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Laboratory surveillance of invasive pneumococcal disease in New South Wales, Australia, before and after introduction of 7-valent conjugate vaccine: reduced disease, but not antibiotic resistance rates

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SUMMARY

We compared serotype distributions of *Streptococcus pneumoniae* isolates from patients aged <5 and $\geqslant 5$ years with invasive pneumococcal disease in New South Wales, Australia, and antibiotic susceptibilities of isolates from the <5 years age group only, before (2002–2004) and after (2005–2009) introduction of the 7-valent pneumococcal conjugate vaccine (PCV7). Overall, there were significant decreases in the mean annual number of referred isolates (770 *vs.* 515) and the proportion belonging to PCV7 serotypes (74% *vs.* 38%), but non-PCV7 serotypes, particularly 19A, increased (5% *vs.* 18%). All changes were more marked in the <5 years age group. Susceptibility testing of isolates from the <5 years age group showed variation in resistance between serotypes, but significant overall increases in penicillin non-susceptibility (23% *vs.* 31%), ceftriaxone resistance (2% *vs.* 12%) and multidrug resistance (4% *vs.* 7%) rates; erythromycin resistance fell (32% *vs.* 25%). Continued surveillance is needed to monitor changes following the introduction of 13-valent PCV in 2012.

Key words: Antibiotic susceptibility, broth microdilution, serotype distribution, 7-valent pneumococcal conjugate vaccine, *Streptococcus pneumoniae*.

INTRODUCTION

Streptococcus pneumoniae is one of the most significant childhood pathogens. A systematic review of disease burden in children aged <5 years estimated that in 2000 there were 14·5 million cases of severe pneumococcal disease, causing 821 000 deaths worldwide [1]. In Australia, in 2002, the incidence of invasive pneumococcal disease (IPD) was 11·5/100 000 overall (or 57·3/100 000 in children aged <5 years). Of 2271 cases notified, 44% presented with pneumonia, 5% with meningitis and 35% with unlocalized bacteraemia [2, 3].

The 7-valent pneumococcal conjugate vaccine (Prevnar®, Wyeth; PCV7), which contains serotype antigens 4, 6B, 9V, 14, 18C, 19F and 23F, has been available in Australia since 2001, when it was introduced for routine use in Aboriginal children and other high-risk groups. There was limited uptake in the non-Aboriginal population until it was added to the routine immunization schedule in January 2005. Since then, it has been available free of charge for all

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infants as a three-dose schedule given at ages 2, 4 and 6 months [4]. In October 2011, a 13-valent pneumococcal conjugate vaccine [Prevenar 13® (PCV13), containing the seven PCV7 antigens plus serotype 1, 3, 5, 6A, 7F and 19A antigens] replaced PCV7. Since 1999, all isolates from IPD cases have been referred for serotyping to one of three Pneumococcal Reference Laboratories (PRL) in Australia, including one in New South Wales (NSW) where the population in June 2009 was 7·1 million, or nearly one third of the total Australian population. Based on the number of notified cases of IPD, we estimate that isolates are referred from at least 95% of cases in NSW each year.

Studies in many countries have documented the impact of routine use of PCV7 in infants on *S. pneumoniae* serotype distribution but few have examined corresponding changes in antibiotic resistance rates and results are varied [5–8]. There is little recent information on pneumococcal resistance in Australia [9, 10]. The aim of this study was to compare serotype distributions of *Streptococcus pneumoniae* isolates from patients aged <5 and $\geqslant 5$ years with IPD in NSW, Australia before and after introduction of PCV7 and also to determine rates of antibiotic resistance, in relation to changes in serotype distribution, in IPD isolates from children aged <5 years old in NSW before and after the introduction of PCV7, and before the introduction of PCV13, into the routine infant immunization schedule.

METHODS

Specimens

Between 2002 and 2009, 5177 *S. pneumoniae* isolates from patients with IPD were referred to the NSW Pneumococcal Reference Laboratory at the Centre for Infectious Diseases and Microbiology, Westmead Hospital. After exclusion of isolates that were duplicates ($n=102$), not viable on receipt ($n=75$), not *S. pneumoniae* ($n=26$), non-typable ($n=45$) or for which no corresponding patient date of birth was available ($n=43$) there were 4886 unique isolates with known serotype and patient age, of these, 1170 (23·5%) were from children aged <5 years. Antibiotic susceptibility testing could not be performed on 166 (14%) of these 1170 isolates because the isolate could not be located from storage ($n=22$), was not viable on subculture ($n=43$) or, in the early years of surveillance, had been sent as a formalized specimen ($n=101$), leaving 1004 isolates from children aged <5 years for susceptibility testing.

Serotypes were grouped as: (*a*) those included in PCV7; (*b*) those included in the PCV13, excluding PCV7 serotypes; and (*c*) non-PCV serotypes.

Serotyping

Pneumococcal isolates were serotyped by Neufeld's Quellung reaction using pool, type and factor-specific antisera (Statens Serum Institut, Copenhagen, Denmark).

Antibiotic susceptibility testing

Commercial broth microdilution minimum inhibitory concentration (MIC) panels (Sensititre, TREK Diagnostic Systems Ltd, UK) were used for quantitative susceptibility testing. This panel includes 18 antibiotics and three blank cells as growth controls. Antibiotics (range of concentrations in mg/l) used in this panel were: azithromycin (0·25–2), amoxicillin/clavulanate (2/1–16/8), cefuroxime (0·5–4), meropenem (0·25–2), erythromycin (0·25–2), chloramphenicol (2–16), cotrimoxazole (0·5/9·5–4/76), vancomycin (0·5–4), telithromycin (0·06–2), ceftriaxone (0·06–2), levofloxacin (0·5–16), clindamycin (0·06–2), cefipime (0·12–8), penicillin (0·03–80), tetracycline (0·5–8), linezolid (0·25–4), moxifloxacin (0·25–8), and gatifloxacin (0·5–8).

MICs corresponding to susceptible and resistant were classified as recommended by the Clinical and Laboratory Standards Institute (CLSI) for all antibiotics except penicillin. The CLSI MIC cut-offs for penicillin-susceptible, -intermediate and -resistant (S/I/R) isolates were changed, in 2008, depending on disease type. However, for consistency with previous data we retained the previous National Committee for Clinical Laboratory Standards (NCCLS) categories namely susceptible (S) $\leqslant 0{\cdot}06$ mg/l, intermediate (I) 0·12–1 mg/l, and resistant $\geqslant 2$ mg/l (R).

Statistical analysis

Changes in the distribution of serotypes and antibiotic susceptibility within serotypes between periods were compared using χ^2 or Fisher's exact test as appropriate. Because not all isolates with a known serotype could be tested for antibiotic susceptibility, to obtain susceptibility estimates for groups of serotypes we weighted the serotype-specific susceptibility patterns by the distribution of serotypes in the groups. For

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Table 1. *Changes in numbers and proportions of isolates referred in different serotype groups and age groups before and after introduction of the 7-valent pneumococcal conjugate vaccine*

	Referred isolates (% of total)			
	<5 years age group		⩾5 years age group	
Serotype group: serotypes	2002–2004	2005–2009	2002–2004	2005–2009
PCV7 total referred:	630 (88)†	146 (32)†	1074 (67)	824 (39)
Mean referred p.a.	210 (88)	29·2 (32)	358 (67)	164·8 (39)
14‡	85·3 (36)	7·8 (9)	111·7 (21)	30·6 (8)
19F	30·3 (13)	8·8 (10)	33 (6)	24·8 (6)
6B	29·3 (12)	4·3 (5)	32 (6)	20·4 (5)
4	18·3 (8)	1·4 (1·5)	84 (16)	31·6 (7)
23F	14·3 (6)	2 (2)	38 (7)	21·4 (5)
9V	13·7 (6)	1·2 (1)	41 (8)	23 (5)
18C	18·7 (8)	3·8 (4)	18·3 (3)	13·4 (3)
All non-PCV7*: total	84 (12)	310 (68)	521 (33)	1297 (61)
Mean referred p.a.	28	62	173·6	259·4
PCV13 excl. PCV7: total	51 (7)	198 (43)	234 (25)	630 (44)
PCV13 excl. PCV7: mean p.a.	17 (7)	39·6 (43)	78	126
19A†	8·7 (4)	30·4 (33)	18·7 (4)	51·6 (12)
6A	5·7 (2)	3·2 (4)	13·7 (3)	14·8 (3)
3‡	2·3 (1)	3·4 (4)	31·3 (6)	30·2 (7)
1§	0	1·4 (2)	5·3 (1)	16·6 (4)
7F‡	0·3	1·2 (1)	9 (2)	12·8 (3)
5	0	0	0	0
Non-PCV: total	33 (5)	112 (25)	287 (18)	667 (31)
Non-PCV: mean p.a.	11 (5)	22·4 (25)	95·6 (18)	133·4 (31)
22F†	2 (1)	4·2 (5)	22 (4)	29·4 (7)
15B§	0·7 (<1)	2·2 (2)	3·7 (<1)	4·6 (1)
15C	1·7 (<1)	3·6 (4)	2·7 (<1)	7 (2)
38	1·7 (<1)	2·2 (2)	2·3 (<1)	6·2 (1)
6C‡	0·3 (<1)	1·8 (2)	2 (<1)	12·2 (3)
33F‡	0·3 (<1)	1·4 (2)	4 (<1)	6·4 (2)
11A	0	1·2 (1)	10 (2)	10 (2)
Others	4·3 (2)	5·8 (6)	49 (9)	57·6 (14)
Grand total isolates (*N*)	714	456	1595	2121
Average referred p.a.	238	91·2	531·7	424·2

* Non-PCV7 group includes all serotypes except those serotypes in the PCV7 vaccine.
The following differences in proportions were statistically significant between time periods: † $P<0{\cdot}0001$, ‡ $P=0{\cdot}01$, § $P=0{\cdot}001$.

these adjusted proportions, variance was calculated using methods for a standardized proportion [11] and the z approximation to the binomial distribution was used to make comparisons. P values $<0{\cdot}05$ were considered statistically significant for all comparisons.

RESULTS

Changes in serotype distribution between time periods

The mean annual numbers and cumulative percentages of the main serotypes in referred isolates, in both age groups and time periods, are shown in Table 1 and Figures 1 and 2. The average numbers of isolates referred per annum fell significantly between 2002–2004 and 2005–2009, due to decreases in PCV7 serotypes, which fell by 86% (from 210 to 29·2) in the <5 years age group and by 54% (from 358 to 164·8) in the ⩾5 years age group (Table 1).

This was partly offset by significant increases in the average numbers of isolates referred each year in non-PCV7 serotypes which increased 121% (from 28 to 62) and 49% (from 173·6 to 259·4) in the <5 and ⩾5 years age groups, respectively. This was

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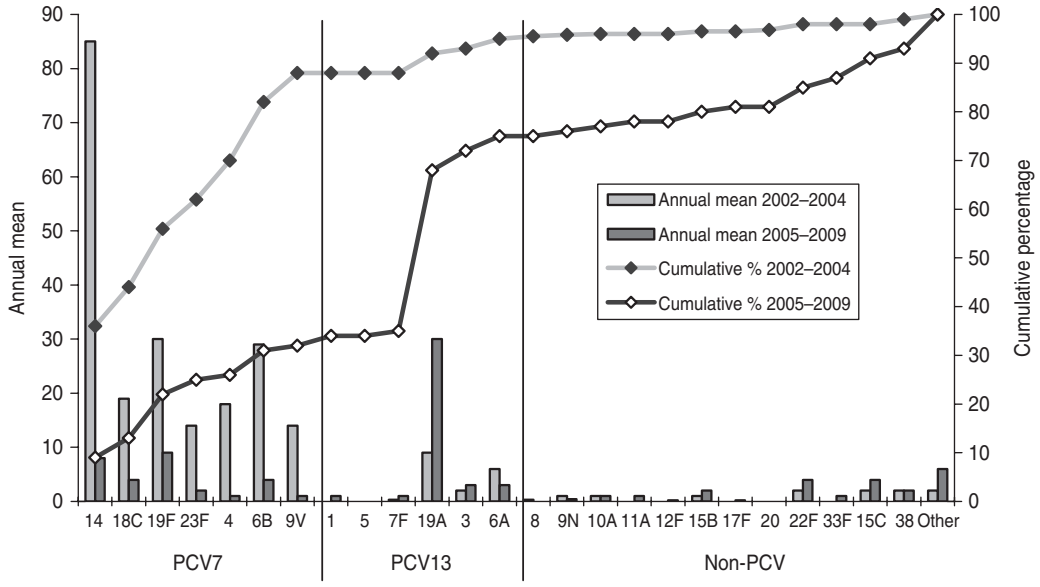

Fig. 1. Mean annual number and cumulative percentages of serotype groups and selected serotypes identified in invasive pneumococcal disease isolates referred to the NSW Pneumococcal Reference Laboratory, from children aged <5 years before and after introduction of 7-valent pneumococcal conjugate vaccine into the routine childhood immunization schedule (in 2005).

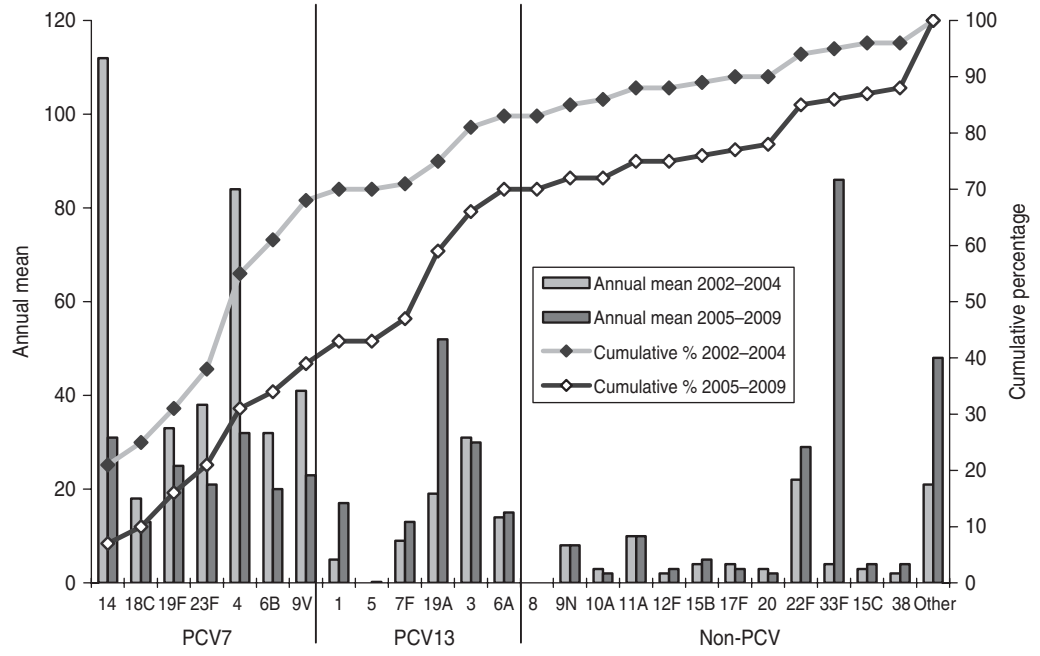

Fig. 2. Mean annual number and cumulative percentages of serotype groups and selected serotypes identified in invasive pneumococcal disease isolates referred to the NSW Pneumococcal Reference Laboratory, from children and adults aged ⩾5 years before and after introduction of 7-valent pneumococcal conjugate vaccine into the routine childhood immunization schedule (in 2005).

mainly due to serotype 19A, which rose from 4% to 33% of the total in the <5 years age group and from 4% to 12% in the ⩾5 years age group (Table 1). Although numbers are small, even when the two age groups were combined, there were significant proportional increases between periods in serotypes 1, 3, 6C, 7F, 15B, 22F and 33F (Table 1 and Figs. 1 and 2).

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Table 2. *Numbers and proportions of referred isolates from <5-year-olds tested for antibiotic susceptibility before (2002–2004) and after (2005–2009) the introduction of 7-valent pneumococcal conjugate vaccine, by serotype group*

Serotype group: serotypes	Tested isolates/referred isolates (%)	
	2002–2004	2005–2009
PCV7	506/630 (80)	139/146 (95)
14	204/256 (80)	36/39 (92)
19F	77/91 (85)	44/44 (100)
6B	65/88 (74)	19/21 (90)
4	47/55 (85)	6/7 (86)
23F	39/43 (91)	10/10 (100)
9V	37/41 (90)	6/6 (100)
18C	37/56 (66)	18/19 (95)
PCV13 excl. PCV7	38/51 (75)	186/198 (94)
19A	17/26 (65)	140/152 (92)
6A	15/17 (88)	16/16 (100)
3	5/7 (71)	17/17 (100)
1	0	7/7 (100)
7F	1/1 (100)	6/6 (100)
5	0	0
Non-PCV	28/33 (85)	107/112 (96)
22F	5/6 (83)	20/21 (95)
15B	2/2 (100)	10/11 (91)
15C	3/5 (60)	16/18 (89)
38	5/5 (100)	11/11 (100)
6C	1/1 (100)	9/9 (100)
33F	1/1 (100)	7/7 (100)
11A	0	6/6 (100)
Others*	11/13 (85)	28/29 (97)
Grand total	572/714 (80)	432/456 (95)

* Other non-PCV serotypes: 23A, 10A, 9N, 35F, 24F, 25A, 35B (<5 isolates each); 12F, 20, 8, 2, 5, 17F, 9A/L, 18A/B/F, 19B/C, 23B, 13, 16F, 21, 33A, 35A, 22A, 7C, 15A (⩽1 isolate each).

Antibiotic susceptibility

The distribution of 1170 isolates from children aged <5 years and the 1004 isolates available for testing, between serotype groups (PCV7, PCV13 and selected non-PCV serotypes) and time periods is shown in Table 2. Of the 1004 isolates available for testing, 415 (41%) were susceptible to all antibiotics tested. Table 3 shows the numbers and proportions (weighted to represent all referred isolates with known serotype) of PCV7, 19A and other serotypes, which were penicillin intermediate (pen-I; MIC 0·12–1 mg/l) or resistant (pen-R; MIC ⩾2 mg/l) by time period. As expected, there were major differences between serotypes. Pen-R isolates were more prevalent in PCV7, than in non-PCV7 serotypes in both time periods; with the highest resistance rates in 19F and 9V. Pen-R rates did not change significantly between time periods overall, or in either PCV7 or non-PCV7 serotype groups, but there was a significant increase in pen-R serotype 14 only. Pen-I was less common than pen-R in PCV7 isolates but more common in non-PCV7 isolates. The combined rates of pen-I and pen-R in PCV7 isolates did not change, significantly, but increased, in non-PCV7 isolates, from 24% to 34% between time periods, largely due to the increase in proportion of serotype 19A isolates, most of which were pen-I.

There was a significant overall decrease in the rate of erythromycin resistance between time periods (Table 4). Of PCV7 isolates, the relatively high resistance rates, especially in serotype 14 isolates, did not change between time periods. In contrast, in non-PCV7 serotypes, an initially low rate of erythromycin resistance in 2002–2004 increased significantly in 2005–2009, due to increases in the overall proportion and erythromycin resistance rate of serotype 19A.

All isolates were susceptible to vancomycin and linezolid, all but one to telithromycin and >99% to gatifloxacin, levofloxacin and moxifloxacin (data not shown). The weighted numbers and proportions of isolates resistant to other antibiotics are shown in Table 5. Two of 1004 isolates tested (0·2%, both serotype 19F) were resistant to all antibiotics tested except vancomycin, linezolid, telithromycin, gatifloxacin, levofloxacin and moxifloxacin. The highest rates of resistance in both periods were to macrolides (azithromycin, erythromycin), cotrimoxazole and penicillin, although macrolide resistance decreased significantly between periods. Ceftriaxone resistance was uncommon, but increased significantly (from 2% to 6%). All but one of the ceftriaxone-resistant isolates were also resistant to penicillin (data not shown). Clindamycin and tetracycline resistance rates also increased (Table 5).

There was also a significant increase in multidrug resistance (MDR) (i.e. resistance to three or more antibiotic classes) between time periods. For example penicillin, erythromycin and cotrimoxazole (Pen/Ery/Cot) MDR increased from 4% to 7% (25/572 *vs.* 31/432, $P=0·04$). In 2002–2004, nearly all Pen/Ery/Cot (21/25, 84%) MDR isolates belonged to serotype 19F compared to only 35% (11/31, $P<0·001$) after 2005, when 19F was replaced as the predominant MDR serotype by 19A, which increased from 4% (1/25) to 39% (12/31) of all MDR

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Table 3. *Weighted numbers and proportions of referred isolates from <5-year-olds that were penicillin-intermediate (pen-I) and penicillin-resistant (pen-R) before (2002–2004) and after (2005–2009) the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7), by serotype group*

	2002–2004			2005–2009		
Serotype	Referred	Pen-I*, *n* (%)	Pen-R†, *n* (%)	Referred	Pen-I, *n* (%)	Pen-R, *n* (%)
PCV7	630	70 (11)	80 (13)	146	13 (9)	25 (17)
14	256	24 (9)	11 (4)‡[a]	39	4 (10)	7 (18)‡[a]
19F	91	13 (14)	26 (29)	44	5 (11)	12 (27)
6B	88	20 (23)	4 (5)	21	0	3 (14)
4	55	1 (2)	1 (2)	7	0	0
23F	43	3 (7)	2 (5)	10	1 (10)	1 (10)
9V	41	4 (10)	28 (68)	6	1 (17)	2 (33)
18C	56	5 (9)	3 (5)	19	1 (5)	0
Non-PCV7	84	15 (18)	3 (6)	310	89 (29)	16 (5)
19A	26	12 (46)	3 (12)	152	84 (55)	15 (10)
All others	58	4 (7)	0	158	7 (5)	1 (1)
Total	714	84 (12)‡[b]	82 (12)	456	101 (22)‡[b]	41 (9)

* Penicillin minimum inhibitory concentration (MIC): I = 0·12–1 mg/l.
† Penicillin MIC: R = ⩾2 mg/l.
‡ Significant differences between time periods were: [a] increased proportion of penicillin-resistant serotype 14 isolates ($P<0·005$); [b] increased proportion of penicillin-intermediate isolates ($P<0·001$).

Table 4. *Weighted numbers and proportions of referred isolates from <5-year-olds that were erythromycin-resistant before (2002–2004) and after (2005–2009) the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7), by serotype group*

	2002–2004		2005–2009	
Seorotype group: serotype	Referred	Ery-R* (%)	Referred	Ery-R* (%)
PCV7	630	220 (35)	146	50 (34)
14	256	142 (55)	39	20 (51)
19F	91	35 (38)	44	15 (34)
6B	88	27 (31)	21	9 (43)
4	55	1 (2)	7	1 (14)
23F	43	8 (19)	10	3 (30)
9V	41	1 (2)	6	3 (50)
18C	56	8 (14)	19	0
Non-PCV7	84	5 (6)†	310	61 (20)†
19A	26	3 (12)‡	152	51 (34)‡
All others	58	2 (4)	158	11 (7)
Total	714	226 (32)§	456	112 (25)§

* Ery-R = erythromycin resistant; minimum inhibitory concentration >0·05 mg/l.
† Increased erythromycin resistance in non-PCV7 isolates ($P=0·005$).
‡ Increased erythromycin resistance in serotype 19A ($P=0·04$).
§ Decreased erythromycin resistance rate in all isolates ($P=0·01$).

isolates ($P<0·01$). Other serotypes represented in Pen/Ery/Cot MDR isolates were 14 (11%, no change between time periods), 6A, 6B, 9V and 23F (1–2 isolates each).

DISCUSSION

This is the first study in Australia and one of the few anywhere, in which the changes in serotype

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Table 5. *Weighted numbers and proportions of isolates from <5-year-olds that were resistant to individual antibiotics before (2002–2004) and after (2005–2009) the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7)*

	2002–2004 Referred ($n=714$)	2005–2009 Referred ($n=456$)
Antibiotics	Resistant isolates, *n* (%)	
Amoxicillin/clavulanate	21 (3)	23 (5)
Azithromycin	216 (30)	101 (22)‡[a]
Cefepime	29 (4)	30 (6)
Ceftriaxone	17 (2)	26 (6)‡[b]
Cefuroxime	105 (15)	56 (12)
Chloramphenicol	27 (4)	19 (4)
Clindamycin	51 (7)	61 (14)‡[c]
Cotrimoxazole	157 (22)	79 (17)
Erythromycin	226 (32)	112 (25)‡[d]
Meropenem	24 (3)	23 (5)
Penicillin (R&I)†	166 (23)	143 (31)‡[a]
Tetracycline	72 (10)	79 (17)‡[e]

† Penicillin-resistant and penicillin-intermediate (non-susceptible) isolates combined.

‡ Statistically significant differences in resistance rates between 2002–2004 and 2005–2009: [a] $P=0{\cdot}002$, [b] $P=0{\cdot}005$, [c] $P=0{\cdot}0005$, [d] $P=0{\cdot}01$, [e] $P=0{\cdot}0004$.

distribution have been directly correlated with serotype-specific and overall antibiotic susceptibility in a comprehensive collection of IPD isolates, routinely referred for serotyping. In common with previous studies [12–15], we confirmed a rapid and marked fall in the mean annual numbers and proportions of PCV7 serotypes referred after the introduction of routine childhood immunization, in Australia, particularly in the vaccine target group. This was also apparent to a lesser extent in older age groups due to herd effect. In contrast, there was a significant increase in absolute numbers and proportions of non-PCV7 serotypes in both age groups, to which the main contributor was serotype 19A with significant, but less marked, contributions from 1, 3, 6C, 7F, 15B, 22F and 33F.

Before introduction of PCV7, penicillin resistance was common in several of the most common 'childhood' serotypes [14], especially 19F and 9V. As expected, antibiotic resistance of IPD isolates fell in many countries, at least initially, following widespread vaccine use [16, 17], because of the marked fall in the numbers of cases due to PCV7 serotypes. However, this was offset by increases in antibiotic-resistant non-PCV7 serotypes, especially 19A, which spread throughout North America and many other countries [7, 13, 18–21].

There has been little previous information about antibiotic resistance in invasive pneumococci in Australia. A national survey in 2005, of all age groups, showed that 16·5% of 351 invasive isolates were penicillin non-susceptible (MIC $\geqslant 0{\cdot}12$ mg/l), including 5·4% that were pen-R [22]. Our results (24% non-susceptible overall, and 12% pen-R in 2002–2004, in the <5 years age group) are somewhat higher, but the post-PCV7 changes (to 31% and 9%, respectively) are broadly consistent with those reported in international studies of antibiotic resistance following introduction of PCV7. Some studies have shown an initial reduction in penicillin non-susceptibility (pen-I plus pen-R) rates, later followed by increases [12, 14, 18] and others, no significant change [5]. Although penicillin non-susceptibility rates have increased after introduction of PCV7 in many countries, the actual rates vary widely in different regions from 54% to 74% in parts of Africa and Asia to 20–30% in many European countries (MIC >0·06) [14] and 31% Australia (present study).

Most previous studies, like ours, have reported very low rates of resistance to vancomycin, telithromycin,

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linezolid and gatifloxacin [23] and high rates of macrolide (16–32%) and cotrimoxazole (17–22%) resistance [24–26]; changes in resistance rates in response to PCV7 use vary [27, 28].

As reported elsewhere [29], we found that resistance to third-generation cephalosporins, while generally still low, increased after introduction of PCV7. This is of concern, since this class of antibiotics is the mainstay of meningitis treatment and could lead to increased reliance on vancomycin, which is now recommended, in addition to a third-generation cephalosporin, for empirical therapy of suspected pneumococcal meningitis. Fortunately, pneumococci are still universally susceptible to vancomycin [30, 31]. Most cases of IPD, other than meningitis, (including those due to pen-I and pen-R pneumococci) will respond to parenteral penicillin, which should be the initial treatment of choice in community-acquired pneumonia.

Recent increases in serotype 19A prevalence have been reported previously in Australia [15, 32, 33] and elsewhere [7, 15, 34–37], as have smaller increases in other non-PCV7 serotypes, including 1, 3, 7F, 15, 22 and 33 [38]. In NSW, serotype 19A isolates had a high pen-I rate, which increased after the introduction of PCV7 and was the major contributor to the unchanged overall levels of penicillin non-susceptibility. Serotype 19A was generally uncommon before the introduction of PCV7. In NSW, it represented only 4% of isolates from <5-year-olds in 2002–2004 but increased to 33% in 2005–2009, with a fivefold increase in number of isolates referred. This is most likely due to a combination of antibiotic use and immune pressure [39].

While serotype 19A has increased in many countries, there have been differences in the dynamics of change. For example, in Canada a high proportion of the increased number of serotype 19A isolates were pen-R and MDR and belonged to clonal complex (CC)271/320. This was attributed to a capsule-switching recombination event (with the predominant pre-vaccine pen-R serotype 19F) and subsequent clonal expansion [40]. In NSW, the predominant pre-vaccine pen-R serotype, 19F, also mostly belonged to CC271/320 [41]. However, post-vaccine serotype 19A isolates in NSW are mainly pen-I and the increase in their numbers is more likely to be due to expansion of a pre-existing pen-I 19A clone, than to capsule switching. This is consistent with the findings of Hanage *et al.* [42], who showed that increases in pen-R non-PCV7 serotypes were due to expansion of pre-existing clones rather than acquisition of *de novo* resistance or serotype switching. Further work is underway to identify the predominant clonal complexes in 19A isolates in NSW.

In the USA [43], the increase in pen-R serotype 19A began before and continued after the introduction of PCV7 (in 2001), but stabilized in 2005. As in Canada, this was largely due to expansion of pen-R CC271/320, but in the USA it replaced the predominantly pen-I CC199 as the most prevalent 19A CC, suggesting that antibiotic use, rather than vaccine pressure, was the major driver of the increase in 19A. However, a concomitant increase in a putative 'vaccine escape', pen-S/I ST695 serotype 19A (a variant of CC199, which switched capsule and adjacent penicillin-binding protein genes with serotype 4) would be better explained by vaccine pressure. Vaccine pressure would also explain the finding, in an infant PCV7 immunization study, that acquisition of serotype 19A was significantly higher in fully immunized (with three doses) than unimmunized children [44]. Thus the global emergence of serotype 19A appears to have been driven by both antibiotic use and immune pressure (and probably other factors), to varying degrees, and to result from recombination involving capsular and/or antibiotic resistance loci, clonal expansion or both [45].

Our study was limited by the fact that we were unable to test the susceptibility of IPD isolates in all age groups. However, given the major differences in serotype-specific resistance, we believe that serotype distribution is a reasonable predictor of antibiotic resistance. As expected, the changes in serotype distribution in the $\geqslant 5$ years age group was qualitatively similar to but less marked than that in the vaccine target age group, reflecting the well-described herd effect. The study demonstrates the complexity of changes in pneumococcal antibiotic resistance. The predicted major decrease in β-lactam antibiotic resistance, following the introduction of PCV7, has not been realized despite the marked fall in overall incidence of IPD. Although PCV13 includes serotype 19A and other serotypes that have increased since the introduction of PCV7, any attempt to predict its impact on future serotype or antibiotic resistance distributions of IPD isolates would be premature. Therefore, continued laboratory surveillance of IPD isolates (at least) is essential to inform future treatment guidelines and vaccine development.

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DECLARATION OF INTEREST

None.

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