Australian Government
Department of Health

COMMUNICABLE DISEASES INTELLIGENCE

2018 Volume 42
PII:S2209-6051(18)00021-0

Australian Group on Antimicrobial-resistance (AGAR) Australian Staphylococcus aureus Sepsis Outcome Programme (ASSOP) Annual Report 2016

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on behalf of the Australian Group on Antimicrobial-resistance

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COMMUNICABLE DISEASES INTELLIGENCE

ISSN: 2209-6051 Online

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This journal is indexed by Index Medicus and Medline.

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Australian Group on Antimicrobial-resistance (AGAR) Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP) Annual Report 2016

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Abstract

From 1st January to 31st December 2016, 32 institutions around Australia participated in the Australian *Staphylococcus aureus* Sepsis Outcome Programme (ASSOP). The aim of ASSOP 2016 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. A total of 2,540 *S. aureus* bacteraemia episodes were reported, of which 19.7% were methicillin-resistant. The 30-day all-cause mortality associated with methicillin-resistant SAB was 23.1% which was significantly higher than the 15.3% mortality associated with methicillin-susceptible SAB. With the exception of the β-lactams and erythromycin, antimicrobial-resistance in methicillin-susceptible *S. aureus* (MSSA) was rare. However, in addition to the β-lactams approximately 45% of methicillin-resistant *S. aureus* (MRSA) were resistant to erythromycin and ciprofloxacin and approximately 14% 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 2 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. Seventy two percent of methicillin-resistant SAB were due to community associated clones. Although polyclonal almost 60% of community associated clones were characterised as ST93-IV [2B] (Queensland CA-MRSA), ST5-IV [2B] and ST1-IV [2B]. CA-MRSA in particular the ST45-V_T [5C2&5] clone has acquired multiple antimicrobial-resistance determinants including ciprofloxacin, erythromycin, clindamycin, gentamicin and tetracycline. Twelve percent of CA-MRSA were ST45-V_T [5C2&5]. As CA-MRSA is well established in the Australian community it is important antimicrobial-resistance patterns in community- and healthcare-associated SAB is monitored as this information will guide therapeutic practices in treating *S. aureus* sepsis.

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 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 Staphylococcal Sepsis Outcome Programme (ASSOP).[11] The primary objective of ASSOP 2016 was to determine the proportion of SAB isolates demonstrating antimicrobial-resistance with particular emphasis on:

- assessing susceptibility to methicillin
- molecular epidemiology of methicillin-resistant *S. aureus* (MRSA).

Methodology

Participants

Thirty-two laboratories from all 8 Australian states and territories.

Collection Period

From 1st January to 31st December 2016, the 32 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 AMRID Research 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 3 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 2 proportions and calculation of 95% confidence intervals (95%CI) were performed using MedCalc for Windows, version 12.7 (MedCalc Software, Ostend Belgium).

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

Results

From 1st January to the 31st December 2016, 2,540 unique episodes of *S. aureus* bacteraemia were identified. A significant difference (p<0.0001) was seen in patient sex with 65.4% (1,661) being male (95% CI 63.5 – 67.3). The average age of patients was 58 years ranging from 0 – 102 years with a median age of 62 years. Overall 76.2% (1,936/2,540) of episodes were community onset (95% CI 74.5% – 77.9%). All-cause mortality at 30-days was 16.7% (95% CI 15.3 – 18.2). Methicillin-resistant SAB mortality was 23.1% (95% CI 19.5 – 27.0) which was significantly higher than for methicillin-susceptible SAB mortality (15.3%, 95% CI 13.8 – 16.9) (p=0.0003).

Methicillin-Susceptible *Staphylococcus aureus* (MSSA) Antimicrobial Susceptibility

Overall 80.3% (2,040) of the 2,540 isolates were methicillin-susceptible of which 77.2% (1,571) were penicillin-resistant (MIC >0.12 mg/L). However, as β-lactamase was detected in 66 phenotypically penicillin susceptible isolates, 80.4% 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 3.2% (Table 1). There were 7 isolates reported by Vitek2® as non-susceptible to daptomycin (MIC >1.0mg/L). By Etest® three of the isolates were considered susceptible (MICs 0.25, 0.38 and 0.5mg/L). Four isolates had Etest® MICs of 1.5, 2.0 (2 isolates) and 3.0 mg/L and therefore were considered non-susceptible. By Vitek2® two isolates were linezolid-resistant (MIC >4 mg/L). However by Etest® the isolates had an MIC ≤4 mg/L (0.5 and 1.5 mg/L) and were therefore considered linezolid-susceptible. All MSSA were vancomycin and teicoplanin and susceptible. Twenty four (1.2%) of 2,036 isolates had high level mupirocin-resistance of which 15 isolates were referred from Queensland. Inducible resistance to clindamycin was determined by the Vitek2® susceptibility system. Of the 1,798 isolates tested, 10.1% (181) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI breakpoints) of which 87.9% (159) were classified as having inducible clindamycin resistance. Multi-resistance was uncommon in MSSA (0.7%, 14/1,922).

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

MRSA Antimicrobial Susceptibility

The proportion of *S. aureus* that were MRSA was 19.7% (95%CI 18.2 – 21.3). The 500 MRSA identified were either cefoxitin screen positive by Vitek2® (417) or had a cefoxitin MIC >4 by Phoenix™ (83). All 500 MRSA isolates were

Table 1: The number and proportion of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2016

Antimicrobial	Tested	Breakpoint (mg/L)	Non-Susceptible	
			n	%
Penicillin	2,035	>0.12*	1,571	77.2
Vancomycin	2,035	>2*	0	0.0
Teicoplanin	2,037	>8†	0	0.0
		>2‡	0	0.0
Rifampicin	1,981	>1†	3	0.2
		>0.5‡	3	0.2
Fusidic Acid	2,036	>1‡	65	3.2
Gentamicin	2,038	>4†	9	0.4
		>1‡	16	0.8
Erythromycin	2,036	>0.5†	225	11.1
		>2‡	190	9.3
Clindamycin	2,035	>0.5*	20	1.0
Tetracycline	1,814	>4†	40	2.2
		>2‡	40	2.2
Co-trimoxazole	2,038	>2/38†	50	2.5
		>4/76‡	46	2.3
Ciprofloxacin	2,036	>1*	59	2.9
Nitrofurantoin	1,930	>32†	7	0.4
		>64‡	1	0.1
Daptomycin	2,037	>1*	4	0.2
Linezolid	2,038	>4*	0	0

*CLSI and EUCAST non-susceptible breakpoint
†CLSI non-susceptible breakpoint
‡EUCAST non-susceptible breakpoint

phenotypically penicillin-resistant. Amongst the MRSA isolates, non-susceptibility to non-β-lactam antimicrobials was common except for rifampicin, fusidic acid and nitrofurantoin where resistance was below 3.2% (Table 2). There were 4 isolates reported by Vitek2® as non-susceptible to daptomycin (MIC >1.0mg/L). By Etest® the isolates had MICs of 1.5 (2 isolates) and 2 mg/L (2 isolates). When using the EUCAST resistant breakpoint of >2 mg/L one isolate was teicoplanin resistant (MIC = 4 mg/L). However, using the CLSI resistant breakpoint of >8 mg/L the isolate was classified as susceptible. One MRSA had a vancomycin MIC of 4.0mg/L by both Vitek2® and Etest® however no *van* genes were present. Four (0.8%) of 498 MRSA isolates tested had high level mupirocin-resistance. Inducible resistance to clindamycin was determined by the Vitek2® susceptibility system. Of the 417 isolates tested by Vitek2®, 31.2% (130) were erythromycin non-susceptible/clindamycin intermediate/susceptible (CLSI and EUCAST breakpoints) of which 83.9% (109) were classified as having inducible clindamycin resistance. Multi-resistance was seen in 11.8% of MRSA. This was a significant decrease from the 2015 study (24.3%, p<0.0001).

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

MRSA Molecular Epidemiology

Whole genome sequencing was performed on 93.6% (468/500) of the MRSA. Based on molecular typing, 27.6% (129) and 72.4% (339) of isolates

Table 2: The number and proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates non-susceptible to penicillin and the non-β-lactam antimicrobials, Australia, 2016

Antimicrobial	Tested	Breakpoint (mg/L)	Non-Susceptible (%)	
			n	%
Penicillin	500	>0.12*	500	100
Vancomycin	500	>2*	1	0.2
Teicoplanin	500	>8†	0	0
		>2‡	1	0.2
Rifampicin	498	>1†	13	2.6
		>0.5‡	13	2.6
Fusidic Acid	500	>1‡	16	3.2
Gentamicin	500	>4†	70	14.0
		>1‡	76	15.2
Erythromycin	500	>0.5†	219	43.8
		>2‡	209	41.8
Clindamycin	500	>0.5*	69	13.8
Tetracycline	417	>4†	63	15.1
		>2‡	63	15.1
Co-trimoxazole	500	>2/38†	56	11.2
		>4/76‡	54	10.8
Ciprofloxacin	500	>1*	217	43.5
Nitrofurantoin	488	>32†	1	0.2
		>64‡	0	0
Daptomycin	500	>1*	4	0.8
Linezolid	500	>4*	0	0

*CLSI and EUCAST non-susceptible breakpoint
†CLSI non-susceptible breakpoint
‡EUCAST non-susceptible breakpoint

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 129 HA-MRSA isolates, 42.6% (55) were epidemiologically classified as hospital onset and 57.4% (74) were classified as community onset. Five HA-MRSA clones were identified: 97 isolates of ST22-IV [2B] (EMRSA-15) (20.7% of MRSA typed and 3.9% of *S. aureus*); 29 isolates of ST239-III [3A] (Aus -2/3 EMRSA) (6.2% and 1.2%) and single isolates of ST5-II [2A] (USA100/New York Japan), ST8-II [2B] (Irish-1) and ST8-III [2B] (EMRSA-17).

ST22-IV [2B] (EMRSA-15) was the dominant HA-MRSA clone in Australia accounting for 75% of HA-MRSA ranging from 0% in the Northern Territory to 100% in Tasmania (Table 4). ST22-IV [2B] (EMRSA-15) was PVL negative and using CLSI breakpoints 98.0% and 47.5% were ciprofloxacin and erythromycin non-susceptible respectively.

ST239-III [3A] (Aus-2/3 EMRSA) accounted for 22.5% of HA-MRSA ranging from 0% in Tasmania to 100% in the Northern Territory (Table 4). PVL negative ST239-III [3A] (Aus-2/3 EMRSA) were typically resistant to erythromycin (96.6%), co-trimoxazole (82.8%), ciprofloxacin (96.6%), gentamicin (93.1%), tetracycline (88.0%) and clindamycin (69.0%).

Table 3: Proportion of healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus*, Australia, 2016 by clone, healthcare and community onset, and Panton-Valentine leucocidin carriage

Strain	Total		Onset				PVL Positive	
			Healthcare		Community			
	n	%*	n	%†	n	%†	n	%†
Healthcare Associated MRSA								
ST22-IV [2B] (EMRSA-15)	97	20.7	40	41.2	57	58.8	0	0
ST239-III [3A] (Aus-2/3)	29	6.2	13	44.8	16	55.2	0	0
ST5-II (NY/Japan/USA100 variant)	1	0.2	1	100	0	0	0	0
ST8-II (Irish-1)	1	0.2	0	0	1	100	0	0
ST8-III (EMRSA 17)	1	0.2	1	100	0	0	0	0
Total HA-MRSA	129	27.6	55	42.6	74	57.4	0	0
Community Associated MRSA								
ST93-IV [2B] (Queensland)	101	21.6	12	11.9	89	88.1	97	96.0
ST5-IV	50	10.7	10	20.0	40	80.0	13	26.0
ST1-IV	45	9.6	12	26.7	33	73.3	3	6.7
ST45-Vt	41	8.8	10	24.4	31	75.6		0
ST30-IV	19	4.1	6	31.6	13	68.4	12	63.2
ST78-IV	17	3.6	3	17.6	14	82.4		0
ST97-IV	7	1.5	1	14.3	6	85.7		0
ST188-IV	4	0.9	2	50.0	2	50.0		0
ST72-IV	4	0.9	1	25.0	3	75.0		0
ST1-I	3	0.6		0	3	100		0
ST59-IV	3	0.6		0	3	100		0
ST59-Vt	3	0.6		0	3	100	2	66.7
ST5-Vt	3	0.6	1	33.3	2	66.7		0
ST872-IV	3	0.6		0	3	100		0
ST8-IV	3	0.6	1	33.3	2	66.7	3	100
ST953-IV	3	0.6	3	100		0		0
ST1-Vt	2	0.4	1	50.0	1	50.0		0
ST45-IV	2	0.4		0	2	100		0
ST73-IV	2	0.4	1	50.0	1	50.0		0
ST772-Vt	2	0.4	1	50.0	1	50.0	1	50.0
ST834-IV	2	0.4	1	50.0	1	50.0		0
ST835-IV	2	0.4		0	2	100		0
ST1232-Vt	1	0.2		0	1	100	1	100
ST1420-IV	1	0.2		0	1	100	1	100
ST149-IV	1	0.2		0	1	100		0
ST1850-IV	1	0.2	1	100		0	1	100
ST1slv-IV	1	0.2		0	1	100		0
ST30-V	1	0.2		0	1	100	1	100
ST30-Vt	1	0.2		0	1	100	1	100
ST338-Vt	1	0.2		0	1	100	1	100
ST45-V	1	0.2	1	100		0		0
ST5slv-IV	1	0.2		0	1	100		0
ST5-V	1	0.2		0	1	100		0
ST5-VI	1	0.2		0	1	100		0
ST672-Vt	1	0.2	1	100		0		0
ST6-IV	1	0.2	1	100		0		0
ST6slv-Vt	1	0.2		0	1	100		0
ST762-IV	1	0.2		0	1	100		0
ST88-IV	1	0.2		0	1	100		0
ST8-Vt	1	0.2		0	1	100		0
Total CA-MRSA	**339**	**72.4**	**70**	**20.6**	**269**	**79.4**	**137**	**40.4**
Grand Total	468	100	125	26.7	343	73.3	137	40.4

*Percentage of all MRSA typed
†Percentage of the strain

Table 4: The number and proportion of healthcare associated methicillin-resistant *Staphylococcus aureus* (MRSA) multilocus sequence types, Australia, 2016, by region

Type	ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aus	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ST22-IV	4	66.7	34	68.0			3	60.0	22	81.5	8	100	16	80.0	10	90.9	97	75.2
ST239-III	2	33.3	14	28.0	2	100	2	40.0	5	18.5			3	15.0	1	9.1	29	22.5
ST5-II													1	5.0			1	0.8
ST8-II			1	2.0													1	0.8
ST8-III			1	2.0													1	0.8
Total	6	100	50	100	2	100	5	100	27	100	8	100	20	100	11	100	129	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), 2016, by region

Type	ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aus	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ST93-IV			16	17.8	26	66.7	19	32.8	8	23.5	1	33.3	10	25.3	21	31.8	101	29.8
ST5-IV			11	12.2	8	20.5	14	24.1	4	11.8			6	14.0	7	10.6	50	14.7
ST1-IV	1	16.7	11	12.2	3	7.7	5	8.6	11	32.4	2	66.7	3	7.0	9	13.6	45	13.3
ST45-Vt	1	16.7	29	32.2					4	11.8			7	16.3			41	12.1
ST30-IV			9	10			4	6.9					6	14.0			19	5.6
ST78-IV	1	16.7							3	8.8					13	19.7	17	5.0
Other	3	50	14	15.6	2	5.1	16	27.6	4	11.8			11	25.6	17	24.2	66	19.5
Total	6	100	90	100	39	100	58	100	34	100	3	100	43	100	66	100	339	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

Community-associated methicillin-resistant *Staphylococcus aureus*

For the 339 CA-MRSA isolates, 20.6% (70) of episodes were epidemiologically classified as hospital-onset and 79.4% (269) classified as community-onset. Based on the multi locus sequence type and the *SCCmec* type 40 CA-MRSA clones were identified (Table 3). Overall 80.5% of CA-MRSA were classified into six clones each having more than ten isolates: ST93-IV [2B] (Queensland CA-MRSA) (21.6% of MRSA typed and 4.2% of *S. aureus*); ST5-IV (10.7% and 2.1%); ST1-IV (9.6% and 1.9%); ST45-VT (8.8% and 1.7%); ST30-IV (4.1% and 0.8%); and ST78-IV (3.6% and 0.7%).

ST93-IV [2B] (Queensland CA-MRSA) accounted for 29.8% of CA-MRSA ranging from 0% in the Australian Capital Territory to 66.7% in the Northern Territory (Table 5). Typically PVL positive, 78.2% (79/101) of ST93-IV [2B] (Queensland CA-MRSA) were resistant to the β-lactams only or additionally resistant to erythromycin (14.9%, 15/101) or erythromycin and clindamycin (6.9%, 7/101).

ST5-IV accounted for 14.7% of CA-MRSA and was isolated in all regions of Australia except the Australian Capital Territory and Tasmania ranging from 0% to 24.1% in Queensland (Table 5). ST5-IV, 26% PVL positive, was typically resistant to the β-lactams only 40% (20/50) β-lactams and co-trimoxazole (26%, 13/50), or additionally resistant to erythromycin (10%, 5/50), fusidic acid (5.7%, 5/50), ciprofloxacin and erythromycin (6.0% 3/50), and one (2.0%) each of clindamycin and erythromycin, erythromycin and fusidic acid, erythromycin and tetracycline or erythromycin, clindamycin and tetracycline.

ST1-IV accounted for 13.3% of CA-MRSA ranging from 7.7% in the Northern Territory to 35.3% in South Australia (Table 5). Typically PVL negative, 64.4% of isolates were resistant to the β-lactams only (29/45) or additionally resistant to erythromycin (11.1%, 5/45), fusidic acid (4.4%, 2/45), erythromycin and fusidic acid (4.4%, 2/45) erythromycin, clindamycin and tetracycline (4.4%, 2/45) and one (2.2%) each of ciprofloxacin, ciprofloxacin and erythromycin, ciprofloxacin and gentamicin, erythromycin and tetracycline, and erythromycin, clindamycin, fusidic acid and tetracycline.

ST45-VT accounted for 12.1% of CA-MRSA and was isolated primarily in New South Wales (Table 5). All isolates were PVL negative and were resistant to the β-lactams. Isolates were additionally non-susceptible to erythromycin, ciprofloxacin, gentamicin and tetracycline (29.3%, 12/41), ciprofloxacin (19.5%, 8/41) erythromycin, ciprofloxacin and tetracycline (14.6%, 6/41), erythromycin, ciprofloxacin, clindamycin, gentamicin and tetracycline (9.8%, 4/41), erythromycin, ciprofloxacin and gentamicin (7.3%, 3/41) two (4.9%) of erythromycin, ciprofloxacin and clindamycin, and ciprofloxacin, gentamicin and tetracycline, and one (2.4%) each of co-trimoxazole, erythromycin and ciprofloxacin, ciprofloxacin and gentamicin, ciprofloxacin and tetracycline.

ST30-IV accounted for 5.6% of CA-MRSA and was isolated only in New South Wales, Victoria and Queensland (Table 5). Typically PVL positive 89.5% of isolates were resistant to the β-lactams only (17/19). One isolate (5.3%) was resistant to clindamycin and erythromycin and one isolate to clindamycin and co-trimoxazole.

ST78-IV accounted for 5.0% of CA-MRSA and was predominantly in Western Australia (Table 5). Isolates were resistant to the β-lactams and erythromycin (100%, 17/17).

Overall 92.3% of CA-MRSA were non-multiresistant including 49.3% resistant to the β-lactams only. However 26 (7.7%) CA-MRSA isolates were multiresistant.

Panton-Valentine leucocidin

Overall 137 (29.3%) MRSA were PVL positive, including 40.4% of 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 Australia antimicrobial-resistance data with other countries is possible. (http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx).

In the 2016 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 13.7% (95% CI 13 - 14), ranging from 1.2% (95% CI 1 – 2) in the Netherlands and Norway to 50.5% (95% CI 51 – 63) in Romania. (http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-europe-2016.pdf)

In ASSOP 2016, 19.7% (95% CI 18.2 – 21.3) of the 2,540 SAB episodes were methicillin-resistant.

This compares to 19.1% (95% CI 17.5 – 21.0) in ASSOP 2013[11], 18.8% (95%CI 17.2 – 20.5) in ASSOP 2014[17] and 18.2% (95% CI 16.7– 19.8) in ASSOP 2015.[31] However, for 20 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 2016. Similar to Europe, which has seen the EU/EEA population-weighted mean percentage decrease significantly from 23.2% in 2009 to 13.7% in 2016, the percentage of methicillin-resistant SAB in Australia has decreased from 23.8% (95% CI 21.4 – 26.4) in 2007 to 19.1% (95%CI 17.5 -21.0) in 2016 (P<0.0001). 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 2016, the all-cause mortality at 30-days was 16.7% (95% CI 15.3 – 18.2). 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 (23.1%, 95% CI 19.5 – 27.0) and was significantly higher than MSSA-associated SAB mortality (15.3%, 95% CI 13.8 – 16.9) p=0.0003. 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 2 healthcare associated MRSA clones, ST22-IV [2B] (EMRSA-15), which is typically ciprofloxacin and erythromycin resistant, and ST239-III [3A] (Aus-2/3 EMRSA) which is typically erythromycin, clindamycin, ciprofloxacin, co-trimoxazole, tetracycline and gentamicin-resistant. From the early 1980s until recently, the multi-resistant ST239-III [3A]

(Aus-2/3 EMRSA) was the dominant HA-MRSA clone in Australian hospitals. However, ST22-IV [2B] (EMRSA-15) has replaced 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 2016, approximately 21% of MRSA were characterised as ST22-IV [2B] (EMRSA-15). CA-MRSA, in particular the ST45-V_T clone (8.8% 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, one isolate was teicoplanin non-susceptible when EUCAST criteria were applied.

Approximately 20.6% of SAB caused by CA-MRSA were healthcare-onset. Although, in several parts of the United States the CA-MRSA clone USA300 has replaced the HA-MRSA clone ST5-II [2A] (USA100) as a cause of healthcare associated MRSA infection,[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 2016 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] 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 2016 has demonstrated antimicrobial-resistance in SAB in Australia continues to be 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.

Acknowledgments

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

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