Game of clones:

Staphylococcus aureus infections

in New Zealand

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THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES

MAURICE WILKINS CENTRE
FOR MOLECULAR BIODISCOVERY

Outline

- **Clinical epidemiology of *S. aureus* in NZ**
  - National trends and demographics in *S. aureus* infections
  - Comparative demographics of MSSA and MRSA infections
- **Causes of increasing rates of *S. aureus* skin infections**
  - Colonisation in Auckland children
  - MSSA – clonality and virulence
- **Impact of high burden of *S. aureus* skin infections**
  - Topical antimicrobial prescribing
  - Emergence of fusidic acid-resistant clones

National trends in *S. aureus* infections

Epidemiology of *S. aureus* in New Zealand

- In certain settings, particularly North America, the clinical and molecular epidemiology of *S. aureus* infections has changed considerably over the past two decades
- Comparatively little is known about the trends and demographics of *S. aureus* infections in other geographic settings, particularly in the Southern Hemisphere
- To date, most studies in New Zealand have focused on one aspect of *S. aureus* disease (e.g. geographic region; bacteraemia; MRSA) [1]

[1] **Williamson DA et al. *Clin Microbiol Infect, 2014***

Epidemiology of *S. aureus* in New Zealand

- Aims:
  - To describe longitudinal trends in the incidence of invasive and non-invasive *S. aureus* disease in New Zealand
  - To describe the comparative demographics (age; sex; ethnicity; geographic region) of *S. aureus* infections in New Zealand

Epidemiology of *S. aureus* in New Zealand

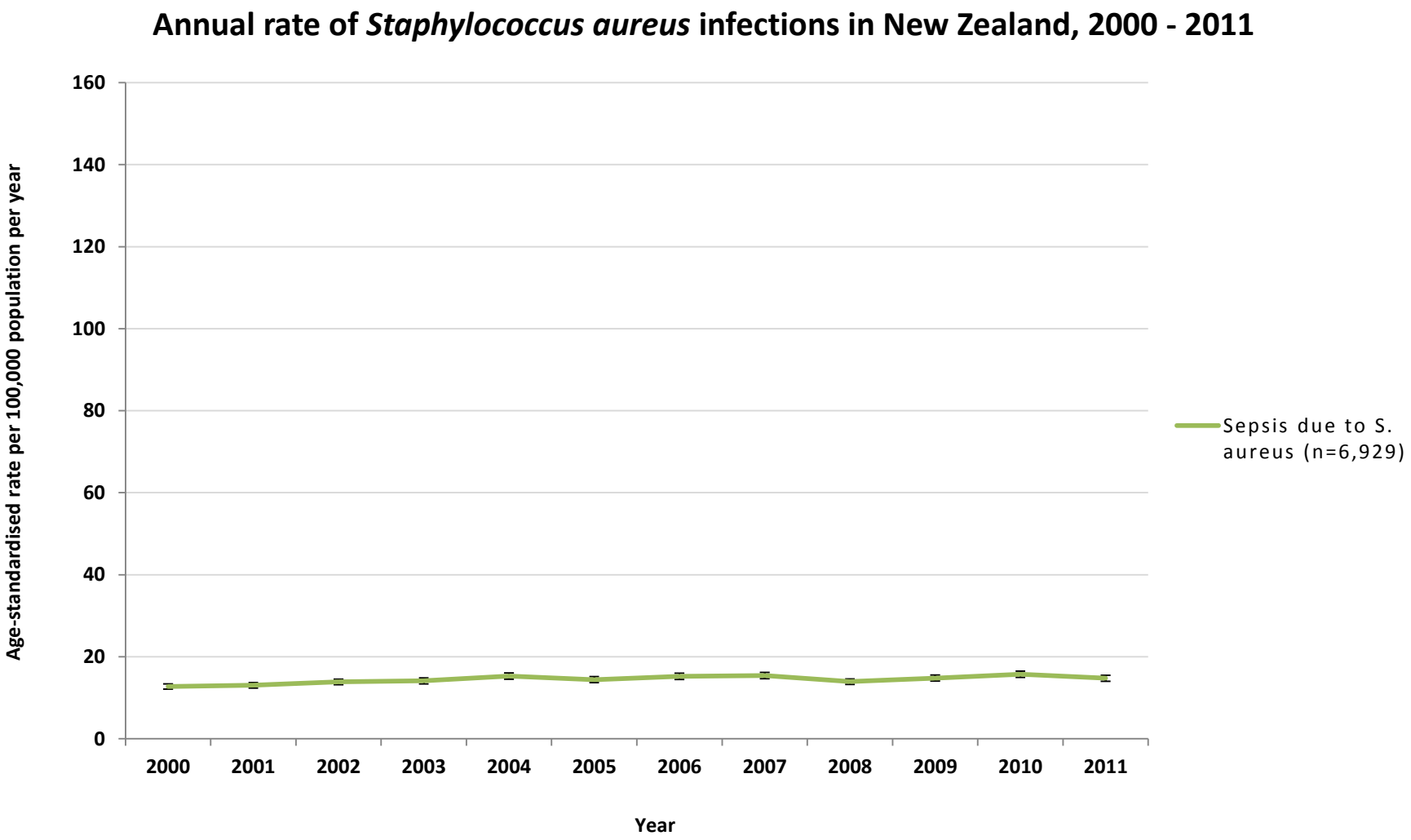
- Patients with *S. aureus*-related overnight hospitalizations between 2000 and 2011 were identified on the basis of *S. aureus*-specific *ICD-10-AM* discharge codes:

Invasive *S. aureus* infections		**Non-invasive *S. aureus* infections**
A410: 'Sepsis due to *S. aureus*'	**J152**: 'Pneumonia due to *S. aureus*'	**B956**: 'Other *S. aureus* disease' plus codes relating to skin infection [1, 2]

[1] **O'Sullivan CE & Baker MG. *J Paediatr Child Health* 2010**

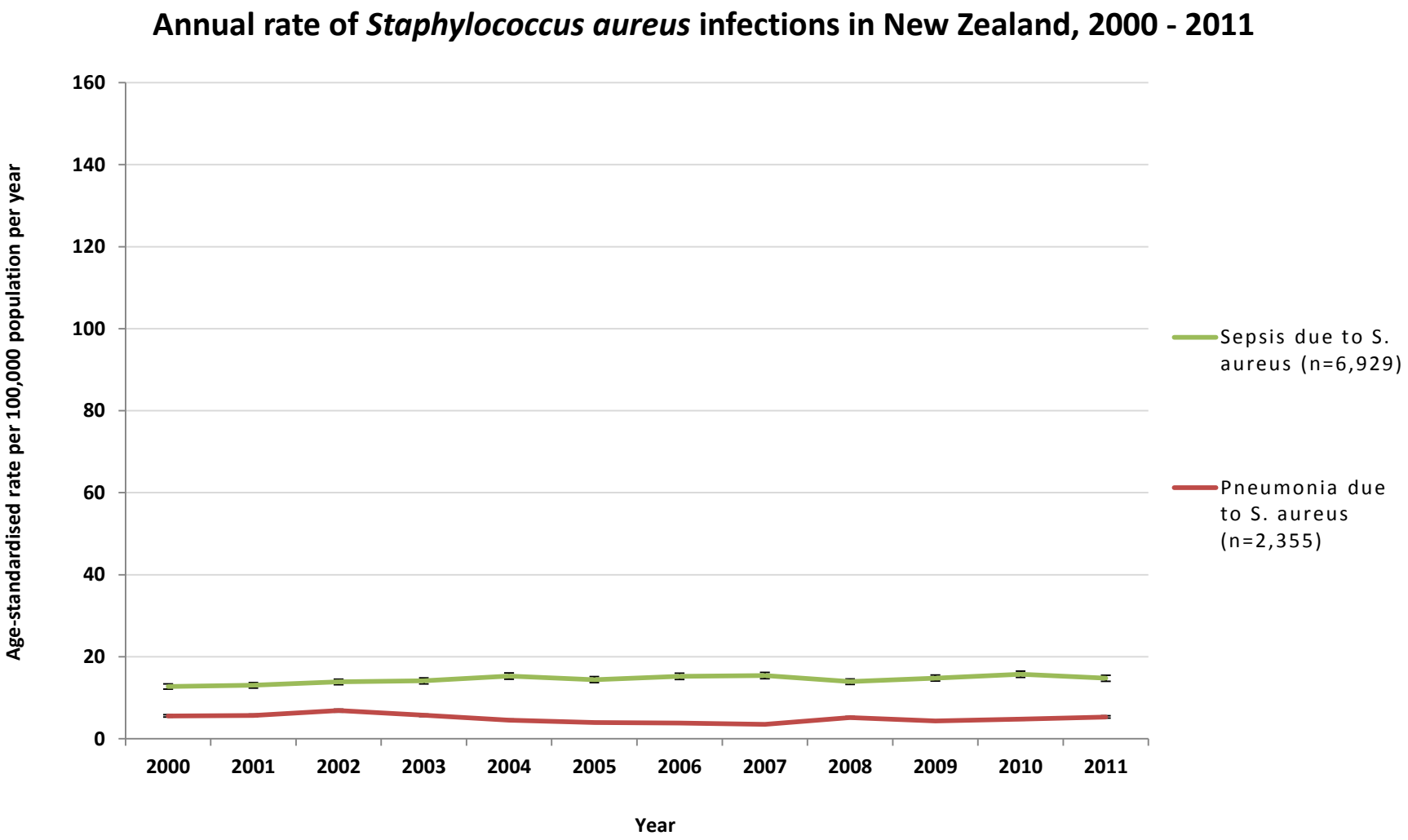
[2] **Williamson DA et al. *Pediatric Infect Dis J* 2013**

Epidemiology of *S. aureus* in New Zealand



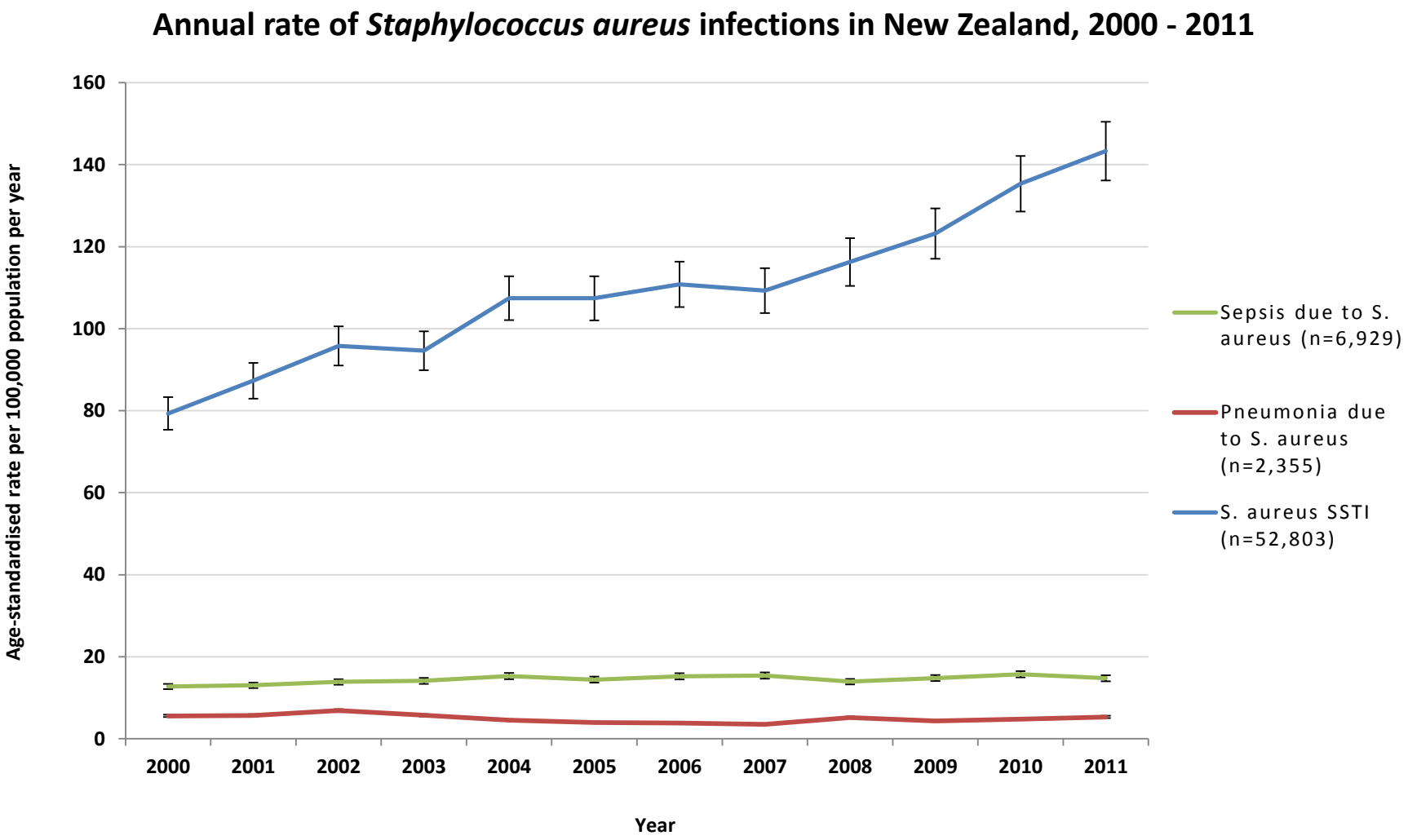
Williamson DA et al. *Emerg Infect Dis, 2014*

Epidemiology of *S. aureus* in New Zealand



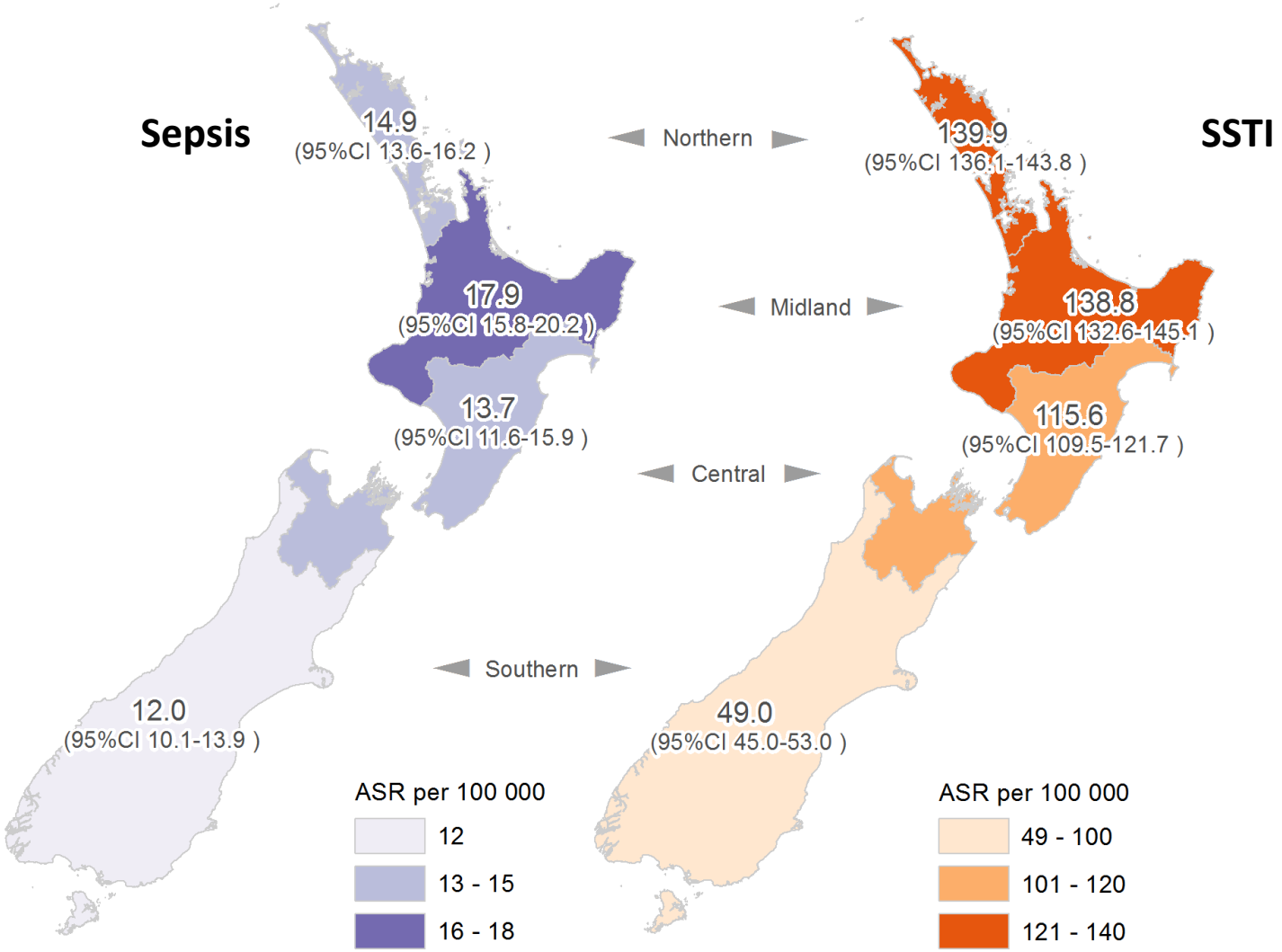
Williamson DA et al. *Emerg Infect Dis, 2014*

Epidemiology of *S. aureus* in New Zealand



Williamson DA et al. *Emerg Infect Dis, 2014*

Epidemiology of *S. aureus* in New Zealand



Williamson DA et al. *Emerg Infect Dis, 2014*

Epidemiology of *S. aureus* in New Zealand

Sepsis due to *Staphylococcus aureus*

Characteristic	Number of patients	Age-standard rate (per 100 000)	Relative Risk (95% CI)	*P*
Age, median, years				
<5 years	301	9.13	0.68 (0.60 – 0.76)	<0.001
5-14 years	315	4.44	0.33 (0.29 – 0.37)	<0.001
15-29 years	521	5.38	0.40 (0.36 – 0.44)	<0.001
30-69 years	3,213	13.51	1.00	
≥70 years	2,579	62.37	4.62 (4.38 – 4.86)	<0.001
Sex	6,929			
Male	4,338	19.70	1.96 (1.91 – 2.00)	<0.001
Female	2,591	10.07	1.00	
Ethnicity	6,928			
European	4,557	12.06	1.00	
Māori	1,322	31.01	2.57 (2.48 – 2.66)	<0.001
Pacific peoples	672	38.08	3.16 (3.01 – 3.31)	<0.001
Other	377	8.63	0.72 (0.68 – 0.76)	<0.001
Total	**6,929**	**14.48**		

Williamson DA et al. *Emerg Infect Dis, 2014*

Epidemiology of *S. aureus* in New Zealand

SSTI due to *Staphylococcus aureus*

Characteristic	Number of patients	Age-standard rate (per 100 000)	Relative Risk (95% CI)	*P*
Age, median, years				
<5 years	8,068	244.80	2.76 (2.69 – 2.84)	<0.001
5-14 years	7,528	106.16	1.20 (1.17 – 1.23)	<0.001
15-29 years	10,396	107.29	1.21 (1.18 – 1.24)	<0.001
30-69 years	21,019	88.41	1.00	
≥70 years	5,792	140.07	1.58 (1.54 – 1.63)	<0.001
Sex				
Male	29,818	127.48	1.37 (1.36 – 1.38)	<0.001
Female	22,985	93.29	1.00	
Ethnicity				
European	24,386	74.33	1.00	
Māori	15,629	225.83	3.04 (3.00 – 3.07)	<0.001
Pacific peoples	9,813	363.38	4.89 (4.83 – 4.95)	<0.001
Other	2,969	50.09	0.67 (0.66 – 0.69)	<0.001
Total	**52,803**	**109.98**		

Williamson DA et al. *Emerg Infect Dis, 2014*

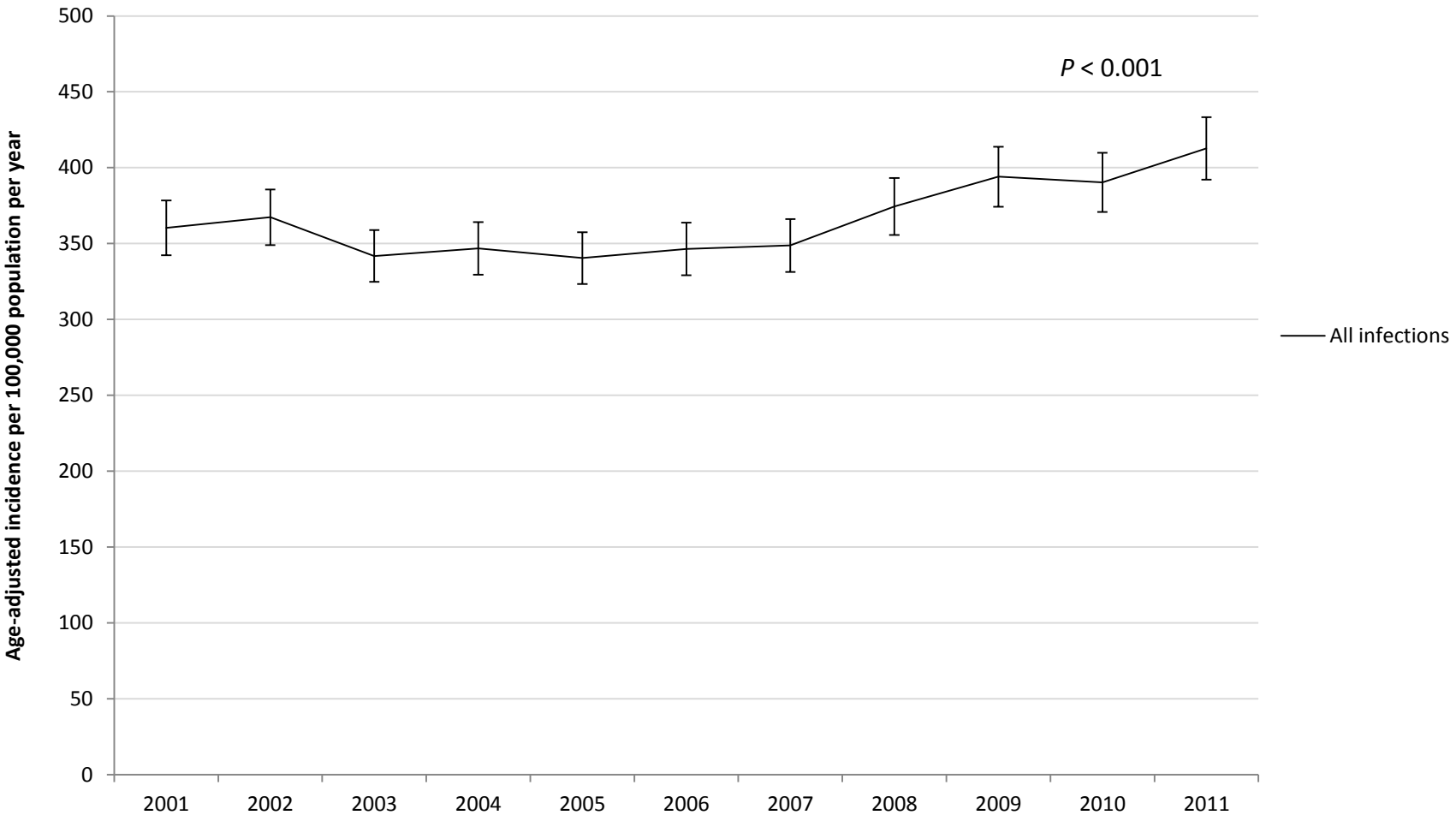
Comparative demographics of *S. aureus* infections

S. aureus infections in Auckland, 2001 - 2011

Characteristic	Number of patients (%) n = 16,249
Type of infection	
Invasive	3,752 (23)
Non-invasive	12,479 (77)
Place of acquisition	
Community-onset	8,754 (54)
Healthcare-associated, community-onset	5,523 (34)
Hospital-onset	1,972 (12)
Resistance phenotype	
MSSA	14,290 (88)
nmMRSA	1,804 (11)
mMRSA	117 (1)

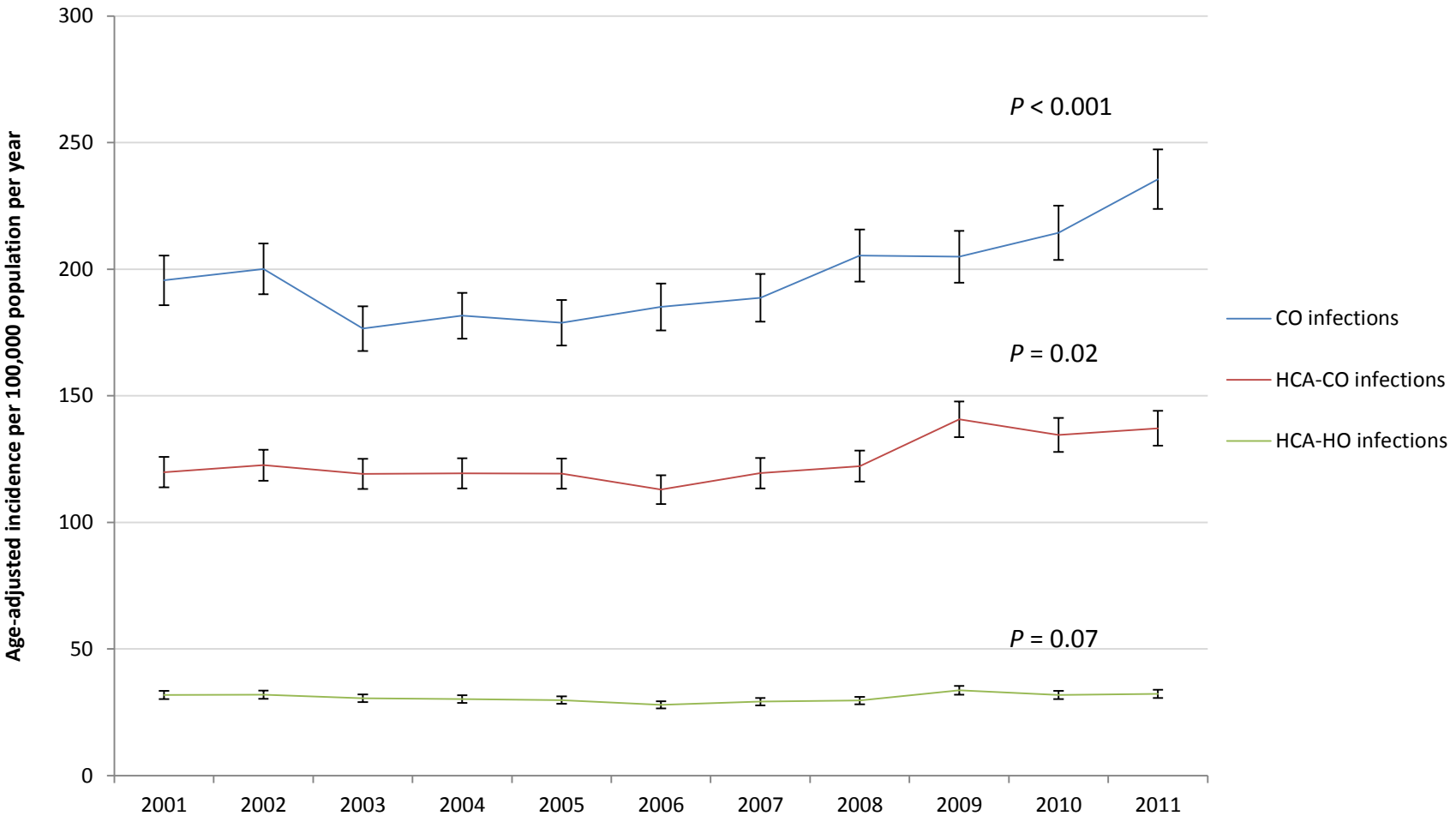
Williamson DA et al. *BMC Infect Dis, 2013*

S. aureus infections in Auckland, 2001 - 2011



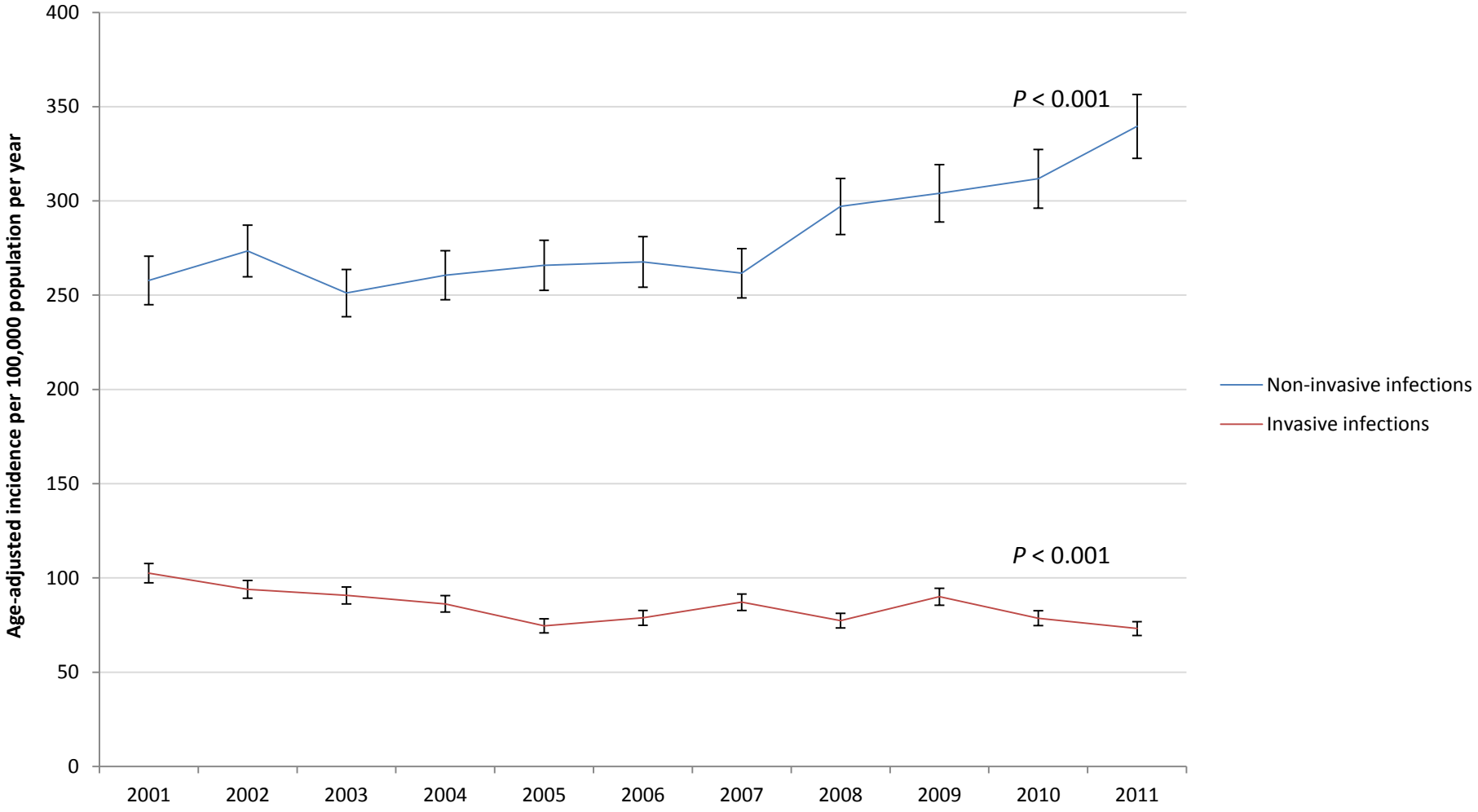
Williamson DA et al. *BMC Infect Dis, 2013*

S. aureus infections in Auckland, 2001 - 2011



Williamson DA et al. *BMC Infect Dis, 2013*

S. aureus infections in Auckland, 2001 - 2011



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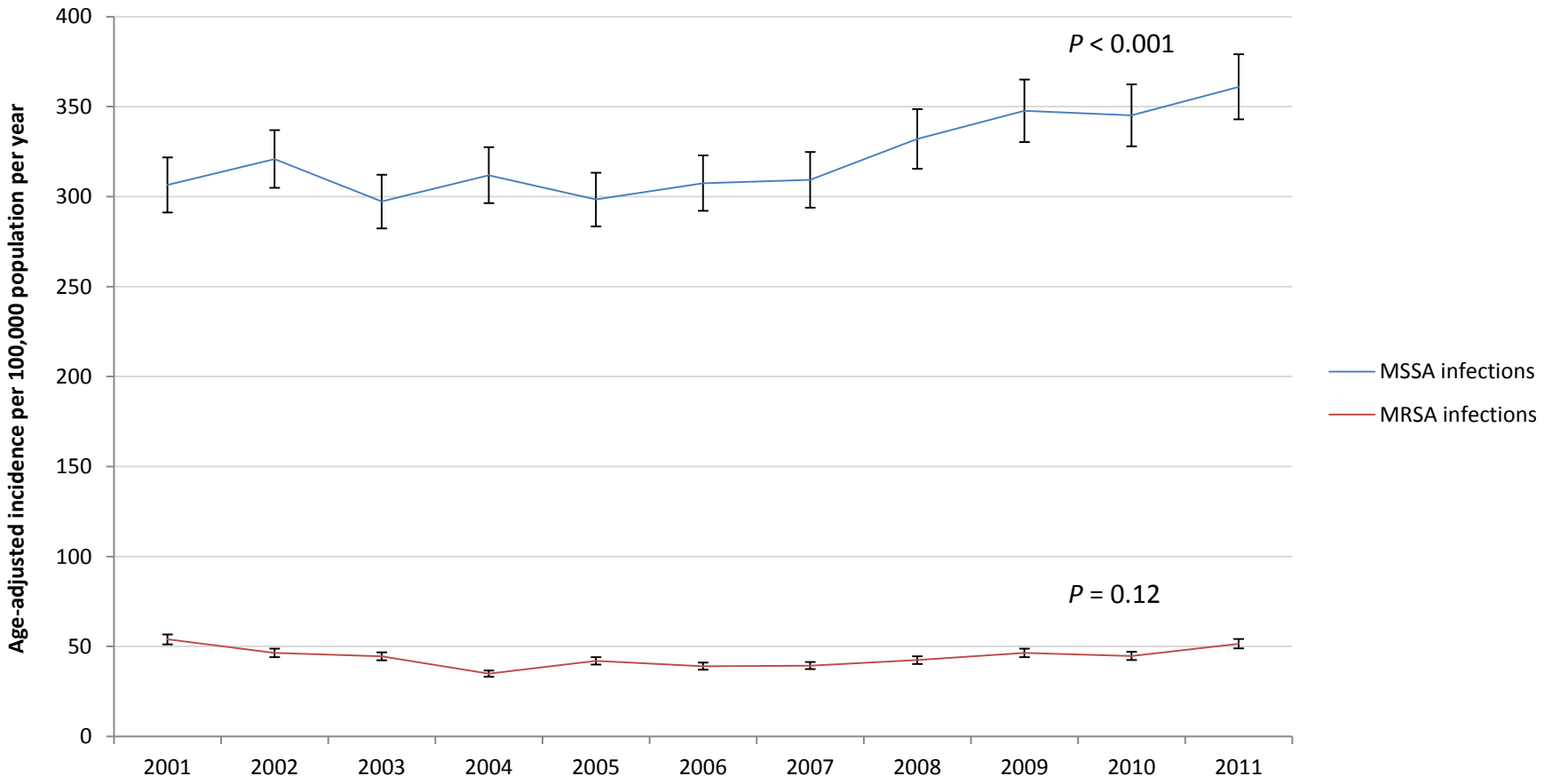
S. aureus infections in Auckland, 2001 - 2011



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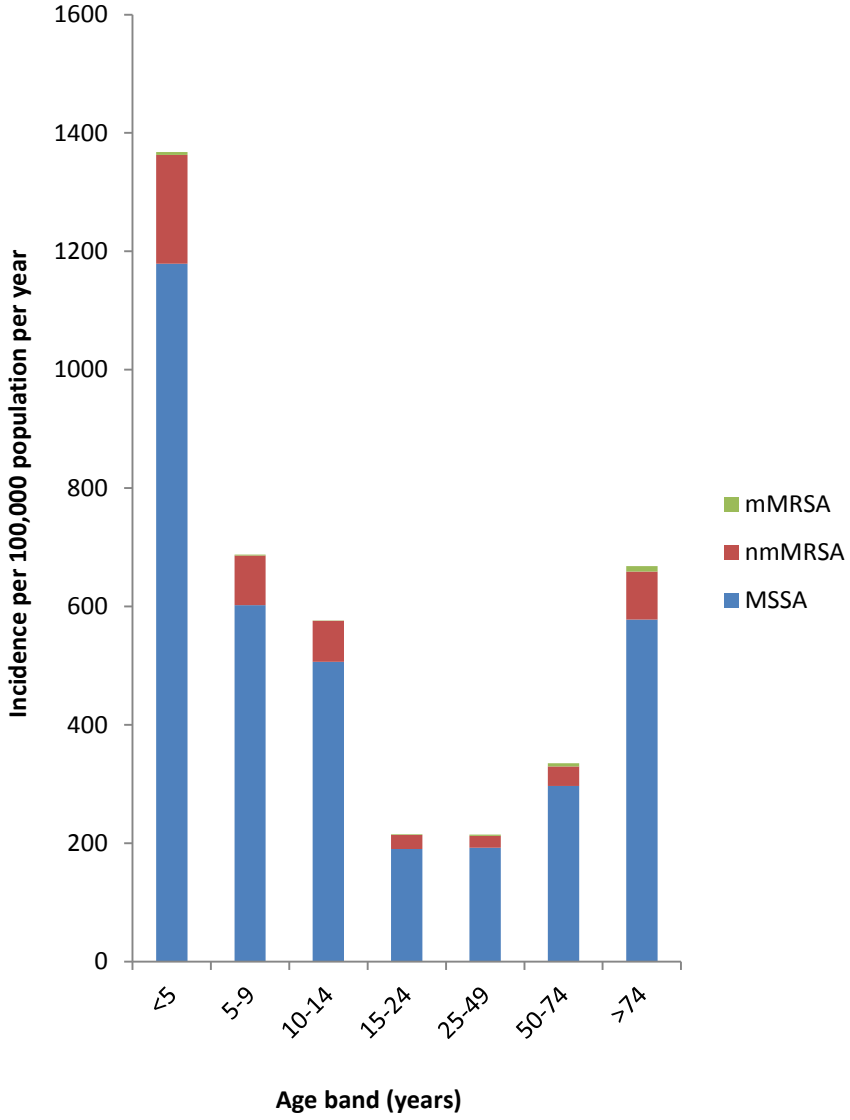
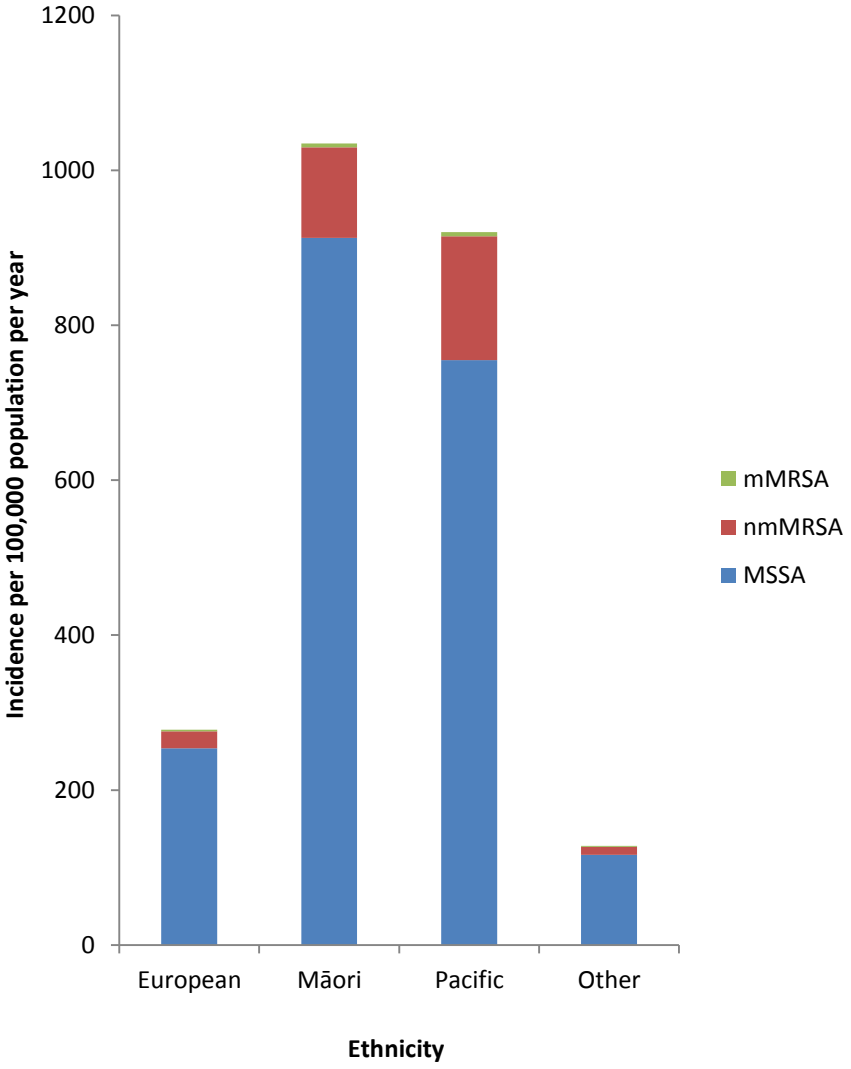




Williamson DA et al *BMC Infect Dis, 2013*

Epidemiology of *S. aureus* in New Zealand

- **The incidence of *S. aureus* SSTI has risen significantly in New Zealand over the past decade, with significant socio-demographic disparity**
  - Likely due to combination of host, bacterial and environmental factors
- **The 'knock-on' effects of this increase are likely to be substantial**

Understanding the causes of *S. aureus* SSTI in New Zealand

Understanding the causes of *S. aureus* SSTI in NZ

- **Highest burden of *S. aureus* SSTI in Māori and Pacific children**
- **Possible causes of increasing rates of *S. aureus* SSTI**
  - High colonisation pressure?
  - Differences in virulence of circulating strains?

S. aureus carriage in Auckland children

SA carriage in Auckland children

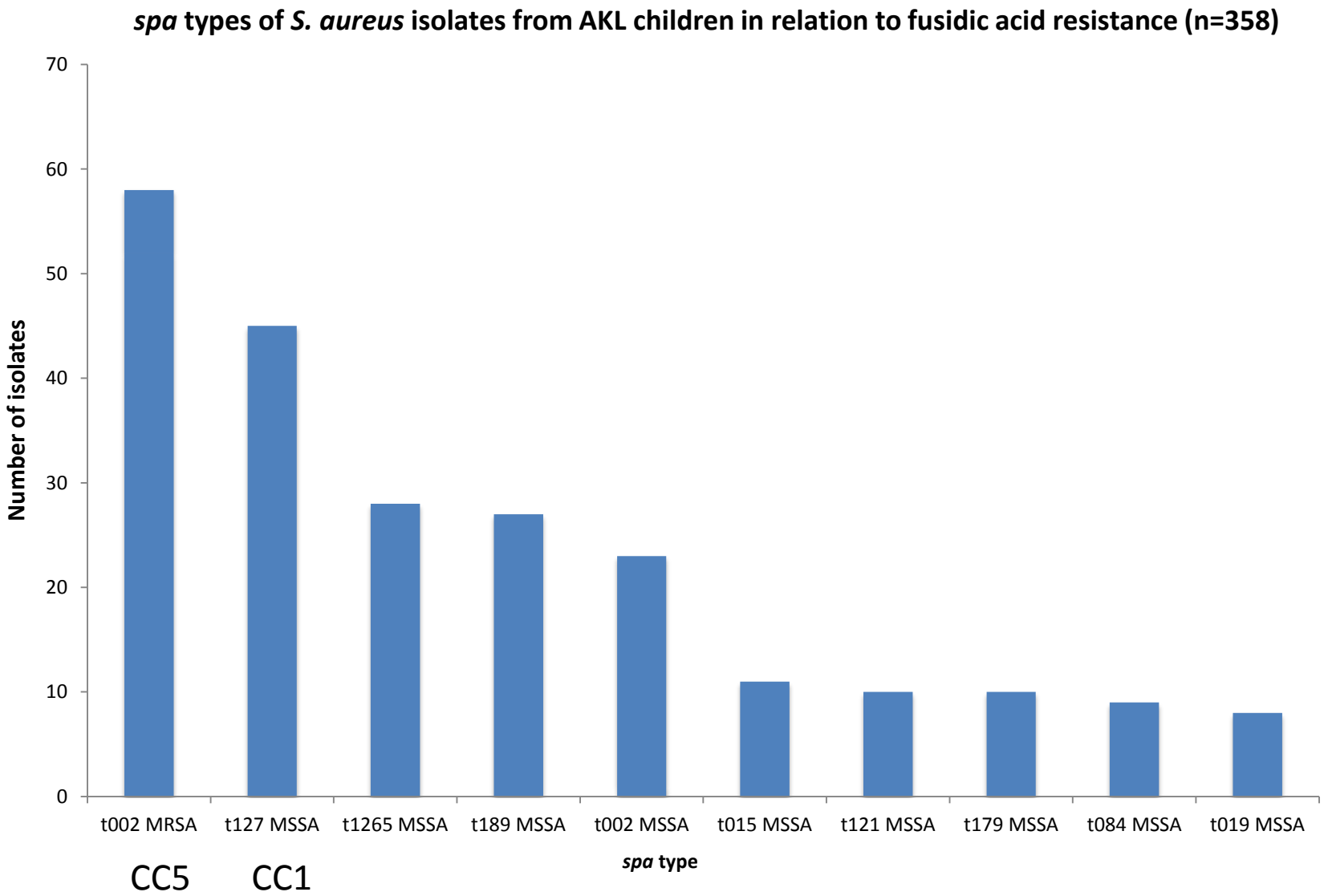
- The anterior nares is considered the primary site of *S. aureus* carriage (prevalence of 20-35% in developed world)
- More recent studies have highlighted the importance of other anatomical sites in *S. aureus* carriage, particularly the oropharynx
- Cross-sectional study of approximately 1,100 primary school-age children in October 2013:
  - Anterior nares swab (*S. aureus*)
  - Oropharyngeal swab (GAS; *S. aureus*)
  - Open skin lesions

Collaborator: Dr Alison Leversha, ADHB

SA carriage in Auckland children

Location	Number of patients	Overall SA carriage rate (%)	Nasal carriage (%)	Throat carriage (%)	Nasal and throat carriage (%)	Exclusive nasal carriage (%)	Exclusive throat carriage (%)
Auckland, NZ (2013)	923 (Children)	55.5	30.4	41.2	15.7	14.7	24.8

SA carriage in Auckland children



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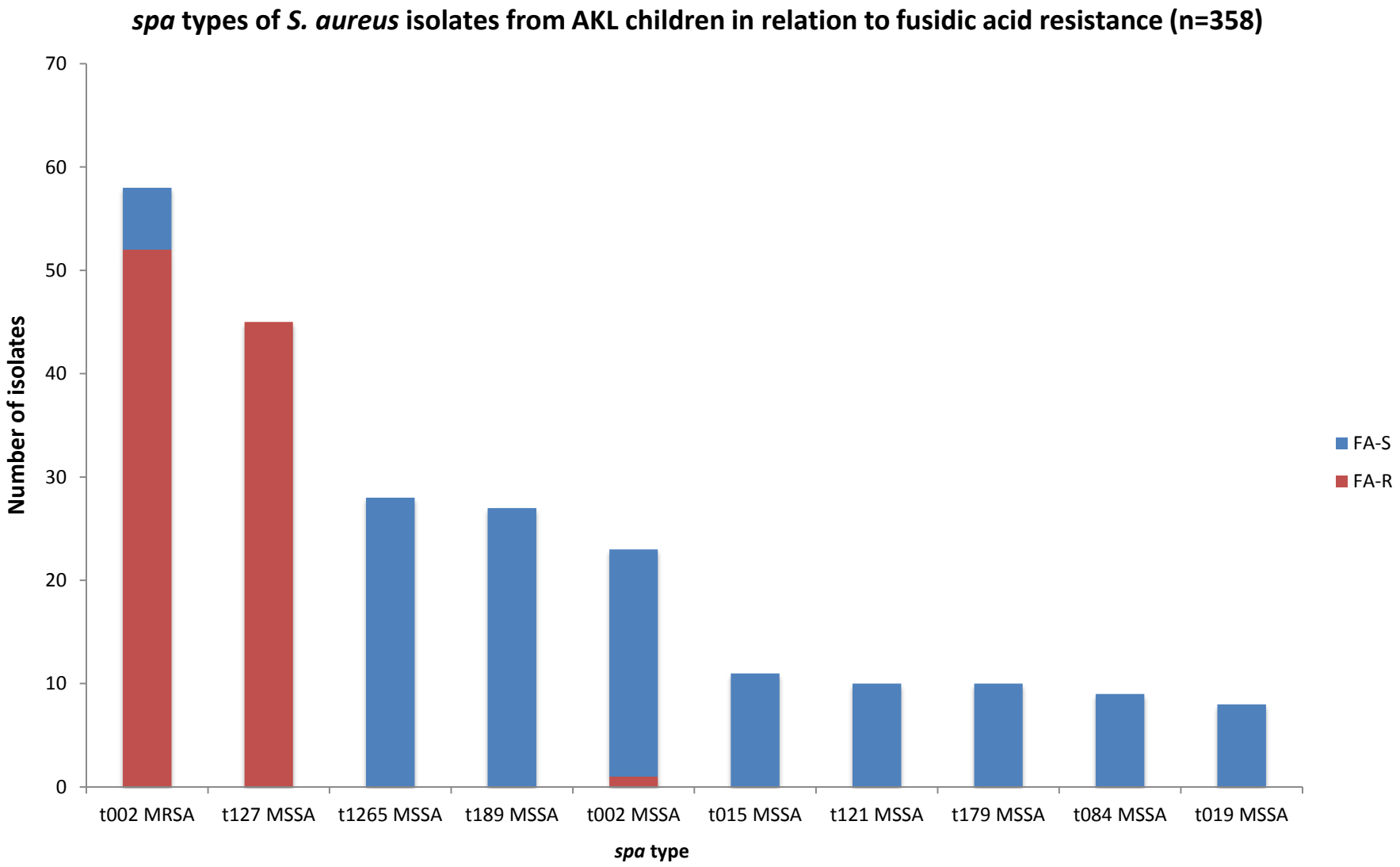


Clonality and virulence in MSSA

Clonality and virulence of MSSA

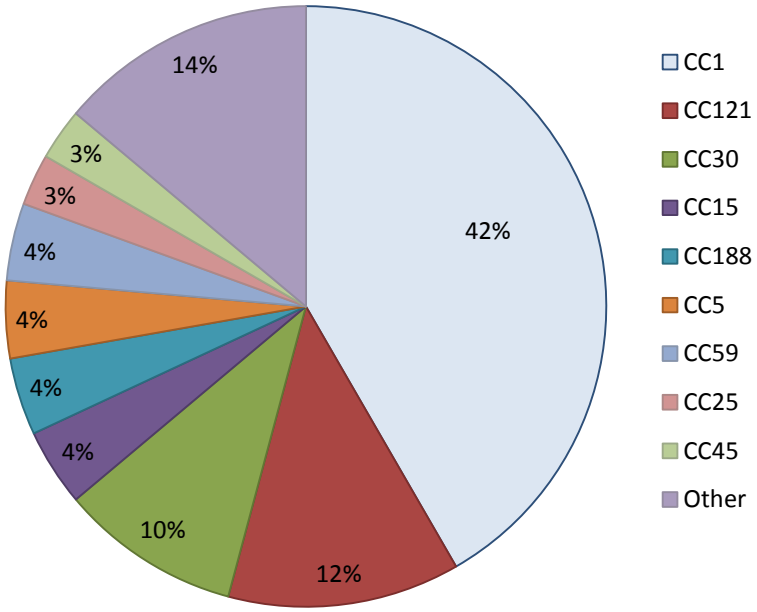
- The vast majority of *S. aureus* disease in New Zealand is caused by MSSA
- The extent to which the increase in MSSA disease in New Zealand is driven by an increase in one or several MSSA clones is unknown
- Limited data exist on the clonal structure and virulence gene profile of MSSA strains in New Zealand

Clonality and virulence of MSSA

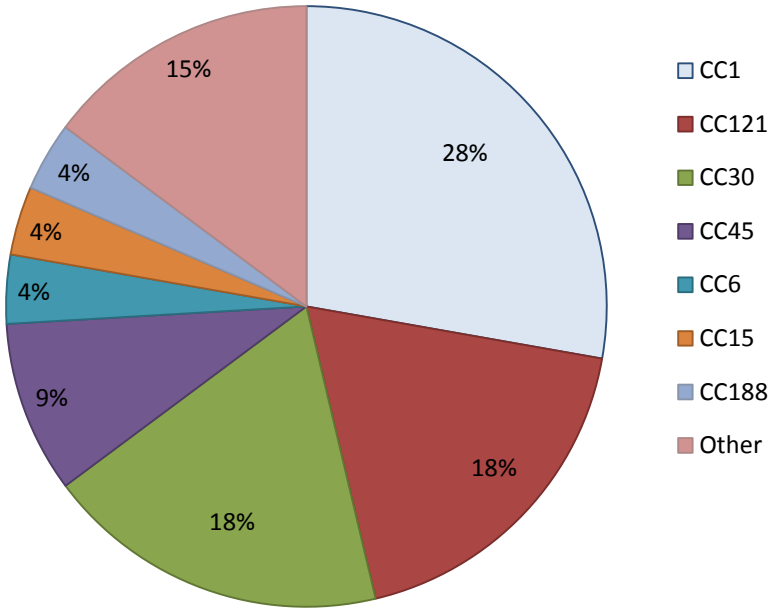
- Representative sample of 72 invasive and 54 non-invasive MSSA isolates from paediatric patients between 2007 and 2010
- DNA microarray analysis performed on all isolates
- Strain types and virulence gene repertoire compared between invasive and non-invasive isolates
- **Null hypothesis** = no difference in strain types or VFs between invasive and non-invasive MSSA strains

S. aureus clonal complexes

Invasive *S. aureus* isolates (n = 72)



Non-invasive *S. aureus* isolates (n = 54)



- Three MSSA clones predominated: CC1, CC121 and CC30
- Unexpectedly high prevalence of *lukF-PV* and *lukS-PV* genes (53%)

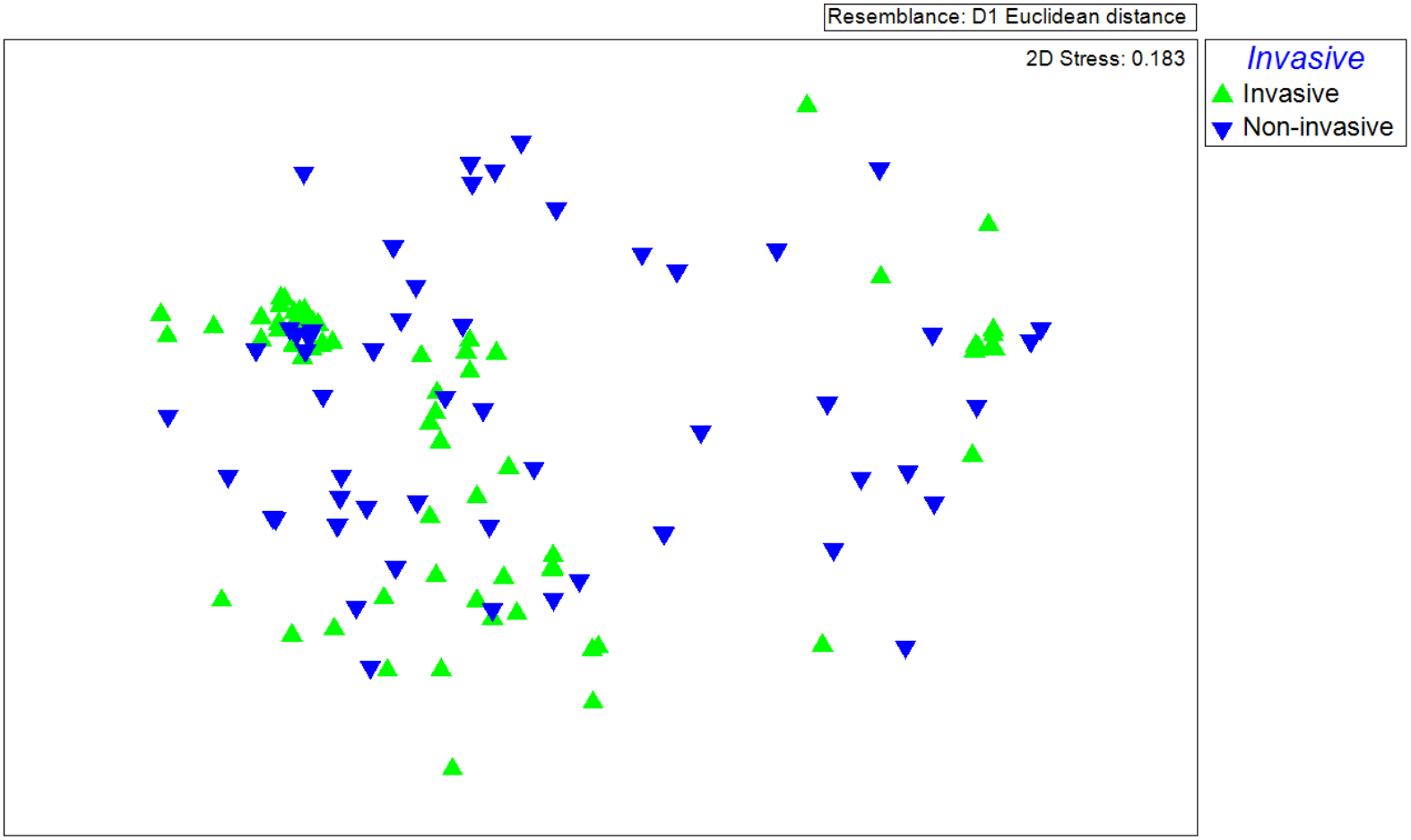
Williamson DA et al, Epidemiol Infect, 2014

Distribution of virulence-associated genes

Gene(s)	Invasive *S. aureus* (n = 72)	Non-invasive *S. aureus* (n = 54)	*P*
Toxin genes	4 (6)	5 (9)	0.49
tst	25 (35)	26 (48)	0.14
egc	28 (39)	21 (39)	1.0
sea	1 (1)	4 (7)	0.16
etA	0	2 (4)	0.18
etB			
Haemolysin and leucocidin genes			
lukF-PV / lukS-PV	42 (58)	29 (54)	1.0
hla	68 (94)	54 (100)	0.93
hlb	62 (86)	45 (83)	0.80
MSCRAMMS and biofilm-associated genes			
bbp	68 (94)	53 (98)	0.39
clfA	71 (98)	54 (100)	1.0
clfB	71 (98)	54 (100)	1.0
cna	54 (75)	45 (83)	0.28
fin	60 (83)	41 (76)	0.37
fnbA	72 (100)	54 (100)	1.0
fnbB	72 (100)	54 (100)	1.0
sdrC	65 (90)	52 (96)	0.29
sdrD	65 (90)	51 (94)	0.51
Other genes			
edinA	2 (3)	0	1.0
edinB	3 (4)	1 (2)	0.63
sak	64 (89)	50 (93)	0.55
chp	35 (49)	25 (46)	0.86
scn	69 (96)	52 (96)	1.0

Williamson DA et al, Epidemiol Infect, 2014

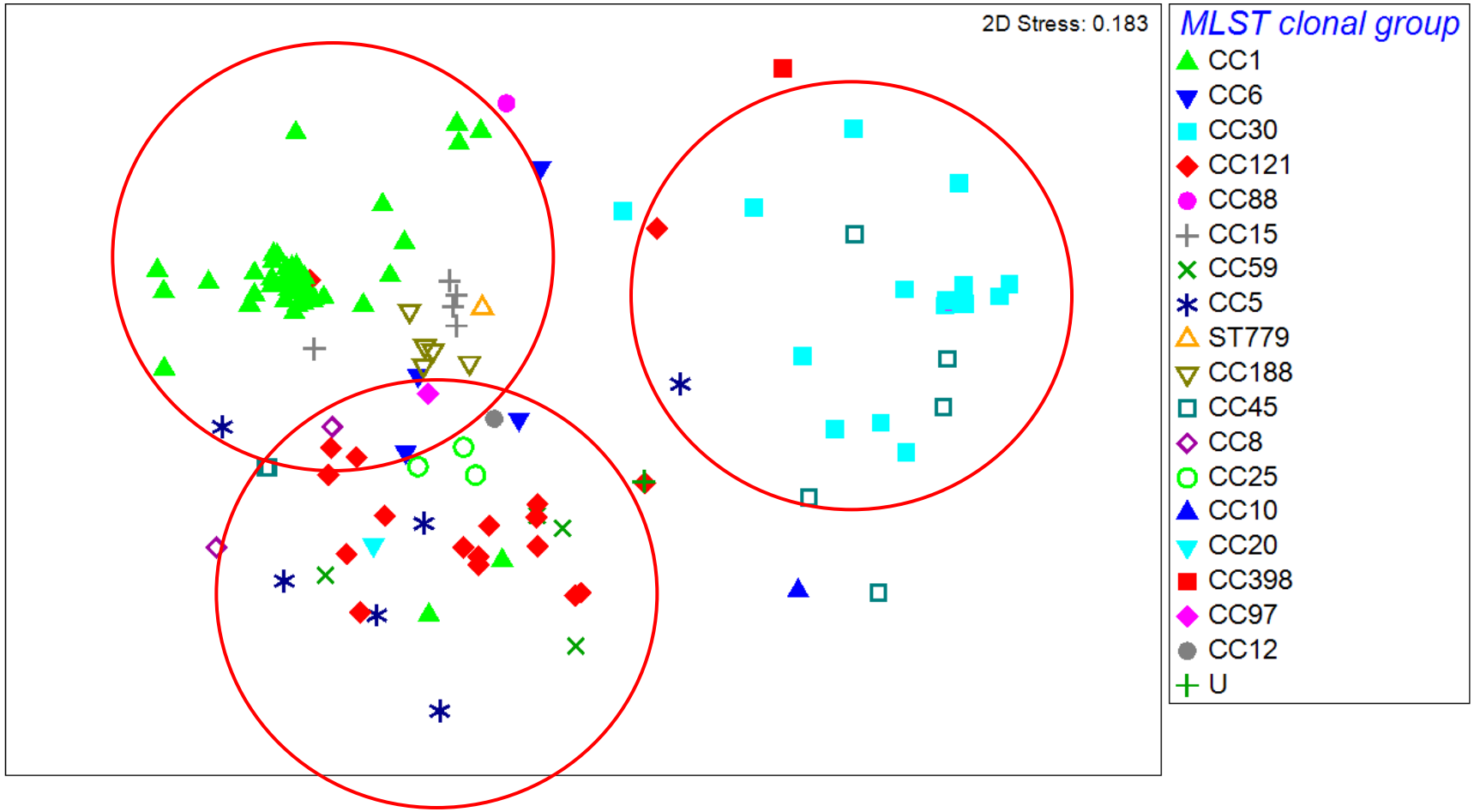
PERMANOVA analysis of virulence factors



- No overall significant difference between invasive and non-invasive strains

Williamson DA et al, Epidemiol Infect, 2014

PERMANOVA analysis of virulence factors



Significant interaction between MLST group and invasive status, which could suggest that different genes may be responsible for invasiveness in different MLST groups

Williamson DA et al, Epidemiol Infect, 2014

Clonality and virulence in MSSA

Potential Associations between Hematogenous Complications and Bacterial Genotype in *Staphylococcus aureus* Infection

Vance G. Fowler, Jr.,[1,3] Charlotte L. Nelson,[3] Lauren M. McIntyre,[4] Barry N. Kreiswirth,[5] Alastair Monk,[6] Gordon L. Archer,[6] Jerome Federspiel,[1] Steven Naidich,[7] Brian Remortel,[9] Thomas Rude,[1] Pamela Brown,[1] L. Barth Reller,[1,2] G. Ralph Corey,[1,3] and Steven R. Gill[8]

[1]Division of Infectious Diseases, Department of Medicine, [2]Clinical Microbiology Laboratory, and [3]Duke Clinical Research Institute, Duke University Medical Center, Durham, North Carolina; [4]University of Florida, Gainesville; [5]Public Health Research Institute, Newark, New Jersey; [6]Virginia Commonwealth University Medical Center, Richmond; [7]eGenomics, Inc., New York, and [8]State University of New York, Buffalo; [9]Institute for Genomic Research, Bethesda, Maryland

Fowler VG et al. *J Infect Dis* 2007; 196: 738-47

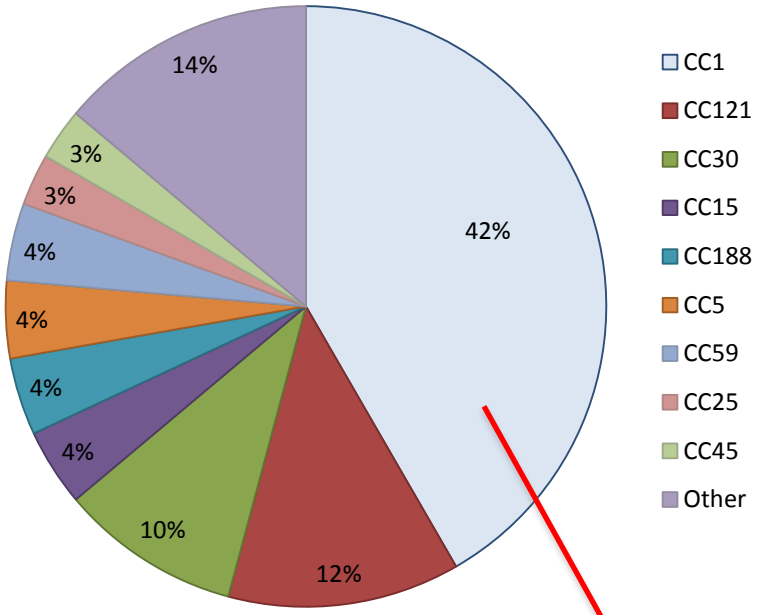
Subpopulations of *Staphylococcus aureus* Clonal Complex 121 Are Associated with Distinct Clinical Entities

Kevin Kurt[1], Jean-Philippe Rasigade[2], Frederic Laurent[2], Richard V. Goering[3], Helena Žemličková[4], Ivana Machova[4], Marc J. Struelens[5], Andreas E. Zautner[6], Silva Holtfreter[7], Barbara Bröker[7], Stephen Ritchie[8], Sin Reaksmey[9], Direk Limmathurotsakul[10], Sharon J. Peacock[10,11], Christiane Cuny[1], Franziska Layer[1], Wolfgang Witte[1], Ulrich Nübel[1]*

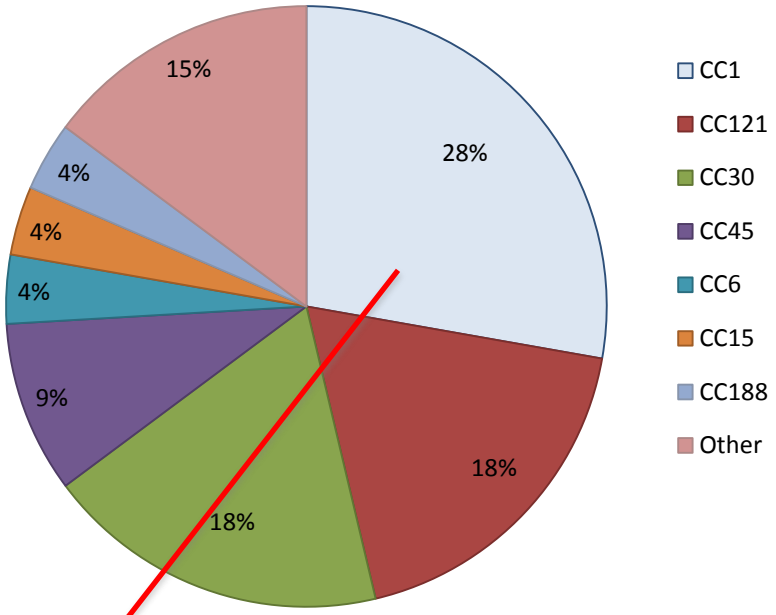
Kurt K et al. *PLoS One* 2013;8(3):e58155

S. aureus clonal complexes

Invasive *S. aureus* isolates (n = 72)



Non-invasive *S. aureus* isolates (n = 54)



All CC1 fusidic acid-resistant, due to *fusC* gene

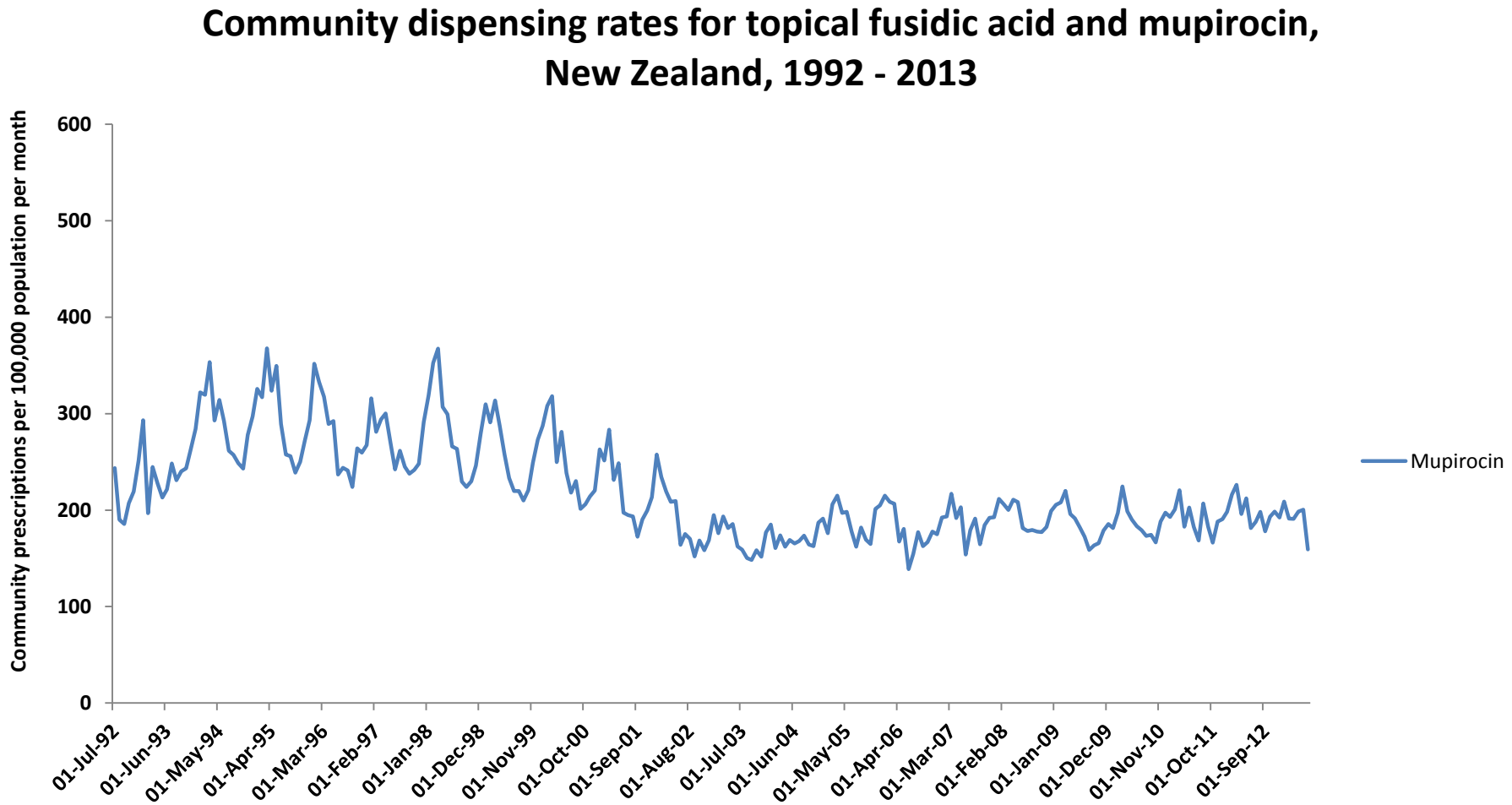
Williamson DA et al, Epidemiol Infect, 2014

Topical antimicrobial usage in NZ

Pharmacoepidemiology of topical antimicrobial dispensing in NZ

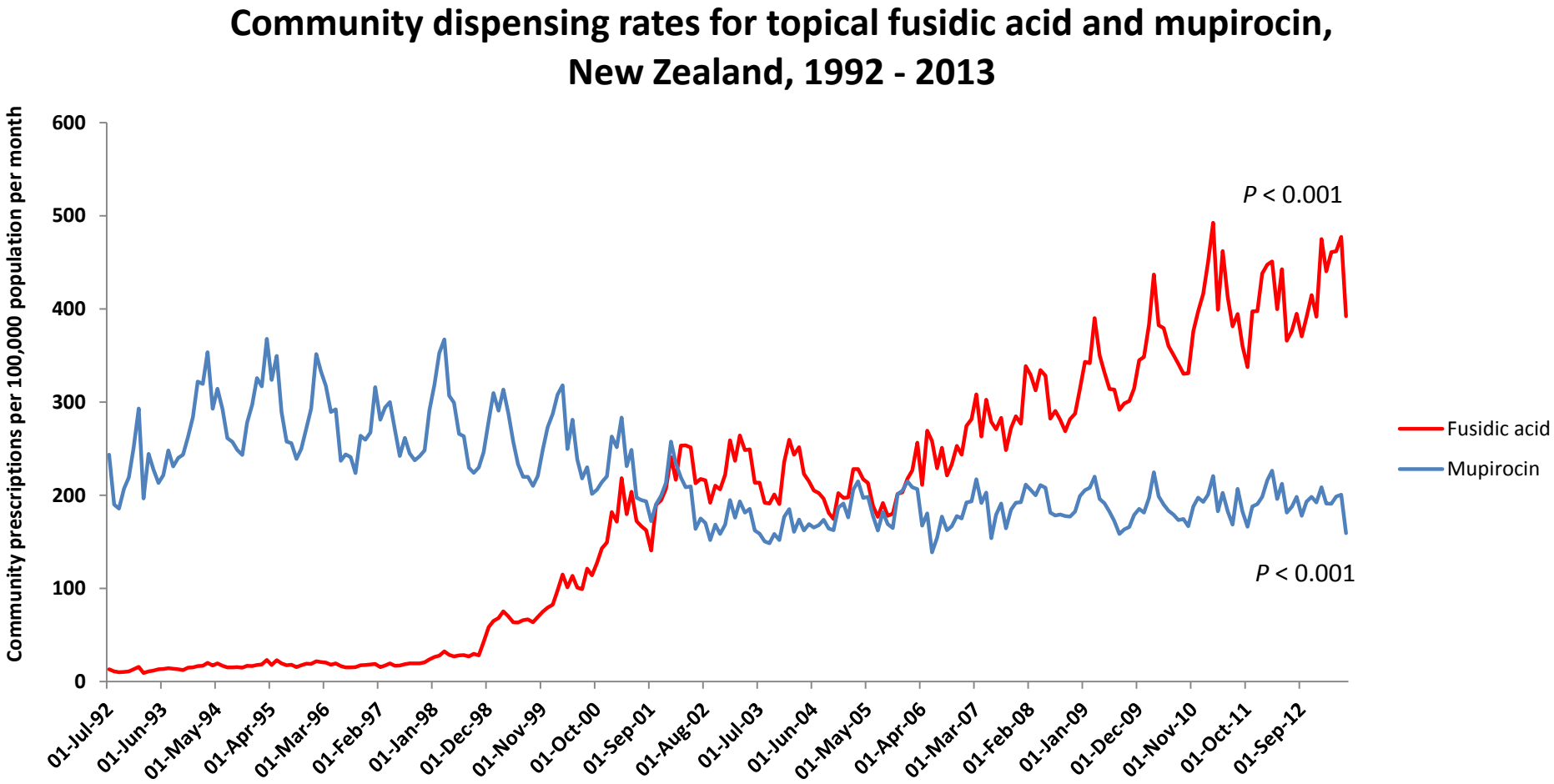
- Topical antimicrobials, such as mupirocin and fusidic acid, are commonly prescribed for a number of dermatological conditions, although evidence-based prescribing supports their use for only a few specific indications
- To date, most antimicrobial stewardship policies have focused exclusively on systemic antimicrobials
- Few studies have assessed the trends and demographics of prescribing for topical antimicrobials

Topical antimicrobial dispensing in NZ



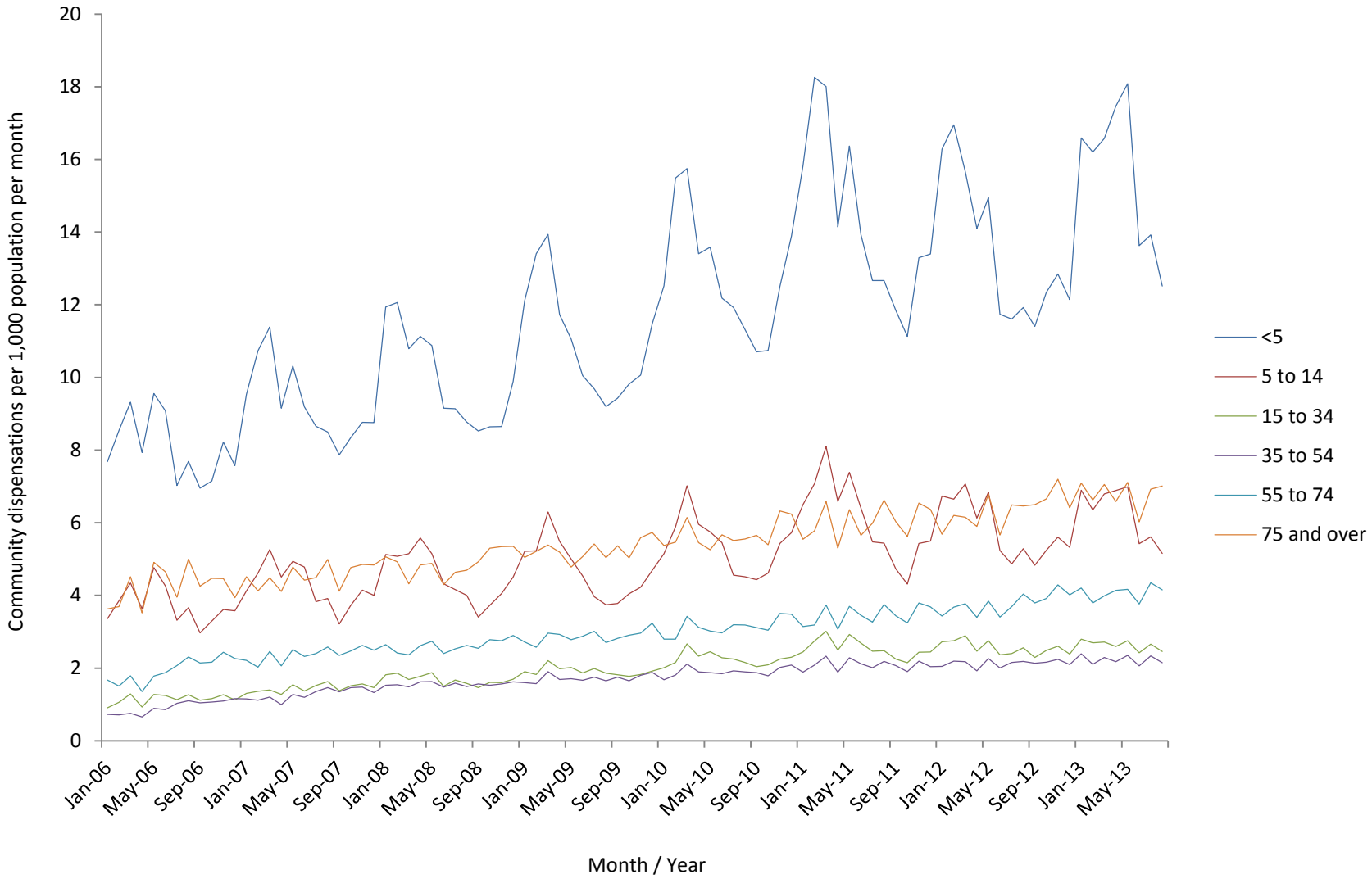
Williamson DA et al. Clinical Infectious Diseases, 2014

Topical antimicrobial dispensing in NZ



Williamson DA et al. Clinical Infectious Diseases, 2014

Demographics of topical FA dispensing



Williamson DA et al. submitted

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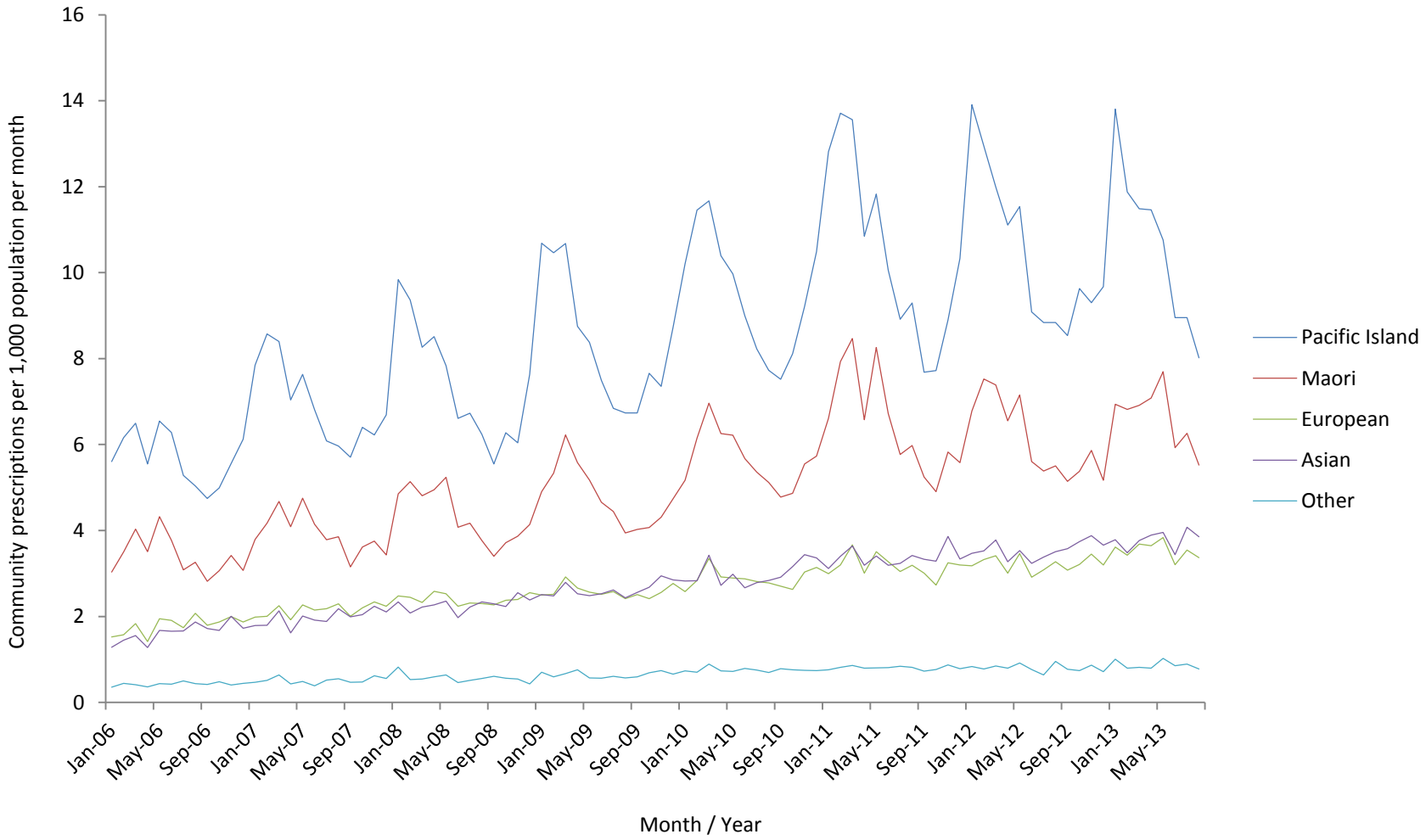

Williamson DA et al. submitted

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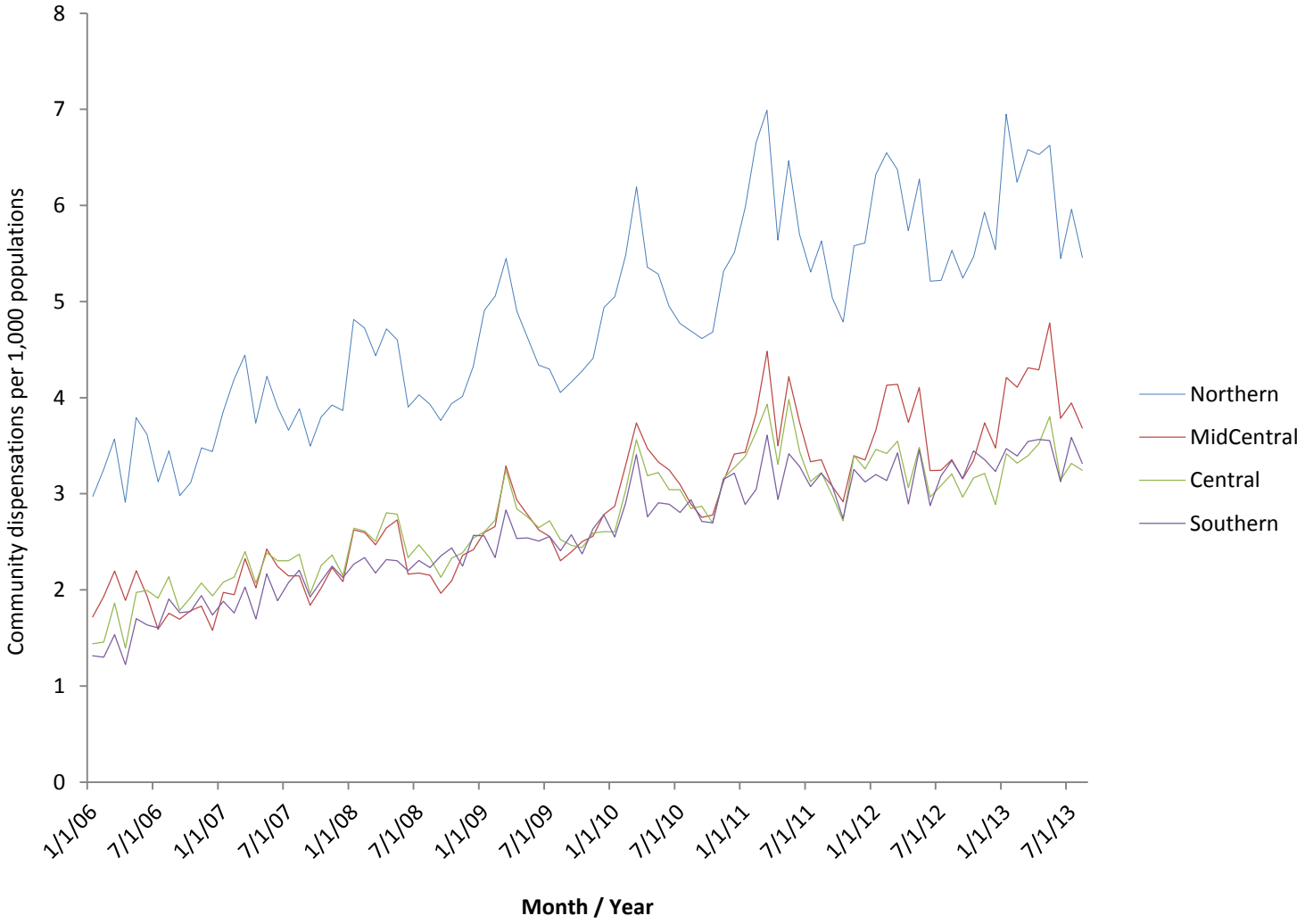
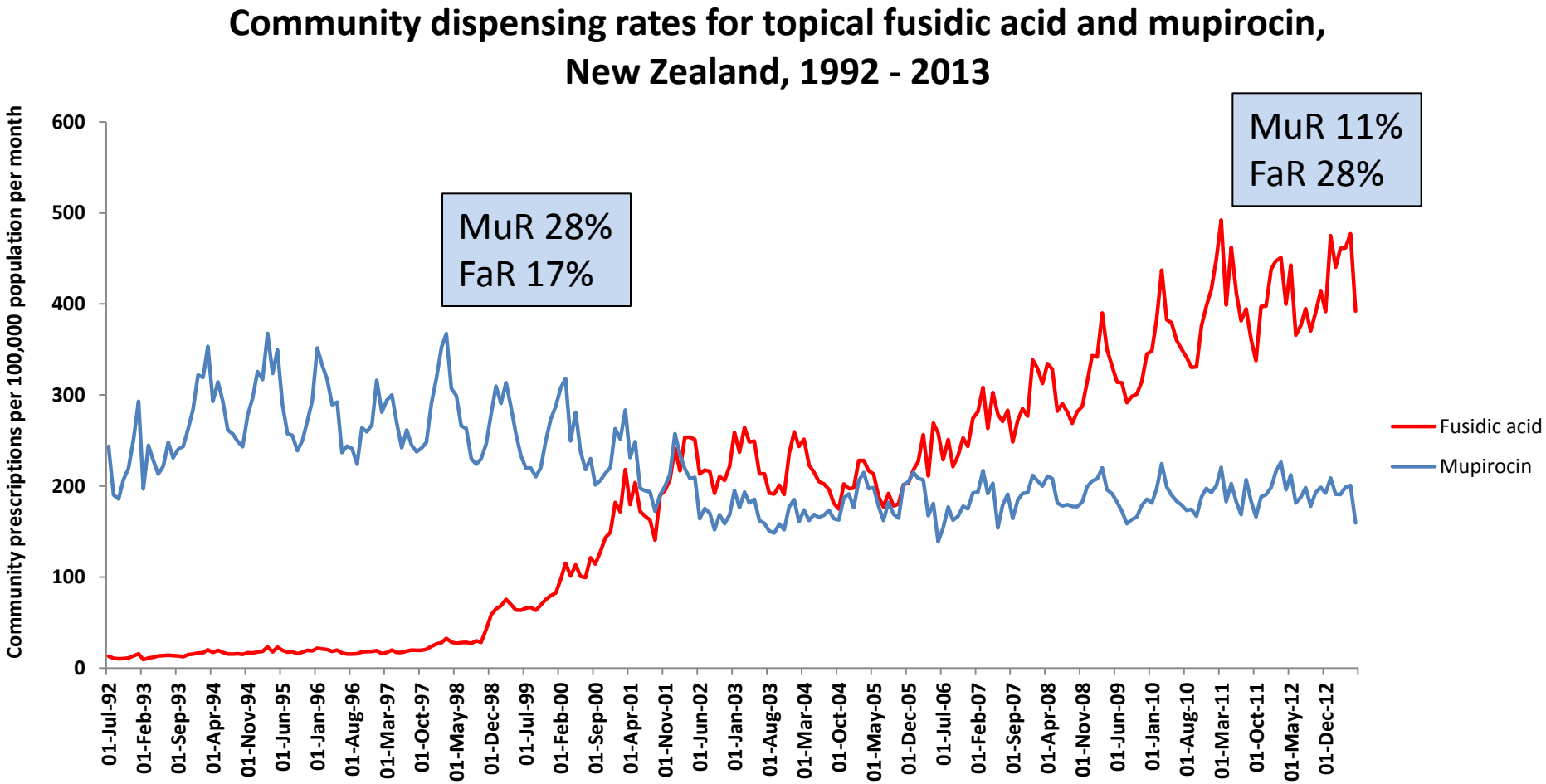


Williamson DA et al. submitted

Fusidic acid resistance in *S. aureus*

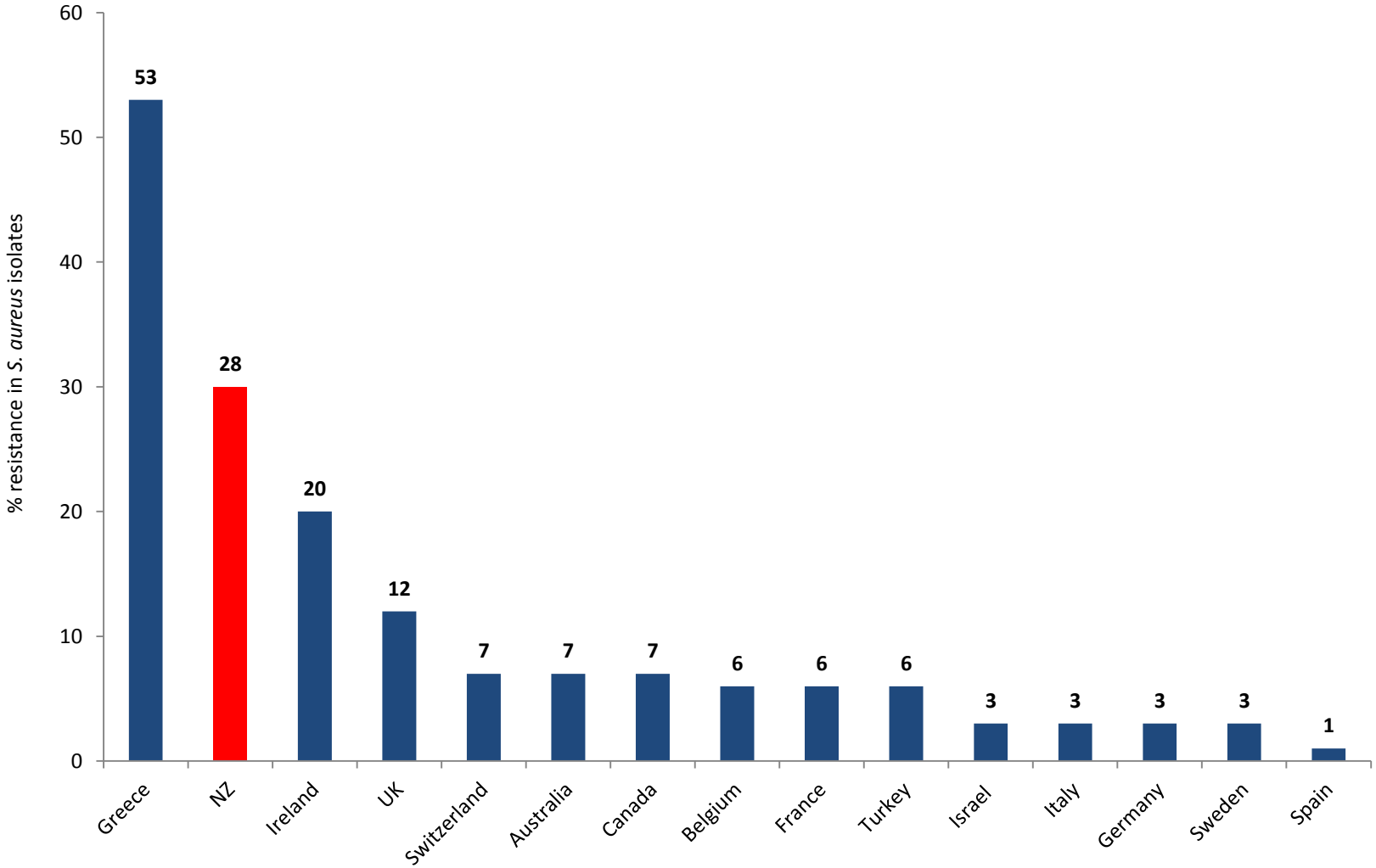
- **What is the impact (if any) of increased fusidic acid prescribing on resistance rates in *S. aureus*?**
- One previous unbiased study of *S. aureus* resistance in NZ in 1999:
  - Mupirocin resistance – 28%
  - Fusidic acid resistance – 17%
  - (MRSA – 2%)
- **2013**: 500 *S. aureus* isolates collected over a two month period in Aug / Sep (random 20 / day)

Topical antimicrobial prescriptions in NZ



Williamson DA et al. Clinical Infectious Diseases, 2014

Fusidic acid resistance rates globally



Castanheira et al, AAC, 2010
Castanheira et al, JAC, 2010

Antimicrobial Susceptibility of *Staphylococcus aureus* in New Zealand in 1999

It is reassuring that the prevalence of MRSA was less than 2%. However, the high prevalence of mupirocin and fusidic acid resistance is of concern as mupirocin is an important topical antibiotic for the eradication of MRSA and renewed interest has been shown in the use of fusidic acid for treating MRSA.

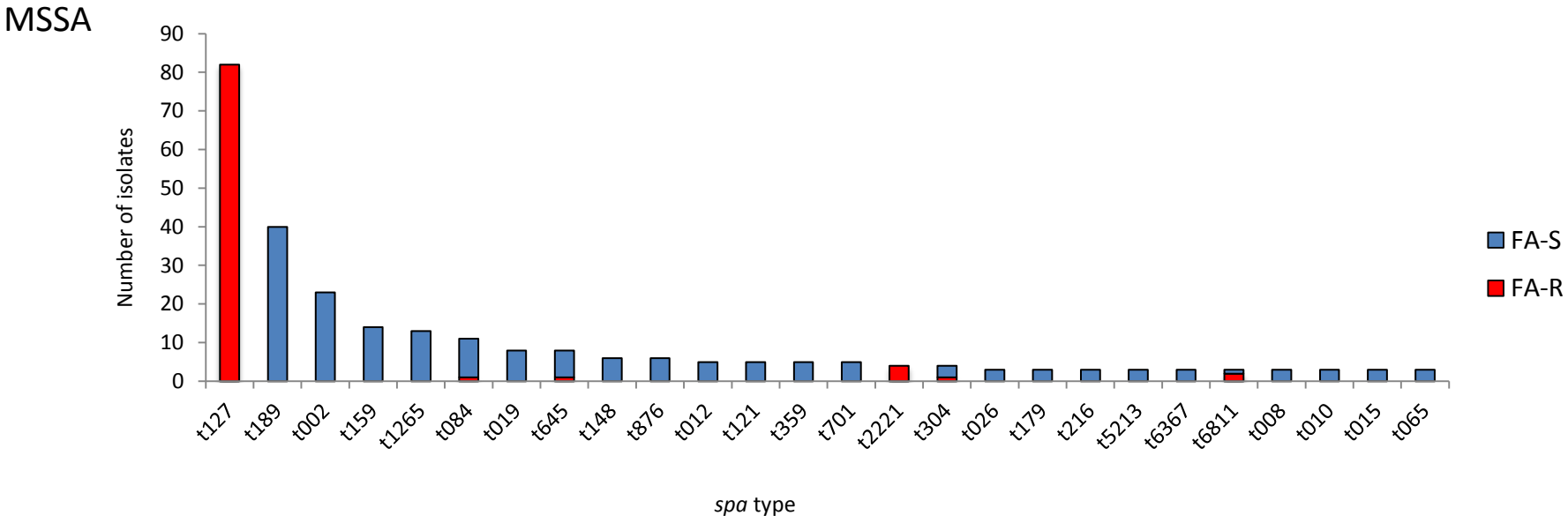
A report prepared for the Ministry of Health as part of the 1998 / 1999 contract (Project C8)

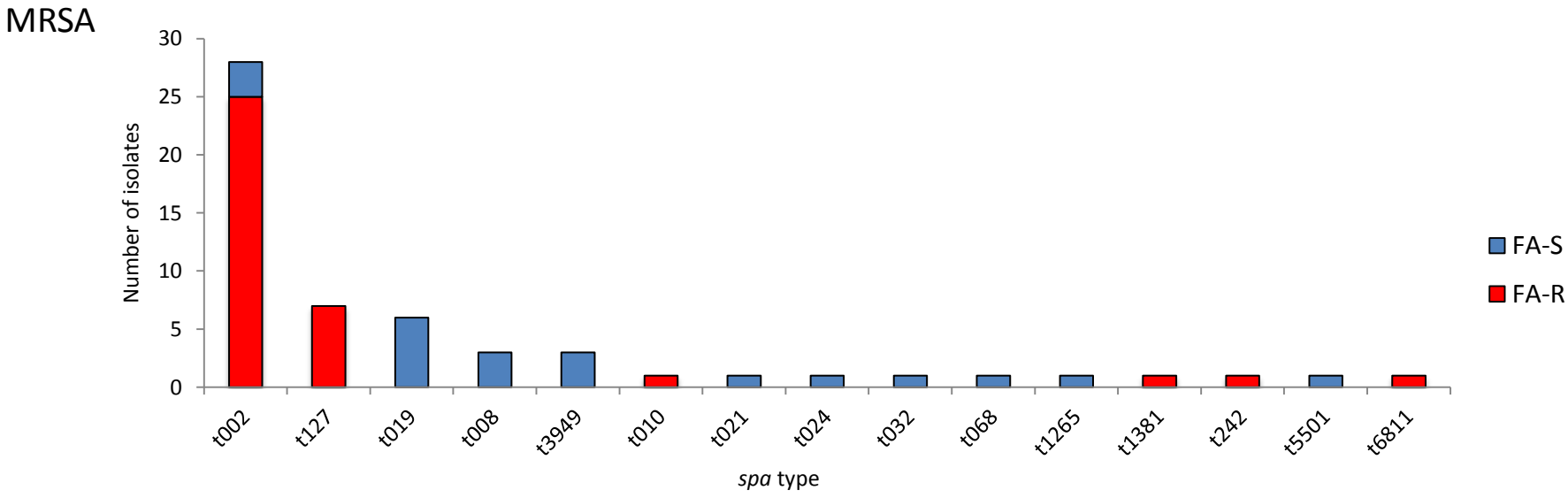
Dumb and Dumber—The Potential Waste of a Useful Antistaphylococcal Agent: Emerging Fusidic Acid Resistance in *Staphylococcus aureus*

Benjamin P. Howden[1,2] and M. Lindsay Grayson[1,3,4]

[1]Infectious Diseases Department, Austin Health, Heidelberg, and Departments of [2]Microbiology and [3]Epidemiology and Preventive Medicine, Monash University, and [4]Department of Medicine, University of Melbourne, Melbourne, Australia

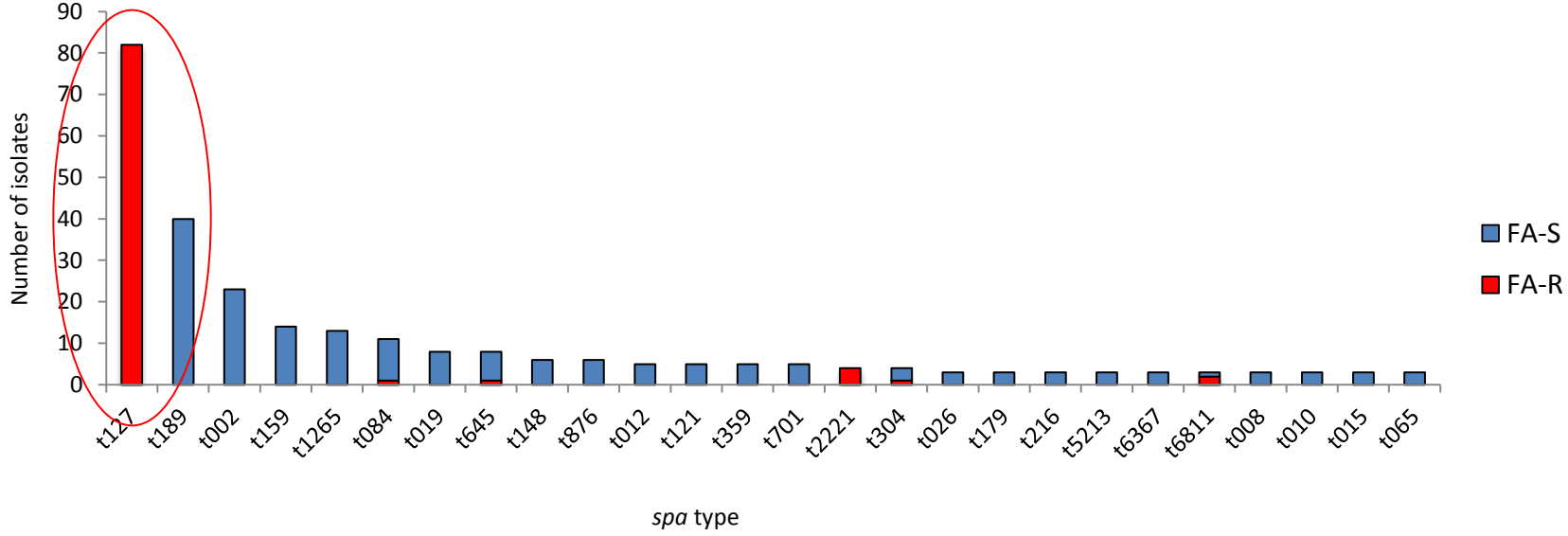
Clinical Infectious Diseases 2006; 42:394–400



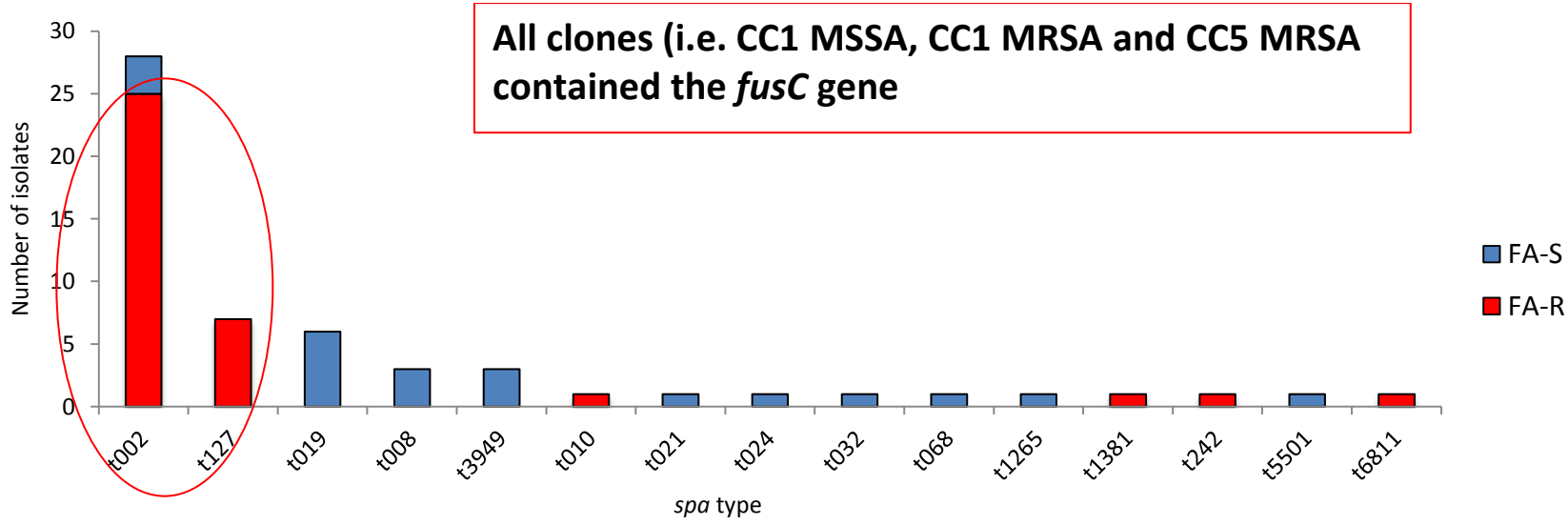


Williamson DA et al. *Clinical Infectious Diseases, 2014*

MSSA



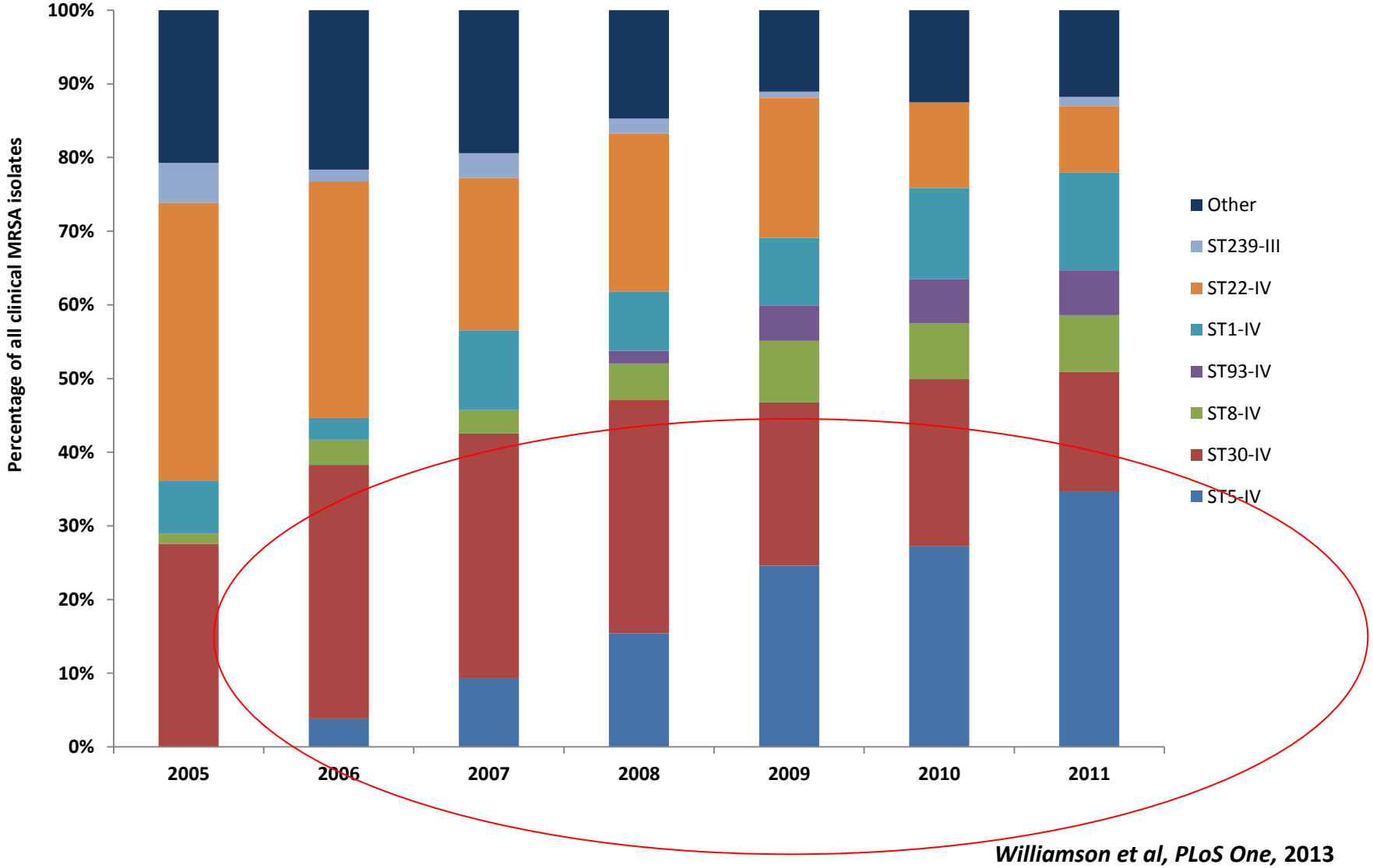
MRSA



All clones (i.e. CC1 MSSA, CC1 MRSA and CC5 MRSA contained the *fusC* gene

Genetic context of *fusC* gene in NZ *S. aureus* clones

Relative proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) clones circulating in New Zealand, 2005–2011 (n=3,323)



***Williamson et al, PLoS One*, 2013**

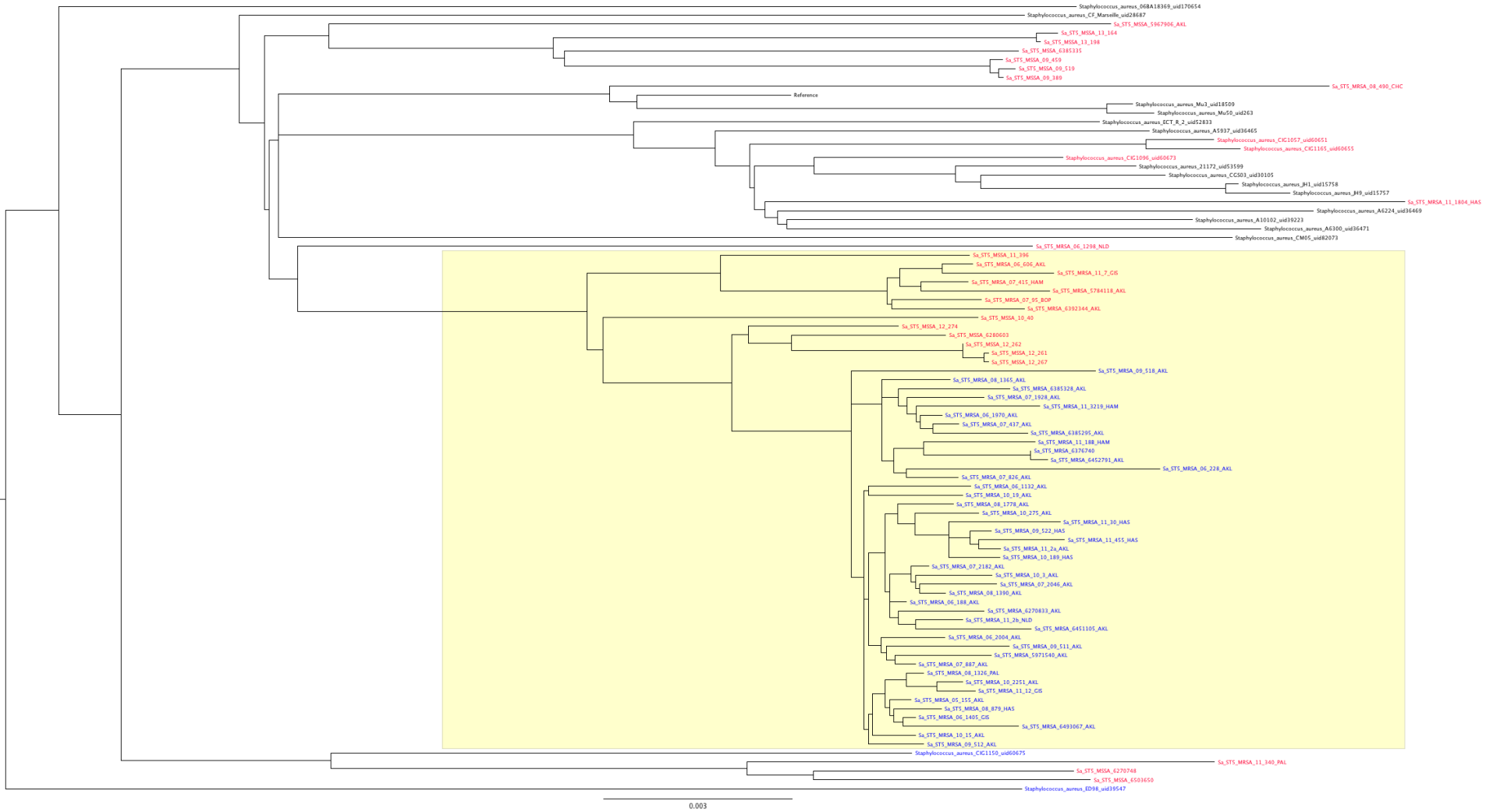
Emergence of ST5 CA-MRSA in NZ

- Where has this clone come from:
  - Imported from overseas?
  - Emerged locally from circulating ST5 MSSA?
- Are infections caused by this clone any different from those caused by other CA-MRSA clones?
- **Why has this clone emerged so rapidly and what are the factors driving the emergence and spread?**

Emergence of ST5 CA-MRSA in NZ

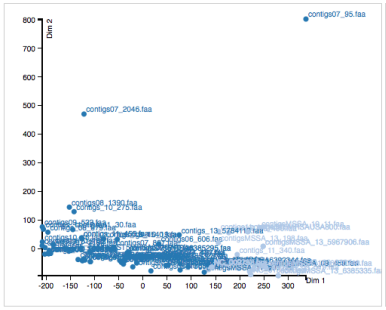
SNP-based phylogeny of ST5 *S. aureus*

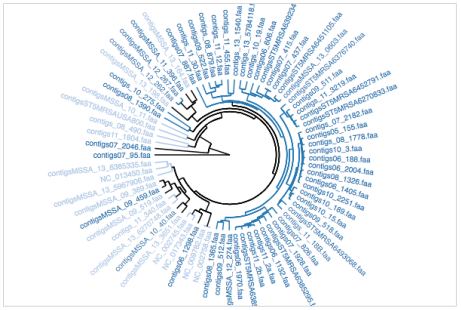
Emergence of ST5 CA-MRSA in NZ



AK3 pangenome

Loaded 73 strains and 3520 orthologs clusters



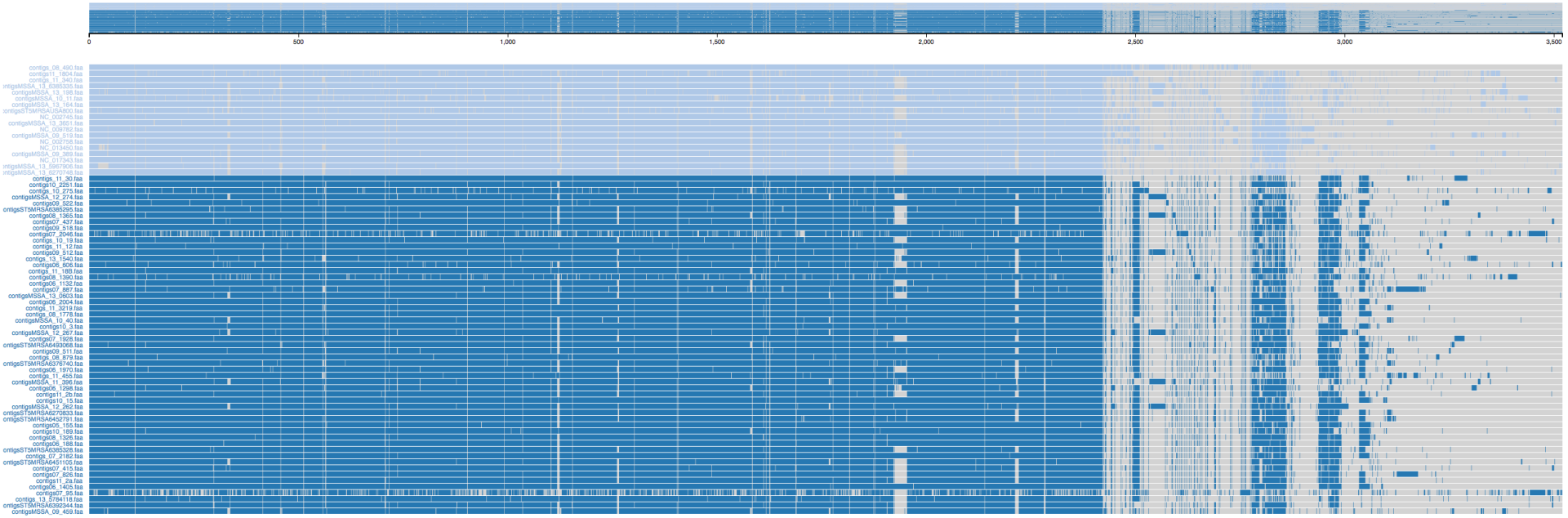


Dendrogram: Radial
Vertical scale: 1.0
Strain sort: CORE
Strain colour: CORE
Gene search:

Colour legend : CORE

0

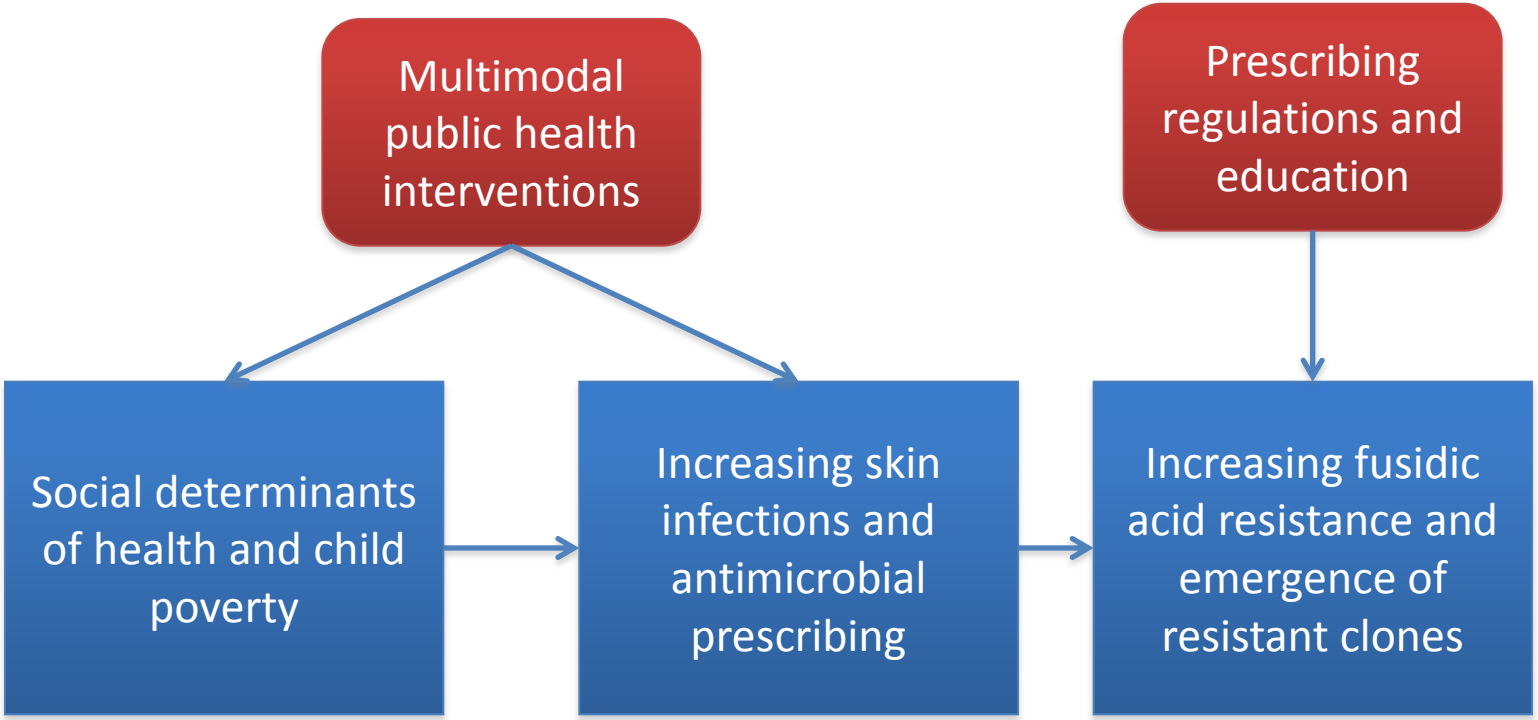
1



Conclusions

- There has been a significant increase in community-onset *S. aureus* skin infections in NZ over the past decade
- This is concurrent with an increase in dispensing of topical fusidic acid, and a subsequent increase in fusidic acid resistance
- This has resulted in dramatic changes in the genomic epidemiology of endemic *S. aureus* clones in New Zealand:
  - Rapid emergence of fusidic-acid resistant ST5 MRSA strain
  - Clonal expansion of fusidic acid resistant CC1 MSSA

What can be done??



Acknowledgements and collaborators

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ESR

AUCKLAND DISTRICT HEALTH BOARD Te Toka Tumai

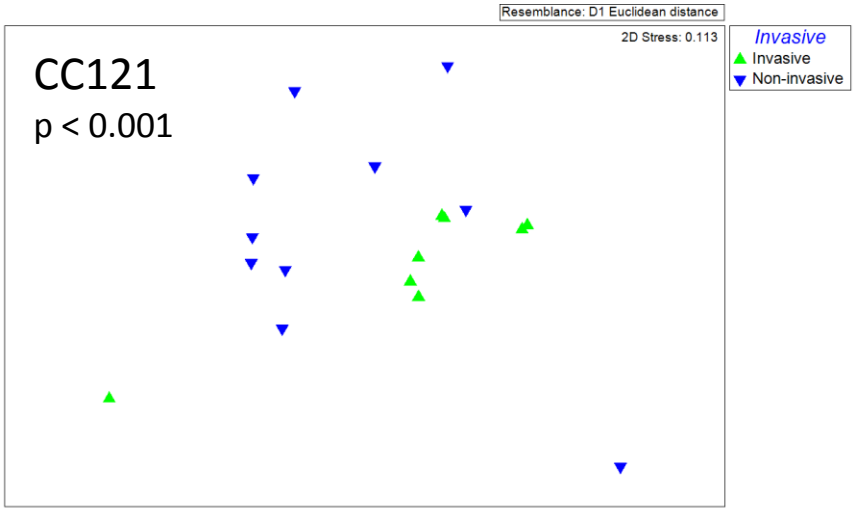
THE UNIVERSITY OF AUCKLAND FACULTY OF MEDICAL AND HEALTH SCIENCES

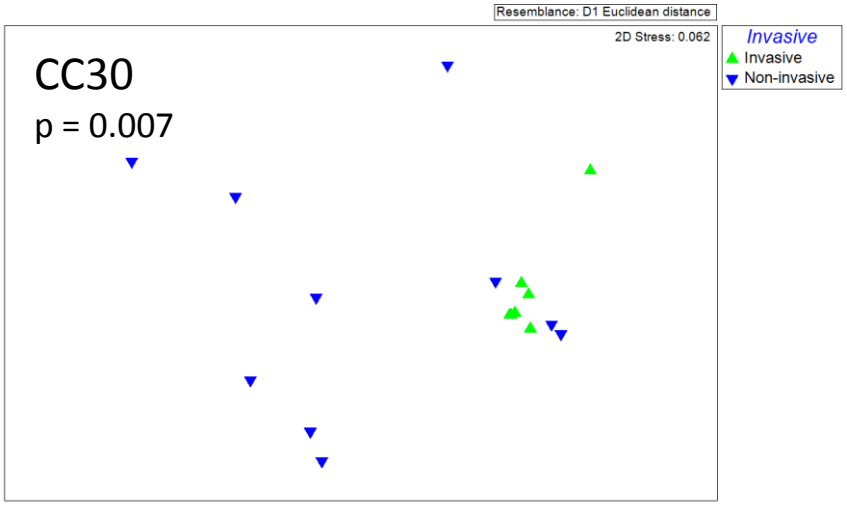
Questions

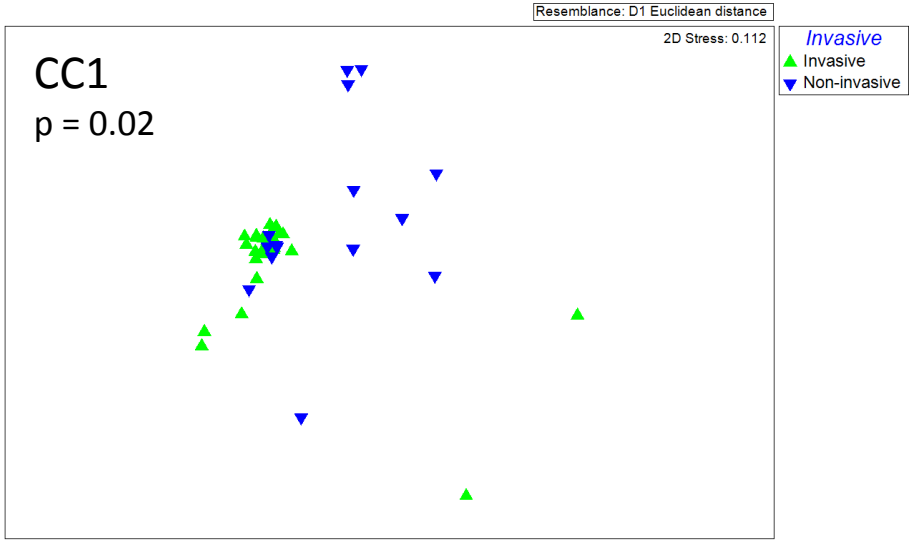
Pharmacoepidemiology of topical antimicrobial dispensingin NZ

- New Zealand has a predominantly publicly funded healthcare system, although patients aged over six years pay a fee to access primary care, and a fee for prescription medications
- The exact fee depends on whether the medication is fully or partially subsidised by the New Zealand Government
- Data on government-subsidised community dispensing (both fully and partially funded) is maintained in a central data warehouse, the 'National Pharmaceutical Collection'
- Dispensing data for topical formulations of fusidic acid and mupirocin were obtained from January 1992 to August 2013

PERMANOVA analysis of virulence factors







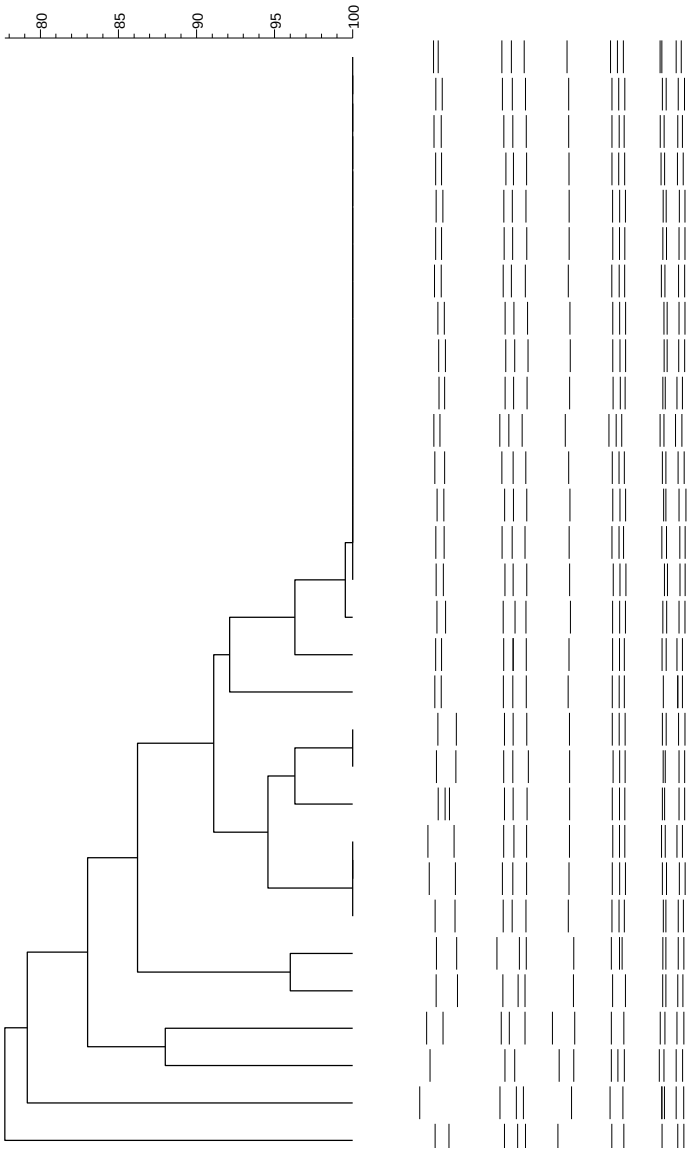
Emerging MRSA in New Zealand: CC398

- First recognised in swine farmers in early 2000s (LA-MRSA)
- Accounts for approximately 25% MRSA cases in Netherlands
- Found in diverse livestock hosts around the globe
- New Zealand:
  - Twelve (known) isolations in New Zealand
  - Eleven cases from same geographic region
  - Evidence of contact with pigs or pig carcasses in three patients
  - *lukF-PV* / *lukS-PV* - positive CC398 in two patients

J Antimicrob Chemother, 2013

Dice (Opt:0.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]

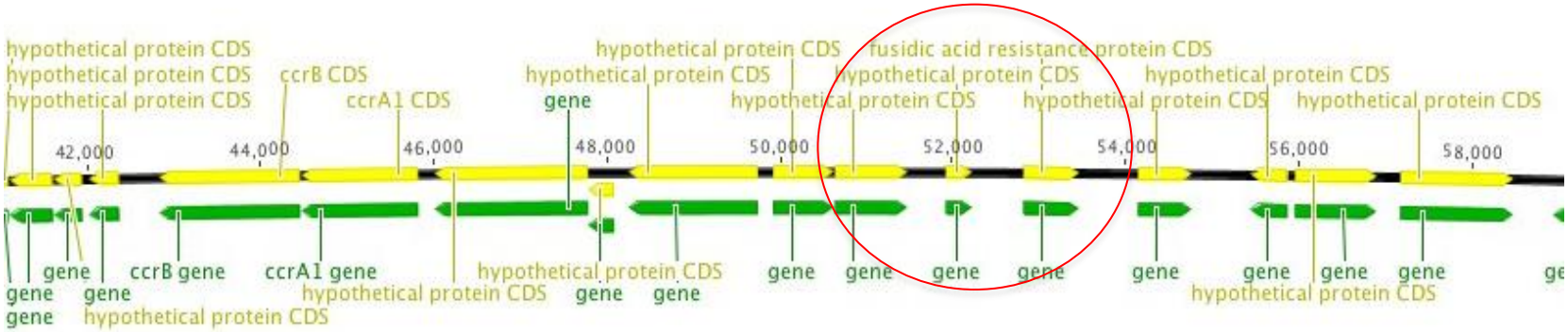
SmaI **SmaI**

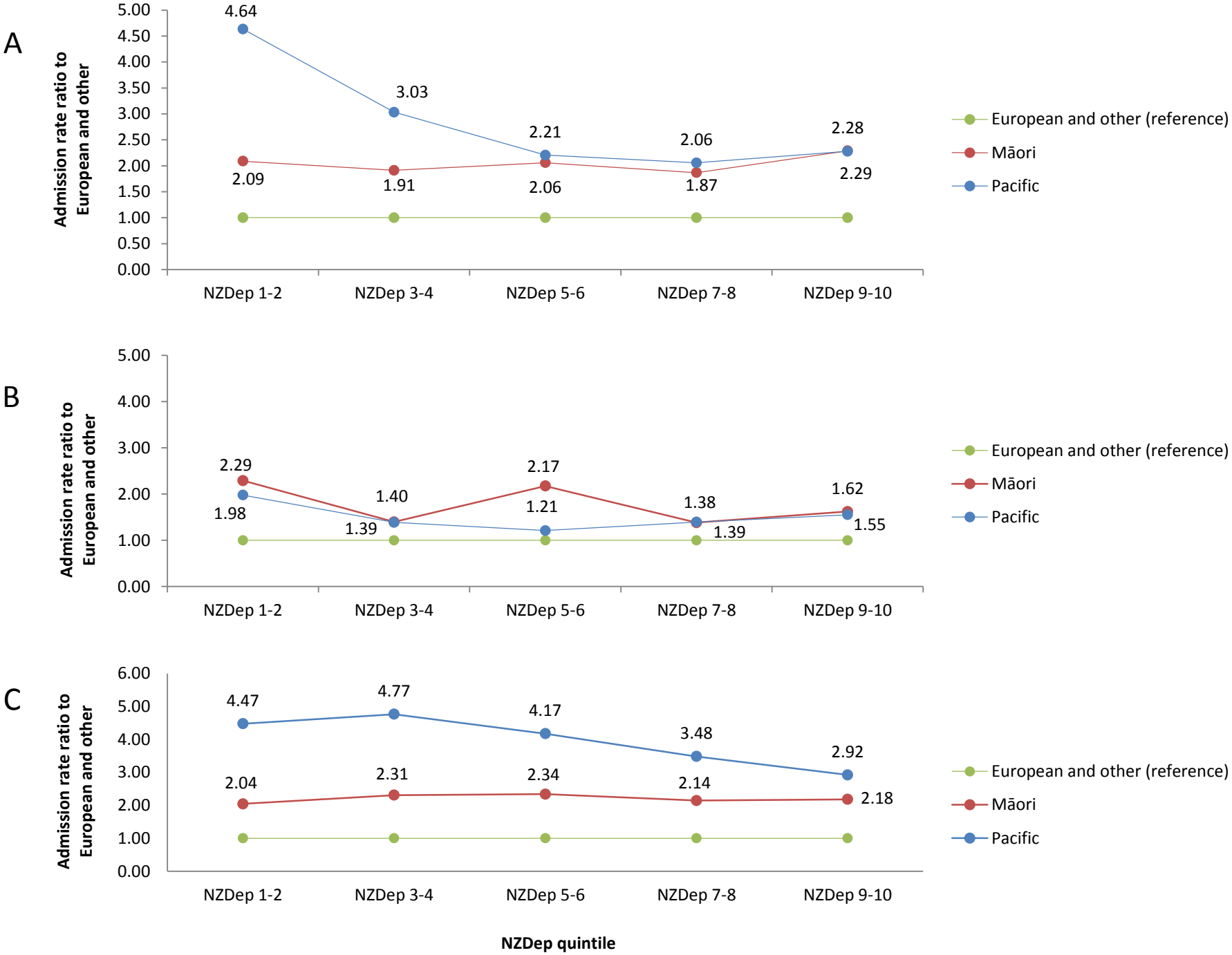


MRS05/0115	t002	AK3 index	FaR
MRS08/0879	t062	AK3	FaR
MRS08/1326	t002	AK3	CipS FaR
MRS08/1390	t002	AK3	CipS ClR EmR FaR
MRS08/1778	t045	AK3	CipS FaR
MRS09/0518	t002	AK3	ClR EmR FaR
MRS09/0522	t045	AK3	FaR
MRS10/0008	t002	AK3	FaR
MRS10/0015	t002	AK3	FaR
MRS10/0189	t045	AK3	CipS FaR
MRS10/2251	t6787	AK3	CipS CoS EmS FaR GmS MuS RfS
MRS11/0012	t002	AK3	CipS FaR
MRS11/3219	t002	AK3	CipR EmR FaR
MRS12/0001	t002	AK3	CipS FaR
MRS12/0021	t002	AK3	CipS FaR
MRS10/0003	t002	AK3	ClR EmR FaR
MRS09/0511	t002	AK3	FaR
MRS12/0029	t045	AK3	CipS FaR
MRS11/0002	t002	AK3	CipS ClR EmR FaR
MRS12/0004	t002	AK3	CipS FaR
MRS11/0030	t045	AK3	CipR FaR GmR MuR
MRS08/1365	t002	AK3	CipS FaR
MRS09/0512	t002	AK3	FaR
MRS11/0263	t045	AK3	CipS FaR
MRS11/0007	t002	AK3	CipS FaR
MRS11/0340	t5677	AK3	CipS ClS CoS EmS FaS MuS TeS
MRS08/0490	t002		CipR EmR GmR
WA MRSA-3		WA MRSA-3 refere.	
NRS/387		USA800	FaS MuS
MRS11/1804	t045		CipR EmR FaS MuS

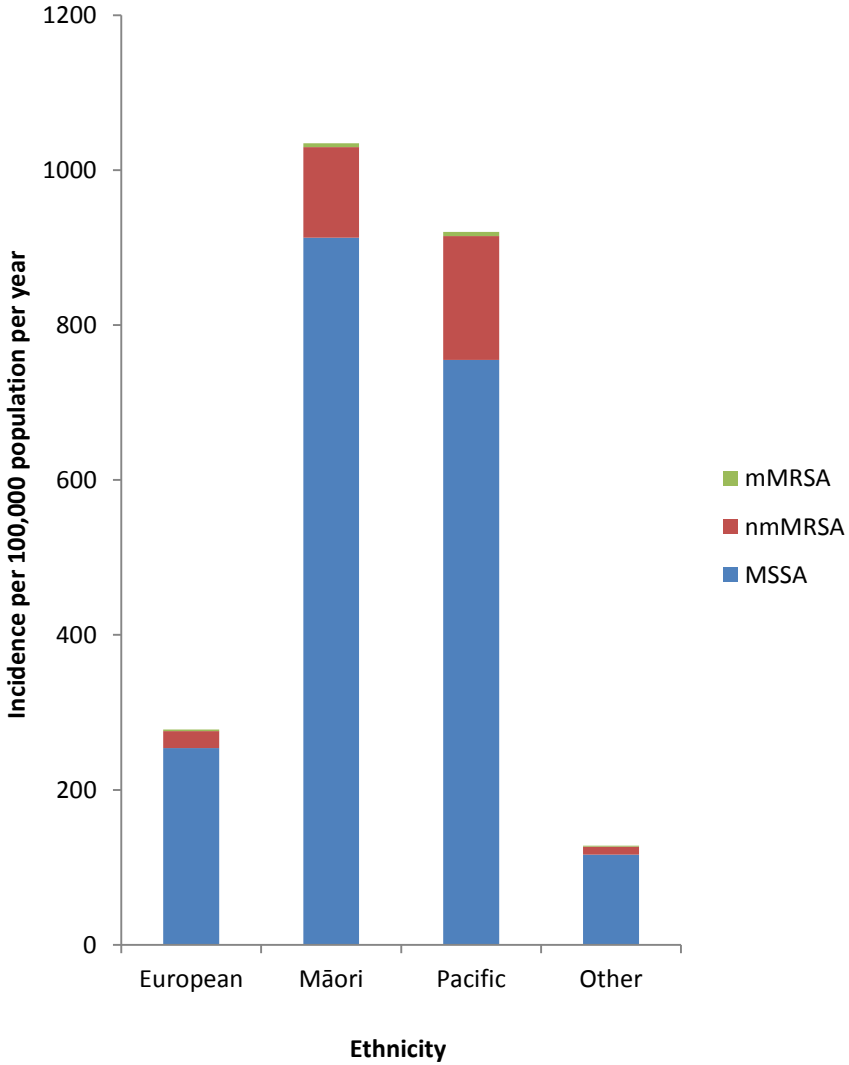
Genetics of fusidic acid resistance

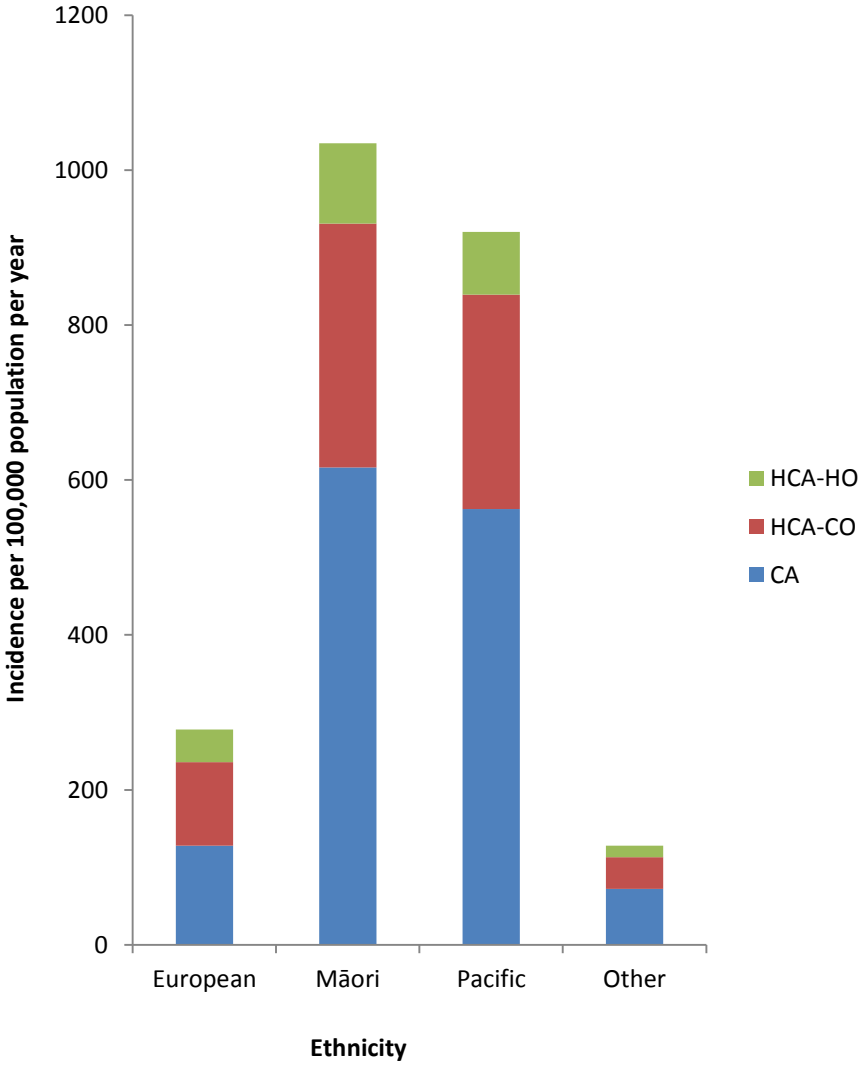
- All CC1 (*spa* t127) isolates identified to date contain *fusC* gene (including isolates from 1999)
- *fusC* gene in CC1 contained in SCC (SCC*fus*)
- Also associated with specific recombinases (*ccrA1* and *ccrB1*)
- ? genetic background of *fusC* gene in CC5 MRSA
-




A
Admission rate ratio to European and other
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
4.64
3.03
2.21
2.06
2.28
2.09
1.91
2.06
1.87
2.29
NZDep 1-2
NZDep 3-4
NZDep 5-6
NZDep 7-8
NZDep 9-10
European and other (reference)
Māori
Pacific
B
Admission rate ratio to European and other
5.00
4.00
3.00
2.00
1.00
0.00
2.29
1.98
1.40
1.39
2.17
1.21
1.38
1.39
1.62
1.55
NZDep 1-2
NZDep 3-4
NZDep 5-6
NZDep 7-8
NZDep 9-10
European and other (reference)
Māori
Pacific
C
Admission rate ratio to European and other
6.00
5.00
4.00
3.00
2.00
1.00
0.00
4.47
4.77
4.17
3.48
2.92
2.04
2.31
2.34
2.14
2.18
NZDep 1-2
NZDep 3-4
NZDep 5-6
NZDep 7-8
NZDep 9-10
European and other (reference)
Māori
Pacific
NZDep quintile

B.



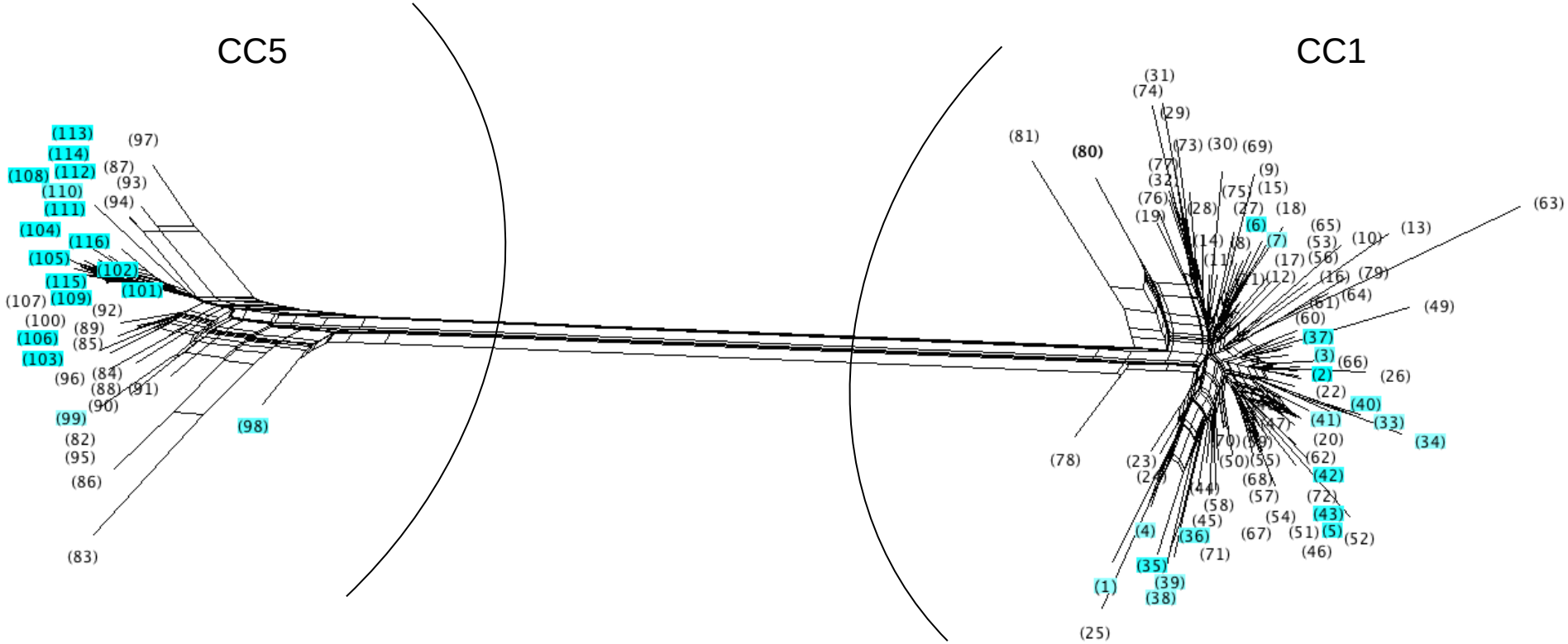


Williamson DA et al BMC Infect Dis, 2013

Pharmacoepidemiology of topical antimicrobial prescriptions in NZ

- Dispensing data for topical formulations of fusidic acid and mupirocin were obtained from January 1992 to August 2013
- Demographic data available from 2006 onwards
- Since 1997, fusidic acid has been fully subsidised, meaning that patients (aged >6 years) pay a nominal prescription charge
- Until 2000, mupirocin was available to purchase 'over the counter'. From 2001, it has been partially subsidised, meaning that in addition to the nominal prescription charge, patients (aged >6 years) pay an additional charge (approximately NZ$3)

S1.



SCC*mec-fusC* (CC5 MRSA)