Australian Government
Department of Health

COMMUNICABLE DISEASES INTELLIGENCE

2018 Volume 42
PII: S2209-6051(18)00016-7

Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP) Annual Report 2015

Geoffrey W Coombs, Denise A Daley, Yung Thin Lee, Stanley Pang , Jan M Bell, John D Turnidge and Stanley Pang for the Australian Group on Antimicrobial Resistance

www.health.gov.au/cdi

COMMUNICABLE
DISEASES
INTELLIGENCE

ISSN: 2209-6051 (Online)

Communicable Diseases Intelligence aims to disseminate information on the epidemiology and control of communicable diseases in Australia. Communicable Diseases Intelligence invites contributions dealing with any aspect of communicable disease epidemiology, surveillance or prevention and control in Australia. Submissions can be in the form of original articles, short reports, surveillance summaries, reviews or correspondence. Instructions for authors can be found in Commun Dis Intell 2016;40(1):E189–E193.

Communicable Diseases Intelligence contributes to the work of the Communicable Diseases Network Australia.

http://www.health.gov.au/cdna

Website
http://www.health.gov.au/cdi

Contacts
Communicable Diseases Intelligence is produced by:
Health Protection Policy Branch
Office of Health Protection
Australian Government Department of Health
GPO Box 9848, (MDP 6) CANBERRA ACT 2601

Email: cdi.editor@health.gov.au

This journal is indexed by Index Medicus and Medline.

Disclaimer
Opinions expressed in Communicable Diseases Intelligence are those of the authors and not necessarily those of the Australian Government Department of Health or the Communicable Diseases Network Australia. Data may be subject to revision.

Submit an Article
You are invited to submit your next communicable disease related article to the Communicable Diseases Intelligence (CDI) for consideration. More information regarding CDI can be found at: http://health.gov.au/cdi. Further enquiries should be directed to: cdi.editor@health.gov.au.

Annual Report

Australian Group on Antimicrobial Resistance (AGAR) Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP) Annual Report 2015

Geoffrey W Coombs, Denise A Daley, Yung Thin Lee, Stanley Pang , Jan M Bell, John D Turnidge and Stanley Pang for the Australian Group on Antimicrobial Resistance

Abstract

From 1 January to 31 December 2015, 31 Australian institutions participated in the Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP). The aim of ASSOP 2015 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 methicillin-resistant isolates. Overall 18.2% of the 2,399 SAB episodes were methicillin-resistant. The 30-day all-cause mortality associated with methicillin-resistant SAB was 18.8% which was not significantly higher than the 15.1% mortality associated with methicillin-sensitive SAB. With the exception of the β-lactams and erythromycin, antimicrobial resistance in methicillin sensitive *S. aureus* (MSSA) remains rare. However, in addition to the β-lactams, approximately 50% of methicillin-resistant *S. aureus* (MRSA) were resistant to erythromycin and ciprofloxacin and approximately 15% resistant to co-trimoxazole, tetracycline and gentamicin. When applying the EUCAST breakpoints teicoplanin resistance was detected in two *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 clones; ST22-IV [2B] (EMRSA-15) and ST239-III [3A] (Aus-2/3 EMRSA). ST22-IV [2B] (EMRSA-15) is the predominant healthcare-associated clone in Australia. Sixty-seven percent of methicillin-resistant SAB were due to community-associated clones. Although polyclonal, almost 43% of community-associated clones were characterised as ST93-IV [2B] (Queensland CA-MRSA) and ST1-IV [2B] (WA1). CA-MRSA in particular the ST45-V [5C2&5] (WA84) clone has acquired multiple antimicrobial resistance determinants including ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline. As CA-MRSA is well established in the Australian community it is important antimicrobial resistance patterns in community and healthcare-associated SAB are monitored 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 sensitive *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 great majority of these cases the organism can be detected in blood cultures. Therefore, *S. aureus* bacteraemia (SAB) is considered a very useful marker for serious invasive infection.[2]

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

The Australian Group on Antimicrobial Resistance (AGAR), a network of laboratories located across Australia, commenced surveillance of antimicrobial resistance in *S. aureus* in 1986.[10] In 2013, AGAR commenced the Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP).[11] The primary objective of ASSOP 2015 was to determine the proportion of SAB isolates demonstrating antimicrobial resistance with particular emphasis on:

- Assessing susceptibility to methicillin; and
- Molecular epidemiology of methicillin-resistant *S. aureus* (MRSA).

Methodology

Participants

Thirty-one laboratories from all eight Australian states and territories.

Collection Period

From 1 January to 31 December 2015, the 31 participating 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 (MALDI) biotyper (Bruker Daltonics, Germany), slide coagulase, tube coagulase, appropriate growth on chromogenic agar and demonstration of deoxyribonuclease production. Additional tests such as fermentation of mannitol, growth on mannitol-salt agar or polymerase chain reaction (PCR) for the presence of the *nuc* gene may have been performed for confirmation.

Minimum inhibitory concentration (MIC) data and isolates were referred to the Antimicrobial Resistance and Infectious Diseases Laboratory at the School of Veterinary and Life Sciences, Murdoch University. 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 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 MiSeq platform (Illumina, San Diego, USA). Sequencing results were analysed using the Nullarbor pipeline.[14] Spa types were determined using the online *spa* typing tool described by Bartels *et al.*[15] SCC*mec* elements were identified using SCC*mec* sequences described by Monecke *et al.*[16]

Chi-square tests for comparison of two proportions and calculation of 95% confidence intervals (95% CI) were performed using MedCalc for Windows, version 12.7 (MedCalc Software, Ostend Belgium).

Table 1: The number and proportion of methicillin sensitive *Staphylococcus aureus* (MSSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2015

Antimicrobial	Number tested	Breakpoint (mg/L)	Non-Susceptible	
			n	%
Penicillin	1,962	>0.12*	1,585	80.8
Vancomycin	1,962	>2*	0	0.0
Teicoplanin	1,963	>8†	0	0.0
		>2‡	0	0.0
Rifampicin	1,915	>1†	2	0.1
		>0.5‡	2	0.1
Fusidic Acid	1,963	>1‡	59	3.0
Gentamicin	1,963	>4†	15	0.8
		>1‡	19	1.0
Erythromycin	1,963	>0.5†	209	10.7
		>2‡	147	7.5
Clindamycin	1,963	>0.5*	16	0.8
Tetracycline	1,745	>4†	31	1.8
		>2‡	31	1.8
Co-trimoxazole	1,963	>2/38†	29	1.5
		>4/76‡	29	1.5
Ciprofloxacin	1,963	>1*	49	2.5
Nitrofurantoin	1,865	>32†	34	1.8
		>64‡	1	0.05
Daptomycin	1,963	>1*	0	0
Linezolid	1,963	>4*	0	0

*CLSI and EUCAST non-susceptible breakpoint

†CLSI non-susceptible breakpoint

‡EUCAST non-susceptible breakpoint

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

Results

From 1 January to the 31 December 2015, participating laboratories identified 2,399 unique episodes of *S. aureus* bacteraemia. A significant difference (P<0.0001) was seen in patient sex with 64.9% (1,558) being male (95% CI 63 - 66.8). The average age of patients was 57 years ranging from 0 – 105 years with a median age of 60 years. Overall 77.4% (1,855) of 2,398 episodes were community onset (95% CI 75.7% – 79.1%). All-cause mortality at 30-days was 15.8% (95% CI 14.4 – 17.5). Methicillin resistant SAB mortality was 18.8% (95% CI 14.9 – 23.3) which was not significantly higher than methicillin susceptible SAB mortality (15.1%, 95% CI 13.4 – 16.9, p=0.6).

MSSA Antimicrobial Susceptibility

Overall 81.8% (1,963) of the 2,399 isolates were methicillin sensitive of which 76.8% (1,507) were penicillin resistant (MIC >0.12 mg/L). However, as β-lactamase was detected in 78 phenotypically penicillin susceptible isolates, 80.8% of MSSA were considered penicillin resistant. Apart from erythromycin non-susceptibility, resistance to the non-β-lactam antimicrobials amongst MSSA was rare, ranging from <0.1% to 3.0% (Table 1).

Two isolates were reported as non-susceptible to daptomycin by Vitek2®. By Etest® both isolates had MICs ≤1 mg/L and were therefore considered susceptible. All MSSA were vancomycin, teicoplanin and linezolid susceptible. Twenty-two (1.1%) of 1,962 isolates had high level mupirocin resistance of which 10 isolates were referred from Queensland. Inducible resistance to clindamycin was determined by the Vitek2® susceptibility system. Of the 1,751 isolates tested, 10% (175) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI breakpoints) of which 90.9% (159) were classified as having inducible clindamycin resistance. Multi-resistance was uncommon in MSSA (0.7%, 14/1,963).

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

MRSA Antimicrobial Susceptibility

The proportion of *S. aureus* that were MRSA was 18.2% (95%CI 16.7 – 19.8). The 436 MRSA identified were either cefoxitin screen positive by Vitek2® (399) or had a cefoxitin MIC >4 by Phoenix™ (13). All 436 MRSA isolates were phenotypically penicillin resistant. Amongst the MRSA isolates, non-susceptibility to non-β-lactam antimicrobials was common except for rifampicin, fusidic acid and nitrofurantion where resistance was below 4.9% (Table 2). There were four isolates reported by Vitek2® as non-susceptible to daptomycin. By Etest® two isolates had MICs ≤0.125 mg/L and were therefore considered susceptible. Two isolates had MICs = 3.0 mg/L and were considered non-susceptible. By Vitek2® three isolates were linezolid resistant (MIC >4 mg/L). However, by Etest® the isolates had an MIC ≤4 mg/L (1.5, 2.0 and 2.0 mg/L) and were therefore considered linezolid susceptible. When using the EUCAST resistant breakpoint of >2 mg/L, two isolates were teicoplanin resistant (MIC = 4 mg/L). However, using the CLSI resistant breakpoint of >8 mg/L both isolates were classified susceptible. All MRSA were vancomycin susceptible. Ten (2.3%) of the 436 MRSA isolates had high level mupirocin. Inducible resistance to clindamycin was determined by the Vitek2® susceptibility system. Of the 371 isolates tested by Vitek2®, 29.9% (111) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI and EUCAST breakpoints) of which 89.2% (99) were classified as having inducible clindamycin resistance. Multi-resistance was common in MRSA (24.3%, 106/436).

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

Table 2: The number and proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2015

Antimicrobial	Number tested	Breakpoint (mg/L)	Non-Susceptible (%)	
			n	%
Penicillin	436	>0.12*	436	100
Vancomycin	436	>2*	0	0
Teicoplanin	436	>8†	0	0
		>2‡	2	0.5
Rifampicin	433	>1†	13	3.0
		>0.5‡	13	3.0
Fusidic Acid	436	>1‡	21	4.8
Gentamicin	436	>4†	71	16.3
		>1‡	78	17.9
Erythromycin	436	>0.5†	189	43.4
		>2‡	181	41.5
Clindamycin	436	>0.5*	62	14.2
Tetracycline	367	>4†	73	19.9
		>2‡	73	19.9
Co-trimoxazole	436	>2/38†	67	15.4
		>4/76‡	62	14.2
Ciprofloxacin	436	>1*	205	47.0
Nitrofurantoin	427	>32†	9	2.1
		>64‡	1	0.2
Daptomycin	436	>1*	2	0.5
Linezolid	436	>4*	0	0

*CLSI and EUCAST non-susceptible breakpoint

†CLSI non-susceptible breakpoint

‡EUCAST non-susceptible breakpoint

MRSA Molecular Epidemiology

Whole genome sequencing was performed on 427 of the 436 MRSA. Based on molecular typing, 33.7% (144) and 66.3% (283) of isolates were classified as healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) clones respectively (Table 3).

Healthcare-associated methicillin-resistant *Staphylococcus aureus*

For the 144 HA-MRSA isolates, 36.8% (53) were epidemiologically classified as hospital onset and 63.2% (91) were classified as community onset. Four HA-MRSA clones were identified: 108 isolates of ST22-IV [2B] (EMRSA-15) (25.3% of MRSA and 4.5% of *S. aureus*); 34 isolates of ST239-III [3A] (Aus -2/3 EMRSA) (8.0%

Table 3: Proportion of healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus* (MRSA), Australia, 2015 by clone, healthcare and community onset, and Panton-Valentine leucocidin (PVL) carriage

Strain	Total		Onset				PVL Positive	
			Healthcare		Community			
	n	%*	n	%†	n	%†	n	%†
Healthcare Associated MRSA								
ST22-IV [2B] (EMRSA-15)	108	25.3	34	31.5	74	68.5	2	1.9
ST239-III [3A] (Aus-2/3)	34	8.0	19	55.9	15	44.1	0	0
ST225-II (NY/Japan/USA100 variant)	1	0.2			1	100	0	0
ST36-II (EMRSA-6/USA 200)	1	0.2			1	100		
Total HA-MRSA	144	33.7	53	36.8	91	63.2	2	1.4
Community Associated MRSA								
ST93-IV [2B] (Queensland)	90	21.1	14	15.6	76	84.4	74	82.2
ST45-V	41	9.6	16	39.0	25	61.0	0	0
ST5-IV	35	8.2	12	34.3	23	65.7	18	51.4
ST1-IV	31	7.3	5	16.1	26	83.9	1	3.2
ST30-IV	17	4.0	2	11.8	15	88.2	15	88.2
ST78-IV	13	3.0	1	7.7	12	92.3	1	7.7
ST5-V	7	1.6	1	14.3	6	85.7	0	0
ST8-IV	6	1.4	2	33.3	4	66.7	0	0
ST872-IV	5	1.2	3	60.0	2	40.0	0	0
ST1-I	4	0.9	2	50.0	2	50.0	0	0
ST762-IV	3	0.7	2	66.7	1	33.3	1	33.3
ST152-V	2	0.5	0	0	2	100	2	100
ST188-IV	2	0.5	1	50.0	1	50.0	1	50.0
ST30-V	2	0.5	0	0	2	100	1	50.0
ST45-IV	2	0.5	0	0	2	100	0	0
ST59-V	2	0.5	0	0	2	100	2	100
ST6-IV	2	0.5	0	0	2	100	0	0
ST73-IV	2	0.5	0	0	2	100	0	0
ST88-IV	2	0.5	0	0	2	100	0	0
ST953-IV	2	0.5	0	0	2	100	0	0
ST97-IV	2	0.5	0	0	2	100	0	0
ST12slv-IV	1	0.2	0	0	1	100	0	0
ST149-IV	1	0.2	0	0	1	100	0	0
ST1slv-V	1	0.2	1	100	0	0	0	0
ST45slv-IV	1	0.2	0	0	1	100	0	0
ST47-V	1	0.2	1	100	0	0	0	0
ST573-V	1	0.2	1	100	0	0	0	0
ST584-IV	1	0.2	1	100	0	0	0	0
ST5-novel	1	0.2	0	0	1	100	0	0
ST612-IV	1	0.2	1	100	0	0	0	0
ST627dlv-IV	1	0.2	0	0	1	100	0	0
ST923-IV	1	0.2	0	0	1	100	1	100
Total CA-MRSA	283	66.3	66	23.3	217	76.7	117	41.3
Grand Total	**427**	**100**	**119**	**27.9**	**308**	**72.1**	**119**	**27.9**

*Percentage of all MRSA

†Percentage of the strain

and 1.4%) and single isolates of ST225-II [2A] (USA100/New York Japan variant) and ST36-II (EMRSA-16/USA 200).

ST22-IV [2B] (EMRSA-15) was the dominant HA-MRSA clone in Australia accounting for 75% of HA-MRSA ranging from 12.5% in the Northern Territory to 100% in Tasmania and the Australian Capital Territory (Table 4). ST22-IV [2B] (EMRSA-15) was typically PVL negative and using CLSI breakpoints 99.1% and 50.9% were ciprofloxacin and erythromycin resistant respectively.

ST239-III [3A] (Aus-2/3 EMRSA) accounted for 23.6% of HA-MRSA ranging from 0% in Tasmania, Western Australia and the Australian Capital Territory to 87.5% in the Northern Territory (Table 4). PVL negative ST239-III [3A] (Aus-2/3 EMRSA) were typically resistant to erythromycin (100%), co-trimoxazole (100%), ciprofloxacin (97.1%), gentamicin (94.1%), tetracycline (82.4%) and clindamycin (76.5%).

Community-associated methicillin-resistant *Staphylococcus aureus*

For the 283 CA-MRSA isolates, 23.3% (66) of episodes were epidemiologically classified as hospital onset and 76.7% (217) classified as community onset. Based on the multi locus sequence type and the *SCCmec* type, 32 CA-MRSA clones were identified (Table 3). Overall 80.2% of CA-MRSA were classified into six clones each having more than ten isolates: ST93-IV [2B] (Queensland CA-MRSA) (21.1% of MRSA and 3.8% of *S. aureus*); ST45-V (9.6% and 1.7%); ST5-IV (8.2% and 1.5%); ST1-IV (7.3% and 1.3%); ST30-IV (4.0% and 0.7%); and ST78-IV (3.0% and 0.5%).

ST93-IV [2B] (Queensland CA-MRSA) accounted for 31.8% of CA-MRSA ranging from 0.0% in Tasmania to 81.5% in the Northern Territory (Table 5). Typically PVL positive, 83.3% (75/90) of ST93-IV [2B] (Queensland CA-MRSA) were resistant to the β-lactams only or additionally resistant to erythromycin (11.1%, 10/90) or erythromycin and clindamycin (5.6%, 5/90).

ST45-V accounted for 14.5% of CA-MRSA and was isolated primarily in New South Wales and Victoria (Table 5). All isolates were PVL negative and were resistant to the β-lactams and with one exception to ciprofloxacin. Isolates were additionally non-susceptible to erythromycin, gentamicin and tetracycline (31.7%, 13/41), gentamycin and tetracycline (14.6%, 6/41) erythromycin and gentamicin (12.2%, 5/41), erythromycin and tetracycline (7.3%, 3/41), erythromycin, clindamycin and tetracycline (7.3%, 3/41) tetracycline (4.9%,2/41) and one (2.4%) each of erythromycin or erythromycin, gentamicin and rifampicin or gentamicin or clindamycin and erythromycin.

ST5-IV accounted for 12.4% of CA-MRSA and was isolated in all mainland regions of Australia ranging from 0.0% in Tasmania to 28.6% in South Australia (Table 5). ST5-IV, approximately 50.0% PVL positive, was typically resistant to the β-lactams and co-trimoxazole (42.9%, 15/35), the β-lactams only (28.6% (10/35) or additionally resistant to erythromycin (8.6%, 3/35), ciprofloxacin (5.7%, 2/35), ciprofloxacin, erythromycin and tetracycline (5.7% 2./35), and one (2.9%) each of ciprofloxacin and erythromycin, clindamycin and erythromycin or erythromycin and co-trimoxazole.

ST1-IV accounted for 11% of CA-MRSA ranging from 0.0% in the Australian Capital Territory to 50.0% in Tasmania (Table 5). Typically PVL negative, 67.7% of isolates were resistant to the β-lactams only (21/31) or additionally resistant to erythromycin and fusidic acid (16.1%, 5/31), erythromycin (9.7%, 3/31), or fusidic acid (6.5%, 2/31).

ST30-IV accounted for 6.0% of CA-MRSA and was isolated primarily isolated in Queensland (Table 5). Typically PVL positive 64.7% of isolates were resistant to the β-lactams only (11/17). Four isolates were non-susceptible to nitrofurantoin (23.5%). One isolate (5.9%) was resistant to

Table 4: The number and proportion of healthcare-associated methicillin-resistant *Staphylococcus aureus* (MRSA) multilocus sequence types, Australia, 2015, by region

Type	ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aus	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ST22-IV	4	100	49	72.1	1	12.5	11	91.7	7	77.8	1	100	18	78.3	17	89.5	108	75.0
ST239-III			19	27.9	7	87.5	1	8.3	2	22.2			5	21.7			34	23.6
ST225-II															1	5.3	1	0.7
ST36-II															1	5.3	1	0.7
Total	**4**	**100**	**68**	**100**	**8**	**100**	**12**	**100**	**9**	**100**	**1**	**100**	**23**	**100**	**19**	**100**	**144**	**100**

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

Table 5: The number and proportion of the major community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) multilocus sequence types, Australia (>10 isolates), 2015, by region

Type	ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aus	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ST93-IV	1	12.5	11	16.9	22	66.7	19	34.5	12	34.3			12	30.8	13	28.3	90	31.8
ST45-V	2	25.0	31	47.7				2.9	1	2.9			7	179			41	14.5
ST5-IV	3	37.5	3	4.6	3	9.1	10	22.9	8	22.9			1	2.6	7	15.2	35	12.4
ST1-IV			10	15.4	1	3.0	4	14.3	5	14.3	1	50.0	3	7.7	7	15.2	31	11.0
ST30-IV			1	1.5	1	3.0	10	2.9	1	2.9			3	7.7	1	2.2	17	6.0
ST78-IV	1	12.5	2	3.1			2	2.9	1	2.9	1	50.0			6	13.0	13	4.6
Other	1	12.5	7	10.8	6	18.2	10	20.0	7	20.0			13	33.3	12	26.1	56	19.8
Total	8	100	65	100	33	100	55	100	35	100	2	100	39	100	46	100	283	100

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

clindamycin, erythromycin and nitrofurantoin and one isolate to ciprofloxacin, erythromycin and co-trimoxazole.

ST78-IV accounted for 4.6% of CA-MRSA and was predominantly in Western Australia (Table 5). Isolates were resistant to the β-lactams and erythromycin (76.9%, 10/13). One isolate (7.7%) was additionally resistant to tetracycline, one isolate to ciprofloxacin and one to clindamycin and co-trimoxazole.

Overall 82.3% of CA-MRSA were non-multiresistant including 47.3% resistant to the β-lactams only. However, 50 (12.7%) CA-MRSA isolates were multiresistant.

Panton-Valentine leucocidin

Overall, 119 (29.7%) MRSA were PVL positive, including 41.3% of the CA-MRSA (Table 3).

Discussion

The AGAR surveillance programmes 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 programmes are similar to those conducted in Europe, comparison of Australian antimicrobial resistance data with other countries is possible.[31]

In the 2015 European Centre for Disease Prevention and Control and Prevention (ECDC) SAB surveillance program, the European Union/European Economic Area (EU/EEA) population-weighted mean percentage of *S. aureus* resistant to methicillin was 16.8% (95% CI 16 - 17), ranging from 0% (95% CI 0 – 4) in Iceland to 57.2% (95% CI 51 – 63) in Romania.[32]

In ASSOP 2015, 18.2% (95% CI 16.7– 19.8) of the 2,399 SAB episodes were methicillin-resistant. This compares to 19.1% (95% CI 17.5 – 21.0) in ASSOP 2013 and 18.8% (95%CI 17.2 – 20.5) in ASSOP 2014.[17] In 2015, Ireland reported a similar percentage to Australia: 18.1%, (95% CI 16 – 21). However, for 19 of the 30 European countries (primarily the northern European countries, Germany, France and the United Kingdom) the percentage of SAB isolates resistant to methicillin was less than that reported in ASSOP 2015. Similar to Europe, which has seen the EU/EEA population-weighted mean percentage decrease significantly from 23.2% in 2009 to 16.8% in 2015, the percentage of methicillin-resistant SAB in Australia has decreased from 23.8% (95% CI 21.4 – 26.4) in 2007 to 18.2% (95%CI 16.7 – 19.8) in 2015 (P<0.0001).[18] The decrease in methicillin-resistant SAB is consistent with what has been reported elsewhere[19,20] 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.[21-25] 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 2015, the all-cause mortality at 30-days was 15.8% (95% CI 14.4 – 17.5). In comparison, the 2008 Australian New Zealand Cooperative on Outcomes in Staphylococcal Sepsis (ANZCOSS) reported a significantly higher figure of 20.6% (95% CI 18.8 - 22.5, P<0.0001), and when adjusted for Australian institutions only was 25.9% (personal communication). MRSA-associated SAB mortality remains high (18.8%, 95% CI 14.9 – 23.3) but was not significantly higher than MSSA-associated SAB mortality (15.1%, 95% CI 13.4 – 16.9). 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.[26]

With the exception of the β-lactams and erythromycin, antimicrobial resistance in MSSA remains rare. However, in addition to the β-lactams approximately 50% of MRSA were resistant to erythromycin and ciprofloxacin and approximately 15% resistant to co-trimoxazole, tetracycline and gentamicin. Resistance was largely attributable to two healthcare-associated MRSA clones, ST22-IV [2B] (EMRSA-15), which is typically ciprofloxacin and erythromycin resistant, and ST239-III [3A] (Aus-2/3 EMRSA) which is typically erythromycin, clindamycin, ciprofloxacin, co-trimoxazole, tetracycline and gentamicin resistant. 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 ST239-III [3A] (Aus-2/3 EMRSA) as the most prevalent HA-MRSA isolated from clinical specimens and this change has occurred throughout most of the country.[27] In ASSOP 2015, approximately 25% of MRSA were characterised as ST22-IV [2B] (EMRSA-15). CA-MRSA, in particular the ST45-V clone (9.6% of MRSA), 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 23% of SAB caused by CA-MRSA were of healthcare onset. Although in several parts of the United States of America the CA-MRSA clone USA300 has replaced the HA-MRSA clone ST5-II [2A] (USA100) as a cause of healthcare-associated MRSA infection,[28] transmission of CA-MRSA in Australian hospitals is thought to be rare.[29,30] Consequently, it is likely that many of the healthcare onset CA-MRSA SAB infections reported in ASSOP 2015 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 2015 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.

Author Details

Prof Geoffrey W Coombs[1,2],Ms Denise A Daley[3], Ms Yung Thin Lee[1], Dr Stanley Pang[1,2], Ms Jan M Bell[4], Prof John D Turnidge[5] for the Australian Group on Antimicrobial Resistance

1. Antimicrobial Resistance and Infectious Diseases Laboratory, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia

2. Department of Microbiology, PathWest Laboratory Medicine-WA, Fiona Stanley Hospital, Murdoch, Western Australia, Australia

3. Australian Group on Antimicrobial Resistance, Fiona Stanley Hospital, Murdoch, Western Australia, Australia

4. School of Biological Sciences, University of Adelaide, Adelaide, South Australia, Australia

5. Departments of Molecular and Cellular Biology, University of Adelaide, Adelaide, South Australia, Australia

Corresponding Author

Prof Geoffrey Coombs

Antimicrobial Resistance and Infectious Diseases Laboratory, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia

Telephone: +61 8 6152 2397

Email: g.coombs@murdoch.edu.au

Acknowledgments

This study was funded by a grant from the Australian Commission on Safety and Quality in Health Care.

Members of the AGAR in 2015 were:

Australian Capital Territory

Peter Collignon and Susan Bradbury, The Canberra Hospital

New South Wales

Thomas Gottlieb and Graham Robertson, Concord Hospital

James Branley and Donna Barbaro, Nepean Hospital

George Kotsiou and Peter Huntington, Royal North Shore Hospital

Sebastian van Hal and Bradley Watson, Royal Prince Alfred Hospital

Jon Iredell and Andrew Ginn, Westmead Hospital

Rod Givney and Ian Winney, John Hunter Hospital

Peter Newton and Melissa Hoddle, Wollongong Hospital

Northern Territory

Rob Baird and Jann Hennessy, Royal Darwin Hospital

James McLeod, Alice Springs Hospital

Queensland

Enzo Binotto and Bronwyn Thomsett, Pathology Queensland Cairns Base Hospital

Graeme Nimmo and Narelle George, Pathology Queensland Central Laboratory

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

Robert Horvath and Laura Martin, Pathology Queensland Prince Charles Hospital

Naomi Runnegar and Joel Douglas, Pathology Queensland Princess Alexandra Hospital

Jenny Robson and Georgia Peachey, Sullivan Nicolaides Pathology

South Australia

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

Morgyn Warner and Kija Smith, SA Pathology (Royal Adelaide Hospital and Women's and Children's Hospital)

Tasmania

Louise Cooley and David Jones, Royal Hobart Hospital

Victoria

Denis Spelman and Amanda Dennison, The Alfred Hospital

Benjamin Howden and Peter Ward, Austin Hospital

Tony Korman and Despina Kotsanas,
Monash Medical Centre

Andrew Daley and Gena Gonis,
Royal Women's Hospital

Mary Jo Waters and Lisa Brenton,
St Vincent's Hospital

Western Australia

David McGechie and Denise Daley,
PathWest Laboratory Medicine –
WA Fiona Stanley Hospital

Ronan Murray and Jacinta Bowman,
PathWest Laboratory Medicine –
WA Queen Elizabeth II Hospital

Michael Leung and Jacinta Bowman, PathWest Laboratory Medicine – Northwest WA

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

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

Shalinie Perera and Ian Meyer, Western Diagnostic Pathology, Joondalup Hospital

References

1. Laupland KB. Incidence of bloodstream infection: a review of population-based studies. *Clin Microbiol Infect* 2013; 19: 492-500.

2. Johnson AP, Pearson A, Duckworth G. Surveillance and epidemiology of MRSA bacteraemia in the UK. *J Antimicrob Chemothera* 2005; 56: 455-62.

3. Thwaites GE, Edgeworth JD, Gkrania-Klotsas E et al. Clinical management of Staphylococcus aureus bacteraemia. *Lancet Infect Dis* 2011; 11: 208-22.

4. Collignon P, Nimmo GR, Gottlieb T et al. Staphylococcus aureus bacteremia, Australia. *Emerg Infect Dis* 2005; 11: 554-61.

5. Frederiksen MS, Espersen F, Frimodt-Moller N et al. Changing epidemiology of pediatric Staphylococcus aureus bacteremia in Denmark from 1971 through 2000. *Pediatr Infect Dis J* 2007; 26: 398-405.

6. Benfield T, Espersen F, Frimodt-Moller N et al. Increasing incidence but decreasing in-hospital mortality of adult Staphylococcus aureus bacteraemia between 1981 and 2000. *Clin Microbiol Infect* 2007; 13: 257-63.

7. van Hal SJ, Jensen SO, Vaska VL et al. Predictors of mortality in Staphylococcus aureus Bacteremia. *Clin Microbiol Rev* 2012; 25: 362-86.

8. Kaasch AJ, Barlow G, Edgeworth JD et al. Staphylococcus aureus bloodstream infection: a pooled analysis of five prospective, observational studies. *J Infect* 2014; 68: 242-51.

9. Turnidge JD, Kotsanas D, Munckhof W et al. Staphylococcus aureus bacteraemia: a major cause of mortality in Australia and New Zealand. *Med J Aust* 2009; 191: 368-73.

10. Nimmo GR, Bell JM, Collignon PJ. Fifteen years of surveillance by the Australian Group for Antimicrobial Resistance (AGAR). *Commun Dis Intell* 2003; 27 Suppl: S47-54.

11. Coombs GW, Nimmo GR, Daly DA et al. Australian Staphylococcus aureus Sepsis Outcome Programme annual report, 2013. *Commun Dis Intell* 2014; 38: E309-19.

12. CLSI. Performance standards for antimicrobial susceptibility testing. *Twenty-fourth informational supplement M100-S24*. Villanova, PA, USA, 2014.

13. European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints. 2014.

14. Seemann T, Goncalves da Silva A, Bulach DM, Schultz MB, Kwong JC, Howden BP. Nullarbor. San Francisco; Github. [Accessed: 03 Jun 2016]. Available from: https://github.com/tseemann/nullarbor

15. Bartels MD, Petersen A, Worning P, Nielsen JB, Larner-Svensson H, Johansen HK, Andersen LP, Jarløv JO, Boye K, Larsen AR, Westh H.Comparing whole-genome sequencing with Sanger sequencing for *spa* typing of methicillin-resistant Staphylococcus aureus. *J. Clin. Microbiol*. 2014. 52(12): 4305-8.

16. Monecke, S., Slickers, P. and Ehricht, R. (2008), Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. *FEMS Immunology & Medical Microbiology,* 53: 237–251.

17. Australian Group on Antimicrobial Resistance Australian Staphylococcus aureus Sepsis Outcome Programme annual report, 2014. Coombs GW, Daley DA, Thin Lee Y, Pearson JC, Robinson JO, Nimmo GR, Collignon P, Howden BP, Bell JM, Turnidge JD; Australian Group on Antimicrobial Resistance. Commun Dis Intell Q Rep. 2016 Jun 30;40(2):E244-54.PMID: 27522136 Free Article

18. Turnidge JD, Nimmo GR, Pearson J et al. Epidemiology and outcomes for Staphylococcus aureus bacteraemia in Australian hospitals, 2005-06: report from the Australian Group on Antimicrobial Resistance. *Commun Dis Intell* 2007; 31: 398-403.

19. Johnson AP, Davies J, Guy R et al. Mandatory surveillance of methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia in England: the first 10 years. *J Antimicrob Chemothera* 2012; 67: 802-9.

20. de Kraker ME, Davey PG, Grundmann H et al. Mortality and hospital stay associated with resistant Staphylococcus aureus and Escherichia coli bacteremia: estimating the burden of antibiotic resistance in Europe. *PLoS Med* 2011; 8: e1001104.

21. Johnson PD, Martin R, Burrell LJ et al. Efficacy of an alcohol/chlorhexidine hand hygiene program in a hospital with high rates of nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection. *Med J Aust* 2005; 183: 509-14.

22. Vos MC, Behrendt MD, Melles DC et al. 5 years of experience implementing a methicillin-resistant Staphylococcus aureus search and destroy policy at the largest university medical center in the Netherlands. *Infect Control Hosp Eepidemiol* 2009; 30: 977-84.

23. Grayson ML, Jarvie LJ, Martin R et al. Significant reductions in methicillin-resistant Staphylococcus aureus bacteraemia and clinical isolates associated with a multisite, hand hygiene culture-change program and subsequent successful statewide roll-out. *Med j Aust* 2008; 188: 633-40.

24. Kim YC, Kim MH, Song JE et al. Trend of methicillin-resistant Staphylococcus aureus (MRSA) bacteremia in an institution with a high rate of MRSA after the reinforcement of antibiotic stewardship and hand hygiene. *Am J Infect Control* 2013; 41: e39-43.

25. Lawes T, Edwards B, Lopez-Lozano JM et al. Trends in Staphylococcus aureus bacteraemia and impacts of infection control practices including universal MRSA admission screening in a hospital in Scotland, 2006-2010: retrospective cohort study and time-series intervention analysis. *BMJ Open* 2012; 2.

26. Chua KY, Monk IR, Lin YH et al. Hyperexpression of alpha-hemolysin explains enhanced virulence of sequence type 93 community-associated methicillin-resistant Staphylococcus aureus. *BMC Microbiol* 2014; 14: 31.

27. Coombs GW PJ, Nimmo GR, Collignon PJ,

Bell JM, McLaws M-L, Christiansen KJ, Turnidge JD, on behalf of the Australian Group on Antimicrobial Resistance. Antimicrobial susceptibility of Staphylococcus aureus and molecular epidemiology of meticillin-resistant *S. aureus* isolated from Australian hospital inpatients: Report from the Australian Group on Antimicrobial Resistance 2011 Staphylococcus aureus Surveillance Programme. *J Glob Antimicrob Resis* 2013; 1: 149-56.

28. Nimmo GR. USA300 abroad: global spread of a virulent strain of community-associated methicillin-resistant Staphylococcus aureus. *Clin Microbiol Infect* 2012; 18: 725-34.

29. O'Brien FG, Pearman JW, Gracey M et al. Community strain of methicillin-resistant Staphylococcus aureus involved in a hospital outbreak. *J Clin Mmicrobiol* 1999; 37: 2858-62.

30. Schlebusch S, Price GR, Hinds S et al. First outbreak of PVL-positive nonmultiresistant MRSA in a neonatal ICU in Australia: comparison of MALDI-TOF and SNP-plus-binary gene typing. *Eurp J Clin Microbiolo Infect Dis* 2010; 29: 1311-4.

31. http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx.

32. http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-europe-2014.pdf