

## Age-Specific Cluster of Cases of Serotype 1 *Streptococcus pneumoniae* Carriage in Remote Indigenous Communities in Australia<sup>∇</sup>

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**Seven-valent pneumococcal conjugate vaccination commenced in 2001 for Australian indigenous infants. Pneumococcal carriage surveillance detected substantial replacement with nonvaccine serotypes and a cluster of serotype 1 carriage. Our aim was to review *Streptococcus pneumoniae* serotype 1 carriage and invasive pneumococcal disease (IPD) data for this population and to analyze serotype 1 isolates. Carriage data were collected between 1992 and 2004 in the Darwin region, one of the five regions in the Northern Territory. Carriage data were also collected in 2003 and 2005 from four regions in the Northern Territory. Twenty-six cases of serotype 1 IPD were reported from 1994 to 2007 in the Northern Territory. Forty-four isolates were analyzed by BOX typing and 11 by multilocus sequence typing. In the Darwin region, 26 children were reported carrying serotype 1 (ST227) in 2002 but not during later surveillance. Scattered cases of serotype 1 carriage were noted in two other regions. Cocolonization of serotype 1 with other pneumococcal serotypes was common (34% serotype 1-positive swabs). In conclusion, pneumococcal carriage studies detected intermittent serotype 1 carriage and an ST227 cluster in children in indigenous communities in the Northern Territory of Australia. There was no apparent increase in serotype 1 IPD during this time. The rate of serotype 1 cocolonization with other pneumococcal serotypes suggests that carriage of this serotype may be underestimated.**

Seven-valent pneumococcal conjugate vaccine (PCV7) immunization for infants and catch-up immunization programs commenced in 2001 for indigenous Australian children. The incidence of invasive pneumococcal disease (IPD) in indigenous children less than 2 years of age in the Northern Territory fell from approximately 600 cases/100,000 individuals to 264 cases/100,000 individuals after the introduction of PCV7 (9). Serotype replacement in IPD is not confirmed in this population as reported elsewhere (21). However, replacement with nonvaccine serotypes is evident in carriage, and it is responsible for continued pneumococcal carriage rates in excess of 80% among young indigenous children (15). Following the introduction of PCV7, our carriage surveys of children detected a shift to a serotype hierarchy led by serotype 16F (14) and a cluster of serotype 1 carriage.

*Streptococcus pneumoniae* serotype 1 is an important contributor to IPD in children and adults in much of the world (6). There can be substantial variations in the proportion of serotype 1 IPD in studies from the same population in different

years and from the same country at different sites. This is due to the serotype's propensity to cause outbreaks of disease (7). Serotype 1 is associated with complicated pneumonia, pulmonary empyema, peritonitis, and salpingitis (reviewed in reference 8) and was identified in a highly lethal pneumococcal meningitis epidemic (27).

Serotype 1 is not commonly detected in studies of colonization, presumably due to its short duration and low density of carriage (7). However, serotype 1 carriage can be evident during outbreaks of serotype 1 IPD. For example, a central Australian community outbreak of predominantly adult serotype 1 bacteremic pneumonia was related to nasopharyngeal (NP) colonization of 13 of 75 (17.3%) children with acute lower respiratory tract infection (4). Furthermore, in a closed community in southern Israel, serotype 1 carriage in 26 of 650 (4%) residents across all age groups was reported during an outbreak of serotype 1 disease in adults and children (2). We found few reports of NP serotype 1 carriage in the absence of a reported serotype 1 IPD outbreak, for example, among young children at an outpatient clinic in Mozambique (0.5% of 192 pneumococcal isolates) (24), among outpatients presenting with acute otitis media or pneumonia in Switzerland (1.7% of 1,540 isolates) (10), and among healthy children 7 years old or older in Turkey (4.3% of 117 isolates) (1).

The aim of this study was to determine the molecular characteristics of serotype 1 pneumococci detected in various carriage studies and those from cases of IPD in indigenous Australians in remote communities in the Northern Territory of

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TABLE 1. Carriage study details

Study	Description (reference)	Study yr	No. enrolled	No. of swabs obtained	Median age at enrollment (mo)	Age range at enrollment (mo)
1a	Longitudinal study of otitis media in one community (11)	1992–1993	41	208 NP (2–4 weekly)	0.17	0.07–2.5
1b	Pilot antibiotic trial in a subset of study 1a (not published)	1993–1994	20	273 NP (2–4 weekly)	4.1	0.66–20.6
2	Randomized controlled trial of long-term amoxicillin for treatment of otitis media with effusion in three communities (12)	1996–2001	125	1,032 NP (2–4 weekly, then 1 every 6 mo)	2.8	0.2–19
3	Carriage study of infants receiving PCV7 and 23PPV in three communities (not published)	2001–2004	97	914 NP (2–4 weekly to 12 mo, then 1 at 18 and 24 mo)	1.13	0.23–7.96
4	Cross-sectional carriage surveys in four indigenous communities (13) <sup>a</sup>	2002 <sup>b</sup>	192	206	85.7	24–188
		2002 <sup>c</sup>	306	307	414	195–945
		2004 <sup>b</sup>	159	166	89.6	25–176
		2004 <sup>c</sup>	232	234	431	197–962
5	Cross-sectional carriage surveillance in children 0 to 6 yr old in 29 (2003) and 17 (2005) communities (15)	2003	902	902	21.3	0.76–75
		2005	818	818	33.7	0.36–72

<sup>a</sup> Some participants were swabbed twice; serotype 1 carriage was assessed on the first swab.

<sup>b</sup> Children less than 16 years of age.

<sup>c</sup> Adults 16 years and over.

Australia. This included a 2002 cluster of serotype 1 carriage in children.

**MATERIALS AND METHODS**

**Ethics.** The human research ethics committees of the Northern Territory Department of Health and Community services and the Menzies School of Health Research granted approval for the original studies and the analysis of serotype 1 isolates.

**Definition of studies and regions.** The Northern Territory of Australia can be divided into five administrative regions: the Darwin, Arnhem, Katherine, Barkly, and Alice regions.

The primary focus of this report was serotype 1 carriage in four closely situated communities in the Darwin region. Pneumococcal carriage in children and adults in these communities was studied between 1992 and 2004 (Table 1, studies 1 to 4). Data from a nasal pneumococcal carriage surveillance study in children 0 to 6 years of age that was undertaken in 29 communities across the Arnhem, Katherine, Darwin, and Alice regions of the Northern Territory and the Pitjantjatjara lands (included in the Alice region) in 2003 and 2005 were also included (Table 1, study 5).

**Pneumococcal immunization schedule for indigenous Australians.** For indigenous infants, vaccination with PCV7 is recommended at 2, 4, and 6 months of age, followed by the 23-valent pneumococcal polysaccharide vaccine (23PPV) at 18 to 24 months of age. 23PPV is also recommended for indigenous adults who are 15 to 49 years of age and medically at risk and for those who are 50 years of age and older (17).

**Carriage data.** NP or nasal swabs were collected from indigenous children (less than 16 years of age) and adults enrolled in a series of longitudinal or cross-sectional studies between 1992 and 2005 (Table 1). Pneumococcal carriage data were collected from the databases of the specific projects listed in Table 1. All projects were undertaken by trained research staff using standardized data collection methods including WHO-recommended swab collection, storage, and microbiological analysis techniques for nasal or NP swabs (19, 23). At least one pneumococcal colony and any that were morphologically distinct were selected from each swab for serotyping by the quellung method (Statens Serum Institut, Denmark). Serotype 1 isolates were sent to a reference laboratory (Queensland Health) for confirmation.

**IPD data.** IPD cases reported from Northern Territory hospital patients identifying the Northern Territory as their place of residence, and where the serotype was known, were included. A case of IPD was defined as the isolation or detection of *S. pneumoniae* from a blood, cerebrospinal fluid, or other sterile site sample by nucleic acid testing. Enhanced surveillance and notification of IPD

began in 1994. Collection and analysis of IPD data and isolates have been described previously (20, 26).

**Molecular typing.** All serotype 1 isolates were analyzed by BOX typing (25). Selected isolates of each BOX type and region were further analyzed by multilocus sequence typing (MLST) (3) using primers found in the MLST database (<http://spneumoniae.mlst.net/misc/info.asp#experimental>), with the exception of the *spi* forward primer (*spiF3*, 5'-CAGATTTTGCAAGCCTATGG), the *recP* reverse primer (*recPR2*, 5'-GCCGTGTACAGCATTAGTTC), and the *ddl* forward primer (*ddlF2*, 5'-GATGGCTCTGTTC AAGGATT).

**RESULTS**

**Serotype 1 carriage in the four Darwin region communities.**

The earliest study, conducted from 1992 to 1993 (study 1 [11]), identified three infant pneumococcal serotype 1 carriers and, furthermore, two carriers from among the siblings of study participants (66 NP swabs were collected from siblings of 31

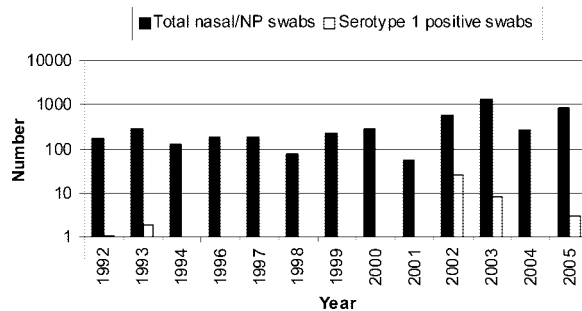


FIG. 1. Serotype 1 carriage in all studies. The number of serotype 1-positive swabs (the first serotype 1-positive swab for each individual) and the total number of nasal or NP swabs collected each year in study participants listed in Table 1 (excluding household contacts). Years 1992 to 2004 represent carriage in four Darwin region communities (studies 1 to 4), whereas years 2003 and 2005 include cross-sectional carriage surveillance data from 29 Northern Territory communities, including Darwin region communities (study 5).

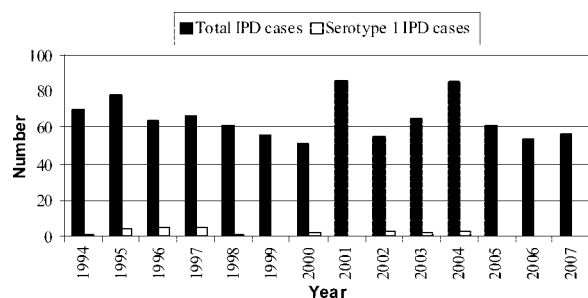


FIG. 2. Cases of all-serotype IPD and serotype 1 IPD in the Northern Territory. The total number of cases of IPD where serotype was known and the number of cases of serotype 1 IPD cases in the Northern Territory between 1994 and 2007 are shown.

infants (Table 1; Fig. 1). Longitudinal studies of infants and some household contacts conducted from 1996 to 2001 (study 2 [12]) detected serotype 1 carriage in one parent but not in children.

Subsequently, a 2002 cross-sectional pneumococcal carriage survey (study 4 [13]) of 193 children (2 to 15 years of age) and 305 adults detected a cluster of serotype 1 carriage among non-PCV7-vaccinated children 2 to 11 years of age in three of the four participating communities. Serotype 1 was the second most common serotype (after serotype 16F) carried by 13% (21/158) of children in this age group. Another carriage study of infants less than 2 years of age in the same communities (study 3 [unpublished]) detected two further serotype 1 carriers in 2002 and in early 2003. In 2004, a carriage surveillance follow-up with study 4 participants failed to detect serotype 1 carriage. Since IPD surveillance began in 1994, there have been no reported cases of serotype 1 IPD in these communities.

**Serotype 1 carriage across the Northern Territory.** Cross-sectional surveillance of pneumococcal nasal carriage in 903 children living in 29 communities in 2003 (study 5 [15, 16]) detected serotype 1 carriage in three Arnhem region communities (seven isolates from 461 swabs in this region) (Fig. 1). Further surveillance of 818 children in 17 communities in 2005 detected serotype 1 in two Katherine region communities (three isolates from 154 swabs in this region). Serotype 1 pneumococci were also detected in ear discharge samples from children with serotype 1-negative nasal swabs: 1 child in 2003 and 1 child in 2005.

**All IPD and serotype 1 IPD cases in the Northern Territory.** Twenty-six cases of serotype 1 IPD were recorded in the Northern Territory between 1994 and 2007 (Fig. 2). These cases were not from communities that had concomitant carriage studies. The median age of patients with serotype 1 IPD was 26.5 years, and 86% of cases were indigenous Australians. The serotype 1 IPD cases were associated with 20 cases of (blood culture-positive) pneumonia, 3 cases of meningitis, and 1 case each of bacteremia, peritonitis, and septic arthritis. There was a single death of an adult patient with bacteremia.

**Duration and density of serotype 1 carriage.** In longitudinal studies with 2 to 4 weekly samplings, serotype 1 strains were generally detected at single examinations. Only one child was found to carry a serotype 1 pneumococcus strain at two consecutive examinations. This was followed by detection of sero-

type 1 in bilateral ear discharge samples 1 month later. Serotype 1 isolates were detected at low and very high densities, as determined by a semiquantitative culture measure (22). Simultaneous colonization with other pneumococcal serotypes was detected in 34% of serotype 1-positive nasal swabs, compared with 14% of swabs with multiple colonization detected for all serotypes, based on colony morphology (5). Cocolonization with nonserotypeable pneumococci was evident in another 5% of serotype 1-positive swabs.

**Molecular typing.** The serotype 1 isolates from NP and ear discharge swabs collected in study 1 (1992 to 1993) were designated BOX type H (Table 2). MLST results from representative isolates corresponded to ST304 from the MLST database. Serotype 1 carriage isolates from study 4 (2002) were BOX type I, which corresponded to ST227 from the MLST database. Serotype 1 isolates detected in other Northern Territory communities in 2003 and 2005 were also BOX type I, which corresponded to ST227.

Serotype 1 isolates from cases of IPD were available from four regions in 2003 to 2004. The Darwin and Arnhem region isolates were BOX type I (ST227), whereas the Katherine region BOX type I isolate was ST3079. A BOX type J (ST3079) was found in the Darwin region, and BOX type H (ST304) was found in the Barkly region.

## DISCUSSION

Pneumococcal carriage studies in adults and children detected intermittent episodes of serotype 1 carriage and an age-specific cluster in indigenous communities in the Northern Territory of Australia. In the Darwin region, serotype 1 ST304 carriage was detected in five children in 1992 to 1993, and a decade later, we identified a cluster of serotype 1 ST227 carriage. During the following 3 years, carriage of serotype 1 ST227 was detected in children in other communities in the Darwin, Arnhem, and Katherine regions of the Northern Territory. There was no apparent increase in serotype 1 IPD cases during this time. Cases of serotype 1 ST227 IPD were not from communities with concomitant carriage studies.

The apparent restriction of serotype 1 carriage to children under 11 years of age during the carriage cluster may be related to the serotype 1 carriage detected 10 years earlier in these communities. We can speculate that serotype 1 carriage results in a protective immune response and that high-fre-

TABLE 2. Molecular types of serotype 1 pneumococci from carriage and IPD studies

Study yr	Study no.	Region	Specimen	BOX type (no. tested)	MLST (no. tested)
1992, 1993	1	Darwin	NP	H (5)	304 (1)
1992, 1993	1	Darwin	Ear discharge	H (1)	304 (1)
2002, 2003	3, 4	Darwin	NP	I (26)	227 (2)
2003	5	Arnhem	NP	I (3)	227 (1)
2005	5	Katherine	NP	I (4)	227 (1)
2003	IPD	Arnhem	Blood	I (1)	227 (1)
2003	IPD	Katherine	Blood	I (1)	3079 (1)
2004	IPD	Darwin	Blood	I (1)	227 (1)
2004	IPD	Darwin	Blood	J (1)	3079 (1)
2004	IPD	Barkly	Blood	H (1)	304 (1)



quency carriage will occur when a large proportion of the population is susceptible. The apparent age specificity may also relate to the overall reduction in pneumococcal carriage with age: 72 to 89% in children less than 9 years of age, 45% at age 10, and 30% at age 15.

A study in a Portuguese day-care center demonstrated an increase in the prevalence of serotype 1 carriage between 2001 (0%) and 2006 (3.1%). The authors suggested an association between carriage and the introduction of PCV7 (18). It is unclear from our study whether the observed cluster was a replacement phenomenon related to the 2001 commencement of infant immunization and catch-up PCV7 immunization programs. Furthermore, our data cannot ascertain whether the absence of serotype 1 carriage among adults during the carriage cluster in children is related to expanded 23PPV immunization recommendations (17).

In conclusion, we have detected serotype 1 strains circulating in carriage in indigenous children living in remote communities in the Northern Territory of Australia. The sequence types of these strains are the same as internationally disseminated IPD strains, including those found in the Northern Territory. It is generally believed that serotype 1 carriage is rarely detected because it is short-lived. Our longitudinal data support this belief, and we suggest that carriage rates are further underestimated because carriage may be "hidden" during colonization with other serotypes. We can further speculate from our data that serotype 1 carriage is more likely to be detected among young children who have higher pneumococcal acquisition rates and who are less likely than adults to have had previous exposure to serotype 1.

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