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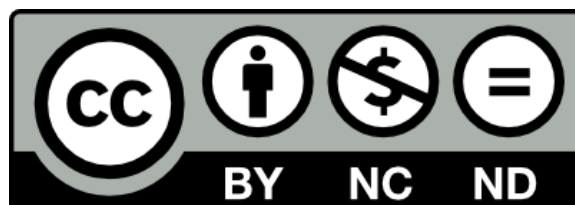
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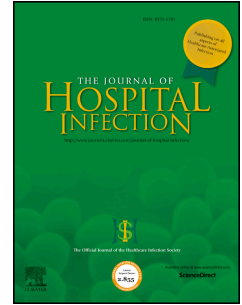


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Differing epidemiology of two major healthcare-associated meticillin-resistant *Staphylococcus aureus* clones

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SUMMARY

Background: Two methicillin-resistant *Staphylococcus aureus* (MRSA) clones, sequence type (ST) 22 and ST239, have successfully spread globally. Across Australia, ST22 has supplanted ST239 as the main healthcare-associated MRSA. To understand the reasons underlying this shift, the epidemiology and clinical features of infections due to ST22 and ST239 MRSA isolates from a tertiary hospital in Melbourne, Australia were compared

Methods: Over six months, consecutive MRSA isolates with clinical data were collected from specimens referred to Alfred Health Pathology (AHP). Isolates were genotyped by a multi-locus-sequence-typing-based high-resolution melting method.

Findings: Three hundred and twenty-eight of 1079 (30%) *S. aureus* isolated by AHP were MRSA. Of these, 313 were genotyped; 78 (25%) were clonal complex (CC) 22 (representing ST22) and 142 (45%) were CC239 (representing ST239). Common clinical syndromes included skin or soft tissue, respiratory tract and osteo-articular infections. On multi-variate logistic regression, compared with CC239, CC22 was associated with older patients [adjusted odds ratio (aOR) 1.04 for each year increase, 95% confidence interval (CI) 1.02–1.07], from subacute hospitals (aOR 2.7, 95% CI 1.2–5.8) or long-term care facilities (LTCFs; aOR 5.5, 95% CI 2.0–14.5). Median time from patient admission to MRSA isolation was nine days for CC239 and one day for CC22 ($P < 0.01$). MRSA strain epidemiology varied according to hospital unit.

Conclusions: CC22 and CC239 MRSA have differing ecological niches. CC22 is associated with elderly patients in LTCFs, and CC239 is associated with nosocomial acquisition. Infection control strategies involving LTCFs and their residents will likely be required to achieve continued MRSA control.

Keywords:

MRSA

Epidemiology

Strains

Healthcare-associated

Genotype

ST22

ST239

ACCEPTED MANUSCRIPT

<A>Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) first emerged in healthcare settings in the 1960s, and has subsequently spread through hospitals worldwide.¹ The acquisition of antimicrobial resistance provides a selective advantage in the nosocomial environment, and has complicated treatment regimens significantly. Today, MRSA is a major cause of morbidity and mortality in hospitals and the community.²⁻⁷

Circulating MRSA clones vary between hospital and community settings. A small number of MRSA clones have dominated globally in hospital settings, and progressive waves of different clones have occurred over time.^{8,9} Currently, sequence type (ST) 22 (EMRSA-15) has been growing in importance in the UK, Europe, South-East Asia (i.e. Singapore) and Australia, and is replacing other MRSA clones (ST36 or EMRSA-16 in the UK, ST239 in Singapore and Australia).¹⁰⁻¹³

This study was performed to determine the relative prevalence of the healthcare-associated MRSA (HA-MRSA) clones ST22 and ST239 in a tertiary referral centre and affiliated hospitals in Melbourne, Australia. Clinical features were compared and differences were identified between these two clones to further our epidemiological understanding of why ST22 is increasingly prevalent.

<A>Methods**Setting**

Alfred Health Pathology (AHP) services the three hospitals of Alfred Health (The Alfred Hospital, Caulfield Hospital and Sandringham Hospital), all located in the inner south-east of Melbourne, with a total of approximately 580 acute inpatient beds and 220 subacute beds. The Alfred Hospital is a tertiary referral centre, while Caulfield and Sandringham Hospitals are smaller healthcare facilities with a large number of rehabilitation and long-term care facility (LTCF) beds (including aged care facility beds). Consecutive MRSA isolates were collected from clinical specimens referred to AHP between 1 July and 31 December 2010. Repeat isolates from a patient with the

same antibiogram within 30 days were excluded. Samples collected for screening purposes were not included in the study.

Microbiology and typing

Isolates resembling Gram-positive cocci that were latex agglutination positive (Pastorex Staph-Plus, Bio-Rad, Hercules, CA, USA) were confirmed as *S. aureus* by the Vitek 2 Gram-positive identification card (bioMérieux, Marcy-l'Étoile, France). A DNase plate was used to confirm isolate identification as *S. aureus* if latex agglutination and Vitek 2 gave discordant results. Meticillin resistance was identified by cefoxitin disc diffusion (using the breakpoints of the Clinical and Laboratory Standards Institute) and the Vitek 2 AST-P612 Gram-positive susceptibility card. Evaluation for penicillin-binding protein (PBP2') by the Thermo Scientific Oxoid PBP2' Latex Agglutination Test (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to delineate discordant Vitek 2 and cefoxitin susceptibility results. Susceptibility to other antimicrobials was performed by the Vitek 2 AST-P612 Gram-positive susceptibility card (see Table A, online supplementary material). MRSA isolates resistant to at least three non-beta-lactam antibiotics in different antibiotic classes were defined as multi-resistant MRSA (lincosamides and macrolides were considered a single antibiotic class); all other isolates were non-multi-resistant MRSA.¹⁴

Isolates were typed using a multi-locus sequence typing (MLST)-based high-resolution melting scheme that provides inferred MLST clonal complexes (CC), as described previously.¹⁵ Isolates typed as CC22 and CC239 have previously been determined to represent ST22 and ST239 accurately in this context.¹⁵ It was confirmed that the antibiograms of those typed as CC22 or CC239 were consistent with the typical antibiograms of known ST22 and ST239.¹³

Clinical details and definitions

Demographic and clinical data were collected on all patients by chart review. For the purposes of this study, patients occupying LTCF beds at Alfred Health were considered as community LTCF

residents rather than Alfred Health inpatients. MRSA infections were defined as healthcare-associated if any of the following criteria were met:¹⁶ (a) discharge from a healthcare facility within the previous 30 days; (b) resident of a LTCF; (c) current haemodialysis, day oncology, home nursing or hospital in the home patient; or (d) if MRSA was isolated from a specimen collected >48 h after hospital admission. All other infections were considered to be community-associated. Healthcare-associated infections were further divided into nosocomial and non-nosocomial. Nosocomial healthcare-associated infections represented MRSA acquired in the hospital setting (i.e. history of acute hospital admission within the last 30 days, or MRSA isolation >48 h after current hospital admission) and non-nosocomial healthcare-associated infections represented all other healthcare-associated infections. An implant-related infection was assumed if the isolate was recovered from a site directly involving a foreign body (e.g. intravascular catheter, indwelling urethral catheter, orthopaedic fixation device). For the purposes of this study, isolates for which a clinical syndrome was documented and specific treatment was provided were deemed to be clinically significant. Isolates that were not treated were deemed to be clinically non-significant.

Epidemiology

Temporal trends of CC239 and CC22 MRSA epidemiology at hospital (Alfred Health), regional (Victoria and Tasmania) and national (Australia) levels were assessed by collating the 2003–2011 MRSA typing results from the Australian Group on Antimicrobial Resistance hospital-onset *S. aureus* programmes.^{13,17–20} The proportions of MRSA among *S. aureus* sterile site isolates at AHP in 2010 and 2014 were compared to evaluate temporal trends in meticillin-susceptible *S. aureus*/MRSA epidemiology.

Ethics and statistics

Ethical approval for this study was granted by the Alfred Health Ethics Committee (Project No.: 25/13).

Statistical analysis was performed using Stata Version 12 (StataCorp, College Station, TX, USA) and Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Categorical data were compared using Chi-squared or Fisher's exact tests, and continuous data were compared using Student's *t*-test or Mann-Whitney *U* test, as appropriate, with $P < 0.05$ used to determine statistical significance. Logistic regression analysis was performed to determine independent associations with CC22 or CC239. Variables that were significant at a 0.10 level on bivariate comparison were included in the initial model, with backwards-stepwise removal of variables at a 0.05 level using the likelihood ratio test.

<A>Results

Antibiotic susceptibility and typing

Of 1079 *S. aureus* isolated by AHP, 328 (30%) were identified as MRSA, and 313 (95%) were genotyped (15 isolates were unable to be typed on repeat attempts). Seventy percent of MRSA were characterized as either CC22 (78 isolates, 25% of MRSA) or CC239 (142 isolates, 45% of MRSA; Figure 1).

<insert Figure 1 near here>

The antibiogram for the 78 CC22 isolates was typical for ST22 EMRSA-15, with all isolates resistant to ciprofloxacin and 71% of isolates resistant to erythromycin. Almost all CC239 isolates were multi-resistant (139/142, 98%), typically to tetracycline, erythromycin, co-trimoxazole, gentamicin and ciprofloxacin (see Table B, online supplementary material).

Although the proportion of sterile site *S. aureus* isolates that were MRSA decreased from 26% to 19% between 2010 and 2014, this did not reach statistical significance ($P=0.3$).

Clinical features of CC22 and CC239 MRSA

Complete clinical data were available for 208 of 220 (95%) patients with CC22 or CC239 MRSA. In 126 of these patients, the isolate was considered to be a clinically significant pathogen. The clinical syndromes associated most commonly with CC22 or CC239 were skin and soft tissue infections (SSTIs; 74/126, 59%), respiratory tract infections including pneumonia (26/126, 21%), osteo-articular infections (17/126, 13%) and urinary tract infections (5/126, 4%). Seventy-one of the 220 (32%) CC22/CC239 patients had blood cultures performed, and six of these patients were CC22/CC239 blood culture positive.

The key differences between patients from whom CC22 or CC239 MRSA were isolated are presented in Table I. For the subgroup of inpatients, multi-variate logistic regression showed that, in comparison with CC239, CC22 was associated with older patients [adjusted odds ratio (aOR) 1.04 for each year increase, 95% confidence interval (CI) 1.02–1.07] and resident in LTCFs (aOR 5.5, 95% CI 2.0–14.5). When outpatients were included in the model, CC22 was also independently associated with patients from subacute hospitals relative to CC239 (aOR 2.7, 95% CI 1.2–5.8).

<insert Table I near here>

CC22 and CC239 have different epidemiological niches

Both CC22 and CC239 were predominantly healthcare associated (Table I); however, the nature of the healthcare association differed for each strain. The healthcare association of CC22 was more likely to be LTCF residence compared with CC239 (42% vs 6%, $P<0.0001$).

CC239 was the major nosocomial MRSA, representing 58% of all nosocomial isolates, although this proportion varied between inpatient units (Table II). For inpatients, CC239 was isolated a median of nine days [interquartile range (IQR) 1–29] after admission, whereas CC22 was isolated a median of one day (IQR 0–14) after admission ($P=0.01$). CC239 accounted for most

MRSA in the intensive care unit (ICU) (68% of ICU MRSA isolates compared with 38% of non-ICU isolates; $P < 0.0001$), and for >80% of MRSA isolates in the respiratory and vascular surgery units.

CC239 was isolated frequently from respiratory tract specimens and patients with respiratory tract infections (Table D). Analysis of all 65 MRSA isolates from respiratory tract samples (sputum, bronchoalveolar lavage) demonstrated that 46 (71%) were CC239. Further, a large number of CC239 isolates were from patients with cystic fibrosis (CF) (23/46 CC239 respiratory tract isolates were from patients with CF), and nearly all isolates from patients with CF were CC239 (23/25 isolates from patients with CF were CC239). Similarly, in 31 patients with respiratory tract infections, 17 were patients with CF, 21 had CC239 MRSA isolated from the respiratory tract, and 14/17 patients with CF had CC239 MRSA.

In contrast, CC22 was commonly recovered from patients presenting from LTCFs, and accounted for 38% of MRSA from LTCFs compared with 11% for CC239. Additionally, CC22 represented 47% of MRSA isolates recovered from patients admitted under general medicine, which typically cares for older patients, commonly from LTCFs, with multiple comorbidities.

Rise of CC22 and fall of CC239 from 2003 to 2011

To determine if epidemiological shifts seen over time at Alfred Health reflected broader changes in Australia, the proportions of MRSA due to CC22 and CC239 were compared at hospital (Alfred Health), regional level (Victoria/Tasmania) and national (Australia) level. At Alfred Health, proportions of MRSA due to CC239 decreased from 94% to 45%, and proportions of MRSA due to CC22 increased from 0% to 40% between 2003 and 2011. Nationally, similar trends were evident, with a decreasing proportion of MRSA characterized as CC239 (65% in 2003 to 30% in 2011), while the proportion of MRSA characterized as CC22 increased (9% in 2003 to 30% in 2011). Regional data reflected similar changes (Figure 2).

<insert Figure 2 near here>

<A>Discussion

CC22 MRSA is replacing CC239 MRSA as the predominant healthcare-associated MRSA clone in Australia and elsewhere in the world (e.g. Singapore, Portugal and UK). Although differences between CC22 MRSA and other MRSA clones have been investigated at microbiological, antimicrobial resistance and genomic levels, there are few published data comparing the detailed clinical epidemiology of CC22 with other clones.^{10,13,21-23} This study identified significant risk factors for CC22 relative to CC239, including older age, LTCF residence and admission to a subacute healthcare facility. In contrast, CC239 is primarily a nosocomial pathogen, with acquisition and transmission occurring within the acute care hospital system. These findings provide a key insight into the reasons for the growing prominence of CC22 MRSA, particularly in the context of an ageing demographic and expanding LTCF resident population. In Australia, the proportion of those aged >65 years has increased from 13% to 15% over the past 10 years.²⁴

In Europe and Australia, there has been a gradual decline in the proportion of MRSA among all *S. aureus* isolates.^{12,25} These trends likely reflect the impact of a number of initiatives (e.g. antimicrobial stewardship, handwashing, patient isolation) rather than any single intervention. The limited local data for invasive infection isolates demonstrate a similar trend, with a reduction in the proportion of *S. aureus* being MRSA from 26% to 19% over a five-year period (not statistically significant). Clearly, ongoing surveillance of MRSA rates and circulating MRSA clones is important to monitor the dynamic epidemiology of MRSA, and identify the key drivers behind MRSA reservoirs and spread.

In this study, many of the CC22 MRSA patients admitted to the general medicine units at Alfred Health and the subacute hospitals were from LTCFs. As the median time for recovery of CC22 MRSA was only one day after admission, it appears that many elderly LTCF patients harbour CC22 MRSA prior to hospital admission, and LTCFs have become CC22 MRSA reservoirs. This is

consistent with the UK, where CC22 has been reported to be the predominant MRSA clone in LTCF residents, and circulating hospital MRSA clones often reflect those in neighbouring LTCFs.^{26,27}

The epidemiology of CC239 MRSA stands in stark contrast. Patients with CC239 MRSA were younger and the median time for recovery of CC239 was nine days after admission. Furthermore, certain units appear to be foci for acquiring CC239; in this case, CC239 MRSA was over-represented in ICUs and respiratory and vascular surgery units compared with elsewhere in the hospital. Together, this suggests that CC239 MRSA is a more strictly nosocomial clone that circulates and is acquired within the acute care hospital system.

The recent reduction in CC239 MRSA in Australia has coincided with marked improvements in hand hygiene compliance and improvements in infection control.^{12,28,29} While this may help to explain the relative reduction in CC239 MRSA compared with CC22 MRSA, it also highlights the potential difficulties of addressing the issue of rising levels of CC22 MRSA. Although patients with CC22 harboured this CC for a prolonged period in the hospital (median time from admission to isolation: one day; median length of stay: 20 days), the number of nosocomial acquisitions of CC22 was low. This suggests that hospital-based infection control procedures are likely to be effective within the nosocomial setting in preventing the transmission of CC22. However, established infection control strategies directed at MRSA are difficult to implement in LTCFs.²⁶ Notably, screening for MRSA colonization is not routine in Australian LTCFs, nor is screening of patients admitted from LTCFs to the hospitals in this study.

The burden of CC22 MRSA is large and represents a potential target for infection control strategies. While inferences on the direction of spread of CC22 MRSA between LTCFs and hospital cannot be made definitively on the basis of this study, a group of patients that may be acting as a reservoir for CC22 MRSA was identified. This group is therefore appropriate for interventions targeted at reducing the CC22 MRSA burden. However, the optimal strategy for screening, intervening and reducing the MRSA burden remains controversial ('search and destroy' approach,

'bundles of care', decolonization, barrier precautions, terminal cleaning and hand hygiene have all been employed with varying success), particularly in LTCFs.³⁰⁻³⁴

Interestingly, CC239 was frequently isolated from patients with CF. Alfred Health is a specialist treatment and referral centre for CF and lung transplant patients. MRSA infection has been linked independently to poorer outcomes in patients with CF, but determination of MRSA strain types and dynamics in adult patients with CF has been limited.³⁵⁻³⁷ Available data show a predominance of healthcare MRSA strains, as seen in this study, with minimal temporal strain variation in individuals. In lung transplant recipients, MRSA also leads to increased morbidity, and healthcare-associated MRSA strain types similar to those described in this study predominate.^{38,39} Whether or not these CC239 are clonal and represent patient-to-patient transmission in the CF and lung transplant patient population warrants further investigation with higher resolution typing methods.

Although this study concentrated on a single healthcare system in Melbourne, the patterns of change in MRSA clones at the Alfred Hospital reflect those seen elsewhere in Australia. Similarly, there are parallels in the replacement of other MRSA clones by CC22 MRSA elsewhere in the world.^{10,11} Furthermore, the diversity of services offered by the three hospitals studied provides a useful counterpoint to elucidate differences in the populations affected by CC22 compared with CC239. This study's a-priori definition of clinically significant infections, that included information on whether or not antibiotics were prescribed, may have underestimated the number of clinically significant infections, as a minority of patients may have been deemed unsuitable for treatment and therefore the infection was not treated. In contrast, given that antibiotic over-prescribing is well recognized and that a large number of isolates were from non-sterile sites, the surrogate of antibiotic prescription may have actually overestimated true infections. Although the typing method used in this study provides a rapid and cost-effective means of assigning isolates to the CC level, this study would have been able to draw stronger inferences regarding transmission and hospital acquisition if higher resolution typing, such as whole genome sequencing, had been performed.

<A>Conclusions

Healthcare MRSA strains CC22 and CC239 accounted for the majority of MRSA morbidity, but each clone has a particular epidemiological niche. CC22 is common in the elderly and LTCF residents, and was typically introduced into the hospital system by these patient groups. CC239 MRSA transmission was predominantly nosocomial and was identified as a major MRSA strain in ICUs and respiratory and vascular surgery units. These findings suggest that targeted interventions effective at reducing transmission for one clone are unlikely to be as effective for the other clone. As CC22 gains in prominence, infection control strategies involving LTCFs and their residents will become increasingly important to achieve continued reductions in rates of MRSA infections.

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Conflict of interest statement

None declared.

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Table 1

Clinical correlates of patients from whom CC22 or CC239 methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated

		All MRSA N=328 (%)	CC22 N=78 (%)	CC239 N=142 (%)	P-value
Median age (IQR)		64 (49–81)	78 (69–85)	61 (45–74)	<0.001*
Female sex		115 (35)	33 (42)	46 (32)	0.2
Clinically significant		200/319 (63)	45/72 (63)	81 (57)	0.4
Relevant clinical syndrome	SSTI	121/200 (61)	24/45 (53)	50/81 (62)	0.4
	Osteo-articular infection	30/200 (15)	11/45 (24)	6/81 (7)	0.007
	Respiratory tract	18/200 (9)	0/45 (0)	15/81 (19)	0.002
	Pneumonia	13/200 (7)	5/45 (11)	6/81 (7)	0.5
	Urinary tract	13/200 (7)	3/45 (7)	2/81 (3)	0.3
	Intravascular	5/200 (3)	2/45 (4)	2/81 (3)	0.6
	Prosthetic-material-related infection	49/200 (25)	11/45 (24)	16/81 (20)	0.5
	Bacteraemic	9/200 (5)	3/45 (7)	3/81 (4)	0.5
Inpatient at time of MRSA isolation		252/327 (77)	54 (69)	122 (86)	0.005
ICU admission		56 (17)	8 (10)	38 (27)	0.004
30-day survival		289/309 (94)	65/71 (92)	133/137 (97)	0.09
Median length of stay in days (IQR)		27.5 (1–42)	19.5 (5–42)	36 (15–66)	0.004*
Comorbidities	Diabetic	109/318 (34)	29/75 (39)	62 (44)	0.6
	Dialysis	10/325 (3)	3 (4)	6 (4)	1.0
	HIV	4/326 (1)	0 (0)	3 (2)	0.6
	Transplant recipient	23/326 (7)	1 (1)	21 (15)	0.001
	IVDU	5/325 (2)	0 (0)	1 (0.7)	1.0
	CVC	95/326 (29)	15 (19)	60 (42)	0.001
	Healthcare associations	LTCF	65/322 (20)	25/76 (33)	7/141 (5)
	Haemodialysis	8/322 (3)	4/76 (5)	3/141 (2)	0.2

	Day oncology	6/322	(2)	2/76	(3)	3/141	(2)	1.0
	HITH/home nursing	4/322	(1)	0/76	(0)	2/141	(1)	0.5
	HCW	2/322	(1)	0/76	(0)	0/141	(0)	1.0
Acquisition	Healthcare associated	252/325	(78)	60/76	(79)	111	(78)	0.2
	Community associated	73/325	(23)	16/76	(21)	31	(22)	0.2

SSTI, skin or soft tissue infection; osteo-articular infection, septic arthritis or osteomyelitis; pneumonia differentiated from respiratory tract infection by the presence of a new chest x-ray infiltrate; ICU, intensive care unit; HIV, human immunodeficiency virus; IVDU, intravenous drug use; CVC, central vascular catheter; LTCF, long-term care facility; HITH, hospital in the home; HCW, healthcare worker; IQR, interquartile range; clinically significant, treated with antimicrobials and compatible syndrome documented in patient record.

Where data were unavailable or not applicable, relevant denominator is shown.

Unless indicated, all statistical comparisons were made using Chi-squared test (*comparison by Mann-Whitney U-test); *P*-values refer to comparison of CC22 with CC239.

Table II

CC22 and CC239 meticillin-resistant *Staphylococcus aureus* (MRSA) epidemiology according to inpatient unit and hospital

		No. of isolates (% of all MRSA)	CC22	CC239	P-value
All MRSA isolates		328 (100)	78 (24)	142 (43)	
Requesting unit (N=321)	General medicine	49 (15)	23 (47)	9 (18)	<0.001
	Respiratory	33 (10)	1 (3)	28 (85)	<0.001
	Burns	27 (8)	3 (11)	19 (70)	0.03
	GEM	25 (8)	3 (12)	12 (48)	0.3
	Plastic surgery	24 (7)	5 (21)	11 (46)	0.8
	Emergency department	19 (6)	6 (32)	1 (5)	0.009
	Vascular surgery	18 (6)	1 (6)	16 (89)	0.007
	Orthopaedics	14 (4)	5 (36)	3 (21)	0.1
	Rehabilitation	12 (4)	5 (42)	4 (33)	0.3
	General surgery	11 (3)	1 (9)	8 (73)	0.2
	Infectious diseases	11 (3)	2 (18)	6 (55)	0.7
Other	85 (27)	19 (22)	25 (29)	0.3	
Hospital where isolated (N=318)	Alfred Hospital	249 (78)	47 (19)	124 (50)	< 0.001
	Caulfield Hospital	45 (14)	14 (31)	14 (31)	0.09
	Sandringham Hospital	23 (7)	11 (48)	4 (17)	0.003
	Other	1 (0.3)	0 (0)	0 (0)	1.0

GEM, geriatric evaluation unit.

Data expressed as N (% or row) unless otherwise specified.

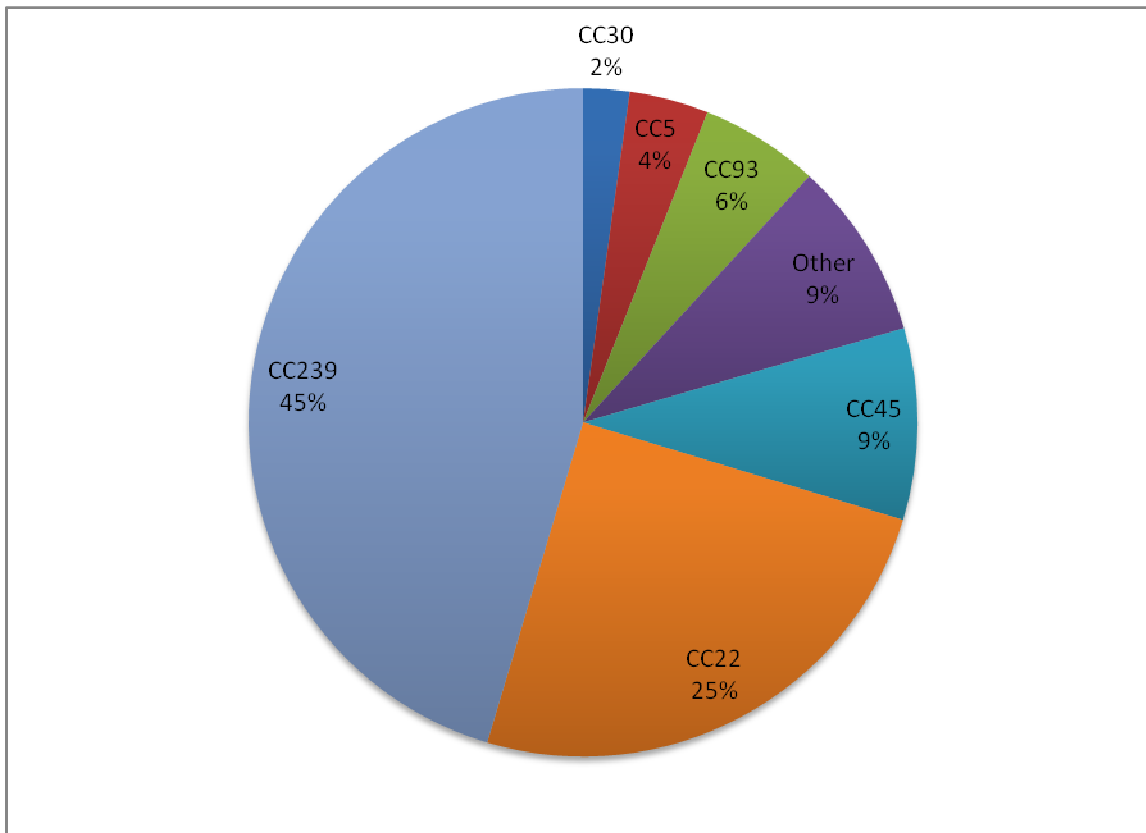
P-values refer to comparison of respective proportions of CC22 and CC239 by Fisher's exact test.

Author queries

Refs 12, 13, 17, 18, 19, 20 – need city

Ref 24 – need city and year

Figure 1. Distribution of clonal complexes (CC) among 313 meticillin-resistant *Staphylococcus aureus* (MRSA) isolates.



'Other' includes CC1, CC8, CC15 and MRSA that could not be typed definitively by the multi-locus-sequence-typing-based high-resolution melting scheme.

Figure 2. Relative burden of CC22 and CC239 over time in Australia. (a) Australian hospitals, (b) Victoria/Tasmanian hospitals, (c) Alfred Health. Red, ST239; green, ST22; purple, other.

