

Staphylococcus aureus Programme 2003 (SAP 2003) Hospital/Community Survey

MRSA Epidemiology and Typing Report

PREPARED BY:

Mr Geoffrey Coombs Department of Microbiology and Infectious Diseases, Royal Perth Hospital.

Associate Professor Keryn Christiansen Department of Microbiology and Infectious Diseases, Royal Perth Hospital.

Ms Julie Pearson Department of Microbiology and Infectious Diseases, Royal Perth Hospital.

Dr Frances O'Brien Molecular Genetics Research Unit, Curtin University

Dr Graeme Nimmo Princess Alexandra Hospital, Queensland Health Pathology.

Associate Professor Peter Collignon Department of Microbiology, The Canberra Hospital

On behalf of the Australian Group for Antimicrobial Resistance (AGAR)

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Epidemiology and Typing Report of MRSA Isolated in the Australian Group on Antimicrobial Resistance (AGAR) 2003 *Staphylococcus aureus* Surveillance Programme (SAP 2003)

Commencement Date

1st November 2003

Isolates

Approximately 100 consecutive isolates of *Staphylococcus aureus* from 100 different patients at each site were tested by 23 laboratories located across Australia (total number of isolates = 2,184). Isolates were collected from hospital inpatients including patients attending the emergency department.

Participating Laboratories (23)

Australian Capital Territory (1)

The Canberra Hospital

South Australia (4)

Gribbles Pathology Flinders Medical Centre Institute of Medical Veterinary Science Women's and Children's Hospital

New South Wales (5)

Concord Hospital Nepean Hospital Royal North Shore Hospital South West Area Pathology Services Westmead Hospital

Northern Territory (1)

Royal Darwin Hospital

Queensland (3)

Princess Alexandra Hospital Royal Brisbane Hospital Sullivan Nicolaides Pathology

Tasmania (1)

Royal Hobart Hospital

Victoria (4)

Alfred Hospital Gribbles Pathology Royal Children's Hospital St Vincent's Hospital

Western Australia (4)

Fremantle Hospital PathCentre Royal Perth Hospital Saint John of God Pathology

Methicillin Susceptibility Testing

Breakpoint agar dilution (NCCLS) (1)

- Mueller Hinton agar (BBL Mueller Hinton II, Cat No. 11438, Acumedia, Cat No. 7101) supplemented with 2% (w/v) NaCl and oxacillin 2mg/L
- Plates incubated at 35°C for 24 hours

Epidemiological Typing

Performed by the Gram-positive Bacteria Typing and Research Unit Department of Microbiology and Infectious Diseases, Royal Perth Hospital Molecular Genetics Research Unit, Curtin University

MRSA Nomenclature

The Gram-positive Bacteria Typing and Research Unit employs the international MRSA nomenclature system described by Dr Mark Enright *et al.* (2). This system provides a universally standardised MRSA nomenclature allowing MRSA clones to be readily compared between laboratories and countries. It is based upon the combination of seven housekeeping genes sequence types (STs) using multilocus sequence typing (MLST) and the SCC*mec* type using multiplex PCR. The MRSA genotype is therefore the sum of the SCC*mec* type and the type of its recipient chromosome. For example, an MRSA clone of ST22 and SCC*mec* type IV is referred to as ST22-MRSA-IV (previously known as UK EMRSA-15).

MLST

MLST is a highly discriminatory method of characterising MRSA. For each of the seven housekeeping gene fragments, different sequences are assigned as distinct alleles, and an isolate is defined by the alleles of each of the seven housekeeping loci (the allelic profile or ST). The ST can be compared with other strains using the program BURST located on the MLST website (<u>www.saureus.mlst.net</u>). As there are many alleles for each loci, isolates are highly unlikely to have identical ST by chance, and therefore isolates with the same ST are considered members of the same clone.

SCC*mec*

The gene for methicillin resistance, *mecA*, is contained within a mobile element known as the *mec* region or staphylococcal cassette chromosome mec (SCC*mec*). The SCC*mecs* differ depending on variations in the the *mecA* regulatory region (*mec* complex), the type of cassette chromosome recombinases (*ccr* genes), and the resistance determinants they have acquired due to the integration of plasmids and transposons.

Five SCC*mec* types have been identified globally. Types I, II and III are associated with "health-care-associated MRSA" while Types IV and V are normally associated with "community associated MRSA".

In this report MRSA are identified as either "epidemic" or "community" and are assigned an MLST/SCC*mec* type. The previous nomenclature applied to epidemic and community MRSA clones will also be reported.

Epidemiological Typing Methods

• Antibiogram

Breakpoint Agar Dilution (NCCLS) (1)

oxacillin (2mg/L) tetracycline (4mg/L), erythromycin (0.5mg/L), trimethoprim (8mg/L), gentamicin (4mg/L), ciprofloxacin (1mg/L), rifampicin (1mg/L), fusidic acid (1mg/L), mupirocin (1mg/L)

Resistance was defined as growth on the concentration tested; a fine haze was ignored for tetracycline and trimethoprim.

• Resistogram

Disk Diffusion (3, 4)

mercuric chloride (HgCl₂) (0.4µM) phenylmercuric acetate (PMA) (5 mM)

• Urea Slope (5)

Christensen's Urea slop incubated for 24hrs at 37°C.

• Coagulase Gene Typing

Coagulase gene restriction fragment length polymorphism typing was performed as previously described (6).

• Pulsed Field Gel Electrophoresis

Electrophoresis of chromosomal DNA was performed as previously described (7) using the CHEF DR III System (Bio-Rad Laboratories Pty Ltd). Chromosomal patterns were examined visually, scanned with a Fluor-S Multimager and digitally analysed using Multi-Analyst/PC (Bio-Rad Laboratories). PFGE patterns were grouped according to the criteria of Tenover *et al.* (8) and using a dendogram similarity of 80% or greater to assign strain relatedness. *S aureus* NCTC 8325 was used as the size marker (9).

• Chromosomal DNA Preparation

Chromosomal DNA for MLST and SCC*mec* typing was prepared using the DNeasy Tissue kit (Qiagen Pty Ltd, Clifton Hill, Victoria, Australia 3068).

• Multilococcus Sequence Typing (MLST

MLST was performed on selected isolates as specified by Enright *et al.* (2). The sequences obtained were compared with the sequences at the MLST web site at <u>http://www.mlst.net/</u>, to assign a sequence type (ST).

• Staphylococcal Chromosomal Cassette mec (SCCmec)

The staphylococcal cassette chromosome *mec* (SCC*mec*) was typed as previously reported (10) and also by using multiplex primers as described previously (11, 12).

Identification of Epidemic MRSA Clones

• ST239-MRSA-III (Aus-2 and Aus-3 EMRSA)

Antibiogram Resistogram Pulsed-field Gel Electrophoresis

• ST22-MRSA-IV (UK EMRSA-15)

Antibiogram Urea Slope Coagulase PCR/RFLP

• ST36-MRSA-II (UK EMRSA-16)

Antibiogram Urea Slope Coagulase PCR/RFLP Pulsed-field Gel Electrophoresis

• ST250-MRSA-I (Classic EMRSA)

Antibiogram Coagulase PCR/RFLP Pulsed-field Gel Electrophoresis Multilocus Sequence Typing SCC*mec* PCR

• Sporadic Multiresistant MRSA

Antibiogram Coagulase PCR/RFLP Pulsed-field Gel Electrophoresis Multilocus Sequence Typing SCC*mec* PCR

Identification of Community MRSA Clones

• ST30-MRSA-IV (WSPP)

Antibiogram Coagulase PCR/RFLP Pulsed-field Gel Electrophoresis

• "WA MRSA"

ST1-MRSA-IV (WA-1) ST129-MRSA-IV (WA-2) ST5-MRSA-IV (WA-3) ST45-MRSA-V (WA-4) ST75-MRSA-IV (WA-8) ST5-MRSA-V (WA-11)

> Antibiogram Coagulase PCR/RFLP Pulsed-field Gel Electrophoresis

• ST93-MRSA-IV (Queensland MRSA)

Antibiogram Coagulase PCR/RFLP Pulsed-field Gel Electrophoresis

- ST8 MRSA-IV (WA-12)
- ST8-MRSA-V
- STnovel-MRSA-IV
- STnovel-MRSA-novel

Antibiogram Coagulase PCR/RFLP Pulsed-field Gel Electrophoresis Multilocus Sequence Typing SCC*mec* PCR

RESULTS

In SAP 2003, 536 (24.5%) of *Staphylococcus aureus* were classified as MRSA. 526 MRSA were forwarded to the Gram-positive Typing and Research Unit for epidemiological typing

City	Epidemic MRSA (%)	Community MRSA (%)	Total
Canberra	10 (71.4)	4 (28.6)	14
Sydney	177 (90.8)	20 (9.2)	195
Darwin	10 (32.3)	21 (67.7)	31
Brisbane	48 (82.8)	10 (17.2)	58
Adelaide	45 (67.2)	22 (32.8)	67
Hobart	4 (80)	1 (20)	5
Melbourne	87 (90.6)	9 (9.4)	96
Perth	12 (20)	48 (80)	60
TOTAL	393 (74.7)	133 (25.3)	526

Proportion of Epidemic and Community MRSA from each City

Typing Tests Performed

Routine Antibiogram (8 antibiotics)	526
Coagulase Gene Polymerase Chain Reaction (PCR) Assay	195
Extended Antibiogram / Resistogram (23 antibiotics and chemicals)	340
Pulsed – Field Gel Electrophoresis (PFGE)	484
Urease Reaction	63
Multi Locus Sequencing Typing (MLST)	15
SCCmec PCR	15

	LAB	ST239-MRSA-III Aus 2 EMRSA	ST239-MRSA-III Aus 3 EMRSA	ST22-MRSA-IV UK EMRSA 15	ST36-MRSA-II UK EMRSA 16	ST250-MRSA-I Classic MRSA	STnovel- MRSA-III	TOTAL
ACT (10)	TCH	7	2	1				10
NSW (177)	СН	23		7				30
	HN	27		4				31
	RNSH	27	1	10				38
	SWAPS	36		9			1	42
	НМ	33		3				36
NT (10)	RDH	10						10
QLD (48)	PAH	8	1	1				10
	RBH	8						8
	NS	23	3	4				30
SA (45)	FMC	1	6	1				11
	IMVS	3	21	3				27
	WCH							0
	GP-SA		6	1				7
TAS (4)	RHH	3		1				4
VIC (87)	АН	9	39				1	49
	GP	2	4					6
	RCH	2		1				3
WA	SVH	3	26					29
Perth (12)	FH		1	2	1			4
	PC	1	1					2
	RPH	1		4		1		6
	SJOG							0
TOTAL		227	114	49	1	1	1	393

2003 Staphylococcus aureus Antimicrobial Programme (SAP 2003) – Epidemic MRSA

	LAB	STI MRSA IV (WA1)	ST129 MRSA IV (WA2)	ST5 MRSA IV (WA3)	ST45 MRSA V (WA4)	STnovel MRSA IV (WA8)	ST5 MRSA V (WA11)	ST8 MRSA IV (WA12)	ST93 MRSA IV (QLD)	ST30 MRSA IV (WSPP)	ST8 MRSA V	STnovel MRSA IV	STnovel MRSA novel	TOTAL
ACT (4)	TCH	1	1						2					4
NSW (20)	СН	3		1										4
	HN							1	6	2				6
	RNSH			1										1
	SWAPS	1							1					2
	HM									2				2
NT (21)	RDH	8	2	1		3				6	1			21
QLD (10)	PAH	2												2
	RBH	2							2	1				S
	NS	3												3
SA (22)	FMC	5			1									9
	IMVS	1	2	2								1		9
	WCH	3		1					1	1			1	7
	GP-SA			1					1		1			3
TAS (1)	RHH	1												1
VIC (9)	HY				1		1							2
	GP	1		1					1			1		4
	RCH								1	1				2
	HAS									1				1
WA (48)	FH	5	4		1					1				11
	PC	4	4	3										11
	RPH	11	2	2			2							17
	SJOG	9	2		1									6
TOTAL		57	17	13	4	3	3	1	15	15	2	2	1	133

2003 Staphylococcus aureus Antimicrobial Programme (SAP 203) – Community MRSA

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Epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA)

Certain strains of MRSA are known to spread easily between and within hospitals and are designated epidemic MRSA (EMRSA).

CLONE	ALTERNATIVE NAME	n (%)
ST239-MRSA-III	Aus -2 and Aus -3 EMRSA	341 (86.8%)
ST22-MRSA-IV	UK EMRSA-15	49 (12.5%)
ST36-MRSA-II	UK EMRSA-16	1 (<1%)
ST250-MRSA-I	Classic MRSA	1 (<1%)
STnovel-MRSA-III	Multiresistant MRSA	1 (<1%)
TOTAL		393

In SAP 2003 four international epidemic MRSA clones (393 isolates) were identified

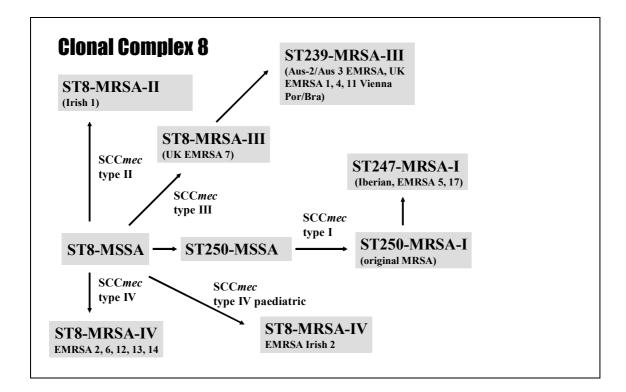
Percentage figures relate to the epidemic MRSA isolates

ST239-MRSA-III

In Australia ST239-MRSA-III has been classified into two subclones: Aus -2 and Aus- 3 EMRSA. This classification is based on the mercuric acetate and phenylmercuric chloride resistogram. ST239-MRSA-III has evolved from the "Eastern Australian EMRSA" clone described in the 1980s.

ST239-MRSA-III has emerged as one of the most commonly encountered and internationally disseminated multidrug-resistant EMRSA clones. It is also known as "UK EMRSA-1", the "Portuguese/Brazilian" clone or the "Vienna" clone. SCC*mec* type III is a health care associated SCC*mec* which has several transposons, integrated plasmids and other antibiotic resistance genes. Hence ST239-MRSA-III is typically resistant to multiple antibiotics including erythromycin, tetracycline, trimethoprim, ciprofloxacin and gentamicin.

ST239 belongs to clonal complex 8. Within this clonal complex there are three other major EMRSA clones: ST8-MRSA-II (Irish-1 EMRSA), ST8-MRSA-IV (UK EMRSA –2, –6, -12, -13 and -14) and ST247-MRSA-I (Iberian or UK EMRSA-17). The original MRSA clone ST250-MRSA-I, and ST8-MRSA-IV^{paediatric} (Irish-2 EMRSA) are also located within this clonal complex.

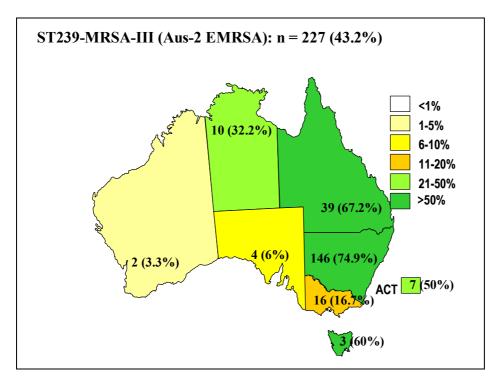


Phenotypic Characteristics

Antibiogram:	Aus-2 EMRSA (n = 227)	Aus-3 EMRSA (n = 114)
Erythromycin ^R Tetracycline ^R Trimethoprim ^R Gentamicin ^R	> 99% 96% 100% 97%	100% 97% > 99% 97%
Ciprofloxacin ^R Fusidic Acid ^R Rifampicin ^R Mupirocin ^R	98% < 1% < 1% 3%	> 99% < 1% 4% 0%
Resistogram: Mercuric Acetate ^R Mercuric Chloride ^R	< 1% < 1%	> 99% > 99%
Urease:	Positive	Positive

Epidemiology

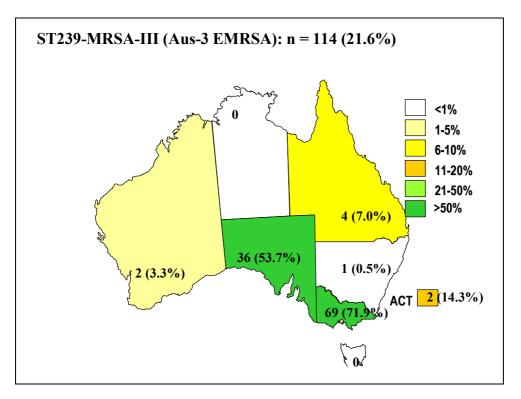
Aus-2 EMRSA



227 (43.2%) of MRSA isolated in SAP 2003 were characterised as Aus-2 EMRSA which accounted for 57.8% of EMRSA. Although reported in all Australian capital cities Aus-2 EMRSA was the dominate MRSA in Sydney, Brisbane, Hobart and Canberra. In the previous hospital/community *Staphylococcus aureus* survey, SAP 2001, 48.1% of MRSA (n=255) were characterised as Aus-2 EMRSA.

	SAP 2001	SAP 2003
Canberra	19 (82.6%)	7 (50%)
Sydney	149 (74.9%)	146 (74.9%)
Darwin	5 (31.2%)	10 (32.2%)
Brisbane	26 (57.8%)	39 (67.2%)
Adelaide	12 (14.6%)	4 (6%)
Hobart	10 (100%)	3 (60%)
Melbourne	30 (28.0%)	16 (16.7%)
Perth	4 (8.3%)	2 (3.3%)
Total	255 (48.1%)	227 (43.2%)

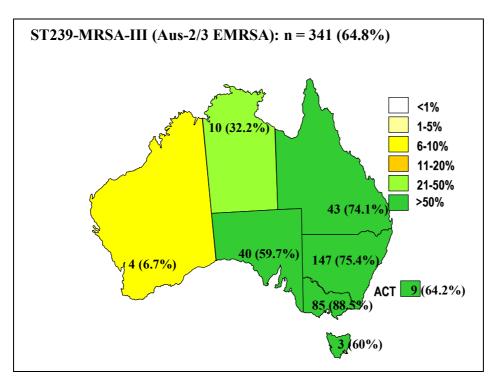
Aus-3 EMRSA



114 (21.6%) of MRSA isolated in SAP 2003 were characterised as Aus-3 EMRSA which accounted for 29.0% of EMRSA. Although reported in several Australian capital cities Aus-3 EMRSA was the dominant EMRSA clone isolated in Melbourne (71.9%) and Adelaide (53.7%). In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 20.9% of MRSA (n=111) were characterised as Aus-3 EMRSA.

	SAP 2001	SAP 2003
Canberra	0	2 (14.3%)
Sydney	6 (3%)	1 (0.5%)
Darwin	5 (31.2%)	0
Brisbane	5 (11.1%)	4 (7.0%)
Adelaide	35 (42.7%)	36 (53.7%)
Hobart	0	0
Melbourne	59 (55.1%)	69 (71.9%)
Perth	1 (2.1%)	2 (3.3%)
Total	111 (20.9%)	114 (21.6%)

341 (64.8%) of MRSA were characterised as either Aus-2 or Aus-3 which accounted for more than 86% of EMRSA. This clone however is infrequently isolated on the western coast.



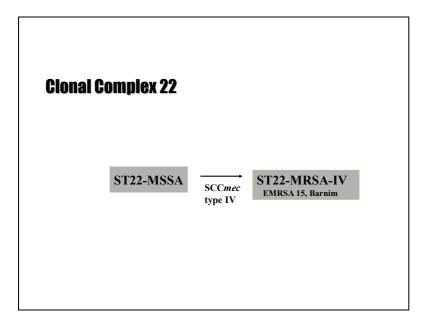
In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 69.1% of MRSA (n=266) were characterised as ST239-MRSA-III.

	SAP 2001	SAP 2003
Canberra	19 (82.6%)	9 (64.2%)
Sydney	155 (77.9%)	147 (75.4%)
Darwin	10 (62.5%)	10 (32.2%)
Brisbane	31 (68.9%)	43 (74.1%)
Adelaide	47 (57.3%)	40 (59.7%)
Hobart	10 (100%)	3 (60%)
Melbourne	89 (83.2%)	85 (88.5%)
Perth	5 (10.4%)	4 (6.7%)
Total	366 (69.1%)	341 (64.8%)

ST22-MRSA-IV

Also known as "UK EMRSA-15" or the "German Barnim" strain, ST22-MRSA-IV has become a major epidemic MRSA clone in many parts of the world including Australia, United Kingdom, New Zealand and several European countries. First identified in the Midlands and South-East England in the early 1990s it accounts for over half of UK isolates sent to the Laboratory of Hospital Infection in Colindale for typing. It is non multiresistant (typically resistant to ciprofloxacin and erythromycin only) and is staphylococcal enterotoxin C, G and I positive. In New Zealand and Australia ST22-MRSA-IV is frequently isolated from patients in long term care facilities and is associated with pre employment screening of health staff from the United Kingdom.

ST22 belongs to clonal complex 22. Although it is considered to be a hospital associated MRSA it has acquired the type IV community SCC*mec* which lacks transposons, integrated plasmids and other antibiotic resisance genes. Clonal complex 22 has a single epidemic clone that is believed to have evolved from ST22 MSSA



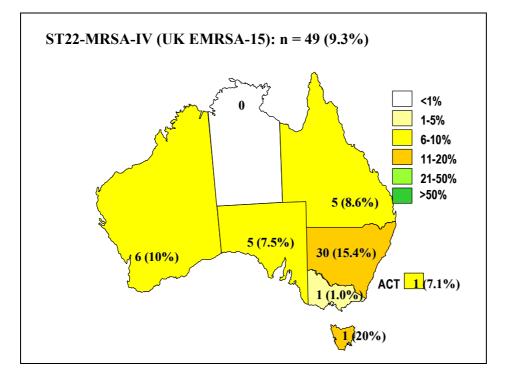
Phenotypic Characteristics

Antibiogram:	Erythromycin ^R (69%) Ciprofloxacin ^R (100%)
	Fusidic Acid ^R (0%) Tetracycline ^R (0%) Trimethoprim ^R (0%) Rifampicin ^R (0%) Mupirocin ^R (0%) Gentamicin ^R (0%)

Urease:

Negative

Epidemiology



49 (9.3%) of MRSA isolated in SAP 2003 were characterised as ST22-MRSA-IV which accounted for 12.5% of EMRSA. Although reported in most Australian capital cities this clone was predominantly isolated in Sydney and Perth (15.4% and 10% of MRSA respectively).

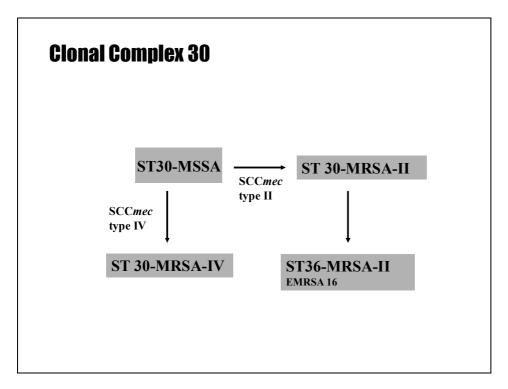
In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 6.8% (n=36) of MRSA were characterised as ST22-MRSA-IV.

	SAP 2001	SAP 2003
Canberra	0	1 (7.1%)
Sydney	30 (15.1%)	30 (15.4%)
Darwin	0	0
Brisbane	0	5 (8.6%)
Adelaide	1 (1.2%)	5 (7.5%)
Hobart	0	1 (20%)
Melbourne	0	1 (1.0%)
Perth	5 (11.1%)	6 (10%)
Total	36 (6.8%)	48 (9.3%)

ST36-MRSA-11

Also known as "UK EMRSA-16", ST36-MRSA-II was first identified in a single hospital outbreak in London in 1991-2. It now accounts for almost a quarter of UK isolates sent to the Laboratory of Hospital Infection in Colindale for typing. ST36-MRSA-II has been isolated in several European countries including Denmark, Finland, Sweden and Turkey, and in the USA. ST36-MRSA-II is resistant to ciprofloxacin, erythromycin and variably resistant to the aminoglycosides. It carries staphylococcal enterotoxin A, G and I and TSST-1.

ST36 belongs to clonal complex 30 and is thought to have evolved from ST30-MRSA-II. ST36-MRSA-II is the only EMRSA in this complex. *SCCmec* type II is a health care associated SCC*mec* which carries *aadD*, the gene for tobramycin and kanamycin resistance, Tn554 (erythromycin resistance-encoding transposon) and *ermA*.



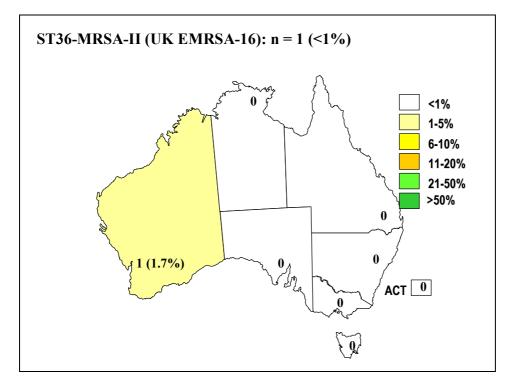
Phenotypic Characteristics

Antibiogram:	Erythromycin ^R (100%) Ciprofloxacin ^R (100%)
	Fusidic Acid ^R (0%) Tetracycline ^R (0%) Trimethoprim ^R (0%) Rifampicin ^R (0%) Mupirocin ^R (0%) Gentamicin ^R (0%)

Urease:

Positive

Epidemiology

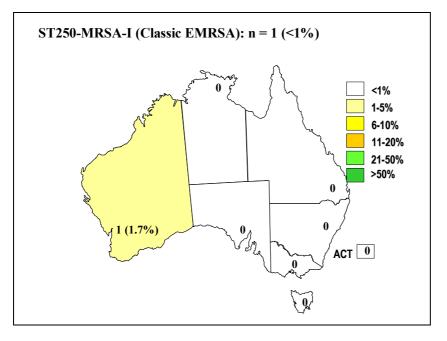


Only one isolate of ST36-MRSA-II was isolated in SAP 2003. In SAP 2001 two isolates of ST-MRSA-II were reported.

	SAP 2001	SAP 2003
Canberra	0	0
Sydney	2 (1%)	0
Darwin	0	0
Brisbane	0	0
Adelaide	0	0
Hobart	0	0
Melbourne	0	0
Perth	0	1 (1.7%)
Total	2 (<1%)	1 (<1%)

ST250-MRSA-1

ST250-MRSA-I is the original MRSA clone reported in 1961 and forms part of clonal complex 8. ST250 is thought to have evolved from the very successful MSSA lineage ST8-MSSA. Although SCC*mec* type I is considered a health acquired *SCCmec* it is smaller than SCC*mec* types II and III and lacks other antibiotic resistance genes. Subsequently ST250-MRSA-I is typically non-multiresistant. Although ST250-MRSA-I is now rarely isolated, ST247-MRSA-I (known as UK EMRSA-17 or the Iberian clone) has become a major global EMRSA clone.



One isolate of ST250-MRSA-I was isolated in Western Australia in SAP 2003.

	SAP 2001	SAP 2003
Canberra	0	0
Sydney	0	0
Darwin	0	0
Brisbane	0	0
Adelaide	0	0
Hobart	0	0
Melbourne	0	0
Perth	0	1 (1.7%)
Total	0	1 (<1%)

Community Methicillin-resistant *Staphylococcus aureus*)

Community MRSA were first reported in Australia in the early 1980s in aboriginal communities living in the Kimberley region of Western Australia (WA). Known collectively as "WA MRSA" they were subsequently isolated in other remote communities in WA, South Australia and Northern Territory. In SAP 2000 "WA MRSA" were identified in most Australian capital cities. These strains are usually susceptible to most non- β -lactams antibiotics. "WA MRSA" have acquired the community associated *SCCmec* types IV and V, which lack transposons, integrated plasmids and other antibiotic resistance genes. Although they have been introduced into teaching hospitals they rarely cause outbreaks. In the 1990s non-multiresistant MRSA were isolated on the eastern seaboard in suburban/regional areas of south east Queensland, Sydney and Canberra. They were frequently isolated in people of Pacific Island descent and were subsequently identified as "Western Samoan Phage Pattern MRSA" (WSPP MRSA). WSPP MRSA have previously been reported in New Zealand and several Pacific islands. Although these strains initially caused skin infections they have now been associated with serious invasive disease.

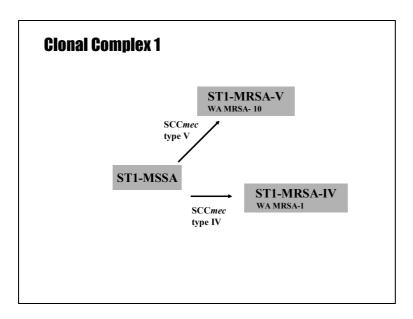
CLONE	ALTERNATIVE NAME	n (%)
ST1-MRSA-IV	WA-1 MRSA	57 (42.9%)
ST129-MRSA-IV	WA-2 MRSA	17 (12.8%)
ST5-MRSA-IV	WA-3 MRSA	13 (9.8%)
ST45-MRSA-V	WA-4 MRSA	4 (3.0%)
ST93-MRSA-IV	Queensland MRSA	15 (11.3%)
ST75-MRSA-IV	WA-8 MRSA	3 (2.2%)
ST5-MRSA-V	WA-11 MRSA	3 (2.2%)
ST8-MRSA-IV	WA-12 MRSA	1 (0.8%)
ST30-MRSA-IV	WSSP MRSA	15 (11.3%)
ST8-MRSA-V		2 (1.5%)
STnovel-MRSA-IV		1 (0.8%)
STnovel-MRSA-IV		1 (0.8%)
STnovel-MRSA-novel		1 (0.8%)
TOTAL		133

In SAP 2003 thirteen community MRSA clones (133 isolates) were identified

Percentage figures relate to the epidemic MRSA isolates

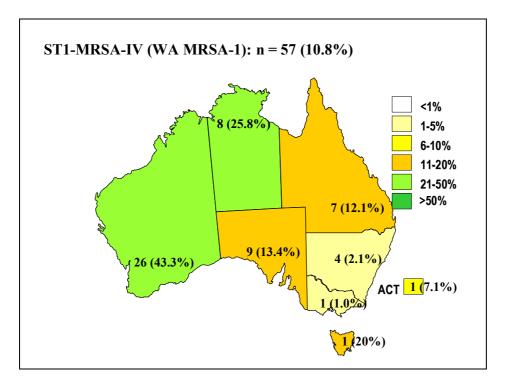
ST1-MRSA-IV

Also known as "WA MRSA-1", ST1-MRSA-IV forms part of clonal complex 1. Within this complex two community MRSA have been identified having acquired either SCC*mec* IV or V. ST1-MRSA-IV has been reported in several European countries and in the USA.



Epidemiology

ST1-MRSA-IV is the most frequently isolated community MRSA in Australia. 57 (10.8%) of MRSA isolated in SAP 2003 were characterised as ST1-MRSA-IV which accounted for 42.9% of community MRSA.



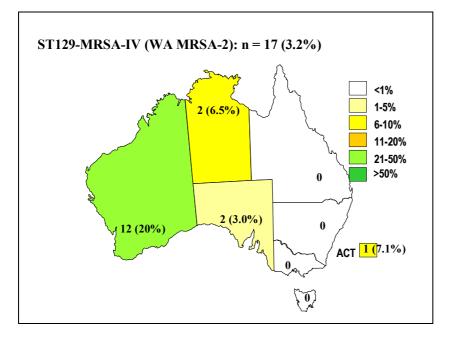
Although reported in all Australian capital cities this clone was predominantly isolated in Perth and Darwin (43.3% and 25.8% of MRSA respectively).

In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 7.5% (n=40) of MRSA were characterised as ST1-MRSA-IV.

	SAP 2001	SAP 2003
Canberra	0	1 (7.1%)
Sydney	1 (0.5%)	4 (2.1%)
Darwin	0	8 (25.8%)
Brisbane	6 (13.3%)	7 (12.1%)
Adelaide	11 (13.4%)	9 (13.4%)
Hobart	0	1 (20%)
Melbourne	0	1 (1.0%)
Perth	22 (45.8%)	26 (43.3%)
Total	40 (7.5%)	57 (10.8%)

ST129-MRSA-IV

Also known as "WA MRSA-2", ST129-MRSA-IV forms part of clonal complex 298. Within this complex several community MRSA clones have been identified including ST78, ST255 and ST257. Clonal complex 298 is a small clonal complex that includes strains from Australia, Portugal and Japan.



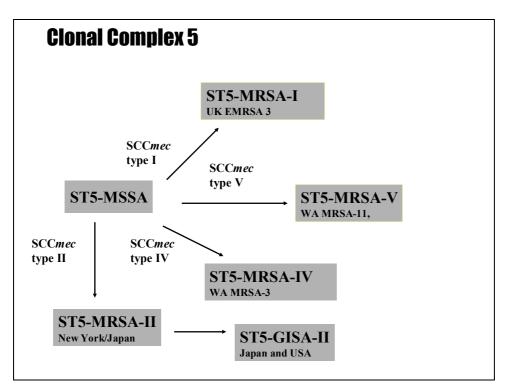
17 (3.2%) of MRSA isolated in SAP 2003 were characterised as ST129-MRSA-IV which accounted for 12.8% of community MRSA. ST129-MRSA-IV was predominately isolated in Perth (12% of MRSA). In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 1.7% (n=9) of MRSA were characterised as ST129-MRSA-IV.

	SAP 2001	SAP 2003
Canberra	0	1 (7.1%)
Sydney	0	0
Darwin	0	2 (6.5%)
Brisbane	1 (2.2%)	0
Adelaide	0	2 (3.0%)
Hobart	0	0
Melbourne	0	0
Perth	8 (16.7%)	12 (20%)
Total	9 (1.7%)	17 (3.2%)

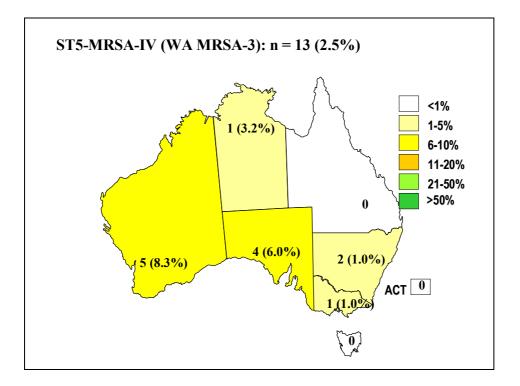
ST5-MRSA-IV

Also known as "WA MRSA-3", ST5-MRSA-IV forms part of clonal complex 5.

This clonal complex has two community MRSA clones, ST5-MRSA-IV and ST5-MRSA-V, and two epidemic MRSA clones, ST-MRSA-II, also known as the "New York/Japan EMRSA", and ST-MRSA-I also known as "UK EMRSA-3". The original hVISA, ST5-GISA-II, is thought to have evolved from the New York/Japan EMRSA clone.



13 (2.5%) of MRSA isolated in SAP 2003 were characterised as ST5-MRSA-IV which accounted for 9.8% of community MRSA. ST5-MRSA-IV was predominately isolated in Perth and Adelaide (8.3% and 6.0% of MRSA respectively).

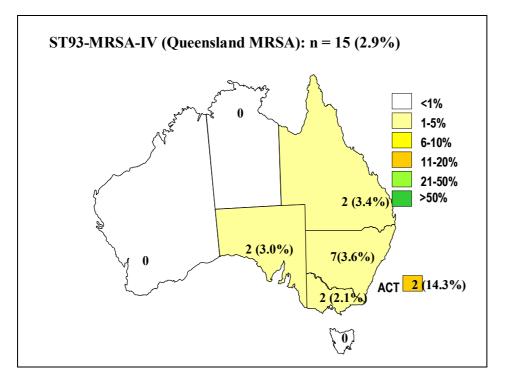


In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 1.5% (n=8) of MRSA were characterised as ST5-MRSA-IV.

	SAP 2001	SAP 2003
Canberra	0	0
Sydney	2 (1.0%)	2 (1.0%)
Darwin	0	1 (3.2%)
Brisbane	0	0
Adelaide	3 (1.9%)	4 (6.0%)
Hobart	0	0
Melbourne	2 (1.9%)	1 (1.0%)
Perth	1 (2.1%)	5 (8.3%)
Total	8 (1.5%)	13 (2.5%)

ST93-MRSA-IV

Also known as the "Queensland MRSA" clone, ST93-MRSA-is a singleton (does not form part of a clonal complex) first reported in Queensland (13). ST93-MRSA-IV is Panton-Valentine leukocidin (PVL) positive, a toxin that has been associated with virulence and community MRSA isolated outside Australia.



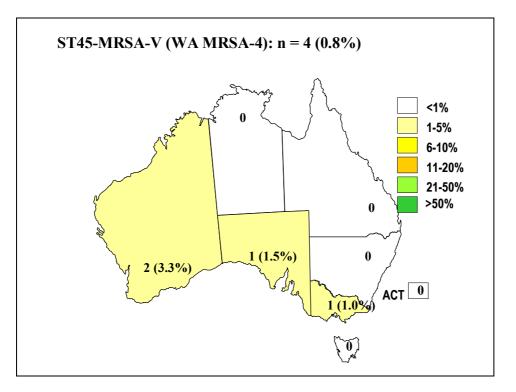
15 (2.9%) of MRSA isolated in SAP 2003 were characterised as ST93-MRSA-IV which accounted for 11.3% of community MRSA. ST93-MRSA-IV was predominantly isolated on the eastern seaboard particularly in Canberra, Sydney, and Brisbane.

In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 0.6% (n=3) of MRSA were characterised as ST93-MRSA-IV.

	SAP 2001	SAP 2003
Canberra	0	2 (14.3%)
Sydney	2 (1.0%)	7 (3.6%)
Darwin	0	0
Brisbane	0	2 (3.4%)
Adelaide	1 (1.2%)	2 (3.0%)
Hobart	0	0
Melbourne	0	2 (2.1%)
Perth	0	0
Total	3 (0.6%)	15 (2.9%)

ST45-MRSA-V

Also known as "WA MRSA-4", ST45-MRSA-V is a singleton (ie does not form part of a complex clone) and has acquired community SCC*mec* type V. SCC*mec* V lacks transposons, integrated plasmids and other antibiotic resistance genes and therefore strains are characteristically non-multiresistant.



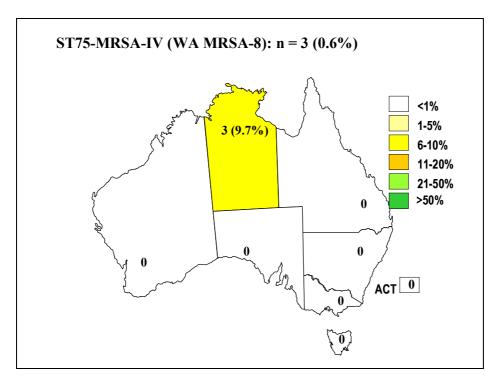
4 (0.8%) of MRSA isolated in SAP 2003 were characterised as ST45-MRSA-V which accounted for 3.0% of community MRSA. Strains of ST45-MRSA-V were only isolated in Perth and Adelaide.

In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 0.6% (n=3) of MRSA were characterised as ST45-MRSA-V.

	SAP 2001	SAP 2003
Canberra	1	0
Sydney	0	0
Darwin	0 (4.3%)	0
Brisbane	0	0
Adelaide	1 (1.2%)	1 (1.5%)
Hobart	0	0
Melbourne	0	1 (1%)
Perth	1 (2.1%)	2 (3.3%)
Total	3 (0.6%)	4 (0.8)

ST75-MRSA-IV

Also known as "WA MRSA-8", ST75-MRSA-IV is a singleton (ie does not form part of a complex clone) with a novel sequence type.

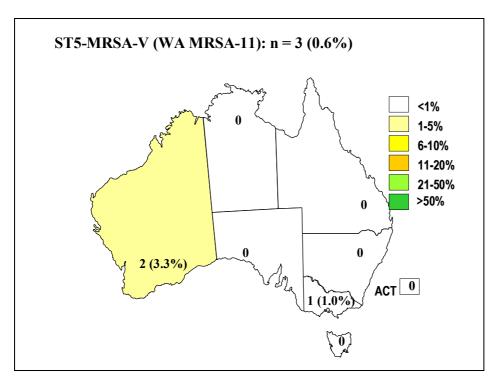


3 (0.6%) of MRSA isolated in SAP 2003 were characterised as ST75-MRSA-IV which accounted for 2.3% of community MRSA ST75-MRSA-IV were only isolated in Darwin. In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 0.8% (n=4) of MRSA were characterised as ST75-MRSA-IV.

	SAP 2001	SAP 2003
Canberra	0	0
Sydney	0	0
Darwin	3 (18.8%)	3 (9.7%)
Brisbane	0	0
Adelaide	0	0
Hobart	0	0
Melbourne	0	0
Perth	1 (2.1%)	0
Total	4 (0.8%)	3 (0.6%)

ST5-MRSA-V

Also known as "WA MRSA-11" ST5-MRSA-V forms part of clonal complex 5. This clone has acquired community SCC*mec* V.

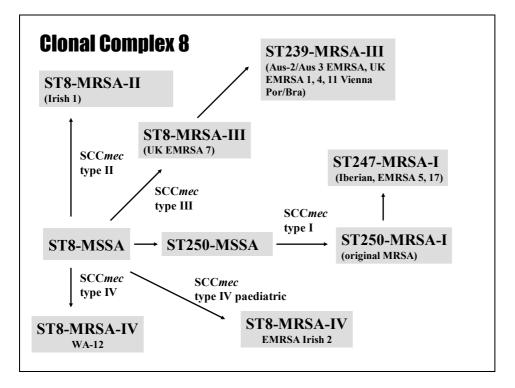


3 (0.6%) of MRSA isolated in SAP 2003 were characterised as ST5-MRSA-V which accounted for 2.3% of community MRSA. ST5-MRSA-V was only isolated in Perth and Melbourne. ST5-MRSA-V was not isolated in the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001.

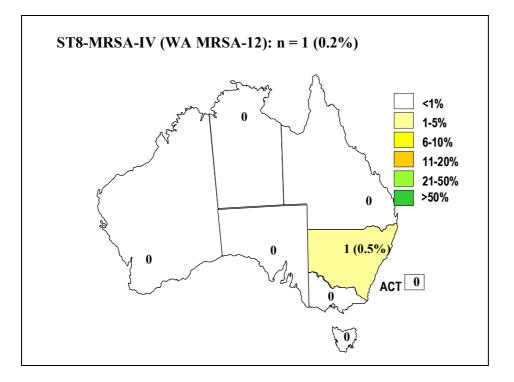
	SAP 2001	SAP 2003
Canberra	0	0
Sydney	0	0
Darwin	0	0
Brisbane	0	0
Adelaide	0	0
Hobart	0	0
Melbourne	0	1 (1.0%)
Perth	0	2 (3.3%)
Total	0	3 (0.6%)

ST8-MRSA-IV

Also known as "WA MRSA-12", ST8-MRSA-IV forms part of clonal complex 8.



1 (0.2%) MRSA isolated in SAP 2003 was characterised as ST8-MRSA-IV which accounted for 0.8% of community MRSA. This strain was isolated in Sydney.



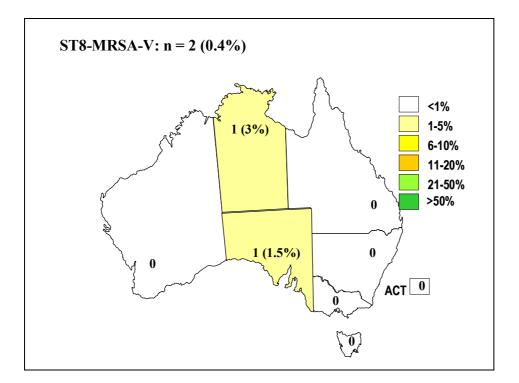
	SAP 2001	SAP 2003
Canberra	1 (4.3%)	0
Sydney	0	1 (0.5%)
Darwin	0	0
Brisbane	0	0
Adelaide	1 (1.2%)	0
Hobart	0	0
Melbourne	0	0
Perth	0	0
Total	2 (0.4%)	1 (0.2%)

In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 0.8% (n=2) of MRSA were characterised as ST8-MRSA-IV.

ST8-MRSA-V

ST8-MRSA-V forms part of clonal complex 8.

2 (0.4%) of MRSA isolated in SAP 2003 were characterised as ST8-MRSA-V which accounted for 1.5% of community MRSA. Strains of ST8-MRSA-V were isolated in Darwin and Adelaide.

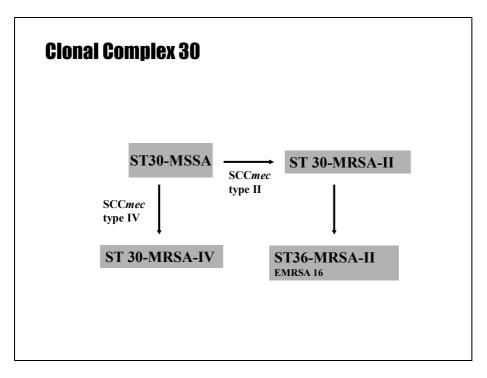


ST8-MRSA-V was not isolated in the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001.

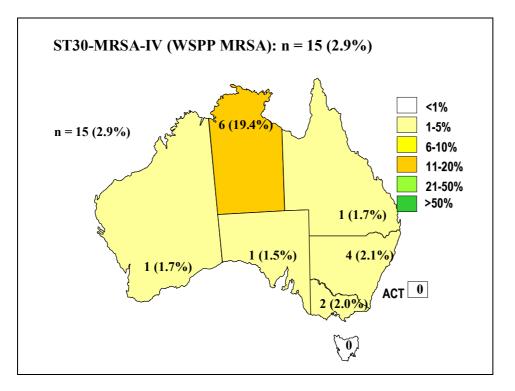
	SAP 2001	SAP 2003
Canberra	0	0
Sydney	0	0
Darwin	0	1 (3%)
Brisbane	0	0
Adelaide	0	1 (1.5%)
Hobart	0	0
Melbourne	0	0
Perth	0	0
Total	0	2 (0.4%)

ST30-MRSA-IV

Also known as "WSPP MRSA", ST30-MRSA-IV forms part of clonal complex 30 which includes ST36-MRSA-II (UK EMRSA-16). ST30-MRSA-IV originally described in Polynesians living in New Zealand and the Pacific islands and is PVL toxin positive.



15 (2.9%) of MRSA isolated in SAP 2003 were characterised as ST30-MRSA-IV accounting for 11.3% of community MRSA. Strains of ST30-MRSA-IV were isolated throughout Australia.



	SAP 2001	SAP 2003
Canberra	1 (4.3%)	0
Sydney	3 (1.5%)	4 (2.1%)
Darwin	2 (12.5%)	6 (19.4%)
Brisbane	3 (6.7%)	1 (1.7%)
Adelaide	2 (2.4%)	1 (1.5%)
Hobart	0	0
Melbourne	3 (2.8%)	2 (2.0%)
Perth	0	1 (1.7%)
Total	14 ((2.6%)	15 (2.9%)

In the previous hospital/community *Staphylococcus aureus* Survey, SAP 2001, 2.6% (n=14) of MRSA were characterised as ST30-MRSA-IV.

Novel Community MRSA

STnovel-MRSA-IV

Single strain isolated in Adelaide.

STnovel-MRSA-IV

Single strain isolated in Melbourne.

STnovel-MRSA-novel

Single strain isolated in Adelaide.

DISCUSSION

From the AGAR SAP 2003, 526 MRSA were forwarded to the Gram-positive Bacteria Typing and Research Unit for epidemiological typing.

393 (74.7%) MRSA were classified as EMRSA. Using the international nomenclature, MLST/SCC*mec*, 392 of these strains could be classified into four international clones:

ST239-MRSA-III (previously known as Aus-2/3 EMRSA, UK EMRSA-1, Portuguese/Brazilian clone or the Vienna clone)

ST22-MRSA-IV (previously known as UK EMRSA-15 or the German Barnim strain)

ST36-MRSA-II (previously known as UK EMRSA-16)

ST250-MRSA-I (original MRSA clone)

Although ST250-MRSA-I is now rarely reported internationally, the other three EMRSA clones are classified as major international epidemic clones and have been identified in many countries.

ST239-MRSA-III, a multiresistant MRSA, was the major EMRSA isolated in Australian hospitals. In SAP 2003, 64.8% of MRSA and 86.8% of EMRSA were identified as ST239-MRSA-III. Although ranging from 32.2% to 88.5% of MRSA in the central and east coasts of Australia, only 6.7% of MRSA isolated in Western Australia were identified as ST239-MRSA-III

In Australia, based on their susceptibility to mercuric chloride and phenylmercuric acetate, ST239-MRSA-III has been classified into two subclones, Aus-2 EMRSA and Aus-3 EMRSA. Aus-2 EMRSA is predominantly isolated in Brisbane, Sydney, Canberra, Hobart and Darwin, while Aus-3 EMRSA is the predominant clone in Melbourne and Adelaide.

ST22-MRSA-IV an international clone of non multiresistant MRSA associated with hospital infection, was first documented in Australia in 1997 in Perth where it was detected in pre-employment screening of healthcare workers coming from the United Kingdom, Ireland and eastern Australia (14) From SAP 2003, it has become apparent that this clone has become established in most cities throughout Australia. Overall 9.3% of MRSA and 12.5% of EMRSA were identified as ST22-MRSA-IV. Although not isolated in the Northern Territory, 1 - 20% of MRSA isolated throughout Australia were ST22-MRSA-IV.

ST36-MRSA-III is a major EMRSA isolated in the United Kingdom. Although only a single strain was reported in SAP 2003, this clone has been shown to spread rapidly in the hospital environment.

In SAP 2003, 133 (25.3%) MRSA were classified as community MRSA. Epidemiological typing has shown these strains have emerged from diverse genetic backgrounds. Overall the thirteen different clones identified could be grouped either as singletons or into 5 clonal complexes. Both community SCC*mec* types IV and V and a novel SCC*mec* were detected.

Clonal Complex 1 ST1-MRSA-IV (WA MRSA-1) Clonal Complex 8 ST8-MRSA-IV (WA MRSA-12) ST8-MRSA-V Clonal Complex 5

ST5-MRSA-IV (WA MRSA-3) ST5-MRSA-V (WA MRSA-11)

Clonal Complex 30 ST30-MRSA-IV (WSPP MRSA)

Clonal Complex 298 ST129-MRSA-IV (WA MRSA-2)

Singletons

ST93-MRSA-IV (Queensland MRSA) ST45-MRSA-V (WA MRSA-4) ST75-MRSA-IV (WA MRSA-8) STnovel-MRSA-IV STnovel-MRSA-IV STnovel-MRSA-novel

88.2% of community MRSA can be classified into five major community clones:

ST1-MRSA-IV (42.9%) Isolated throughout Australia ranging from 1.0% in Melbourne to 43.3% in Perth.

ST129-MRSA-IV (12.8%) Isolated primarily in the central and western coasts of Australia ranging from 3.0% in Adelaide to 20% in Perth.

ST93-MRSA-IV (11.3%) Isolated on the eastern coast of Australia ran

Isolated on the eastern coast of Australia ranging from 2.1% in Melbourne to 14.3% in Canberra.

ST30-MRSA-IV (11.3%)

Isolated in most cities in Australia ranging from 1.5% in Adelaide to 19.4% in Darwin

ST5-MRSA-IV (9.8%)

Isolated in most cities in Australia ranging from 1.0% in Sydney to 8.3% in Perth

Although MRSA in most Australian cities are EMRSA, this study has shown several "WA MRSA" clones have spread throughout the country. Furthermore, non "WA MRSA" community clones have now emerged. ST93-MRSA-IV originally reported in Queensland and New South Wales has become the major community MRSA clone isolated in Sydney. Unlike the "WA MRSA" clones, ST93-MRSA-IV is PVL toxin positive.

Another non "WA MRSA" clone, ST30-MRSA-IV (also known as WSPP MRSA) has also become a prominent community MRSA in several Australian cities including Darwin (19.4% of MRSA). ST30-MRSA-IV is also PVL toxin positive.

In addition several 'sporadic' community MRSA were reported in several Australian cities including:

ST75-MRSA-IV (Darwin) ST8-MRSA-V (Darwin and Adelaide) STnovel-MRSA-IV (Adelaide) STnovel-MRSA-IV (Melbourne) STnovel-MRSA-novel (Adelaide)

The presence of SCC*mec* types IV and V and a novel SCC*mec* in multiple clones also supports the diverse genetic background of community MRSA isolated in Australia.

The ability of some of these clones to spread widely is cause for public health concern and may require modification of guidelines for treatment and control of community-acquired infection due to *S aureus*.

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Gram-positive Bacteria Typing and Research Unit

Curtin University, WA Royal Perth Hospital, WA Phu Huynh Mary Malkowski and Rebecca Lee