Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP) Annual Report 2013

Author Details

[1,2]Dr Geoffrey W Coombs, [3,4]Prof Graeme R Nimmo, [5]Ms Denise A Daley, [1]Ms Tam T Le, [2]Ms Julie C Pearson, [2]Ms Hui-Leen Tan, [1,2]Dr J Owen Robinson, [6,7]Prof Peter Collignon, [8] Prof Mary-Louise McLaws, [9,10]Prof John D Turnidge for the Australian Group on Antimicrobial Resistance

[1]Australian Collaborating Centre for *Enterococcus* and *Staphylococcus Species* (***ACCESS***) Typing and Research, School of Biomedical Sciences, Curtin University, Perth, Western Australia, Australia

[2]Department of Microbiology and Infectious Diseases, PathWest Laboratory Medicine-WA, Royal Perth Hospital, Perth, Western Australia, Australia

[3]Division of Microbiology, Pathology Queensland Central Laboratory, Queensland, Australia

[4]Griffith University School of Medicine, Gold Coast, Queensland, Australia

[5]Australian Group on Antimicrobial Resistance, Royal Perth Hospital, Perth, Western Australia, Australia

[6]Department of Microbiology and Infectious Diseases, The Canberra Hospital, Australian Capital Territory

[7]School of Clinical Medicine, Australian National University, Australian Capital Territory

[8] School of Public Health and Community Medicine, UNSW Medicine, UNSW Australia, New South Wales, Australia

[9]SA Pathology, Department of Microbiology and Infectious Diseases, Women's and Children's Hospital, North Adelaide, South Australia, Australia

[10]Departments of Pathology, Paediatrics and Molecular and Biomedical Sciences, University of Adelaide, Adelaide, South Australia, Australia

Corresponding Author

Dr Geoffrey Coombs

Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (***ACCESS***) Typing and Research, School of Biomedical Sciences, Curtin University, Perth, Western Australia, Australia

Telephone: +61 8 9224 2446

Facsimile: +61 8 9224 1989

Email: Geoff.Coombs@curtin.edu.au

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Abstract

From 1st January to 31st December 2013 26 institutions around Australia participated in the Australian Staphylococcal Sepsis Outcome Programme (ASSOP). The aim of ASSOP 2013 was to determine the proportion of *Staphylococcus aureus* bacteraemia (SAB) isolates in Australia that are antimicrobial resistant, with particular emphasis on susceptibility to methicillin and to characterise the molecular epidemiology of the isolates. Overall 19.1% of the 2,010 SAB episodes were methicillin resistant which is significantly higher than that reported in most European countries. Although the SAB 30-day all cause mortality appears to be decreasing in Australia, methicillin-resistant SAB associated mortality remains high (20.1%) and was significantly higher than methicillin-sensitive SAB associated mortality (13%) ($P<0.0001$). With the exception of the β-lactams and erythromycin, antimicrobial resistance in methicillin sensitive *S. aureus* (MSSA) remains rare. However in addition to the β-lactams approximately 50% of methicillin resistant *S. aureus* (MRSA) were resistant to erythromycin and ciprofloxacin and approximately 20% resistant to co-trimoxazole, tetracycline and gentamicin. Linezolid, daptomycin and teicoplanin resistance was detected in a small number of *S. aureus* isolates. Resistance was not detected for vancomycin. Resistance was largely attributable to two healthcare associated MRSA clones; ST22-IV [2B] (EMRSA-15) and ST239-III [3A] (Aus-2/3 EMRSA). ST22-IV [2B] (EMRSA-15) has now become the predominate healthcare associated clone in Australia. Approximately 60% of methicillin-resistant SAB were due to community

associated clones. Although polyclonal almost 50% of community associated clones were characterised as ST93-IV [2B] (Queensland CA-MRSA) and ST1-IV [2B] (WA1). CA-MRSA in particular the ST45-V [5C2&5] (WA84) clone has acquired multiple antimicrobial resistance determinants including ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline. As CA-MRSA is well established in the Australian community it is important antimicrobial resistance patterns in community and healthcare associated SAB is monitored as this information will guide therapeutic practices in treating *S. aureus* sepsis.

Background

Globally *Staphylococcus aureus* is one of the most frequent causes of hospital-acquired and community-acquired blood stream infections.[1] Although there are a wide variety of manifestations of serious invasive infection caused by *S. aureus,* in the great majority of these cases the organism can be detected in blood cultures. Therefore, *S. aureus* bacteraemia (SAB) is considered a very useful marker for serious invasive infection.[2]

Although prolonged antimicrobial therapy and prompt source control are used to treat SAB,[3] mortality ranges from as low as 2.5% to as high as 40%.[4-6] Mortality rates however are known to vary significantly with patient age, clinical manifestation, co-morbidities and methicillin resistance.[7, 8] A recent prospective study of SAB conducted in 27 laboratories in Australia and New Zealand found a 30-day all cause mortality of 20.6%.[9] On univariate analysis increased mortality was significantly associated with older age, European ethnicity, methicillin resistance, infections not originating from a medical device, sepsis syndrome, pneumonia/empyema and treatment with a glycopeptide or other non-β-lactam antibiotic.

The Australian Group on Antimicrobial Resistance (AGAR), a network of laboratories located across Australia, commenced surveillance of antimicrobial resistance in *S. aureus* in 1986.[10] The use of an active surveillance strategy with

standard methodology for collection and examination of clinically significant isolates has produced longitudinal data accurately reflecting the changing prevalence of antimicrobial resistance in healthcare-acquired and community-acquired *S aureus* infections.[11, 12] In 2013 AGAR commenced the Australian Staphylococcal Sepsis Outcome Programme (ASSOP). The primary objective of ASSOP 2013 was to determine the proportion of SAB isolates demonstrating antimicrobial resistance with particular emphasis on:

1. Assessing susceptibility to methicillin
2. Molecular epidemiology of methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA).

Methodology

Participants: Twenty-six laboratories from all eight Australian states and territories.

Collection Period: From 1st January to 31st December 2013, the 26 laboratories collected all *S. aureus* isolated from blood cultures. *S. aureus* with the same antimicrobial susceptibility profiles isolated from a patient's blood culture within 14 days of the first positive culture were excluded. A new *S. aureus* sepsis episode in the same patient was recorded if it was confirmed by a further culture of blood taken more than 14 days after the initial positive culture. Data were collected on age, sex, date of admission and discharge (if admitted), and

mortality at 30 days from date of first positive blood culture. To avoid interpretive bias, no attempt was made to assign attributable mortality. Each episode of bacteraemia was designated healthcare onset if the first positive blood culture(s) in an episode were collected >48 hours after admission.

Laboratory Testing: Participating laboratories performed antimicrobial susceptibility testing using the Vitek2® (bioMérieux, France) or the Phoenix™ (BD, USA) automated microbiology systems according to the manufacturer's instructions. *S. aureus* was identified by morphology and positive results of at least one of the following tests: Vitek MS® (bioMérieux, France), matrix-assisted laser desorption ionization (MALDI) biotyper (Bruker Daltonics, Germany), slide coagulase, tube coagulase, appropriate growth on chromogenic agar and demonstration of deoxyribonuclease production. Additional tests such as fermentation of mannitol, growth on mannitol-salt agar or polymerase chain reaction (PCR) for the presence of the *nuc* gene may have been performed for confirmation.

Minimum inhibitory concentration (MIC) data and isolates were referred to the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (*ACCESS*) Typing and Research. Clinical and Laboratory Standards Institute (CLSI)[13] and European Committee on Antimicrobial Susceptibility Testing (EUCAST)[14] breakpoints were utilised for interpretation. Isolates with a resistant or an intermediate category were classified as non-susceptible. High level

mupirocin resistance was determined using a mupirocin 200µg disk according to CLSI guidelines on all isolates with a mupirocin MIC >8 by Vitek2® or >256 by Phoenix™.[13] Multi-resistance was defined as resistance to three or more of the following non-β-lactam antimicrobials: vancomycin, teicoplanin, erythromycin, clindamycin, tetracycline, ciprofloxacin, gentamicin, co-trimoxazole, fusidic acid, rifampicin, high level mupirocin, linezolid and daptomycin.

Electrophoresis of chromosomal DNA was performed as previously described on all MRSA using contour-clamped homogeneous electric field (CHEF) DR III system (Bio-Rad Laboratories Pty Ltd, USA).[15] Chromosomal patterns were examined visually, scanned with a Quantity One software (Bio-Rad Laboratories Pty Ltd, USA), and digitally analysed using FPQuest (Applied Maths NV, Belgium). Multilocus sequence typing (MLST) was performed on all unique pulsed-field types as previously described.[16] The sequences were submitted to http://www.mlst.net where an allelic profile was generated and an ST assigned.

SCC*mec* typing was performed on all MRSA with a unique pulsed-field pattern using the Clondiag *S. aureus* Genotyping Array Hybridisation Kit (Alere, USA) as previously described.[17]

Detection of Panton-Valentine leucocidin determinants (PVL) and *mecA* was performed by PCR on all MRSA as previously described.[18, 19]

A chi-square test for comparison of two proportions was performed and 95% confidence intervals (95%CI) were determined using MedCalc for Windows, version 12.7 (Medcalc Software, Ostend Belgium).

Approval to conduct the prospective data collection was given by the research ethics committee associated with each participating laboratory.

Results

From 1 January to 31 December 2013 2,010 unique episodes of *S. aureus* bacteraemia were identified. A significant difference (P<0.0001) was seen in patient sex with 65.4% (1,314) being male (95% CI 63.3% – 67.5%). The average age of patients was 58 years ranging from 0 – 102 years with a median age of 62 years. Place of onset was recorded for 1,960 of the 2,010 episodes, of which 71.6% (1,404) were hospital onset (95% CI 69.6% – 73.6%). All cause mortality at 30-days was 14.4% (95% CI 12.8% – 16.2%). Methicillin resistant SAB mortality was 20.1% (95% CI 15.9% – 24.7%, 67/334) which was significantly higher than methicillin susceptible SAB mortality (13%, 95% CI 11.3% – 14.9%, 179/1378, P<0.0001).

MSSA Antimicrobial Susceptibility: Overall 80.9% (1,626) of the 2,010 isolates were methicillin sensitive of which 79.6% (1,294) were penicillin resistant (MIC

>0.12 mg/L). However as β-lactamase was detected in 69 phenotypically penicillin susceptible isolates, 83.8% of MSSA were considered penicillin resistant. Apart from erythromycin non-susceptibility (11.0%) resistance to the non-β-lactam antimicrobials amongst MSSA was rare, ranging from 0% to 3.9% (Table 1). A single isolate was linezolid resistant (MIC >8 mg/L), five isolates were non-susceptible to daptomycin (MIC 2 - 4 mg/L), and using the EUCAST resistant breakpoint of >2 mg/L one isolate was teicoplanin resistant (MIC = 4 mg/L). Vancomycin non susceptibility was not detected. Twenty (1.2%) of the 1,626 isolates had high level mupirocin resistance of which 16 isolates were referred from Queensland. Inducible resistance to clindamycin was determined by the Vitek2® susceptibility system. Of the 1,478 isolates tested, 8.6% (127) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI and EUCAST breakpoints) of which 89.8% (114) were classified as having inducible clindamycin resistance. Multi-resistance was uncommon in MSSA (1.7%, 28/1626)

There were no significant differences in interpretation for any drug when CLSI or EUCAST non susceptibility breakpoints were utilised (P>0.05).

MRSA Antimicrobial Susceptibility: The proportion of *S. aureus* that were MRSA was 19.1% (95%CI 17.5% – 21.0%). Of the 384 MRSA identified 97.9% were either cefoxitin screen positive by Vitek2® (363/384) or had a cefoxitin MIC >8 by Phoenix™ (13/384). Eight isolates that were either cefoxitin screen

negative (4/8) or had a cefoxitin MIC ≤2 mg/L (4/8) were oxacillin resistant (MIC >2 mg/L) and *mecA* positive by PCR. Although two of the 384 isolates were phenotypically penicillin susceptible, both isolates were β-lactamase positive. Amongst the MRSA isolates, non-susceptibility to non-β-lactam antimicrobials was common except for rifampicin (MIC 2 - ≥32 mg/L), fusidic acid (MIC 2 - ≥32 mg/L), nitrofurantion (MIC ≥64 mg/L) and daptomycin (MIC 2 - 4 mg/L) where resistance was below 3% nationally (Table 2). Resistance was not detected for vancomycin, teicoplanin or linezolid. Of the 384 MRSA isolates 1.8% (7/384) had high level mupirocin resistance. Of the 327 isolates tested by Vitek2®, 27.8% (91) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI and EUCAST breakpoints) of which 89.0% (81) were classified as having inducible clindamycin resistance. Multi-resistance was common in MRSA (25.8%, 99/384).

There were no significant differences in interpretation for any drug when CLSI or EUCAST non susceptibility breakpoints were utilised (P>0.05).

MRSA Molecular Epidemiology: Of the 384 MRSA identified, 368 were referred to *ACCESS* Typing and Research for strain characterisation. Based on molecular typing, 41.0% (151) and 59.0% (217) isolates were classified as healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) clones respectively (Table 3).

Healthcare-associated-methicillin-resistant Staphylococcus aureus

For the 151 HA-MRSA strains, 50.3% (76) were epidemiologically classified as hospital onset (blood culture collected >48 hours after admission) and 47.7% (72) were classified as community onset. Date of hospital admission was not available for three patients. Three HA-MRSA clones were identified: 88 isolates of ST22-IV [2B] (EMRSA-15) (23.9% of MRSA and 4.4% of *S. aureus*); 59 isolates of ST239-III [3A] (16.0% and 2.9%) and four isolates of ST5-II [2A] (USA100/New York Japan MRSA).

ST22-IV [2B] (EMRSA-15) was the dominant HA-MRSA clone in Australia accounting for 58.3% of HA-MRSA ranging from 42.9% in the Australian Capital Territory to 100% in Tasmania (Table 4). ST22-IV [2B] was typically PVL negative and using CLSI breakpoints 100% and 67% were ciprofloxacin and erythromycin resistant respectively.

ST239-III [3A] (Aus-2/3 EMRSA) accounted for 39.1% of HA-MRSA ranging from 0% in Tasmania to 100% in the Northern Territory (Table 4). PVL negative ST239-III [3A] (Aus -2/3 EMRSA) were typically resistant to erythromycin (100%), co-trimoxazole (100%), ciprofloxacin (97%), gentamicin (97%), tetracycline (84%) and clindamycin (81%).

Community-associated-methicillin-resistant Staphylococcus aureus

For the 217 CA-MRSA strains, 28.6% (62) episodes were epidemiologically classified as hospital onset and 70.5% (153) were classified as community onset. Date of hospital admission was not available for two patients. Twenty-seven different CA-MRSA clones were identified by pulsed-field gel electrophoresis (PFGE) corresponding to 19 MLST/SCC*mec* clones (Table 3). Overall 80.7% of CA-MRSA were classified into six clones each having more than ten isolates: ST93-IV [2B] (Queensland CA-MRSA) (13.6% of MRSA and 2.5% of *S. aureus*); ST1-IV [2B] (WA1) (12.2% and 2.2%); ST5-IV [2B] (WA3) (6.8% and 1.2%); ST78-IV [2B] (WA2) (5.4% and 1.0%); ST30-IV [2B] (South West Pacific [SWP] CA-MRSA) (4.9% and 0.9%); and ST45-V [5C2&5] (WA84) (4.6% and 0.8%).

ST93-IV [2B] (Queensland CA-MRSA) accounted for 23.0% of CA-MRSA ranging from 15.4% in Western Australia to 100% in Tasmania (Table 5). PVL positive ST93-IV [2B] (Queensland CA-MRSA) was typically resistant to the β-lactams only (77.1%, 37/48) or additionally resistant to erythromycin (10.4%, 5/48). Four isolates were resistant to erythromycin and clindamycin. A single isolate was non-susceptible to ciprofloxacin with an MIC of 2 mg/L. One isolate exhibited high-level mupirocin resistance.

ST1-IV [2B] (WA1) accounted for 20.7% of CA-MRSA ranging from 0% in the Australian Capital Territory and Tasmania to 26.9% in Western Australia (Table 5). Typically PVL negative, 66.7% of isolates were resistant to the β-lactams only (30/45) or additionally resistant to erythromycin (8.9%, 4/45) or fusidic acid

(6.7%, 3/45) or both (2.2%, 1/45). Four isolates were non–susceptible to ciprofloxacin and additionally resistant to erythromycin and clindamycin (1); erythromycin, gentamicin, daptomycin and tetracycline (1); erythromycin, fusidic acid, and co-trimoxazole (1); or erythromycin, clindamycin and nitrofurantoin (1). Single isolates were non-susceptible to nitrofurantoin; or resistant to gentamicin; gentamicin, erythromycin and high-level mupirocin; erythromycin or fusidic acid and tetracycline.

ST5-IV [2B] (WA3) accounted for 11.5% of CA-MRSA ranging from 0% in the Australian Capital Territory and Tasmania to 17.9% in South Australia (Table 5). PVL negative ST5-IV [2B] (WA3) was typically resistant to the β-lactams only (44%, 11/25) or additionally resistant to erythromycin (20%, 5/25). Three isolates were non-susceptible to ciprofloxacin including one isolate additionally resistant to erythromycin and clindamycin. Two isolates exhibited high-level mupirocin resistance. Two isolates were resistant to erythromycin and clindamycin. Single isolates were resistant to erythromycin and co-trimoxazole, or rifampicin.

ST78-IV [2B] (WA2), PVL negative, accounted for 9.2% of CA-MRSA and was isolated predominately in Western Australia (Table 5). Isolates were resistant to the β-lactams only (50%, 10/20) or additionally resistant to erythromycin (45%, 9/20). One isolate was additionally resistant to erythromycin and clindamycin.

ST30-IV [2B] (SWP CA-MRSA) and ST45-V [5C2&5] (WA84) accounted for 8.3% and 7.8% of CA-MRSA respectively and were isolated primarily in the eastern regions of Australia (Table 5). Typically PVL positive, ST30-IV [2B] (SWP CA-MRSA) was typically resistant to the β-lactams only (50%, 9/18). Isolates were additionally non-susceptible to nitrofurantoin (6 isolates), resistant to cotrimoxazole (1); erythromycin (1); clindamycin (1); tetracycline and nitrofurantoin (1); or clindamycin, fusidic acid, nitrofurantoin and high-level mupirocin (1). All PVL negative ST45-V [5C2&5] (WA84) isolates were resistant to the β-lactams and ciprofloxacin. Isolates were additionally resistant to erythromycin and tetracycline (2 isolates), erythromycin, gentamicin and tetracycline (2), erythromycin and clindamycin (2), erythromycin (1), erythromycin and gentamicin (1), erythromycin, clindamycin and tetracycline (1), erythromycin, clindamycin and gentamicin (1), or erythromycin, clindamycin, gentamicin and tetracycline (1).

Overall 90.8% of CA-MRSA were non-multiresistant and 51.6% were resistant to the β-lactams only. However, twenty CA-MRSA isolates were multiresistant.

Panton-Valentine leucocidin: Overall 20.9% (77) of MRSA were PVL positive, all were CA-MRSA. (Table 3). PVL positive CA-MRSA clones included the international CA-MRSA clone ST8-IV [2B] USA300.

Discussion

The Australian Group on Antimicrobial Resistance Targeted Resistance Surveillance program (AGAR-TRS) collects data on antimicrobial resistance, focussing on bloodstream infections caused by *S. aureus*, *Enterococcus* and *Enterobacteriaceae*. All data being collected in the AGAR-TRS programs are generated as part of routine patient care in Australia with most being available through laboratory and hospital bed management information systems. Isolates are referred to a central laboratory where strain and antimicrobial resistance determinant characterisation is performed. As the programs are similar to those conducted in Europe (http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx) comparison of Australia antimicrobial resistance data with other countries is possible.

In the 2012 European Centre for Disease Prevention and Control and Prevention (ECDC) SAB surveillance program, the European Union/European Economic Area (EU/EEA) population-weighted mean percentage of *S. aureus* resistant to methicillin was 17.8%, ranging from 0.7% in Sweden to 53.9% in Romania (http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2012.pdf). In ASSOP 2013, 19.1% (95% CI 17.5% – 21.0%) of the 2,010 SAB episodes were methicillin resistant. Five European countries reported a similar percentage to Australia including Bulgaria (19.8%; 95% CI,

15%– 26%), Croatia (22%, 95% CI 18% – 26%), France (19.2%, 95% CI 18% – 20%), Ireland (22.6%, 95% CI 20% – 25%), and Slovakia (21.7%, 95% CI 18% – 26%). However for 16 of the 30 European countries (primarily northern Europe countries including Germany and the United Kingdom) the percentage of SAB isolates resistant to methicillin was less than that reported in ASSOP 2013. Similar to Europe, which has seen the EU/EEA population-weighted mean percentage decrease significantly from 23.2% in 2009 to 17.8% in 2012, the percentage of methicillin resistant SAB in Australia has decreased from 23.8% (95% CI 21.4% – 26.4%) in 2007 to 19.1% (95%CI 17.5% – 21.0%) in 2013 (P<0.0001).[20] The decrease in methicillin resistant SAB is consistent with what has been reported elsewhere[21, 22] and is believed to be attributed to the implementation of antimicrobial stewardship and a package of improved infection control procedures including hand hygiene, MRSA screening and decolonisation, patient isolation and infection prevention care bundles.[23-27] However, unlike Europe, Australia has a high prevalence of CA-MRSA and so further reduction in the proportion of SAB due to MRSA may prove problematic.

In ASSOP 2013 the all cause mortality at 30-days was 14.4% (95% CI 12.8% – 16.2%). In comparison, the 2008 Australian New Zealand Cooperative on Outcome in Staphylococcal Sepsis (ANZCOSS) reported a significantly higher figure of 20.6% (95% CI 18.8% - 22.5%, P<0.0001), and when adjusted for Australian institutions only was 25.9% (personal communication). Although SAB 30-day mortality appears to be falling in Australia, MRSA-associated SAB

mortality remains high (20.1%, 95% CI,15.9% – 24.7%, 67/334) and was significantly higher than MSSA-associated SAB mortality (13%, 95% CI 11.3% – 14.9%, 179/1378, P<0.0001). Although it has recently been shown that invasive MRSA infection may be more life-threatening partially because of the inferior efficacy of the standard treatment, vancomycin,[9] the emergence of hyper-virulent multi-resistant CA-MRSA clones such as ST93-IV [2B] (Queensland CA-MRSA), causing healthcare-associated SAB is of concern.[28]

With the exception of the β-lactams and erythromycin, antimicrobial resistance in MSSA remains rare. However, in addition to the β-lactams approximately 50% of MRSA were resistant to erythromycin and ciprofloxacin and approximately 20% resistant to co-trimoxazole, tetracycline and gentamicin. Resistance was largely attributable to two healthcare associated MRSA clones, ST22-IV [2B] (EMRSA-15), which is typically ciprofloxacin and erythromycin resistant, and ST239-III [3A] (Aus-2/3 EMRSA) which is typically erythromycin, clindamycin, ciprofloxacin, co-trimoxazole, tetracycline and gentamicin resistant. Since the early 1980s the multi-resistant ST239-III [3A] (Aus-2/3 EMRSA) was the dominant HA-MRSA clone in Australian hospitals. However, ST22-IV [2B] (EMRSA-15) has recently replaced it as the most prevalent HA-MRSA isolated from clinical specimens and this change has occurred throughout the country.[29] In the current survey, ST239-III [3A] was the only HA-MRSA clone in the Northern Territory. In ASSOP 2013 approximately 24% of MRSA were characterised as ST22-IV [2B] (EMRSA-15). CA-MRSA, in particular the ST45-V [5C2&5] (WA84) clone, has acquired multiple

antimicrobial resistance determinants including ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline. Linezolid, daptomycin and teicoplanin resistance was detected in a small number of *S. aureus* isolates. Resistance was not detected for vancomycin.

Approximately 30% of SAB caused by CA-MRSA were healthcare onset. Although in several parts of the United States the CA-MRSA clone USA300 has replaced the HA-MRSA clone ST5-II [2A] (USA100) as a cause of healthcare associated MRSA infection,[30] transmission of CA-MRSA in Australian hospitals is thought to be rare.[31, 32] Consequently it is likely that many of the healthcare onset CA-MRSA SAB infections reported in ASSOP 2013 were caused by the patient's own colonising strains acquired prior to admission. In Australia CA-MRSA clones such as PVL-positive ST93-IV [2B] (Queensland CA-MRSA) and PVL-negative ST1-IV [2B] (WA1) are well established in the community and therefore it is important to monitor antimicrobial resistance patterns in both community and healthcare associated SAB as this information will guide therapeutic practices in treating *S. aureus* sepsis.

In conclusion ASSOP 2013 has demonstrated antimicrobial resistance in SAB in Australia is a significant problem and continues to be associated with a high mortality. This may be due, in part, to the high prevalence of methicillin resistant SAB in Australia, which is significantly higher than most EU/EEA countries. Consequently MRSA must remain a public health priority and continuous

surveillance of SAB and its outcomes and the implementation of comprehensive MRSA strategies targeting hospitals and long-term care facilities are essential.

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Members of the AGAR in 2013 were:

Australian Capital Territory

Peter Collignon and Susan Bradbury, The Canberra Hospital

New South Wales

Thomas Gottlieb and Graham Robertson, Concord Hospital

James Branley and Donna Barbaro, Nepean Hospital

George Kotsiou and Peter Huntington, Royal North Shore Hospital

Sebastian van Hal and Bradley Watson, Royal Prince Alfred Hospital

David Mitchell and Lee Thomas, Westmead Hospital

Northern Territory

Rob Baird and Jann Hennessy, Royal Darwin Hospital

Queensland

Enzo Binotto and Bronwyn Thomsett, Pathology Queensland Cairns Base Hospital

Graeme Nimmo and Narelle George, Pathology Queensland Central Laboratory

Petra Derrington and Sharon Dal-Cin, Pathology Queensland Gold Coast Hospital

Chris Coulter and Robert Horvath, Pathology Queensland Prince Charles Hospital

Naomi Runnegar and Joel Douglas, Pathology Queensland Princess Alexandra Hospital

Jenny Robson and Georgia Peachey, Sullivan Nicolaides Pathology

South Australia

Kelly Papanoum and Nicholas Wells, SA Pathology (Flinders Medical Centre)

Morgyn Warner and Fleur Manno, SA Pathology (Royal Adelaide Hospital)

John Turnidge and Jan Bell, SA Pathology (Women's and Children's Hospital)

Tasmania

Louise Cooley and Rob Peterson, Royal Hobart Hospital

Victoria

Denis Spelman and Christopher Lee, The Alfred Hospital

Benjamin Howden and Peter Ward, Austin Hospital

Tony Korman and Despina Kotsanas, Monash Medical Centre

Andrew Daley and Gena Gonis, Royal Women's Hospital

Mary Jo Waters and Linda Joyce, St Vincent's Hospital

Western Australia

David McGechie and Rebecca Wake, PathWest Laboratory Medicine – WA Fremantle Hospital

Barbara Henderson and Ronan Murray, PathWest Laboratory Medicine – WA Queen Elizabeth II Hospital

Owen Robinson, Denise Daley and Geoffrey Coombs, PathWest Laboratory Medicine – WA Royal Perth Hospital

Sudha Pottumarthy-Boddu and Fay Kappler, St John of God Pathology

Table 1: The number and proportion of methicillin sensitive *Staphylococcus aureus* (MSSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2013

Antimicrobial	Tested	Breakpoint (mg/L)	Non-Susceptible (%)
Penicillin	1,626	>0.12[a]	1,342 (82.5)
Vancomycin	1,626	>2[a]	0
Teicoplanin	1,626	>8[b]	0
		>2[c]	1 (<0.1)
Rifampicin	1,587	>1[b]	0
Fusidic Acid	1,480	>1[c]	61 (3.9)
Gentamicin	1,626	>4[b]	15 (0.9)
	1,480	>1[c]	16 (1.1)
Erythromycin	1,626	>2[b]	178 (11.0)
		>1[c]	179 (11.0)
Clindamycin	1,626	>0.5[b]	39 (2.4)
	1,480	>0.25[c]	36 (2.4)
Tetracycline	1,553	>4[b]	31 (2.0)
		>1[c]	35 (2.3)
Co-trimoxazole	1,626	>2/38[a]	33 (2.0)
Ciprofloxacin	1,626	>1[a]	46 (2.8)
Nitrofurantoin	1,550	>32[b]	30 (1.9)
Linezolid	1,626	>4[a]	1 (<0.1)
Daptomycin	1,552	>1[a]	4 (0.3)

[a]CLSI and EUCAST non-susceptible breakpoint
[b]CLSI non-susceptible breakpoint
[c]EUCAST non-susceptible breakpoint

Table 2: The number and proportion of methicillin resistant *Staphylococcus aureus* (MRSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2013

Antimicrobial	Tested	Breakpoint (mg/L)	Non-Susceptible (%)
Penicillin	384	>0.12[a]	381 (99.2)
Vancomycin	384	>2[a]	0
Teicoplanin	384	>8[b]	0
		>2[c]	0
Rifampicin	381	>1[b]	7 (1.8)
Fusidic Acid	327	>1[c]	9 (2.3)
Gentamicin	384	>4[b]	68 (17.7)
	327	>1[c]	52 (16.0)
Erythromycin	384	>2[b]	192 (49.9)
		>1[c]	192 (49.9)
Clindamycin	384	>0.5[b]	84 (21.9)
	327	>0.25[c]	67 (20.5)
Tetracycline	363	>4[b]	63 (17.4)
		>1[c]	79 (21.8)
Co-trimoxazole	384	>2/38[a]	71 (18.5)
Ciprofloxacin	384	>1[a]	195 (50.8)
Nitrofurantoin	378	>32[b]	11 (2.9)
Linezolid	384	>4[a]	0
Daptomycin	362	>1[a]	4 (1.1)

[a]CLSI and EUCAST non-susceptible breakpoint
[b]CLSI non-susceptible breakpoint
[c]EUCAST non-susceptible breakpoint

Table 3: Proportion of healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus*, Australia, 2013 by clone, healthcare and community onset, and Panton-Valentine leucocidin carriage

Strain	Total (%)[a]	Onset (%)[b]			PVL Positive (%)[b]
		Healthcare	Community	Unknown	
Healthcare Associated MRSA					
ST22-IV [2B] (EMRSA-15)	88 (23.9)	38 (43.2)	49 (55.7)	1 (1.1)	0
ST239-III [3A] (Aus-2/3)	59 (16.0)	35 (59.3)	22 (37.3)	2 (3.4)	0
ST5-II [2A] (USA100)	4 (1.1)	3 (75)	1 (25.0)	0	0
Total	151 (41.0)	76 (50.3)	72 (47.7)	3 (2.0)	0
Community Associated MRSA					
ST93-IV [2B] (Queensland)	50 (13.6)	10 (20.0)	40 (80.0)	0	48 (96.0)
ST1-IV [2B] (WA1)	45 (12.2)	17 (37.8)	28 (62.2)	0	4 (8.9)
ST5-IV [2B] (WA3)	25 (6.8)	9 (36.0)	16 (64.0)	0	0
ST78-IV [2B] (WA2)	20 (5.4)	6 (30.0)	14 (70.0)	0	0
ST30-IV [2B] (SWP)	18 (4.9)	7 (38.9)	11 (61.1)	0	14 (77.8)
ST45-V [5C2&5] (WA84)	17 (4.68)	4 (23.5)	13 (76.5)	0	0
ST8-IV [2B] (USA300)	7 (1.9)	2 (28.6)	3 (42.9)	2 (28.6)	6 (85.7)
ST73-IV [2B] (WA65)	6 (1.6)	1 (16.7)	5 (83.3)	0	0
ST835-IV [2B] (WA48)	4 (1.1)	1 (25)	3 (75)	0	0
ST72-IV [2B] (Korean)	3 (0.8)	1 (33.3)	2 (66.7)	0	0
ST953-IV [2B] (WA54)	3 (0.8)	0	3 (100)	0	0
ST5-IV [2B] (WA121)	2 (0.5)	0	2 (100)	0	2 (100)
ST923-IV [2B] (WA62)	2 (0.5)	0	2 (100)	0	2 (100)
ST5-IV [2B] (WA71)	2 (0.5)	2 (100)	0	0	1 (50)
ST45-V [5C2] (WA4)	1 (0.3)	1 (100)	0	0	0
ST45-IV [2B] (WA23)	1 (0.3)	0	1 (100)	0	0
ST6-IV [2B] (WA66)	1 (0.3)	0	1 (100)	0	0
ST5-V [5C2] (WA90)	1 (0.3)	0	1 (100)	0	0
ST5-IV [2B] (WA96)	1 (0.3)	0	1 (100)	0	0
ST8-IV [2B] (WA101)	1 (0.3)	0	1 (100)	0	0

STnovel-IV [2B] (WA114)	**1 (0.3)**	**0**	**1 (100)**	**0**	**0**
ST5-V [5C2] (WA109)	**1 (0.3)**	**0**	**1 (100)**	**0**	**0**
ST612-IV [2B] (WA20)	**1 (0.3)**	**0**	**1 (100)**	**0**	**0**
ST1-V [5C2]	**1 (0.3)**	**0**	**1 (100)**	**0**	**0**
ST45-V [5C2]	**1 (0.3)**	**0**	**1 (100)**	**0**	**0**
ST59-V [5C2]	**1 (0.3)**	**0**	**1 (100)**	**0**	**0**
ST5-V [5C2]	**1 (0.3)**	**1 (100)**	**0**	**0**	**0**
Total	**217(59.0)**	**62 (28.6)**	**153 (70.5)**	**2 (0.9)**	**77 (35.5)**
Grand Total	**368**	**138 (37.5)**	**225 (61.1)**	**5 (1.4)**	**77 (20.9)**

[a]Percentage of all MRSA
[b]Percentage of the strain

Table 4: The number and proportion of healthcare associated methicillin resistant *Staphylococcus aureus* (MRSA) multilocus sequence types, Australia, 2013, by region

Type	ACT	NSW	NT	Qld	SA	Tas	Vic	WA	Aus
ST22-IV [2B] (EMRSA-15)	3 (42.9)	35 (56.5)	0	14 (70)	13 (81.3)	1 (100)	14 (45.2)	8 (88.9)	88 (58.3)
ST239-III (3A] (Aus-2/3 EMRSA)	4 (47.1)	26 (41.9)	5 (100)	6 (30)	3 (18.7)	0	14 (45.2)	1 (11.1)	59 (39.1)
ST5-II [2A] (USA100)	0	1 (1.6)	0	0	0	0	3 (9.6)	0	4 (2.6)
Total	7	62	5	20	16	1	31	9	151

ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; Qld = Queensland; SA = South Australia; Tas = Tasmania; Vic = Victoria; WA = Western Australia; Aus = Australia

Table 5: The number and proportion of the major community associated methicillin resistant *Staphylococcus aureus* (MRSA) multilocus sequence types, Australia (>10 isolates), 2013, by region

Type	ACT	NSW	NT	Qld	SA	Tas	Vic	WA	Aus
ST93-IV [2B] (Qld)	1 (25)	10 (30.3)	9 (47.4)	8 (17.1)	9 (32.1)	1 (100)	4 (12.1)	8 (15.4)	50 (23.0)
ST1-IV [2B] (WA1)	0	4 (12.1)	4 (21.1)	12 (25.5)	5 (17.9)	0	6 (18.2)	14 (26.9)	45 (20.7)
ST5-IV [2B] (WA3)	0	2 (6.1)	1 (5.3)	6 (12.8)	5 (17.9)	0	3 (9.1)	8 (15.4)	25 (11.5)
ST78-IV [2B] (WA2)	0	1 (3.0)	0	1 (2.1)	2 (7.1)	0	1 (3.0)	15 (28.8)	20 (9.2)
ST30-IV [2B] (SWP)	0	3 (9.1)	3 (15.8)	6 (12.8)	2 (7.1)	0	3 (9.1)	1 (1.9)	18 (8.3)
ST45-V [5C2&5] (WA84)	2 (50)	6 (18.2)	0	0	1 (3.6)	0	8 (24.2)	0	17 (7.8)
Other	1 (25)	7 (21.2)	2 (10.5)	14 (29.7)	4 (14.3)	0	8 (24.2)	6 (11.5)	42 (19.4)
Total	4	33	19	47	28	1	33	52	217

ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; Qld = Queensland; SA = South Australia; Tas = Tasmania; Vic = Victoria; WA = Western Australia; Aus = Australia

References

1. Laupland KB. Incidence of bloodstream infection: a review of population-based studies. *Clin Microbiol Infect* 2013; **19**: 492-500.
2. Johnson AP, Pearson A, Duckworth G. Surveillance and epidemiology of MRSA bacteraemia in the UK. *J Antimicrob Chemother* 2005; **56**: 455-62.
3. Thwaites GE, Edgeworth JD, Gkrania-Klotsas E *et al*. Clinical management of *Staphylococcus aureus* bacteraemia. *Lancet Infect Dis* 2011; **11**: 208-22.
4. Collignon P, Nimmo GR, Gottlieb T *et al*. *Staphylococcus aureus* bacteremia, Australia. *Emerg Infect Dis* 2005; **11**: 554-61.
5. *Frederiksen MS, Espersen F, Frimodt-Moller N et al. Changing epidemiology of pediatric Staphylococcus aureus* bacteremia in Denmark from 1971 through 2000. *Pediatr Infect Dis J* 2007; **26**: 398-405.
6. Benfield T, Espersen F, Frimodt-Moller N *et al*. Increasing incidence but decreasing in-hospital mortality of adult *Staphylococcus aureus* bacteraemia between 1981 and 2000. *Clin Microb Infect* 2007; **13**: 257-63.
7. van Hal SJ, Jensen SO, Vaska VL *et al*. Predictors of mortality in *Staphylococcus aureus* Bacteremia. *Clin Microbiol Rev* 2012; **25**: 362-86.
8. Kaasch AJ, Barlow G, Edgeworth JD *et al*. *Staphylococcus aureus* bloodstream infection: a pooled analysis of five prospective, observational studies. *J Infect* 2014; **68**: 242-51.
9. Turnidge JD, Kotsanas D, Munckhof W *et al*. *Staphylococcus aureus* bacteraemia: a major cause of mortality in Australia and New Zealand. *Med J Aust* 2009; **191**: 368-73.
10. Nimmo GR, Bell JM, Collignon PJ. Fifteen years of surveillance by the Australian Group for Antimicrobial Resistance (AGAR). *Commun Dis Intell* 2003; **27 Suppl**: S47-54.
11. Coombs GW Daley DA, Pearson JC, *et al*. Community-Onset *Staphylococcus aureus* Surveillance Programme Annual Report, 2012. *Commun Dis Intell* 2014; **38**: 26 - 36.
12. Coombs GW Nimmo GR, Pearson JC, *et al*.Australian Group on Antimicrobial Resistance Hospital-Onset *Staphylococcus aureus* Surveillance Programme Annual Report, 2011. *Commun Dis Intell* 2013; **37**: 210 - 8.
13. CLSI. Performance standards for antimicrobial susceptibility testing. *Twenty-fourth informational supplement M100-S24*. Villanova, PA, USA, 2014.
14. European Committee on Antimicrobial Susceptibility Testing (2014). Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0, Januray 2014. Available at http://www.eucast.org/clinical_breakpoints/. Accessed January 1, 2014.
15. O'Brien FG, Udo EE, Grubb WB. Contour-clamped homogeneous electric field electrophoresis of *Staphylococcus aureus*. *Nat Protoc* 2006; **1**: 3028-33.
16. Enright MC, Day NP, Davies CE *et al*. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38**: 1008-15.
17. Coombs GW, Monecke S, Pearson JC *et al*. Evolution and diversity of community-associated methicillin-resistant *Staphylococcus aureus* in a geographical region. *BMC microbiology* 2011; **11**: 215.
18. Fey PD, Said-Salim B, Rupp ME *et al*. Comparative molecular analysis of community- or hospital-acquired methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agent Chemother* 2003; **47**: 196-203.
19. Costa AM, Kay I, Palladino S. Rapid detection of *mecA* and *nuc* genes in staphylococci by real-time multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2005; **51**: 13-7.
20. Turnidge JD, Nimmo GR, Pearson J *et al*. Epidemiology and outcomes for *Staphylococcus aureus* bacteraemia in Australian hospitals, 2005-06: report from the Australian Group on Antimicrobial Resistance. *Commun Dis Intell* 2007; **31**: 398-403.
21. Johnson AP, Davies J, Guy R *et al*. Mandatory surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia in England: the first 10 years. *J Antimicrob Chemother* 2012; **67**: 802-9.

22. de Kraker ME, Davey PG, Grundmann H *et al*. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. *PLoS Med* 2011; **8**: e1001104.
23. Johnson PD, Martin R, Burrell LJ *et a*l. Efficacy of an alcohol/chlorhexidine hand hygiene program in a hospital with high rates of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection. *Med J Aust* 2005; **183**: 509-14.
24. Vos MC, Behrendt MD, Melles DC *et a*l. 5 years of experience implementing a methicillin-resistant *Staphylococcus aureus* search and destroy policy at the largest university medical center in the Netherlands. *Infect Control and Hosp Epidemiol* 2009; **30**: 977-84.
25. Grayson ML, Jarvie LJ, Martin R *et a*l. Significant reductions in methicillin-resistant *Staphylococcus aureus* bacteraemia and clinical isolates associated with a multisite, hand hygiene culture-change program and subsequent successful statewide roll-out. *Med J Aust* 2008; **188**: 633-40.
26. Kim YC, Kim MH, Song JE *et al*. Trend of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia in an institution with a high rate of MRSA after the reinforcement of antibiotic stewardship and hand hygiene. *Am J Infect Control* 2013; **41**: e39-43.
27. Lawes T, Edwards B, Lopez-Lozano JM *et al*. Trends in *Staphylococcus aureus* bacteraemia and impacts of infection control practices including universal MRSA admission screening in a hospital in Scotland, 2006-2010: retrospective cohort study and time-series intervention analysis. *BMJ Open* 2012; **2**.
28. Chua KY, Monk IR, Lin YH *et al*. Hyperexpression of alpha-hemolysin explains enhanced virulence of sequence type 93 community-associated methicillin-resistant *Staphylococcus aureus*. *BMC microbiology* 2014; **14**: 31.
29. Coombs GW PJ, Nimmo GR, Collignon PJ, *et al*. Antimicrobial susceptibility of *Staphylococcus aureus* and molecular epidemiology of meticillin-resistant *S. aureus* isolated from Australian hospital inpatients: Report from the Australian Group on Antimicrobial Resistance 2011 *Staphylococcus aureus* Surveillance Programme. *J Glob Antimicrob Resis* 2013; **1**: 149-56.
30. Nimmo GR. USA300 abroad: global spread of a virulent strain of community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* **18**: 725-34.
31. O'Brien FG, Pearman JW, Gracey M *et al*. Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. *J Clin Microbiol* 1999; **37**: 2858-62.
32. Schlebusch S, Price GR, Hinds S *et a*l. First outbreak of PVL-positive nonmultiresistant MRSA in a neonatal ICU in Australia: comparison of MALDI-TOF and SNP-plus-binary gene typing. *Europ J Clin Microbiol Infect Dis* 2010; **29**: 1311-4.