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Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP) Bloodstream Infection Annual Report 2023

Geoffrey W Coombs, Denise A Daley, Princy Shoby, Sruthi Mamoottil Sudeep,
Shakeel Mowlaboccus, on behalf of the Australian Group on Antimicrobial Resistance

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Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP) Bloodstream Infection Annual Report 2023

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Abstract

From 1 January to 31 December 2023, fifty-seven institutions across Australia participated in the Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP). The aim of ASSOP 2023 was to determine the proportion of *Staphylococcus aureus* bacteraemia (SAB) isolates in Australia that were antimicrobial resistant, with particular emphasis on methicillin resistance, and to characterise the methicillin-resistant *S. aureus* (MRSA) molecular epidemiology. A total of 3,422 SAB episodes were reported, of which 77.0% were community-onset. Overall, 16.1% of *S. aureus* were methicillin resistant. The 30-day all-cause mortality associated with methicillin-resistant SAB was 14.8%, which was not significantly different to the 16.5% all-cause mortality associated with methicillin-susceptible SAB ($p = 0.44$). With the exception of the β -lactams and erythromycin, antimicrobial resistance in methicillin-susceptible *S. aureus* (MSSA) was infrequent. However, in addition to the β -lactams, approximately 33% of MRSA were resistant to ciprofloxacin; 30% to erythromycin; 13% to tetracycline; 13% to gentamicin; and 3% to co-trimoxazole. Two New South Wales daptomycin-resistant MRSA, with minimum inhibitory concentrations (MICs) of 3.0 and 4.0 mg/L, were identified as ST22-IV, with a V351E *mprF* mutation, and ST45-V with a T345I *mprF* mutation respectively. Three daptomycin-resistant MSSA were identified. One from Tasmania, with a daptomycin MIC of 1.5 mg/L, identified as ST9295 with a L341I *MprF* mutation; one from New South Wales, with a daptomycin MIC of 3.0 mg/L, identified as ST97 with a L776S *mprF* mutation; and one from Western Australia, with a daptomycin MIC of 2.0 mg/L, identified as ST5. No previously reported mutations in known loci were detected in the Western Australian isolate. When applying the European Committee on Antimicrobial Susceptibility Testing breakpoints, teicoplanin resistance was detected in three MSSA isolates and one MRSA isolate. Vancomycin or linezolid resistance was not detected. Resistance to non- β -lactam antimicrobials was largely attributable to the healthcare-associated MRSA (HA-MRSA) clone ST22-IV [2B] (EMRSA-15), and the community-associated MRSA (CA-MRSA) clone ST45-V [5C2&5] which has acquired resistance to multiple antimicrobials including ciprofloxacin, clindamycin, erythromycin, gentamicin, and tetracycline. ST22-IV [2B] (EMRSA-15) was the predominant HA-MRSA clone in Australia. Overall, 85% of methicillin-resistant SAB were caused by community-associated MRSA (CA-MRSA) clones. Although polyclonal, approximately 70.3% of CA-MRSA clones were characterised as ST93-IV [2B] (Queensland clone); ST5-IV [2B]; ST1-IV [2B]; ST45-V [5C2&5]; ST30-IV [2B]; ST8-IV [2B]; ST6-IV [2B]; ST97-IV [2B]; and ST953-IV [2B]. 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* bacteraemia.

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 blood stream infections.¹ Although there are a wide variety of manifestations of serious invasive infection caused by *S. aureus*, in the majority of 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 In 2009, the Infectious Diseases Society of America highlighted *S. aureus* as one of the key problem bacteria or ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) requiring new therapies.³ In 2024, the World Health Organisation listed methicillin-resistant *S. aureus* (MRSA) in its bacterial priority list of pathogens.⁴

Although prolonged antimicrobial therapy and prompt source control are used to treat SAB,⁵ mortality ranges from as low as 2.5% to as high as 40%.^{6–9} Mortality rates, however, are known to vary significantly with patient age, clinical manifestation, comorbidities and methicillin resistance. A prospective study of SAB conducted in 27 laboratories in Australia and New Zealand found a 30-day all-cause mortality of 20.6%.¹⁰ 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 1986.¹¹ In 2013, AGAR commenced the Australian *Staphylococcus aureus* Sepsis Outcome Program, now known as the Australian *Staphylococcus aureus* Surveillance Outcome Program (ASSOP).¹² The primary objective of ASSOP 2023 was to determine the proportion of SAB isolates displaying antimicrobial resistance, with particular emphasis on:

1. susceptibility to methicillin; and
2. the molecular epidemiology of MRSA.

Methodology

Participants

Thirty-three laboratories servicing 57 institutions from all Australian states and mainland territories.

Collection period

From 1 January to 31 December 2023, the 33 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* with the same antimicrobial susceptibility profiles were excluded. A new SAB 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, dates 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 SAB episode was designated healthcare onset if the first positive blood culture(s) in the episode were collected > 48 hours after admission.

Laboratory testing

Participating laboratories performed antimicrobial susceptibility testing using the Vitek2[®] (bioMérieux, France) or the BD Phoenix[™] (Becton Dickinson, USA) automated microbiology systems according to the manufacturer's instructions. Identification of *S. aureus* was achieved by matrix-assisted laser desorption ionisation (MALDI) using either the Vitek MS[®] (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 may have been performed for confirmation.

Minimum inhibitory concentration (MIC) data and isolates were referred to the ASSOP reference laboratory at Murdoch University. The European Committee on Antimicrobial Susceptibility Testing (EUCAST)¹³ MIC breakpoints were utilised for interpretation. Linezolid, teicoplanin and daptomycin non-susceptible isolates were retested by Etest[®] (bioMérieux) as recommended by the manufacturer. The control strain used was *S. aureus* ATCC[®] 29213.

All *S. aureus* with a penicillin MIC \leq 0.12 mg/L, without a β -lactamase result provided by the referring laboratory, were confirmed by disk diffusion using a 10U penicillin disk. High-level mupirocin resistance was determined by the BD Phoenix™ or by using a mupirocin 200 μ g disk on all isolates with a mupirocin MIC $>$ 8 mg/L by Vitek2®. Cotrimoxazole-resistant isolates were confirmed using a 25 μ g disk. All disk susceptibility testing was performed using Clinical and Laboratory Standards Institute (CLSI) methodology and breakpoints.¹⁴

Multi-resistance was defined as resistance to three or more of the following non- β -lactam antimicrobials: ciprofloxacin, co-trimoxazole, erythromycin/clindamycin, fusidic acid, gentamicin, linezolid, high-level mupirocin, rifampicin, tetracycline, teicoplanin, and vancomycin.

Whole genome sequencing (WGS) was performed by the ASSOP Research Laboratory at Murdoch University using the NextSeq 500 platform (Illumina, San Diego, USA). Sequence reads were analysed using the Nullarbor pipeline.¹⁵ The SCCmec type was determined using KmerFinder v3.2 and the SCCmec database curated from the Center for Genomic Epidemiology database.^{16–19}

Confidence intervals for proportions, Fisher's exact test for categorical variables, and chi-square test for trend were calculated, if 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 2023, there were 3,422 unique episodes of SAB identified. A significant difference ($p < 0.0001$) was observed in patient sex, with 2,260 (66.0%) being male (95% confidence interval [95% CI]: 63.4–68.8). The mean age of patients was 58 years, ranging from 0 to 103 years, with a median age of 63 years. Overall, 2,636 episodes (77.0%) were community-onset (95% CI: 74.1–80.0). All-cause mortality at 30 days (where known) was 16.2% (95% CI: 14.7–17.8). Methicillin-resistant SAB mortality was 14.8% (95% CI: 11.5–18.9); methicillin-susceptible SAB mortality was 16.5% (95% CI: 14.9–18.3).

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

Overall, 2,872 of the 3,422 isolates (83.9%) were methicillin susceptible. Where results were available, 2,089/2,848 MSSA isolates (73.3%) were penicillin resistant (MIC $>$ 0.12 mg/L). All available penicillin-susceptible isolates (MIC \leq 0.12 mg/L) were retested by penicillin disc diffusion (zone-edge test). On testing, a further 67 phenotypically penicillin-susceptible isolates were considered penicillin-resistant. Forty-seven penicillin-susceptible isolates were not available for confirmation. Apart from erythromycin resistance (15.6%), resistance to the non- β -lactam antimicrobials amongst MSSA was infrequent (Table 1).

Nine isolates were identified by Vitek2® as resistant to daptomycin (MIC $>$ 1.0 mg/L). Of these nine isolates, two were unavailable for confirmation. By Etest®, four isolates were considered daptomycin susceptible (MICs 0.125–1.0 mg/L). The remaining three isolates, one from Tasmania, with a daptomycin MIC of 1.5 mg/L, was identified as ST9295 with a L341I *mprF* mutation; one from New South Wales, with a daptomycin MIC of 3.0 mg/L, was identified as ST97 with a L776S *mprF* mutation; and one from Western Australia, with a daptomycin MIC of 2.0 mg/L, was identified as ST5. No previously reported mutations in known loci were detected in the Western Australian isolate.

Table 1: The number and proportion of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates non-susceptible to penicillin and the non- β -lactam antimicrobials, EUCAST breakpoints,^a AGAR, 2023

Antimicrobial	Isolates (n)	Susceptible, increased exposure % (n)	Resistant % (n)
Benzylpenicillin	2,848	— ^b	73.3 (2,089)
Benzylpenicillin ^c	2,819	— ^b	76.8 (2,165)
Cefoxitin (methicillin) ^d	3,422	— ^b	16.1 (550)
Ciprofloxacin	2,855	97.1 (2,772)	2.9 (83)
Clindamycin (constitutive)	2,851	0.0 (0)	1.9 (53)
Clindamycin (inducible + constitutive resistance)	2,854	0.0 (0)	12.8 (364)
Daptomycin	2,862	— ^b	0.1 (3)
Erythromycin	2,828	— ^b	15.6 (442)
Fusidic acid	2,828	— ^b	2.5 (71)
Gentamicin	2,841	— ^b	4.5 (129)
Linezolid	2,864	— ^b	0.0 (0)
Mupirocin (high-level) ^e	2,018	— ^b	1.7 (34)
Rifampicin	2,851	— ^f	0.4 (11)
Teicoplanin	2,861	— ^b	0.1 (3)
Tetracycline/doxycycline ^g	2,851	— ^b	3.2 (91)
Trimethoprim/sulfamethoxazole ^h	2,839	0.1 (2)	0.4 (12)
Vancomycin	2,861	— ^b	0.0 (0)

a EUCAST: European Committee on Antimicrobial Susceptibility Testing.

b No category defined.

c Beta-lactamase adjusted.

d Resistance as determined by cefoxitin screen (Vitek) or cefoxitin MIC (Phoenix).

e Mupirocin high-level resistance screen (CLSI).

f The rifampicin concentration range on cards restricts category interpretation to non-resistant or resistant.

g Doxycycline concentration range (Phoenix panel) restricts ability to accurately identify intermediate and resistant category.

h SXT resistant isolates by MIC confirmed by disc diffusion (CLSI).

Fourteen isolates were identified by Vitek2[®] or BD Phoenix[™] as teicoplanin resistant (MIC >2.0 mg/L). By Etest[®], eight isolates had a teicoplanin MIC \leq 2.0 mg/L and were therefore considered teicoplanin susceptible. Three isolates with teicoplanin MICs of 3.0 mg/L were confirmed as resistant. The three remaining isolates were unavailable for confirmation of teicoplanin resistance. All MSSA were linezolid and vancomycin susceptible. Overall, 2,018 of the 2,872 MSSA (70.3%) had mupirocin susceptibility testing performed, of which 34 (1.7%) were high-level mupirocin resistant. Seventeen of the 34 high-level mupirocin-resistant isolates were referred from Queensland.

The remainder of the isolates were from New South Wales (n = 9); Western Australia (n = 5); Victoria (n = 1); Tasmania (n = 1) and the Australian Capital Territory (n = 1). Eighteen of the 34 mupirocin-resistant MSSA were also resistant to fusidic acid. Of the 2,851 MSSA isolates tested, 53 (1.9%) were constitutively resistant to clindamycin; however, 364 (12.8%) were classified as having both constitutive and inducible clindamycin resistance. Only 4.4% of MSSA were multi-resistant. By Vitek2[®] or BD Phoenix[™], 69 isolates were reported as cotrimoxazole resistant (69/2,850; 2.4%). By disc susceptibility testing, only 12 were confirmed as cotrimoxazole resistant (12/2,839; 0.4%).

MRSA antimicrobial susceptibility

The proportion of *S. aureus* identified as MRSA was 16.1% (95% CI: 14.8–17.5). Of the 550 MRSA identified, 488 were ceftioxin-screen positive by Vitek2[®] and 62 had a ceftioxin MIC > 4 mg/L by BD Phoenix[™]. All MRSA isolates were penicillin resistant. Amongst the MRSA isolates, resistance to non- β -lactam antimicrobials was common (Table 2). All MRSA were susceptible to vancomycin and linezolid. Three isolates were reported by Vitek2[®] as daptomycin resistant (MIC > 1.0 mg/L). By Etest[®], one isolate was considered daptomycin susceptible (MIC 0.5 mg/L). The remaining two isolates were confirmed as daptomycin resistant (MIC 3.0 and 4.0 mg/L). Both isolates were from New South Wales; one was identified as

ST22-IV with a V351E *mprF* mutation and the other as ST45-V with a T345I *mprF* mutation.

By Vitek2[®], four isolates were reported as teicoplanin resistant (MIC = 4.0 mg/L). All four isolates were available for testing by Etest[®]. Three had teicoplanin MICs of 2.0 mg/L and were therefore considered susceptible. The remaining isolate, with a teicoplanin MIC of 3.0 mg/L, was confirmed as resistant. Of the 550 MRSA, 354 (64.4%) had mupirocin testing performed. Twelve MRSA had high-level mupirocin resistance (2.0%). The isolates were from Queensland (n = 8), Western Australia (n = 2), Victoria (n = 1), and New South Wales (n = 1).

Table 2: The number and proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates non-susceptible to penicillin and the non- β -lactam antimicrobials, EUCAST breakpoints,^a AGAR, 2023

Antimicrobial	Isolates (n)	Susceptible, increased exposure % (n)	Resistant % (n)
Benzylpenicillin	544	— ^b	99.6 (542)
Benzylpenicillin ^c	549	— ^b	100.0 (549)
Ceftioxin (methicillin) ^d	3,422	— ^b	16.1 (550)
Ciprofloxacin	546	67.2 (367)	32.8 (179)
Clindamycin (constitutive)	546	0.0 (0)	13.6 (74)
Clindamycin (inducible + constitutive resistance)	546	0.0 (0)	24.9 (136)
Daptomycin	550	— ^b	0.4 (2)
Erythromycin	539	— ^b	30.2 (163)
Fusidic acid	538	— ^b	4.1 (22)
Gentamicin	542	— ^b	13.3 (72)
Linezolid	548	— ^b	0.0 (0)
Mupirocin (high-level) ^e	354	— ^b	3.4 (12)
Rifampicin	545	— ^f	1.1 (6)
Teicoplanin	550	— ^b	0.2 (1)
Tetracycline/doxycycline ^g	546	— ^b	13.5 (74)
Trimethoprim/sulfamethoxazole ^h	540	0.2 (1)	3.1 (17)
Vancomycin	548	— ^b	0.0 (0)

a EUCAST: European Committee on Antimicrobial Susceptibility Testing.

b No category defined.

c Beta-lactamase adjusted.

d Resistance as determined by ceftioxin screen (Vitek) or ceftioxin MIC (Phoenix).

e Mupirocin high-level resistance screen (CLSI).

f The rifampicin concentration range on cards restricts category interpretation to non-resistant or resistant.

g Doxycycline concentration range (Phoenix panel) restricts ability to accurately identify intermediate and resistant category.

h SXT resistant isolates by MIC confirmed by disc diffusion (CLSI).

Of the 546 MRSA tested, 74 (13.6%) were constitutively resistant to clindamycin by EUCAST criteria, whilst 136 (24.9%) were classified as constitutive and inducible clindamycin resistance.

By Vitek2® or BD Phoenix™, 77/545 MRSA (14.1%) were reported as cotrimoxazole resistant. By disc susceptibility testing, only 17/545 (3.1%) were confirmed as cotrimoxazole resistant.

Multi-resistance was identified in 100/524 MRSA (19.1%).

MRSA molecular epidemiology

WGS was performed on 491 of the 550 MRSA (89.3%). Based on molecular typing, 74 (15.1%) and 417 (84.9%) of the isolates were identified as healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) clones respectively (Table 3).

Table 3: Proportion of healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus*, AGAR, 2023 by clone, onset, and Panton-Valentine leucocidin carriage

Clone ^a	Clonal complex	Total, <i>n</i>	Community onset, % (<i>n</i>) ^b	Hospital onset, % (<i>n</i>) ^b	PVL positive, % (<i>n</i>) ^b
Healthcare-associated					
ST22-IV (EMRSA-15)	22	64	62.5 (40)	37.5 (24)	0.0 (0)
ST239-III (Aus2/3 EMRSA)	239	9	— ^c (9)	— ^c (0)	— ^c (0)
ST9276-III	239	1	— ^c (1)	— ^c (0)	— ^c (0)
Total HA-MRSA		74	67.6 (50)	32.4 (24)	0.0 (0)
Community-associated					
ST93-IV	93	107	83.2 (89)	16.8 (18)	96.3 (103)
ST5-IV	5	41	85.4 (35)	14.6 (6)	43.9 (18)
ST1-IV	1	35	82.9 (29)	17.1 (6)	5.7 (2)
ST45-V	45	34	67.6 (23)	32.4 (11)	0.0 (0)
ST30-IV	30	20	70.0 (14)	30.0 (6)	90.0 (18)
ST8-IV	8	17	70.6 (12)	29.4 (5)	82.4 (14)
ST6-IV	6	15	86.7 (13)	13.3 (2)	6.7 (1)
ST97-IV	97	13	76.9 (10)	23.1 (3)	0.0 (0)
ST953-IV	97	11	72.7 (8)	27.3 (3)	0.0 (0)
ST22-IV (PVL positive)	22	9	— ^c (7)	— ^c (2)	— ^c (9)
ST78-IV	78	8	— ^c (6)	— ^c (2)	— ^c (0)
ST45-IV	45	5	— ^c (4)	— ^c (1)	— ^c (0)
ST5-VI	5	4	— ^c (4)	— ^c (0)	— ^c (0)
ST1232-V	398	4	— ^c (3)	— ^c (1)	— ^c (4)
ST59-V	59	3	— ^c (2)	— ^c (1)	— ^c (1)
ST88-IV	88	3	— ^c (1)	— ^c (2)	— ^c (0)
ST72-IV	72	3	— ^c (2)	— ^c (1)	— ^c (0)
ST149-IV	5	3	— ^c (1)	— ^c (2)	— ^c (0)
ST5-V	5	3	— ^c (1)	— ^c (2)	— ^c (0)
ST6145-V	45	3	— ^c (1)	— ^c (2)	— ^c (0)

Clone ^a	Clonal complex	Total, n	Community onset, % (n) ^b	Hospital onset, % (n) ^b	PVL positive, % (n) ^b
ST9286-IV	93	2	— ^c (2)	— ^c (0)	— ^c (2)
ST8536-IV	1	2	— ^c (2)	— ^c (0)	— ^c (0)
ST3628-V	5	2	— ^c (1)	— ^c (1)	— ^c (0)
ST872-IV	1	2	— ^c (2)	— ^c (0)	— ^c (0)
ST9272-IV	5	2	— ^c (0)	— ^c (2)	— ^c (0)
ST6151-IV	93	2	— ^c (2)	— ^c (0)	— ^c (2)
ST9290-IV	93	2	— ^c (1)	— ^c (1)	— ^c (2)
ST30-V	30	2	— ^c (2)	— ^c (0)	— ^c (2)
ST5-novel	5	2	— ^c (0)	— ^c (2)	— ^c (0)
ST8-novel	8	2	— ^c (1)	— ^c (1)	— ^c (0)
ST9268-IV	5	2	— ^c (1)	— ^c (1)	— ^c (0)
ST772-V	1	2	— ^c (1)	— ^c (1)	— ^c (2)
ST9277-IV	30	1	— ^c (1)	— ^c (0)	— ^c (1)
ST9296-IV	5	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9285-IV	93	1	— ^c (1)	— ^c (0)	— ^c (1)
ST762-IV	672	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9271-IV	6	1	— ^c (1)	— ^c (0)	— ^c (0)
ST6963-IV	22	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9281-IV	30	1	— ^c (1)	— ^c (0)	— ^c (0)
ST152-IV	152	1	— ^c (1)	— ^c (0)	— ^c (0)
ST22-V	22	1	— ^c (0)	— ^c (1)	— ^c (0)
ST1535-V	15	1	— ^c (0)	— ^c (1)	— ^c (0)
ST1223-IV	1223	1	— ^c (1)	— ^c (0)	— ^c (0)
ST72-V	22	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9274-V	45	1	— ^c (0)	— ^c (1)	— ^c (0)
ST7613-V	5	1	— ^c (0)	— ^c (1)	— ^c (0)
ST9279-IV	1	1	— ^c (0)	— ^c (1)	— ^c (0)
ST7696-V	45	1	— ^c (0)	— ^c (1)	— ^c (0)
ST9283-IV	93	1	— ^c (1)	— ^c (0)	— ^c (0)
ST7709-IV	97	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9288-IV	1	1	— ^c (1)	— ^c (0)	— ^c (0)
ST100-V	59	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9293-IV	78	1	— ^c (1)	— ^c (0)	— ^c (0)
ST7891-IV	1	1	— ^c (1)	— ^c (0)	— ^c (0)
ST2689-IV	5	1	— ^c (1)	— ^c (0)	— ^c (0)
ST3921-IV	30	1	— ^c (1)	— ^c (0)	— ^c (1)
ST1-V	1	1	— ^c (1)	— ^c (0)	— ^c (0)
ST78-V	78	1	— ^c (1)	— ^c (0)	— ^c (0)

Clone ^a	Clonal complex	Total, <i>n</i>	Community onset, % (<i>n</i>) ^b	Hospital onset, % (<i>n</i>) ^b	PVL positive, % (<i>n</i>) ^b
ST2250-IV	2250	1	— ^c (1)	— ^c (0)	— ^c (0)
ST80-IV	80	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9275-IV	22	1	— ^c (0)	— ^c (1)	— ^c (1)
ST835-I	5	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9278-IV	93	1	— ^c (1)	— ^c (0)	— ^c (1)
ST835-novel	5	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9280-IV	2250	1	— ^c (1)	— ^c (0)	— ^c (0)
ST8454-IV	1	1	— ^c (0)	— ^c (1)	— ^c (0)
ST9282-IV	5	1	— ^c (1)	— ^c (0)	— ^c (1)
ST8534-IV	8	1	— ^c (0)	— ^c (1)	— ^c (1)
ST9284-V	45	1	— ^c (1)	— ^c (0)	— ^c (0)
ST188-IV	1	1	— ^c (1)	— ^c (0)	— ^c (0)
ST1482-IV	30	1	— ^c (1)	— ^c (0)	— ^c (1)
ST1-I	1	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9289-novel	1	1	— ^c (1)	— ^c (0)	— ^c (1)
ST508-IV	45	1	— ^c (0)	— ^c (1)	— ^c (0)
ST9292-IV	2250	1	— ^c (1)	— ^c (0)	— ^c (0)
ST88-V	88	1	— ^c (1)	— ^c (0)	— ^c (1)
ST9294-IV	5	1	— ^c (1)	— ^c (0)	— ^c (1)
ST5213-IV	1	1	— ^c (1)	— ^c (0)	— ^c (0)
ST5-VIII	5	1	— ^c (1)	— ^c (0)	— ^c (0)
ST12-novel	12	1	— ^c (0)	— ^c (1)	— ^c (0)
ST965-IV	5	1	— ^c (0)	— ^c (1)	— ^c (0)
ST9266-IV	1	1	— ^c (0)	— ^c (1)	— ^c (0)
ST672-IV	672	1	— ^c (1)	— ^c (0)	— ^c (0)
ST9267-IV	1	1	— ^c (1)	— ^c (0)	— ^c (0)
Total CA-MRSA		417	76.5 (319)	23.5 (98)	45.8 (191)
MRSA typed		491	75.2 (369)	24.8(122)	38.9 (191)

a MRSA: methicillin-resistant *Staphylococcus aureus*; HA-MRSA: healthcare-associated MRSA; CA-MRSA: community-associated MRSA; PVL: Panton-Valentine leucocidin.

b Percentage of the clone.

c Insufficient numbers (< 10) to calculate percentage.

Healthcare-associated methicillin-resistant *Staphylococcus aureus*

For the 74 HA-MRSA isolates, 24 episodes (32.4%) were classified as hospital-onset and 50 (67.6%) were classified as community-onset. Based on the multi locus sequence type and the SCC_{mec} type, three HA-MRSA clones were identified: 64 isolates of ST22-IV [2B] (EMRSA-15) (13.1 % of MRSA typed and 1.9% of *S. aureus*); nine isolates of ST239-III [3A] (Aus-2/3 EMRSA) (1.8% and 0.3%); and one isolate of ST9276-III [3A] (a single locus variant of ST239-III) (Table 3).

ST22-IV [2B] (EMRSA-15) is the dominant HA-MRSA clone in Australia, and in 2023 accounted for 86.5% of HA-MRSA and was identified in all states and territories (Table 4). ST22-IV [2B] (EMRSA-15) is Panton-Valentine Leucocidin (PVL) negative and 96.8% and 33.9% were ciprofloxacin and erythromycin resistant, respectively. Overall, 38.7% of ST22-IV [2B] (EMRSA-15) isolates were hospital-onset.

ST239-III [3A] (Aus-2/3 EMRSA) accounted for 12.2% of HA-MRSA and was identified in New South Wales and Queensland (Table 4). PVL-negative ST239-III [3A] (Aus-2/3 EMRSA) are typically resistant to ciprofloxacin, clindamycin, cotrimoxazole, erythromycin, gentamicin, and tetracycline. All nine ST239-III [3A] (Aus-2/3 EMRSA) isolates were community-onset.

Community-associated methicillin-resistant *Staphylococcus aureus*

For the 417 CA-MRSA isolates, 98 episodes (23.5%) were classified as hospital-onset and 319 (76.5%) as community-onset. Based on the multi locus sequence type and the SCC_{mec} type, 66 CA-MRSA clones were identified (Table 3). Overall, 70.3% of CA-MRSA were classified into nine clones each represented by ten or more isolates: 107 isolates of ST93-IV [2B] (Queensland clone) (21.8% of MRSA typed and 3.1% of *S. aureus*); 41 isolates of ST5-IV [2B] (8.4% and 1.2%); 35 isolates of ST1-IV [2B] (7.1% and 1.0%); 34 isolates of ST45-V [5C2&5] (6.9% and 1.0%); 20 isolates of ST30-IV [2B] (4.1% and 0.6%); 17 isolates of ST8-IV [2B] (3.5% and 0.5%); 15 isolates of ST6-IV [2B] (3.1% and 0.4%); 13 isolates of ST97-IV [2B] (2.7% and 0.4%); and 11 isolates of ST953-IV[2B] (2.2% and 0.3%).

ST93-IV [2B] (Queensland clone) accounted for 25.7% of CA-MRSA, ranging from 10% in Tasmania and the Australian Capital Territory to 51.0% of CA-MRSA in the Northern Territory (Table 5). Typically PVL positive, 83.2% of ST93-IV [2B] isolates were community-onset.

ST5-IV [2B] accounted for 9.9% of CA-MRSA and was isolated in all regions of Australia except Tasmania (Table 5). Overall, 43.9% of the isolates were PVL positive and 85.4% were community-onset.

ST1-IV [2B] accounted for 8.4% of CA-MRSA and was isolated in all regions of Australia except the Australian Capital Territory (Table 5). Overall, 5.7% of the isolates were PVL positive and 83.2% were community-onset.

Table 4: The number and proportion of healthcare-associated methicillin-resistant *Staphylococcus aureus* (MRSA) clones, AGAR, 2023, by state and territory

Clone	Percentage (n) ^a								
	ACT	NSW	NT	Qld	SA	Tas.	Vic.	WA	Australia
ST22-IV (EMRSA-15)	— ^b (2)	80.0 (32)	— ^b (1)	— ^b (4)	— ^b (5)	— ^b (2)	100.0 (10)	— ^b (8)	86.5 (64)
ST239-III (Aus2/3 EMRSA)	— ^b (0)	17.5 (7)	— ^b (0)	— ^b (2)	— ^b (0)	— ^b (0)	0.0 (0)	— ^b (0)	12.2 (9)
ST9276-III	— ^b (0)	2.5 (1)	— ^b (0)	— ^b (0)	— ^b (0)	— ^b (0)	0.0 (0)	— ^b (0)	1.4 (1)
Total	2	40	1	6	5	2	10	8	74

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 Insufficient numbers (< 10) to calculate percentage.

Table 5: The number and proportion of the major community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) clones (> 10 isolates), AGAR by state and territory and PVL carriage, ^a 2023

Clone	PVL	Percentage (n) ^b									
		ACT	NSW	NT	Qld	SA	Tas.	Vic.	WA	Australia	
ST93-IV	10.0 (1)	16.5 (19)	51.0 (25)	34.2 (25)	33.3 (7)	10.0 (1)	13.4 (9)	27.8 (20)	25.7 (107)		
	Number PVL-positive	1	17	25	24	7	1	9	19	103	
ST5-IV	0	2	0	1	0	0	0	0	4		
	Number PVL-negative	0	2	0	1	0	0	0	4		
ST1-IV	10.0 (1)	6.1 (7)	18.4 (9)	11.0 (8)	14.3 (3)	0.0 (0)	6.0 (4)	12.5 (9)	9.8 (41)		
	Number PVL-positive	0	1	9	1	0	0	1	6	18	
ST1-IV	0	6	0	7	3	0	3	3	23		
	Number PVL-negative	0	6	0	7	3	0	3	23		
ST1-IV	0.0 (0)	7.0 (8)	8.2 (4)	12.3 (9)	4.8 (1)	20.0 (2)	4.5 (3)	11.1 (8)	8.4 (35)		
	Number PVL-positive	0	0	0	1	0	0	0	1	2	
ST45-V	0	8	4	8	1	2	3	7	33		
	Number PVL-negative	0	8	4	8	1	2	3	33		
ST45-V	10.0 (1)	13.0 (15)	0.0 (0)	5.5 (4)	4.8 (1)	20.0 (2)	11.9 (8)	4.2 (3)	8.2 (34)		
	Number PVL-positive	0	0	0	0	0	0	0	0		
ST30-IV	1	15	0	4	1	2	8	3	34		
	Number PVL-negative	1	15	0	4	1	2	8	34		
ST30-IV	0.0 (0)	8.7 (10)	0.0 (0)	5.5 (4)	0.0 (0)	10.0 (1)	6.0 (4)	1.4 (1)	4.8 (20)		
	Number PVL-positive	0	10	0	2	0	1	4	1	18	
ST8-IV	0	0	0	2	0	0	0	0	2		
	Number PVL-negative	0	0	0	2	0	0	0	2		
ST8-IV	0.0 (0)	7.8 (9)	0.0 (0)	2.7 (2)	4.8 (1)	0.0 (0)	7.5 (5)	0.0 (0)	4.1 (17)		
	Number PVL-positive	0	8	0	2	1	0	3	0	14	
ST6-IV	0	1	0	0	0	0	2	0	3		
	Number PVL-negative	0	1	0	0	0	0	2	3		
ST6-IV	0.0 (0)	8.7 (10)	0.0 (0)	4.1 (3)	0.0 (0)	0.0 (0)	1.5 (1)	1.4 (1)	3.6 (15)		
	Number PVL-positive	0	1	0	0	0	0	0	1		
ST6-IV	0	9	0	3	0	0	1	1	14		
	Number PVL-negative	0	9	0	3	0	0	1	14		

Clone	Percentage (n) ^b										
	PVL	ACT	NSW	NT	Qld	SA	Tas.	Vic.	WA	Australia	
ST97-IV		0.0 (0)	4.3 (5)	2.0 (1)	2.7 (2)	0.0 (0)	0.0 (0)	7.5 (5)	0.0 (0)	3.1 (13)	
	Number PVL-positive	0	0	0	0	0	0	0	0	0	
	Number PVL-negative	0	5	1	2	0	0	5	0	13	
ST953-IV		0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	15.3 (11)	2.6 (11)	
	Number PVL-positive	0	0	0	0	0	0	0	0	0	
	Number PVL-negative	0	0	0	0	0	0	0	11	11	
Other clones (n = 75)		70.0 (7)	27.8 (32)	20.4 (10)	21.9 (16)	38.1 (8)	40.0 (4)	41.8 (28)	26.4 (19)	29.7 (124)	
	Number PVL-positive	3	7	5	4	1	2	10	3	35	
	Number PVL-negative	4	25	5	12	7	2	18	16	89	
Total		10	115	49	73	21	10	67	72	417	

a PVL: Panton-Valentine leucocidin.

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

Table 6: Antimicrobial resistance combinations for the most predominant CA-MRSA clones,^{a,b} AGAR, 2023

Resistance pattern ^c	ST93-IV	ST5-IV	ST1-IV	ST45-V	ST30-IV	ST8-IV	ST6-IV	ST97-IV	ST953-IV
Single resistance									
β-lactams only	84	30	15	15	10	13	11	3	
Resistance to methicillin and one antimicrobial									
Cip			2	5	1	1	1	1	1
Ery	3	2							
Fus		7							
Gen					2				
Rif	1								
Tet		2	1						
Resistance to methicillin and two antimicrobials									
CipGen				3					
ClinSxt		1							
EryCip		1	1	1					
EryClin	17	3	8	1	1		1	6	
EryGen								1	
GenFus		1							
Resistance to methicillin and three antimicrobials									
CipTetGen				8					
EryCipGen		1							
EryCipGly	1								
EryClinTet		1						1	
EryClinCip				2				2	

Resistance pattern ^c	ST93-IV	ST5-IV	ST1-IV	ST45-V	ST30-IV	ST8-IV	ST6-IV	ST97-IV	ST953-IV
Resistance to methicillin and four antimicrobials									
ClinCipTetGen				1					
EryCipTetGen				1					
EryClinCipGen				2					
EryClinCipTet		1		3					
Resistance to methicillin and five antimicrobials									
EryClinCipTetFus				1					
EryClinCipTetGen				5					
EryClinCipSxtTet				1					
Resistance to methicillin and six antimicrobials									
EryClinCipTetGenDap				1					
Total	106	40	34	34	19	16	14	13	11

a CA-MRSA: community-acquired methicillin-resistant *Staphylococcus aureus*.

b Only data from isolates tested against all antimicrobial groups were included (n = 287).

c Cip: ciprofloxacin; Clin: clindamycin; Dap: daptomycin; Ery: erythromycin; Fus: fusidic acid; Gen: gentamicin; Gly: glycopeptide; Rif: rifampicin; Sxt: cotrimoxazole; Tet: tetracycline.

ST45-V [5C2&5] accounted for 8.2% of CA-MRSA and was isolated in all regions except the Northern Territory (Table 5). All isolates were PVL negative and 67.6% were community-onset.

ST30-IV [2B] accounted for 4.8% of CA-MRSA and was isolated in all regions of Australia except South Australia and the Northern Territory (Table 5). Overall, 90.0% of the isolates were PVL positive and 70.0% were community-onset.

ST8-IV [2B] accounted for 4.1% of CA-MRSA and was isolated in all regions except Western Australia, Tasmania, the Northern Territory, and the Australian Capital Territory (Table 5). Overall, 82.4% of the isolates were PVL positive and 70.6% of ST97-IV [2B] isolates were community-onset.

ST6-IV [2B] accounted for 3.6% of CA-MRSA and was isolated in New South Wales, Victoria, Queensland, and Western Australia (Table 5). Overall, 6.7% isolates of the isolates were PVL positive and 86.7% were community-onset.

ST97-IV [2B] accounted for 3.1% of CA-MRSA and was isolated in New South Wales, Victoria, Queensland, and the Northern Territory. All ST97-IV [2B] isolates were PVL negative and 76.9% were community-onset.

ST953-IV [2B] accounted for 2.6% of CA-MRSA and was isolated only in Western Australia. All ST953-IV [2B] isolates were PVL negative and 72.7% were community-onset.

Overall, 66.7% of CA-MRSA were non-multi-resistant with 56.1% of CA-MRSA resistant to the β -lactams only. A significant increase was observed ($p < 0.01$) in multi-resistant CA-MRSA isolates in ASSOP 2023 (33.3%), from 9.2% in ASSOP 2013. Multi-resistance was primarily due to the ST45-V [5C2&5] clone.

The resistance profiles of the nine predominant CA-MRSA clones are shown in Table 6.

Panton-Valentine leucocidin

Overall, 191 MRSA isolates (38.9% of MRSA and 45.8% of CA-MRSA) were PVL positive. The PVL associated genes were not detected in the HA-MRSA isolates (Table 3).

Discussion

The AGAR surveillance programs collect data on antimicrobial resistance, focussing on bloodstream infections caused by *S. aureus*, *Enterococcus* and gram-negative bacilli including the *Enterobacteriales*, *Pseudomonas aeruginosa* and *Acinetobacter* species. All data collected in the AGAR programs are generated as part of routine patient care in Australia, with most data available through laboratory and hospital bed management information systems. Isolates are referred to a central laboratory where strain and antimicrobial resistance determinant characterisation are performed. As the programs are similar to the antimicrobial surveillance programs conducted in Europe, comparison of Australian antimicrobial resistance data with other countries is possible.^{20,21}

In ASSOP 2023, methicillin resistance was found in 16.1% (95% CI: 14.8–17.5) of the 3,422 SAB episodes. In the 2022 European Centre for Disease Prevention and Control (ECDC) SAB surveillance program, methicillin resistance ranged from 1.1% (95% CI: 0.7–1.7) in Norway to 50.8% (95% CI: 41.3–61.8) in Cyprus.²¹

A decrease in methicillin-resistant SAB has been reported in several parts of the world,^{22,23} 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.^{24–27} The percentage of methicillin-resistant SAB in Australia has decreased significantly over the last ten years of ASSOP, ranging from 18.9% in 2014 to 16.1% in 2023 (X^2 for linear trend = 27.65; $p \leq 0.01$).ⁱ There have also been significant decreases in HA-MRSA from 40.2% in 2014 to 15.1% in 2023 (X^2 for linear trend = 157.74; $p \leq 0.01$) and in hospital-onset MRSA from 34.1% to 25.3% ($p < 0.01$) over the last ten ASSOP surveys.^{12,28–36} Over the same time period, significant increases have been observed in CA-MRSA from 59.8% to 84.9% ($p = 0.03$) and in community-onset MRSA from 65.9% to 74.7% ($p < 0.01$). Because of the increased burden of CA-MRSA bacteraemia in the Australia community, a significant reduction in the overall proportion of SAB due to MRSA may prove problematic.

i Rates include only those laboratories that participated in all years 2014–2023.

In ASSOP 2023, the all-cause mortality at 30 days was 16.2% (95% CI: 14.7–17.8). There was no significant difference in mortality observed between methicillin-resistant SAB (14.8%) and methicillin-susceptible SAB (16.5%) ($p = 0.44$).

With the exception of the β -lactams and erythromycin, antimicrobial resistance in MSSA remains rare. However, for MRSA, in addition to the β -lactams, approximately 33% of isolates were resistant to ciprofloxacin; 30% to erythromycin; 13% to tetracycline; and 13% to gentamicin. Antimicrobial resistance was identified in the two major 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 clone and this change has occurred throughout most of the country. In ASSOP 2023, 13.1% of MRSA were characterised as ST22-IV [2B] (EMRSA-15), and 1.8% as ST293-III [3A].

In ASSOP 2023, ST93-IV [2B] (Queensland clone) remained the predominant CA-MRSA clone (25.7% of CA-MRSA) in Australia. CA-MRSA, in particular the ST45-V [5C2&5] clone (6.9% of MRSA), has acquired multiple antimicrobial resistance determinants including resistance to ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline.

Approximately 23.5% of SAB episodes caused by CA-MRSA were hospital-onset. As transmission of CA-MRSA in Australian hospitals is thought to be rare,^{37,38} it is likely that many of the hospital-onset CA-MRSA SAB infections reported in ASSOP 2023 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 clone) 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 2023 has demonstrated 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 in 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 management strategies targeting hospitals and long-term care facilities are essential.

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