Molecular epidemiology of multiple drug resistant type 6B *Streptococcus pneumoniae* in the Northern Territory and Queensland, Australia

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SUMMARY

The emergence of type 6B *Streptococcus pneumoniae* resistant to five antibiotics (penicillin, chloramphenicol, trimethoprim–sulphamethoxazole, erythromycin and tetracycline) in both the Northern Territory and Queensland prompted an investigation of the genetic relatedness and patterns of migration of the isolates. Pulsed field gel electrophoresis of genomic DNA of 74 multiple drug-resistant (MDR) isolates cultured in both regions between August 1988 and June 1997 showed that 100% of MDR isolates from the Northern Territory and 96% of MDR strains from Queensland were genetically indistinguishable or closely related to the index strain. None of a further 65 type 6B isolates that were resistant to one or two, or susceptible to all of the above antibiotics, were clonally related to the MDR pneumococci. The geographical distribution of the MDR type 6B clone increased over time. The index strain, first isolated in Darwin in August 1988, was identified in Brisbane, 2900 km distant, less than 4 years later and subsequently in other Queensland centres. Surveillance programmes are important to monitor the emergence and spread of potentially invasive MDR pneumococcal clones in countries that are well serviced by air and road transport.

INTRODUCTION

Streptococcus pneumoniae resistant to three or more classes of antibiotics were first recognized in South Africa in 1977 [1] and subsequently elsewhere [2]. Several serotypes, including types 6B and 23F, readily develop multiple resistance [3]. The ability of such types to migrate to geographically distant regions is associated with their predilection for colonizing the upper respiratory tract (URT) of young children and family contacts [4–6]. The clonality of multiply drug resistant pneumococci has been established by analysing macrorestriction fragment digests of genomic DNA by procedures such as pulsed-field gel electrophoresis (PFGE) [4, 7, 8]. In Australia, type 6B pneumococci resistant to penicillin, chloramphenicol,

trimethoprim–sulphamethoxazole (TMP–SMX), erythromycin and tetracycline were first identified in Darwin, Northern Territory in August. Further strains with the same type and antibiogram were recovered from the nasopharynx of Northern Territory Aboriginal infants by Leach and co-workers in 1992–4 [9] and from hospitalized subjects in Darwin from 1991. In the course of a Queensland-based pneumococcal surveillance programme [10], phenotypically similar pneumococci were first encountered in Brisbane (South East Queensland) and Cairns (North Queensland) in 1992 and 1995 respectively and subsequently, in other centres in Queensland. It was decided to investigate the clonality of these multiple drug resistant type 6B (MDR6B) pneumococci and their migration patterns by examining isolates, using PFGE, from Queensland and the three sources in the Northern Territory described above.

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METHODS

Bacterial strains

Seventy-four isolates of MDR6B pneumococci were studied. Of these, 28 strains represented 64% of the MDR6B isolates from the Darwin region of the Northern Territory and 46 strains, represented 85% of the MDR isolates from Queensland. These strains were cultured from August 1988 to April 1994 (Darwin region, Northern Territory) and from May 1992 to June 1997 (Queensland). Fifteen isolates were recovered from normally sterile sites (blood and/or CSF = 14, joint aspirate = 1) or other sites (sputum = 29, upper respiratory tract (URT) secretions = 18, ear = 6, eye = 3, lung post mortem = 1, bronchus = 1). The source of one isolate was unknown. Two isolates were recovered from the same patient admitted on separate occasions to different hospitals.

Further type 6B isolates including 25 strains susceptible to penicillin, chloramphenicol, TMP–SMX, erythromycin and tetracycline and 40 strains resistant to 1 (TMP–SMX, $n = 25$) or 2 (penicillin and TMP–SMX, $n = 15$) were also examined in order to assess the genetic heterogeneity within and between antibiogram categories. These strains were cultured from normally sterile sites (blood and/or CSF = 52) and other sources (sputum = 2, URT secretions = 7, ear = 2, eye = 2) from patients in South East Queensland (39 isolates), North Queensland (18) and the Darwin region (8) from June 1991 to September 1997.

Identification

Pneumococci were identified presumptively by Gram stain, colonial morphology on blood agar, and optochin susceptibility. They were typed by the Quellung reaction with antisera raised at Statens Seruminstitut, Copenhagen, Denmark as described elsewhere [11].

Antibiotic susceptibility testing

The Etest (AB Biodisk, Solna, Sweden) was used as described elsewhere [12] and interpreted according to the National Committee for Clinical Laboratory Standards performance standards [13].

Pulsed-field gel electrophoresis

PFGE is the preferred strain typing procedure for *S. pneumoniae* [14]. The method used follows those as described by Lefevre et al. [15] and Matushek et al. [16] with the following modifications.

Penumococcal isolates were grown at 36·5 °C in 10 ml of brain heart infusion broth (Becton Dickinson and Co., Cockeysville, MD) supplemented with 10% non-inactivated donor horse serum (CSL Biosciences, Parkville, Victoria, Australia) to an optical density of 0·4–0·8 at 590 nm. The agarose gel was electrophoresed using the Gene Navigator System (Pharmacia Biotech AB) for 24 h at 170 V with pulse times stepped at 5 s (9 h), 20 s (9 h) and 40 s (6 h). The interpretation of banding profiles and categories of genetic relatedness followed the recommendations of Tenover et al. [17].

RESULTS

Antimicrobial susceptibility

The minimal inhibitory concentration (MIC) ranges (μg/ml) of 5 antibiotics for 74 MDR6B isolates were penicillin, 0·5–2·0; chloramphenicol, 16–64; TMP–SMX, 4–32/76–608; erythromycin, 256; tetracycline, 16–128. The tetracycline MIC for one isolate was not done. Of a further 65 type 6B pneumococci, 25 strains were susceptible to all antibiotics, 25 strains were resistant to TMP-SMX only (MIC range 1–19 to 32–608 μg/ml), and 15 strains were resistant to both penicillin and TMP-SMX (MIC ranges 0·125–1·0 μg/ml and 1–19/4–76 μg/ml, respectively).

Pulsed-field gel electrophoresis

For the genomic DNA fragment analysis of MDR6B isolates the initial MDR6B strain recognized in Darwin in August 1988 was selected as the index strain with which other MDR6B isolates were compared. Comparison of the fragment profiles of 74 strains demonstrated a high level of homogeneity (Fig. 1). Overall, 97% were genetically indistinguishable (27%) or closely related (70%) to the index isolate. The remaining 3% of isolates were possibly related to the index isolate. Regionally, 59% of Darwin pneumococci were indistinguishable from the index strain. While only 9% of Queensland isolates were indistinguishable to the index strain, 87% were closely related to it genetically. None of 65 type 6B pneumococci with broad-spectrum antibiotic susceptibility (25 strains) or resistance to 1 drug (25 strains) or 2 drugs (15 strains), was genetically related to the index strain.
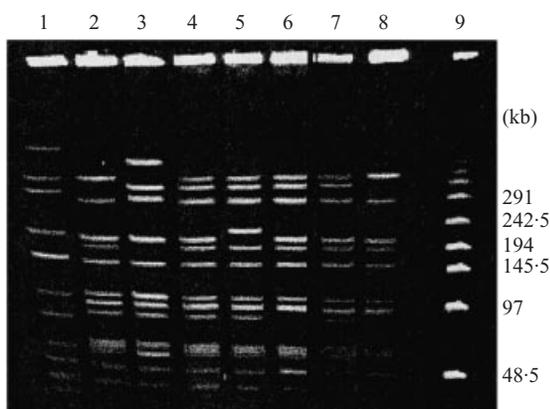
Fig. 1. Genetically related pulsed field gel electrophoresis profiles of DNA *Sma*1 digests of type 6B *S. pneumoniae* strains from the Darwin region, Northern Territory (lanes 2, 3, 4, 7) and Queensland (lanes 5, 6, 8). Lane 1 (*S. pneumoniae*, ATCC 49619) is the reference strain. Lane 4 is the index strain, NT 1. Lane 9 is the λ-DNA ladder with the sizes (in kilobases) indicated to the right.

Migration patterns of clonal MDR6B pneumococci

The first MDR6B pneumococcus was isolated in Darwin in August 1988. A strain with a genotype similar to that of the index case was first identified in Queensland in a 72-year-old patient hospitalized with bacterial meningitis in Brisbane in May 1992. Other initial MDR6B isolates in Queensland, by major centre, followed: Cairns (North Queensland) February 1995, Mackay (Central Queensland) October 1995, Gold Coast (south of Brisbane) March 1996, and Townsville (North Queensland) October 1996. The Townsville isolate was genetically indistinguishable from a strain cultured from the same patient while hospitalized in Brisbane in October 1995. The possible migration routes are outlined in Figure 2. Regular air services exist between Darwin and Brisbane then north from Brisbane to other major Queensland centres. There is also an excellent road network interstate.

DISCUSSION

This study shows that a clone of multiple drug-resistant type 6B pneumococci, first isolated in Darwin in 1988, subsequently emerged in major Queensland centres during 1992–7. The inter-country migration of antibiotic resistant pneumococcal clones, such as types 6B and 23F, to geographically distant areas is well documented [4, 5]. The spread of a type 6B clone from the Darwin region to Queensland is thus not surprising since the greatest distance between Darwin

and any major Queensland centre does not exceed 4000 km. The Northern Territory and Queensland are geographically contiguous and well served by air and road services. While precise routes of clonal migration are unclear, the identification in Brisbane of a strain which was genetically indistinguishable from that of the index case, before other isolations elsewhere in Queensland, suggests direct spread from Darwin to Brisbane.

The dispersal of pneumococci such as group 6 types is favoured since upper airway carriage by these organisms is common, particularly in young children in both industrialized and developing countries. Carriage is of high density and of long duration [18, 19]. The epidemiology of meningitis due to multiple drug-resistant 6B pneumococci has also been investigated in an infant day care centre. This study determined the susceptibility for upper respiratory tract carriage of the index strain by close contacts including other children, staff and family contacts of the colonized children [6]. It is clear that once established in Queensland a 6B clone with the epidemiological characteristics of a common and tenacious carriage organism is likely to spread readily between cities in the highly populated eastern seaboard of Queensland.

Evidence of random genetic events which have altered the PFGE profiles of multiple drug-resistant 6B strains isolated during the course of this study have been noted. Genetic diversity increased as the study progressed. While 59% of 27 Northern Territory strains isolated during 1988–94 were indistinguishable to the index strain, minimal fragment differences were observed in other Northern Territory strains and in 87% of 46 genetically related Queensland isolates cultured during 1992–7. Such changes are common, are known to occur naturally over time and result from point mutations and insertions and deletions of DNA [17]. Nonetheless, the general stability of multiple drug resistant type 6B clones over time and in widely separated geographic locations has been reported [20].

Type 6B pneumococci with different antibiograms from those of the test strains were included in the current study for comparative purposes. All strains were shown to be genetically diverse and unrelated to the multiple drug-resistant type 6B strains.

Multiple drug resistant pneumococci such as type 6B create a clinical dilemma because of their ability, not only to colonize the upper respiratory tract, but also to invade normally sterile sites. In our study 20%
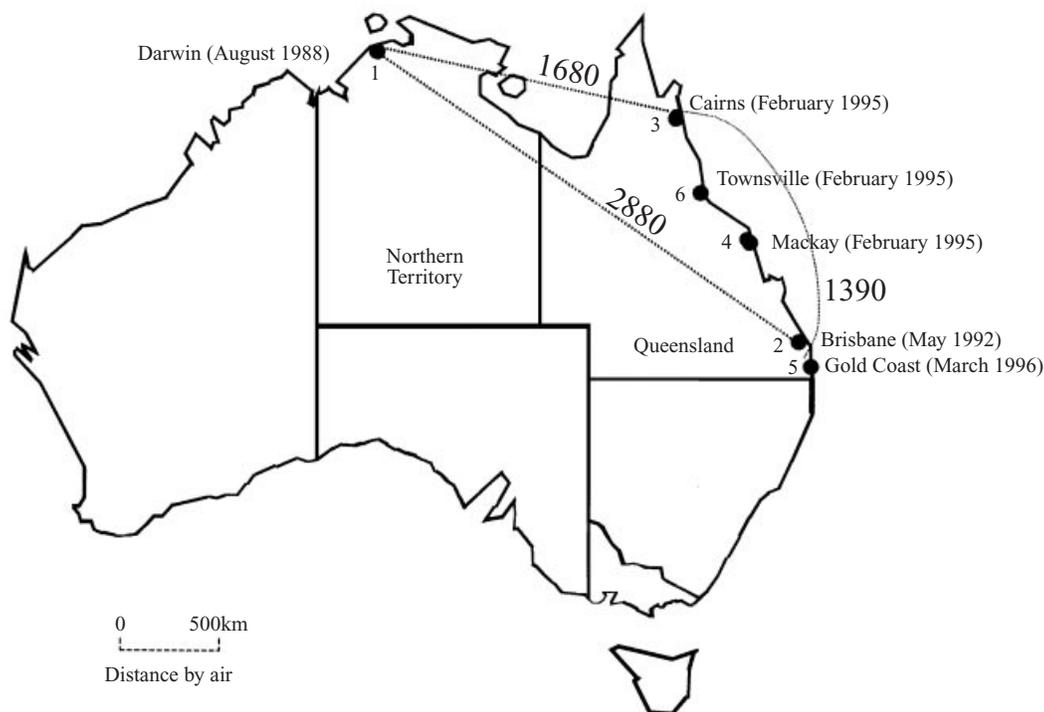
Fig. 2. Dates and referral centres of the first isolations of clonally related multiple drug-resistant type 6B *Streptococcus pneumoniae*, and inter-centre distances (km), in the Northern Territory and Queensland, Australia. The numbers 1–6 adjacent to each referral centre represents the chronological order of migration of the MDR6B clones.

of 74 isolates were cultured from blood (12 strains), CSF (2 strains) and joints (1 strains). The ability of multiple drug resistant pneumococcal clones to spread widely highlights the importance of surveillance programmes that monitor the type distribution and drug resistance of both carriage and invasive types. The use of conjugate pneumococcal vaccines in young children may reduce the emergence and spread of multiple drug resistant clones [21].

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