin such as tea tree oil, medicinal honey and octenidine dihydrochloride are now showing promising results.

How to best control *S. aureus* transmission and infection remains debatable. Questions about when it is a suitable time to screen for *S. aureus* carriage in hospitals, whether selective or universal decolonisation is efficient to minimise nosocomial infections by *S. aureus*/MRSA and
whether such strategies are cost effective remain unknown. However, thus far the strategy MRSA “search and destroy” (i.e. screening 100% of admissions and decolonisation of identified carriers) has been applied in health care centres in developed countries with some success. For example, in an inpatient rehabilitation facility in the USA screening all admissions for MRSA carriage with subsequent decolonisation significantly reduced the mean monthly MRSA infection rate from 1.0714 per 1000 patient days to 0.6557 per 1000 patient days.

### 2.8 S. aureus vaccination

Since nasal carriage is a risk factor for subsequent infections, vaccine clinical trials could target S. aureus nasal carriage reduction or eradication – which should reduce the risk of infection- or prevent infection independent of carriage. Table 2.2 summarizes the vaccine candidates that have been developed up until the year 2013 and their status. There have been seven vaccine trials conducted thus far. These vaccine trials used different antigenic targets, such as the Iron Surface Determinant B (IsdB) in V710, and the Capsular Polysaccharide proteins (CPS 5 and 8) in the StaphVAX. In a clinical trial on adults scheduled for cardiac surgery, the V710 vaccine performed poorly, with lack of efficacy, significant number of side effects, and a high mortality rate among the vaccine group compared to the placebo group. In addition, no data are available on the effect of this vaccine on carriage. When determining the impact of the S. aureus conjugate vaccine (StaphVAX) on nasal carriage, it was found that following vaccination, high titres of anti-CP5 and CP8 antibodies were observed, however, nasal carriage rates did not change accordingly. Of the existing trials, only one trial of a neonatal passive vaccine (Altastaph) was conducted. This vaccine targeted infants with very low birth-weight up to seven days of age. This Trial was completed and Altastaph did not show any significant difference between the vaccine and the placebo group.

Selection of inappropriate targets could be one explanation for vaccines failure, for example, the StaphVAX which is a conjugate vaccine targeted the capsular polysaccharides CP5 and 8, combined with Pseudomonas protein A (Table 2.2). However, it has been shown that not all S.
*S. aureus* strains produce capsular proteins. In addition, Abs elicited against CP5 and CP8 cross-react with naturally produced Abs against some staphylococcal cell-surface Ags, therefore, these Abs may neutralize the opsonic activity of the CP vaccine. Moreover, *S. aureus* possesses a very high antigenic variability, with expression of antigens depending on whether the strain is colonizing or invading. For example, the *agr* system which regulates expression of enterotoxins is down-regulated during carriage, while the microbial surface components recognizing adhesives matrix molecules are activated during colonisation. Similarly, the Immune Evasion Cluster (IEC) is present in 90% of carriage strains and in few invasive strains. Furthermore, *S. aureus* produces immune evasion factors that may block or prevent the appropriate immune response against the vaccine. Although animal models may increase our understanding on the interplay between the microbes and the host, they may not fully predict the success of a vaccine in human. For example, the use of immune adjuvants such as Complete Freund’s Adjuvant (which is not applicable for use in humans) may elicit immune responses in animals that cannot be elicited in humans using other adjuvants.

Most targeted Ags in these vaccines only induced humoral immunity, except for the rAls3p-N (N terminus of the candida Als3p adhesin with aluminium hydroxide adjuvant). The vaccine rAls3p-N elicited T-mediated responses (Th-1/Th-17) that recruited and activated phagocytes at sites of infections, resulting in effective clearance of both *S. aureus* and *Candida albicans* from mice tissues. Additionally, in a mouse model, T-cell dependant immunity mediated by Th-17 and neutrophil influx has shown to play a role in clearance of *S. aureus* nasal carriage. Therefore, the vaccine rAls3p-N seem to be promising in protection against both *S. aureus* colonisation and infection. New approaches have been introduced for vaccine development such as the use of attenuated *S. aureus* strains. Also, a “convergent immunity” (use of antigenic determinants from non-target organism which share homologous epitopes with the targeted organism) approach may have relevance for novel vaccines. A good example is the cross-kingdom convergent immunogens shared between Candida and Staphylococcus; despite the divergence, structural and functional homology was observed in agglutinin-like sequence (Als) proteins in *C. albicans* and the microbial
surface components of *S. aureus* such as clumping factor A (clfA) which mediate adhesion of these organisms into host tissues or cells. *S. aureus* and *C. albicans* share similar features like occupation of epidermal and mucosal tissues, and similar pathogenesis strategies in human - mucocutaneous colonisation/ opportunism/ immune evasion/ hematogenous dissemination. This explains why the vaccine rAlS3p-N had efficacy in mouse models of both *C. albicans* and *S. aureus* (including MRSA) infections

Recent vaccine trials targeted specific high risk populations such as haemodialysis, or surgical patients (Table 2.2). However, with the increased burden of infections associated with CA-MRSA in the general population, in addition to the blurred definition of HA-and CA-MRSA strains, the notion of an “at-risk” population should be revisited. For example, a population with significant exposure to health care settings and persistent *S. aureus* carriage could potentially represent an interesting target for vaccine trials.
Table 2.2: Summary of *Staphylococcus aureus* active vaccine trials reported by Botelho-Nevers et al.

<table>
<thead>
<tr>
<th>Company</th>
<th>Targeted antigen</th>
<th>Target population</th>
<th>Outcome</th>
<th>Trial.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nabi (StaphVAX)</td>
<td>CPS 5/8 + pseudomonal exoprotein A</td>
<td>Haemodialysis</td>
<td>Failed in phase III</td>
<td>NCT00071214</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiovascular surgery</td>
<td>Completed phase III</td>
<td>NCT00211913</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orthopaedic joint surgery</td>
<td>Phase III</td>
<td>NCT00211965</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orthopaedic implant</td>
<td>Completed 2006</td>
<td>NCT00211926</td>
</tr>
<tr>
<td>GlaxoSmith Kline Pfizer</td>
<td>Nabi vaccine + WTA + PVL(rLukS-PV/rAT) + Hla</td>
<td>Volunteers</td>
<td>Completed phase I</td>
<td>NCT01160172</td>
</tr>
<tr>
<td></td>
<td>Type 5 and 8 CPS + ClfA = SA3Ag</td>
<td>Volunteers</td>
<td>Completed phase I</td>
<td>NCT01018641</td>
</tr>
<tr>
<td></td>
<td>SA4Ag</td>
<td>Haemodialysis</td>
<td>Completed phase II</td>
<td>NCT00572910</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patients with bacteraemia, wound infections and cardiothoracic surgery</td>
<td>Failed phase I/II</td>
<td>NCT00518687</td>
</tr>
<tr>
<td>Merck</td>
<td>IsdB (V710)</td>
<td>Volunteers</td>
<td>Completed phase I</td>
<td>NCT01447407</td>
</tr>
<tr>
<td>Novadigm</td>
<td>rAls3p-N*</td>
<td>Volunteers</td>
<td>Completed phase I</td>
<td>NCT01011335</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eap, GST-Can, His-Clf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VRI</td>
<td></td>
<td>Volunteers</td>
<td>Completed phase I</td>
<td></td>
</tr>
<tr>
<td>Uniformed Services University of Health Sciences / Nabi</td>
<td>Mon-valent (rAT or rLukS-PV)</td>
<td>Volunteers</td>
<td>Completed phase I/II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bi-valent (rAT and rLukS-PV)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: ATP: Adenosine triphosphate; C. albicans: Candida albicans; ClfA: Clumping factor A; CPS: Capsular polysaccharide; VRI: Vaccine Research International; Eap: Extracellular adherence protein; GST-Can: Collagen-binding protein; His-Clf: Clumping factor; Hla: A-toxin (alpha-hemolysin); IsdB: Iron surface determinant B; LTA: Lipoteichoic acid; SA4Ag: 4-antigen *Staphylococcus aureus* vaccine.

*C. albicans* surface protein that cross reacts with *S. aureus*.
References

Literature review


36 Kwambana B A, Barer MR, Bottomley C, et al. Early acquisition and high nasopharyngeal co-colonisation by Streptococcus pneumoniae and three respiratory pathogens amongst Gambian new-
Literature review

bourns and infants. *BMC Infect Dis* 2011; **11**: 175.


Literature review


Walsh EJ, O’Brien LM, Liang X, Hook M, Foster TJ. Clumping factor B, a fibrinogen-binding...


Literature review


Literature review


Literature review


Simor AE. Staphylococcal decolonisation: An effective strategy for prevention of infection? Lancet


Harbarth S, Dharan S, Liassine N, et al. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant Staphylococcus
Literature review


Pier GB. Will there ever be a universal *Staphylococcus aureus* vaccine? *Hum vaccines Immunother* 2013; 9: 1865–76.


Chapter Three

Molecular epidemiology of Methicillin-resistant *S. aureus* in Africa: a systematic review

*Shima M. A. Abdulgader¹, Adebayo O. Shittu², Mark Nicol¹.³.⁴, and Mamadou Kaba¹.³* (2015) Front. Microbiol. 6:348. doi: 10.3389/fmicb.2015.00348

¹Division of Medical Microbiology, Department of Clinical Laboratory Sciences, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
²Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria
³Institute for Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
⁴National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa
3.1 Summary

Methicillin-resistant *S. aureus* (MRSA) infections are a serious global problem, with considerable impact on patients and substantial health care costs. This systematic review provides an overview on the clonal diversity of MRSA, as well as the prevalence of Panton-Valentine leucocidin (PVL)-positive MRSA in Africa. A search on the molecular characterisation of MRSA in Africa was conducted by two authors using predefined terms. We screened for articles published in English and French through to October 2014 from five electronic databases. A total of 57 eligible studies were identified. Thirty-four reports from 15 countries provided adequate genotyping data. The hospital-associated MRSA ST239/ST241-III [3A] was identified in nine African countries. This clone was also described with SCC*mec* type IV [2B] in Algeria and Nigeria, and type V [5C] in Niger. In Africa, the European ST80-IV [2B] clone was limited to Algeria, Egypt and Tunisia. The clonal types ST22-IV [2B], ST36-II [2A] and ST612-IV [2B] were only reported in South Africa. No clear distinctions were observed between MRSA responsible for hospital and community infections. The community clones ST8-IV [2B] and ST88-IV [2B] were reported both in the hospital and community settings in Angola, Cameroon, Gabon, Ghana, Madagascar, Nigeria, and São Tomé and Príncipe. The proportion of PVL-positive MRSA carriage and/or infections ranged from 0.3% to 100% in humans. A number of pandemic clones were identified in Africa. Moreover, some MRSA clones are limited to specific countries or regions. We strongly advocate for more surveillance studies on MRSA in Africa.

**Keywords:** *Staphylococcus aureus*, MRSA, Molecular epidemiology, Africa, Systematic review.
3.2 Background

Methicillin-resistant *S. aureus* (MRSA) are a major public health concern and are responsible for both hospital- and community-associated infections worldwide. It is estimated that MRSA infections within the health care setting alone affected more than 150,000 patients annually in the European Union, with an additional cost of 380 million Euros. In the United States of America, 80,461 invasive MRSA infections and 11,285 related deaths occurred in 2011, and an estimated annual burden of between $1.4 billion - 13.8 billion was attributed to community-acquired MRSA. Besides, MRSA has been established as a pathogen for domestic animals and linked with livestock-associated infections.

Methicillin resistance is usually due to the *mecA* gene, borne on the staphylococcal cassette chromosome *mec* (SCCmec) that codes for a 78-kDa penicillin binding protein (PBP2a), with decreased affinity to methicillin and all β-lactam antibiotics. To date, 11 SCCmec types have been identified. Some cassettes, for example, SCCmec II (53kb) and SCCmec III (67 kb), are large and possess mobile genetic elements (MGE), such as integrated plasmids (pUB110, pI258 and pT181) and transposons (e.g. Tn554), and are frequently associated with hospital-acquired MRSA. In contrast, SCCmec IV (21-24 kb) and V (27kb) are shorter elements, generally susceptible to non-beta-lactam antibiotics, and linked with community MRSA.

However, the spread of various MRSA clones between the hospital and community settings has made the dichotomous ranking difficult. Recently, a variant *mecA* gene (named *mecC*) which is situated on an SCCmec XI element has been described. It has a higher relative affinity for oxacillin as compared with cefoxitin, and exhibits only 69% sequence similarity at the nucleotide level and 63% amino-acid identity to *mecA/PBP2a*. Furthermore, based on whole genome sequencing, mutations of the endogenous penicillin-binding proteins (PBP) 1, 2 and 3 in *meca* and *mecC* negative strains have been postulated as a possible alternative mechanism for beta-lactam resistance in MRSA.

There is great interest in tracking, identifying and understanding the diversity of MRSA in various settings. Currently, the most widely used molecular techniques include *Staphylococcus* protein A
gene typing (spa) and multilocus sequence typing (MLST). Studies (particularly using MLST) have provided evidence that a small set of lineages, clonal complex (CC)5, CC8, CC22, CC30 and CC45, are associated with most of the MRSA infections in hospitals. Besides, a number of different geographically distinct lineages, CC1, CC8, CC30 and CC80, have also been associated with community MRSA infections, while CC8 and CC30 have been identified as pandemic lineages both in the hospital and community setting. Furthermore, regional clones have been described in Australia (sequence type [ST] 93), India (ST772), South Korea (ST72), Taiwan and China (ST59).

The distribution of MRSA clones in Africa is not well-described. Understanding the molecular epidemiology of MRSA in Africa is important as a recent review indicated that since the year 2000, the prevalence of MRSA appears to be increasing in many African nations and pose a visible threat to the continent. Furthermore, there is evidence of the replacement of existing MRSA clones with different and new clonal types in a number of countries, but information on this trend is lacking in Africa. The occurrence and changes in clonal identities, and their geographic spread is important to understand the spread and evolution of MRSA.

Panton-Valentine leuckocidin (PVL) is a two-component pore-forming toxin with cytolytic activity on defined cells of the immune system (neutrophils, macrophages and monocytes). It is encoded by the lukS-PV and lukF-PV genes, and PVL-producing S. aureus exhibit a propensity for causing mainly severe and often recurrent skin and soft tissue infections. In addition, PVL-positive MRSA are associated with community onset-pneumonia. Although the PVL genes are mainly carried by community-associated MRSA (CA-MRSA), data from West and Central Africa showed that at least 40% of clinical methicillin-susceptible S. aureus (MSSA) isolates in this region are PVL-positive. Therefore, the acquisition of the meca gene by PVL-positive MSSA and the possible dissemination of PVL-positive CA-MRSA could present a significant challenge in disease management and infection control in resource-limited countries in Africa.

This systematic review examined published literature on the molecular epidemiology of MRSA in Africa. By summarizing currently available data on the continent, our objective is to describe the
distribution of MRSA clones, the prevalence of PVL-positive MRSA, and to highlight the need to
develop more comprehensive surveillance and reporting systems for multi-resistant organisms
such as MRSA in Africa.

3.3 Method

This systematic review was conducted in accordance with the preferred reporting items for
systematic reviews and meta-analyses (PRISMA) guidelines.

3.3.1 Literature search strategy

The relevant English and French articles available in five electronic databases (MEDLINE,
EBSCOhost, ISI Web of knowledge, Scopus, and African Journals Online) were retrieved by two
authors using predefined search terms (Table 3.1). The literature search was conducted until 31
October 2014.
Table 3.1: Keywords used to identify eligible studies available in four biomedical databases

<table>
<thead>
<tr>
<th>Database</th>
<th>Search period</th>
<th>Search strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDLINE via PubMed</td>
<td>1974 - October 2014</td>
<td>(MRSA OR Methicillin-resistant <em>Staphylococcus aureus</em>) AND (Algeria OR Angola OR Benin OR Botswana OR Burkina Faso OR &quot;Burkina Faso&quot; OR Burkina Fasso OR Upper Volta OR &quot;Upper Volta&quot; OR Burundi OR Cameroon OR Cape Verde OR &quot;Cape Verde&quot; OR Central African Republic OR Chad OR Comoros OR &quot;Iles Comores&quot; OR Iles Comores OR Comoro Islands OR “Comoro Islands” OR Congo OR Democratic Republic Congo OR &quot;Democratic Republic of the Congo&quot; OR Zaire OR Djibouti OR Egypt OR Equatorial Guinea OR &quot;Equatorial Guinea&quot; OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea Bissau OR &quot;Guinea Bissau&quot; OR &quot;Ivory Coast&quot; OR Cote d’Ivoire OR &quot;Cote d’Ivoire&quot; OR Kenya OR Lesotho OR Liberia OR Libya OR Libia OR Jamahiriya OR Jamahiryia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Ile Maurice OR “Ile Maurice” OR Morocco OR Mozambique OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR Sao Tome OR &quot;Sao Tome&quot; OR Senegal OR Seychelles OR Sierra Leone OR &quot;Sierra Leone&quot; OR Somalia OR South Africa OR &quot;South Africa&quot; OR Sudan OR South Sudan OR “South Sudan” OR Swaziland OR Tanzania OR Tanganyika OR Zanzibar OR Togo OR Tunisia OR Uganda OR Western Sahara OR &quot;Western Sahara&quot; OR Zambia OR Zimbabwe OR Africa OR Africa* OR Southern Africa OR West Africa OR Western Africa OR Eastern Africa OR East Africa OR North Africa OR Northern Africa OR Central Africa OR Sub Saharan Africa OR Subsaharan Africa OR Sub-Saharan Africa OR Sub-Saharan Africa) NOT (Guinea pig* OR &quot;Guinea pig*&quot; OR Aspergillus niger OR &quot;Aspergillus niger&quot; OR Europe* OR America* OR Asia*)</td>
</tr>
<tr>
<td>EBSCOhost via Academic Search premier, Africa-Wide information and CINAHL</td>
<td>1982 - October 2014</td>
<td>(MRSA OR methicillin-resistant <em>Staphylococcus aureus</em>) AND (Algeria OR Angola OR Benin OR Botswana OR Burkina Faso OR &quot;Burkina Faso&quot; OR Burkina Fasso OR Upper Volta OR &quot;Upper Volta&quot; OR Burundi OR Cameroon OR Cape Verde OR &quot;Cape Verde&quot; OR Central African Republic OR Chad OR Comoros OR &quot;Iles Comores&quot; OR Iles Comores OR Comoro Islands OR “Comoro Islands” OR Congo OR Democratic Republic Congo OR &quot;Democratic Republic of the Congo&quot; OR Zaire OR Djibouti OR Egypt OR Equatorial Guinea OR &quot;Equatorial Guinea&quot; OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea Bissau OR &quot;Guinea Bissau&quot; OR &quot;Ivory Coast&quot; OR Cote d’Ivoire OR &quot;Cote d’Ivoire&quot; OR Kenya OR Lesotho OR Liberia OR Libya OR Libia OR Jamahiriya OR Jamahiryia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Ile Maurice OR “Ile Maurice” OR Morocco OR Mozambique OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR Sao Tome OR &quot;Sao Tome&quot; OR Senegal OR Seychelles OR Sierra Leone OR &quot;Sierra Leone&quot; OR Somalia OR South Africa OR &quot;South Africa&quot; OR Sudan OR South Sudan OR “South Sudan” OR Swaziland OR Tanzania OR Tanganyika OR Zanzibar OR Togo OR Tunisia OR Uganda OR Western Sahara OR &quot;Western Sahara&quot; OR Zambia OR Zimbabwe OR Africa OR Africa* OR Southern Africa OR West Africa OR Western Africa OR Eastern Africa OR East Africa OR North Africa OR Northern Africa OR Central Africa OR Sub Saharan Africa OR Subsaharan Africa OR Sub-Saharan Africa OR Sub-Saharan Africa OR Sub-Saharan Africa) NOT (Guinea pig* OR &quot;Guinea pig*&quot; OR Aspergillus niger OR &quot;Aspergillus niger&quot; OR Europe* OR America* OR Asia*)</td>
</tr>
<tr>
<td>ISI web of knowledge</td>
<td>1950 – October 2014</td>
<td>(MRSA OR methicillin-resistant <em>Staphylococcus aureus</em>) AND (Africa)†</td>
</tr>
<tr>
<td>Scopus from SciVerse</td>
<td>1982 – October 2014</td>
<td>(MRSA OR methicillin-resistant <em>Staphylococcus aureus</em>) AND (Africa)†</td>
</tr>
</tbody>
</table>

† In order to exclude studies from other continents, the African countries have been manually selected as recommended by Scopus database.
3.3.2 Eligible articles identification

Figure 3.1 summarizes the study selection process. All duplicate articles were removed and data on MSSA as well as in-vitro studies were also excluded. The eligibility of published reports in this review was based primarily on polymerase chain reaction (PCR) detection of the mecA gene, and the use of at least one molecular tool for genotyping of MRSA strains (Table 3.2). In addition, worldwide surveys that covered African countries were also included. An MRSA clone was defined based on the combination of MLST sequence type (ST) and SCCmec typing data as previously reported. The nomenclature of the SCCmec types was as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements. SCCmec elements that could not be classified were indicated as non-typeable (SCCmec-NT). In this study, we categorized MRSA into various CCs according to the current eBURST scheme, Version 3 (accessed 30 October 2014).

3.3.3 Data extraction and synthesis

The relevant data were extracted from each of the articles as stated in Table 3.2. Separate articles that analysed the same S. aureus isolates but answered different questions (in Table 3.2 highlighted in grey) were considered as a single study.

3.3.4 eBURST analysis

The relationship between the MRSA STs described in this review and other lineages reported world-wide was analysed using the eBURST scheme. The allelic profiles were downloaded from the MLST website (http://saureus.mlst.net/) which included the African MRSA STs as well as 223 representative and randomly selected STs (from each CC) based on the differences in their allelic profiles. The minimum spanning tree was constructed by the goeBURST algorithm using the Phyloviz software v1.1 (http://www.phyloviz.net/).
3.4 Results

3.4.1 Literature search

The systematic search of the five electronic databases yielded 1775 articles (Figure 3.1). No studies were identified from AJOL. After the removal of duplicate studies and assessment of titles and abstracts, 110 full-text articles were screened, of which 57 studies were considered eligible for the qualitative analysis according to our inclusion criteria.

![Flowchart](https://example.com/flowchart.png)

**Figure 3.1: Preferred reporting item for systematic reviews.**

*Note: CONS - coagulase negative staphylococci; MSSA - methicillin susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*

3.4.2 Characteristics of the studies included in the systematic review

Most of the data analysed were obtained from single centre studies conducted mainly in five countries; Tunisia (n=13), Nigeria (n=9), South Africa (n=7), Algeria (n=5), and Egypt (n=3) (Table 3.2). Multicentre studies were only reported in two articles ⁵⁰,⁵¹, including a survey which comprised five African countries, Cameroon, Madagascar, Morocco, Niger and Senegal ⁵⁰, and an
inter-continental multicentre study, which included South Africa. Only three studies investigated the detection of MRSA in animals (Table 3.2).

In most of the reports included in this study, *S. aureus* was identified by phenotypic and culture characteristics, while molecular identification (16SrRNA, detection of the thermonuclease and the elongation factor tu - nuc, tuf - genes) was performed in only 12.3% (7/57). The screening for antibiotic resistance and toxin/virulence genes were carried out in seven and 22 studies, respectively (Table 3.2). Furthermore, all the eligible studies analysed MRSA using at least one genotyping technique, and 59.6% (34/57) provided adequate genotyping data on MRSA clones from 15 African countries (Tables 3.2 and 3.3). Studies included in this systematic review did not investigate on the *mecC* gene.

![Figure 3.2: Methicillin-resistant *S. aureus* clones reported in Africa](image)

Each clonal complex is annotated with a coloured circle. The number of studies conducted in each country is also indicated.
### Table 3.2: Characteristics of eligible articles that studied Methicillin-resistant *S. aureus*

<table>
<thead>
<tr>
<th>Country</th>
<th>Study period</th>
<th>Study population (sample type)</th>
<th>No. of <em>S. aureus</em> isolates</th>
<th>No. of MRSA (^a)</th>
<th>Setting (no.)</th>
<th>Genotyping tools</th>
<th>pvl</th>
<th>Detection of genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCCmec coa agr spa typing</td>
<td>MLST PFGE</td>
<td>Antibiotic resistance</td>
<td>Toxin/ Virulence</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algeria</td>
<td>2003-2004</td>
<td>Clinical samples from hospitals and community</td>
<td>614</td>
<td>204</td>
<td>HA (40)/CA (21)</td>
<td>✓ - ✓ - ✓ ✓ ✓ ✓</td>
<td>- -</td>
<td>- -</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>2004-2007</td>
<td>Human infections (in-and-out patients)</td>
<td>843</td>
<td>23</td>
<td>NR</td>
<td>✓ - - ✓ ✓ ✓ -</td>
<td>- -</td>
<td>- -</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>2005-2007</td>
<td>From military hospital (Pus, venous catheter, tracheal aspirates, lumbar puncture fluid, blood culture and urine)</td>
<td>NR</td>
<td>64</td>
<td>HA (50)/CA (14)</td>
<td>✓ - - - - - ✓</td>
<td>✓ -</td>
<td>- ✓</td>
<td>54,55</td>
</tr>
<tr>
<td></td>
<td>2006-2007</td>
<td>Healthy and hospitalized individuals</td>
<td>221(^a) 52(^b) gyrA PCR 99(^a) 23(^b)</td>
<td>64</td>
<td>HA (65)/CA (84)</td>
<td>✓ - ✓ ✓ ✓ ✓</td>
<td>- -</td>
<td>- ✓</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>2010-2011</td>
<td>Children and neonates (SSTI, bacteraemia, otitis and bone/joint infections)</td>
<td>129</td>
<td>25</td>
<td>HA (15)/CA (10)</td>
<td>✓ - - - ✓ ✓ -</td>
<td>✓ -</td>
<td>- -</td>
<td>57</td>
</tr>
<tr>
<td>Angola</td>
<td>2012</td>
<td>Nasal swabs from inpatients and health care workers (HCW)</td>
<td>117</td>
<td>77</td>
<td>NR</td>
<td>✓ - ✓ ✓ ✓ ✓ ✓</td>
<td>- -</td>
<td>- ✓</td>
<td>58</td>
</tr>
<tr>
<td>Egypt</td>
<td>2007-2008</td>
<td>Pus, sputum, wounds, abscess, blood, urine, and discharge</td>
<td>NR</td>
<td>21</td>
<td>CA (4)</td>
<td>✓ ✓ ✓ ✓ ✓ NR</td>
<td>✓ -</td>
<td>- ✓</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>SSTI and nasal swabs</td>
<td>38</td>
<td>18</td>
<td>CA (18)</td>
<td>✓ - - - - - ✓</td>
<td>- -</td>
<td>- -</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>Septic wounds, UTI and RTI (nasal swabs)</td>
<td>10</td>
<td>7</td>
<td>-</td>
<td>- ✓ - - - - -</td>
<td>- -</td>
<td>- -</td>
<td>61</td>
</tr>
<tr>
<td>Gabon</td>
<td>2008-2010</td>
<td>asymptomatic carriers (nares, axillae, inguinal swabs) and patients (abcess, wound, blood and others)</td>
<td>217</td>
<td>12</td>
<td>HA (6)/CA (6)</td>
<td>✓ - ✓ ✓ ✓ ✓ -</td>
<td>✓ -</td>
<td>- ✓</td>
<td>46,62</td>
</tr>
<tr>
<td></td>
<td>2010-2013</td>
<td>swabs from <em>S. aureus</em> carrier mothers (nasal and mammillary) and their infants (Nasal and pharyngeal)</td>
<td>460</td>
<td>9</td>
<td>NR</td>
<td>✓ - - ✓ ✓ ✓ ✓</td>
<td>- -</td>
<td>- ✓</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>Blood culture of one patient</td>
<td>1</td>
<td>1</td>
<td>NR</td>
<td>- - ✓ ✓ ✓ ✓</td>
<td>- -</td>
<td>- ✓</td>
<td>64</td>
</tr>
<tr>
<td>Ghana</td>
<td>2011-2012</td>
<td>In-patients and hospital staff</td>
<td>105</td>
<td>6</td>
<td>NR</td>
<td>✓ - ✓ ✓ ✓ ✓ ✓</td>
<td>- -</td>
<td>- -</td>
<td>65</td>
</tr>
</tbody>
</table>

\(\alpha\) SCCmec, \(\beta\) coa, agr, spa, coa, spa, typhing, SCCmec, coa, agr, spa, coa, spa, typhing; \(\gamma\) SCCmec, coa, agr, spa, coa, spa, typhing; \(\delta\) SCCmec, coa, agr, spa, coa, spa, typhing
<table>
<thead>
<tr>
<th>Country</th>
<th>Study period</th>
<th>Study population (sample type)</th>
<th>No. of S. aureus isolates</th>
<th>S. aureus molecular identification</th>
<th>No. of MRSA†</th>
<th>Setting (no.)</th>
<th>Genotyping tools</th>
<th>pvl</th>
<th>Detection of genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghana</td>
<td>2010-2012</td>
<td>SSTI and blood samples from six hospitals</td>
<td>308</td>
<td>-</td>
<td>9</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>2011-2012</td>
<td>Nasal swabs from apparently healthy carriers</td>
<td>124</td>
<td>-</td>
<td>2</td>
<td>HA (2)</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>66</td>
</tr>
<tr>
<td>Kenya</td>
<td>2005-2007</td>
<td>In and outpatients with SSTI boil, abscess, cellulitis and ulcer</td>
<td>84</td>
<td>-</td>
<td>69</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>Nasal and axillary skin swabs from hospitalized patients</td>
<td>85</td>
<td>-</td>
<td>6</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>68</td>
</tr>
<tr>
<td>Libya</td>
<td>2009-2010</td>
<td>Nasal swabs from in-patient children, their mothers, out-patient children and HCW</td>
<td>758</td>
<td>-</td>
<td>70</td>
<td>HA (12)</td>
<td>CA (6)</td>
<td>-</td>
<td>-</td>
<td>69</td>
</tr>
<tr>
<td>Mali</td>
<td>2005</td>
<td>Asymptomatic nasal carriers</td>
<td>88</td>
<td>-</td>
<td>1</td>
<td>CA (1)</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>70</td>
</tr>
<tr>
<td>Mozambique</td>
<td>2010-2011</td>
<td>Post-operative, burn wound infections, skin and soft tissue abscesses</td>
<td>99</td>
<td>-</td>
<td>9</td>
<td>HA (8),</td>
<td>CA (1)</td>
<td>-</td>
<td>-</td>
<td>71</td>
</tr>
<tr>
<td>Nigeria</td>
<td>1998-2002</td>
<td>Wounds, aspirate, amniotic fluid</td>
<td>276</td>
<td>-</td>
<td>4</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>2002-2004</td>
<td>Wound samples, blood cultures, urine, otitis media and ocular related infections</td>
<td>200</td>
<td>-</td>
<td>3</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>2007-2012</td>
<td>Clinical specimen</td>
<td>150</td>
<td>-</td>
<td>12</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>74,75</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Surgical and paediatric patients wound samples, corneal, conjunctival, auricular, genital</td>
<td>346</td>
<td>-</td>
<td>70</td>
<td>HA (42),</td>
<td>CA (28)</td>
<td>-</td>
<td>-</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>2008-2010</td>
<td>HIV-positive and healthy individuals nasal swabs</td>
<td>202</td>
<td>-</td>
<td>26</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>Wound infections, semen, UTI, chronic ulcer, conjunctivitis, throat infections</td>
<td>68</td>
<td>-</td>
<td>11</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>2009-2011</td>
<td>Patients and carriers</td>
<td>62</td>
<td>-</td>
<td>22</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>78</td>
</tr>
<tr>
<td>Country</td>
<td>Study period</td>
<td>Study population (sample type)</td>
<td>No. of S. aureus isolates</td>
<td>S. aureus molecular identification</td>
<td>No. of MRSA*</td>
<td>Setting (no.)</td>
<td>Genotyping tools</td>
<td>pvl</td>
<td>Detection of genes</td>
<td>Antibiotic resistance</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>---------------------------</td>
<td>-----------------------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>------------------</td>
<td>-----</td>
<td>-------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Nigeria</td>
<td>2010</td>
<td>Clinical samples from patients with burns, sepsicaemia, wound infections, osteomyelitis, bronchitis and GIT</td>
<td>51</td>
<td>tuf gene PCR</td>
<td>15</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>Urine, blood and aspirates, wound, eye and ear, urethral and endocervical swab</td>
<td>116</td>
<td>-</td>
<td>48</td>
<td>HA (40), CA (8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2001-2003</td>
<td>Wound samples, sputum, otitis media and blood culture</td>
<td>227</td>
<td>nuc gene PCR</td>
<td>61</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2001-2003</td>
<td>Isolates from 16 laboratories in KZN</td>
<td>241</td>
<td>-</td>
<td>24</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2001-2003</td>
<td>Wounds, sputum, otitis media, urine and blood culture</td>
<td>NR</td>
<td>-</td>
<td>61</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>South Africa</td>
<td>2005-2006</td>
<td>Bacteraemia, SSTI, urine, catheter tip, cerebrospinal and drainage fluids</td>
<td>NR</td>
<td>-</td>
<td>320</td>
<td>HA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2006-2007</td>
<td>Clinical samples</td>
<td>NR</td>
<td>-</td>
<td>97</td>
<td>HA (79), CA (4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2007-2008</td>
<td>Pus and pus swabs, urine, blood, RTS and CVCT</td>
<td>NR</td>
<td>-</td>
<td>100</td>
<td>CA (10)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2009-2010</td>
<td>A wide range of clinical specimens mostly SSTI</td>
<td>NR</td>
<td>-</td>
<td>367</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>São Tomé and Príncipe</td>
<td>2010-2012</td>
<td>Patients and healthy carriers</td>
<td>52</td>
<td>-</td>
<td>14</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2008</td>
<td>Wound, nasal swab and pus</td>
<td>160</td>
<td>-</td>
<td>24</td>
<td>HA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>Apparently healthy children under 5 years nasal swabs</td>
<td>114</td>
<td>nuc gene PCR</td>
<td>12</td>
<td>CA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tunisia</td>
<td>1998-2007</td>
<td>Clinical specimens from neutropenic patients</td>
<td>72</td>
<td>nuc gene PCR</td>
<td>13</td>
<td>HA (13)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>Patients who developed MRSA infections</td>
<td>NR</td>
<td>-</td>
<td>6</td>
<td>HA (6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Country</td>
<td>Study period</td>
<td>Study population (sample type)</td>
<td>No. of S. aureus isolates</td>
<td>Setting (no.)</td>
<td>Genotyping tools</td>
<td>pvl</td>
<td>Detection of genes</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>--------------</td>
<td>------------------------------</td>
<td>--------------------------</td>
<td>--------------</td>
<td>-----------------</td>
<td>-----</td>
<td>-----------------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SCCmec</td>
<td>coa</td>
<td>agr</td>
<td>spa</td>
<td>MLST</td>
<td>PFGE</td>
</tr>
<tr>
<td>Tunisia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>typing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003-2004</td>
<td>Pus, blood, pleural fluid, venous catheter</td>
<td>NR</td>
<td>-</td>
<td>72</td>
<td>NR</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2003-2004</td>
<td>Pathological samples from different wards</td>
<td>147</td>
<td>-</td>
<td>19</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2003-2005</td>
<td>Pus and associated with cutaneous infections</td>
<td>NR</td>
<td>-</td>
<td>64</td>
<td>CA (64)</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>2004</td>
<td>Cutaneous pus, blood cultures, urine and puncture fluids</td>
<td>NR</td>
<td>-</td>
<td>34</td>
<td>HA (32), CA (2)</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2004-2005</td>
<td>Cutaneous pus, RTS, urine, blood culture,</td>
<td>475</td>
<td>-</td>
<td>57</td>
<td>NR</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2004-2008</td>
<td>Samples from hospitals and community</td>
<td>NR</td>
<td>-</td>
<td>69</td>
<td>HA (41), CA (28)</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>2006-2008</td>
<td>Children with CA invasive infections bacteraemia and osteomyelitis</td>
<td>36</td>
<td>-</td>
<td>8</td>
<td>CA (8)</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>2007</td>
<td>Pus and skin infections</td>
<td>NR</td>
<td>-</td>
<td>11</td>
<td>CA (11)</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>2008</td>
<td>Pus and blood culture case report</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>NR</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>2008-2009</td>
<td>Humans in contact with animals</td>
<td>55</td>
<td>-</td>
<td>1</td>
<td>CA (1)</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Uganda</td>
<td>2009-2010</td>
<td>Swabs from patients, HCW and from hospital environment sinks, door handles, surgical trays, bed and table surfaces</td>
<td>41</td>
<td>-</td>
<td>41</td>
<td>NR</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2011-2012</td>
<td>SSI</td>
<td>64</td>
<td>nuc gene PCR</td>
<td>24</td>
<td>NR</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Multicentre</td>
<td>2007-2008</td>
<td>SSTI, bacteraemia/septicaemia, urine, wounds osteomyelitis and myositis</td>
<td>NR</td>
<td>-</td>
<td>86</td>
<td>CA (9), HA (77)</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Multicentre</td>
<td>2004-2005</td>
<td>Uncomplicated skin infections</td>
<td>292</td>
<td>-</td>
<td>105</td>
<td>HA (3)</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
</tbody>
</table>
Table 3.2 (Continued)

<table>
<thead>
<tr>
<th>Country</th>
<th>Study period</th>
<th>Study population (sample type)</th>
<th>No. of S. aureus isolates</th>
<th>S. aureus molecular identification</th>
<th>No. of MRSA‡</th>
<th>Setting (no.)</th>
<th>Genotyping tools</th>
<th>pvl</th>
<th>Detection of genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pigs (nasal swabs)</td>
<td>73</td>
<td>NR</td>
<td>6</td>
<td>NR</td>
<td>SCCmeC coa agr spa typing</td>
<td>MLST PFGE</td>
<td>Antibiotic resistance Toxin/Virulence</td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>NR</td>
<td>Cows and buffaloes milk, cattle septic wounds</td>
<td>9</td>
<td>-</td>
<td>5</td>
<td>NR</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>61*</td>
</tr>
<tr>
<td>Senegal</td>
<td>2009-2011</td>
<td>Pigs (nasal swabs)</td>
<td>73</td>
<td>-</td>
<td>6</td>
<td>NR</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>108</td>
</tr>
<tr>
<td>Tunisia</td>
<td>2010</td>
<td>Healthy sheep (nasal swabs)</td>
<td>73</td>
<td>-</td>
<td>5</td>
<td>CA (6)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>109</td>
</tr>
</tbody>
</table>


† MRSA as confirmed by mecA PCR; ✓ test was conducted; - test was not conducted; α hospitalized individuals; β: nasal carrier study; # African multicentre study which included Cameroon, Madagascar, Morocco, Niger and Senegal; * An international multicentre study which included only South Africa; * Study was conducted in both animal and human.
3.4.3 Community- and hospital- associated MRSA

Overall, 51% (29/57) of the eligible studies provided the potential source (hospital- or community-associated) of the MRSA strains. Only 17.5% (10/57) reported MRSA from community settings (Table 3.2). USA300 (ST8-IV [2B]) and other related sequence types were noted both in health care and community settings in nine African countries (Tables 3.2 and 3.4). The “Brazilian/Hungarian clone” (ST239-III [3A]) was associated with hospital-acquired infections in nine countries (Tables 3.2 and 3.4). Furthermore, the “West Australia MRSA-2” (ST88-IV [2B]) was reported in community- and hospital-acquired infections in several African countries (Table 3.2 and 3.4).

3.4.4 Detection of Panton-Valentine leuckocidin (PVL) genes

The screening for PVL-associated genes (lukF-PV and lukS-PV) was carried out in 44 studies, and the detection of PVL genes were only reported in 32 studies (Table 3.3). In animals, PVL-positive MRSA (ST5) was described in nasal samples of pigs from Senegal. In humans, the proportion of PVL-positive MRSA carriage and/or infections ranged from 0.3% to 100%. Studies from Algeria and Tunisia reported higher PVL prevalence while investigations from South Africa reported the lowest prevalence (Table 3.3). Overall, PVL-positive MRSA were more frequently reported with skin and soft tissue infections, and community-associated clones (Tables 3.2 and 3.4). There was no report on the role of PVL in necrotizing pneumonia caused by MRSA in Africa.
Table 3.3: Panton-Valentine Leukocidin prevalence as reported by the eligible studies with sample size of 30 or above

<table>
<thead>
<tr>
<th>Country</th>
<th>PVL positive (no. positive/total tested)</th>
<th>Prevalence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algeria</td>
<td>46/61</td>
<td>75</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>19/64</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>94/122</td>
<td>77</td>
<td>56</td>
</tr>
<tr>
<td>Kenya</td>
<td>14/69</td>
<td>20</td>
<td>67</td>
</tr>
<tr>
<td>Libya</td>
<td>10/35</td>
<td>29</td>
<td>69</td>
</tr>
<tr>
<td>Nigeria</td>
<td>33/70</td>
<td>47</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>1/320</td>
<td>0.3</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>4/97</td>
<td>4</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>5/56</td>
<td>9</td>
<td>89</td>
</tr>
<tr>
<td>South Africa</td>
<td>68/72</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td>Tunisia</td>
<td>64/64</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>43/69</td>
<td>62</td>
<td>100</td>
</tr>
<tr>
<td>Uganda</td>
<td>30/41</td>
<td>73</td>
<td>105</td>
</tr>
<tr>
<td>Multicentre*</td>
<td>20/86</td>
<td>23</td>
<td>50</td>
</tr>
</tbody>
</table>

Note: * Multicentre study which included Cameroon, Madagascar, Morocco, Niger and Senegal

PVL: Panton-Valentine Leukocidin; no: number

3.4.5 MRSA clones reported in Africa using the current eBURST scheme

Figures 3.2 and 3.3 summarize the MRSA clones identified in Africa based on MLST CCs. By the current eBURST scheme, six main CCs were identified: CC5, CC22, CC30, CC45, CC80, and CC88. In addition, a number of diverse spa types were identified among the MRSA clones in Africa (Table 3.4), but the distribution of spa types t042 and t044 (associated with CC80-IV [2B]) appear to be limited to three North African (Algeria, Egypt and Tunisia) countries (Table 3.4).

3.4.5.1 Clonal complex 5

This clonal complex is considered the largest group based on the eBURST scheme (Figure 3.4). It was subdivided into three main clusters and designated as CC5-ST1, ST5 and ST8.

MRSA CC5 with sequence type 1

This group was reported in Nigeria and Tunisia. The clonal type included the PVL-positive ST1-V [5C] isolated from patients in a tertiary hospital in Nigeria, and the PVL-negative ST1 with a non-
typeable SCCmec element (spa type t035 and agr type III) identified in Tunisia. In addition, an ST1 related sequence (ST772-V [5C]), “the Bengal-Bay clone” has been described in Nigeria.

**MRSA CC5 with sequence type 5**

This clone was documented in 14 studies and consisted of diverse SCCmec elements (Table 3.4). The ST5-I [1B]/III [3A] were identified from clinical samples in health care institutions in South Africa. ST5-II [2A] has been described in Nigeria, and Senegal. ST5-IV [2B]-PVL-positive was the dominant clone in hospitalized patients with skin and soft tissue infections in Dakar, Senegal. In addition, ST5-IV [2B] was detected from nasal samples of pigs in the same geographical area. ST5-IV [2B] has also been identified in Algeria, Gabon, Morocco, and South Africa, while the SCCmecIVA [2B] variant was recovered from hospitalized patients in Algeria, Angola, and São Tomé and Príncipe. Moreover, ST5-IVa [2B] was reported from nasal samples of apparently healthy-hospital workers in Angola. Other ST5 and related clones identified are ST5-V [5C] in Angola, Cameroon, and Nigeria. ST72-SCCMec-NT in South Africa, ST72-V [5C] in Angola and Ghana, and ST105-II [2A] from a patient in São Tomé and Príncipe. Furthermore, ST650-IV [2B] was detected from clinical samples in health care institutions in South Africa. Finally, ST2629-V [5C] was described in Angola.

**MRSA CC5 with sequence type 8**

MRSA assigned to this clone are widespread and diverse across Africa as evidenced in 27 studies (Table 3.4). The first known early or ancestral clone, ST250-I [1B], was mainly associated with hospital-acquired infections in Ibadan, South-West Nigeria, and recently observed in Ghana. ST8-II [2A] was only described in the KwaZulu-Natal region of South Africa, while a number of investigations reported ST8-IV [2B] in Angola, Cameroon, Gabon, Ghana, Madagascar, Nigeria, São Tomé and Príncipe, and South Africa. The MRSA strains from Angola possessed the SCCmec type IVd element. ST612-IV [2B], a double locus variant (dlv) of ST8-IV [2B], and only recently reported as PVL-positive, is widespread across South Africa, alongside other variants such as ST1173/ST1338-IV [2B]. The ST8-IV [2B] clone in South Africa was identified from a variety of clinical infections, in particular, bacteraemia, skin and soft tissue and wound...
Molecular epidemiology of MRSA in Africa

infections $^{84,85,87,89}$. An ST8-IVc [2B] strain (PVL-positive; spa type t062) was identified from a four day old male child with community-acquired invasive infection in Tunisia $^{101}$. Furthermore, ST8-V [5C] was described in Algeria $^{56}$, Angola $^{58}$, Ghana $^{65}$, Nigeria $^{43,78}$, São Tomé and Príncipe $^{90}$, and South Africa $^{89}$. Other STs observed within the CC5-ST8 cluster include ST8-SCCmec-NT in Gabon $^{63}$ and Nigeria $^{111}$, ST94-IV [2B] described in Nigeria $^{43}$ and ST97-SCCmec-NT in Tunisia $^{100}$. In addition, ST247-I [1B] was reported only in Tunisia $^{100}$ and Nigeria $^{78}$, ST637-III [3A] in Algeria $^{52}$, ST1819-I [1B] in Tunisia $^{100}$, and ST2021-V [5C] in Ghana $^{45}$.

The “Brazilian/Hungarian clone” (ST239-III [3A]) is an hybrid of CC30 and CC8 based on a single large chromosomal replacement $^{19,112}$, and ST241-III [3A] is a slv of ST239-III [3A]. These two STs were identified concurrently in Algeria $^{52}$, Morocco, Niger and Senegal $^{50}$, and Tunisia $^{100}$. ST239-III [3A] has also been described in Ghana $^{45}$ and Kenya $^{68}$, and consistently since 2001 in South Africa $^{83-85,87,89}$. A recent study detected ST239 with the SCCmec type III_mercury [3A] in a tertiary health care facility in South-West Nigeria $^{78}$. ST241-III [3A] is the dominant clone in North-East Nigeria $^{43,75,79}$. Interestingly, three SCCmec variants, ST239-IVa [2B], ST239/ST241-V [5C], and ST241-IV [2B], and associated with hospital-acquired infections were reported in Algeria $^{52}$, Niger $^{50}$ and Nigeria $^{76}$.

3.4.5.2 Clonal complex 22

In Africa, ST22 was identified only in Algeria $^{52}$, South Africa $^{85,87,89}$, and Tunisia $^{100}$. ST22-IV [2B] was related with hospital-associated infections in the Western Cape and KwaZulu-Natal provinces of South Africa. A variant of ST22 (ST22-V [5C]-PVL-positive) was also reported in an hospital in Western Cape, South Africa $^{89}$. The ST22 identified in Tunisia possessed a non-typeable SCCmec element $^{100}$. Besides, an ST636-SCCmec-NT strain has also been reported in Algeria $^{52}$. 

75
Figure 3.3: A minimum spanning showing MRSA clones circulating in Africa clustered with 223 randomly selected MLST ST circulating world-wide. Allelic profiles were downloaded from the MLST website (http://saureus.mlst.net/) which included the MRSA sequence types (STs) described in this review as well as 223 randomly selected STs (from each CC) based on the differences in their allelic profiles. The Group founder is coloured in green and the related STs are in blue. The six main CCs described in this review are indicated by the dotted lines and the STs reported in Africa are indicated by the red colour.
3.4.5.3 Clonal complex 30

ST30-IVa [2B]-PVL-positive, also known as “South-West Pacific clone”, has been noted in Egypt 59, and a multicentre African study identified ST30-V [5C] only in Antananarivo, Madagascar 50. The hospital associated ST36-II [2A] (UK-EMRSA-16), was described only in South Africa 85,87,89,107, while ST39-II [2A] (double locus variant (dlv)) was identified in an hospital in Algiers, Algeria 57, and Ile-Ife, South-West Nigeria 79. MRSA assigned with these groups (ST36-II [2A] and ST39-II [2A]) were PVL-negative. Furthermore, two SCCmec variants, ST37-IVa [2B] and ST37-III [3A], were reported in Algeria 52, and Nigeria 52,79, respectively.

3.4.5.4 Clonal complex 45

ST45-IV [2B], the “Berlin clone”, was detected in an hospital in the KwaZulu-Natal (South Africa) during a multicentre surveillance study 83 and ST45-V [5C] was reported in mother-infant pairs in Gabon 63. An MRSA with a non-typeable SCCmec associated with community-acquired infections has been identified in Tunisia 100. Finally, ST508-V [5C], a slv to ST45, and also associated with community-acquired infections was described in Ghana 66.

3.4.5.5 Clonal complex 80

The CC80 was limited to three North African countries: Algeria, Egypt, and Tunisia (Table 3.4). The European clone, ST80-IV [2B]-PVL-positive, was first described in Algeria from both hospitalized and outpatients 52, and has continued to be the leading clone in the country 52,53,56,57. ST80-IVc [2B] has been identified in Egypt 59, and Tunisia 100,104. In addition, sequence types related to ST80 have been recovered from human clinical samples (ST153-SCCmec-NT, ST728-IVc [2B], ST1440-IVc [2B] and ST2563-IVc [2B]) 100,101, and nasal specimen of healthy sheep (ST153-SCCmec-NT) 109 in Tunisia. The afore-mentioned sequence types, ST728, ST1440 and ST2563 belonged to agr type III and were PVL-positive. Moreover, a PVL-negative ST80-IVc [2B] with agr type II has also been detected in Tunisia 100, and a PVL-negative ST635-IV [2B] in Algeria 52.
3.4.5.6 Clonal complex 88

The "West Australia MRSA-2 clone" (WA-MRSA-2), ST88-IV [2B], was reported in both hospital and community settings in eight African countries; Angola, Cameroon, Gabon, Ghana, Madagascar, Niger, Nigeria, and Senegal. The MRSA strains from Angola possessed an SCCmec IVa [2B] element. PVL-positive ST88-IV [2B] were detected in Nigeria, and an SCCmec subtype ST88-IVa [2B] was identified among three health care workers and a patient in São Tomé and Príncipe. The ST88-IV [2B] with spa type t3489 was also recovered from nasal samples of swine in Senegal. In addition, an SCCmec nontypeable ST88 was described from an out-patient in Algeria, and a strain related to WA-MRSA-2 (ST1289-IV [2B]) was identified in Yaoundé, Cameroon.

3.4.5.7 Other clonal complexes

CC7, CC121, and CC152 have been reported in Africa. The PVL-negative ST789 (assigned with CC7) was identified in Angola (with SCCmec IV [2B]). However, in Ghana, ST789 was PVL-positive and carried an SCCmec IV element [2B]. An ST1010-PVL-positive (CC121) with nontypeable SCCmec element has only been described in Egypt. Furthermore, PVL-positive MRSA assigned to CC152 (ST152-SCCmec-NT) was reported in Nigeria.
Table 3.4: Methicillin-resistant *Staphylococcus aureus* clones reported in 34 eligible studies

<table>
<thead>
<tr>
<th>Country</th>
<th>Clonal type</th>
<th>Clonal complex</th>
<th>spa type</th>
<th>PVL status</th>
<th>agr status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algeria</td>
<td>ST80-IV [2B]</td>
<td>80 ND</td>
<td>+</td>
<td>III</td>
<td></td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>ST37-IVA [2B]</td>
<td>30 ND</td>
<td>+</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST239-III [3A]</td>
<td>5 ND</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST239-IVA [2B]</td>
<td>5 ND</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST241-III [3A]</td>
<td>5 ND</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST637-III [3A]</td>
<td>5 ND</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST5-IV, IVA [2B]</td>
<td>5 ND</td>
<td>+,-</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST635-IV [2B]</td>
<td>80 ND</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST636-NT</td>
<td>22 ND</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST80-IV [2B]</td>
<td>80 t044</td>
<td>+</td>
<td>ND</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>ST239-III [3A]</td>
<td>5 t037</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST5-IVA [2B]</td>
<td>5 t111, t1450</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST88-NT</td>
<td>88 t188, t267</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST80-IV [2B]</td>
<td>80 t044, t143</td>
<td>+</td>
<td>III</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>ST241-III [3A]</td>
<td>5 ND</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST6-V [5C]</td>
<td>5 ND</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST80-IVc [2B]</td>
<td>80 ND</td>
<td>+,-</td>
<td>ND</td>
<td></td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>ST39-IV [2A]</td>
<td>39 ND</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angola</td>
<td>ST5-IVA [2B]</td>
<td>5 t104, t311, t1167</td>
<td>-</td>
<td>II</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>ST8-IVd, V [2B]</td>
<td>5 t104, t1774</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST72-V [5C]</td>
<td>5 t3092</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST88-IVA [2B]</td>
<td>88 t186, t325, t786, t951, t3869</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST5-V [5C]</td>
<td>5 t6065</td>
<td>-</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST2629-V [5C]</td>
<td>5 t6065</td>
<td>-</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST789-V [5C]</td>
<td>7 t192</td>
<td>-</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cameroon</td>
<td>ST5-V [5C]</td>
<td>5 t131</td>
<td>+</td>
<td>II</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>ST88-IV [2B]</td>
<td>88 t186</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST8-IV [2B]</td>
<td>5 t24, t451</td>
<td>+</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST1289-V [5C]</td>
<td>88 t1339</td>
<td>+</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>ST80-IVc [2B]</td>
<td>80 t042, t1044</td>
<td>+</td>
<td>III</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>ST30-IVA [2B]</td>
<td>30 t18</td>
<td>+</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST1010-Xa</td>
<td>121 t159, t312</td>
<td>+</td>
<td>IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gabon</td>
<td>ST8-IV [2B]</td>
<td>5 t121</td>
<td>+</td>
<td>I</td>
<td></td>
<td>44,62</td>
</tr>
<tr>
<td></td>
<td>ST88-IV [2B]</td>
<td>88 t186</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST5-IV [2B]</td>
<td>5 t53</td>
<td>-</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST5-NF</td>
<td>5 t112, t121</td>
<td>+</td>
<td>ND</td>
<td></td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>ST45-V [5C]</td>
<td>45 t37, t186</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghana</td>
<td>ST72-V [5C]</td>
<td>5 t537</td>
<td>-</td>
<td>ND</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>ST8-V [5C]</td>
<td>5 t64</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST88-IV [2B]</td>
<td>88 t325, t1951, t2649</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST8-IV [5C]</td>
<td>5 t121</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST8-IV [2B]</td>
<td>5 t307</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST239-III [3A]</td>
<td>5 t037</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST2504-IB</td>
<td>5 t028</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST2021-V [5C]</td>
<td>5 t024</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST88-IV [2B]</td>
<td>88 t186</td>
<td>-</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST789-IV [2B]</td>
<td>7 t547</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST508-V [5C]</td>
<td>45 t5132</td>
<td>-</td>
<td>ND</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>Kenya</td>
<td>ST239-III [3A]</td>
<td>5 t037</td>
<td>-</td>
<td>ND</td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>Madagascar</td>
<td>ST8-IV [2B]</td>
<td>5 t121</td>
<td>+</td>
<td>I</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>ST30-V [5C]</td>
<td>30 t4686</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST88-IV [2B]</td>
<td>88 t186</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morocco</td>
<td>ST239, ST241-III [3A]</td>
<td>5 t037, t138</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST5-IV [2B]</td>
<td>5 t311</td>
<td>+</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niger</td>
<td>ST239, ST241-III [3A]</td>
<td>5 t138</td>
<td>+</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST239, ST241-V [5C]</td>
<td>5 t037</td>
<td>+</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST88-IV [2B]</td>
<td>88 t186</td>
<td>+</td>
<td>III</td>
<td></td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>ST88-IV [2B]</td>
<td>88 t186</td>
<td>+</td>
<td>III</td>
<td></td>
<td>76</td>
</tr>
</tbody>
</table>
### Table 3.4 (Continued)

<table>
<thead>
<tr>
<th>Country</th>
<th>Clonal type ST-SCmec</th>
<th>Clonal complex</th>
<th>spa type</th>
<th>PVL status</th>
<th>agr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigeria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>ST241-IV [2B]</td>
<td>5</td>
<td>t037</td>
<td>-</td>
<td>-</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>ST250-I [1B]</td>
<td>5</td>
<td>t194, t292</td>
<td>-</td>
<td>-</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>ST241-III [3A]</td>
<td>5</td>
<td>t037</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>61</td>
</tr>
<tr>
<td>ST37-IV [3A]</td>
<td>5</td>
<td>t064</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST94-IV [2B]</td>
<td>5</td>
<td>t008</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST5-V [5C]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST241-III [3A]</td>
<td>5</td>
<td>t037</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>79</td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>88</td>
<td>t729, t1603</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST39-II [2A]</td>
<td>30</td>
<td>t074</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST94-IV [2B]</td>
<td>5</td>
<td>t008</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST5-V [5C]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST152-NT</td>
<td>152</td>
<td>t4690</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST1-I [5C]</td>
<td>5</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>78</td>
</tr>
<tr>
<td>ST2-IV [2B]</td>
<td>5</td>
<td>t311</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>88</td>
<td>t423</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST239, ST241-III [3A]</td>
<td>5</td>
<td>t037, t138</td>
<td>-</td>
<td>-</td>
<td>I</td>
<td>50</td>
</tr>
<tr>
<td>ST5-II [2A]</td>
<td>5</td>
<td>t311</td>
<td>+</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST5-II [2A]</td>
<td>5</td>
<td>t311</td>
<td>+</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>88</td>
<td>t160</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST241-III [3A]</td>
<td>5</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>75</td>
</tr>
<tr>
<td>ST612-IV [2B]</td>
<td>5</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>107</td>
</tr>
<tr>
<td>ST113-IV [2B]</td>
<td>5</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>64</td>
</tr>
<tr>
<td>ST113-IV [2B]</td>
<td>5</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST239-II [2A]</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST45-IV [2B]</td>
<td>45</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST612-IV [2B]</td>
<td>5</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>107</td>
</tr>
<tr>
<td>ST5-I [1B]</td>
<td>5</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST605-IV [2B]</td>
<td>5</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST612-IV [2B]</td>
<td>5</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST72-NT</td>
<td>5</td>
<td>t092</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST22-IV [2B]</td>
<td>22</td>
<td>t032</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST36-II [2A]</td>
<td>30</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST239-II [2A]</td>
<td>5</td>
<td>t037</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>87</td>
</tr>
<tr>
<td>ST614-IV [2B]</td>
<td>5</td>
<td>t045</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST94-IV [2B]</td>
<td>5</td>
<td>t045</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST22-IV [2B]</td>
<td>22</td>
<td>t032</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST113-IV [2B]</td>
<td>30</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST36-II [2A]</td>
<td>30</td>
<td>t012, t021</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST5-I [1B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td>69</td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST22-IV [2B]</td>
<td>22</td>
<td>t032</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST36-II [2A]</td>
<td>30</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST5-I [1B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td>69</td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST22-IV [2B]</td>
<td>22</td>
<td>t032</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST36-II [2A]</td>
<td>30</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST5-I [1B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td>69</td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST22-IV [2B]</td>
<td>22</td>
<td>t032</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST36-II [2A]</td>
<td>30</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ST5-I [1B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td>69</td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST88-IV [2B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>-</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>ST22-IV [2B]</td>
<td>22</td>
<td>t032</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST36-II [2A]</td>
<td>30</td>
<td>t012</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

**São Tome and Príncipe**

<table>
<thead>
<tr>
<th>Clonal type ST-SCmec</th>
<th>Clonal complex</th>
<th>spa type</th>
<th>PVL status</th>
<th>agr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST5-Iva [2B]</td>
<td>5</td>
<td>t015</td>
<td>-</td>
<td>II</td>
<td>90</td>
</tr>
</tbody>
</table>

**South Africa**

<table>
<thead>
<tr>
<th>Clonal type ST-SCmec</th>
<th>Clonal complex</th>
<th>spa type</th>
<th>PVL status</th>
<th>agr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST5-I [1B]</td>
<td>5</td>
<td>t002</td>
<td>-</td>
<td>II</td>
<td>69</td>
</tr>
</tbody>
</table>

**Senegal**

<table>
<thead>
<tr>
<th>Clonal type ST-SCmec</th>
<th>Clonal complex</th>
<th>spa type</th>
<th>PVL status</th>
<th>agr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST5-Iva [2B]</td>
<td>5</td>
<td>t015</td>
<td>-</td>
<td>II</td>
<td>90</td>
</tr>
</tbody>
</table>
Table 3.4 (Continued)

<table>
<thead>
<tr>
<th>Country</th>
<th>Clonal type</th>
<th>Clonal complex</th>
<th>spa type</th>
<th>PVL status</th>
<th>agr</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>São Tomé and Príncipe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST8-V [5C]</td>
<td>5</td>
<td>t451</td>
<td>-</td>
<td>I</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>ST8-IV [2B]</td>
<td>5</td>
<td>t451</td>
<td>-</td>
<td>I</td>
<td></td>
<td>81</td>
</tr>
<tr>
<td>ST88-IVa [2B]</td>
<td>88</td>
<td>t186, t786</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tunisia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST80-IV [2B]</td>
<td>80</td>
<td>t044</td>
<td>+</td>
<td>III</td>
<td>102</td>
<td>97</td>
</tr>
<tr>
<td>ST72-IVc [2B]</td>
<td>80</td>
<td>t042, t044</td>
<td>+</td>
<td>III</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>ST8-IVc [2B]</td>
<td>5</td>
<td>t062</td>
<td>+</td>
<td>III</td>
<td></td>
<td>104</td>
</tr>
<tr>
<td>ST80-IVc [2B]</td>
<td>80</td>
<td>t203</td>
<td>+</td>
<td>III</td>
<td></td>
<td>104</td>
</tr>
<tr>
<td>ST1-NT</td>
<td>5</td>
<td>t035</td>
<td>-</td>
<td>III</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>ST247-I [1B]</td>
<td>5</td>
<td>t040</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST249-III [3A]</td>
<td>5</td>
<td>t003</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST241-III [3A]</td>
<td>5</td>
<td>t125</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST97-NT</td>
<td>5</td>
<td>t003</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1819-I [1B]</td>
<td>5</td>
<td>NS</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST80-IVc [2B]</td>
<td>80</td>
<td>t070</td>
<td>+</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST2563-IVc [2B]</td>
<td>80</td>
<td>t070</td>
<td>+</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1440-IVc [2B]</td>
<td>80</td>
<td>t070</td>
<td>+</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST80-IVc [2B]</td>
<td>80</td>
<td>t1021</td>
<td>-</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST80-IVc [2B]</td>
<td>80</td>
<td>ND</td>
<td>-</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST22-NT</td>
<td>22</td>
<td>t998</td>
<td>-</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST45-NT</td>
<td>45</td>
<td>ND</td>
<td>-</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST153-NT</td>
<td>80</td>
<td>NS</td>
<td>+</td>
<td>III</td>
<td></td>
<td>109</td>
</tr>
<tr>
<td>ST153-NT</td>
<td>80</td>
<td>t044</td>
<td>ND</td>
<td>III</td>
<td></td>
<td>109</td>
</tr>
</tbody>
</table>

Note: ST: sequence type; GC: Clonal complex; SCCmec: staphylococcal chromosomal cassette mec element; spa: Staphylococcus aureus protein A gene; agr: accessory gene regulator gene; PVL: Panton-Valentine Leukocidin genes; NT: non typeable; NR: not reported; ND: not determined; NS: new spa type; n: number of isolates; A: Antananarivo; C: Casablanca; D: Dakar; N: Niamey; Y: Yaoundé; *clones isolated from pigs; +: PVL positive; -: PVL negative; X*: unknown SCCmec type other than I, II, III, IV or V.
3.5 Discussion

MRSA has been reported in Africa, at least since 1978. This systematic review showed that adequate data on the molecular epidemiology of MRSA are limited, with reports from only 15 of the 54 African countries. No spa type was dominant, however, t042 and t044 were the major spa types identified in three North African countries (Table 3.4). Moreover, we did not observe a clear distinction between hospital- and community-associated MRSA clones in Africa which is in agreement with other investigations worldwide. In this systematic review, the use of the current eBURST scheme grouped several African MRSA CCs (CC1, CC5, CC8, and CC97) into a single cluster, (CC5). This raises some concern on a suitable method for discrimination and grouping of S. aureus strains. To overcome the above mentioned issue, whole genome sequencing approach might be the alternative.

Although a combination of factors could be responsible for the dissemination of clones between continents, increased movement of human population within or between countries might be one of the potential factors. International travel could play a significant role in the transmission of MRSA, particularly the replacement of existing MRSA with fitter and more transmissible clones. We observed that the predominant hospital-associated epidemic clones, EMRSA-15 [ST22-IV (2B)] and (EMRSA-16 [ST36-II (2A)], in the United Kingdom (UK) were reported only in South Africa. Moreover, ST80-IV (2B) (the European clone) has consistently been recognised as the predominant PVL-positive MRSA clone in North Africa. A recent report based on whole genome analysis provided strong evidence that the European ST80-IV (2B) was derived from a PVL-positive MSSA ancestor in sub-Saharan Africa that acquired the SCCmec IV element, and clonal spread was enhanced by increased transnational movement. However, the factors responsible for the limited spread of the ST80-IV (2B) only in North Africa observed so far are unclear.

The SCCmec IV (and its subtypes) and SCCmec V were identified in several MRSA clones, and ST5 and ST8 clearly showed more diversity in terms of SCCmec types compared with other STs in Africa. The success of these SCCmec types (IV and V) could be due to their small sizes and low
fitness costs \cite{29,40,123}. It is also noteworthy that the SCC\textit{mec} types IVa and IVc were identified in genetically unrelated clones, e.g. ST5-IVa [2B] (CC5) in Algeria \cite{52,53}, São Tomé and Príncipe \cite{90}, ST8-IVc [2B] in Tunisia (CC5) \cite{101}, and ST37-IVa [2B] (CC30) in Algeria \cite{52}. This might suggest horizontal gene transfer or independent acquisition \cite{124}. Another interesting observation was the detection of the SCC\textit{mec} type IVa and V in the hospital-associated ST239/ST241-III [3A] in Algeria \cite{52}, Nigeria \cite{76}, and Niger \cite{50}. Since ancient MSSA strains for this ST have not been reported \cite{123}, our observation suggests that acquisition of these SCC\textit{mec} types by MSSA is less likely, and points to the possible replacement of SCC\textit{mec} type III with IV and V on the ST239/241 genome \cite{125}.

Data on the epidemiology of MRSA in animals are limited in Africa \cite{108,109,126}. Moreover, the genetic relatedness between human and animal MRSA has not been investigated (Table 3.4). It should be noted, however, that human-associated ST5-IV [2B], ST88-IV [2B] and ST153-SCC\textit{mec}-NT have been reported from animals in Tunisia \cite{109} and Senegal \cite{108}. Recently, human-associated \textit{S. aureus} lineages were described in captive Chimpanzees in Gabon, Madagascar, Uganda and Zambia \cite{127,128,129}. Notably, a likely case of \textit{S. aureus} transmission from a veterinarian to a chimpanzee from the same sanctuary was demonstrated \cite{129}. Zoonotic transmission may constitute a major concern in Africa, where there is often substantial exposure to domesticated animals \cite{108,109,130}. Furthermore, animal-adapted clones might undergo further host-adaptive evolutionary changes, which could result in an epidemic spread of new and more virulent strains in the human population \cite{131,132,133}.

Other risk factors for animal to human MRSA transmission, which include contaminated environment \cite{8,9} and meat products \cite{134,135,136}, have not been investigated in Africa. Livestock-associated MRSA are widespread in Europe, but the transmission of these strains to humans is either rare or limited to people with direct contact with MRSA infected/carrier animals \cite{9}. Using whole genome sequencing, evidence of zoonotic transmission of MRSA harbouring \textit{mecC} was reported in Denmark \cite{137}. The \textit{mecC}-positive MRSA, initially known as a livestock MRSA belonging to the CC130, is recognised in both animals and humans in Europe \cite{138}. In addition, this clone has been implicated in severe infections in humans \cite{22}, resulting in one death \cite{139}. The clinical importance of \textit{mecC}-positive MRSA is not yet clear in Africa as data is unavailable. Therefore we
suggest that surveillance for MRSA should include detection of the \textit{mecC} gene where \textit{mecA} is not detected in resistant strains.

This systematic review did not seek to provide information on the burden of PVL-positive MRSA in Africa. However, it provided some interesting observations on their epidemiology in Africa such as the identification of PVL-positive isolates assigned with CC7 (originally classified with CC152) in Ghana\textsuperscript{45}, CC88-IV [2B] in Cameroon\textsuperscript{50} and Nigeria\textsuperscript{76}, ST612-IV [2B] in South Africa\textsuperscript{89}, and CC152 in Nigeria\textsuperscript{79}. Until now, CC152 was only described in the Balkan region\textsuperscript{140,141}. The mode of acquisition of the \textit{mecA} gene by ST152 is still unknown, but it might be explained by either its introduction through international travel or the acquisition of the methicillin resistance gene by PVL-positive MSSA, which is prevalent in West and Central Africa\textsuperscript{70}. These observations highlight the need for further surveillance data (including information on community-acquired necrotizing pneumonia) to understand the epidemiology of PVL-associated \textit{S. aureus} in both hospital and community settings on the African continent.

### 3.6 Conclusion

A number of pandemic MRSA clones were identified in Africa. In contrast, some MRSA clones are limited to specific countries or regions. Although the eBURST snapshot provided a description of the relationship between the MRSA clones reported in Africa and other lineages submitted into the MLST database from other continents, the objective of this review was not to understand the origin of MRSA clones in Africa, as this will require in depth analysis like whole genome sequencing. However, it did show that CC5 is the largest group and predominant in Africa. Nevertheless, the limited data available on MRSA in Africa draw attention to the need for increased surveillance of MRSA and molecular epidemiological studies. We strongly recommend improved co-operation between clinicians and microbiologists in Africa. This synergy could provide an understanding on the local epidemiology of MRSA. In addition, we strongly advocate the establishment of effective diagnostic microbiology facilities that will incorporate high-throughput technologies for monitoring the clonal expansion and dissemination of MRSA. In the meantime, increased networking through collaboration with \textit{S. aureus} reference centres could
provide support for genotyping services to African countries with limited resources. Finally, population-based surveillances for MRSA are needed to evaluate the situation of community associated MRSA as well as studies on MRSA from animal hosts. To understand the origin of the newly emerged clones, MSSA genotyping needs to be incorporated with MRSA surveillance studies.
References

16. Ito T, Ma XX, Takeuchi F, Okuma K. Novel Type V Staphylococcal Cassette Chromosome mec Driven by a Novel Cassette Chromosome Recombinase , ccrC Novel Type V Staphylococcal Cassette
Molecular epidemiology of MRSA in Africa


32 Conceição T, Aires-de-Sousa M, Füzi M, *et al.* Replacement of methicillin-resistant *Staphylococcus*
Molecular epidemiology of MRSA in Africa


Molecular epidemiology of MRSA in Africa


Molecular epidemiology of MRSA in Africa


Bouchami O, Achour W, Ben Hassen A. Typing of staphylococcal cassette chromosome *mec* encoding methicillin resistance in *Staphylococcus aureus* strains isolated at the bone marrow transplant...
Molecular epidemiology of MRSA in Africa


108 Fall C, Seck A, Richard V, *et al*. Epidemiology of *Staphylococcus aureus* in pigs and farmers in the


Molecular epidemiology of MRSA in Africa


Chapter Four

Prevalence, incidence and determinants of *S. aureus* nasopharyngeal carriage during infancy
4.1 Summary

Data on the prevalence and determinants of *Staphylococcus aureus* nasopharyngeal (NP) carriage during the first year of life in low and middle income countries are lacking. We collected NP swabs from 137 mother-infant pairs at birth and followed up the infants every two weeks for 12 months. *S. aureus* was identified using conventional culture methods. Binomial logistic regression models were used to investigate the association between the possible predictive variables and *S. aureus* NP carriage.

*S. aureus* NP carriage declined with increasing age. *S. aureus* acquisition was highest during the first 2 weeks of life. The overall acquisition incidence at the species level was (0.24 events per 100 child-days). Multivariate analysis revealed that male gender, maternal carriage, day care attendance, large family size (> 5 individuals), high socioeconomic status and hot season were risk factors for *S. aureus* NP carriage during the first year of life. On the other hand, having an animal and either maternal or parental smoking had a negative association with *S. aureus* NP carriage in this cohort. The estimated odds ratios of carrying *S. aureus* changed over time among HIV-exposed and *S. pneumoniae* carrier infants. The association between *S. aureus* carriage and feeding practices was site-dependent.
Prevalence, Incidence and Determinants of *S. aureus* NP Carriage

4.2 Background

The presence of *S. aureus* is associated with a number of diseases such as skin and soft tissue infections, necrotizing pneumonia and secondary bacterial infections among infants with atopic dermatitis. However, *S. aureus* may asymptotically be carried in different human body sites, of which the nasal cavity, and more recently the nasopharynx, are considered frequent carriage sites. The prevalence of *S. aureus* nasal carriage in adults varies regionally. In Africa, the prevalence ranges from 13% in Tunisia, 18% in Kenya and 29% in Gabon. Carriage in Europe ranges from 15%-25.4%, in the United States 30.4% and Asia 24%-35%; and in South and Southeast Asia 9%-14%. *S. aureus* colonisation is more prevalent in children and young adolescents under 20 years old compared to adults. The highest nasopharyngeal carriage prevalence is observed during the first year of life, ranging from 45% to 60% within the first two weeks and decreasing to 12% - 20% by 12 months.

Demographical, behavioural and environmental factors may influence the carriage. *S. aureus* carriage in childhood has a U-shaped relationship with age; with higher rates reported for infants younger than six months, decreasing subsequently and increasing again in children between five and seven years old. In addition, males are more likely to carry *S. aureus* across different age groups compared to females. Evidence suggest that infants can acquire the same *S. aureus* strain from their mothers. However, some infants who are born to non-carrier mothers acquire *S. aureus* soon after birth, suggesting other sources of transmission early in life.

Other factors such as big family size (> 5 individuals), presence of young siblings, low socio-economic conditions, exposure to health care settings and passive smoke are associated with an increased risk of *S. aureus* nasal/nasopharyngeal carriage. In addition, nose picking, having companion animals, and animal husbandry are also associated with high *S. aureus* nasal carriage rates and subsequent infections among humans.

Factors associated with reduced risk of *S. aureus* carriage are antibiotic therapy within the previous three months, day care attendance pneumococcal conjugate vaccination (PCV)
namely PCV-13 34, and Streptococcus pneumoniae NP carriage 35,36. Carriage with Streptococcus pneumoniae, particularly vaccine serotypes, is inversely associated with S. aureus nasopharyngeal carriage in infants during the first year of life. This inverse association is lacking in HIV infected children, suggesting involvement of immune-related mechanisms 37,38. The effect of breastfeeding on nasal carriage is debatable, since it was found to have a protective effect against S. aureus nasopharyngeal carriage among Taiwanese children, whereas Peacock et al. found that breast-feeding at the time of sampling, was associated with increased nasal carriage rates among infants 39,40.

With all the determinants mentioned above, data on the prevalence and risk factors associated with S. aureus nasopharyngeal carriage are scarce in Africa. Only a few studies investigated S. aureus nasopharyngeal carriage longitudinally in the healthy community, including infants during the first year of life 8,23. In this chapter we aim to i) describe the longitudinal epidemiology of S. aureus nasopharyngeal carriage in infants during their first year of life; and ii) to identify the possible determinants associated with carriage of S. aureus in the nasopharynx.

4.3 Methods

4.3.1 Study design and population

The study population represents two different ethnic groups; Black African and Coloured who were enrolled at Mbekweni and TC Newman clinics, respectively (Table 4.1). Nasopharyngeal swabs (NP) were collected from 137 mother-infant pairs at birth, and from infants approximately every two weeks until 12 months of age. Infants received pneumococcal conjugate vaccine 13 (PCV13) at 6, 14 and 36 weeks according to the South African childhood vaccination program. All HIV-positive mothers and their infants received Prevention of Mother to Child Transmission (PMTCT) treatment of HIV. The clinical data including hospitalisation were derived from the case report forms at seven time points (birth, 6-10 weeks, 10 weeks, 14 weeks, 6 months, 9 months and 12 months) throughout the 12 month in line with the larger study (Table 4.1).
4.3.2 Important definitions used in this study

The main aim of the larger study (DCHS) is to investigate the risk factors and aetiological agents of lower respiratory tract infection during infancy and childhood. NP swabs were collected to study the NP carriage with four main bacterial commensals of this anatomical site during infancy. This includes *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Staphylococcus aureus*. It is established that the anterior nares is the main niche for *S. aureus*. During the collection of the NP swab, it is likely that the swab tip might touch the mucosal/epithelial surfaces of the nares (anterior part of the nasal cavity), especially if the infant resists due to discomfort during swab collection. Therefore, *S. aureus* nasopharyngeal carriage in our study may represent *S. aureus* strains carried in both the anterior (nose) and posterior (nasopharynx) parts of the nasal cavity.

4.3.3 Nasopharyngeal (NP) swab collection

NP swabs (Ultra mini-tip Copan flocked swab, FLOQSwabs™, COPAN Diagnostics, Murrieta, CA) were obtained by a trained nurse and immediately placed into Skimmed milk Tryptone Glucose Glycerine medium (STGG) for bacterial culture and storage. Samples were kept at 4°C, delivered to the laboratory within 3-4 hours, and stored at -80°C until further processing.

4.3.4 Characterisation of *S. aureus* isolates

4.3.4.1 Bacterial culture

In total, 3417 NP swabs were collected from the mother-infant pairs. STGG samples were allowed to thaw at room temperature then vortexed briefly. Thereafter, 20μl was inoculated onto Mannitol Salt Agar (MSA) (National Health Laboratory Services, Green Point Media Laboratory Cape Town, South Africa), and incubated at 37°C in ambient air. After 24 hours, growth characteristics and colony morphologies were assessed. For each plate, mannitol fermenting colonies were selected for identification. For morphologically similar mannitol fermenting colonies, only one colony was selected for further processing. Morphologically different colonies
were treated independently. Non-mannitol fermenting colonies suspected to be *S. aureus* (i.e. produced yellow/golden colonies on the MSA plate), were also included for further identification.

4.3.4.2 Deoxyribonuclease (DNase) test

Suspected colonies of *S. aureus* were inoculated onto DNase agar (National Health Laboratory Services, Green Point Media Laboratory Cape Town, South Africa), and incubated at 37°C for 24 hours. An excess volume of 1 N HCL was applied. A DNase positive result identified by a clear zone around the bacterial streak. DNase-positive (mannitol-fermenting and non-fermenting) isolates were stored in STGG in -80°C for further laboratory investigations.

4.3.5 Statistical analysis

The point prevalence and incidence (the number of new carriage events/the sum of person-time at risk) of *S. aureus* NP carriage were calculated for the 137 infants that were available for follow-up during their first year of life. Data on maternal *S. aureus* NP carriage were available for 125 mothers. The gglplot2 package of the statistical software R was used to plot the incidence rate with a fitted smoothing function to provide an overall trend over time.

Logistic regression models for binomial outcomes (presence/absence of *S. aureus* in the nasopharynx) was used to investigate the effect of demographical, epidemiological and clinical determinants on *S. aureus* NP carriage. The variables listed in Table 4.1 as well as season, infant’s wellbeing as reported by infant’s caregiver and *S. pneumoniae* carriage were included in the multivariate analysis. For the purpose of this analysis we considered "any hospitalisation" as a risk factor. The socioeconomic status (SES) was derived from a validated methods that was defined by Myer et al. 2008. This method takes into consideration an aggregate of factors: household income, household material and financial resources, individual education and employment status. Two logistic regression analyses were performed based on the questions asked; the first model was for longitudinal data (i.e. repetitive measurements per child), and the second was a case-control model. In the first model a time interaction (age in weeks) was added to the longitudinally collected measurements. A sub analysis was further performed by enrolment site (Mbekweni and TC Newman). In the second model a case-control analysis (cross-
sectional) based on *S. aureus* carrier status was performed; the cases are defined as 1) all infants carried *S. aureus* in the nasopharynx at any given time point throughout the study period, and 2) the controls are defined as infants who never carried *S. aureus* at any time point during the study period. Pearson's chi-square test was used to measure the association between maternal and infant carriage during the first two weeks (only a few observations were available at birth, therefore, we extended the analysis to two weeks). All analyses were performed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) 42. The significance of the difference between two independent proportions was tested using the z-ratio.

### 4.4 Results

#### 4.4.1 Participants characteristics

Table 4.1 summarizes the demographic, clinical and epidemiological characteristics of the study participants. A total number of 394 NP swabs were missing over the study period (12 from mothers at birth and 382 swabs from infants over the 12 months period (Appendix 1). The missing swabs were either not collected or were excluded because they did not fall within the 2-weekly follow-up window. Of the 137 infants, 77 (56.2%) were females (44 from Mbekweni and 33 from TC Newman). The majority of infants were born via normal vaginal delivery (80%) and 25% were delivered preterm (gestational age ≤ 37 weeks). Regarding infants’ feeding during the first six months, 43.7% were exclusively formula fed; 26% were exclusively breast fed; and 27% were mixed-fed (both formula and breast-feeding). Fifty nine percent of the infants received day care outside their homes with the majority (65%, 53/81) from TC Newman. The median family size was five and ranged from 1-20 individuals per household. Infants from Mbekweni had higher HIV exposure (30 infants, 40%) compared to infants from TC Newman (3 infants, 5%, $p=0.0001$). In general, high cigarette smoke exposure was observed among infants from TC Newman; 72% (43/60) had a smoker father, 60% (36/60) had a smoker mother and 45% (27/60) were exposed to smoke from both parents (total % exposed = 100). During the study period, 15 infants were hospitalized (27% were hospitalized for pneumonia, 20% for diarrhoea, 20% for wheezing, 6% for each of jaundice, upper respiratory tract infection), and 18 infants received TB treatment.
Table 4.1: Participant characteristics

<table>
<thead>
<tr>
<th>Cohort Characteristics</th>
<th>% (no.) Mbekweni (n=77)</th>
<th>TC Newman (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBekweni (n=77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56.2 (77)</td>
<td>57 (44)</td>
<td>55 (33)</td>
</tr>
<tr>
<td>Male</td>
<td>43.8 (60)</td>
<td>43 (56)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black African</td>
<td>56.2 (77)</td>
<td>100 (77)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Coloured</td>
<td>43.7 (60)</td>
<td>0 (0)</td>
<td>100 (60)</td>
</tr>
<tr>
<td>Gestational age:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>39 (27-42)</td>
<td>39 (30-42)</td>
<td>39 (27-42)</td>
</tr>
<tr>
<td>Premature birth*</td>
<td>25 (34)</td>
<td>23 (18)</td>
<td>27 (16)</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>80 (109)</td>
<td>81 (63)</td>
<td>77 (46)</td>
</tr>
<tr>
<td>Caesarean</td>
<td>20 (28)</td>
<td>18 (14)</td>
<td>23 (14)</td>
</tr>
<tr>
<td>Baby feeding method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>during 6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusive breast feeding</td>
<td>26 (36)</td>
<td>25 (19)</td>
<td>28 (17)</td>
</tr>
<tr>
<td>Exclusive formula</td>
<td>43.7 (60)</td>
<td>51 (39)</td>
<td>35 (21)</td>
</tr>
<tr>
<td>feeding</td>
<td>27 (37)</td>
<td>20 (15)</td>
<td>37 (22)</td>
</tr>
<tr>
<td>Day care attendance</td>
<td>59 (81)</td>
<td>36 (28)</td>
<td>88 (53)</td>
</tr>
<tr>
<td>HIV exposure</td>
<td>24 (33)</td>
<td>40 (30)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Smoke exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker father</td>
<td>61 (84)</td>
<td>53 (41)</td>
<td>72 (43)</td>
</tr>
<tr>
<td>Smoker mother</td>
<td>32 (45)</td>
<td>12 (9)</td>
<td>60 (36)</td>
</tr>
<tr>
<td>Both parents</td>
<td>25 (34)</td>
<td>10 (7)</td>
<td>45 (27)</td>
</tr>
<tr>
<td>Any smoker exposure</td>
<td>83 (114)</td>
<td>70 (54)</td>
<td>100 (60)</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>54 (74)</td>
<td>70 (54)</td>
<td>33 (20)</td>
</tr>
<tr>
<td>Moderate-high</td>
<td>45 (63)</td>
<td>30 (23)</td>
<td>67 (40)</td>
</tr>
<tr>
<td>Animals in the house</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 (62)</td>
<td>30 (23)</td>
<td>65 (39)</td>
<td></td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>10 (13)</td>
<td>10 (8)</td>
<td>8 (5)</td>
</tr>
<tr>
<td>Secondary</td>
<td>86 (115)</td>
<td>(62)</td>
<td>88 (53)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>4 (6)</td>
<td>5 (4)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Hospitalization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>during the first year</td>
<td>11 (15)</td>
<td>10 (8)</td>
<td>12 (7)</td>
</tr>
<tr>
<td>Infants treated for TB</td>
<td>13 (18)</td>
<td>10 (7)</td>
<td>18 (11)</td>
</tr>
<tr>
<td>Family size &gt; 5</td>
<td>36 (50)</td>
<td>35 (27)</td>
<td>38 (23)</td>
</tr>
<tr>
<td>Number of people</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in household</td>
<td>5 (1-20; 3-6)</td>
<td>4 (1-17; 3-7)</td>
<td>5 (1-20; 4-6)</td>
</tr>
<tr>
<td>Having a sibling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>younger than 5 yrs.</td>
<td>34 (47)</td>
<td>38 (29)</td>
<td>30 (18)</td>
</tr>
</tbody>
</table>

Note: HIV: Human immune deficiency virus; IQR: interquartile range; TB: Tuberculosis; yrs.: years * ≤ 37 weeks.
4.4.2 Prevalence and incidence of \textit{S. aureus} nasopharyngeal carriage

\textit{S. aureus} was isolated from 21\% (725/3417) of NP swabs collected from both mothers and infants (21 positive swabs from mothers, and 704 from infants). The prevalence of \textit{S. aureus} NP carriage in mothers was 17\% (21/125) on the day of birth; 20\% (15/72) of mothers from Mbekweni were \textit{S. aureus} carriers, compared to 11\% (6/53) were from TC Newman (\(p=0.09\)). \textit{S. aureus} was isolated from the nasopharynx in 2.3\% of infants at birth (within 72 hours), 45\% at two weeks peaking at 54\% at four weeks. The rate subsequently declined gradually with increasing age to 6.6\% at week 52 (Figure 4.1). During the study period, 88\% of infants (121/137) carried \textit{S. aureus} at least once; in only 16 infants \textit{S. aureus} was not detected at any of the sampling periods. Of these 16 infants, eleven were from TC Newman (\(p=0.03\)).

![Figure 4.1: The point prevalence of \textit{S. aureus} NP carriage during the first year of life. The error bars represent a 95\% confidence interval.](image)

One hundred and twenty one new \textit{S. aureus} acquisition events were observed during the study period, translating into an acquisition incidence of 0.24 events per 100 child-days. The incidence varied between time points, with a maximum 2.45 events per 100 child-days between 0-2 weeks and decreased to 0.63 events per 100 child-days during weeks 50-52. The lowest incidence was 0.16 events per 100 child-days which occurred between weeks 14-16 and 44-46(Figure 4.2).
Figure 4.2: Incidence of acquisition of *S. aureus* NP carriage during the first year of life.

We calculated incidence per 100 child-days at two-weekly intervals. Each data point represents the number of new acquisition events occurred in the time between the previous and the indicated time point. We fitted a smoothing function showed by the blue line with a confidence interval for the smoothing function -the area shaded in grey-to describe the trend of the incidence over time.

### 4.4.3 Determinants of *S. aureus* nasopharyngeal carriage during infancy

#### 4.4.3.1 Longitudinal analysis for determinants of *S. aureus* nasopharyngeal carriage

Multivariate logistic regression analysis included the variables listed in Table 4.1, as well as season, infant’s health status on the day of sample collection as reported by the infant’s caregiver, and *S. pneumoniae* NP carriage. For the general carriage model –which included participants from both study sites, mode of delivery, preterm, and feeding method during the first six month did not show any association with *S. aureus* NP carriage during the first year of life (Figure 4.4). Increasing age showed a significant negative association with *S. aureus* NP carriage in all infants (odds ratio (OR), 0.96; 95%CI, 0.95-0.97). The presence of an animal in the household OR, 0.77;
Prevalence, Incidence and Determinants of *S. aureus* NP Carriage

95%CI, 0.61-0.96), as well as cigarette smoke exposure (from either mothers or fathers) was negatively associated with *S. aureus* NP carriage (Figure 4.4).

Males were at higher risk for carrying *S. aureus* compared to females (OR, 1.25; 95%CI, 1.01-1.54; \( p=0.03 \)). Moreover, maternal *S. aureus* carriage was significantly associated with increased risk of infant carriage at any time throughout the study period (OR, 1.94; 95%CI, 1.45-2.60). However, no significant association was observed between maternal and infant carriage status within the first two weeks (\( p=0.14 \)). The odds of *S. aureus* NP carriage increased with moderate to high socioeconomic conditions (OR, 1.54; 95%CI 1.21-1.97), day care attendance (OR, 1.33; 95%CI 1.03-1.71), family size of ≥5 individuals (OR, 1.54; 95%CI 1.20-1.97), and hospital admission (OR, 1.39; 95%CI 1.02-1.90, \( p=0.04 \)). While, having a young sibling (OR, 1.24; 95%CI, 0.97-1.57) have a marginally positive association. TB treatment (OR, 1.22; 95%CI, 0.87-1.71) was not statistically significantly associated with *S. aureus* NP carriage. Regarding the effect of season on *S. aureus* NP carriage, the rate of *S. aureus* detection (corrected for age) was highest during summer as oppose to autumn, winter and spring (Figure 4.4). Although not statistically significant, the odds of *S. aureus* NP carriage was lowest during spring compared to winter (OR, 1.14; 95%CI, 0.85-1.52), and increased slightly during autumn compared to winter (OR, 1.2; 95%CI, 0.91-1.6). The risk of *S. aureus* NP carriage during summer was 61% more than the risk during winter (OR, 1.61; 95%CI, 1.21-2.14) (Figure 4.4).

![Figure 4.3: Changes in the detection rates of *S. aureus* over different seasons.](image-url)
Figure 4.4: Multivariate analysis of variables associated with *S. aureus* NP carriage during the first year of life.

This model included ALL the study participants from both clinical sites (Mbekweni and TC Newman). The figure features all statistically significant and non-significant variables. Variable with more than two categories are shown as gradients of the same colour. The legend on the right include the variable name and the reference category, [odds ratio (95%CI), p-value]. An Odds ratio of **1.00** is where there no association between the variable and the outcome (dotted line).
In the general model, the effect of both the variables HIV-exposure and *S. pneumoniae* NP carriage on *S. aureus* NP carriage was significant only when interacted with time (age), therefore, the main effect of both variables cannot be interpreted apart from the change in time. The interaction with time showed that HIV-exposed infants were less likely than HIV-unexposed infants to carry *S. aureus* at birth (OR =0.72, *p* = 0.02) but became progressively more likely than unexposed infants to carry *S. aureus* as age increased (OR=2.03, *p*= 0.02 at the age of 52 weeks) (Figures 3.5 and 3.6a). Considering the *S. pneumoniae* carriage (*S. pneumoniae* carrier was defined as one who was carrying the bacterium at any time during the 12 month period) the reverse was true when considering carriage of *S. pneumoniae* as a risk factor; *S. pneumoniae* carriers were more likely to carry *S. aureus* at birth (OR= 1.85, *p* = 0.0001) but as from week 22 onwards, the odds of carrying *S. aureus* for *S. pneumoniae* carriers decreased relative to the odds of carrying *S. aureus* among *S. pneumoniae* non carriers (OR= 0.39, *p*=0.0001 at age 52 weeks) (Figure 4.6b). We repeated the logistic regression model on HIV-exposed and unexposed infants separately. In this analysis, the inverse association between carriage of *S. aureus* and *S. pneumoniae* was absent among HIV-exposed (OR=1.01, *p*=0.24) as opposed to HIV-unexposed infants (OR=0.95, *p*=0.0000).

![Figure 4.5: The proportion of *S. aureus* NP carriage by HIV-exposure during four age intervals.](image-url)
Figure 4.6 estimated odds ratio of *S. aureus* carriage for both HIV-exposure and *S. pneumoniae* carriage overtime.

(a) Estimated odds ratio of *S. aureus* carriage for HIV-exposure vs HIV unexposed over time as infants grow older the odds of carrying *S. aureus* among HIV-exposed infants’ increased relative to the odds of *S. aureus* carriage for unexposed infants. (b) Estimated odds ratio of *S. aureus* carriage for *S. pneumoniae* carriers’ vs *S. pneumoniae* non-carriers over time. The odds of *S. aureus* carriage among *S. pneumoniae* carriers’ changed with time relative to the odds of *S. aureus* carriage among *S. pneumoniae* non-carriers.

### 4.4.3.2 Longitudinal determinants of *S. aureus* nasopharyngeal carriage as stratified by the clinical site

The clinical sites Mbekweni and TC Newman represent two different ethnic groups. Therefore, we explored differences in *S. aureus* NP carriage determinants between the two groups. Both day care attendance and family size of ≥5 individuals were significantly associated with increased risk of carrying *S. aureus* in infants from both sites (Figure 4.7). Infants from both sites who were reported to be well at the time of sample collection, as indicated by their caregiver were less likely to carry *S. aureus* compared to those who were not well, however this was only significant in infants from TC Newman (OR, 0.57, 95%CI 0.41-0.82). The presence of young siblings was associated with reduced carriage of *S. aureus* at TC Newman only (Figure 4.7). Normal birth was a risk factor for *S. aureus* NP carriage compared to caesarean; this was significant at TC Newman only. Infants from TC Newman who had exclusive formula feeding as well as those who had mixed feeding during the first six months of life were significantly more likely to carry *S. aureus* compared
to exclusive breast feeding, but the reverse was true for infants from Mbekweni, although this was not statistically significant. Since 100% of the infants from TC Newman were exposed to cigarette smoke, it was not possible to determine the effect of the variable “any smoker exposure” due to the singularity (only exposed) of this variable. Exposure to either paternal (OR, 0.73, 95%CI 0.74-1.14) or maternal smoking (OR, 0.74, 95%CI 0.48-1.15) similarly showed negative association with *S. aureus* NP carriage for infants from TC Newman, but only paternal smoking was negatively associated with *S. aureus* NP carriage for infants from Mbekweni (OR, 0.53, 95% CI 0.31-0.89). The effect of season on *S. aureus* NP carriage varied between the two sites and was most marked among infants from Mbekweni.
Figure 4.7: Multivariate analysis for *S. aureus* NP carriage stratified by study site.

*S. aureus* carriage determinants for infants from (a) Mbekweni and (b) TC Newman. The figures include all significant and non-significant variables. An odds ratio of **1.00** means there is no association between the variable and *S. aureus* carriage. The legend shows the variable name (tested vs reference categories), odds ratio (OR), 95% confidence interval and the *p*-value. * The infant health status: whether the infants was feeling well on the day of sample collection as reported by the infant’s caregiver.

(a) Mbekweni

- Age [0.96 [0.94-0.97], *p* = 0.0002]
- Gender (male vs female) [1.06 [0.69-1.66]], *p* = 0.7
- Preterm [0.34 [0.05-2.19], *p* = 0.4]
- Maternal *S. aureus* carriage [0.77 [0.42-1.41], *p* = 0.41]
- Delivery mode (normal vs cesarean) [1.6 [1.02-2.5], *p* = 0.04]
- Day care attendance [1.55 [1.13-2.13], *p* = 0.01]
- Infant's health status [0.57 [0.41-0.82], *p* = 0.001]
- *K. pneumoniae* carriage (yes vs no) [0.60 [0.42-0.87], *p* = 0.01]
- HIV-exposure (yes vs no) [1.22 [0.50-2.98], *p* = 0.66]
- Having animals [1.26 [0.79-2.01], *p* = 0.3]
- Autumn vs winter [0.92 [0.59-1.44], *p* = 0.7]
- Spring vs winter [0.71 [0.44-1.15], *p* = 0.2]
- Summer vs winter [1.03 [0.65-1.63], *p* = 0.9]
- Paternal smoking [0.73 [0.47-1.14], *p* = 0.18]
- Maternal smoking (yes vs no) [0.74 [0.48-1.15], *p* = 0.2]
- Exclusive formula vs exclusive breastfeeding [3.8 [1.42-7.37], *p* = 0.01]
- Mixed vs exclusive breastfeeding [4.8 [2.11-6.6], *p* = 0.0001]
- Mod-high vs low SES [1.24 [0.76-2.03], *p* = 0.4]
- Family size > 5 vs ≤5 [1.65 [1.13-2.42], *p* = 0.01]
- Having young (< 5 years) sibling (yes vs no) [0.46 [0.32-0.69], *p* = 0.001]
- Hospital admission (yes vs no) [1.71 [1.09-2.66], *p* = 0.02]
- TB treatment (yes vs no) [0.96 [0.56-1.65], *p* = 0.9]

(b) TC Newman

- Age [0.96 [0.94-0.97], *p* = 0.0002]
- Gender (male vs female) [0.90 [0.62-1.16], *p* = 0.3]
- Preterm [1.28 [0.87-1.86], *p* = 0.2]
- Maternal *S. aureus* carriage [2.31 [1.64-3.25], *p* = 0.0001]
- Delivery mode (normal vs cesarean) [1.09 [0.72-1.65], *p* = 0.7]
- Day care attendance [1.55 [1.13-2.13], *p* = 0.01]
- Infant's health status [0.85 [0.56-1.29], *p* = 0.5]
- *S. pneumoniae* carriage (yes vs no) [0.82 [0.62-1.09], *p* = 0.17]
- HIV-exposure (yes vs no) [1.27 [0.90-1.77], *p* = 0.16]
- Having animals [0.67 [0.47-0.94], *p* = 0.02]
- Autumn vs winter [1.49 [1.02-2.18], *p* = 0.04]
- Spring vs winter [1.48 [1.22], *p* = 0.05]
- Summer vs winter [2.2 [1.46-3.12], *p* = 0.0001]
- Paternal smoking [0.52 [0.31-0.99], *p* = 0.02]
- Maternal smoking (yes vs no) [1.03 [0.59-1.79], *p* = 0.9]
- Exclusive formula vs exclusive breastfeeding [0.39 [0.12-1.23], *p* = 0.3]
- Mixed vs exclusive breastfeeding [0.45 [0.11-1.61], *p* = 0.26]
- Mod-high vs low SES [2.3 [1.62-3.4], *p* = 0.0001]
- Family size > 5 vs ≤5 [1.73 [1.18-2.53], *p* = 0.004]
- Having young (< 5 years) sibling (yes vs no) [1.24 [0.80-1.75], *p* = 0.2]
- Hospital admission (yes vs no) [1.37 [0.92-2.05], *p* = 0.12]
- TB treatment (yes vs no) [1.06 [0.58-1.93], *p* = 0.0]
4.4.4 Determinants of any carriage of *S. aureus* during the first year of life

We performed a case-control analysis where all *S. aureus* carriers (infants with any event of *S. aureus* carriage during the first year of life) were considered as cases, while all *S. aureus* non-carriers (infants who never carried *S. aureus* during the first year of life) were considered as controls. No association was noted for most of the variables as shown in Table 4.2. However, day care attendance (*p*=0.06) and maternal smoking (*p*=0.06) showed marginal statistical significance.

**Table 4.2: *S. aureus* carriage determinants between carriers and non-carriers.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender (vs females)</td>
<td>1.48</td>
<td>(0.40-5.45)</td>
<td>0.55</td>
</tr>
<tr>
<td>HIV-exposure (yes vs no)</td>
<td>0.71</td>
<td>(0.12-4.10)</td>
<td>0.70</td>
</tr>
<tr>
<td>Maternal <em>S. aureus</em> carriage (yes vs no)</td>
<td>2.90</td>
<td>(0.30-28.14)</td>
<td>0.36</td>
</tr>
<tr>
<td>TB treatment (yes vs no)</td>
<td>3.20</td>
<td>(0.25-40.67)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Day care (yes vs no)</strong></td>
<td><strong>4.31</strong></td>
<td><strong>(0.96-19.39)</strong></td>
<td><strong>0.06</strong></td>
</tr>
<tr>
<td><strong>Maternal smoking (yes vs no)</strong></td>
<td><strong>0.22</strong></td>
<td><strong>(0.05-1.04)</strong></td>
<td><strong>0.06</strong></td>
</tr>
<tr>
<td>Paternal smoking (yes vs no)</td>
<td>1.93</td>
<td>(0.49-7.60)</td>
<td>0.35</td>
</tr>
<tr>
<td>Exclusive formula (vs breast-feeding)</td>
<td>0.47</td>
<td>(0.09-2.60)</td>
<td>0.39</td>
</tr>
<tr>
<td>Mixed (vs breast-feeding)</td>
<td>0.35</td>
<td>(0.07-1.85)</td>
<td>0.22</td>
</tr>
<tr>
<td>Family size &gt;5 vs ≤5</td>
<td>4.35</td>
<td>(0.71-26.44)</td>
<td>0.11</td>
</tr>
<tr>
<td>Having a young sibling (yes vs no)</td>
<td>0.70</td>
<td>(0.34-1.42)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Variables highlighted in bold showed weak significant associations.
4.5 Discussion

Studying bacterial carriage in newborns provides insights into how bacterial and host factors influence carriage since newborns are microbiologically and immunologically naïve \(^{39}\). Carriage studies during infancy are useful in providing baseline data on the coverage of childhood vaccinations and may predict changes in respiratory and invasive diseases \(^{34}\). This study has provided a comprehensive longitudinal description of \textit{S. aureus} nasopharyngeal carriage during the first year of life, and explored possible determinants that may have an influence on carriage in healthy infants.

In this study, the overall \textit{S. aureus} prevalence in all NP swabs was 21\% which was comparable to a detection rate of 26\% reported in a South African cohort study in Soweto involving infants and their mothers \(^{44}\). In infants, the carriage rate decreased over time from 54\% at the age of 4 weeks to 6.6\% by 52 weeks. The carriage rates observed in our study were higher compared to the rates in similar African studies on infants of the same age, for example, at eight weeks we observed a 45\% carriage rate compared to 10\% at the same age in a study from the Gambia \(^8\). However, a study by Kinabo et al.\(^{45}\) which focused on the nasopharyngeal colonisation by HIV status in Tanzanian infants reported 66\% overall \textit{S. aureus} carriage rate at 6 weeks, 36\% at 12 weeks and 24\% at 24 weeks \(^{45}\). The carriage was higher in the Tanzanian study at age 6 and 24 weeks compared to our study, however, in our study the rate was 45\% at age 12 weeks. By HIV status, Kinabo et al reported significantly higher \textit{S. aureus} carriage among HIV-infected infants in all three time points compared to HIV-uninfected children \(^{45}\). Carriage rates comparable to ours were reported in a similar birth cohort study from the Netherlands \(^{19}\).

A possible explanation for the decline in carriage rate after the eighth week of age might be the development of immune responses to \textit{S. aureus} which may lead to its eradication in some individuals \(^{39}\). Another possible explanation for this decline could be due to microbial interference \(^{46\text{-}50}\). A good example is the well documented inverse association between nasopharyngeal carriages of \textit{S. aureus} and \textit{S. pneumoniae} in healthy infants during the first year of life \(^{8,35,36}\). In this
Prevalence, Incidence and Determinants of *S. aureus* NP Carriage

study, the estimated odds of carrying *S. aureus* changed over time: *S. pneumoniae* carriers were twice as likely to carry *S. aureus* compared to *S. pneumoniae* non carriers at birth but 60% less likely to carry *S. aureus* at age 52 weeks. An explanation for this early life positive association between carriage of *S. aureus* and *S. pneumoniae* is not known. Nonetheless, the presence of other bacteria such as *Haemophilus influenzae* in the nasopharynx early in life has been shown to shift the interactions between *S. aureus* and *S. pneumoniae* [44]. In pneumococcal vaccine-naïve South African infants, the negative association between *S. aureus* and *S. pneumoniae* shifted to synergistic when *H. influenzae* was present [44]. This shift was also evident in an animal model study where carriage by *H. influenzae* was more likely when prior *S. aureus* or *S. pneumoniae* carriage was present [51]. Although the mechanism for the inverse association between *S. aureus* and *S. pneumoniae* is still under investigation [25,48,52,53]. Lijek et al. showed, in a mouse model, involvement of cross-reactive streptococcal antibodies that may protect from subsequent staphylococcal carriage. However, this has not been shown in humans [30]. In our study, this inverse relationship was not significant in infants from Mbekweni but clear in infants from TC Newman.

A South African study that targeted HIV-infected children, concluded that the inverse association between carriage of *S. aureus* and *S. pneumoniae* was lacking among HIV-infected infants [37]. Considering our clinical data, none of the infants who participated in this study was HIV-positive, but 30 out of the total 33 infants who were exposed to HIV, were from Mbekweni. This might, to some extent, explain the absence of the inverse association between the carriage of *S. aureus* and *S. pneumoniae* in infants from Mbekweni, however, more investigation is warranted.

In the logistic regression model, HIV-exposure only showed an association with *S. aureus* NP carriage when interacted with time; the estimated odds for carrying *S. aureus* for HIV-exposed infants increased over time and they were twice as likely to carry *S. aureus* by 52 weeks of age. This was also supported by the increased proportion of *S. aureus* NP carriage among HIV-exposed infants over time. None of the infants included in this study was HIV-infected. Similar *S. aureus* detection rates were described among HIV-exposed and unexposed South African infants, where a significantly lower *S. aureus* prevalence (25%) was observed among HIV-exposed compared to
44.3% among HIV-unexposed within 6-12 weeks of age followed by a trend of increasing *S. aureus* prevalence among HIV-exposed infants towards 52 weeks (15% vs 12%) \(^{54}\). Since it is known that *S. aureus* carriage is a risk factor for subsequent infections, especially among hospitalized and immune suppressed individuals (including HIV-infected)\(^{14}\), Madhi *et al.* suggested that understanding the ecology of the nasopharynx may extend our understanding on the factors leading to increased risk of these invasive diseases \(^{54}\).

The incidence of *S. aureus* acquisition during the first year of life was 0.24 events per 100 child-days. To our knowledge, no published data are available on the incidence of acquisition of *S. aureus* carriage among healthy infants. The high incidence of acquisition observed in our study within the first four weeks suggests high exposure to various sources of *S. aureus*. Coming into close contact with *S. aureus* carriers is a critical determinant for *S. aureus* carriage, since *S. aureus* can easily be transmitted between individuals by hands or direct skin contact \(^{12,14,55}\). Our data showed that maternal *S. aureus* carriage was associated with increased risk of carrying *S. aureus* at any time among their infants, this is in agreement with previous \(^{20,23,56}\). However, when we tested the association between maternal and infant carriage during the first two weeks, no significant association was noted (\(p=0.14\)), suggesting sources other than maternal-infant transmission during the first few weeks or that this study was underpowered to determine this significance of this association specifically at this age period. In keeping with this, our results suggest that family size of \(\geq 5\) individuals increased the risk of *S. aureus* carriage in infants. Sharing the household with asymptomatic carriers may facilitate transmission across individuals including infants \(^{57}\). This cross transmission may also lead to spread of drug resistant strains as well as community associated diseases \(^{58}\). Interestingly, having a young sibling was protective against *S. aureus* NP carriage, particularly in infants from TC Newman. Of note, Cohen *et al.* discussed that in children younger than two years, the factors which are associated with increased *S. pneumoniae* carriage are often associated with decreased *S. aureus* carriage, and the presence of a sibling(s) in the household is reported as a risk factor for *S. pneumoniae* carriage during the first year of life \(^{59}\). This may in part account for this finding.
The association between carriage and feeding practices was site-dependent. Exclusive formula feeding and mixed feeding were significantly associated with increased risk of \textit{S. aureus} carriage in infants from TC Newman, suggesting that exclusive breast feeding may be protective in this setting. In Mbekweni, on the other hand, these formula/mixed feeding were non-significantly associated with \textit{reduced} risk of carriage, perhaps because mothers also had higher carriage rates in comparison with those from TC Newman. The high maternal \textit{S. aureus} carriage rate may be related to HIV status since almost all HIV-infected mothers were from Mbekweni. Breast feeding by a carrier mother has previously been shown to be a risk factor for \textit{S. aureus} nasal carriage during infancy \textsuperscript{19,39}.

Higher socioeconomic status and living conditions have been shown to promote compliance with hygiene and health control measures \textsuperscript{60}. In contrast, our data indicate that moderate to high socioeconomic status was associated with increased \textit{S. aureus} carriage. Jourdain \textit{et al.} observed similar findings among healthy preschool children in Belgium, and suggested that children with high socioeconomic status often attend day care centres where they get exposed more to \textit{S. aureus} \textsuperscript{9}. Attending day care centres was also a risk factor for \textit{S. aureus} carriage in our cohort.

The unique longitudinal design of this study provides time series data to investigate the effect of seasonal changes on \textit{S. aureus} NP carriage. \textit{S. aureus} NP carriage was highest during summer (Figures 3.3 and 3.4). In keeping with our data, higher prevalence of \textit{S. aureus} carriage occurred during the hot season Among healthy Japanese children attending day care centres \textsuperscript{61}. However, seasonal changes had no significant effect on \textit{S. aureus} NP carriage rates among infants in the United Kingdom with a more temperate climate \textsuperscript{62}. Some studies explained the seasonality of staphylococcal skin and soft tissue infections by seasonal variation in \textit{S. aureus} carriage rates \textsuperscript{63,64}. \textit{S. aureus} was heavily and frequently isolated during summer from lesions of atopic dermatitis patients as oppose to winter \textsuperscript{63}. A recent study by Wang and co-workers, explained this increase during hot seasons by the favourable conditions through skin’s warm temperatures and humidity due to perspiration. These conditions may increase the chances of skin colonisation and also facilitate the spread of skin and soft tissue infections \textsuperscript{64}. 
In our study we found that male gender, and hospital admission were risk factors for *S. aureus* NP carriage. Previous studies identifying *S. aureus* carriage determinants during childhood have yielded inconsistent findings, possibly due to variations in study designs and targeted population. Male gender has been associated with increased risk of carrying *S. aureus* in other studies. Studies that investigated *S. aureus* carriage determinants in the adult population explored possible reasons for this gender difference. For example, it has been suggested that women are protected against *S. aureus* infections by the sex hormone oestrogen which binds to oestrogen receptors on immune effector cells and regulate the expression of protective cytokines. Also, differences in physical activities, occupations as well as behavioural practices were shown to influence carriage and infections by *S. aureus*. However, the reasons for the differences during infancy are not known and require further investigation.

It is well recognized that hospitals are efficient settings for the acquisition and transmission of microbes including *S. aureus*. Environmental contamination and close contact with *S. aureus* carrier healthcare workers and/or patients are among the reasons facilitating this. Since preterm infants often require postnatal hospitalisation, they are at a high risk for acquiring and subsequently being infected by *S. aureus* or MRSA from the neonatal intensive units. Our findings correlate well with previous studies; hospital admission was a risk factor for *S. aureus* NP carriage. Preterm birth was a risk factor only in infants from Mbekweni. We also showed that cigarette smoke exposure, presence of an animal in the household as well as infants being healthy at the time of sample collection were negatively associated with *S. aureus* NP carriage. A study which included healthy infants and their mothers during the first year of life in Europe, found that prolonged colonisation by *S. aureus* was high among infants with non-smoking mothers suggesting the protective effect of the passive smoking among infants. An old in-vitro study investigated the effect of smoke exposure on bacterial binding epithelial cells and found that high non-dilute smoke extract reduced binding of bacteria supporting the protective effect of smoke exposure. Furthermore, a meta-analysis on the effect of passive smoke exposure on paediatric invasive bacterial disease and pharyngeal carriage revealed that this exposure was associated...
Prevalence, Incidence and Determinants of *S. aureus* NP Carriage

with increased invasive disease caused by *H. influenzae, N. meningitides*, and *S. pneumoniae*, but *S. aureus* was not included in this analysis. However, clearly the effect of smoking or smoke exposure on *S. aureus* colonisation requires further investigation. Previous studies showing positive associations between *S. aureus* carriage and animal exposure were limited to populations at professional risk of zoonotic transmission such as veterinarians and farmers. However, in our context it is not clear as to why animals have a protective effect on *S. aureus* carriage. A direct effect of inhaling dust from an animal enriched environment on *S. aureus* carriage has not been shown, but, in a mouse model Fujimura et al showed that the immune responses which result from inhaling animal enriched dust down regulate the secretion of mucin (mucous protein). Mucin is a key component for *S. aureus* to establish its niche in the mucosal epithelial tissues, therefore, alteration in secretion of this protein might disturb the *S. aureus* colonisation process. However, further investigation is warranted to proof this theory.

Pneumococcal conjugate vaccine uptake and revaccination may have an impact on *S. aureus* NP carriage during the first year of life. However, we could not assess this impact since all infants included in this study received PCV-13 according to South African childhood vaccination program. A randomised clinical trial investigated the effect of seven-valent pneumococcal conjugate vaccine (PCV7) among healthy children showed a temporal increase in *S. aureus* carriage only at 11 months of age after the booster dose among vaccinated infants. However, in the Netherlands, and after seven years of introduction of PCV-13, *S. aureus* carriage rate decreased and reached the pre PCV-7 era (especially among 11 months old infants). In this study, small number of *S. aureus* non-carriers was identified (only 16 infants). This limited our ability to identify differences in carriage determinants between *S. aureus* carriers and non-carriers. Nonetheless, we observed a weak association between *S. aureus* carriage and day care attendance (positive) and maternal smoking (negative). Similar effects for the day care attendance and smoke exposure were observed in the general model (Figures 4.4 and 4.6).
4.6 Conclusions

Among healthy South African infants, *S. aureus* NP carriage rate was higher compared to that found in most other studies of African infants at the same age. The overall incidence of acquisition was 0.24 episodes per 100 child-days. Male gender, higher socioeconomic status, maternal carriage, large family size and hospitalization were significantly associated with *S. aureus* NP carriage during the first year of life. The effect of HIV-exposure and *S. pneumoniae* varied by age. The variation in some *S. aureus* carriage determinants between infants from TC Newman and Mbekweni emphasise the likely complex interactions between variables impacting on carriage.
References


Prevalence, Incidence and Determinants of *S. aureus* NP Carriage


Prevalence, Incidence and Determinants of S. aureus NP Carriage


Prevalence, Incidence and Determinants of S. aureus NP Carriage


Prevalence, Incidence and Determinants of \textit{S. aureus} NP Carriage


Chapter Five

The population structure of *S. aureus* isolates from the nasopharynx of mothers and children in the Drakenstein cohort
5.1 Summary

To study the population structure of *Staphylococcus aureus* in a South African birth cohort, nasopharyngeal (NP) swabs were collected at birth from 137 mother-infant pairs and two-weekly from infants for the first year of life.

*S. aureus* isolates were identified using standard microbiological methods. All isolates were genotyped using *spa* typing, and screened for the *pvl* gene by real-time PCR. Isolates which showed phenotypic resistance to oxacillin were screened for *mecA* gene, and were further characterized by Staphylococcal Cassette Chromosome *mec* (*SCCmec*) typing using multiplex endpoint PCR. ‘Based Upon Repeat Pattern’ (BURP) clustering analysis was performed on all assigned *spa* types. A modified *spa* PCR was performed on *S. aureus* isolates which failed to amplify any product using the standard *spa* PCR.

*S. aureus* was detected in 21% (725/3417) of the NP swabs. A total of 11 *spa*-clonal complexes (*spa-CC*) (n=578 isolates) were identified and 11 *spa* types were singletons (n=116). Three *spa* types (n=27) were excluded from the clustering analysis due to small number of repeats. The *mecA* gene was present in 12 isolates; 11 isolates represented a single clone (PVL-negative-t045-*SCCmec* type I [1A]) and the remaining isolate belonged to a distinct clone which harboured a nontypeable (NT) *SCCmec* element (PVL-positive-t891-*SCCmec*-NT). A total of eight isolates showed genetic rearrangements within the *spa* locus which affected their typeability using the standard *spa* PCR primers. In addition, four isolates remained untypeable after submission to the Ridom StaphType server. The prevalence of PVL was 35% and all PVL-positive isolates except for one, were MSSA.

In conclusion, low prevalence of MRSA was reported among infants, and high level of genetic diversity was observed among the MSSA strains as determined by *spa* typing. PVL was mainly associated with MSSA strains.
5.2 Background

*Staphylococcus aureus* is genetically a clonal organism compared to other species such as *Neisseria gonorrhoea*. Its genome (approximately 2.8kb) consists of three components: a conserved core genome which is > 97% conserved across strains; core variable genes (> 700 genes) which generate the variations between lineages; and mobile genetic elements (MGEs) which encode a variety of virulence and resistance determinants. Investigating the diversity of *S. aureus* lineages and the genetic relatedness between these lineages is crucial to understand the epidemiology of *S. aureus* within a setting. Molecular typing, based on *S. aureus* multi-locus sequencing of seven house-keeping genes (MLST), revealed that methicillin susceptible *S. aureus* (MSSA) strains make up more clonal complexes (CCs) in terms of numbers with diverse genetic backgrounds compared to the limited number of epidemic methicillin-resistant *S. aureus* (MRSA) CCs observed world-wide. Up to 82 *S. aureus* MLST clonal complexes (CCs) and 384 singleton sequence types (STs) have been identified thus far. Of these clusters, CC5, CC9, CC12, CC22, CC25, CC30, CC45, and CC51 are the major *S. aureus* lineages associated with humans. CC5 is considered the largest clonal complex. Not all MSSA lineages provide a suitable genetic background for acquiring the Staphylococcal Cassette Chromosome mec (SCCmec) element, hence, some MSSA lineages such as CC15 have not been associated with MRSA strains as yet.

*S. aureus* is associated with asymptomatic carriage as well as infections both in community and healthcare settings regardless of its methicillin resistance. However, the challenge with MRSA infections is the limited availability of therapeutic options. Thus far, there are eleven SCCmec types that are linked to MRSA strains associated with both humans and animals. SCCmec-types I [1B], II [2A] and III [3A] are larger in size and carry resistance genes to a wide range of antibiotics, and were originally typically associated with healthcare settings, whereas, IV [2B] and V [5C] are smaller, less number of resistance genes and were associated more with community settings. However, the
distinction between the strains related to community or hospital settings is increasingly blurring. The MRSA clone STS-SCCmec IV [2B] was first described as a paediatric clone, however, it achieved pandemic spread and significant clinical relevance. Harbouring the large sized SCCmec elements such as types I [1B] and II [2A] is considered as a fitness cost, which may be compensated for by the reduction in toxin production. Conversely, harbouring SCCmec type IV does not have an effect on the relative fitness and toxicity, which explains the success of the CA-MRSA strains both in community and healthcare settings.

Panton-Valentine Leuckocidin (PVL) is a bi-component pore-forming toxin that causes lysis of host immune cells, namely, monophages, macrophages and neutrophils. It is encoded by the genes lukS-PV and lukF-PV which are carried by several icosahedral-or elongated-head-shape temperate bacteriophages e.g. φSa2MW, φPVL, φ108PVL. PVL is also a potent virulence factor that is associated with skin and soft tissues infections and necrotizing pneumonia in the community especially among children under five years old. According to the Centres for Disease Control and Prevention (CDC), both epidemiological data (detection of MRSA in outpatient clinics or within 48 hours after hospital admission, with no clear risk factors for acquiring MRSA) and the molecular structure (SCCmec element, antibiotic susceptibility and harbouring PVL) should be used to distinguish between hospital associated and community-associated (CA) MRSA strains. However, previously designated community-associated (CA) clones are now circulating in the healthcare settings and vice versa, therefore strain genotype cannot be used as a proxy for hospital vs. community acquisition. PVL-positive strains have been associated with skin and soft tissue infections and to lesser degree with invasive diseases among individuals within the community settings. Population-based studies on S. aureus strains carrying PVL are scarce, and circulation of PVL-positive S. aureus strains within the community may impose a health threat and an infection control challenge in resource-limited regions. In Africa, the PVL prevalence among MRSA strains ranges from 0.3% (in South Africa) to 100% (in Northern Africa), however, PVL is more prevalent among African MSSA.
There are a number of typing tools which helped in understanding the molecular epidemiology of *S. aureus* 
4,12: phenotypic methods are based on detection of a biochemical material produced by the bacterium under specific conditions, while genotypic tools determine the variation at the genomic DNA level 24–26. Sequence-based methods such as MLST (a seven house-keeping genes strategy in *S. aureus*) and *S. aureus* protein A (*spa*) typing are considered robust and reproducible across laboratories 3,6,27. Protein A is involved in the immune evasion process by binding to the Fc-fragment of immune globulins and inhibiting phagocytosis 17,28. The variable number of tandem repeat structure of the X-region (located downstream of the *spa* locus and flanked with the IgG binding domain), has been utilised successfully as a typing tool in epidemiological investigations 5,27,29. This single locus sequence-based typing tool-*spa* typing is reliably used to study the molecular epidemiology and population structure of *S. aureus* in different settings 12.

*Spa* typing has advantages over MLST in particular, speed, accuracy, and is less expensive. However, 1-2% of *S. aureus* strains remain non-typeable using this method 30. This is due to the genetic rearrangements that affect the annealing site of the standard forward primer in IgG binding 30,31.

It is important to understand the population structure of MSSA because the structure of MSSA in a geographic region predicts the potential MRSA strains that might emerge in future following an acquisition of SCCmec element by a local MSSA strain 32–34. Taking note that some MRSA strains seem to be imported to a geographical region through travel activities, acquisition of a SCCmec element by a local MSSA strain also contributes largely to emergence of new MRSA clones 35. A systematic review of the molecular epidemiology of MRSA in Africa observed that data on *S. aureus* population structure is limited to certain countries, with less attention on MSSA and in particularly within the community. Furthermore, MSSA genotypic data from South Africa are also lacking 22,23,36–38. Nonetheless, it appears that distinct PVL-positive MSSA lineages are circulating in Central and Western Africa e.g. CC15, CC121 and CC152, and are not yet linked to any successful MRSA clones 38–42. MRSA strains belonging to CC5 - the largest *S. aureus* MLST cluster- are the most prevalent and common strains in many African regions, and are mainly linked to healthcare
settings. However, some clones showed limited geographical distribution of specific clones, such as the ST612-IV [2B] clone (CC8) in South Africa.

In view of the importance of understanding the population structure and the molecular epidemiology of *S. aureus* and the lack of data in South Africa, particularly from the community and healthy population, this study was proposed. The specific aims of the experiments reported in this chapter are to: (i) confirm the presence of the *mecA* gene and characterise the SCCmec element in isolates that showed phenotypic resistance to oxacillin using the disc diffusion method; (ii) describe the *spa* types of *S. aureus* isolates; (iii) define the genetic relatedness between the identified *spa* types and describe the genetic evolution affecting the *spa* locus; and (iv) determine the prevalence and epidemiology of PVL in this population.

### 5.3 Methods

Nasopharyngeal (NP) swabs were collected from 137 mother-infants pairs at birth and then solely from infants every two weeks for the first year of life. *S. aureus* was isolated and identified from NP swabs as described in chapter 4 (section 4.3.4). The nucleic acid was extracted from all isolates to perform the molecular characterisation.

#### 5.3.1 Nucleic acid extraction

The stored *S. aureus* isolates were sub-cultured onto Columbia based blood agar BA (2% agar and 5% sheep blood) and incubated in ambient air at 37°C overnight. DNA was extracted using the heat-lysis protocol with a slight modification i.e. Tris buffer was used to re-suspend the colonies instead of phosphate-buffered-saline. A loopful from an 18-24 hour old culture on BA was suspended in 200µl of the Tris buffer, heated at 95°C for 15 minutes and then centrifuged at 10,000g for 5 minutes. A volume of 50ul of the supernatant was kept at -20°C for molecular characterisation.

#### 5.3.2 Confirmation of resistance to β-lactams by *mecA* PCR

*S. aureus* isolates that showed resistance to oxacillin (described in Chapter 6) were screened for the *mecA* gene using end-point PCR as described previously. A final volume of 50µl was used
for the PCR reactions which contained a final concentration of 1X Super-Therm buffer, 1.5 mM MgCl$_2$, 1.5U Super-Therm Taq polymerase (JMR holdings, London, UK), and 0.2 mM deoxynucleotide triphosphate mix (dNTPs) (Thermo Scientific, Wilmington, USA), and a primer concentration of 0.25µM for each primer. A volume of 5µl template was added to the mixture. The PCR amplification was performed in the Applied Biosystems 2720 Thermal cycler (Applied Biosystems, Carlsbad, USA). The thermal cycling temperatures were as follows: denaturation at 94°C for 4 minutes, followed by 30 amplification cycles of 94°C, 53°C and 72°C for 30 seconds each and final elongation at 72°C for 3 minutes. The control strain COL and PCR grade water were used as a positive and a non-template controls respectively in each PCR run. The PCR products were resolved on 2% agarose gel in 1% Tris-acetic acid-EDTA buffer at 80V for 1.5h and visualized with ethidium bromide.

5.3.3 Characterisation of SCCmec element by a multiplex end-point PCR assay

The mecA-positive $S.\ aureus$ strains were further characterized based on the SCCmec-element using the multiplex PCR as described previously $^{44}$. A minor limitation of this assay is that it only detects SCCmec-types I-VI, however, the other types are rare within the human population (as discussed in section 2.4). Only primers and control strains for SCCmec-types I-VI were used in this PCR. The multiplex reaction was performed in a 50µl final volume, which contained a final concentration of 1X Super-Therm buffer, 1.5 mM MgCl$_2$, 1.5U Super-Therm Taq polymerase (JMR holdings, London, UK), and 0.2 mM dNTPs (Thermo Scientific, Wilmington, USA). The mecA gene was amplified as an internal control using the primers mecAP4 and mecAP7 (0.4µM concentration). The primers CIF-F2, CIF-R2, RIF-F10, RIF-R13 were added at a final concentration of 0.6µM, while the primers ccrB-F2, ccrB-R2, ccrC-F2, ccrC-R2, dcs-F2, dcs-R1, kdp-F1, kdp-R1, mecIP3, mecIP2, SCCmec III J1F, SCCmec J1R, SCCmec V J1F, SCCmec V J1R were added to a final concentration 0.8µM $^{44}$. Three microliters that contained 5ng of DNA of each of the samples was added to the PCR mix. The prototypic MRSA control strains for SCCmec types I-VI were used as
Population Structure of *S. aureus*

positive controls: COL (type I [1B]), BK2464 (type II [2A]), ANS46 (type III [3A]), MW2 (type IV [2B]), WIS (Type V [5C]) and HDE288 (type VI [4B])

The PCR amplification was performed in a Applied Biosystems 2720 Thermocycler (Applied Biosystems, Carlsbad, USA) and the thermal cycling temperatures were as follows: denaturation at 94°C for 4 minutes, followed by 30 amplification cycles of 94°C, 53°C and 72°C for 30 seconds each and final elongation at 72°C for 3 minutes. The PCR products were resolved on 3% agarose gel in Tris-acetic-EDTA buffer and electrophoresed at 80V for 2h and visualized with ethidium bromide. The different banding patterns (SCCmec-types) were determined for the MRSA strains.

5.3.4  **spa typing**

All identified *S. aureus* isolates were characterized by amplifying the variable X-region of the spa locus. A PCR reaction with 50µl final volume, contained a final concentration of 1X KAPA Taq Ready Mix (1U of KAPA Taq DNA polymerase, 0.2mM dNTPs, 1.5mM MgCl₂ and Kapa Taq buffer (KAPA Biosystems)). The primers spa-1113F and spa-1517R were added at a final concentration of 0.25uM in each reaction. Three microliters of DNA were added to the PCR mix, and 3µl of DNA of *S. aureus* strain MW2 and PCR grade water were included as PCR positive and non-template negative controls, respectively. PCR amplification was performed in the Applied Biosystems 2720 Thermocycler (Applied Biosystems, Carlsbad, USA) using the following thermal cycling conditions: denaturation at 80°C for 5 minutes, followed by 35 amplification cycles of 94°C 45 seconds, 60°C for 45 seconds and 72°C for 45 seconds, and a final elongation step of 72°C for 10 minutes. Gel electrophoresis (2% agarose gel) was performed to visualize the PCR products. Amplicon purification was performed using EXOSAP-IT (Affymetrix, Inc. Cleveland, USA) according to the manufacturer’s recommendations. A mixture of 0.5µl Exonuclease I and 2µl of Alkaline Phosphatase were added to 10 µl of the PCR product as this step removes the excess primers and dNTPs. The mixture was incubated at 37°C for 15 minutes, where after, it was heated at 85°C for 15 minutes to stop the reaction. Sequencing of the purified PCR product was done following the manufacturer’s instructions on the ABI3500XL with the ABI BigDye v3.1 (Applied
Population Structure of S. aureus

Biosystems, Carlsbad, USA). A previously spa-typed strain was re-sequenced alongside each new batch as a sequencing control. The forward and reverse sequences for each isolate were analysed using the StaphType Ridom software version 2.1.1 (Ridom, Münster, Germany), which assigns a spa type to each isolate. Novel spa types were assigned after synchronization of the local database with the spa server (http://spaserver.ridom.de/) 27. Cluster analysis was performed using a ‘Based Upon Repeat Patterns’ (BURP) algorithm, which is implemented in the Staph Type Ridom software. The default cost setting (cost 4) were used to when performing the BURP clustering and spa types with ≤5 repeats were excluded from the cluster analysis.

5.3.4.1 Re-amplification of spa PCR (X-region)-negative isolates

S. aureus isolates with typical morphological characteristics on mannitol salt agar medium and DNase positive, but did not yield a PCR amplicon for the spa X-region, were subjected to two biochemical tests: the staphaurex latex agglutination test (BioMerieux, Marcy l’Etoile, France) as a positive confirmatory test; and pyrrolidonyl arylamidase (PYR) as a negative confirmatory test (Mast Diagnostics, Bootle, United Kingdom)45,46. These tests were carried out according to the manufacturer’s recommendations. The control strains S. aureus ATCC 23925 and Streptococcus pyogenes ATCC 19615 were used as positive controls for the staphaurex latex and PYR tests, respectively. S. aureus was confirmed as staphaurex positive, PYR negative. The DNA was extracted from the confirmed S. aureus isolates as described previously (section 5.3.1) and used as templates in a modified spa PCR. A newly designed forward primer spa-T3-F was used along with the standard reverse primer 1517R (section 5.3.4) to amplify a larger region which includes sequence upstream the X-region 30. As recommended, only the reverse read was sequenced since the forward primer has multiple annealing sites in the IgG binding domains, which may result in multiple PCR products of different length 30. Mastermix concentrations and cycling conditions similar to the conventional spa PCR were used. The resulting sequences were compared to the complete spa locus sequence control strain N513 (Genbank ID: 29165615). The sequences annotation was done using the software SnapGene (GSL Biotech).
5.3.5 Screening for pvl genes

A previously published real-time PCR assay was adopted to screen for the pvl genes (lukS-PV and lukF-PV) in the S. aureus isolates. The final reaction volume of 20µl contained 1X SensiFAST™ Probe No-ROX readymade buffer, 0.5µM primers (PVL1-F and PVL1-R) concentration, and 0.2µM probe (SPVL1-FAM). The PCR amplification was performed using the Bio-Rad CFX96 Touch™ Real-Time PCR amplification system (Bio-Rad Laboratories, Hercules, CA, USA), and thermal cycling conditions were as follow: 95°C for 5 minutes for denaturation followed by 40 amplification cycles of 94°C and 58°C for 15 seconds. Two microliters of the extracted DNA (section 5.3.1) were added to each reaction. The S. aureus prototypic strain MW2 and PCR grade water were included as positive and non-template negative controls, respectively in each run. Only unique, non-duplicated strains carried by each infant were used to calculate the PVL prevalence.

5.4 Results

5.4.1 PCR Confirmation of the presence of the mecA gene

In total, 12 (1.6%) S. aureus isolates exhibited resistance to oxacillin and cefoxitin (Chapter 6). They amplified a 162 bp product of the mecA gene, and were further characterised using SCCmec typing (Figure 5.1).

![Figure 5.1: Amplification of mecA gene (162bp) from methicillin-resistant S. aureus (MRSA) strains.](image-url)

Lanes 2-13 contain MRSA strains, lane 14 is a mecA negative S. aureus strain, lane 15 is a non-template negative control and lane 16 is the MRSA control strain COL. Lanes 1 and 17 contain GeneRuler™ DNA molecular weight ladder (Thermo Scientific).
5.4.2 Characterisation of the SCCmec element in MRSA strains

The PCR amplification profiles for the 11 MRSA strains were similar to, but distinct from the amplification profile of the SCCmec type I [1B] control strain COL (Figure 5.2). COL amplified three bands sized 495bp, 342bp and 162bp, and the identified MRSA strains showed an additional band (449bp) which corresponds to a band amplified by ccrC primers from SCCmec type V [5C] control strains (Figure 5.2). The other MRSA strain was non-typeable as amplification of a 209 bp fragment which corresponds to the mecI region in SCCmec type II and III control strains was observed.

![Figure 5.2: Characterisation of the SCCmec-types for methicillin-resistant S. aureus (MRSA) control strains and the 12 MRSA strains identified from infants](image)

Lanes 2-14 contain MRSA strains from infants except for lane 9 which contains a susceptible isolate. Lanes 16-21 contain MRSA control strains carrying SCCmec types I-VI; lane 16, COL (type I [1B]), lane 17, BK2464 (type II [2A]), lane 18, ANS46 (type III [3A]), lane 19, MW2 (type IV [2B]), lane 20, WIS (Type V [5C]) and lane 21, HDE288 (type VI [4B]). Lane 22 contains a non-template negative control, lanes 1, 15 and 23 contain GeneRuler™ DNA molecular weight ladder (ThermoFisher Scientific Inc., MA, USA). Samples 2-8, and 11-14 amplified similar banding patterns as the control strain COL (162bp, 342bp, and 495bp). Sample 10 amplified two bands (162bp (mecA) and 209bp (mecI)).

5.4.3 spa Typing and ‘Based Upon Repeat Pattern’ (BURP) clustering

Genotyping of 725 S. aureus isolates by targeting the spa gene resulted in 85 spa types. BURP analysis clustered 71 spa types (n=578) into 11 spa-clonal complexes (spa-CCs). Eleven spa types (n=116) were singletons and three spa types (n=27) were excluded from the cluster analysis due to the small number of repeats (Figure 5.3). Four isolates were un-typeable. The spa-CC15 was
the largest cluster; consisting of 22 spa types (n= 212 strains), followed by spa-CC701 with 12 spa types (81 strains). Three spa-CCs each consisted of two spa types, and had no founder spa type for the group (CC no founder (NF) 1, 2, and 3) as shown in Figure 5.3. Seven novel spa types t14704, t14705, t14709 (CC15), t14706 and t14710 (spa-CCNF2), t14707 (spa-CC701), and t14708 (spa-CC2), were identified. The MRSA strains belonged to only two spa types: 11 with spa type t045 (spa-CC2), and one strain with spa type t891 (CC891) (Figure 5.3). The spa types carried by mothers were represented by six CCs (spa-CC15; 8 strains, spa-CC701; 3 strains, spa-CC891; 2 strains, spa-CC2, 21 and 84 were represented by 1 strain each, and five singletons.
Figure 5.3: ‘Based Upon Repeat Pattern’ clustering analysis on identified **spa** types.

Using a cost of four and a minimum number of repeats of five were used to perform the cluster analysis. The analysis resulted in 11 **spa**-CCs and 11 singletons. **Spa** types with five or fewer repeats were excluded from this analysis. The blue circles represent the founder of the cluster (i.e. the **spa** type with the highest finder score), the yellow circle is the group sub-founder. The circle size is proportional to the number of isolates with the same **spa** type. The novel **spa** types identified in this study are highlighted with the dotted circles. The distance between the **spa** types does not reflect degree of genetic difference.
Figure 5.4 shows the distribution of the different *spa*-CCs for each sampling point during the first year of life. *spa*-CC15 was the most frequently reported within the first 32 weeks while it was not detected at week 46. The relative proportions of each *spa*-CC among the range of isolates at each time point are shown in appendix 4.

Figure 5.4: Genotype distribution of the 11 *spa*-clonal complexes and the singleton isolates identified from the 137 infants at the 27 time points.

### 5.4.4 Molecular epidemiology of MRSA among carriers

MRSA strains were identified from six infants; four harboured the same MRSA clone (t045-SCC*meC*-I [1A]-PVL negative) within the first four weeks of life, and one at 26 weeks (Figure 5.5). An MRSA strain was recovered from the remaining infant at the age of 8 weeks. A genetically distinct strain (t891-SCC*meC* non-typeable) was identified at the age of 52 weeks from an infant who previously carried the predominant MRSA clone. Two infants carried MRSA on three consecutive occasions within the first six weeks. None of the mothers carried MRSA on the day of delivery.
Figure 5.5: The molecular epidemiology of S. aureus among MRSA carriers analysed by time.
Two different MRSA clones were carried by six infants: t045-MRSA SCCmeC I-like [1B]; PVL negative (yellow) and t891-SCCmeC non-typeable (NT); PVL-positive (Red). Other MSSA strains carried by infants during the study period were showed in grey.

5.4.5 Genetic rearrangements in the spa locus

5.4.5.1 S. aureus isolates which were untypeable by spa:

Following the analysis of the spa sequences on the Ridom StaphType software, four isolates (isolated from the same infant at four different time points) had 10 repeats in total and were untypeable after submission to the spa server (http://spaserver.ridom.de/). An atypical 26bp repeat was identified in all the isolates and the phred quality scoring for the 26 nucleotides ranged between 87 and 116. However, a repeat identifier number (ID) was not communicated when submitted individually to the spa server (Figure 5.6). A spa type (t1994) was assigned to these isolates when the atypical repeat (indicate the sequence) was not included in the sequence submitted.
5.4.5.2 *S. aureus* isolates negative for the X-region PCR:

The hypervariable X-region of the *spa* locus was not amplified in 8 isolates. All the isolates were confirmed to be *S. aureus* based on the biochemical tests; positive for the staphaurex latex agglutination and negative for the PYR tests. Nevertheless, the newly designed forward primer (*spa*T3-F), which amplified a larger region, successfully amplified two bands in each isolate corresponding to the number of binding sites. Annotation of the reverse sequences revealed mutations/deletions in the position where the standard forward primer *spa*1113-F anneal compared to the control strain N315 (Figure 5.7).
Figure 5.7: Schematic diagram of the rearrangements identified in the *spa* hypervariable region in eight isolates compared to prototyped control strains. (a) Alignment of the standard *spa* PCR primers (*spa* 1113-F and *spa* 1517-R), as well as the previously newly modified forward primer (*spa* T3-F) with four prototyped strains (COL, MW2, Mu3 and TCH1516) showing perfect alignment with all primers. (b) Alignment of *spa* PCR primers with the eight strains with rearrangements in the annealing site of the standard forward primer annealing site affecting their typeability. (c) Shows at the nucleotide level the alignment of the standard forward primer, as well as the genetic rearrangements for all 8 isolates resulting in negative *spa* PCR results.
5.4.6 Prevalence and epidemiology of Panton-Valentine Leukocidin (PVL)

Using the non-duplicated S. aureus strains, the PVL prevalence was 35% (104/294), and consisted mainly of MSSA. Twenty one percent of PVL-positive strains belonged to CC15, (with 11 different spa types). Similarly, 18% of MSSA in singletons (n=6 spa types) were PVL positive (Table 5.1).

Half (54%) of the 121 S. aureus carriers (infants who carried S. aureus at any time during the first year of life) carried a PVL-positive strain at least once. Nine of the 21 mothers carried a PVL-positive strain.

Table 5.1: The prevalence of PVL in each spa-clonal complex (CC) and its distribution among spa-types

<table>
<thead>
<tr>
<th>spa-clonal complex (CCs)</th>
<th>Total no. of spa types % (n)</th>
<th>No. of non-duplicated strains % (n)</th>
<th>PVL-positive strains % (n)</th>
<th>PVL-positive spa types (no. of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC15</td>
<td>25 (21)</td>
<td>24 (71)</td>
<td>19 (20)</td>
<td>t015 (6), t050 (1), t073 (3), t589 (2), t1156 (2), t1619 (1), t2794 (1), t3219 (1), t8282 (1), t13519 (1), t14709 (1)</td>
</tr>
<tr>
<td>CC701</td>
<td>14 (12)</td>
<td>12 (35)</td>
<td>7 (7)</td>
<td>t701 (3), t2360 (3), t14707 (1)</td>
</tr>
<tr>
<td>CC84</td>
<td>12 (10)</td>
<td>8 (23)</td>
<td>11 (12)</td>
<td>t084 (3), t346 (3), t774 (2), t7071 (3), t8079 (1)</td>
</tr>
<tr>
<td>CC2</td>
<td>9 (8)</td>
<td>11 (31)</td>
<td>11 (11)</td>
<td>t002 (7), t071 (2), t4352 (1), t14708 (1)</td>
</tr>
<tr>
<td>CC891</td>
<td>5 (4)</td>
<td>9 (25)</td>
<td>13 (14)</td>
<td>t891 (8), t7185 (3), t2251 (1), t2570 (2)</td>
</tr>
<tr>
<td>CC21</td>
<td>5 (4)</td>
<td>4 (11)</td>
<td>9 (9)</td>
<td>t012 (2), t021 (5), t1053 (1), t2018 (1)</td>
</tr>
<tr>
<td>CC174</td>
<td>3.5 (3)</td>
<td>3 (10)</td>
<td>3 (4)</td>
<td>t174 (2), t127 (2)</td>
</tr>
<tr>
<td>CC349</td>
<td>3.5 (3)</td>
<td>1.7 (5)</td>
<td>2 (2)</td>
<td>t1054 (1), t2645 (1)</td>
</tr>
<tr>
<td>CCNF1</td>
<td>2 (2)</td>
<td>0.7 (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CCNF2</td>
<td>2 (2)</td>
<td>0.7 (2)</td>
<td>1 (1)</td>
<td>t14710 (1)</td>
</tr>
<tr>
<td>CCNF3</td>
<td>2 (2)</td>
<td>0.7 (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Singletons</td>
<td>13 (11)</td>
<td>16 (48)</td>
<td>18 (19)</td>
<td>t148 (1), t272 (3), t317 (6), t2763 (7), t5468 (1), t14791 (1)</td>
</tr>
<tr>
<td>Strain excluded from clustering</td>
<td>3.5 (3)</td>
<td>10 (28)</td>
<td>5 (5)</td>
<td>t026 (5)</td>
</tr>
<tr>
<td>Non-typeable strains</td>
<td>No spa-type</td>
<td>0.3 (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>294</td>
<td>35 (104)</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: The proportion of the PVL-positive strains was calculated for each spa-CC (no. non-duplicated PVL positive strains/total no. of the non-duplicated strains carried by each infant in the CC).
PVL: Panton-valentine leukocidin; CC: clonal complex; (-): no PVL-positive strains.
5.5 Discussion

This chapter provides an overview on the population structure of \textit{S. aureus} isolated from apparently healthy infants during the first year of life, as well as from their mothers at delivery. The twelve MRSA strains identified in this study were represented by only two spa types: eleven strains belonged to \textit{spa} type t045 and one assigned with \textit{spa} type t891. The eleven strains harboured SCC\textit{mec}-type I and were PVL negative. A similar MRSA clone - reported to be ST5 (based on MLST) - harbouring the same \textit{spa} and SCC\textit{mec} types was described as the second most prevalent clone in hospitals in Cape Town, particularly, in Mowbray Maternity Hospital where it accounted for 37\% of MRSA strains \textsuperscript{37}. In understanding the evolutionary history of MRSA clones belonging to ST5, Nübel et al. (2008)\textsuperscript{34} indicated that ST5-MRSA strains might have limited geographical dispersal and their emergence is associated with acquisition of SCC\textit{mec} element by a local MSSA strain. Therefore, it is possible that this clone may have emerged after the acquisition of SCC\textit{mec} I from a locally established MSSA clone that is circulating in hospitals in Cape Town, as previously discussed by Jansen van Rensburg et al. \textsuperscript{37}. All MRSA strains with \textit{spa} type t045 and SCC\textit{mec}-I were isolated between August 2012 and July 2013, while four of the six MRSA carriers acquired the strain within the first 4 weeks of their life. Also since none of the mothers carried MRSA, suggest that there is a common source for transmission to infants, such as a shared hospital ward or a healthcare worker. Considering our data, together with the previous study from hospitals in Cape Town, it appears that this clone is associated with maternity wards in Cape Town \textsuperscript{37}.

The PVL-positive MRSA strain with a unique non-typeable SCC\textit{mec} element was detected using a multiplex PCR \textsuperscript{44}. It amplified only the mecI region which is a regulator gene in the \textit{mec} complex, in addition to the mecA region (Figure 5.2). The mecI region is truncated in all the SCC\textit{mec} types associated with humans except for types II, III and VII. In addition, these SCC\textit{mec} elements (II, III, and VII) carry transposons that confer resistance to glycopeptides as well as erythromycin \textsuperscript{48,49}. However, the MRSA strain identified in our study showed susceptibility to all non-beta-lactam antimicrobials (described in Chapter 6) implying that it is not SCC\textit{mec} type II, III or VII. Moreover,
harbouring PVL and resistance to β-lactams antimicrobials were considered as classical features for CA-MRSA strains. Additionally, this MRSA strain was detected a later time (at age 52 weeks), compared to the other MRSA strains. Nevertheless, it is important to note that the distinction between CA-MRSA and HA-MRSA is not clear since HA-MRSA are circulating in the community and vice versa. Carrying large SCCmec elements such as types I, II, and III by MRSA strains is considered to confer a fitness cost and may be associated with reduced virulence. This reduced fitness and virulence is not seen among MRSA strains carrying the smaller SCCmec elements (e.g., type-IV), which explains the overall success of CA-MRSA strains to disseminate and cause infections both in community and healthcare settings.

The prevalence of PVL-positive S. aureus in this study was 35%, which is lower than the 57% PVL prevalence reported among S. aureus isolates identified in a birth cohort study from Gabon. Panton-Leukocidin-producing S. aureus is associated with recurrent skin and soft tissue infections (SSTI) as well as severe infections such as necrotizing pneumonia especially in young children. In Africa, the prevalence of PVL-positive clinical MSSA strains ranges between 25.9% and 100% of isolates, with the highest prevalence in North African countries. In South Africa, 97% of MSSA strains isolated from paediatric and adult patients in Tygerberg hospital, Cape Town, were PVL-positive and mainly identified from SSTI, and bone and joint infections. On the other hand, MRSA less frequently harbour pvl genes, particularly HA clones, for example, the prevalence of PVL in clinical MRSA strains in South Africa ranges from 0.3%-9%. The clinical relevance of PVL-positive strains in this study is not clear, since these were carriage rather than disease-associated strains.

Based on spa genotyping data, MSSA strains in this cohort exhibited considerable heterogeneity which is similar to findings from other similar studies as well as data from the MLST database (http://saureus.mlst.net/). The spa-CC15 was the largest spa-CC included only MSSA strains accounting for 24% of isolates, with 19% of the strains identified as PVL-positive. It has been shown to be associated with the MLST CC45 - one of the main lineages associated with epidemic MRSA clones. In a community-based multicentre study which included Gabon, Mozambique and
Tanzania, CC45 was overrepresented (45%) in nasal swabs as oppose to other clinical samples such as blood. Almost 81% (9/11) of the isolates belonging to CC21 were PVL-positive. This spa-CC is associated with the MLST CC30, which represents the MSSA ancestor of the world-wide dominant endemic CA-MRSA clone referred to as the “South-West Pacific” clone. Only a single strain with the spa type t064 (CC701) was identified in our study. This spa type (t064) was previously reported to be associated with the dominant local MRSA clone (ST612-IV [2B]-t064, which is a double-locus variant of ST8-IV [2B]) in healthcare settings across South Africa. We did not perform MLST on S. aureus isolates in this study, but, considering the frequency of spa type t064, it appears that the MSSA lineages which are genetically related to the dominant “South African” local MRSA clone (ST612) are relatively uncommon in this community. The rate of PVL-positive isolates belonging to CC891 was 13%. CC891 was predominantly identified among the MSSA strains causing a wide range of infections in Tygerberg hospital in Cape Town. CC891 was not detected in an African birth cohort study in Gabon. Based on a systematic review on molecular epidemiology of MRSA in Africa, t891 was mainly associated with PVL-positive epidemic clone ST22-IV [2B] which was mainly limited to South Africa. In this study, only a single MRSA strain was reported as part of CC891, but this requires close monitoring.

The identification of seven novel spa types belonging to different MSSA genetic backgrounds (spa-CCs) provide evidence for the frequent genetic evolution that could occur in S. aureus particularly within the spa locus. In this study, eight MSSA strains with spa type t021 had genetic rearrangements in the annealing site of the standard forward primers used for spa genotyping. These rearrangements affected their typeability and could have been overlooked if identification of S. aureus had been performed using the standard protocol for spa typing. In a study in which the genetic rearrangements that affect the spa locus using a selection of S. aureus strains from both healthy carriers and patients was investigated, Votintseva et al. (2014) estimated that 2% of S. aureus carriers are colonised with completely untypeable spa types, and 13% of the population are colonised with “hidden” S. aureus strains with deletions/insertions throughout the spa gene. Another set of five MSSA strains were untypeable despite the amplification of the spa variable.
Population Structure of *S. aureus*

X-region using the standard primer set. Submission of the repeat succession to the Ridom SpaServer could not resolve it, however, resubmission of the other nine repeats (excluding the atypical repeat) classified the strains as t1994. This suggests that the *spa* type was subjected to an insertion of the atypical repeat which resulted in this untypeable genotype.

A limitation in this study was that MLST was not performed on *S. aureus* strains which hampered comparing the population structure *S. aureus* strains in our cohort with other global strains. In addition; a number of “hidden” carriage strains or co-carriage events, could have been overlooked due to the use of the standard *spa* primers. Performing whole genome sequencing on a select set of MRSA and MSSA strains will provide better understanding of the genetic background of these strains.

### 5.6 Conclusion

A low prevalence of MRSA was observed among healthy infants. A diverse MSSA population with relatively high PVL prevalence was observed. The standard *spa* PCR primers failed to amplify eight isolates which highlight the importance of regularly updating the *spa* protocol to overcome the frequent rearrangements occurring in the *spa* locus.
Population Structure of *S. aureus*

References

14. Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC. The role of the Panton-Valentine leucocidin
Population Structure of *S. aureus*


Population Structure of *S. aureus*


Chapter Six

*S. aureus* antibiotic susceptibility patterns
6.1 Summary

This chapter provides a description of the antibiotic susceptibility of *Staphylococcus aureus* obtained from nasopharyngeal (NP) samples of South African infants during the first year of life and their mothers. A total of 3417 NP swabs were collected from mothers (n=125) and infants (n=3292) throughout the study period. The antibiotic susceptibility testing was performed using disc diffusion method. Isolates that indicated resistance to oxacillin were confirmed by *mecA* PCR. MRSA strains were screened for vancomycin resistance using the VITEK2 system. *S. aureus* genotyping data (*spa*-clonal complexes (CC)) were compared with the antibiotic susceptibility profiles to establish any relationship with the various CCs. The overall rate of *S. aureus* was 22% (725/3292) corresponding to 21 isolates from mothers at birth and in 704 isolates from infants. Resistance rates were calculated on unique strains carried by each infant and their mothers (n=294). All the isolates were susceptible to tigecycline, linezolid and mupirocin. Eighty three percent of *S. aureus* isolates were resistant to penicillin; 9.5% to gentamicin; 4.1% to tetracycline and co-trimoxazole, and 2.4% to rifampicin. The inducible MLS$_B$ phenotype (ICR) was observed in 3% (n=8) of isolates, while, constitutive erythromycin resistance was identified in 1.3% (n=4). The prevalence of methicillin-resistant *S. aureus* (MRSA) was 2% (6/294) and none of the mothers carried MRSA at birth. All the MRSA strains were susceptible to vancomycin. A strong association was observed between resistance to gentamicin and *spa*-CC891, and tetracycline resistance with *spa*-CC84, *spa*-CC701 and *spa*-CC-no founder (NF) 2. Although the resistance rates were generally low, the frequency of resistance was high within the first six months of life and declined over time. A total of eight events in the antimicrobial resistance profile within the same *spa* type were noted in six infants.

In conclusion, the studied *S. aureus* isolates showed low resistance rates to all tested antibiotics except for penicillin. In addition, a very low MRSA prevalence was reported among healthy infants. The frequency of antibiotic resistance was relatively increased during the first six month of life. Gentamicin, erythromycin and clindamycin should be used with caution for empiric presumptive *S. aureus* infections in our settings.
6.2 Background

*Staphylococcus aureus* is an established pathogen that is associated with superficial self-limiting and invasive infections \(^1\). Prior to the antibiotic era, the mortality rate in patients with *S. aureus* bacteraemia reached 80\% \(^2\). Major improvements in outcome were noted among patients infected with *S. aureus* after the introduction of penicillin in 1940 \(^2,3\). However, only two years later, penicillin-resistant *S. aureus* strains were first described in hospitals, and 20 years after the introduction of penicillin, 80\% resistance of *S. aureus* isolates were reported \(^2\). In 1961, methicillin - a penicillinase-resistant penicillin - was introduced for clinical use, but resistance was reported two years later in England \(^4\). The spread of methicillin-resistant *S. aureus* (MRSA) strains is a serious global health concern. In particular, resistance to methicillin is often accompanied by resistance to other classes of antibiotics \(^2,5,6\).

Resistance to antibiotics emerged as a result of chromosomal mutations as well as horizontal transfer of resistance genes from a donor organism. Selective pressure associated with inappropriate antibiotic use and sub-optimal dosing also favour the selection of resistant strains \(^6\). Oxacillin (Methicillin) resistance is mediated by expression of an alternative penicillin-binding protein (PBP2a) encoded by *mecA* or *mecC* genes. PBP2a has low affinity to methicillin and all \(\beta\)-lactam antimicrobials, hence methicillin (and other \(\beta\)-lactam antibiotics) cannot bind to the PBP2a efficiently. This low efficient binding of \(\beta\)-lactams leads to resistance \(^7\). The *mecA* gene is encoded in a mobile genetic element called the Staphylococcal Cassette Chromosome *mec* (SCCmec) element, which also harbour genes conferring resistance to other antimicrobial classes (other than \(\beta\)-lactam antibiotics) \(^8\). The prevalence of MRSA across the African continent varies from 16\% to 55\%, and a recent review indicated that since 2005, MRSA rates appear to be increasing in most countries, except for South Africa \(^9\). The review also observed high susceptibility of MRSA in Africa to glycopeptides (93\%-100\%), fusidic acid (33\%-100\%), linezolid (85\%-100\%) and rifampicin (22\%-100\%). In contrast, high rates of resistance to gentamicin, co-trimoxazole, tetracycline and erythromycin were also noted \(^9\).
Classically, MRSA emerged within hospitals - hospital-associated (HA)-MRSA affecting at risk populations such as elderly and hospitalized patients. However, since 1990, genetically distinct strains rapidly spread within the community - community-associated (CA)-MRSA - affecting otherwise healthy individuals with no previous exposure to healthcare settings including children. Based on their molecular structure, CA-MRSA confer resistance only to β-lactam antimicrobials compared with HA-MRSA. However, the distinction based on susceptibility and epidemiological data is increasingly blurred, as CA-MRSA have emerged in healthcare settings. The prevalence of the CA-MRSA is increasing world-wide, gradually replacing the HA-MRSA in hospitals. A number of reports have indicated that MRSA carriage rates among healthy children in developed and developing countries ranged from 0.2% to 32% 14–18. Among healthy children, CA-MRSA are mainly associated with skin and soft tissue infections (SSTIs) and community acquired pneumonia. In South Africa, data on MRSA are mainly linked with healthcare settings. Moreover, only few studies focused on MRSA related infections among children. Among children admitted to The Red Cross War Memorial Children’s Hospital, 6.3% of MRSA infections were attributed to CA-MRSA causing bacteraemia, and were mainly isolated from SSTIs. Another study of paediatric bacteraemia from Tygerberg Hospital showed that approximately 15% of CA *S. aureus* bacteraemia were MRSA. Overall, data on the prevalence of MRSA carriage among healthy children in South Africa are sparse.

In Africa, the majority of studies that investigated *S. aureus*/MRSA in Africa were conducted in clinical settings and may not reflect the community setting. In this chapter, the aims are i) to describe the antibiotic susceptibility patterns of *S. aureus* strains isolated from healthy South African infants during their first year of life and their mothers; ii) identify the multi drug resistance profiles of community-associated MSSA and MRSA; iii) determine the relationship between the resistance profiles and *S. aureus* genotypes; and iv) investigate longitudinal changes in antibiotic susceptibility patterns for each infant.
6.3 Methodology

6.3.1 Study participants and *S. aureus* isolates

Nasopharyngeal swabs (NP) were collected from 137 mother-infant pairs at birth, and every two weeks from infants throughout their first year of life. *S. aureus* was isolated and identified using standard culture methods described in Chapter 3. The isolates were stored in Skimmed milk Tryptone Glucose Glycerine medium (STGG) medium at -80°C until further laboratory investigations. Data on antimicrobial use, hospitalization, HIV-exposure, maternal *S. aureus* carriage, and concurrent *Mycobacterium tuberculosis* (TB) treatment of infants were collected at seven time points in the first year of life using a standardized questionnaire by a healthcare provider.

6.3.2 Antibiotic susceptibility testing

The antibiotic susceptibility profiles were determined for 725 *S. aureus* isolates identified from the 137 mother-infant pairs during the first year of life. The stored *S. aureus* isolates were sub-cultured onto Columbia blood agar BA (2% agar and 5% sheep blood) and incubated in ambient air at 37°C overnight. The susceptibility testing was performed using the Kirby-Bauer disc diffusion method employing a digital caliper tool for measuring zone diameter and interpreted according to the Clinical Laboratory Standard Institute. The antibiotic discs included penicillin G (10U), oxacillin (1 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), trimethoprim/sulphamethoxazole (will be referred to as Co-trimoxazole) (25 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), fusidic acid (10 µg), rifampicin (30 µg), linezolid (30 µg), and tigecycline (15 µg) (Mast group ltd., Merseyside, UK).

Screening for mupirocin resistance was performed using 5 µg and 200 µg discs to detect low and high level resistance, respectively. Interpretative zone diameter for tigecycline (15 µg), fusidic acid (10 µg) and mupirocin (5 and 200 µg), not included in CLSI guidelines, were interpreted using the European Committee on Antibiotic Susceptibility Testing (EUCAST) guidelines. *S. aureus* ATCC 25923 and BAA-976 were used as quality control strains for methicillin-susceptible
and resistant strains respectively, and were included in each experiment. Isolates showing resistance to oxacillin and cefoxitin were further confirmed by PCR targeting the meca gene (Chapter 5). Susceptibility testing to vancomycin was performed for MRSA strains using the VITEK2 system (BioMerieux, Marcy l’Etoile, France) at the National Health Laboratory Service at Groote Schuur Hospital. To identify inducible macrolide-lincosamide-streptogramin B (MLS\(_B\)) resistance phenotype, the D-test was performed according to the CLSI guidelines \(^{27,29}\). A “D-shaped” inhibition zone confirmed inducible resistance to clindamycin using erythromycin (ICR) \(^{29}\). The ATCC strain BAA-977 was used as a quality control strain for ICR and was included with each experiment. An isolate was considered multi-drug resistant if it exhibited resistance to three or more classes of antibiotics.

### 6.3.3 Confirmation of Tigecycline non-susceptible strains using E-test

The agar diffusion E-test for tigecycline (BioMerieux, Marcy l’Etoile, France) was used to determine the minimum inhibitory concentration (MIC) for \(S.\) \(aureus\) isolates that indicated reduced susceptibility to tigecycline by the disc diffusion method. MICs were interpreted using the EUCAST guidelines for reporting susceptibility to tigecycline. \(S.\) \(aureus\) ATCC 25923 was included for quality control.

### 6.3.4 Calculation of the antibiotics resistance rate

Due to the longitudinal nature of this study, only the unique strains carried by each infant (i.e. excluded duplicated strains carried by the same infant) were selected to calculate the resistance rate of the tested antibiotics.

### 6.4 Results

#### 6.4.1 Characteristics of participants

A total of 725 \(S.\) \(aureus\) isolates (704 from infants and 21 from mothers) were obtained from 3417 NP swabs (125 mothers on the day of delivery and 3292 samples from 137 infants) in the first year of life. NP swabs were not collected from 12 mothers. Antibiotic consumption rate was 31% (43/137) among infants during the first year of life. The proportion of infants who consumed
Antibiotic Susceptibility patterns

at least one antibiotic during the first year of life was 31% (43/137). A total of 58 NP swabs were collected at the time antibiotic consumption. For all the 43 infants who consumed antibiotic, the total number of NP swabs collected longitudinally throughout the study period was 1449, this corresponds to 44% of the total number of NP swabs collected in the study. Of the 43 infants, antimicrobial agents were administered to 26% (11/43) at multiple time points: eight infants received antibiotics at two time points; two at three time points; and one infant at four time points. Only 11 S. aureus isolates were identified from 10 infants out of the 43 infants in which antimicrobial consumption was reported. The detection rate of S. aureus in swabs from infants with antimicrobial consumption was 17% (242/1449) vs 25% (463/1843) for those who had none (p= 0.0001). Data on antimicrobial use were not available for all mothers.

The S. aureus carriage rate among mothers at birth was 17% (21/125). A quarter of the infants were preterm infants, while the rate of HIV-exposure among infants was 24% (33/137). A total of 13% (18/137) were on TB treatment (of which 2 were HIV-exposed), and 11% (15/137) were hospitalised at least once during the first year of life.

6.4.2 Antibiotic susceptibility patterns for S. aureus isolates

A total of 273 non-repeated S. aureus strains were carried by the 137 infants throughout the study period, in addition to the 21 strains carried by mothers at the day of delivery. Among infants, 16% (n=45) were susceptible to all tested antibiotics, while only one of the 21 maternal isolates was susceptible to all antibiotics (p=0.12). Overall, eighty three percent of S. aureus strains were resistant to penicillin, 9.5% to gentamicin, 4% to tetracycline and co-trimoxazole, and 2.3% to rifampicin. Constitutive erythromycin resistance was identified in 1.3% (n=4), whereas the inducible MLSB phenotype (ICR) was observed in 3% (n=8) of isolates. In addition, reduced susceptibility (intermediate) was noted for ciprofloxacin (4%), chloramphenicol (1%) and erythromycin (2%) and clindamycin (0.4%). Only five isolates were resistant to fusidic acid and three to tigecycline (Figure 6.1). However, based on the E-test method, the three isolates had MICs of 0.064 (1 isolate) and 0.094 (2 isolates) confirming their susceptibility to tigecycline according to EUCAST guidelines 28.
The identification of MRSA strains was based on resistance to oxacillin and cefoxitin according to the CLSI guidelines\(^27\). Only 2% (n=6) of the isolates exhibited phenotypic resistance to oxacillin and cefoxitin, and were positive for \textit{mecA} gene (section 5.4.1). Both MSSA and MRSA strains were susceptible to linezolid, and neither low nor high level mupirocin resistance was identified in the isolates investigated (Figure 6.1). All the 11 \textit{S. aureus} isolates which were identified from infants who had taken antibiotics at the time of sample collection showed resistance only to penicillin except for one MRSA strain.

![Figure 6.1: Antibiotic non-susceptibility for the 294 non-duplicated \textit{Staphylococcus aureus} isolates from NP swabs.](image)

### 6.4.3 Antibiotic susceptibility patterns of methicillin-resistant \textit{S. aureus} strains

The twelve MRSA strains were identified from six infants within the first month of life, and three infants carried MRSA at least twice (Table 6.1). None of the mothers carried MRSA strains on the day of delivery. Of the 12 strains, nine were resistant to gentamicin and ten showed the ICR phenotype. All MRSA strains were susceptible to vancomycin (MICs were \(<=1\) for all MRSA strains) using the VITEK2 system. None of the HIV-exposed infants carried MRSA, however, two preterm infants were MRSA carriers of which one was hospitalised at birth. Only two of the 18 TB
treated infants carried MRSA, and only one MRSA carrier was on antibiotics at one time point (Table 6.1).

Table 6.1: Clinical characteristics of MRSA carriers.

<table>
<thead>
<tr>
<th>PID</th>
<th>Isolation time point</th>
<th>spa type</th>
<th>Gender</th>
<th>Clinical site</th>
<th>Preterm</th>
<th>Mode of delivery</th>
<th>Maternal carriage</th>
<th>Hospital admission</th>
<th>TB treatment</th>
<th>HIV-exposure</th>
<th>Antimicrobial use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1193</td>
<td>2 weeks</td>
<td>045</td>
<td>Male</td>
<td>Mbekweni</td>
<td>Vaginal</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>At 4 weeks of age</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>045</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>045</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1282</td>
<td>26 weeks</td>
<td>045</td>
<td>Female</td>
<td>Mbekweni</td>
<td>Vaginal</td>
<td>√</td>
<td>X</td>
<td></td>
<td>From 9 to 12 month</td>
<td>√</td>
<td>X</td>
</tr>
<tr>
<td>1296</td>
<td>52 weeks</td>
<td>045</td>
<td>Female</td>
<td>Mbekweni</td>
<td>Caesarean</td>
<td>√</td>
<td>X</td>
<td>at birth</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>1304</td>
<td>2 weeks</td>
<td>045</td>
<td>Male</td>
<td>Mbekweni</td>
<td>Caesarean</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>3167</td>
<td>2 weeks</td>
<td>045</td>
<td>Female</td>
<td>TC Newman</td>
<td>Vaginal</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>045</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>045</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 weeks</td>
<td>045</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3207</td>
<td>4 weeks</td>
<td>045</td>
<td>Female</td>
<td>TC Newman</td>
<td>Caesarean</td>
<td></td>
<td></td>
<td></td>
<td>From 6 to 9 months</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Note: PID: participant identifier number.

6.4.4 Antibiotypes of multi drug resistant of S. aureus isolates

Overall, 16 different multi-drug-resistance profiles (3 and 13 profiles for MRSA and MSSA, respectively) were identified from 32 S. aureus isolates (Table 6.2). Nine of the 12 MRSA strains were categorised in antibiotype 2, while, six isolates were grouped with antibiotype 15 (resistance to penicillin, chloramphenicol, co-trimoxazole, and rifampicin) (Table 6.2).
Table 6.2: Multi-drug resistance profiles of *Staphylococcus aureus* strains.

<table>
<thead>
<tr>
<th>Antibiotype No.</th>
<th>Resistance profile</th>
<th>No. isolates</th>
<th>No. infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>PEN OXA CLIN ERY</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>PEN OXA CLIN ERY</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>PEN OXA CLIN ERY</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MSSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PEN CLIN ERY</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>PEN CLIN ERY</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>PEN ERY CIP</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>PEN GEN CIP</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>PEN GEN CIP</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>PEN GEN CIP RIF</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>PEN TCY CIP</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>PEN TCY SXT RIF</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>PEN TCY CHL SXT RIF</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>PEN TCY CHL SXT RIF</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>PEN CHL SXT RIF</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>PEN CHL SXT RIF</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>PEN CIP SXT RIF</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>


6.4.5 Antibiotic resistance trends over time

The longitudinal analysis of the antibiotic resistance patterns at each time point over the first year of life showed that penicillin resistance was observed at all the time points, and ranged between 46% - 100%, while resistance to chloramphenicol was observed only in weeks six and 18. Furthermore, no antibiotic resistance (except penicillin) was observed at birth, 34 and 50 week, and resistance to clindamycin and gentamicin was observed consistently in the first six months of life. Tetracycline resistance rates among MSSA were observed at the same time points in which MRSA strains were identified, while concurrent resistance to both rifampicin and cotrimoxazole were noted at nine time points. Although the *S. aureus* resistance rates were generally low, the frequency of resistance peaked during the first six months and thereafter declined (Figure 6.2).
Figure 6.2: The longitudinal resistance patterns in the 725 S. aureus isolates identified from the 137 mother-infant pairs.
The resistance patterns are shown for 11 antimicrobial agents (all isolates were susceptible to tigecycline, linezolid, and mupirocin). Proportions of antimicrobial resistance are shown for each antimicrobial agent at the respective time points (dark colours correspond to higher proportions per antimicrobial agent).
6.4.6 Relationship between antibiotic resistance and spa-clonal complexes

The spa types identified from both mothers and infants clustered into eleven spa-clonal complexes (spa-CCs) and 21 singletons (section 5.4.3). MRSA strains belonged exclusively to spa-CC2 (n=11) and spa-CC891 (n=1) (Figure 6.3). Concurrent resistance to both cotrimoxazole and rifampicin, and to tetracycline clustered mainly in spa-CC84, and spa-CC701. The singleton group (n=145) were collectively resistant to nine antibiotic classes. Most of the strains (73%) grouped in spa-CC15 – being the predominant cluster, spa-CC174 and spa-CC349 were resistant only to penicillin. In addition, 80% of S. aureus isolates resistant to gentamicin in this study clustered with spa-CC891 (Figure 6.3).

Figure 6.3: The relationship between antibiotic resistance and spa-clonal complexes (spa-CC). Analysis was done separately for MSSA and MRSA isolates. * Both singleton as well as the isolates excluded from the clustering analysis (small number of repeats) are grouped together in this category.
6.4.7 Changes in antibiotic susceptibility patterns over time within the same infant

In general, the antibiotic susceptibility patterns changed with variation in the *spa* types carried by each infant during the first year (section 7.4.2). The focus of this section is on the longitudinal changes in resistance profiles within the same *spa* types carried by an infant. Overall, eight shifts in resistance profile were identified in six infants with the same *spa* type (Figure 6.4). Most of these events were changes from susceptible to resistant profiles for certain antibiotics. This included a shift from an MSSA to MRSA within two weeks (acquisition of resistance to oxacillin and gentamicin). On the other hand, two infants experienced a shift back to a susceptible profile: loss of resistance to ciprofloxacin and co-trimoxazole for one infant (PID 1324); and loss to resistant to penicillin, tetracycline and co-trimoxazole for another infant (PID 1249) (Figure 6.4).
Figure 6.4: Longitudinal *Staphylococcus aureus* carriage profiles showing the shifts in resistance profiles for antibiotics which occurred in six infants during the first year of life.

The *spa* types carried by each infant are described with a red colour when there is resistance, and with green where the strain is susceptible. All cells in white mean that no *S. aureus* was identified at the corresponding time point. PID: participant identifier.
6.5 Discussion

The antibiotic susceptibility patterns of *S. aureus* in the community setting are not well described in South Africa. The vast majority of *S. aureus* strains observed in this cohort were MSSA strains (98%). Methicillin-resistant strains were identified from six infants, while none of the mothers harboured MRSA. Similarly low MRSA prevalence have been noted from healthy and outpatient infants from Brazil (0.2%)\(^\text{30}\), the United States of America (0.7%)\(^\text{31}\), Gabon (<1.9%)\(^\text{32}\) and Libya (2.2%)\(^\text{18}\). However, higher MRSA rates (ranged between 8.3 and 9.2%) have been reported from infants hospitalized in neonatal intensive care units in some developed and developing countries\(^\text{18,33,34}\). In this study, *S. aureus* resistance rates for penicillin and gentamicin were 83% and 9.5%, respectively. A higher penicillin resistance rate (95%) was reported in infants in an African birth cohort study in the Gabon\(^\text{32}\). Also, a higher (27%) gentamicin resistance was observed among healthy children in Tanzania\(^\text{15}\). Gentamicin is administered as a first line drug for children presenting with sepsis in South Africa\(^\text{25}\). However, in this study, a possible reason for gentamicin resistance may be due to other antibiotic pressure giving rise to clonal expansion of multi-resistant clones (CC891 for MSSA and t045 for MRSA)\(^\text{9}\).

All the strains identified in this study were susceptible to linezolid and mupirocin. In addition, all MRSA strains were susceptible to vancomycin as determined by the Vitek2 system. However, the broth microdilution method for screening heterogeneous vancomycin intermediate *S. aureus* (hVISA) among MRSA strains was not performed in this study. Nevertheless, only two cases of hVISA have been reported in South Africa\(^\text{35}\). However, across the African continent, resistance rates to vancomycin and linezolid as mainly reported by disc diffusion were 0%-18% and 0%-15% respectively\(^\text{9}\). Since the CLSI guidelines indicate that disc diffusion method is not reliable to screen for vancomycin resistance, the rates described above are questionable and require further confirmation\(^\text{27}\).
The inducible clindamycin resistance was observed in 92% (11/12) of MRSA strains and in 1.4% (10/693) of MSSA strains. Furthermore, constitutive erythromycin resistance was found only in 1.6% of the MSSA strains. The resistance rates for clindamycin and erythromycin in this study were very low compared to a rate of 17% inducible clindamycin and 14% constitutive erythromycin resistance among MRSA identified in healthy Tanzanian children under five years old. However, in the Tanzanian study nasal but not nasopharyngeal swabs were assessed. Resistance to macrolide antibiotics (e.g. erythromycin) may occur due to changes in the ribosomal target or defect in the protein synthesis. These changes may affect the activity of both clindamycin and erythromycin, which is encoded by the erythromycin methylase (erm) gene, and referred to as the macrolide-lincosamide-streptogramin B (MLSb) phenotype. However, the constitutively expressed macrolide efflux pump is mediated by the msrA gene.

The prevalence of MRSA seem to be increasing throughout the African continent, except for South Africa, which may be attributed to suboptimal infection prevention and control capacity. The small number of MRSA identified in our study did not provide the opportunity to investigate the risk factors associated with carriage. Nevertheless, three of the six infants with MRSA were exposed to factors which might have facilitated acquisition of MRSA at an early age (Table 6.1). Preterm birth, hospitalisation, and use of antibiotics have been shown in previous studies as potential risk factors for MRSA acquisition. Furthermore, one MRSA strain was PVL positive (t891, SCCmec-NT) (section 5.4.4 and susceptible to all non-β-lactam antibiotics, which is a typical molecular feature MRSA strains circulating in the community setting. This strain was recovered from an infant at a relatively older age (at week 52). The other 11 MRSA strains except for one, shared a common resistance profile i.e. resistance to penicillin, gentamicin, and inducible clindamycin resistance (83%), while the remaining strain was resistant only to gentamicin. This resistance profile together with the genotype (described in Chapter 5) were consistent with MRSA strains identified from hospitals in Cape Town since 2008, suggesting the continued existence of this MRSA clone in Cape Town.
Data from previous studies within South Africa suggest that fusidic acid is an effective agent for the treatment of *S. aureus* infections in South Africa. It was recommended that fusidic acid should be used in combination with other antibiotics such as rifampicin to prevent development of resistance. In this study, only five MSSA strains showed *in-vitro* resistance, which is in agreement with previous investigations indicating *S. aureus* low resistance to fusidic acid in Sub-Saharan Africa compared to North African countries. A low rate of MSSA resistance to rifampicin (2.4%) and co-trimoxazole (4.1%) was also observed despite the wide use of these antibiotics in South Africa. Rifampicin is part of first line treatment of tuberculosis (TB) and co-trimoxazole is used as a prophylactic drug for the prevention of fungal infections among HIV-infected individuals. Nevertheless, this trend should be closely monitored.

All *S. aureus* isolates identified in this study were susceptible to tigecycline. Only three isolates showed reduced susceptibility when tested by disc diffusion method. These isolates were confirmed to be susceptible using the E-test. A few reports on reduced susceptibility to tigecycline have been published, including small numbers of both MSSA and MRSA strains from clinical samples in Brazil, India, Libya, and Taiwan. The use of an appropriate method to screen for resistance to this antibiotic is critical. Therefore, tigecycline resistant strains should be subjected to reference testing and archived until a clinical link is revealed.

In this study, it was observed that the frequency of resistance peaked during the first six months and thereafter declined. This trend correlates with the general trend of *S. aureus* NP carriage i.e. high- and diverse- early in life and declining over time. In addition, the presence of strains in a *spa*-CC groups at various time points was associated with some antibiotic resistance phenotypes. This was clear for gentamicin; besides the MRSA strains, resistance to gentamicin was predominantly found in MSSA strains belonging to *spa*-CC891, while tetracycline resistance was found in strains within *spa*-CC84, *spa*-CC701, and *spa*-CCNF2.

Analysis of the antibiotic susceptibility profiles longitudinally revealed shifts within the same *spa* carried by the same infant. Most of these events were changes from susceptible to resistant profile for certain antibiotics. It was observed that one MSSA strain from an infant (3207) became
methicillin- and gentamicin resistant within a two-week period, suggesting either an acquisition event of the \textit{SCCmec} element by a susceptible strain or acquisition of a new MRSA strain. Although we did not perform molecular detection for the involved resistance genes, acquiring resistance could be attributed to chromosomal mutations (as in \textit{rpoB} for rifampicin resistance); acquisition of plasmid-borne resistance genes (such as \textit{tet} genes which confer resistance to tetracycline; and \textit{dfrrA} gene which confer resistance to cotrimoxazole)\textsuperscript{52–54}. The factors for the loss of resistance events are unclear but, it may occur if resistance becomes a fitness burden on the strain, particularly in healthy individuals with presumably minimal use of antibiotics \textsuperscript{55}. Moreover, loss of the mobile genetic elements carrying resistance genes through \textit{in-vitro} serial passage should also be considered \textsuperscript{56}. Performance of whole genome sequencing would be ideal to better understand the genetic factors involved in these shifts.

### 6.6 Conclusion

This study provides baseline data on the antimicrobial susceptibility patterns of \textit{S. aureus} strains carried by a healthy population in South Africa. We report very low MRSA carriage among infants. Generally, very low levels of resistance was noted to all tested antibiotics expect for penicillin. The resistance phenotype to gentamicin was mainly related to a specific genotype cluster. Also, resistance to rifampicin and cotrimoxazole was associated with \textit{spa}-CCNF1 and \textit{spa}-CCNF2. Erythromycin and clindamycin should be used with caution for empiric presumptive treatment of \textit{S. aureus} infections in settings with high resistance to these antibiotics.
References

Antibiotic Susceptibility patterns


34 Huang YC, Chou YH, Su LH, Lien RI, Lin TY. Methicillin-resistant Staphylococcus aureus colonization and its association with infection among infants hospitalized in neonatal intensive care units.
Antibiotic Susceptibility patterns

*Pediatrics* 2006; **118**: 469–74.


Antibiotic Susceptibility patterns


Chapter Seven

Longitudinal assessment of the dynamics of *S. aureus* nasopharyngeal carriage in healthy infants during the first year of life
7.1 Summary

*Staphylococcus aureus* nasopharyngeal (NP) carriage dynamics during infancy have not been comprehensively studied at the genotype level.

NP swabs were collected at birth from 137 mother-infant pairs, and every two weeks from infants for the first year of life. All *S. aureus* isolates were characterized by *spa* typing. In the analyses described in this chapter, *spa* genotyping data were used to describe recurring *S. aureus* acquisition events using a gap-time conditional model. The incidence of *S. aureus* acquisition at the genotype level was also assessed. The Cox proportional hazards model was used to determine the risk factors associated with *S. aureus* first acquisition events. Likely transmission events between mothers and their infants were also determined using the genotyping data. The median carriage duration for each *spa*-clonal complex (CC) was determined, and the difference between these medians was assessed using the median permutation test. The different *S. aureus* NP carriage patterns were determined by combining the carrier index (the proportion of *S. aureus* positive samples collected for each infant during the first year of life) with each of two genotype diversity measurements: the absolute number of *spa*-CCs carried by each infant; or the alpha diversity (Shannon diversity index).

The rate of acquisition at the species level during the first year of life was 1.8 first acquisitions per 137 child-year as oppose to 2.4 first acquisitions per 137 child-year at the genotype level (*p*=0.0006). The overall incidence of acquisition at the species and genotype levels were 0.24 and 0.56 episodes per 100 child-days. Maternal carriage, TB disease, large family size and study site were associated with time to first *S. aureus* acquisition event. No significant difference was observed between the median carriage duration of all 11 *spa*-CCs. A positive correlation was observed between carrier index and each of the genotype diversity measurements. Based on the carrier index values (at the species level) 12% of infants were non-carriers, and 7% had prolonged carriage. At the genotype level, only 2% had prolonged carriage with a single strain.

In conclusion, this chapter showed that *S. aureus* NP carriage is highly dynamic in infants. Based on a carrier index of 0.8 we observed no persistent carriage in this cohort. Prolonged carriage with a single strain occurred rarely. A positive correlation was observed between the increased carrier index and genotype diversity.
S. aureus carriage dynamics

7.2 Background

S. aureus can be carried asymptptomatically on epithelial and mucosal surfaces. This asymptomatic carriage is a risk factor for subsequent infections. In the adult population, longitudinal studies describe three carriage patterns based on the presence of S. aureus in the nasal cavity: persistent carriage occurs in 10%-35% of the adult population; intermittent in 20%-80% and 5%-50% are non-carriers. Among persistent carriers, it is estimated that 80%-90% of staphylococcal infections are caused by the colonizing strain. Previous studies have shown that children have higher carriage rates compared to adults, particularly, during their first year of life. Also, persistent carriage was considered more frequent among children compared to adults. Except for few studies, this concept was not confirmed by genotyping of the carried strains during the first year of life.

The carrier index, which is defined as the number of S. aureus-positive samples in an individual, divided by the total number of samples collected for the same individual during a study period, has been used to define S. aureus carriage patterns. Based on the carrier index values, individuals with indices more than or equal to 0.8 are considered as persistent carriers, while individuals with zero index are non-carriers, and indices between 0 and 0.8 are considered as intermittent carriers. However, some studies used carrier index of 1 (all samples were positive) to define persistent carriage. The number of swabs collected in longitudinal studies ranged from 5-12 swabs collected over several weeks to months. Seven swabs collected over five weeks were shown to reliably distinguish S. aureus non-carriers from intermittent carriers. An algorithm based on the bacterial load in one or two swabs (more than 10^3 colony forming units per swab) was shown to accurately identify persistent nasal carriers with 83% sensitivity and 96% specificity when compared to the carrier index as a reference. Despite all the efforts to determine S. aureus nasal carriage patterns among different populations, a consensus definition for persistent carriage is still lacking.
**S. aureus carriage dynamics**

However, since the carrier index defines *S. aureus* nasal carriage patterns at the species level and not at the genotype level, it provides an incomplete description of the true nature of carriage in individuals. Genotyping of the carried strains gives a better understanding of the dynamics and diversity of the strains identified in the set of collected samples. Few studies have used strain genotyping to support their definitions of persistent vs. intermittent nasal carriage, especially among healthy infants. Describing *S. aureus* carriage in the adult population based on the genotyping data revealed that carriage is dynamic in nature; carriers acquiring and losing different genotypes over time. Similar analysis was not performed among infants. Combining the carrier index and the strain diversity measurements might add more insights to better understand the carriage dynamics in both persistent and intermittent nasal carriers.

The determinants of *S. aureus* carriage are multifactorial; together host, bacterial and environmental factors play key roles in determining carriage status. Maternal *S. aureus* carriage is considered as one of the main risk factors for *S. aureus* acquisition by their infants. However, direct transmission of *S. aureus* from mothers to their infants was evident only in the studies which performed genotyping on the carried strains.

This chapter describes the longitudinal dynamics of *S. aureus* NP carriage among healthy infants during their first year of life, at the genotype level. The specific aims are to i) describe *S. aureus* acquisition both at the species and the genotype levels; ii) calculate the incidence of acquisition at the genotype level (incidence at the species level was described in Chapter 3); iii) determine the risk factors associated with *S. aureus* first acquisition and loss events at any given time point during the study period; iv) utilise genotype data to describe the likely transmission of *S. aureus* between mothers and their infants; v) determine the carriage duration for each of the *spa*-CCs carried by infants during the first year of life and vi) define *S. aureus* carriage patterns using the carrier index combined with genotype diversity measures.
7.3 Methods

7.3.1 Study population and S. aureus strains

A total of 3417 nasopharyngeal swabs (NP) (3292 from infants every two weeks for the first year of life and 125 from mothers at birth) were collected from 137 mother-infant pairs. A minimum number of 18 NP swabs were collected per infant. All S. aureus isolates were identified using standard microbiological methods (section 4.3.4) and further characterised by spa typing (section 5.3.4).

7.3.2 Statistical analysis and graphical representation

7.3.2.1 S. aureus longitudinal carriage dynamics

The spa typing data described in Chapter 5 were used to describe longitudinal S. aureus carriage by different genotypes over time. All the spa types belonging to one CC were assumed to be of the same genotype for purposes of this study. In addition, spa typing data were also utilised to describe the possible transmission of S. aureus between the carrier mothers and their infants. These dynamics were displayed as heat maps which were plotted using the software R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) 21.

S. aureus NP carriage was divided into three elements: acquisition, loss of carriage (clearance) and carriage duration. The definition of these elements was adapted from a previous healthcare-based longitudinal study on an adult population 17. There are a number of difference in terms of the number of swabs collected, the interval between swabs and the study duration between our study and the study by Miller et al 17. Therefore, in this study S. aureus acquisition was defined as either a S. aureus positive swab after at least two confirmed negative swabs or detection of new spa-CC in a child with prior positive swabs with a different spa type. Kaplan-Meier survival analysis was used to describe the time to S. aureus acquisition events (i.e. first acquisition, second, third, etc.) both at the species and the genotype levels. Recurrent acquisition episodes were determined using the conditional gap-time model. This model is a form of survival analysis which assumes that the recurring events (failure) within the same subject are dependent or ordered i.e.
the second event cannot occur before the first event. So in this analysis, the time to the following
*S. aureus* acquisition event from the previous acquisition event within each infant was modelled.
Loss of *S. aureus* carriage was defined as at least two consecutive negative swabs for *S. aureus* or
two consecutive swabs without the previous *spa* type. Both acquisition and loss events were used
to define the carriage duration of each *spa*-CC. The carriage duration was determined by the
difference between the loss date and the acquisition date (i.e. the period between acquisition and
loss). We assumed that *S. aureus* acquisition or loss occurred sometime between the time point
where the event occurred and the previous sampling point, therefore, the mid-point between
these two samples was considered as the start of the event. To determine if there is a significant
difference between median carriage duration of each of the *spa*-CCs, a median permutation test
was performed. In this permutation many replication of the test statistic were created, where
there is no difference by randomly shuffling the carriage duration days between the two CCs.
Then the achieved significance level (ASL) is the proportion of times the permuted test values are
larger than the observed test statistic value. This can be interpreted similar to a *p*-value (values
smaller than 0.05 considered as significantly different). The median carriage duration of PVL-
positive and PVL-negative strains was also calculated.

In chapter 3, the factors associated with *S. aureus* carriage at any time point during the study
period were discussed. In this section, the aim was to determine the factors associated with *S.
aureus* first acquisition event using the Cox proportional hazards regression model (Breslow
method). Although the rationale for identifying the “loss of carriage” events was to determine the
carriage duration, we sought to investigate the possible factors which may have an effect on losing
*S. aureus* carriage using the same model as was used for first acquisition (Cox model).
Determining the risk factors associated with *S. aureus* first acquisition was performed only at the
species level since the study might be under powered to efficiently assess the factors for new
acquisitions at the genotype level. The variables included in the two models were: preterm birth,
delivery mode, maternal *S. aureus* carriage and education, day care attendance, HIV-exposure,
feeding method during the first six months of life, having animals in the household, having a young
sibling (≤5 years old), socioeconomic status, family size (≥ 5 members) and hospital admission. The analysis was done in Stata® version 12.1 (StataCorp, Texas, USA).

7.3.2.3 Defining *S. aureus* nasopharyngeal carriage patterns

In order to define *S. aureus* carriage patterns - persistent, intermittent, and no carriage, the carrier index was calculated - defined as the number of *S. aureus* positive NP swabs over the total number of swabs collected for each infant. Two genotype diversity measures were used, each in combination with the carrier index to assess *S. aureus* NP carriage patterns in this cohort. The diversity measures were: the absolute number of genotypes (spa-CCs) carried by each infant, and the Shannon Diversity Index - also known as alpha diversity. In our context, the alpha diversity measures the variation in the spa-CCs within the sample set of a single infant over the first year of life. The Pearson’s correlation coefficient for the carrier index and the absolute number of spa-CC and also for the carrier index and alpha diversity was estimated. The analysis was collectively performed in R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) 21.

7.4 Results

7.4.1 *S. aureus* NP carriage dynamics

7.4.1.1 *S. aureus* acquisition

Overall, 88% (121/137) of infants acquired *S. aureus* at least once during their first year of life, and 17% (21/125) mothers were *S. aureus* carriers at birth. By 91 days of age 75% of infants had acquired *S. aureus* for the first time (Figure 7.1a). A total of 249 *S. aureus* acquisition events occurred at a rate of 1.8 acquisitions per 137 child-year during the first year of life. Forty-nine percent of these acquisitions (121/249) were first events, which occurred at median age 23 days (95%CI, 19-36 days). Of the 121 carrier infants, 65% acquired *S. aureus* for a second time at a median age 183.5 days (95%CI, 168-253 days); 30% acquired for a third time, and 8% of carrier infants had four acquisition events (Figure 7.1a). Throughout the study period, two infants acquired *S. aureus* at up to five different occasions. At the genotype level, the total acquisition rate was 2.4 acquisitions per 137 child-year. The difference in the acquisition rate at the species and
the genotype levels was highly significant ($p= 0.0006$). The median age for the first genotype acquisition event was 23 days (95%CI, 19 - 36 days). Of the 121 infants who had a first genotype acquisition, 76% had a second genotype acquisition (either identical, related or a different spa type) at a median age 113 days (95%CI, 94 - 154). A total of eight new acquisition events at the genotype level occurred in two infants (Figure 7.1b).

<table>
<thead>
<tr>
<th>Event No.</th>
<th>No. Infants at start</th>
<th>No. acquisition events</th>
<th>Median time (days)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>137</td>
<td>2</td>
<td>23</td>
<td>19-36</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>7</td>
<td>103.5</td>
<td>100-253</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>37</td>
<td>154</td>
<td>125-425</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>18</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>2</td>
<td>140</td>
<td>120-NA</td>
</tr>
</tbody>
</table>

(a)

The table in the figure corner shows the statistics for the four acquisition events. (b) Acquisition events analysed at the genotype level. We plotted six out of the eight (4 infants had 7 events and 2 had 8 events) acquisition events occurred at the genotype level. The table shows the statistics for the acquisition events.

Figure 7.1: Survival time (days) to *S. aureus* acquisition events in 137 healthy infants during the first year of life.
Recurring acquisition events both at the species and the genotype levels were assessed using the conditional gap-time model. (a) Acquisition events analysed at the species level. A total of five acquisition events occurred at the species level, however, we plotted 4 events since only two infants had a fifth event. The table in the figure corner shows the statistics for the four acquisition events. (b) Acquisition events analysed at the genotype level. We plotted six out of the eight (4 infants had 7 events and 2 had 8 events) acquisition events occurred at the genotype level. The table shows the statistics for the acquisition events.
The incidence of acquisition at the genotype level was assessed. The incidence of acquisition of a new genotype was highest between birth and two weeks of age, and declined over time (Figure 7.2). The overall genotype acquisition incidence was 0.65 events per 100 child-days. The highest incidence occurred during the first four weeks; 2.25 events per 100 child-days between 0-2 weeks, and 1.72 events per 100 child-days between 2-4 weeks. The incidence then decreased over time reaching 0.48 events per 100 child-days between weeks 50-52 (Figure 7.2).

**Figure 7.2: Incidence of acquisition of different genotypes during the first year of life.**
We calculated incidence per 100 child-days at two-weekly intervals. Each data point represents the number of new acquisition events occurring in the time between the previous and the indicated time point. We fitted a smoothing function showed by the blue line with a confidence interval for the smoothing function (the area shaded in grey).
7.4.1.2 Determinants for first acquisition and clearance of *S. aureus*

The Cox proportional hazards model was used to determine factors associated with *S. aureus* first acquisition. In this model, the variables preterm birth, delivery mode, maternal education, day care attendance, HIV-exposure, feeding method during the first six months of life, having animals in the household, having a young sibling (≤5 years old), socioeconomic status, family size (≥ 5 members) and hospital admission did not have a significant effect on the rate of *S. aureus* first acquisition at any time point (Table 7.1). However, the hazard of acquiring *S. aureus* at any time point was increased with increasing number of individuals at home (Hazard ratio (HR); 1.24, 95% CI 1.07 – 1.43, \(p = 0.001\)). In addition, an infant with a carrier mother has twice the chance of acquiring *S. aureus* at any time during the first year of life compared to an infant with a non-carrier mother (HR; 2.08, 95%CI 1.07 – 4.07, \(p = 0.03\)). The hazards of acquiring *S. aureus* at any defined time point for infants from the study site TC Newman was lower compared to infants from Mbekweni (HR; 0.50, 95%CI 0.24 – 1.05, \(p = 0.07\)). Infants who were reportedly having TB at any point during the first year of life were at increased risk of acquiring *S. aureus* at any given time point (HR; 2.57, 95%CI 1.24 – 5.33, \(p = 0.01\)). *S. pneumoniae* NP carriage was associated with reduced risk of acquiring *S. aureus* at any given time during the Study period (HR; 0.53, 95%CI 0.31-0.91, \(p = 0.02\)). On the other hand, moderate to high socioeconomic status was associated with loss of *S. aureus* carriage (Table 7.1).

A total of 21 mothers carried *S. aureus* in the nasopharynx at the day of delivery. Only one mother-infant pair carried a similar spa-type (CC15) at birth (Figure 7.3). By the age of two weeks, transmission between mothers and their infants was evident in 47% of the pairs (including the one with a carrier infant at birth). Only one pair carried different spa types at 2 weeks of age. During the first eight weeks, likely transmission between mother-infant pairs was evident in 13 out of the 21 pairs. Four infants never carried their mother’s strain throughout the study period (Figure 7.3). However, as demonstrated in Figure 7.5, infants with *S. aureus* negative mothers at birth (n=116), also harboured *S. aureus* as early as two weeks of age, with one infant being a carrier at birth (Figure 7.5).
Table 7.1: Factors independently associated with *S. aureus* first acquisition and loss.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Outcome for first acquisition of <em>S. aureus</em></th>
<th>Outcome for loss of <em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR [95% Conf. interval]</td>
<td>p-value</td>
</tr>
<tr>
<td>HIV-exposure</td>
<td>0.79 (0.39-1.61)</td>
<td>0.52</td>
</tr>
<tr>
<td>Day care attendance</td>
<td>1.76 (1.03-3.00)</td>
<td>0.04</td>
</tr>
<tr>
<td>Preterm</td>
<td>0.77 (0.42-1.40)</td>
<td>0.39</td>
</tr>
<tr>
<td>Having animal</td>
<td>1.12 (0.66-2.89)</td>
<td>0.68</td>
</tr>
<tr>
<td>Smoking mother</td>
<td>0.86 (0.46-1.63)</td>
<td>0.65</td>
</tr>
<tr>
<td>Smoking father</td>
<td>0.93 (0.58-1.49)</td>
<td>0.78</td>
</tr>
<tr>
<td>No. people in household</td>
<td>1.24 (1.07-1.43)</td>
<td>0.00</td>
</tr>
<tr>
<td>Delivery mode (Vaginal vs caesarean)</td>
<td>0.80 (0.44-1.47)</td>
<td>0.48</td>
</tr>
<tr>
<td>Maternal <em>S. aureus</em> carriage</td>
<td>2.08 (1.07-4.07)</td>
<td>0.03</td>
</tr>
<tr>
<td>Study site (TC Newman vs Mbekweni)</td>
<td>0.50 (0.24-1.05)</td>
<td>0.07</td>
</tr>
<tr>
<td>Hospital admission</td>
<td>0.74 (0.34-1.63)</td>
<td>0.46</td>
</tr>
<tr>
<td>Feeding method at 6month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula feeding (vs breast-feeding)</td>
<td>1.02 (0.51-2.07)</td>
<td>0.95</td>
</tr>
<tr>
<td>Mixed feeding (vs breast-feeding)</td>
<td>1.19 (0.50-2.82)</td>
<td>0.69</td>
</tr>
<tr>
<td>Having TB</td>
<td>2.57 (1.24-5.33)</td>
<td>0.01</td>
</tr>
<tr>
<td>Having younger sibling</td>
<td>0.75 (0.49-1.14)</td>
<td>0.18</td>
</tr>
<tr>
<td>Low-moderate SES</td>
<td>1.04 (0.52-2.07)</td>
<td>0.92</td>
</tr>
<tr>
<td>Moderate-high SES</td>
<td>2.20 (0.85-5.67)</td>
<td>0.10</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> carriage</td>
<td>0.53 (0.31-0.90)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note: HR, hazard ratio; TB; tuberculosis; SES; socioeconomic status. The significant variables are highlighted in **Bold**

Figure 7.3: Transmission of *S. aureus* (spa types) within mother-infant pairs.
7.4.1.3 Carriage duration of the different spa-clonal complexes

Of the 137 infants, 12% never carried *S. aureus* in the nasopharynx throughout the study period. Nineteen percent (137/704) of isolates belonging to all *spa*-CCs were carried for a time interval between 14-21 days (figure 7.4). The longest carriage duration observed was seven months (215 days) by *spa*-CC15 (Figure 7.4). A median carriage duration of 14 days was observed for eight out of the 11 *spa*-CCs: CC2 (Inter-quartile range (IQR); 14-55 days), CC15 (IQR; 14-70 days), CC21 (IQR; 14-34.5 days), CC174 (IQR; 14-45 days), CC701 (IQR; 14-41 days), CCNF1 (IQR; 14-28 days), CCNF2 (IQR; 14-126 days) and CCNF3 (IQR; 14 – 25 days). For the remaining four *spa*-CCs, longer median carriage duration was observed 20.5 days for CC891 (IQR; 14-42 days), 29.5 days for CC84 (IQR; 14-56 days), and 42 days for CC349 (IQR; 14-52). According to the median permutation test, no significant difference was observed between the carriage duration of each of the *spa*-CCs (Table 7.2). A similar median carriage duration was observed from both PVL-positive and PVL-negative strains (14 days).

Figure 7.4: The carriage duration of the different genotypes (*spa*-clonal complexes) carried by the 137 infants distributed over different time intervals.
Figure 7.5: The longitudinal *S. aureus* carriage profiles by different genotypes (*spa*-clonal complexes) for the 137 mother-infant pairs during the first year of life. The first column is showing the nasopharyngeal carriage profiles of mothers at birth. W00 to w52 are the sampling time points throughout the study period. The group “excluded” are the *spa* types with were excluded from the BURP clustering due to the small number of repeats. NF; clonal complexes with No Founder.
### Table 7.2: Summary of the median (carriage duration) permutation for the 11 spa-CCs.

<table>
<thead>
<tr>
<th>Spa-CC</th>
<th>CC2</th>
<th>CC15</th>
<th>CC21</th>
<th>CC84</th>
<th>CC174</th>
<th>CC346</th>
<th>CC701</th>
<th>CC891</th>
<th>CCNF1</th>
<th>CCNF2</th>
<th>CCNF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC2</td>
<td>NA</td>
<td>0.56</td>
<td>0.54</td>
<td>0.29</td>
<td>0.35</td>
<td>0.20</td>
<td>0.06</td>
<td>0.60</td>
<td>0.40</td>
<td>0.38</td>
<td>0.40</td>
</tr>
<tr>
<td>CC15</td>
<td>NA</td>
<td>0.63</td>
<td>0.19</td>
<td>0.46</td>
<td>0.18</td>
<td>0.18</td>
<td>0.70</td>
<td>0.46</td>
<td>0.47</td>
<td>0.47</td>
<td>0.46</td>
</tr>
<tr>
<td>CC21</td>
<td>NA</td>
<td>0.24</td>
<td>0.42</td>
<td>0.05</td>
<td>0.18</td>
<td>0.62</td>
<td>0.53</td>
<td>0.53</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC84</td>
<td>NA</td>
<td>0.47</td>
<td>0.65</td>
<td>0.03</td>
<td>0.54</td>
<td>0.25</td>
<td>0.43</td>
<td>0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC174</td>
<td>NA</td>
<td>0.18</td>
<td>0.08</td>
<td>0.58</td>
<td>0.29</td>
<td>0.30</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC346</td>
<td>NA</td>
<td>0.07</td>
<td>0.17</td>
<td>0.11</td>
<td>0.48</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC701</td>
<td>NA</td>
<td>0.10</td>
<td>0.24</td>
<td>0.25</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC891</td>
<td>NA</td>
<td>0.64</td>
<td>0.43</td>
<td>0.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCNF1</td>
<td>NA</td>
<td>0.39</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCNF2</td>
<td>NA</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCNF3</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CC; Clonal complex, NF; no founder, NA; not applicable. The diagonal line is the comparison with the same spa-CC. Significant difference should be less than 0.05. The table is showing the raw permutation values. If these values were converted to the Achieved Significance level (ASL) all the values in the table would be equal to 1 i.e. no significant difference between the median carriage duration for each of the spa-CCs.

#### 7.4.2 *S. aureus* nasopharyngeal carriage patterns among infants during the first year of life

Figure 7.5 shows the NP carriage profiles by different genotypes for the 137 mother-infant pairs two-weekly for the first year of life. The median number of NP swabs collected per infant was 24 (IQR; 24-27) with a minimum of 18 swabs. Based on the carrier index, 12% (16/137) of infants were non-carriers, while all other 121 infants had indices between 0.03 and 0.74 (Figure 7.6). Using a carrier index of 0.8 as a cut-off to distinguish between persistent and intermittent carriage, we observed no persistent carriers among these infants (Figure 7.6). Therefore, we elected to refer to infants with a carrier index of more than 0.5 as having “prolonged carriage”.

Accordingly, looking at the species level, 6% (7/121) were infants with prolonged carriage. However, at the genotype level, only 2% (2/121) of infants carried a single genotype in more than 50% of their samples (Figure 7.6). Thirty nine percent (47/121) of the infants carried one genotype throughout the first year, but their carrier index ranged from 0.03 to 0.74. Two infants carried five different genotypes and the carrier index was 0.47 and 0.5 for each infant. A positive correlation ($r = 0.5; 95\% CI 0.35 - 0.62, p < 0.0001$) was observed between the carrier index and the absolute number of different spa-CC carried by an infant (Figure 7.7a). The alpha diversity, which measures the diversity of the genotypes identified in the set of samples of one infant over
time, ranged from 0 to 1.73 (Figure 7.7b). The median alpha diversity observed was 1.3 (IQR; 1.03-1.52). Similar to the absolute number of *spa*-CC, a positive correlation was observed between the alpha diversity and the carrier index ($r=0.6; 95\% \text{ CI } 0.46 - 0.70, p < 0.0001$).

**Figure 7.6:** The carrier indices for 137 infants followed up longitudinally every two weeks for the first year of life.

The carrier index is defined as the *S. aureus* positive swabs over the total number of NP swabs collected per infant during the study period. (a) The carrier index values at the species level. (b) The carrier index values analysed by the number of genotypes carried by each infant. The Orange bars represent the number of infants carrying a single genotype in all samples, and the Blue bars represent the number of infants carrying more than one genotypes in their NP samples.
Figure 7.7: A scatter plot for the carrier index and the diversity measures.
(a) The carrier index vs absolute number of spa-CC carried by each infant showing a positive correlation.
(b) The carrier index vs the alpha diversity showing a stronger positive correlation than the absolute number spa-CCs. Each pie chart represent the carriage profile for each infant throughout the first year. The squared dots in both figures indicate infants who carried a single genotype in all positive samples.
7.5 Discussion

Thanks to the intensive longitudinal design of this study, we are able for the first time to describe in details the dynamics of *S. aureus* NP carriage among healthy infants during the first year of life. Using the *spa* typing data to describe these dynamics allowed for discrimination at the genotype level rather than only at species level. The rate of acquisition analysed at the species and the genotype levels varied significantly; 1.8 vs 2.4 acquisitions per 137 child-year respectively. An increased number of acquisition events was revealed when considering the *spa* typing data which would have been unrecognized if only the acquisition at the species level was considered. By 91 days of age 75% of infants acquired *S. aureus* for the first time. In a Gambian birth cohort study, the same proportion of infants (75%) acquired *S. aureus* sooner than observed in this study i.e. by the age of 30 days\(^2^2\). The overall incidence of acquisition at the genotype level was 0.65 episodes per 100 child-days during the first year of life, which was higher than the incidence of *S. aureus* acquisition (0.24 events per 100 child-days) (described in Chapter 3). Studying bacterial carriage at the genotype level will extend our understanding about the different dynamics involved in the carriage process\(^1^7\).

Large family size (>5 individuals), *S. aureus* maternal carriage, and TB disease increased the risk of acquiring *S. aureus* at any given time point. These findings are in line with previous studies which investigated the factors associated with carriage dynamics\(^2^3–^2^5\). It is not clear in this study why TB (at any time during the first year of life) was associated with increased risk of acquiring *S. aureus*. Nonetheless, in TB endemic areas, TB can contribute directly to the burden of childhood pneumonia or it might be an underlying comorbidity which may lead to the development of secondary bacterial infections of the respiratory tract\(^2^6\). In support to this, increased rates in the detection of secondary bacterial infections including *S. aureus* was observed among individuals with pulmonary TB in South Africa\(^2^7,^2^8\). Analysis of the *spa* types carried by the mother-infant pairs revealed that 81% of infants born to a *S. aureus* carrier mother harboured the same maternal strain by the latest eight weeks of age. Majority (47%) of these transmissions occurred by two-weeks of age, and only four infants with carrier mother never shared the maternal strain.
**S. aureus carriage dynamics**

throughout the study period. The fact that 84% (n=116) of mothers were not *S. aureus* carriers and 45% of infants who had samples collected at two weeks carried *S. aureus*, suggested the involvement of other factors, for example, maternal skin carriage or carriage by other family members as possible source of *S. aureus* as shown by previous birth cohort studies 18. The direction of the *S. aureus* transmission might be difficult to determine in this study, however, since all mothers already carried *S. aureus* at birth, while, all infants except for one did immediately after birth, suggest mother-to-infant transmission or a common source. The one infant born to a carrier mother, who carried *S. aureus* at birth may indicate vertical transmission during delivery 18,23.

The median carriage duration for most of the spa-CC was 14 days, and no significant difference was observed in the median carriage duration for all spa-CCs. Also, no association was found between certain genotypes and duration. To our knowledge, there is only one healthcare based study which determined *S. aureus* carriage duration of certain genotypes and found that spa-CC15 was associated with intermittent rather than prolonged carriage 17.

Based on the carrier index, 12% of the infants were non carriers (i.e., carrier index = 0). No bimodal distribution was observed for the other infants consistent with persistent and intermittent carriage. When a carrier index of 0.8 or more was used to define the carriage patterns (as proposed by previous studies), all carriers had indices less than 0.8 (at the species level) suggesting that persistent carriage (based on carrier index 0.8) did not exist in this cohort. Combining the carrier index with the genotyping diversity, it appears that the higher the carrier index the higher the genotype diversity, i.e., infants with relatively prolonged carriage also harboured more diverse genotypes. This observation is in contrast with the notion that persistent carriers carry the same genotype all the time 10,29. The way the carrier index was applied in previous studies, takes into consideration only the frequency at which *S. aureus* was detected in a set of samples and ignores the actual genotype which might result in inaccurate definitions. For example, in this study, considering carrier index of more than 0.5, seven infants carried *S. aureus* and were named “prolonged carriers”, but looking at the genotypes carried by these seven infants,
only two infants carried a single strain throughout the first year. These data show the importance of genotyping when defining different carriage patterns.

The differences between previous longitudinal studies on *S. aureus* carriage in terms of the age of the studied population, number of samples and intervals between samples collected, and the definition of the different carriage patterns, makes it complicated to compare the results between studies. There are few studies which investigated the carriage dynamics among healthy infants during the first year of life. In a longitudinal study in which *S. aureus* NP carriage was investigated in 100 healthy infants from the United Kingdom nine NP swabs were collected per infant during the first six months of life. The authors showed that 25% of infants were never positive for *S. aureus*, and approximately 60% of the infants carried a single genotype in all nine swabs. Another birth cohort study - The Generation R Study - in which three NP swabs were collected from 443 infants over 14 months; only two swabs were collected during the first year of life (at 1.5 and 6 months). In this study, 36% of infants never carried *S. aureus* in all three swabs, while, only two infants carried a single strain in all three swabs. In both studies described above, the relatively higher "no-carriage" rates may be overestimated, since both studies collected a relatively smaller number of swabs over larger time intervals than our study, and *S. aureus* carriage may have been missed between sampling points. Verhoeven and colleagues suggested that a total of seven swabs collected over five weeks are sufficient to distinguish persistent carriage from the other patterns. However, non-carriage and persistent carriage rates tend to decrease with increasing follow-up periods and decreasing culture intervals. In our study, a median number of 24 NP swabs was collected per infant every two weeks for a whole year with a minimum of 18 swabs. Therefore, our data may provide a more accurate estimate of "true" non-carriers due to the increased number of swabs and the shorter intervals between swabs compared to previous studies.

Additionally, a healthcare based longitudinal study which looked at the dynamics of *S. aureus* nasal carriage among adults admitted to a primary care centre in Oxford, also suggested that true non-carriage does not exist. Moreover, based on the continuous new acquisition rates observed
in their study (the cumulative new acquisition probability never plateau), the authors extrapolated that a 5-10 years follow-up is needed to confirm a “never carriage” phenotype.\textsuperscript{17} The fact that the study was carried out within a healthcare setting, where the exposure to pathogens including \textit{S. aureus} is high, may explain the continuous new acquisition rate in the study.\textsuperscript{17,31,32} However, in our study the incidence of acquisition reached stable levels towards 52 weeks of age, suggesting that the 12\% \textit{S. aureus} negative infants we observed were probably true non-carriers. It would be ideal to follow up those \textit{S. aureus} negative infants beyond the first year of life to monitor the carriage dynamics for a longer duration.

Carriage duration as well as the diversity of the genotypes carried by the individual are among the criteria used to distinguish intermittent from persistent carriers; \textit{S. aureus} persistent carriers tend to carry less diverse strains for longer durations.\textsuperscript{11,13,14} In this study, neither the carriage duration nor genotype diversity were able to segregate the infants into two distinct groups. In fact, infants with prolonged carriage harboured more diverse carriage profiles. Although \textit{spa}-CC15 was previously found to be less likely associated with long term compared to intermittent carriage,\textsuperscript{17} in this study no association between carriage duration and genotypes was observed.

A previous study of intermittent and persistent healthy adult carriers who were monitored from one to three years, revealed high genetic relatedness and similar genotype exchange rate over time among these two groups, indicating no preferential colonisation of persistent or intermittent carriers by a specific strain.\textsuperscript{13} The genotypes which were carried transiently in this study were genetically related to those carried for prolonged periods which is in line with previous studies.

Altogether, our data suggest that the true persistent carriage (harbouring a single strain over time) is rare during infancy, however, transient and prolonged carriage do occur.

In this study, it was not possible to analyse the carriage risk factors based on the carriage patterns since only two infants had a “true” prolonged carriage with a single genotype. Other studies have also not reported any specific association between bacterial genotype and certain carriage pattern.\textsuperscript{11,13} But, factors such as bacterial interference between \textit{S. aureus} and other microbes within one niche, regulatory adaptation of \textit{S. aureus} itself, and human DNA polymorphism were
shown to be associated with persistent carriage \textsuperscript{33,34,35}. Of note, the polymorphisms in coagulase and \textit{spa} genes which are used for \textit{S. aureus} genotyping do not contribute to persistent carriage\textsuperscript{10}, which might partly explain the lack of any associations between certain genotypes (mainly \textit{spa} types) and a carriage pattern. In contrast, the genes involved in the carriage process such as clumping factors A and B (involved in adherence) and putative glycosyltransferase (\textit{tagX}; involved in wall teichoic acid biosynthesis) have been shown to accumulate a high proportion of non-synonymous mutations, which could possibly affect the pathogenesis and influence the carriage capability of a given strain \textsuperscript{36,37}.

\section*{7.6 Conclusion}

This longitudinal analysis showed that persistent carriage as defined by a carrier index of 0.8 did not occur in this cohort during the first year of life. However, prolonged carriage did occur, but was uncommon. When comparing our data to previous published studies looked at \textit{S. aureus} carriage among adults, we observed that \textit{S. aureus} NP carriage is more dynamic during infancy compared to adults. Increased carrier index was positively associated with genotype diversity. Similar or genetically related genotypes were observed in infants with transient/intermittent carriage and infants with prolonged carriage. Using genotyping data extended our understanding of the carriage dynamics in healthy infants during their first year of life.
S. aureus carriage dynamics

References

S. aureus carriage dynamics


S. aureus carriage dynamics


Chapter Eight

General discussion

*S. aureus* remains an important human pathogen, both in the community and as the number one cause of hospital associated infections. Colonisation is an important source of these infections and it is estimated that approximately up to two billion healthy individuals may carry *S. aureus* worldwide. The highest *S. aureus* carriage rates are observed during infancy, particularly within the first eight weeks of life. Infants are immunologically and microbiologically naïve, therefore performing carriage studies during infancy provide insights into the bacterial and host factors which influence the colonisation process. However, very little is known regarding *S. aureus* nasopharyngeal carriage during infancy, especially in low and middle income countries. The longitudinal study described in this dissertation can be considered as a baseline community-based study describing the epidemiology, determinants, and dynamics of *S. aureus* nasopharyngeal carriage among healthy infants in South Africa.

In this cohort, *S. aureus* was detected from 21% of the NP swabs collected from 137 mother-infant pairs. The prevalence of nasopharyngeal *S. aureus* carriage among mothers was 17% at birth, which was lower than the rates reported in other similar longitudinal studies. In infants, the prevalence of *S. aureus* was highest during the first eight weeks, and declined sharply with age. A similar trend was noted in other longitudinal studies on *S. aureus* carriage during infancy, despite variation in the geographical regions, indicating the high vulnerability of newborns to *S. aureus* carriage early in life.

In this study, *S. aureus* NP carriage was independently associated with male gender, maternal *S. aureus* carriage status at birth, day care attendance, high socioeconomic status, warm season, and large family size. In contrast, both maternal and paternal smoking, and having an animal in the household were negatively associated with *S. aureus* NP carriage. All these determinants for *S. aureus* carriage correlated with previous studies. The infants included in this study
represented two different ethnic groups; Black African (from Mbekweni) and Coloured (from TC Newman). Feeding methods influenced \(S.\ aureus\) NP carriage differently between these two groups; exclusive breast-feeding was a risk factor among infants from Mbekweni, while this was not the case for infants from TC Newman. Perhaps because mothers from Mbekweni also had higher carriage rates in comparison with those from TC Newman. The high maternal \(S.\ aureus\) carriage rate may be related to HIV status since almost all HIV-infected mothers were from Mbekweni. Breast feeding by a carrier mother has previously been shown to be a risk factor for \(S.\ aureus\) nasal carriage during infancy. HIV-exposed infants were at increasing risk of carrying \(S.\ aureus\) over time; twice as likely to carry \(S.\ aureus\) by one year of age compared to unexposed infants. For infants who carried \(S.\ pneumoniae\) in the NP, a positive correlation between carriage of \(S.\ aureus\) and \(S.\ pneumoniae\) was noted early in life (up to 2 months of age), but this changed to an inverse association over time. The early life positive association between carriage of \(S.\ aureus\) and \(S.\ pneumoniae\) was not reported in previous studies and requires further investigation. In addition, the inverse association between NP carriage of \(S.\ aureus\) and \(S.\ pneumoniae\) was absent among HIV-exposed infants. The absence of this inverse association extends the findings previously reported on HIV-1-infected South African children with 4 month median age 9.

To fill in the gap in knowledge on the population structure of MRSA in Africa, we carried out a systematic review focusing on the molecular epidemiology of MRSA within Africa. Although this review highlighted the scant data available, and proposed the need for more surveillance studies across Africa, it captured the diversity of the MRSA strains circulating within the African continent. The MLST clonal complex 5 was the predominant cluster in Africa, and was mainly related to healthcare settings. While, some MRSA clones such as the Brazilian-Hungarian ST239-III [3A] showed a cross-continent dissemination, some clones such as ST612-IV [2B] were mainly limited to South Africa. Another observation in this review was the small number of community-based studies 10. In the current study MRSA was detected only in 12 (2%) NP swabs collected from six out of the 137 infants, and none of the mothers at birth. The fact that all MRSA strains except for one were detected within a limited time span early in life, belonged to the same clone
(t045-SCCmec I [1A]- PVL negative), and shared similar antibiotic resistance profiles is suggestive of a common source for transmitting this clone to these infants. The one MRSA strain which was genetically distinct; different spa type, PVL-positive, susceptible to all non-β lactam antibiotics, was identified at relatively later time.

Typing of all the identified S. aureus strains allowed for discrimination at the genotype level, however, the continuous genetic rearrangements occurring at the spa locus, particularly, in the X-variable region, affect the typeability of some strains or may lead to miss-identification of mixed carriage with deletions in one or more strains. A total number of eight strains were untypeable using the standard spa primers, due to rearrangements in the forward primer annealing site. They were however, resolved using a previously modified forward primer. Therefore, the use of updated spa typing protocols should always be considered especially among phenotypically confirmed S. aureus isolates with negative spa results. Overall, MSSA strains identified in this study showed a high level of genetic heterogeneity with relatively low level of resistance to most antibiotics, except for penicillin. However, resistance to cotrimoxazole, rifampicin, and fusidic acid was observed, which was not observed among MRSA strains. Spa-CC15 was the predominant cluster circulating in this cohort and included primarily susceptible strains resistant only to penicillin. In addition, a strong relation between certain genotypes and resistance phenotypes was noted, for example, 11 out of the 12 MRSA strains, and 80% of gentamicin resistant MSSA strains were part of CC2 and CC891 respectively. Also, tetracycline resistance was limited to the clonal complexes CC84, CC701, and CCNF1, suggesting a clonal expansion of these genotypes within this cohort. The prevalence of PVL was 21%, and was mostly among susceptible strains, which was comparable to the rates of PVL-positive MSSA previously reported in Sub-Saharan Africa.

The longitudinal design of this study together with genotyping all the identified S. aureus isolates, allowed for detection of changes in the antibiotic resistance profiles within the same spa type carried by the same infant, which to our knowledge was not reported previously. These changes included shifts from a susceptible to a resistant profile antibiotics such as tetracycline and
cotrimoxazole to which resistance is conferred by plasmid-borne resistance genes. Moreover, changes from resistant to susceptible profiles were also noted. Whole genome and plasmid sequencing are planned to better understand the molecular basis of these changes.

To our knowledge, this is the first cohort study that describes in details S. aureus NP carriage intensively during infancy and determines the incidence of S. aureus acquisition as well as carriage dynamics both at the species and the genotype levels. A higher acquisition incidence (0.65 events per 100 child-days) was observed at the genotype level, compared to the species level (0.24 events per 100 child-days). These findings underline the importance of strain genotyping in epidemiological and clinical studies. S. aureus first acquisition was associated with maternal carriage, large family size and TB disease among infants. In addition, infants from the study site Mbekweni were at a higher risk of acquiring S. aureus at any defined time point compared to infants from TC Newman.

Classically, S. aureus persistent nasal carriage was considered to occur more frequently among children as oppose to adults. However, this conclusion was drawn based on the presence or absence of S. aureus rather than the genotype. Studies which used genotyping data to define S. aureus NP carriage patterns during infancy either did a cross-sectional analysis; collected a small number of samples over long periods; or focused only on the first six months of life. However, in this study, a median of 24 NP swabs was collected from infants over the first year of life. Since the carrier index (proportion of S. aureus positive samples) provides an incomplete description of the true nature of carriage in individuals, in this study, we combined the genotype diversity measures (number of spa-CCs and alpha diversity) together with the carrier index to define S. aureus NP carriage patterns in healthy infants during the first year of life. Genotype diversity increased with the increasing carrier index. NP carriage patterns varied according to the analysis level; at the species level, 6% (n=7) of infants had “prolonged” (more than 50% S. aureus positive samples) carriage, while only 2% had prolonged carriage with a single strain at the genotype level. This suggests that persistent carriage with a single strain is rare in this
population. These findings demonstrated that *S. aureus* NP carriage patterns should be refined by incorporating genotype measures.

**In conclusion**, the detailed analysis of *S. aureus* NP carriage both at the species and genotype levels, in this intensively sampled birth cohort adds to current knowledge of the epidemiology and determinants of carriage during infancy. In addition, this study provides a modified strategy to define different *S. aureus* carriage patterns in the healthy population.

**Future outlook**

We propose to further characterise those *S. aureus* strains associated with prolonged and intermittent carriage. This characterisation will be conducted through whole genome analysis to elucidate the possible bacterial determinants associated with a certain carriage pattern. We will also characterize those strains in which loss or acquisition of resistance occurred to determine the genetic basis for these changes. We further propose to study in detail the association between NP carriage of *S. aureus* and other members of the NP microbiome, including other commensal bacteria, fungi and viruses.
References


17. Ciftci IH, Koken R, Bukulmez A, et al. Nasal carriage of Staphylococcus aureus in 4-6 age groups in
Appendices

Appendix 1: The number of missing NP swabs over the 27 time points
Appendix 2: The incidence rate and the confidence intervals for the incidence of *S. aureus* acquisition at the species and the genotype levels

<table>
<thead>
<tr>
<th>week</th>
<th>At the species level</th>
<th>At the genotype level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IR*</td>
<td>Lower CI</td>
</tr>
<tr>
<td>0-2</td>
<td>2.450469</td>
<td>1.84115</td>
</tr>
<tr>
<td>2-4</td>
<td>1.199166</td>
<td>0.79688</td>
</tr>
<tr>
<td>4-6</td>
<td>0.625652</td>
<td>0.35531</td>
</tr>
<tr>
<td>6-8</td>
<td>0.260688</td>
<td>0.10851</td>
</tr>
<tr>
<td>8-10</td>
<td>0.469239</td>
<td>0.24415</td>
</tr>
<tr>
<td>10-12</td>
<td>0.312826</td>
<td>0.14054</td>
</tr>
<tr>
<td>12-14</td>
<td>0.364964</td>
<td>0.17399</td>
</tr>
<tr>
<td>14-16</td>
<td>0.156413</td>
<td>0.05045</td>
</tr>
<tr>
<td>16-18</td>
<td>0.417101</td>
<td>0.20859</td>
</tr>
<tr>
<td>18-20</td>
<td>0.260688</td>
<td>0.10851</td>
</tr>
<tr>
<td>20-22</td>
<td>0.417101</td>
<td>0.20859</td>
</tr>
<tr>
<td>22-24</td>
<td>0.573514</td>
<td>0.31761</td>
</tr>
<tr>
<td>24-26</td>
<td>0.677789</td>
<td>0.39356</td>
</tr>
<tr>
<td>26-28</td>
<td>0.312826</td>
<td>0.14054</td>
</tr>
<tr>
<td>28-30</td>
<td>0.573514</td>
<td>0.31761</td>
</tr>
<tr>
<td>30-32</td>
<td>0.364964</td>
<td>0.17399</td>
</tr>
<tr>
<td>32-34</td>
<td>0.312826</td>
<td>0.14054</td>
</tr>
<tr>
<td>34-36</td>
<td>0.260688</td>
<td>0.10851</td>
</tr>
<tr>
<td>36-38</td>
<td>0.312826</td>
<td>0.14054</td>
</tr>
<tr>
<td>38-40</td>
<td>0.521376</td>
<td>0.28053</td>
</tr>
<tr>
<td>40-42</td>
<td>0.312826</td>
<td>0.14054</td>
</tr>
<tr>
<td>42-44</td>
<td>0.469239</td>
<td>0.24415</td>
</tr>
<tr>
<td>44-46</td>
<td>0.156945</td>
<td>0.05062</td>
</tr>
<tr>
<td>46-48</td>
<td>0.430568</td>
<td>0.21533</td>
</tr>
<tr>
<td>48-50</td>
<td>0.238379</td>
<td>0.08947</td>
</tr>
<tr>
<td>50-52</td>
<td>0.630915</td>
<td>0.2626</td>
</tr>
</tbody>
</table>

IR; incidence rate, CI; confidence interval. * Episodes per 100 child-days.
Appendix 3: Calculations of the DNA concentration for the SCC\textit{mec} multiplex PCR.

According the protocol published by Milheirico et al. 5ng of sample DNA need to be added per reaction.

For each of the MRSA DNA samples, the concentration was standardised to 1.7 ng/ul. Adding 3ul would give 5ng in a final volume of 50ul.

Appendix 4: The relative proportions of each \textit{spa}-CC among the range of isolates at each time point
Appendix 5: Histograms for the carriage duration for each of the spa-clonal complexes.