Australian Group on Antimicrobial Resistance Australian *Staphylococcus aureus* Sepsis Outcome Programme annual report, 2014

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Abstract

From 1 January to 31 December 2014, 27 institutions around Australia participated in the Australian Staphylococcal Sepsis Outcome Programme (ASSOP). The aim of ASSOP 2014 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, 18.8% of the 2,206 SAB episodes were methicillin resistant, which was significantly higher than that reported in most European countries. The 30-day all-cause mortality associated with methicillin-resistant SAB was 23.4%, which was significantly higher than the 14.4% mortality associated with methicillin-sensitive SAB ($P<0.0001$). With the exception of the ß-lactams and erythromycin, antimicrobial resistance in methicillin-sensitive *S. aureus* remains rare. However in addition to the ß-lactams, approximately 50% of methicillin-resistant *S. aureus* (MRSA) were resistant to erythromycin and ciprofloxacin and approximately 15% were resistant to co-trimoxazole, tetracycline and gentamicin. When applying the European Committee on Antimicrobial Susceptibility Testing breakpoints, teicoplanin resistance was detected in 2 *S. aureus* isolates. Resistance was not detected for vancomycin or linezolid. Resistance to non-beta-lactam antimicrobials was largely attributable to 2 healthcare-associated MRSA clones; ST22-IV [2B] (EMRSA-15) and ST239-III [3A] (Aus-2/3 EMRSA). ST22-IV [2B] (EMRSA-15) has become the predominant healthcare associated clone in Australia. Sixty per cent of methicillin-resistant SAB were due to community-associated (CA) clones. Although polyclonal, almost 44% 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 that antimicrobial resistance patterns in community and healthcare-associated SAB is monitored as this information will guide therapeutic practices in treating *S. aureus* sepsis. *Commun Dis Intell* 2016;40(2):E244–E254.

Keywords: Australian Group on Antimicrobial Resistance, antimicrobial resistance surveillance; *Staphylococcus aureus,* methicillin sensitive, methicillin resistant, bacteraemia

Introduction

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, 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 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-ß-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] In 2013 AGAR commenced the Australian Staphylococcal Sepsis Outcome Programme (ASSOP).[11] The primary objective of ASSOP 2014 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 resistant *S. aureus* (MRSA).

Methods

Twenty-seven laboratories from all 8 Australian states and territories participated in the program in 2014.

From 1 January to 31 December 2014, the 27 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 identified by a culture of blood collected more than 14 days after the last 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 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)[12] and European Committee on Antimicrobial Susceptibility Testing (EUCAST)[13] breakpoints were utilised for interpretation. Isolates with a resistant or an intermediate category were classified as non-susceptible. Linezolid and daptomycin non-susceptible isolates were retested by Etest® (bioMérieux) using the Mueller-Hinton agar recommended by the manufacturer. *S. aureus* ATCC 29213 was used as the control strain. High level mupirocin resistance was determined using a mupirocin 200 μg disk according to CLSI guidelines on all isolates with a mupirocin MIC > 8 mg/L by Vitek2® or > 256 mg/L by Phoenix™.[12] Multi-resistance was defined as resistance to 3 or more of the following non-ß-lactam antimicrobials: vancomycin, teicoplanin, erythromycin/clindamycin, tetracycline, ciprofloxacin, gentamicin, co-trimoxazole, fusidic acid, rifampicin, high level mupirocin, or linezolid.

Electrophoresis of chromosomal DNA was performed as previously described on all MRSA using contour-clamped homogeneous electric field DR III system (Bio-Rad Laboratories Pty Ltd, USA).[14] 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.[15] The sequences were submitted to the Multi Locus Sequence Typing on-line database (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.[16]

Detection of Panton-Valentine leucocidin (PVL) determinants and *mecA* was performed by PCR on all MRSA as previously described.[17,18]

Chi-square tests for comparison of 2 proportions and calculation of 95% confidence intervals (95%CI) were performed 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 2014, 2,206 unique episodes of *S. aureus* bacteraemia were identified. A significant difference ($P < 0.0001$) was seen in patient sex with 63.2% (1,395) being male (95% CI 60.6–65.7). The average age of patients was 59 years ranging from 0 to 101 years with a median age of 62 years. Overall, 73.2% (1,615) of the 2,206 episodes were community onset (95% CI 71.0%–75.3%). All-cause mortality at 30-days was 16.1% (95% CI 14.5–17.8). Methicillin-resistant

SAB mortality was 23.4% (95% CI 19.1 to 28.1), which was significantly higher than methicillin-susceptible SAB mortality (14.4%, 95% CI 12.7 to 16.3, $P < 0.0001$).

Methicillin-sensitive *Staphylococcus aureus* antimicrobial susceptibility

Overall, 81.2% (1,792) of the 2,206 isolates were methicillin sensitive of which 77.0% (1,380) were penicillin resistant (MIC > 0.12 mg/L). However as ß-lactamase was detected in 87 phenotypically penicillin susceptible isolates, 81.9% of Methicillin-sensitive *Staphylococcus aureus* (MSSA) were considered penicillin resistant. Apart from erythromycin non-susceptibility, resistance to the non-ß-lactam antimicrobials among MSSA was rare, ranging from < 0.1% to 4.1% (Table 1). Four isolates were reported as non-susceptible to daptomycin by Vitek2®. By Etest® all isolates had MICs ≤ 1 mg/L and were therefore considered susceptible. Two isolates were reported as linezolid resistant (MIC > 8 mg/L) by Vitek2®. However by Etest® both isolates had a MIC ≤ 4 mg/L (1.0 and 2.0 mg/L) and were therefore considered linezolid susceptible. When using the EUCAST resistant breakpoint of > 2 mg/L 1 isolate was teicoplanin resistant (MIC = 4 mg/L). However using the CLSI resistant breakpoint of > 8 mg/L the isolate was classified susceptible. All MSSA were vancomycin susceptible. Twenty-eight (1.6%) of the 1,792 isolates had high level mupirocin resistance, of which 19 isolates were referred from Queensland. Inducible resistance to clindamycin was determined by the Vitek2® susceptibility system. Of the 1,622 isolates tested, 9.1% (147) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI and EUCAST breakpoints) of which 75.5% (111) were classified as having inducible clindamycin resistance. Multi-resistance was uncommon in MSSA (1.7%, 30/1,792).

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

Table 1: The number and proportion of methicillin sensitive Staphylococcus aureus isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2014

Antimicrobial	Tested	Breakpoint (mg/L)	Non-susceptible	
			n	%
Penicillin	1,792	>0.12*	1,467	81.8
Vancomycin	1,792	>2*	0	0.0
Teicoplanin	1,792	>8†	0	0.0
		>2‡	1	0.1
Rifampicin	1,741	>1†	4	0.2
Fusidic acid	1,791	>1‡	74	4.1
Gentamicin	1,792	>4†	14	0.8
		>1‡	18	1.0
Erythromycin	1,790	>2†	177	9.9
		>1‡	181	10.1
Clindamycin	1,790	>0.5†	30	1.7
		>0.25	31	1.7
Tetracycline	1,790	>4†	55	3.1
		>1‡	61	3.4
Co-trimoxazole	1,791	>2/38*	40	2.2
Ciprofloxacin	1,782	>1*	54	3.0
Nitrofurantoin	1,702	>32†	20	1.2
Daptomycin	1,791	>1*	0	0.0
Linezolid	1,792	>4*	0	0.0

* Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) non-susceptible breakpoint.

† CLSI non-susceptible breakpoint.

‡ EUCAST non-susceptible breakpoint.

Methicillin-resistant *Staphylococcus aureus* antimicrobial susceptibility

The proportion of *S. aureus* that were MRSA was 18.8% (95%CI 17.2–20.5). The 414 MRSA identified were either cefoxitin screen positive by Vitek2® (401) or had a cefoxitin MIC > 8 by Phoenix™ (13). All 414 MRSA isolates were phenotypically penicillin resistant. Among the MRSA isolates, non-susceptibility to non-ß-lactam antimicrobials was common except for rifampicin, fusidic acid and nitrofurantion where resistance was below 4.1% (Table 2). There were 6 isolates reported by Vitek2® as non-susceptible to daptomycin. By Etest® 3 isolates had MICs ≤ 1 mg/L and were therefore considered susceptible. Three isolates had MICs > 1 mg/L (1.5, 3 and 4 mg/L) and were considered non-susceptible. By Vitek2®, 2 isolates were linezolid resistant (MIC > 8 mg/L). However by Etest® both isolates had an MIC ≤ 4 mg/L (1 and 1.5 mg/L) and were therefore considered linezolid susceptible. When using the EUCAST resistant breakpoint of > 2 mg/L, 1 isolate was teicoplanin resistant (MIC = 4 mg/L). However, using the CLSI resistant breakpoint of > 8 mg/L the isolate was classified susceptible. All MRSA were vancomycin susceptible. Eight (1.9%) of the 414 MRSA isolates had high level mupirocin resistance of which five isolates were referred from Queensland. Inducible resistance to clindamycin was determined by the Vitek2® susceptibility system. Of the 352 isolates tested by Vitek2®, 31.2% (110) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI and EUCAST breakpoints) of which 88.2% (97) were classified as having inducible clindamycin resistance. Multi-resistance was common in MRSA (24.4%, 101/414).

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

Methicillin-resistant *Staphylococcus aureus* molecular epidemiology

Of the 414 MRSA identified, 403 were referred to *ACCESS* Typing and Research for strain characterisation. Based on molecular typing, 40.4% (163) and 59.6% (240) of isolates were classified

Table 2: The number and proportion of methicillin-resistant Staphylococcus aureus isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2014

Antimicrobial	Tested	Breakpoint (mg/L)	Non-susceptible (%)	
			n	%
Penicillin	414	>0.12*	414	100.0
Vancomycin	414	>2*	0	0.0
Teicoplanin	414	>8†	0	0.0
		>2‡	1	0.2
Rifampicin	412	>1†	4	1.0
Fusidic acid	414	>1‡	17	4.1
Gentamicin	414	>4†	67	16.2
		>1‡	74	17.9
Erythromycin	414	>2†	204	49.3
		>1‡	204	49.3
Clindamycin	414	>0.5†	68	16.4
		>0.25‡	70	16.9
Tetracycline	414	>4†	65	15.7
		>1‡	81	19.6
Co-trimoxazole	413	>2/38*	61	14.8
Ciprofloxacin	414	>1*	212	51.2
Nitrofurantoin	407	>32†	13	3.2
Daptomycin	412	>1*	3	0.7
Linezolid	414	>4*	0	0.0

* Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) non-susceptible breakpoint.

† CLSI non-susceptible breakpoint.

‡ EUCAST non-susceptible breakpoint.

as healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) clones respectively (Table 3).

Healthcare-associated methicillin-resistant *Staphylococcus aureus*

For the 163 HA-MRSA strains, 46.6% (76) were epidemiologically classified as hospital onset and 53.4% (87) were classified as community onset.

Table 3: Proportion of healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus*, Australia, 2014, by clone, healthcare and community onset, and Panton-Valentine leucocidin carriage

Strain	Total		Onset				PVL positive	
			Healthcare		Community			
	n	%*	n	%	n	%†	n	%
Healthcare-associated MRSA								
ST22-IV [2B] (EMRSA-15)	119	29.5	52	43.7	67	56.3	1	0.8
ST239-III [3A] (Aus-2/3)	43	10.7	23	53.5	20	46.5	0	0.0
ST5-II [2A] (USA100)	1	0.2	1	100.0	0	0.0	0	0.0
Total	163	40.4	76	46.6	87	53.4	1	0.6
Community-associated MRSA								
ST93-IV [2B] (Queensland)	60	14.9	13	21.7	47	78.3	56	93.3
ST1-IV [2B] (WA1)	45	11.2	11	24.4	34	75.6	4	8.9
ST45-V [5C2&5] (WA84)	30	7.4	12	40.0	18	60.0	0	0.0
ST30-IV [2B] (SWP)	20	5.0	4	20.0	16	80.0	18	90.0
ST5-IV [2B] (WA3)	20	5.0	6	30.0	14	70.0	0	0.0
ST78-IV [2B] (WA2)	11	2.7	5	45.5	6	54.5	1	9.1
ST188-IV [2B] (WA38)	5	1.2	2	40.0	3	60.0	0	0.0
ST1-V [5C2]	5	1.2	1	20.0	4	80.0	0	0.0
ST8-IV [2B] (USA300)	5	1.2	0	0.0	5	100.0	5	100.0
ST5-IV [2B] (WA71)	4	1.0	0	0.0	4	100.0	0	0.0
ST72-IV [2B] (Korean)	4	1.0	3	75.0	1	25.0	0	0.0
ST5-IV [2B] (WA121)	4	1.0	0	0.0	4	100.0	4	100.0
ST835-IV [2B] (WA48)	4	1.0	0	0.0	4	100.0	0	0.0
ST953-IV [2B] (WA54)	3	0.7	0	0.0	3	100.0	0	0.0
ST5-V [5C2] (WA81)	3	0.7	0	0.0	3	100.0	0	0.0
ST45-IV [2B] (WA75)	3	0.7	0	0.0	3	100.0	0	0.0
ST5-V [5C2] (WA123)	3	0.7	0	0.0	3	100.0	0	0.0
ST59-IV [2B] (WA15)	2	0.5	1	50.0	1	50.0	0	0.0
ST1420-IV [2B] (WA126)	2	0.5	0	0.0	2	100.0	1	50.0
ST6-IV [2B] (WA51)	1	0.2	1	100.0	0	0.0	0	0.0
ST5-IV [2B]	1	0.2	0	0.0	1	100.0	0	0.0
ST5-IV [2B] (WA105)	1	0.2	0	0.0	1	100.0	0	0.0
ST75-IV (2B] (WA8)	1	0.2	1	100.0	0	0.0	0	0.0
ST5-V [5C2] WA14	1	0.2	0	0.0	1	100.0	0	0.0
ST5-V [5C2] WA86	1	0.2	1	100.0	0	0.0	0	0.0
ST2947-V [5C2] (WA129)	1	0.2	0	0.0	1	100.0	1	100.0
Total	240	59.6	61	25.4	179	74.6	90	37.5
Grand total	403	100.0	137	34.0	266	66.0	91	22.6

PVL Panton-Valentine leucocidin.

* Percentage of all methicillin-resistant *Staphylococcus aureus* (MRSA).

† Percentage of the strain.

Three HA-MRSA clones were identified: 119 isolates of ST22-IV [2B] (EMRSA-15) (29.5% of MRSA and 5.4% of *S. aureus*); 43 isolates of ST239-III [3A] (Aus -2/3 EMRSA) (10.7% and 1.9%) and a single isolate of ST5-II [2A] (USA100/New York Japan MRSA).

ST22-IV [2B] (EMRSA-15) was the dominant HA-MRSA clone in Australia accounting for 73% of HA-MRSA ranging from 0% in the Northern Territory to 100% in Tasmania and Western Australia (Table 4). ST22-IV [2B] (EMRSA-15) was typically PVL negative and using CLSI breakpoints 98.3% and 61.3% were ciprofloxacin and erythromycin resistant respectively.

ST239-III [3A] (Aus-2/3 EMRSA) accounted for 26.4% of HA-MRSA ranging from 0% in Tasmania and Western Australia to 100% in the Northern Territory (Table 4). PVL negative ST239-III [3A] (Aus-2/3 EMRSA) were typically resistant to erythromycin (97.7%), co-trimoxazole (100%), ciprofloxacin (97.7%), gentamicin (97.7%), tetracycline (79%) and clindamycin (74.4%).

Community-associated methicillin-resistant *Staphylococcus aureus*

For the 240 CA-MRSA strains, 25.4% (61) of episodes were epidemiologically classified as hospital onset and 74.6% (179) classified as community onset. Twenty-six different CA-MRSA clones were identified by pulsed-field gel electrophoresis (PFGE) corresponding to 19 MLST/SCC*mec* clones (Table 3). Overall, 77.5% of CA-MRSA were classified into 6 clones each having more than 10 isolates: ST93-IV [2B] (Queensland CA-MRSA) (14.9% of MRSA and 2.7% of *S. aureus*); ST1-IV [2B] (WA1) (11.2% and 2%); ST45-V [5C2&5] (WA84) (7.4% and 1.4%); ST30-IV [2B] (South West Pacific [SWP] CA-MRSA) (5.0% and 0.9%); ST5-IV [2B] (WA3) (4.2% and 0.8%); and ST78-IV [2B] (WA2) (2.7% and 0.5%).

ST93-IV [2B] (Queensland CA-MRSA) accounted for 25% of CA-MRSA ranging from 0% in Tasmania and the Australian Capital Territory to 59.1% in the Northern Territory (Table 5). Typically PVL positive, 83.3% of ST93-IV [2B] (Queensland CA-MRSA) were resistant to the ß-lactams only (50/60) or additionally resistant to erythromycin (10%, 6/60), erythromycin and clindamycin (5%, 3/60), or erythromycin, clindamycin and ciprofloxacin (1.7%, 1/60).

ST1-IV [2B] (WA1) accounted for 18.8% of CA-MRSA ranging from 0% in Tasmania to 100% in the Australian Capital Territory (Table 5). Typically PVL negative, 62.2% of isolates were resistant to the ß-lactams only (28/45) or additionally resistant to erythromycin (8.9%, 4/45), erythromycin and fusidic acid (8.9%, 4/45), high level mupirocin (6.7%, 3/45), ciprofloxacin, erythromycin and fusidic acid (4.4%, 2/45), ciprofloxacin, erythromycin and gentamicin (2.2%, 1/45), clindamycin (2.2%, 1/45), erythromycin, fusidic acid and nitrofurantoin (2.2%, 1/45) or nitrofurantoin (2.2%, 1/45).

ST45-V [5C2&5] (WA84) accounted for 12.5% of CA-MRSA and was isolated primarily in the eastern regions of Australia (Table 5). All isolates were PVL negative and were resistant to the ß-lactams and ciprofloxacin. Isolates were additionally non-susceptible to erythromycin and tetracycline (20%, 6/30), erythromycin, gentamicin and tetracycline (16.7%, 5/30), erythromycin and gentamicin (13.3%, 4/30), erythromycin and clindamycin (10%, 3/30) and one (3.3%) each of erythromycin or erythromycin, clindamycin and tetracycline or erythromycin, clindamycin, gentamicin and tetracycline or clindamycin, erythromycin and nitrofurantoin or erythromycin, nitrofurantoin, gentamicin and tetracycline or erythromycin, fusidic acid, gentamicin and tetracycline.

ST30-IV [2B] (SWP CA-MRSA), accounted for 8.3% of CA-MRSA and was primarily isolated in the eastern regions of Australia (Table 5). Typically PVL positive, 70% of isolates were resistant to the ß-lactams only (14/20). Six isolates were non-susceptible to nitrofurantoin (30%).

ST5-IV [2B] (WA3) accounted for 8.3% of CA-MRSA and was primarily isolated in the eastern regions of Australia (Table 5). PVL negative ST5-IV [2B] (WA3) was typically resistant to the ß-lactams only (50%, 10/20) or additionally resistant to erythromycin (20%, 4/20), erythromycin and high level mupirocin (11.5%, 3/20), erythromycin and fusidic acid (5% 1/20), erythromycin and rifampicin (5% 1/20) or high level mupirocin (5%, 1/20).

ST78-IV [2B] (WA2), accounted for 4.6% of CA-MRSA and was isolated in most regions of the Australian mainland (Table 5). Isolates were resistant to the ß-lactams only (27%, 3/11) or additionally resistant to erythromycin (63.6%, 7/11). One isolate was resistant to tetracycline.

Overall, 85.8% of CA-MRSA were non-multiresistant and 50.4% were resistant to the ß-lactams only. However, 34 (14.2%) CA-MRSA isolates were multiresistant.

Table 4: The number and proportion of healthcare associated methicillin resistant *Staphylococcus aureus* multilocus sequence types, Australia, 2014, by state or territory

Type	ACT		NSW		NT		Qld		SA		Tas.		Vic.		WA		Aus.	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ST22-IV [2B] (EMRSA-15)	6	66.7	52	76.5	0	0.0	21	70.0	12	80.0	1	100.0	19	70.4	8	100.0	119	73.0
ST239-III (3A] (Aus-2/3 EMRSA)	3	33.3	15	22.1	5	100.0	9	30.0	3	20.0	0	0.0	8	29.6	0	0.0	43	26.4
ST5-II [2A] (USA100)	0	0.0	1	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.6
Total	9	100.0	68	100.0	5	100.0	30	100.0	15	100.0	1	100.0	27	100.0	8	100.0	163	100.0

Table 5: The number and proportion of the major community associated methicillin resistant *Staphylococcus aureus* multilocus sequence types, Australia (>10 isolates), 2014, by state or territory

Type	ACT		NSW		NT		Qld		SA		Tas.		Vic.		WA		Aus.	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ST93-IV [2B] (Qld)	0	0.0	7	12.3	13	59.1	20	30.3	6	27.3	0	0.0	6	17.6	8	21.6	60	25.0
ST1-IV [2B] (WA1)	1	100.0	7	12.3	4	18.2	10	15.2	5	22.7	0	0.0	6	17.6	12	32.4	45	18.8
ST45-V [5C2&5] (WA84)	0	0.0	16	28.1	1	4.5	0	0.0	4	18.2	0	0.0	8	23.5	1	2.7	30	12.5
ST30-IV [2B] (SWP)	0	0.0	3	5.3	0	0.0	14	21.2	0	0.0	0	0.0	2	5.9	1	2.7	20	8.3
ST5-IV [2B] (WA3)	0	0.0	9	15.8	0	0.0	8	12.1	0	0.0	0	0.0	2	5.9	1	2.7	20	8.3
ST78-IV [2B] (WA2)	0	0.0	2	3.5	0	0.0	2	3.2	2	9.1	0	0.0	2	5.9	3	8.1	11	4.6
Other	0	0.0	13	22.8	4	18.1	12	18.2	5	22.7	1	100.0	8	23.5	11	29.7	54	22.5
Total	1	100.0	57	100.0	22	100.0	66	100.0	22	100.0	1	100.0	34	100.0	37	100.0	240	100.0

Panton-Valentine leucocidin

Overall 91 (22.6%) MRSA were PVL positive, of which 98.9% were CA-MRSA (Table 3). PVL positive CA-MRSA clones included the international CA-MRSA clone ST8-IV [2B] USA300.

Discussion

The AGAR surveillance programs collect data on antimicrobial resistance, focussing on bloodstream infections caused by *S. aureus*, Enterococcus and Enterobacteriaceae. All data being collected in the AGAR 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 comparison of Australia antimicrobial resistance data with other countries is possible.[19]

In the 2013 European Centre for Disease Prevention and Control and Prevention SAB surveillance program, the European Union/European Economic Area (EU/EEA) population-weighted mean percentage of *S. aureus* resistant to methicillin was 18.0% (95% CI 17–20), ranging from 0.0% (95% CI 0–5) in Iceland to 64.5% (95% CI 59–69) in Romania.[20] In ASSOP 2014, 18.8% (95% CI 17.2–20.5) of the 2,206 SAB episodes were methicillin resistant. This compares with 19.1% (95% CI 17.5–21.0) in ASSOP 2013. Two European countries reported a similar percentage to Australia: Bulgaria (19.2%, 95% CI 14–25), and Ireland (19.9%, 95% CI 18–2). However for 18 of the 30 European countries (primarily the northern Europe countries, Germany, France and the United Kingdom) the percentage of SAB isolates resistant to methicillin was less than that reported in ASSOP 2014. Similar to Europe, which has seen the EU/EEA population-weighted mean percentage decrease significantly from 23.2% in 2009 to 18.0% in 2013, the percentage of methicillin-resistant SAB in Australia has decreased from 23.8% (95% CI 21.4–26.4) in 2007 to 18.8% (95%CI 17.2–20.5) in 2014 ($P < 0.0001$).[21] The decrease in methicillin-resistant SAB is consistent with what has been reported elsewhere[22,23] 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.[24–28] 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 2014, the all-cause mortality at 30-days was 16.1% (95% CI 14.5–17.8). In comparison, the 2008 Australian New Zealand Cooperative on Outcomes in Staphylococcal Sepsis 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). MRSA-associated SAB mortality remains high (23.4%, 95% CI 19.1–28.1) and was significantly higher than MSSA-associated SAB mortality (14.4%, 95% CI 12.7–16.3, $P < 0.0001$). 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 CA-MRSA clones such as ST93-IV [2B] (Queensland CA-MRSA), causing healthcare-associated SAB is of concern.[29]

With the exception of the ß-lactams and erythromycin, antimicrobial resistance in MSSA remains rare. However, in addition to the ß-lactams approximately 50% of MRSA were resistant to erythromycin and ciprofloxacin and approximately 15% resistant to co-trimoxazole, tetracycline and gentamicin. Resistance was largely attributable to 2 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. From the early 1980s until recently, 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 replaced it as the most prevalent HA-MRSA isolated from clinical specimens and this change has occurred throughout most of the country.[30] In the current survey, ST239-III [3A] (Aus-2/3 EMRSA) was the only HA-MRSA clone in the Northern Territory. In ASSOP 2014, approximately 30% of MRSA were characterised as ST22-IV [2B] (EMRSA-15), compared with 24% in ASSOP 2013. CA-MRSA, in particular the ST45-V [5C2&5] (WA84) clone, has acquired multiple antimicrobial resistance determinants including ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline.

Resistance was not detected for vancomycin, linezolid or teicoplanin when CLSI interpretive criteria were applied. However two isolates were teicoplanin non-susceptible when EUCAST criteria were applied.

Approximately 25% of SAB caused by CA-MRSA were of healthcare onset. Although in several parts of the United States of Australia the CA-MRSA clone USA300 has replaced the HA-MRSA clone ST5-II [2A] (USA100) as a cause of healthcare-associated

MRSA infection,[31] transmission of CA-MRSA in Australian hospitals is thought to be rare.[32,33] Consequently it is likely that many of the healthcare onset CA-MRSA SAB infections reported in ASSOP 2014 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 2014 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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