Annual survey of methicillin-resistant *Staphylococcus aureus* (MRSA), 2013

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Introduction

ESR conducts annual surveys of methicillin-resistant *Staphylococcus aureus* (MRSA). Each year, all hospital and community microbiology laboratories in New Zealand are asked to refer all MRSA isolated during a one-month period to ESR. Laboratories provide epidemiological information with each isolate referred. At ESR, MRSA are typed to identify MRSA strains. The purpose of these annual surveys is to provide information on the epidemiology of MRSA in New Zealand and to monitor changes over time.

The results of the 2013 MRSA survey are presented in this report, along with the trends in MRSA prevalence.

Previous reports on the annual MRSA surveys are available at http://www.surv.esr.cri.nz/antimicrobial/mrsa_annual.php.

Methods

MRSA isolates and data collection

Hospital and community microbiology laboratories in New Zealand were asked to refer all MRSA isolated during August 2013 to ESR. The Microbiology Laboratory, North Shore Hospital; Medlab Central, Palmerston North; and the Microbiology Laboratory, Nelson Hospital, referred isolates during a 31-day period between mid-August and the end of September 2013. All remaining laboratories referred MRSA during August 2013.

When referring MRSA isolates, laboratories were asked to supply some epidemiological data, including patient age, geographic location, hospitalisation status, MRSA isolation site, infection or colonisation status, and if the MRSA was obtained from a screen or a diagnostic specimen. Laboratories also provided information on the susceptibility of the MRSA isolates to non-β-lactam antibiotics. The two community laboratories in the greater Auckland area, Labtests and Diagnostic Medlab, receive specimens from multiple district health boards (DHBs), Waitemata, Auckland and Counties Manukau, therefore, for MRSA referred from these laboratories, NHI numbers were used to assign people with MRSA to a DHB.

People were classified as hospital patients or hospital staff if (i) they were inpatients or outpatients in a healthcare facility when MRSA was isolated, or had been in the previous three months; (ii) they were in a residential-care facility when MRSA was isolated, or had been in the previous three months; or (iii) they were employed in a healthcare or residential-care facility when MRSA was isolated. Patients or staff were classified as people in the community if (i) MRSA was isolated from patients while in the community and the patients had no history of being in a healthcare or residential-care facility in the previous three months; (ii) MRSA was isolated from healthcare or residential-care facility admission-screening of patients who had no history of being in such facilities in the previous three months; or (iii) MRSA was isolated from pre-employment swabs of healthcare staff with no employment history supplied.

All MRSA isolates received at ESR were assumed to be pure cultures of MRSA and methicillin/oxacillin resistance was not routinely confirmed.

spa *typing and based upon repeat pattern (BURP) analysis*

The polymorphic X region of the staphylococcal protein A gene (*spa*) was amplified as previously described.[1] PCR products were sequenced by the Sequencing Laboratory at ESR using an ABI 3130XL Sequencer. *spa* sequences were analysed using Ridom StaphType software version 2.2.1 (Ridom GmbH, Würzburg, Germany). Sequences were automatically assigned repeats and *spa* types using the software. Clustering of clonal complexes of related *spa* types (Spa-CCs) was performed using the based upon repeat pattern (BURP) algorithm of the Ridom StaphType software and the default settings of the software which exclude *spa* types with less than five repeats and allow a maximum four costs to cluster *spa* types into the same Spa-CC.[2]

Pulsed-field gel electrophoresis (PFGE) and profile analysis

Where necessary to identify strains, PFGE of *Sma*I-digested genomic DNA was performed as previously described.[3] PFGE banding patterns were analysed using BioNumerics software version 6.6 (Applied Maths, St-Martens-Latem, Belgium), with the Dice coefficient and unweighted-pair group method with arithmetic averages, at settings of 0.5% optimisation and

1.5% position tolerance. PFGE banding patterns were interpreted using the criteria proposed by Tenover et al.[4]

Multilocus sequence typing (MLST) and sequence analysis

Where necessary to characterise strains, MLST was performed as previously described.[5] Sequences were analysed using BioNumerics software version 6.6 and sequence types (STs) were assigned using the *S. aureus* database accessible at http://www.mlst.net.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed where necessary to identify strains and to supplement the susceptibility information provided by referring laboratories. Disc susceptibility testing was performed according to the methods of the Clinical and Laboratory Standards Institute (CLSI).[6] Except for fusidic acid and mupirocin, zones of inhibition were interpreted according to CLSI criteria.[7] Fusidic acid zones of inhibition were determined with a 10 µg disc and interpreted as ≥21 mm susceptible, 20 mm intermediate and ≤19 mm resistant.[8] Mupirocin zones of inhibition were determined with a 5 µg disc and interpreted as ≥14 mm susceptible and ≤13 mm resistant.[9]

PCR for staphylococcal-specific 16S rRNA, nuc *and* mecA

Isolates that were not able to be *spa* typed were tested for the genes encoding staphylococcal-specific 16S rRNA, *S. aureus*-specific thermostable nuclease (*nuc*) and methicillin resistance (*mecA*) by triplex PCR as previously described.[10]

Assigning MRSA strains

Isolates were characterised primarily based upon *spa* types and antibiotic susceptibility patterns, with PFGE as a supplementary typing tool where *spa* typing was inconclusive. There were three situations in which *spa* typing was considered inconclusive: (i) when a *spa* type correlated to a known MRSA strain but the antibiotic susceptibility pattern did not; (ii) when an isolate had a novel *spa* type; and (iii) when an isolate had a *spa* type ESR had not yet correlated to an MRSA strain.

Epidemiological analyses

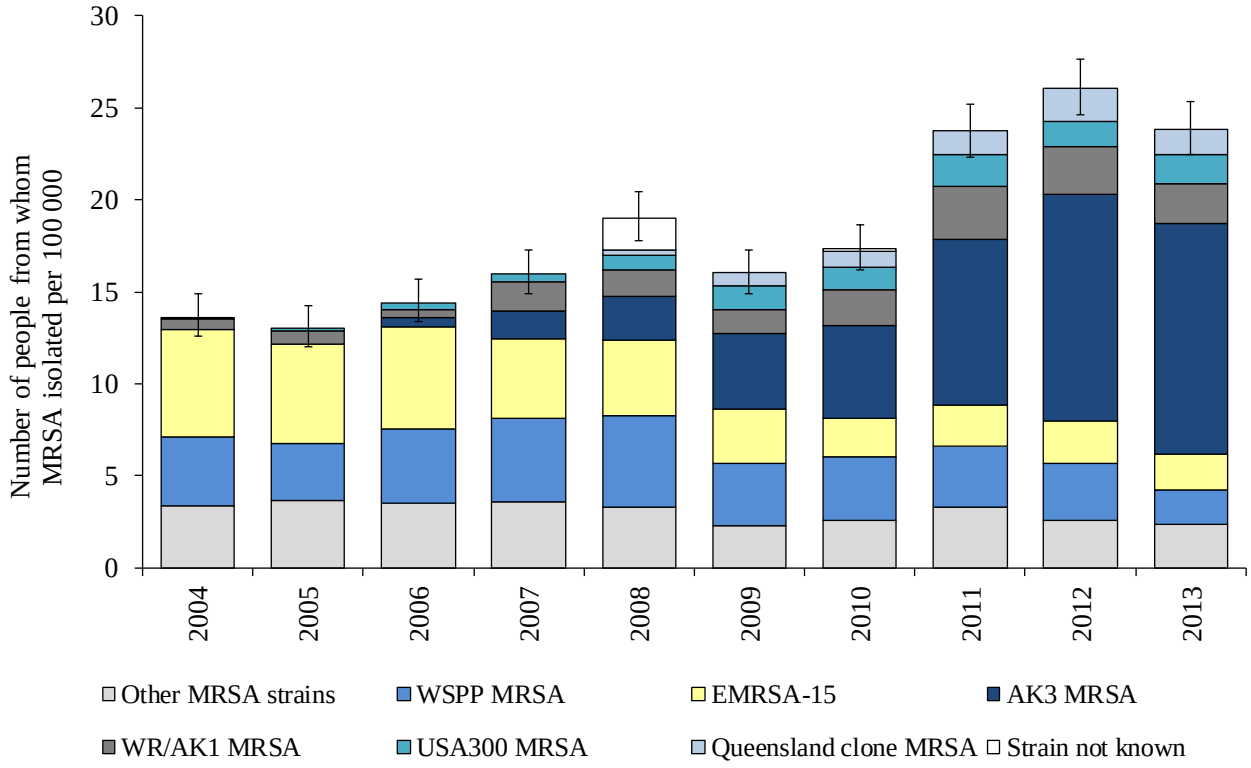
Epidemiological data and results were entered into ESR's laboratory information management system. Data and results were extracted and analysed using customised Microsoft Access queries. Period-prevalence rates were calculated based on the number of MRSA isolated per 100 000 population during the period of the survey. Mid-year New Zealand population estimates were used to calculate prevalence rates. 95% confidence intervals were calculated based on Poisson distribution. The statistical significance of time trends was calculated at a 95% confidence level using Poisson regression and the Mantel-Haenszel test for linear trend.

Results

National period-prevalence rates of MRSA, 2004-2013

During the period of the 2013 MRSA survey, MRSA were isolated from 1067 people, 1055 of whom were patients and 12 of whom were staff. There was a non-significant 8.5% decrease in the MRSA period-prevalence rate between 2012 and 2013, from 26.1 to 23.9 people with MRSA per 100 000 population. However, over the last 10 years, 2004 to 2013, the period-prevalence rate has increased 78% from 13.4 to 23.9 per 100 000 (Figure 1).

Figure 1. MRSA period-prevalence rates, 2004-2013



95% confidence intervals indicated by error bars. The category 'Strain not known' for 2008 and 2010 represents people identified with MRSA during the survey period but from whom no isolate was referred for strain identification.

MRSA infection status, strain prevalence, and strain association with healthcare facilities versus the community and with patient age

In 2013, of the 1055 patients with MRSA, 67.4% were categorised as community patients and 32.6% as hospital patients. MRSA was reported as causing infection in 76.1% of the 903 patients for whom this information was provided.

Six MRSA strains (AK3 MRSA, WR/AK1 MRSA, EMRSA-15, WSPP MRSA, USA300 MRSA and Queensland clone MRSA) were predominant in 2013 and collectively represented 90.1% of all MRSA isolations (Table 1). The dominance of AK3 MRSA evident in recent years increased further in 2013 with this strain accounting for 52.5% of all MRSA included in the survey. The period-prevalence rates for the four most prevalent strains, AK3, WR/AK1, EMRSA-15 and WSPP, were 12.5, 2.2, 1.9 and 1.9 per 100 000 population, respectively (Figure 1).

Table 1. MRSA strain prevalence, association with healthcare facilities versus community, and association with patient age, 2013

Strain	Proportion (%) of all MRSA isolations[a]	Proportion (%) of each strain isolated from:		
		hospital patients or staff	people in the community	patients ≥60 years of age[b]
AK3 MRSA [ST5, SCC*mec* type IV][c]	52.5	25.7	74.3	18.2
WR/AK1 MRSA [ST1, SCC*mec* type IV]	9.2	32.7	67.3	27.6
EMRSA-15 MRSA [ST22, SCC*mec* type IV]	8.1	53.5	46.5	83.3
WSPP MRSA [ST30, SCC*mec* type IV]	7.9	33.3	66.7	12.0
USA300 MRSA [ST8, SCC*mec* type IV]	6.5	52.5	47.8	44.9
Queensland clone MRSA [ST93, SCC*mec* type IV]	6.0	28.1	71.9	18.8

a Other strains accounted for the remaining 9.9% of MRSA.
b Age distribution for patients only, staff not included.
c ST, multilocus sequence type; SCC*mec*, staphylococcal cassette chromosome *mec*.

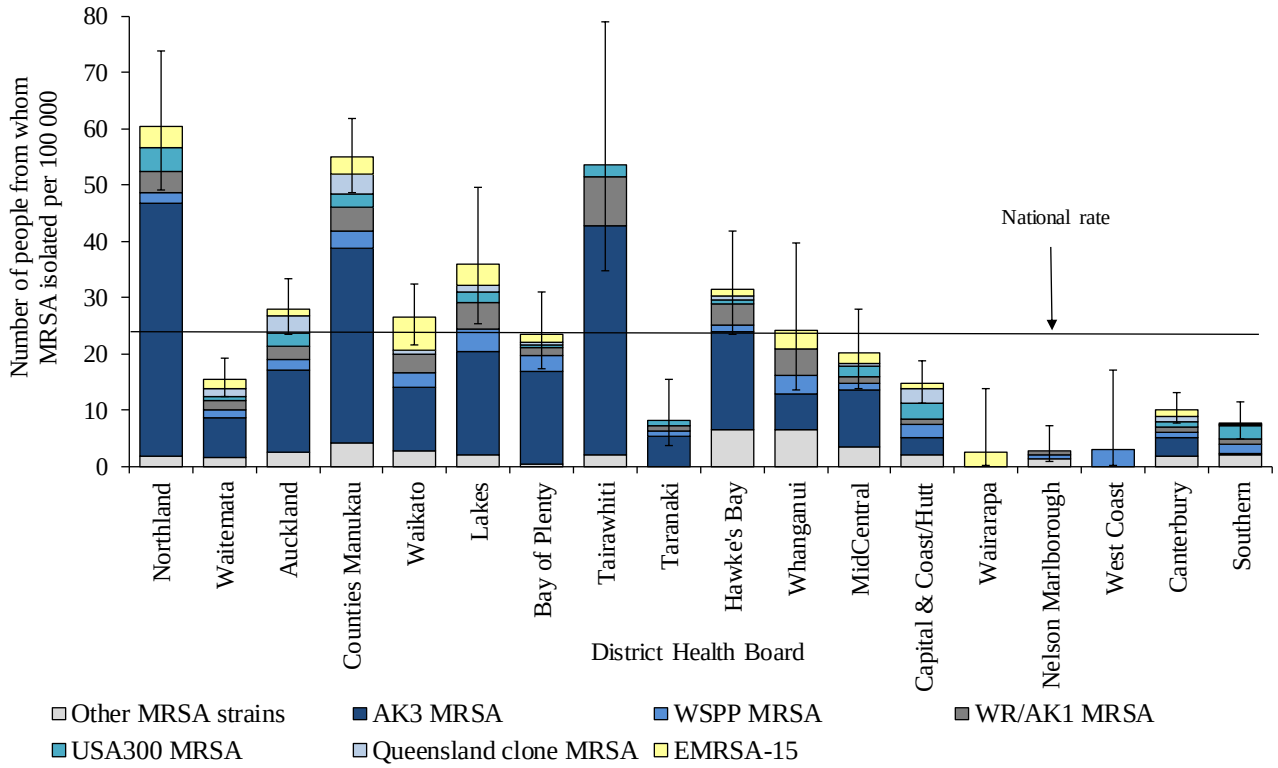
Geographic distribution of MRSA

There were significant geographical differences in the period-prevalence rates of MRSA isolations in 2013. Rates exceeded the national rate of 23.9 people with MRSA per 100 000 population in eight DHBs: Northland (60.5 per 100 000), Counties Manukau (54.9), Tairawhiti (53.5), Lakes (35.9), Hawke's Bay (31.5), Auckland (28.0), Waikato (26.6) and Whanganui (24.0) (Figure 2).

Similar geographical differences were evident in the period-prevalence rates of MRSA isolated only from infection, with the same eight DHBs, and in addition the Bay of Plenty DHB, having rates above the national period-prevalence rate of 15.4 people with an MRSA infection per 100 000 population: Northland (40.3 per 100 000), Tairawhiti (36.4), Counties Manukau (29.7), Auckland (22.7), Hawke's Bay (21.2), Bay of Plenty (20.7), Waikato (19.6), Whanganui (16.0) and Lakes (15.5) (Figure 3).

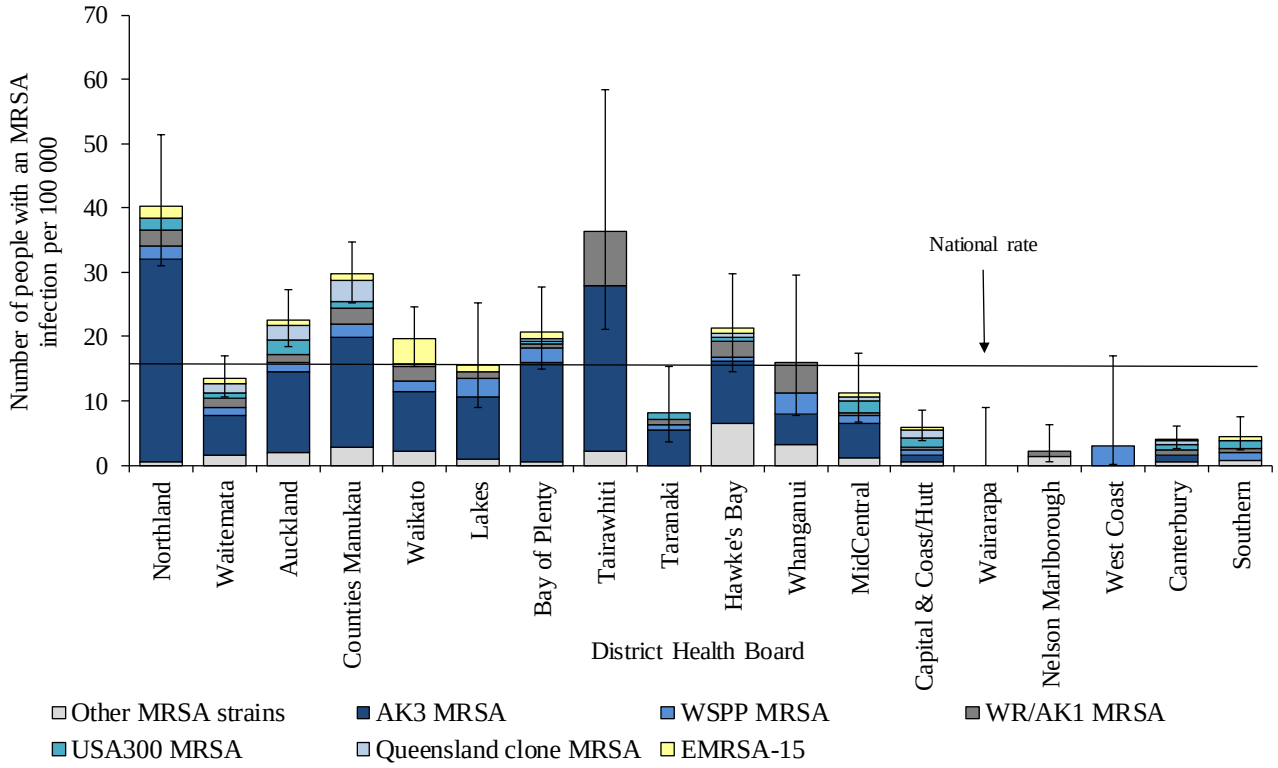
AK3 MRSA was the most prevalent MRSA strain in all North Island DHBs except Wairarapa. It was also the most prevalent strain in the Canterbury/South Canterbury area.

Figure 2. MRSA period-prevalence rates by district health board, 2013



95% confidence intervals indicated by error bars. Data for the Capital & Coast and Hutt DHBs are combined as 'Capital & Coast/Hutt', and data for the Canterbury and South Canterbury DHBs are combined as 'Canterbury'.

Figure 3. MRSA infection period-prevalence rates by district health board, 2013

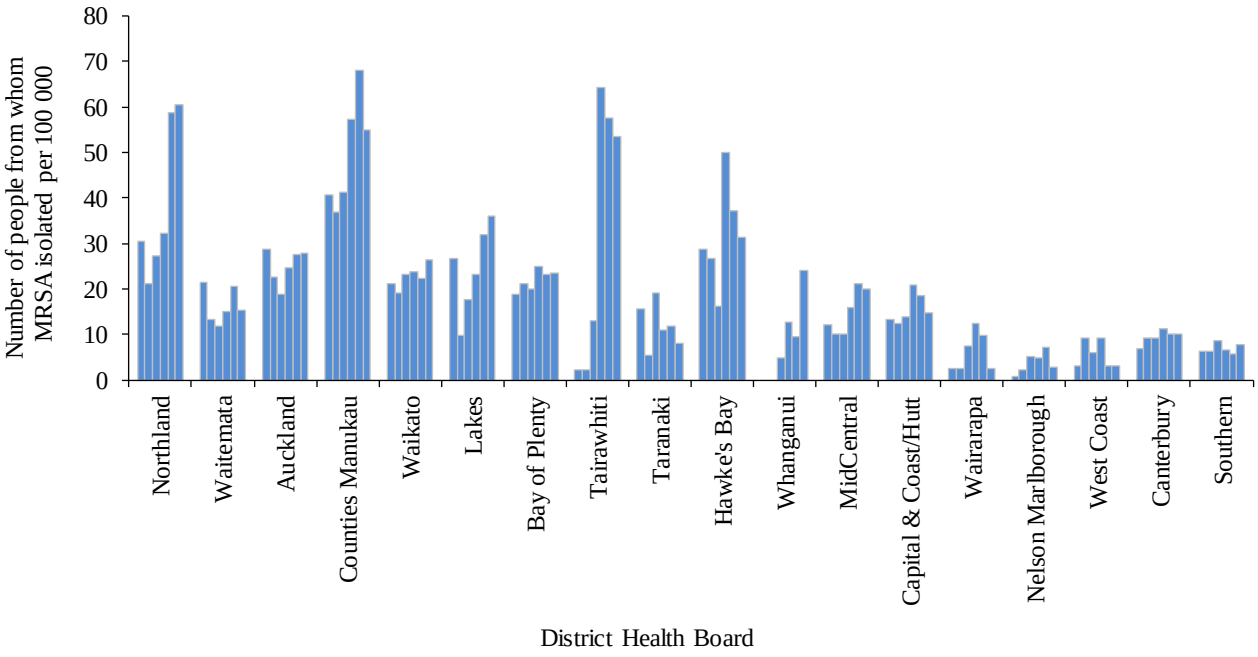


95% confidence intervals indicated by error bars. Data for the Capital & Coast and Hutt DHBs are combined as 'Capital & Coast/Hutt', and data for the Canterbury and South Canterbury DHBs are combined as 'Canterbury'.

Period-prevalence rates of MRSA by DHB, 2008-2013

Over the six-year period 2008 to 2013, there were statistically significant increases in MRSA period-prevalence rates in 6 of the 18 DHB/DHB combinations analysed. These DHBs were, ordered from the DHB with the highest increase to that with the smallest increase: Tairawhiti, Northland, Counties Manukau, Whanganui, Lakes, and MidCentral (Figure 4).

Figure 4. MRSA period-prevalence rates by district health board, 2008-2013



The series of bars for each DHB represent the individual years 2008 to 2013 from left to right. Data for the Capital & Coast and Hutt DHBs are combined as 'Capital & Coast/Hutt', and data for the Canterbury and South Canterbury DHBs are combined as 'Canterbury'.

MRSA strain association with spa *types*

In 2013, the AK3 MRSA strain was most commonly associated with *spa* type t002, WR/AK1 MRSA with t127, EMRSA-15 with t032, WSPP MRSA with t019, USA300 MRSA with t008, and the Queensland clone MRSA with t3949 (Table 2). AK3 MRSA was associated with the greatest variety of *spa* types.

Table 2. *spa* types of the most six most prevalent MRSA strains in 2013

Strain	Number of isolates of the strain	*spa* clonal cluster	*spa* type[a]	Number of isolates of the *spa* type
AK3 MRSA [ST5, SCC*mec* type IV][b]	558[c]	Spa-CC002	t002	486
			t105	11
			t548	9
			t045	8
			t062	5
			t311	4
			t5213	4
			t242	3
			t306	2
			t2069	2
			t6787	2
		Excluded[d]	t1781	3
WR/AK1 MRSA [ST1, SCC*mec* type IV]	96[e]	Spa-CC127	t127	66
			t267	13
			t1418	3
Alternative name: Western Australia (WA) MRSA-1			t7136	3
		Spa-CC008	t701	5
EMRSA-15 [ST22, SCC*mec* type IV]	84[f]	Spa-CC032	t032	49
			t022	7
			t005	3
			t1401	3
			t5538	3
			t852	2
			t1214	2
			t5501	2
WSPP MRSA [ST30, SCC*mec* type IV]	84	Spa-CC019	t019	70
			t1752	3
Alternative names: Southwest Pacific clone and Oceania clone				
USA300 MRSA [ST8, SCC*mec* type IV]	69	Spa-CC008	t008	59
			t024	7
Queensland clone MRSA [ST93, SCC*mec* type IV]	64	Spa-CC202	t3949	47
			t202	15
			t11037	2

Footnotes: see next page.

Table 2 footnotes:

a The *spa* types are only listed in the table if there were ≥2 isolates of the type. In addition to the *spa* types listed in the table:

among the AK3 MRSA isolates there was also 1 isolate of each of the following *spa* types: t003, t010, t067, t088, t179, t214, t535, t601, t688, t1062, t1265, t1594, t4617, t5867, t6571, t7086, t11865, t12932 and t12947;

among the WR/AK1 MRSA isolates there was also 1 isolate of each of the following *spa* types: t359, t591, t3564, t7099, t10753 and t12781;

among the EMRSA-15 MRSA isolates there was also 1 isolate of each of the following *spa* types: t020, t025, t294, t309, t891, t1328, t3107, t5816, t7105, t10279, t12909, t12948 and t13089;

among the WSPP MRSA isolates there was also 1 isolate of each of the following *spa* types: t018, t021, t122, t975, t1836, t2895, t3593, t4672, t5447, t9085 and t11174; and

among the USA300 MRSA isolates there was also 1 isolate of each of the following *spa* types: t622, t1705 and t6442.

b ST, multilocus sequence type; SCC*mec*, staphylococcal cassette chromosome *mec*.

c The total number of AK3 MRSA isolates was 560, but the *spa* type of 2 isolates could not be determined and therefore these isolates were identified solely by PFGE typing.

d An excluded *spa* type does not have sufficient repeat sequences (ie, <5 repeats) to validly include it in the based upon repeat pattern (BURP) cluster analysis.

e The total number of WR/AK1 MRSA isolates was 98, but the *spa* type of 2 isolates could not be determined and therefore these isolates were identified solely by PFGE typing.

f The total number of EMRSA-15 isolates was 86, but the *spa* type of 2 isolates could not be determined and therefore these isolates were identified solely by PFGE typing.

In addition to the six most prevalent MRSA strains listed in Table 2, isolates of several other recognized MRSA strains were identified. These included:

4 isolates of the AKh4 MRSA strain (ST239, SCC*mec* type III);
4 isolates of the Bengal Bay MRSA clone (ST772, SCC*mec* type V);
4 isolates of the WA MRSA-2 strain (ST78, SCC*mec* type IV);
2 isolates of the CC398 MRSA clone (CC398, SCC*mec* type V); and
1 isolate of EMRSA-16 (ST36 SCC*mec* type II)

The AKh4 MRSA is a healthcare-associated MRSA (HA-MRSA) strain that is multiresistant to ciprofloxacin, clindamycin, co-trimoxazole, erythromycin, gentamicin and tetracycline. This strain is a common cause of HA-MRSA infections in many parts of the world including the east coast states of Australia. Its prevalence in New Zealand has decreased in recent years, but it still occasionally causes small outbreaks in healthcare facilities.

The Bengal Bay MRSA clone is a multiresistant MRSA, typically resistant to ciprofloxacin, erythromycin and gentamicin. This strain carries the genes for several virulence factors including the Panton Valentine leukocidin (PVL) genes and the enterotoxin gene cluster.

WA MRSA-2 is a non-multiresistant, community-associated MRSA (CA-MRSA) strain originally recognized in Western Australia.

CC398 MRSA is a livestock-associated MRSA which was originally identified in pigs in Northern European countries and first identified in New Zealand during the 2011 MRSA survey. The two CC398 isolates identified in the 2013 survey were both from people who had had recent contact with farm animals in Europe. One person was a Dutch farmer visiting New

Zealand and the second person had stayed on a farm in Denmark and had contact with pigs during a recent overseas holiday.

There were 91 isolates that were not associated with a recognized MRSA strain, and the most common *spa* types among these isolates were t1853 (15 isolates) and t437 (5 isolates). There were less than five isolates of any other *spa* type not associated with a known MRSA strain.

Discussion

Data from this survey indicates that the period-prevalence rate of MRSA isolation has remained relatively stable between 2011 (23.7 per 100 000 population) and 2013 (23.9 per 100 000), with a notable increase between 2010 and 2011.

It is important to note that the overall increase in MRSA period-prevalence rates over the past decade is likely a reflection of the overall increase in *Staphylococcus aureus* infections in New Zealand, specifically skin and soft tissue infections (SSTIs).[11] One recent study suggested a significant increase in national rates of hospitalizations for *S. aureus* skin infections, from 81 per 100 000 population in 2000 to 140 per 100 000 in 2011 (P <0.001).[11] Similarly using laboratory-based surveillance, a recent Auckland study described a significant (P <0.001) increase in non-invasive *S. aureus* infections between 2001 and 2011, largely driven by community-onset methicillin-susceptible *S. aureus* infections.[12] In addition, the marked geographic difference in MRSA period-prevalence rates is likely to be a reflection of the differential rates of *S. aureus* skin infections across New Zealand, with rates of *S. aureus* SSTI highest in the North and Central parts of New Zealand.[11]

The AK3 MRSA clone continued to predominate in 2013, and accounted for 52.5% of all MRSA isolated in this survey. Although the underlying reasons for the rapid and sustained emergence of the AK3 clone are unclear, it is noteworthy that this clone typically displays resistance to fusidic acid. In keeping with the emergence of the AK3 clone is a corresponding increase in the rate of fusidic acid resistance in MRSA in New Zealand, from 12.1% in 2008 to 37.4% in 2012.[13] Recent data suggests that community prescriptions for fusidic acid have increased significantly in New Zealand over the past decade, and this is likely to provide a strong selective advantage for the AK3 clone in the New Zealand community setting.[14] In addition, recent genotypic analysis of the AK3 clone suggests that fusidic acid resistance is mediated by the *fusC* gene, which is capable of disseminating between multiple lineages of *S. aureus*.[14]

Concurrent with the proportional increase in AK3 MRSA is a decrease in the isolation of previously common MRSA lineages in New Zealand, most notably WSPP MRSA and EMRSA-15. In particular, the proportion of MRSA due to WSPP MRSA fell from 31.5% in 2008 to 7.9% in 2013, reflecting the emergence and dominance of the AK3 clone in the community setting.

In contrast to the diverse range of CA-MRSA clones, MRSA clones in the hospital setting are more genetically restricted and more commonly resistant to a wide range of antimicrobial agents. In New Zealand, the predominant HA-MRSA clone is EMRSA-15, which is typically resistant to several non-β-lactam antimicrobials, particularly ciprofloxacin and erythromycin. In keeping with other countries, recent data suggests an infiltration of CA-MRSA clones into the healthcare setting in New Zealand,[15] and it has been suggested that, due to an increasing community reservoir, CA-MRSA clones will ultimately replace HA-MRSA clones in the hospital setting.[16] This suggestion is supported by data from this survey, with at least a quarter of isolations of each of the major CA-MRSA clones (AK3, WR/AK1, WSPP, USA300 and Queensland clone) coming from the hospital setting. In particular, over half (52.5%) of isolations of the USA300 clone were from hospital patients or staff. Given the apparent high transmissibility of CA-MRSA clones,[17] this finding highlights the need for ongoing systematic molecular surveillance to track MRSA clones in the New Zealand setting.

In conclusion, the period-prevalence rate of MRSA isolation has remained stable over the last three years, but increased over the past decade – an increase that is likely to reflect an overall increase in *S. aureus* disease in New Zealand. The AK3 MRSA strain continues to be the most dominant CA-MRSA strain, and accounted for over half of all MRSA isolated in this survey. The rapid emergence of this clone is likely related to high rates of fusidic acid usage in the New Zealand community setting. Future work should attempt to identify the impact of fusidic acid prescribing on rates of antimicrobial resistance in both MRSA and methicillin-susceptible *S. aureus.*

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