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## **Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP) Annual Report 2020**

Geoffrey W Coombs, Denise A Daley, Nicholas W T Yee, Princy Shoby, Shakeel Mowlaboccus, on behalf of  
the Australian Group on Antimicrobial Resistance

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# Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP) Annual Report 2020

Geoffrey W Coombs, Denise A Daley, Nicholas W T Yee, Princy Shoby, Shakeel Mowlaboccus, on behalf of the Australian Group on Antimicrobial Resistance

## Abstract

From 1 January to 31 December 2020, forty-nine institutions around Australia participated in the Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP). The aims of ASSOP 2020 were to determine the proportion of *Staphylococcus aureus* bacteraemia (SAB) isolates in Australia that were antimicrobial resistant, with particular emphasis on susceptibility to methicillin; and to characterise the molecular epidemiology of the methicillin-resistant isolates. A total of 2,734 SAB episodes were reported, of which 79.7% were community-onset. Of *S. aureus* isolates, 17.6% were methicillin resistant. The 30-day all-cause mortality associated with methicillin-resistant SAB was 14.2%, which was not significantly different from the 13.3% mortality associated with methicillin-susceptible SAB ( $p = 0.6$ ). With the exception of the  $\beta$ -lactams and erythromycin, antimicrobial resistance in methicillin-susceptible *S. aureus* was rare. However, in addition to the  $\beta$ -lactams, approximately 35% of methicillin-resistant *S. aureus* (MRSA) were resistant to erythromycin, 33% to ciprofloxacin, 13% to tetracycline, 13% to gentamicin and 4% to co-trimoxazole. When applying the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, teicoplanin resistance was detected in four *S. aureus* isolates. Resistance was not detected for vancomycin and linezolid. Resistance to non-beta-lactam antimicrobials was largely attributable to two healthcare-associated MRSA (HA-MRSA) clones: ST22-IV [2B] (EMRSA-15) and ST239-III [3A] (Aus-2/3 EMRSA). The ST22-IV [2B] (EMRSA-15) clone is the predominant HA-MRSA clone in Australia. However, 85% percent of methicillin-resistant SAB isolates were community-associated MRSA (CA-MRSA) clones. Although polyclonal, approximately 77% of CA-MRSA clones were characterised as: ST93-IV [2B] (Queensland CA-MRSA); ST5-IV [2B]; ST45-V [5C2&5]; ST1-IV [2B]; ST30-IV [2B]; ST8-IV [2B]; and ST97-IV [2B]. The CA-MRSA clones, in particular ST45-V [5C2&5], have acquired multiple antimicrobial resistance determinants including ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline. The multi-resistant ST45-V [5C2&5] clone accounted for 11.0% of CA-MRSA. As CA-MRSA is well established in the Australian community, it is important to monitor antimicrobial resistance patterns in community- and healthcare-associated SAB as this information will guide therapeutic practices in treating *S. aureus* sepsis.

**Keywords:** Australian Group on Antimicrobial Resistance (AGAR); antimicrobial resistance surveillance; *Staphylococcus aureus*, methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), bacteraemia

## Background

Globally, *Staphylococcus aureus* is one of the most frequent causes of hospital-acquired and community-acquired bloodstream infections.<sup>1</sup> Although there are a wide variety of manifestations of serious invasive infection caused by *S. aureus*, in the 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.<sup>2</sup>

Although prolonged antimicrobial therapy and prompt source control are used to treat SAB,<sup>3</sup> mortality ranges from as low as 2.5% to as high as 40%.<sup>4–6</sup> Mortality rates, however, are known to vary significantly with patient age, clinical manifestation, comorbidities and methicillin resistance.<sup>7,8</sup> A prospective study of SAB conducted in 27 laboratories in Australia and New Zealand found a 30-day all-cause mortality of 20.6%.<sup>9</sup> 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- $\beta$ -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.<sup>10</sup> In 2013, AGAR commenced the Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP).<sup>11</sup> The primary objective of ASSOP 2020 was to determine the proportion of SAB isolates demonstrating antimicrobial resistance, with particular emphasis on:

1. assessing susceptibility to methicillin; and
2. the molecular epidemiology of methicillin-resistant *S. aureus* (MRSA).

## Methodology

### Participants

Thirty laboratories servicing 49 institutions from all Australian states and mainland territories.

### Collection period

From 1 January to 31 December 2020, the 30 laboratories collected all *S. aureus* isolated from blood cultures. When isolated from a patient's blood culture within 14 days of the first positive culture, *S. aureus* isolates with the same antimicrobial susceptibility profiles 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 SAB was designated health-care-onset if the first positive blood culture(s) in an episode were collected more than 48 hours after admission.

### Laboratory testing

Participating laboratories performed antimicrobial susceptibility testing using the Vitek2<sup>®</sup> (bioMérieux, France) or BD Phoenix<sup>™</sup> (Becton Dickinson, USA) automated microbiology systems according to the manufacturer's instructions. Identification of *S. aureus* was achieved by matrix-assisted laser desorption ionization (MALDI) using either the Vitek MS<sup>®</sup> (bioMérieux, France) or the MALDI Biotyper (Bruker Daltonics, Germany). Appropriate growth on chromogenic agar or polymerase chain reaction (PCR) for the presence of the *nuc* gene was performed in some instances for confirmation.

Minimum inhibitory concentration (MIC) data and isolates were referred to the Antimicrobial Resistance and Infectious Diseases (AMRID) Research Laboratory at



Murdoch University. Clinical and Laboratory Standards Institute (CLSI)<sup>12</sup> and European Committee on Antimicrobial Susceptibility Testing (EUCAST)<sup>13</sup> breakpoints were utilised for interpretation. Linezolid and daptomycin non-susceptible isolates were retested by Etest<sup>®</sup> (bioMérieux) using the Mueller-Hinton agar recommended by the manufacturer. The control strain used was *S. aureus* ATCC<sup>®</sup> 29213. High-level mupirocin resistance was determined by the BD Phoenix<sup>™</sup>, or by using a mupirocin 200 µg disk according to CLSI guidelines, on all isolates with a mupirocin MIC > 8 mg/L by Vitek2<sup>®</sup>. 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, and linezolid.

Molecular testing was performed by whole genome sequencing using the NextSeq 500 platform (Illumina, USA). Sequencing results were analysed using the Nullarbor pipeline.<sup>14</sup> SCC<sub>mec</sub> was determined using KmerFinder v3.2,<sup>15</sup> and the SCC<sub>mec</sub> database curated from the CGE database.<sup>16,17</sup>

Confidence intervals (CI) for proportions, Fisher's exact test for categorical variables, and chi-square test for trend were calculated, as appropriate, using MedCalc for Windows, version 12.7 (MedCalc Software, 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 2020, there were 2,734 unique episodes of SAB identified. A significant difference ( $p < 0.0001$ ) was observed in patient sex with 1,823 (66.7%) being male (95% CI: 64.9–68.5). The mean age of patients was 56 years, ranging from 0 to 102 years, with a median age of 61 years. Overall, 2,180 episodes (79.7%) were community-onset (95%

CI: 78.1–81.2). All-cause mortality at 30 days (where known) was 13.5% (95% CI: 12.1–15.0). Methicillin-resistant SAB mortality was 14.2% (95% CI: 12.7–15.7); methicillin-susceptible SAB mortality was 13.3% (95% CI: 11.9–14.8).

## Methicillin-susceptible *Staphylococcus aureus* (MSSA) antimicrobial susceptibility

Overall, 2,253 of the 2,734 isolates (82.4%) were methicillin susceptible, of which 1,714/2,247 (76.3%) were penicillin resistant (MIC > 0.12 mg/L). However, as β-lactamase was detected in 57 phenotypically penicillin-susceptible isolates, 79.0% of MSSA were considered penicillin resistant. Eleven penicillin-susceptible isolates were not available for β-lactamase testing. Apart from erythromycin resistance (12.2% and 12.6% using CLSI and EUCAST breakpoints respectively), resistance to the non-β-lactam antimicrobials amongst MSSA was rare, ranging from 0% to 3.6% (Table 1). There were nine isolates reported by Vitek2<sup>®</sup> as non-susceptible to daptomycin (MIC > 1.0 mg/L). By Etest<sup>®</sup>, five of the nine isolates were considered daptomycin susceptible (MICs 0.19–1.0 mg/L). The four isolates with Etest<sup>®</sup> MICs of 1.5 and 2.0 mg/L were considered non-susceptible by CLSI and resistant by EUCAST interpretive criteria. Polymorphisms in genes encoding *mprF*, *walk*, *walR*, *cls*, *rpoB*, *rpoC*, *pgsA* and *agrA* were investigated. Mutations in *mprF* were identified in three of the four isolates. No known mutations were detected in the remaining isolate.

By Vitek2<sup>®</sup> or BD Phoenix<sup>™</sup>, six isolates were reported as linezolid resistant (MIC > 4 mg/L). By Etest<sup>®</sup>, the six isolates had MICs ranging between 0.5 and 1.0 mg/L and were therefore considered linezolid susceptible. Using EUCAST interpretive criteria, 34 isolates were reported by Vitek2<sup>®</sup> as resistant to teicoplanin (MIC > 2.0 mg/L). By Etest<sup>®</sup>, 32 of the 34 isolates had a teicoplanin MIC of ≤ 2.0 mg/L. The two isolates with MICs of 3.0 and 4.0 mg/L were considered resistant. All MSSA were vancomycin susceptible. Only 1,744 (77.4%) of the 2,253 MSSA had mupirocin susceptibility testing

performed, of which 17 (1.0%) were high-level mupirocin resistant. Twelve of the seventeen isolates were referred from Queensland. Nine of the seventeen mupirocin-resistant MSSA were also resistant to fusidic acid. Of the 2,249 isolates tested, 37 (1.7%) and 40 (1.8%) were constitutively resistant to clindamicin by CLSI and EUCAST criteria respectively. Both constitutive and inducible resistance was identified in 230 (10.2%) and 240 (10.7%) isolates by CLSI and EUCAST criteria respectively. Only 3% of MSSA were multi-resistant. By Vitek2<sup>®</sup> or BD Phoenix<sup>™</sup>, forty-three isolates were reported as non-susceptible to cotrimoxazole. By disc susceptibility testing, 37/43 (86.1%) and 36/43 (83.7%) were found to be susceptible by CLSI and EUCAST criteria respectively.

### MRSA antimicrobial susceptibility

The proportion of *S. aureus* that were MRSA was 17.6% (95% CI: 16.2–19.1). Of the 481 MRSA identified, 425 were ceftioxin-screen positive by Vitek2<sup>®</sup> and 56 had a ceftioxin MIC > 4.0 mg/L by BD Phoenix<sup>™</sup>. Two of the 481 MRSA isolates were phenotypically penicillin susceptible (MIC ≤ 0.125 mg/L). In one of these two isolates, β-lactamase was detected; the other isolate was not available for susceptibility confirmation. Amongst the MRSA isolates, resistance to non-β-lactam antimicrobials was common, except for resistance to rifampicin, nitrofurantoin, cotrimoxazole and fusidic acid which ranged from 0% to 4.4% (Table 2). All MRSA were vancomycin and linezolid susceptible. Four isolates were reported by Vitek2<sup>®</sup> as daptomycin non-susceptible (MIC > 1.0 mg/L). By Etest<sup>®</sup>, two of the four isolates were considered daptomycin susceptible (MICs 0.5 and 1.0 mg/L). The remaining two isolates were confirmed as non-susceptible by CLSI and resistant by EUCAST criteria (MICs 2.0 mg/L). Polymorphisms in genes encoding *mprF*, *walK*, *walR*, *cls*, *rpoB*, *rpoC*, *pgsA* and *agrA* were investigated. Mutations in *mprF* were identified in one isolate. No known mutations were detected in the second isolate.

By Vitek2<sup>®</sup>, six isolates were reported as teicoplanin resistant according to the EUCAST resistant breakpoint of > 2 mg/L, with MICs of 4.0 mg/L (five isolates) and 8.0 mg/L (one isolate). However, using the CLSI resistant breakpoint of > 8 mg/L, the six isolates were all classified as susceptible. By Etest<sup>®</sup>, five of the six isolates were considered susceptible, with MICs of 1.5 mg/L and 2.0 mg/L, and the remaining isolate, with an MIC of 4.0 mg/L, was resistant by EUCAST criteria. Four of 327 MRSA isolates tested (1.2%) had high-level mupirocin resistance.

Of the 480 isolates tested, 53 (11.0%) were constitutively resistant to clindamycin; 132 (27.5%) and 137 (28.5%) were classified as having both constitutive and inducible clindamycin resistance by CLSI and EUCAST criteria respectively.

By Vitek2<sup>®</sup> or BD Phoenix<sup>™</sup>, 69 isolates were reported as non-susceptible to cotrimoxazole. By disc susceptibility testing, 45/66 (68.2%) and 43/66 (65.2%) were found to be susceptible by CLSI and EUCAST criteria respectively. Three isolates were not available for confirmation.

Multi-resistance was seen in 21.4% of MRSA.

### MRSA molecular epidemiology

Whole genome sequencing was performed on 456 of the 481 MRSA (94.8%). Based on molecular typing, 69 (15.1%) and 387 (84.9%) of isolates were identified as healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) clones respectively (Table 3).

**Table 1: The number and proportion of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2020**

Antimicrobial	Tested	Breakpoint guideline	Breakpoint (mg/L) <sup>a</sup>			R	Susceptible (%)	Intermediate (%)	Resistant (%)
			S	I					
Benzylpenicillin	2,247	CLSI/EUCAST	≤0.12		≥0.25	23.7	— <sup>b</sup>	76.3	
Benzylpenicillin β-lactamase adjusted	2,241	CLSI/EUCAST	≤0.12		≥0.25	21.0	— <sup>b</sup>	79.0	
Ciprofloxacin	2,250	CLSI	≤1	2	≥4	97.4	0.5	2.1	
		EUCAST	≤0.001	0.002–1	>1	0.0	96.4	3.6	
Clindamycin (constitutive)	2,249	CLSI	≤0.5	1–2	≥4	98.2	0.1	1.7	
		EUCAST	≤0.25	0.5	>0.5	97.6	0.6	1.8	
Clindamycin (constitutive and inducible)	2,249	CLSI	≤0.5	1–2	≥4	89.7	0.1	10.2	
		EUCAST	≤0.25	0.5	>0.5	88.8	0.5	10.7	
Cotrimoxazole	2,249	CLSI	≤2/38		≥4/76	99.8	— <sup>b</sup>	0.2	
		EUCAST	≤2/38	4/76	>4/76	99.7	0.1	0.2	
Daptomycin	2,249	CLSI/EUCAST	≤1		>1 <sup>c</sup>	99.8	— <sup>b</sup>	0.2	
Erythromycin	2,250	CLSI	≤0.5	1–4	≥8	57.2	30.6	12.2	
		EUCAST	≤1	2	>2	86.5	0.9	12.6	
Fusidic acid	2,249	EUCAST	≤1		>1	97.0	— <sup>b</sup>	3.0	
		CLSI	≤4	8	≥16	98.6	0.6	0.8	
Gentamicin	2,250	EUCAST	≤1		>1	98.0	— <sup>b</sup>	2.0	
		CLSI/EUCAST	<256		≥256	99.0	— <sup>b</sup>	1.0	
High-level mupirocin	2,249	CLSI	≤4		≥8	100.0	— <sup>b</sup>	0.0	
		EUCAST	≤4		>4	100.0	— <sup>b</sup>	0.0	
Nitrofurantoin	2,323	CLSI	≤32	64	≥128	99.2	0.8	1.0	
		CLSI	≤1	2	≤4	99.8	0.0	0.2	
Rifampicin	2,249	EUCAST	≤0.06	0.12–0.5	>0.5	99.7 <sup>d</sup>	— <sup>b</sup>	0.3	
		CLSI	≤8	16	≥32	100.0	0.0	0.0	
Teicoplanin	2,251	EUCAST	≤2		>2	99.9	— <sup>b</sup>	0.1	
		CLSI	≤4	8	≥16	97.7	0.2	2.1	
Tetracycline/doxycycline	2,249	EUCAST	≤1	2	>2	97.1	0.4	2.5	
		CLSI	≤2	4–8	≥16	100.0	0.0	0.0	
Vancomycin	2,251	EUCAST	≤2		>2	100.0	— <sup>b</sup>	0.0	
		CLSI	≤2		>2	100.0	— <sup>b</sup>	0.0	

a S: susceptible; I: intermediate; R: resistant.

b No category defined.

c Non-susceptible; resistance not defined for CLSI guidelines.

d The rifampicin concentration on some cards restricts category interpretation to non-resistant.

**Table 2: The number and proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2020**

Antimicrobial	Tested	Breakpoint guideline	Breakpoint (mg/L) <sup>a</sup>			Susceptible (%)	Intermediate (%)	Resistant (%)
			S	I	R			
Benzylpenicillin	480	CLSI/EUCAST	≤0.12		≥0.25	0.4	— <sup>b</sup>	99.6
Benzylpenicillin β-lactamase adjusted	480	CLSI/EUCAST	≤0.12		≥0.25	0.0	— <sup>b</sup>	100.0
Ciprofloxacin	481	CLSI	≤1	2	≥4	67.0	0.6	32.4
		EUCAST	≤0.001	0.002–1	>1	0.0	66.9	33.1
Clindamycin (constitutive)	480	CLSI	≤0.5	1–2	≥4	89.0	0.0	11.0
		EUCAST	≤0.25	0.5	>0.5	88.6	0.4	11.0
Clindamycin (constitutive and inducible)	480	CLSI	≤0.5	1–2	≥4	72.3	0.2	27.5
		EUCAST	≤0.25	0.5	>0.5	71.3	0.2	28.5
Cotrimoxazole	481	CLSI	≤2/38		≥4/76	96.0	— <sup>b</sup>	4.0
		EUCAST	≤2/38	4/76	>4/76	95.2	0.8	4.0
Daptomycin	481	CLSI/EUCAST	≤1		>1 <sup>c</sup>	99.6	— <sup>b</sup>	0.4
Erythromycin	481	CLSI	≤0.5	1–4	≥8	49.4	17.3	33.3
		EUCAST	≤1	2	>2	65.5	0.0	34.5
Fusidic acid	481	EUCAST	≤1		>1	95.6	— <sup>b</sup>	4.4
Gentamicin	481	CLSI	≤4	8	≥16	89.4	4.2	6.4
		EUCAST	≤1		>1	86.1	— <sup>b</sup>	13.9
High-level mupirocin	327	CLSI/EUCAST	<256		≥256	98.8	— <sup>b</sup>	1.2
		CLSI	≤4		≥8	100.0	— <sup>b</sup>	0.0
Linezolid	481	EUCAST	≤4		>4	100.0	— <sup>b</sup>	0.0
		CLSI	≤32	64	≥128	98.6	1.4	0.0
Nitrofurantoin		CLSI	≤1		≤4	99.8	0.0	0.2
Rifampicin	480	EUCAST	≤0.06	0.12–0.5	>0.5	98.8 <sup>d</sup>	— <sup>b</sup>	0.2
		CLSI	≤8	16	≥32	100.0	0.0	0.0
Teicoplanin	481	EUCAST	≤2		>2	99.8	— <sup>b</sup>	0.2
		CLSI	≤4	8	≥16	86.7	0.4	12.9
Tetracycline/doxycycline	481	EUCAST	≤1	2	>2	84.6	1.9	13.5
		CLSI	≤2	4–8	≥16	100.0	0.0	0.0
Vancomycin	481	EUCAST	≤2		>2	100.0	— <sup>b</sup>	0.0

a S: susceptible; I: intermediate; R: resistant.

b No category defined.

c Non-susceptible; resistance not defined for CLSI guidelines.

d The rifampicin concentration on some cards restricts category interpretation to non-resistant



**Table 3: Proportion of healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus*, Australia, 2020 by clone, onset, and Panton-Valentine leucocidin carriage**

MLST	Total		Onset				PVL positive	
			Hospital		Community			
	n	% <sup>a</sup>	n	% <sup>b</sup>	n	% <sup>b</sup>	n	% <sup>b</sup>
<b>Healthcare-associated MRSA</b>								
ST22-IV [2B] (EMRSA-15)	59	12.9	16	27.1	43	72.9	0	–
ST239-III [3A] (Aus-2/3)	7	1.5	2	28.6	5	71.4	0	–
ST36-II [2A] (EMRSA-16)	1	0.2	0	–	1	100.0	0	–
ST5-II (NY/Japan)	1	0.2	1	100.0	0	–	0	–
ST8-II (EMRSA-1)	1	0.2	1	100.0	0	–	0	–
<b>Total HA-MRSA</b>	<b>69</b>	<b>15.1</b>	<b>20</b>	<b>29.0</b>	<b>49</b>	<b>71.0</b>	<b>0</b>	<b>0.0</b>
<b>Community-associated MRSA</b>								
ST93-IV	100	21.9	12	12.0	88	88.0	99	99.0
ST5-IV	59	12.9	16	27.1	43	72.9	29	49.2
ST45-V	50	11	13	26.0	37	74.0	0	–
ST1-IV	29	6.4	7	24.1	22	75.9	0	–
ST30-IV	21	4.6	2	9.5	19	90.5	17	81.0
ST8-IV	16	3.5	2	12.5	14	87.5	12	75.0
ST97-IV	14	3.1	4	28.6	10	71.4	0	–
ST78-IV	10	2.2	4	40.0	6	60.0	0	–
ST953-IV	8	1.8	1	12.5	7	87.5	0	–
ST6-IV	7	1.5	4	57.1	3	42.9	0	–
ST188-IV	5	1.1	2	40.0	3	60.0	0	–
ST22-IV	5	1.1	1	20.0	4	80.0	5	100.0
ST59-IV	5	1.1	1	20.0	4	80.0	1	20.0
ST59-V	5	1.1	1	20.0	4	80.0	2	40.0
ST872-IV	5	1.1	2	40.0	3	60.0	0	–
ST88-IV	4	0.9	2	50.0	2	50.0	0	–
ST5-V	3	0.7	2	66.7	1	33.3	0	–
ST72-V	3	0.7	0	–	3	100.0	0	–
ST835-I	3	0.7	1	33.3	2	66.7	0	–
ST188-V	2	0.4	1	50.0	1	50.0	0	–
ST398-V	2	0.4	0	–	2	100.0	0	–
ST6145-V	2	0.4	1	50.0	1	50.0	0	–
ST6151-IV	2	0.4	0	–	2	100.0	2	100.0
ST672-V	2	0.4	1	50.0	1	50.0	0	–
ST834-IV	2	0.4	1	50.0	1	50.0	0	–
ST1232-V	1	0.2	1	100.0	0	–	1	100.0
ST12-V	1	0.2	1	100.0	0	–	0	–
ST149-IV	1	0.2	0	–	1	100.0	0	–
ST2250-IV	1	0.2	0	–	1	100.0	0	–

MLST	Total		Onset				PVL positive	
			Hospital		Community			
	n	% <sup>a</sup>	n	% <sup>b</sup>	n	% <sup>b</sup>	n	% <sup>b</sup>
ST3841-IV	1	0.2	1	100.0	0	–	0	–
ST4197-IV	1	0.2	0	–	1	100.0	1	100.0
ST45-IV	1	0.2	0	–	1	100.0	0	–
ST5665-IV	1	0.2	1	100.0	0	–	0	–
ST5669-IV	1	0.2	1	100.0	0	–	0	–
ST6156-IV	1	0.2	0	–	1	100.0	0	–
ST6643-IV	1	0.2	0	–	1	100.0	0	–
ST672-IV	1	0.2	0	–	1	100.0	0	–
ST6957-V	1	0.2	0	–	1	100.0	0	–
ST6959-IV	1	0.2	0	–	1	100.0	0	–
ST6960-IV	1	0.2	0	–	1	100.0	0	–
ST6963-IV	1	0.2	0	–	1	100.0	0	–
ST6965-V	1	0.2	0	–	1	100.0	0	–
ST6967-IV	1	0.2	0	–	1	100.0	0	–
ST6968-IV	1	0.2	0	–	1	100.0	1	100.0
ST6973-V	1	0.2	1	100.0	0	–	0	–
ST6974-IV	1	0.2	0	–	1	100.0	0	–
ST73-IV	1	0.2	1	100.0	0	–	0	–
ST80-IV	1	0.2	0	–	1	100.0	0	–
<b>Total CA-MRSA</b>	<b>387</b>	<b>84.9</b>	<b>88</b>	<b>65.6</b>	<b>299</b>	<b>19.3</b>	<b>170</b>	<b>43.9</b>
<b>Grand total</b>	<b>456</b>	<b>100.0</b>	<b>108</b>	<b>23.7<sup>a</sup></b>	<b>348</b>	<b>72.3<sup>a</sup></b>	<b>170</b>	<b>37.3<sup>a</sup></b>

a Percentage of all MRSA typed.

b Percentage of the strain.

### Healthcare-associated methicillin-resistant *Staphylococcus aureus*

For the 69 HA-MRSA isolates, 20 (29.0%) were classified as hospital-onset and 49 (71.0%) were classified as community-onset. Five HA-MRSA clones were identified: 59 isolates of ST22-IV [2B] (EMRSA-15) (12.9% of MRSA typed and 2.3% of *S. aureus*); seven isolates of ST239-III [3A] (Aus -2/3 EMRSA) (1.5% and 0.3%), and one isolate each of ST5-II [2A] (NY/Japan), ST36-II [2A] (EMRSA-16) and ST8-II (Irish EMRSA-1) (0.2% and 0.04% each).

ST22-IV [2B] (EMRSA-15) was the dominant HA-MRSA clone in Australia in 2020, accounting for 85.5% of HA-MRSA, ranging from 0% in South Australia to 100% in Western Australia,

Tasmania, and the Northern Territory (Table 4). ST22-IV [2B] (EMRSA-15) is Pantone-Valentine leucocidin (PVL) negative and, using CLSI breakpoints, 98.3% and 54.2% were ciprofloxacin and erythromycin non-susceptible respectively. Overall, 27.1% of ST22-IV [2B] (EMRSA-15) were hospital-onset.

ST239-III [3A] (Aus-2/3 EMRSA) accounted for 10.1% of HA-MRSA and was isolated in Victoria (7.1%), New South Wales (11.5%) and Queensland (27.3%) (Table 4). PVL-negative ST239-III [3A] (Aus-2/3 EMRSA) were typically resistant to erythromycin (100%), cotrimoxazole (100%), ciprofloxacin (100%), gentamicin (100%), tetracycline (100%) and clindamycin (85.7%). Overall, 28.6% of ST239-III [3A] (Aus-2/3 EMRSA) were hospital-onset.

## Community-associated methicillin-resistant *Staphylococcus aureus*

For the 387 CA-MRSA isolates, 88 episodes (22.7%) were classified as hospital-onset and 299 (77.3%) as community-onset. Based on the multi-locus sequence type and the *SCCmec* type, 48 CA-MRSA clones were identified (Table 3). Overall, 77.3% of CA-MRSA were classified into eight clones each having ten or more isolates: 100 isolates of ST93-IV [2B] (Queensland CA-MRSA) (21.7% of MRSA typed and 3.6% of *S. aureus*); 59 isolates of ST5-IV [2B] (12.9% and 2.2%); 50 isolates of ST45-V [5C2&5] (11.0% and 1.8%); 29 isolates of ST1-IV [2B] (6.4% and 1.1%); 21 isolates of ST30-IV [2B] (4.6% and 0.8%); 16 isolates of ST8-IV [2B] (3.5% and 0.6%); 14 isolates of ST97-IV [2B] (3.1% and 0.5%) and 10 isolates of ST78-IV [2B] (2.2% and 0.4%).

ST93-IV [2B] (Queensland CA-MRSA) accounted for 25.8% of CA-MRSA, ranging from 0% in Tasmania and the Australian Capital Territory to 52.8% in the Northern Territory (Table 5). Typically PVL positive, ST93-IV [2B] (Queensland CA-MRSA) were resistant to the  $\beta$ -lactams only (78/100; 78.0%) or additionally resistant to erythromycin (12/100; 12.0%) or to erythromycin and clindamycin (9/100; 9.0%) and a single isolate to daptomycin. Overall, 87.9% of ST93-IV [2B] were community-onset.

ST5-IV [2B] accounted for 15.2% of CA-MRSA and was isolated in all jurisdictions of Australia except the Australian Capital Territory, ranging from 10.5% in Queensland to 27.8% in the Northern Territory (Table 5). Overall, 49.2% and 50.8% of ST5-IV [2B] were PVL positive and PVL negative respectively. PVL-positive ST5-IV [2B] was resistant to the  $\beta$ -lactams only (22/29; 75.9%), with other isolates additionally resistant to erythromycin (3/29; 10.3%); to erythromycin, tetracycline and cotrimoxazole (2/29; 6.9%); and single isolates resistant to cotrimoxazole and gentamicin alone. PVL-negative ST5-IV [2B] was resistant to the  $\beta$ -lactams only (16/30; 53.3%) or additionally resistant to fusidic acid (8/30; 26.7%); to erythromycin (3/30; 10.0%);

to tetracycline (2/30; 6.7%); and a single isolate resistant to ciprofloxacin. Overall 72.9% of ST5-IV [2B] were community-onset.

ST45-V [5C2&5] accounted for 12.9% of CA-MRSA and was isolated primarily in New South Wales and Victoria (Table 5). All isolates were PVL negative. In addition to the  $\beta$ -lactams and ciprofloxacin, isolates were resistant to erythromycin, gentamicin and tetracycline (14/50; 28.0%); to erythromycin, and tetracycline (6/50; 12.0%); to erythromycin and gentamicin (5/50; 10.0%); to gentamicin and tetracycline (5/50; 10.0%); to gentamicin (4/50; 8.0%); to clindamycin, erythromycin, gentamicin and tetracycline (4/50; 8.0%); to clindamycin, erythromycin and gentamicin (2/50; 4.0%) and single isolates resistant to clindamycin, erythromycin, tetracycline and cotrimoxazole; to erythromycin, fusidic acid, gentamicin and tetracycline; to erythromycin, fusidic acid, and tetracycline; to clindamycin, erythromycin and tetracycline; to clindamycin and erythromycin; to erythromycin, fusidic acid, and tetracycline; and to tetracycline. Overall, 74.0% of ST45-V [5C2&5] were community-onset.

ST1-IV [2B] accounted for 7.5% of CA-MRSA and was isolated in all regions of Australia except the Australian Capital Territory, ranging from 1.9% in Victoria to 12.5% in South Australia (Table 5). All isolates were PVL negative, 58.6% of isolates were resistant to the  $\beta$ -lactams only (17/29), with others additionally resistant to erythromycin (5/29; 17.4%) or to ciprofloxacin and erythromycin (2/29; 6.9%). Single isolates were resistant to tetracycline; to fusidic acid; erythromycin and clindamycin; to erythromycin and tetracycline; and to erythromycin and cotrimoxazole. Overall, 75.9% of ST1-IV [2B] were community-onset.

ST30-IV [2B] accounted for 5.4% of CA-MRSA and was isolated in all jurisdictions of Australia except Tasmania, ranging from 2.8% in the Northern Territory to 7.0% in Queensland (Table 5). ST30-IV [2B], of which 81% were PVL positive, was typically resistant to the  $\beta$ -lactams only (17/21, 81.0%). Three isolates (14.3%)

**Table 4: The number and proportion of healthcare-associated methicillin-resistant *Staphylococcus aureus* (MRSA) multilocus sequence types (MLST), Australia, 2020, by jurisdiction<sup>a</sup>**

MLST	ACT		NSW		NT		Qld		SA		Tas.		Vic.		WA		Australia	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ST22-IV	3	100	22	84.6	2	100	8	72.7	0	—	4	100	11	78.6	9	100	<b>59</b>	<b>85.5</b>
ST239-III	0	0	3	11.5	0	0	3	27.3	0	—	0	0	1	7.1	0	0	<b>7</b>	<b>10.1</b>
ST36-II	0	0	0	0	0	0	0	0	0	—	0	0	1	7.1	0	0	<b>1</b>	<b>1.4</b>
ST5-II	0	0	1	3.8	0	0	0	0	0	—	0	0	0	0	0	0	<b>1</b>	<b>1.4</b>
ST8-II	0	0	0	0	0	0	0	0	0	—	0	0	1	7.1	0	0	<b>1</b>	<b>1.4</b>
<b>Total</b>	<b>3</b>		<b>26</b>		<b>2</b>		<b>11</b>		<b>0</b>		<b>4</b>		<b>14</b>		<b>9</b>		<b>69</b>	

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

were additionally resistant to erythromycin and clindamycin; a single isolate to erythromycin. Overall, 90.5% of ST30-IV [2B] were community-onset.

ST8-IV [2B] accounted for 4.1% of CA-MRSA and was isolated in New South Wales, Victoria, Queensland and Western Australia (Table 5). Thirteen isolates of ST8-IV [2B] (81.2%) were PVL negative. Nine isolates (56.2%) were resistant to the  $\beta$ -lactams only. Three isolates (18.8%) were also resistant to erythromycin and ciprofloxacin. Single isolates were resistant to ciprofloxacin; to erythromycin; to high-level mupirocin; and to erythromycin, ciprofloxacin and high-level mupirocin. Overall, 87.5% of ST8-IV [2B] were community-onset.

ST97-IV [2B] accounted for 3.6% of CA-MRSA and was isolated from all jurisdictions except South Australia and the Australian Capital Territory, ranging from 2.3% in Western Australia to 5.0% in New South Wales (Table 5). All isolates of ST97-IV [2B] were PVL negative and resistant to the  $\beta$ -lactams only. Overall, 71.4% of ST97-IV [2B] isolates were community-onset.

ST78-IV [2B] accounted for 2.6% of CA-MRSA and was isolated from New South Wales, Victoria and Western Australia (Table 5). All isolates of ST78-IV [2B] were PVL negative. Two isolates were resistant to the  $\beta$ -lactams only. Seven

isolates were additionally resistant to erythromycin (7/10; 70.0%) and one isolate was resistant to erythromycin and tetracycline. Overall 60.0% of ST78-IV [2B] were community-onset.

Overall, 84.5% of CA-MRSA isolates were non-multi-resistant, including 54.5% isolates resistant to the  $\beta$ -lactams only. A significant increase was seen in multi-resistant CA-MRSA isolates in ASSOP 2020 (15.5%) from 9.2% in ASSOP 2013.<sup>11</sup> Multi-resistance was primarily due to the ST45-V [5C2&5] clone.

### Panton-Valentine leucocidin

Overall, 170 (43.9%) of MRSA were PVL positive. All were CA-MRSA (Table 3).

### Discussion

The AGAR surveillance programmes collect data on antimicrobial resistance, focussing on bloodstream infections caused by *S. aureus*, *Enterococcus*, and gram-negative bacilli including the *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter* species. All data collected in the AGAR programs are generated as part of routine patient care in Australia, with most 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 programmes

**Table 5: The number and proportion of the major community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) multilocus sequence types, Australia ( $\geq 10$  isolates), 2020, by jurisdiction<sup>a</sup>**

MLST	ACT <sup>b</sup>		NSW		NT		Qld		SA		Tas. <sup>b</sup>		Vic.		WA		Australia	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
<b>ST93-IV</b>	0	—	11	9.1	19	52.8	13	22.8	8	33.3	0	—	17	31.5	32	36.8	<b>100</b>	<b>25.8</b>
<b>ST5-IV</b>	0	—	16	13.2	10	27.8	6	10.5	3	12.5	1	—	6	11.1	17	19.5	<b>59</b>	<b>15.2</b>
<b>ST45-V</b>	2	—	36	29.8	0	0	0	0	1	4.2	0	—	11	20.4	0	0	<b>50</b>	<b>12.9</b>
<b>ST1-IV</b>	0	—	10	8.3	1	2.8	7	12.3	3	12.5	1	—	1	1.9	6	6.9	<b>29</b>	<b>7.5</b>
<b>ST30-IV</b>	1	—	8	6.6	1	2.8	4	7	1	4.2	0	—	3	5.6	3	3.4	<b>21</b>	<b>5.4</b>
<b>ST8-IV</b>	0	—	8	6.6	0	0	3	5.3	0	0	0	—	1	1.9	4	4.6	<b>16</b>	<b>4.1</b>
<b>ST97-IV</b>	0	—	6	5	1	2.8	2	3.5	0	0	1	—	2	3.7	2	2.3	<b>14</b>	<b>3.6</b>
<b>ST78-IV</b>	0	—	1	0.8	0	0	0	0	0	0	0	—	2	3.7	7	8	<b>10</b>	<b>2.6</b>
<b>Other</b>	2	—	25	20.7	4	11.1	22	38.6	8	33.3	0	—	11	20.4	6	18.4	<b>88</b>	<b>22.7</b>
<b>Total</b>	<b>5</b>		<b>121</b>		<b>36</b>		<b>57</b>		<b>24</b>		<b>3</b>		<b>54</b>		<b>87</b>		<b>387</b>	

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

b Percentages not calculated for jurisdictions with < 10 CA-MRSA isolates in total.

are similar to those conducted in Europe,<sup>18</sup> comparison of Australian antimicrobial resistance data with other countries is possible.

In ASSOP 2020, methicillin resistance was found in 17.6% (95% CI: 16.2–19.1) of the 2,734 SAB episodes. In the 2019 European Centre for Disease Prevention and Control (ECDC) SAB surveillance program, the European Union/European Economic Area (EU/EEA) population-weighted mean percentage of *S. aureus* resistant to methicillin was 15.5% (95% CI: 15–16), ranging from 1.1% (95% CI: 0.6–1.7) in Norway to 46.7% (95% CI: 42.7–50.1) in Romania.<sup>18</sup>

In Europe, the EU/EEA population-weighted mean percentage has significantly decreased from 23.2% in 2009 to 15.5% in 2019. A decrease in methicillin-resistant SAB has been reported in several parts of the world,<sup>19,20</sup> and is believed to be due 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.<sup>21–25</sup> The percentage of methicillin-resistant SAB in Australia, however, has not decreased significantly over the eight years of ASSOP, ranging from 18.3% in 2013 to 17.6% in 2020 ( $p = 0.06$ ). Nonetheless, while a significant decrease in MRSA bacteraemia has not been seen in Australia, significant decreases in HA-MRSA from 41.0% to 15.1% ( $p < 0.0001$ ) and in hospital-onset MRSA from 38.0% to 23.1% ( $p < 0.0001$ ) have been observed over the eight ASSOP surveys.<sup>11,26–31</sup> Over the same time period, significant increases in CA-MRSA from 59.0% to 84.9% ( $p < 0.0001$ ) and in community-onset MRSA from 61.1% to 79.6% ( $p < 0.0001$ ) have been observed. Because of the increased burden of CA-MRSA bacteraemia in Australia, a significant reduction in the overall proportion of SAB due to MRSA may prove problematic.

In ASSOP 2020, the all-cause mortality at 30-days was 13.5% (95% CI: 12.1–15.0%). No significant difference in mortality was observed between methicillin-resistant SAB and methicillin-susceptible SAB ( $p = 0.6$ ).



With the exception of the  $\beta$ -lactams and erythromycin, antimicrobial resistance in MSSA remains rare. However, for MRSA, in addition to resistance to the  $\beta$ -lactams, approximately 33% of isolates were resistant to erythromycin and ciprofloxacin and approximately 13% were resistant to tetracycline and gentamicin. Resistance was largely attributable to two HA-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, cotrimoxazole, tetracycline and gentamicin resistant. In the early 1980s, the multi-resistant ST239-III [3A] (Aus-2/3 EMRSA) was the dominant HA-MRSA clone in Australian hospitals. However, in 2013 the first ASSOP survey showed that ST22-IV [2B] (EMRSA-15) was replacing ST239-III [3A] (Aus-2/3 EMRSA) as the most prevalent HA-MRSA, and this change has occurred throughout most of the country.<sup>32</sup> In ASSOP 2020, approximately 12.9% of MRSA were characterised as ST22-IV [2B] (EMRSA-15).

In ASSOP 2020, ST93-IV [2B] (Queensland CA-MRSA) remained the predominant CA-MRSA clone (25.8%) in Australia. CA-MRSA, in particular the ST45-V [5C2&5] clone (11.0% of MRSA), has acquired multiple antimicrobial resistance determinants including ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline.

Approximately 22.7% of SAB caused by CA-MRSA was hospital-onset. As transmission of CA-MRSA in Australian hospitals is thought to be rare,<sup>33,34</sup> it is likely that many of the hospital-onset CA-MRSA SAB infections reported in ASSOP 2020 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) 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 2020 has demonstrated that antimicrobial resistance in SAB in Australia continues to be a significant problem and is associated with a high mortality. This may be due, in part, to the high prevalence of community-associated methicillin-resistant SAB in Australia, which is higher than most EU/EEA countries. Consequently, MRSA must remain a public health priority; 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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