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Clinical and molecular epidemiology of community-onset invasive *Staphylococcus aureus* infection in New Zealand children

D. A. WILLIAMSON[1,2,3]*, S. R. RITCHIE[1], S. A. ROBERTS[2], G. W. COOMBS[4,5], M. G. THOMAS[1], O. HANNAFORD[6], M. G. BAKER[7], D. LENNON[1] AND J. D. FRASER[1]

[1] *Faculty of Medical and Health Sciences, University of Auckland, New Zealand*
[2] *Department of Clinical Microbiology, Auckland District Health Board, New Zealand*
[3] *Institute of Environmental Science and Research, Wellington, New Zealand*
[4] *Australian Collaborating Centre for Enterococcus and Staphylococcus Species (ACCESS) Typing and Research, PathWest Laboratory Medicine, Perth, Australia*
[5] *School of Biomedical Sciences, Curtin University, Perth, Australia*
[6] *Department of Statistics, NZIAS, Massey University, Albany, New Zealand*
[7] *Department of Public Health, University of Otago, Wellington, New Zealand*

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SUMMARY

Our aim was to describe the epidemiology and incidence of community-onset invasive *S. aureus* disease in children presenting to our hospital, and to compare the clonal complexes and virulence genes of *S. aureus* strains causing invasive and non-invasive disease. The virulence gene repertoire of invasive disease isolates was characterized using DNA microarray and compared with the virulence gene repertoire of non-invasive *S. aureus* isolates. Over the study period, 163 children had an invasive *S. aureus* infection. There was no difference in the distribution of clonal complexes or in the prevalence of genes encoding virulence factors between invasive and non-invasive isolates. Future research should include a strong focus on identifying the host and environmental factors that, along with organism virulence factors, are contributing to the patterns of invasive *S. aureus* disease observed in New Zealand.

Key words: Paediatrics, *Staphylococcus aureus*, statistics.

INTRODUCTION

Staphylococcus aureus is a major human pathogen, both in adults and children [1, 2]. The spectrum of *S. aureus* disease ranges from non-invasive manifestations such as skin and soft tissue infection (SSTI), to serious invasive disease such as pneumonia and endocarditis [3]. Over the past two decades, the incidence of both invasive and non-invasive *S. aureus* disease has increased in several settings, including Europe, North America and New Zealand [1, 4–7]. In many countries, particularly the USA, this increase has been driven by the unprecedented rise in infections caused by a predominant strain of community-associated methicillin-resistant *S. aureus* (CA-MRSA) [8].

New Zealand has a high incidence of both invasive and non-invasive *S. aureus* disease [5, 9–11]. Moreover, similar to reports from North America, the incidence of community-associated paediatric SSTI is increasing in New Zealand [5, 12]. However, in contrast to North America, most *S. aureus* infections in

* Author for correspondence: Dr D. A. Williamson, Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Park Road, Grafton, Auckland, New Zealand.
(Email: deb.williamson@auckland.ac.nz)

New Zealand are caused by methicillin-susceptible *S. aureus* (MSSA) strains, rather than CA-MRSA [10, 11].

Clinical isolates of *S. aureus* harbour an array of virulence factors involved in adhesion, invasion and immune evasion [1, 13, 14]. In particular, the Panton–Valentine leucocidin toxin (PVL) has been extensively studied regarding its role in disease pathogenesis and severity [15–18]. PVL is a bi-component pore-forming cytotoxin and has been implicated as an important virulence factor in both invasive and non-invasive *S. aureus* disease [9, 18]. However, a recent meta-analysis found that, in comparison with isolates causing SSTI, the *lukF-PV* and *lukS-PV* genes encoding PVL were less commonly identified in *S. aureus* isolates causing invasive disease [15]. Moreover, the presence of the *lukF-PV* and *lukS-PV* genes in invasive *S. aureus* isolates was not shown to correlate with adverse clinical outcomes [15]. Similarly, a recent Australian study also found no difference in mortality between patients with 'PVL-positive' and 'PVL-negative' invasive *S. aureus* infections [19]. In addition, a previous study in our setting found that the prevalence of the *lukF-PV* and *lukS-PV* genes in nasal carriage MSSA isolates was the same as the prevalence of the *lukF-PV* and *lukS-PV* genes in disease-causing MSSA isolates (31% *vs.* 37%, respectively; $P = 0{\cdot}33$), suggesting that PVL is not the primary determinant of *S. aureus* disease severity [9]. To date, however, few studies have systematically assessed and compared molecular determinants of virulence, other than PVL, between *S. aureus* isolates causing invasive and non-invasive disease.

Accordingly, we sought to (i) describe the incidence and demographics of invasive *S. aureus* disease in children admitted to our hospital between 2007 and 2010, and (ii) compare the CCs and virulence gene repertoire (including PVL), of *S. aureus* causing invasive and non-invasive disease in these patients.

METHODS

Setting, patients and isolates

Starship Children's Hospital (SCH) in Auckland, New Zealand is a tertiary-level university-affiliated institution, serving a population of ~500 000 inhabitants, within a larger metropolitan population of 1·46 million. We performed a retrospective, cross-sectional analysis of all children (aged < 15 years) admitted to SCH with *S. aureus* infections over the 4-year period from January 2007 and December 2010. All children with *S. aureus* isolated from a clinical specimen were identified from the laboratory database in the Department of Microbiology, Auckland City Hospital. A unique *S. aureus* infection was defined as the first positive *S. aureus* culture taken from the same patient within a 30-day period. All *S. aureus* isolates were phenotypically identified using standard laboratory methods. Antimicrobial susceptibility testing of *S. aureus* isolates was performed by agar dilution, and results were interpreted according to Clinical and Laboratory Standards Institute recommendations [20].

Data collection and case definition

Using the hospital information database, demographic information was obtained about each patient who cultured *S. aureus* from a clinical specimen. The following information was recorded: age, gender, ethnicity, and discharge diagnoses associated with each hospital admission, based on the International Classification of Disease, Tenth Revision (ICD-10) codes [21].

Similar to previously described methodology [22], a list of ICD-10 codes was developed for *S. aureus*-related clinical syndromes (see online Supplementary Table S1). These ICD-10 codes were then grouped into the following broad categories for analysis: (i) skin and soft tissue infection (SSTI), (ii) musculoskeletal infection, (iii) respiratory infection, (iv) endovascular infection, (v) central nervous system infection and (vi) bacteremia, site not specified. For cases that had $\geqslant 1$ of these ICD-10 codes associated with a *S. aureus* culture, the principal discharge diagnosis was regarded as the representative clinical syndrome. A case was described as community-onset if *S. aureus* was isolated from a patient within 48 h of hospital admission.

All cases of *S. aureus* bloodstream infection were classified as invasive infections. In addition, other invasive *S. aureus* infections were defined by the isolation of *S. aureus* from a sterile body site (as defined by the Centers for Disease Control and Prevention Active Bacterial Core surveillance program [4]), plus $\geqslant 1$ ICD-10 discharge codes associated with an appropriate clinical syndrome (Supplementary Table S1).

Molecular characterization and comparison of invasive *vs.* non-invasive *S. aureus* isolates

Detailed molecular characterization of invasive *S. aureus* isolates was performed by DNA microarray

Table 1. *Clinical characteristics of children with community-onset invasive* Staphylococcus aureus *infections admitted to Starship Children's Hospital, Auckland, New Zealand, 2007–2010*

Characteristic	Number of children with invasive *S. aureus* infection, $n = 163$ (%)
Male gender	92 (56·4)
Age, median, years (IQR)	7 (0–00)
Site/type of infection	
Musculoskeletal	91 (56)
Bacteraemia/sepsis	37 (23)
Respiratory	27 (17)
Endovascular	7 (4)
Central nervous system	1 (1)
Ethnicity	
European	49 (30)
Māori	46 (28)
Pacific Islander	53 (33)
Other	15 (9)

IQR, Interquartile; CNS, central nervous system.

analysis (StaphType, Clondiag, Germany) using previously described methods [23]. In brief, this assay detects 334 target sequences in the *S. aureus* genome, corresponding to 186 genes and their allelic variants. This allows for: (i) broad classification of *S. aureus* into major clonal complexes (CCs) [24], (ii) classification of the SCC*mec* complex into distinct allotypes [25], and (iii) detection of genes associated with antimicrobial resistance, virulence and adhesion [24]. In order to compare molecular features of invasive and non-invasive isolates, DNA microarray analysis was also performed on a sample of *S. aureus* isolates obtained from paediatric patients attending SCH with uncomplicated, non-invasive *S. aureus* SSTI. These isolates were obtained during the same study time period (between February 2008 and April 2008), and were collected as part of a previous study conducted in our department [9].

Statistical analysis

Age-standardized incidence rates for children presenting with community-onset invasive *S. aureus* infections were calculated using denominator population information from the 2006 New Zealand census, and from projected population data for the Auckland region [26]. Rates were calculated for the following ethnicities: European, Māori (Indigenous New Zealander), Pacific Peoples, and other ethnicities. Categorical variables were compared using the χ^2 or Fisher's exact tests, and non-parametric variables were compared using the Kruskal–Wallis analysis of variance test. Two-dimensional multi-dimensional scaling (MDS) was used to visualize *S. aureus* subgroups following formation of a distance matrix derived from the DNA array using PRIMER v. 6.1.15 (Primer-E, UK). The null hypothesis that there was no difference in virulence genes between invasive and non-invasive *S. aureus* isolates was tested using permutational analysis of variance (PERMANOVA) [27]. In addition, the interaction between CCs, virulence genes and invasive *vs*. non-invasive status was also assessed using PERMANOVA. Statistical analysis was performed using GraphPad Prism version 5.02 (GraphPad Software, USA), PERMANOVA+ version 1.0.2 (Primer-E, UK) or R version 3.0.1 (R Foundation, Austria). A two-tailed P value of $<0{\cdot}05$ was considered statistically significant.

Ethical approval

The Northern X Ethics Committee, New Zealand, granted ethical approval for this study.

RESULTS

Incidence and clinical features of co-invasive *S. aureus* infections

Between January 2007 and December 2010, 2329 children had *S. aureus* isolated from a clinical specimen plus $\geqslant 1$ ICD-10 discharge code(s) associated with a *S. aureus*-related clinical syndrome. Of these 2329 children, 163 (163/2329, 7%) were classified as having community-onset invasive *S. aureus* infection (Table 1). The average annual incidence of invasive community-onset *S. aureus* disease in children during the study period was 52/100 000 population [95% confidence interval (CI) 44–60/100 000]. When stratified by age, the highest incidence was in the <1 year age group (83/100 000 population, 95% CI 52–130/100 000), and when stratified by ethnicity, the highest incidence was in Māori children (132/100 000 population, 95% CI 98–176/100 000) (Fig. 1). The commonest clinical syndrome associated with invasive *S. aureus* infection was musculoskeletal infection (91/163, 56%), followed by bacteraemia, site not identified (37/163, 23%), respiratory infection (27/163, 17%), endovascular infection (7/163; 4%)

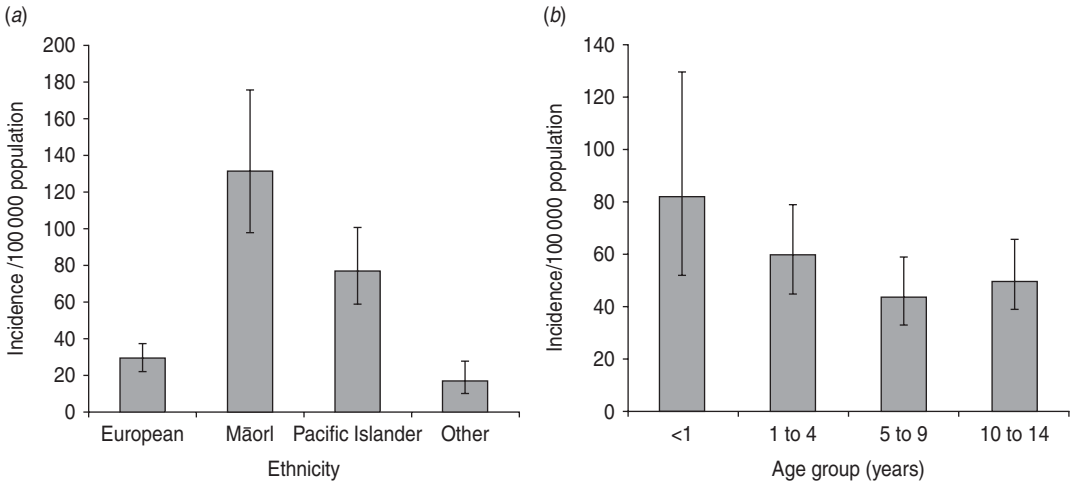

Fig. 1. Incidence of community-onset invasive *Staphylococcus aureus* infection in children admitted to Starship Children's Hospital between 2007 and 2010, stratified by (*a*) ethnicity and (*b*) age.

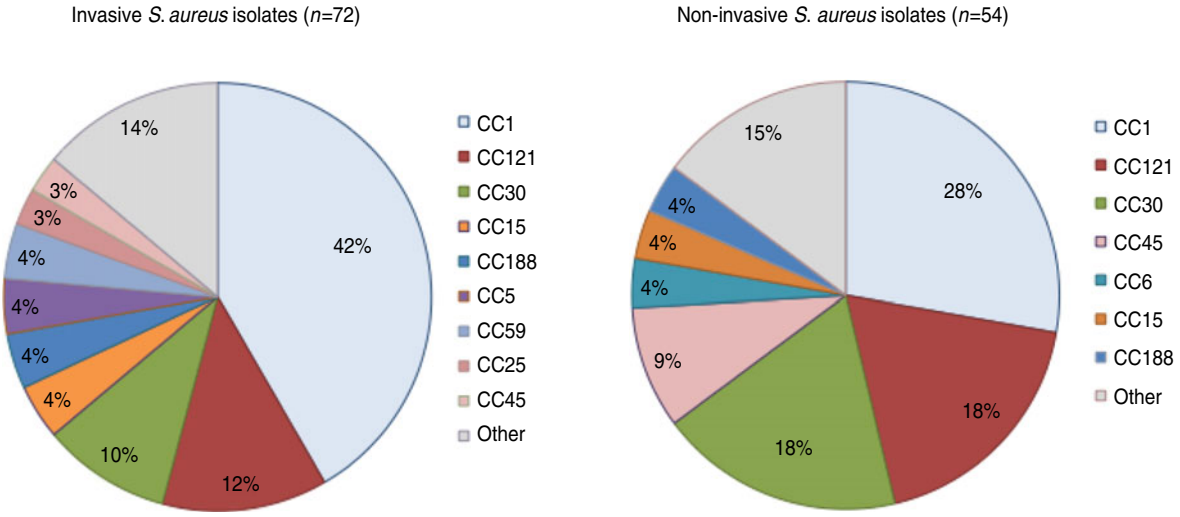

Fig. 2. Distribution of clonal complexes (CC) (as determined by DNA microarray) in invasive and non-invasive isolates of *Staphylococcus aureus.*

and central nervous system infection (1/163, 1%) (Table 1).

Isolates and comparison of molecular features of invasive and non-invasive *S. aureus* isolates

Of the 163 children with invasive *S. aureus* disease, 19 children (19/163, 12%) had isolates that were methicillin-resistant. Of the 163 invasive *S. aureus* disease episodes, isolates from 72 episodes were available from stocked cultures for molecular characterization (68 MSSA, 4 MRSA). Of these 72 isolates, 54 (75%) were associated with musculoskeletal infection, 10 (14%) with respiratory infection, six (8%) with bacteremia and two (3%) with endovascular infection. In order to identify possible genotypic associations with invasiveness, the DNA microarray profiles of these 72 isolates were compared to the DNA microarray profiles of a convenience sample of 54 non-invasive *S. aureus* SSTI isolates from children collected during the study period (50 MSSA, 4 MRSA).

Based on DNA microarray results, a total of 18 different CCs were detected among the invasive and non-invasive isolates (Fig. 2). The DNA microarray was unable to resolve the CCs of two isolates. The three predominant CCs causing SSTI or invasive disease during the study period were CC1 (45/126, 36%),

Table 2. *Prevalence and comparison of regulatory and virulence genes in invasive and non-invasive* Staphylococcus aureus *isolates from children admitted to Starship Children's Hospital, Auckland, New Zealand, 2007–2010*

Gene(s)	Invasive *S. aureus* ($n=72$)	Non-invasive *S. aureus* ($n=54$)	P*
agr group			
agr I	13 (18)	12 (22)	0·65
agr II	10 (14)	4 (7)	0·39
agr III	38 (53)	27 (50)	0·85
agr IV	10 (14)	11 (20)	0·35
Capsule type			
5	13 (18)	4 (7)	0·11
8	59 (72)	50 (93)	0·11
Toxin genes			
tst	4 (6)	5 (9)	0·49
egc	25 (35)	26 (48)	0·14
sea	28 (39)	21 (39)	1·0
seb	13 (18)	14 (26)	0·38
sec	8 (11)	6 (11)	1·0
sel	8 (11)	6 (11)	1·0
etA	1 (1)	4 (7)	0·16
etB	0	2 (4)	0·18
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			
ACME locus	0	2 (4)	0·18
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

MSCRAMMS, Microbial surface components recognizing adhesive matrix molecules; ACME, arginine catabolic mobile element.
All values are numbers of isolates (%) unless otherwise specified.
* Fisher's exact test.

CC121 MSSA (19/126, 15%) and CC30 MSSA (17/126, 13%). The *lukF-PV* and *lukS-PV* genes were found in isolates from eight CCs: CC25 (3/3, 100%), CC88 (2/2, 100%), CC121 (16/19, 84%), CC30 (12/17, 71%), CC1 (34/45, 67%), CC45 (2/7, 29%), CC59 (1/4, 25%) and CC5 (1/6, 17%).

When tested by univariate analyses, there was no significant difference in the prevalence of virulence,

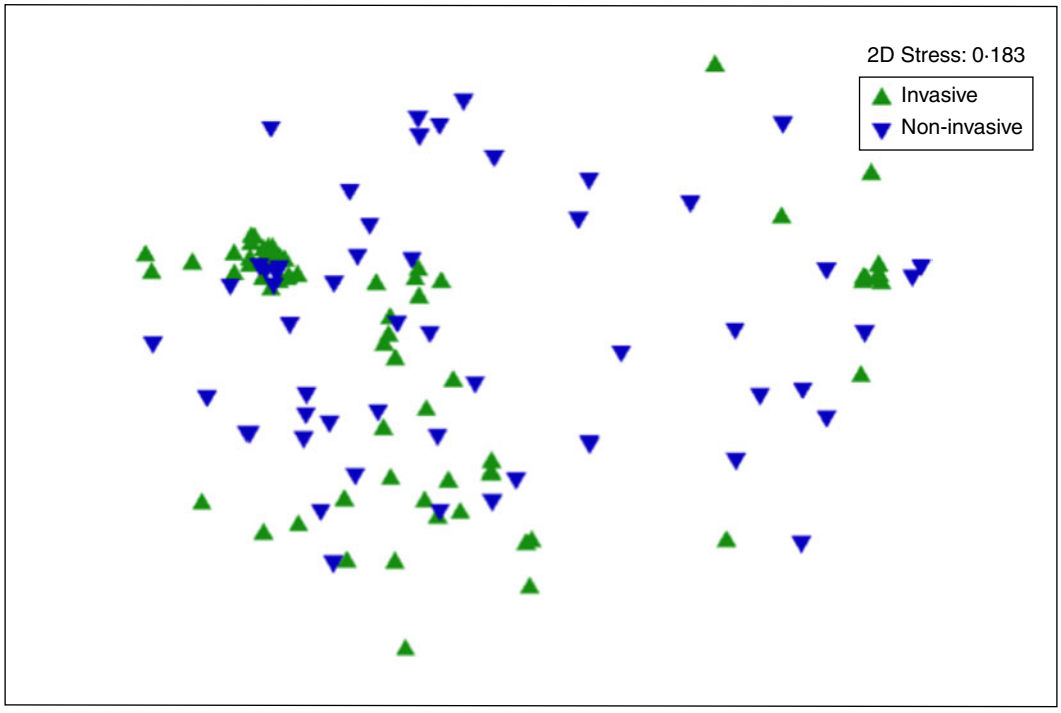

Fig. 3 [*colour online*]. Non-metric multidimensional scaling of virulence genes in invasive and non-invasive *Staphylococcus aureus* isolates based on Euclidean distances.

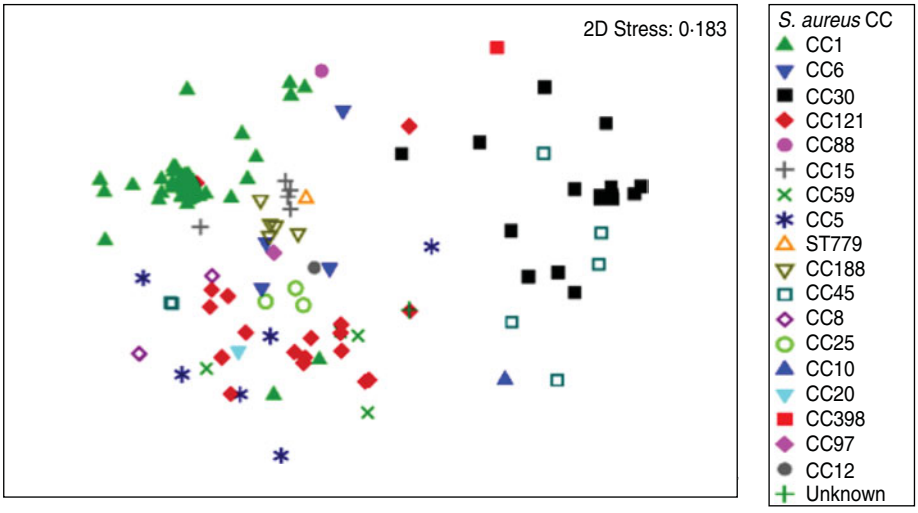

Fig. 4. Non-metric multidimensional scaling of virulence genes in *Staphylococcus aureus* isolates according to clonal complex (CC) (as determined by DNA microarray) based on Euclidean distances.

regulatory or adhesion genes between invasive and non-invasive *S. aureus* isolates (Table 2). Of note, there was no difference in the detection of the *lukF-PV* and *lukS-PV* genes between invasive and non-invasive *S. aureus* isolates (42/72, 58% *vs*. 29/54, 54%; $P = 0{\cdot}72$). The commonest *agr* group detected in both invasive and non-invasive isolates was *agr* group III (54% and 50%, respectively). On PERMANOVA testing, there were no significant differences in the virulence gene repertoire between invasive and non-invasive strains (pseudo-$F = 1{\cdot}3337$, $P = 0{\cdot}1731$) (Fig. 3). Figure 4 shows the MDS map of 126 *S. aureus* isolates differentiated on the basis of their virulence gene repertoire. This MDS map demonstrates that, for example, the virulence gene repertoire of CC1 isolates (green triangles) is distinct

to the virulence gene repertoire of CC30 isolates (black squares). Explored statistically, a significant interaction was observed between CCs and invasive status (pseudo-$F = 2{\cdot}0625$, $P = 0{\cdot}0001$), suggesting that distinct combinations of virulence genes may be present in invasive isolates within specific CCs (Fig. 4). On pairwise testing between invasive and non-invasive strains, a significant difference between invasive and non-invasive status was observed within three CCs: CC1 ($t = 2{\cdot}0853$, $P < 0{\cdot}001$), CC30 ($t = 3{\cdot}2966$, $P = 0{\cdot}007$), and CC121 ($t = 2{\cdot}1472$, $P = 0{\cdot}02$) (Fig. 4). However, our sample size within CCs was insufficient to conclusively determine which combination(s) of genes were responsible for these statistical differences.

DISCUSSION

In this study, we measured the incidence of community-onset invasive *S. aureus* disease in children presenting to our hospital between 2007 and 2010, and systematically compared the genotypic characteristics of invasive and non-invasive *S. aureus* isolates. Using univariate testing, we found no difference in the clonal distribution of *S. aureus* strains or prevalence of tested virulence factors between invasive and non-invasive isolates. However, we observed a significant interaction between CC and invasive status, with three CCs (CC1, CC121, CC30) demonstrating a significant association with invasive *S. aureus* infections. This finding is in keeping with other studies that have also observed an interaction between specific *S. aureus* CCs and clinical syndromes. For example, one previous study found a strong association between CC30 MSSA and infective endocarditis [28] and another recent study found an association between distinct subgroups of CC121 *S. aureus* and disease type [29]. Although we were unable to determine which genes were responsible for the association between CC and invasiveness, our findings suggest that there may be differences in invasive and non-invasive *S. aureus* disease according to phylogenetic grouping. Notably, we found no difference in the prevalence of *lukF-PV* and *lukS-PV* genes between *S. aureus* isolates causing invasive disease and those *S. aureus* isolates causing uncomplicated SSTI. This finding is similar to other recent studies that also found no difference in disease type based on the presence of PVL genes [9, 19]. Interestingly, however, we found an unexpectedly high prevalence of *lukF-PV* and *lukS-PV* genes in both invasive and non-invasive MSSA isolates (57% and 50%, respectively). This rate of PVL-positive MSSA is higher than that reported from several previous studies [9, 30–32], but is comparable to a recent African study, in which the *lukF-PV* and *lukS-PV* genes were detected in 130/228 (57%) clinical MSSA isolates [33]. In addition, our rate of PVL-positive MSSA is higher than that reported from a previous study in New Zealand, which detected the *lukF-PV* and *lukS-PV* genes in 37% of disease-causing MSSA isolates [9]. The reasons for the high rate of PVL-positive MSSA in our study are unclear, but may be partly due to the fact that we only assessed MSSA isolates from paediatric patients. The age-specific incidence of infections caused by PVL-producing *S. aureus* strains has been previously described, with younger patients having a disproportionately higher rate of infections due to PVL-producing *S. aureus* strains [30]. Although this association may be partially mediated by age-related immunological protection, supportive data for this hypothesis remain inconclusive [34, 35].

Our study also provides valuable information on the genotypes of MSSA strains circulating in New Zealand. Despite the fact that about 90% of *S. aureus* infections in New Zealand are caused by MSSA strains [10, 11], only one previous study has provided data on the molecular epidemiology of MSSA strains circulating in our region [9]. Based on DNA microarray data, we found a diverse range of MSSA CCs causing disease, although three MSSA strain types predominated (CC1, CC121, CC30) and together accounted for two-thirds of all MSSA strains identified. Interestingly, in one child with osteomyelitis, we detected a MSSA isolate belonging to CC398. Although CC398 MRSA has recently been described as a cause of CA-MRSA infection in New Zealand [36], to our knowledge, CC398 MSSA has not previously been reported in our country.

Finally, we observed significant demographic variation in the incidence of community-onset invasive *S. aureus* disease among children in our setting. Ethnic variation in invasive *S. aureus* disease has been described previously, most notably in North America, where in one study, the incidence of invasive MRSA infections was more than four times higher in black infants compared to white children from the same population [4]. In addition, Gutierrez *et al.* found that among children in California, black children were 1·5 times more likely to be hospitalized with staphylococcal infection than white children [2]. The reasons for the markedly high disease burden in Māori and Pacific Island children in New Zealand

are unclear, but are likely to be due, in part, to a combination of societal, environmental and host factors, including barriers in accessing healthcare, household crowding, hygiene and nutrition. Future studies should assess modifiable risk factors that may reduce this serious disease burden and ethnic disparity.

There are a number of limitations to our study. In particular, the retrospective nature of analysis limited the amount of data we could collect, particularly information relating to duration of preceding disease course, prior antibiotic usage, medical co-morbidities, and clinical outcomes. In addition, the DNA microarray assay we used for genotypic typing of staphylococcal CCs does not provide the same degree of resolution as other molecular typing methods, such as multi-locus sequence typing or *spa* typing. It is, therefore, possible that there may be additional epidemiological or molecular associations with distinct *S. aureus* sublineages within CCs that we were unable to detect. Finally, the DNA microarray we used only detects the presence of commonly studied virulence genes – it is not a complete representation of virulence genes within *S. aureus*, nor does it quantify virulence gene expression.

In summary, our study provides valuable information on the clinical and molecular epidemiology of invasive *S. aureus* disease in New Zealand children. We observed notable sociodemographic variation in the incidence of *S. aureus* disease in our setting, with rates highest in Māori and Pacific Island children. Most disease was caused by MSSA strains, which were genetically diverse. Based on DNA microarray data, we identified an interaction between invasive disease and distinct *S. aureus* CCs. Future research should include a strong focus on identifying the host and environmental factors that, along with other potential organism virulence factors, are contributing to the patterns of invasive *S. aureus* disease observed in New Zealand.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268814000053.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **David MZ, Daum RS.** Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews* 2010; **23**: 616–687.
2. **Gutierrez K, *et al.*** Staphylococcal infections in children, California, USA, 1985–2009. *Emerging Infectious Diseases* 2013; **19**: 10–20.
3. **Boucher HW, Corey GR.** Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clinical Infectious Diseases* 2008; **46** (Suppl. 5): S344–349.
4. **Klevens RM, *et al.*** Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *Journal of the American Medical Association* 2007; **298**: 1763–1771.
5. **Williamson DA, *et al.*** Increasing incidence and sociodemographic variation in community-onset *Staphylococcus aureus* skin and soft tissue infections in New Zealand children. *Pediatric Infectious Disease Journal* 2013; **32**: 923–925.
6. **Williamson DA, *et al.*** Clinical and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in New Zealand: rapid emergence of sequence type 5 (ST5)-SCC*mec*-IV as the dominant community-associated MRSA clone. *PLoS One* 2013; **8**: e62020.
7. **de Kraker ME, *et al.*** The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clinical Microbiology and Infection* 2012; **19**: 860–868.
8. **Moran GJ, *et al.*** Methicillin-resistant *S. aureus* infections among patients in the emergency department. *New England Journal of Medicine* 2006; **355**: 666–674.
9. **Muttaiyah S, *et al.*** Incidence, risk factors, and outcomes of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* infections in Auckland, New Zealand. *Journal of Clinical Microbiology* 2010; **48**: 3470–3474.
10. **Hill PC, *et al.*** Prospective study of 424 cases of *Staphylococcus aureus* bacteraemia: determination of factors affecting incidence and mortality. *Internal Medicine Journal* 2001; **31**: 97–103.
11. **Hill PC, *et al.*** Prospective study of 125 cases of *Staphylococcus aureus* bacteremia in children in New Zealand. *Pediatric Infectious Disease Journal* 2001; **20**: 868–873.
12. **O'Sullivan CE, Baker MG, Zhang J.** Increasing hospitalizations for serious skin infections in New Zealand children, 1990–2007. *Epidemiology and Infection* 2011; **139**: 1794–1804.
13. **Gordon RJ, Lowy FD.** Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clinical Infectious Diseases* 2008; **46** (Suppl. 5): S350–359.
14. **Tong A, *et al.*** Panton-Valentine leukocidin is not the primary determinant of outcome for *Staphylococcus*

aureus skin infections: evaluation from the CANVAS studies. *PLoS One* 2012; **7**: e37212.

15. **Shallcross LJ, *et al.*** The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. *Lancet Infectious Diseases* 2103; **13**: 43–54.
16. **Tong SY, *et al.*** Clinical correlates of Panton-Valentine leukocidin (PVL), PVL isoforms, and clonal complex in the *Staphylococcus aureus* population of Northern Australia. *Journal of Infectious Diseases* 2010; **202**: 760–769.
17. **Lo WT, Wang CC.** Panton-Valentine leukocidin in the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. *Pediatrics and Neonatology* 2011; **52**: 59–65.
18. **Gillet Y, *et al.*** Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 2002; **359**: 753–759.
19. **Wehrhahn MC, *et al.*** Clinical and laboratory features of invasive community-onset methicillin-resistant *Staphylococcus aureus* infection: a prospective case-control study. *European Journal of Clinical Microbiology & Infectious Diseases* 2010; **29**: 1025–1033.
20. **Clinical and Laboratory Standards Institute.** Performance standards for antimicrobial susceptibility testing; 22nd informational supplement. CLSI document M100-S22, 2012.
21. **World Health Organization.** International statistical classification of diseases and related health problems – 10th revision, 2010.
22. **Tracy LA, *et al.*** Predictive ability of positive clinical culture results and International Classification of Diseases, Ninth Revision, to identify and classify non-invasive *Staphylococcus aureus* infections: a validation study. *Infection Control and Hospital Epidemiology* 2010; **31**: 694–700.
23. **Monecke S, *et al.*** Microarray-based characterisation of a Panton-Valentine leukocidin-positive community-acquired strain of methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection* 2006; **12**: 718–728.
24. **Monecke S, Slickers P, Ehricht R.** Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. *FEMS Immunology and Medical Microbiology* 2008; **53**: 237–251.
25. **Monecke S, *et al.*** Rapid microarray-based identification of different *mecA* alleles in staphylococci. *Antimicrobial Agents and Chemotherapy* 2012; **56**: 5547–5554.
26. **Statistics New Zealand.** 2006 census of populations and dwellings. District Health Board Area summary tables (http://www.stats.govt.nz/Census/about-2006-census/district-health-board-area-summary-tables.aspx). Accessed 30 September 2013.
27. **Anderson MJ.** A new method for non-parametric multivariate analysis of variance. *Australian Ecology* 2001; **82**: 290–297.
28. **Fowler VG Jr., *et al.*** Potential associations between hematogenous complications and bacterial genotype in *Staphylococcus aureus* infection. *Journal of Infectious Diseases* 2007; **196**: 738–747.
29. **Kurt K, *et al.*** Subpopulations of *Staphylococcus aureus* clonal complex 121 are associated with distinct clinical entities. *PLoS One* 2013; **8**: e58155.
30. **Munckhof WJ, *et al.*** Methicillin-susceptible, non-multiresistant methicillin-resistant and multiresistant methicillin-resistant *Staphylococcus aureus* infections: a clinical, epidemiological and microbiological comparative study. *European Journal of Clinical Microbiology & Infectious Diseases* 2008; **27**: 355–364.
31. **Zhao C, *et al.*** Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. *PLoS One* 2012; **7**: e38577.
32. **Orscheln RC, *et al.*** Contribution of genetically restricted, methicillin-susceptible strains to the ongoing epidemic of community-acquired *Staphylococcus aureus* infections. *Clinical Infectious Diseases* 2009; **49**: 536–542.
33. **Breurec S, *et al.*** Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes. *Clinical Microbiology and Infection* 2011; **17**: 633–639.
34. **Croze M.** Serum antibodies against Panton-Valentine leukocidin in a normal population and during *Staphylococcus aureus* infection. *Clinical Microbiology and Infection* 2009; **15**: 144–148.
35. **Hermos CR, Yoong P, Pier GB.** High levels of antibody to panton-valentine leukocidin are not associated with resistance to *Staphylococcus aureus*-associated skin and soft-tissue infection. *Clinical Infectious Diseases* 2010; **51**: 1138–1146.
36. **Williamson DA, *et al.*** Emergence and molecular characterization of clonal complex 398 (CC398) methicillin-resistant Staphylococcus aureus (MRSA) in New Zealand. *Journal of Antimicrobial Chemotherapy*. Published online: 22 December 2013. doi: 10.1093/jac/dkt499.