Characterization of major clones of antibiotic-resistant *Streptococcus pneumoniae* in New Zealand by multilocus sequence typing

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Objectives: To determine the relationship between New Zealand isolates of antibiotic-resistant *Streptococcus pneumoniae* and internationally widespread resistant clones.

Methods: Fifty-nine isolates representing both invasive and non-invasive pneumococci from multiple locations, serotypes and years were analysed by multilocus sequence typing.

Results: Major international clones, including Spain23F-1, France9V-3 and Taiwan19F-14, were found to be present in New Zealand. A one-allele variant of the Taiwan19F-14 clone (*aroE* 15 → 4, ST 271) was particularly prominent.

Conclusions: Antibiotic-resistant pneumococci have not evolved *de novo* in New Zealand, but were introduced to the country during the early 1990s.

Keywords: *S. pneumoniae*, PMEN, MLST

Introduction

*Streptococcus pneumoniae* is an important cause of community-acquired infection and its increase in resistance to antibiotics has complicated therapeutic options worldwide. The increase in prevalence of resistant pneumococci is often attributed to the clonal dissemination of one or more particular pneumococcal strains. Often these strains arise in countries where antibiotic use is not well regulated, and subsequently spread worldwide. Molecular epidemiological methods, particularly multilocus sequence typing (MLST), are well suited to tracking the clonal spread of such strains.

New Zealand, as with many other countries, saw a rapid increase in the prevalence of penicillin non-susceptible *S. pneumoniae* in the late 1990s. In order to explain this observed increase, we sought to determine whether a resistant strain(s) emerged *de novo* in New Zealand, or if a resistant clone(s) from overseas, which subsequently radiated into New Zealand, was responsible.

Materials and methods

Pneumococcal isolates were obtained from those submitted to the central communicable disease laboratory, Institute of Environmental Science and Research (ESR), Porirua. Fifty-nine isolates were obtained from 12 cities throughout New Zealand (Figure 1) during 1992–2000. Representatives of major antibiotic-resistant clones (as assessed by PFGE; data not shown) were included from hospitals and laboratories throughout New Zealand. Thirty-nine isolates (66%) were included from the North Island and 20 (34%) from the South Island. The two major contributing centres were Auckland and Christchurch, with 20 (34%) and 15 (25%) isolates from each, respectively. Forty-three (73%) of the pneumococcal isolates included were from invasive disease; 37 (63%) from blood and six (10%) from other sterile sites. Non-invasive isolates (*n* = 16, 27%) typical of major clones circulating in New Zealand (as determined by PFGE; data not shown) were also characterized. A number of isolates were selected retrospectively in order to establish when major clones were first recovered in New Zealand.

Penicillin and cefotaxime MICs were determined using Etest (AB Biodisk, Solna, Sweden), whereas resistance to other antibiotics (erythromycin, chloramphenicol, tetracycline, co-trimoxazole and vancomycin) was determined by disc diffusion (Oxoid, Basingstoke, UK) in accordance with NCCLS standard guidelines. Serotyping was performed by the Streptococcus Reference Laboratory (ESR Porirua, New Zealand), using the capsular reaction test (Neufeld test) and interpreted using the Danish system of nomenclature. MLST was performed as described by Enright & Spratt.

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sequencing was performed using an ABI Prism 310 automated sequencer (Applied Biosystems) and BigDye terminator mix (Applied Biosystems). Smal macrorestriction profiles were generated and compared using PFGE, as described previously. Amplification of the genes \( pbp2b \) and \( pbp2x \) was performed using PCR, as described previously.

**Results and discussion**

Twenty-nine (49%) of the 59 New Zealand isolates could be assigned to one of five major antibiotic-resistant clones defined by the Pneumococcal Molecular Epidemiology Network (PMEN). The clones identified were Spain\(^{23F-1} \) (ST 81, \( n = 13 \)), Spain\(^{68-2} \) (ST 90, \( n = 2 \)), France\(^{9V-3} \) (ST 156, \( n = 8 \)), England\(^{14-9} \) (ST 9, \( n = 1 \)) and Taiwan\(^{19F-14} \) (ST 236, \( n = 5 \)). A further 18 isolates (31%) were single-allele derivatives of three of the PMEN major antibiotic-resistant clones: Spain\(^{23F-1} \) (spi \( 4 \rightarrow 6 \), ST 83, \( n = 1 \)), France\(^{9V-3} \) (ddl \( 1 \rightarrow 14 \), ST 162, \( n = 3 \)) and Taiwan\(^{19F-14} \) clones (two variants: ddl \( 26 \rightarrow 1 \), ST 237, \( n = 1 \); and aroE \( 15 \rightarrow 4 \), ST 271, \( n = 13 \)). In the current study, one-allele variants were considered to be members of the same clonal group. Data are summarized in Table 1.

Not surprisingly, two of the major drug-resistant pneumococcal clones identified in New Zealand were determined to be members of the Spain\(^{23F-1} \) clone and the France\(^{9V-3} \) clone. Previous MLST studies have shown the widespread global distribution of these clones. The first member of the Spain\(^{23F-1} \) clonal group identified in this study was isolated from a blood culture in Auckland during early 1994. Twelve of the 14 members of this clone were from invasive specimens. Within the Spain\(^{23F-1} \) clonal lineage, \( pbp2b \) and \( pbp2x \) restriction fragment length polymorphism (RFLP) profiles were conserved, as were the corresponding MICs of both penicillin and cefotaxime. A notable exception was observed, however, in the three Spain\(^{23F-1} \) clonal group isolates expressing the 19F capsular type. In two of the three 19F isolates, the MIC was at least one doubling dilution higher for both \( \beta \)-lactams than those isolates expressing the 23F capsule.

All 11 isolates from the France\(^{9V-3} \) clonal group were recovered from blood cultures. All members of this group with ST 156 were isolated during 1995 or thereafter, and exhibited reduced susceptibility to \( \beta \)-lactams. However, three single-allele variants (ST 162, ddl \( 1 \rightarrow 14 \)) were isolated during or prior to 1995 and were all susceptible to penicillin. The \( pbp2b \) gene has been shown to be physically linked to the \( ddl \) gene, and co-transfer has been demonstrated previously. This is supported by the acquisition of \( \beta \)-lactam resistance also being coupled to a change in the \( pbp \) RFLP patterns. None of the New Zealand examples of the France\(^{9V-3} \) clone was found to be multidualt-resistant, although one isolate did express resistance to co-trimoxazole as well as penicillin.

In contrast to the Spain\(^{23F-1} \) clone, which had been prevalent in Spain for at least a decade before its intercontinental spread was first documented, the Taiwan\(^{19F-14} \) clone has emerged relatively recently, but seems equally adept at global dissemination. Nineteen isolates of the Taiwan\(^{19F-14} \) clonal group were noted, all of which were serotype 19F with the exception of a single serotype 14 isolate. Curiously, the single-allele variant (ST 271, aroE \( 15 \rightarrow 4 \)) of the Taiwan\(^{19F-14} \) clone was the most prominent in the current study; of 19 isolates, 13 (68%) were ST 271. This particular variant has previously been described as the major Korean 19F clone (www.mlst.net). Five isolates were from invasive sites (three ST 271 and two ST 236).

The New Zealand Taiwan\(^{19F-14} \) clonal group is resistant to erythromycin, co-trimoxazole and tetracycline and is near uniformly susceptible to chloramphenicol and vancomycin. Two isolates recovered during 1999–2000, however, had acquired resistance to chloramphenicol. Levels of \( \beta \)-lactam resistance are variable among this group. In the case of the five ST 236 isolates, the penicillin and cefotaxime MICs were consistently 2 and 1 mg/L (one instance of a cefotaxime MIC of 0.5 mg/L), respectively; however, among the ST 271 isolates MICs were in the range 2–8 mg/L and 1–32 mg/L of penicillin and cefotaxime, respectively. The observed MIC variability did not correlate with changes in their respective \( pbp \) RFLP profiles (data not shown).

The Taiwan\(^{19F-14} \) clone has emerged only recently. It was first formally identified when multiply antibiotic-resistant \( S. pneumoniae \) from Taiwanese hospitals were examined by MLST, showing this clone was present in Taiwan at least as early as 1993. In 1995, two serotype 19F isolates were recovered from blood cultures in a London hospital that had the same allelic profile. This is the same year that the Taiwan\(^{19F-14} \) clone was observed in New Zealand and coincides with a period of increased prevalence of penicillin-resistant \( S. pneumoniae \) throughout the country. The Taiwan\(^{19F-14} \) clone was identified in the USA as early as 1997–1998. Therefore it seems likely that the Taiwan\(^{19F-14} \) strain did in fact originate in Taiwan, or elsewhere in Asia, and began to disseminate globally as early as 1995.
The remaining 12 isolates yielded allelic profiles that matched eight previously defined STs, but do not belong to any of the currently described PMEN clones. These were predominantly invasive, non-resistant isolates, and were representative of multiple serotypes: 9N (ST 66, \(n=2\)), 14 (ST 124, \(n=2\); ST 129, \(n=1\); and ST 346, \(n=1\)), 19A (ST 199, \(n=2\); and ST 876, \(n=1\)), 19F (ST 146, \(n=1\)) and 23F (ST 36, \(n=2\)). In each instance, these sequence types corresponded to isolates from invasive disease recovered elsewhere in the world. In particular, the ST 124 isolates and one single-allele variant (\(ddl_{14}^{5}\), ST 129) are members of an important clone associated with invasive disease and have been recovered throughout the world, including Sweden, Denmark, Norway, Finland, the UK, Australia and Canada. Another invasive clone, ST 199, and a single-allele variant (\(spi_{17}^{6}\), ST 876), has been recovered previously from invasive disease in the UK. The inclusion of antibiotic-susceptible invasive clones in the PMEN database will better allow the distribution of these strains to be monitored worldwide.

Antibiotic-resistant \(S.\ pneumoniae\) in New Zealand can be attributed mainly to the global spread of international resistant clones; in particular the well-described Spain\(^{23F}\)-1 and France\(^{9V}\)-3 clones, as well as the more recently described Taiwan\(^{9V}\)-14 clone. The use of MLST and the PMEN-described clones\(^3\) allow effective worldwide surveillance of important pneumococcal clones.

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**References**


