Impact of recent antibiotics on nasopharyngeal carriage and lower airway infection in Indigenous Australian children with non-cystic fibrosis bronchiectasis

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Abstract: Indigenous Australian children have the world's highest recorded rate of bronchiectasis. Despite a lack of high-level evidence on effectiveness and antibiotic resistance, long-term antibiotics are often prescribed as part of their clinical management. We determined the impact of recent macrolide (primarily azithromycin) and beta-lactam antibiotic use on nasopharyngeal colonisation, lower airway infection (>10^4 colony-forming units/mL in bronchoalveolar lavage fluid culture) and antibiotic resistance in nontypeable Haemophilus influenzae (NTHi), Streptococcus pneumoniae and Moraxella catarrhalis isolates from 104 Indigenous children with radiographically-confirmed bronchiectasis. Recent antibiotic use was associated with reduced nasopharyngeal carriage of these respiratory bacterial pathogens, especially S. pneumoniae (Odds Ratio [OR] 0.22, 95% Confidence Interval [CI] 0.08, 0.63) and M. catarrhalis (OR 0.33, 95% CI 0.11, 1.01) in 39 children who received macrolides, and S. pneumoniae (OR 0.07, 95% CI 0.01, 0.32) in 26 children who received beta-lactam antibiotics. In the lower airways, only M. catarrhalis infection was reduced in children who received macrolides, but this was not statistically significant (OR 0.18, 95% CI 0.00, 1.75). Children given macrolides were more likely to carry (OR 4.58, 95% CI 1.14, 21.7) and be infected by (OR 8.13, 95% CI 1.47, 81.3) azithromycin-resistant S. pneumoniae. Children receiving beta-lactam antibiotics were more likely to have lower airway infection with ampicillin-resistant NTHi (OR 4.40, 95% CI 0.85, 23.9). Increased risk of lower airway infection by antibiotic-resistant pathogens in children receiving antibiotics is of concern and clinical trials to determine if long-term antibiotic treatments provide an overall clinical benefit in this setting are awaited.

IMPACT OF RECENT ANTIBIOTICS UPON NASOPHARYNGEAL CARRIAGE AND LOWER AIRWAY INFECTION IN INDIGENOUS AUSTRALIAN CHILDREN WITH NON-CYSTIC FIBROSIS BRONCHIECTASIS

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Abstract

Indigenous Australian children have the world's highest recorded rate of bronchiectasis. Despite a lack of high-level evidence on effectiveness and antibiotic resistance, long-term antibiotics are often prescribed as part of their clinical management. We determined the impact of recent macrolide (primarily azithromycin) and beta-lactam antibiotic use on nasopharyngeal colonisation, lower airway infection (>10^4 colony-forming units/mL in bronchoalveolar lavage fluid culture) and antibiotic resistance in nontypeable *Haemophilus influenzae* (NTHi), *Streptococcus pneumoniae* and *Moraxella catarrhalis* isolates from 104 Indigenous children with radiographically-confirmed bronchiectasis. Recent antibiotic use was associated with reduced nasopharyngeal carriage of these respiratory bacterial pathogens, especially *S. pneumoniae* (Odds Ratio [OR] 0.22, 95% Confidence Interval [CI] 0.08, 0.63) and *M. catarrhalis* (OR 0.33, 95% CI 0.11, 1.01) in 39 children who received macrolides, and *S. pneumoniae* (OR 0.07, 95% CI 0.01, 0.32) in 26 children who received beta-lactam antibiotics. In the lower airways, only *M. catarrhalis* infection was reduced in children who received macrolides, but this was not statistically significant (OR 0.18, 95% CI 0.00, 1.75). Children given macrolides were more likely to carry (OR 4.58, 95% CI 1.14, 21.7) and be infected by (OR 8.13, 95% CI 1.47, 81.3) azithromycin-resistant *S. pneumoniae*. Children receiving beta-lactam antibiotics were more likely to have lower airway infection with ampicillin-resistant NTHi (OR 4.40, 95% CI 0.85, 23.9). Increased risk of lower airway infection by antibiotic-resistant pathogens in children receiving antibiotics is of concern and clinical trials to determine if long-term antibiotic treatments provide an overall clinical benefit in this setting are awaited.

Key words: azithromycin, bronchiectasis, microbial drug resistance, nasopharynx, respiratory tract infections.

1. Introduction

The role of bacteria in children with non-cystic fibrosis (CF) bronchiectasis has received surprisingly little attention [1]. Indigenous Australian children have a heavy burden of respiratory disease [2]. Within weeks of birth their upper airways are colonised by *Streptococcus pneumoniae* (pneumococcus), nontypeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* [3]. We recently presented evidence indicating that recurrent pulmonary aspiration of bacterial-laden nasopharyngeal secretions might be important in the pathogenesis of non-CF bronchiectasis in this high-risk group of children [4].

The prescription of long-term maintenance antibiotics, such as azithromycin, is sometimes used to treat adults and children with non-CF bronchiectasis. However, the overall effects of azithromycin in these patients are uncertain. Although a small randomised controlled trial and retrospective reviews from several centres managing adults with non-CF bronchiectasis report that azithromycin improves exacerbation frequency and spirometry, and suppresses sputum bacteria [5-7], higher-level evidence for efficacy is lacking. Moreover, azithromycin can lead to macrolide resistance in respiratory flora [8], an increasing problem worldwide [9]. Consequently, substantial gaps in our knowledge remain over the effects of azithromycin on the upper and lower airway microbiology of children with non-CF bronchiectasis. Moreover, uncertainty surrounds clinically relevant antimicrobial resistance breakpoints for bacterial pathogens acting at separate anatomical sites within the respiratory tract [10].

In this prospective, cross-sectional, observational study we examined the impact of recent antibiotic exposure upon nasopharyngeal colonisation, lower airway infection and antibiotic resistance in common respiratory bacterial pathogens detected in Indigenous children

undergoing bronchoscopy following a radiographically-confirmed diagnosis of bronchiectasis.

2. Methods

2.1 Study participants

Children referred for evaluation of persistent wet cough and/or recurrent lower respiratory infections were enrolled into the study between July 2007 and September 2011 inclusive. All participants had flexible bronchoscopy and high-resolution computed tomography under general anaesthesia for diagnostic evaluation of suspected bronchiectasis at the Royal Darwin Hospital, Northern Territory (NT), Australia. Non-Indigenous children or those not found to have radiographically-confirmed bronchiectasis were excluded from this analysis, as were those found to have cystic fibrosis. The Human Research Ethics Committee of the NT Department of Health and Menzies School of Health Research approved the study. The children's carers provided written, informed consent.

2.2 Data collection

Clinical information was extracted from the medical records using a standardised data collection form. A diagnosis of radiographically-confirmed bronchiectasis was made by the treating paediatric respiratory physician based on the standard criteria for the assessment of high resolution CT scans. Recent antibiotic use was defined as having received any antibiotic within the 2 week period prior to bronchoscopy.

2.3 Specimen collection and storage

Immediately after anaesthetic induction, a rayon-tipped swab was inserted into the nasopharynx, rotated for 2-3 seconds before being withdrawn and placed into a tube

containing 1mL of skim milk tryptone glucose glycerol broth (STGGB) [4]. This was followed by transnasal flexible bronchoscopy as previously outlined [4] so that suction channel use was avoided until the bronchoscope tip extended below the carina. Bronchoalveolar lavage (BAL; 1 mL/kg; maximum 20mL sterile saline) was conducted on the most abnormal appearing lobe identified on radiography or at bronchoscopy in accordance with the European Respiratory Society's guidelines [11], followed by immediate suctioning into a mucus trap. A 0.5 mL aliquot of BAL fluid was added to a cryovial containing 0.5mL of concentrated STGGB for bacterial culture. The BAL fluid and nasopharyngeal swab specimens were transferred to the laboratory for storage at -80^{o}C.

2.4 Laboratory procedures

Specimens were thawed, mixed and 10µL aliquots plated onto selective media. Semi-quantitative colony counts were performed and correlated to density of colony-forming units (CFU)/mL in BAL fluid by serial dilution and quantitative colony counts, and bacterial species were identified according to standard procedures [4]. Lower airway infection was diagnosed when any single respiratory bacterial pathogen was grown in concentrations >10^4 CFU/mL in BAL fluid cultures [4].

From each positive specimen, up to four colonies each of *S. pneumoniae* and NTHi were tested for antibiotic susceptibility using the calibrated disc sensitivity (CDS) method [12]. Minimum inhibitory concentrations (MICs) for penicillin and azithromycin (*S. pneumoniae*) and for ampicillin and azithromycin (NTHi) were determined by Etest® strips (AB Biodisk [now AB bioMérieux], Sweden) when the respective disc annuli were <6mm. Resistance was defined according to the Etest® guidelines, except for *H. influenzae* resistance to azithromycin where the European Committee on Antimicrobial Susceptibility Testing

(EUCAST) breakpoints were used; these include a category for intermediate resistance [13]. NTHi and *M. catarrhalis* isolates were tested for beta-lactamase (BL) activity employing a nitrocephin-based test. *S. pneumoniae* isolates were serotyped by the Quellung reaction with pneumococcal antisera (Statens Serum Institute, Copenhagen, Denmark).

2.5 Statistical analyses

Stata software (Stata Corporation, College Station, TX, Version 10.0) was employed for statistical analyses. Fisher's exact test examined differences in categorical variables. Odds Ratios (OR) were estimated from 2 x 2 tables with confidence intervals (CI) calculated by the exact binomial method. A two-tailed P-value <0.05 was considered significant.

3. Results

3.1 Details of participants

Of 117 children admitted for evaluation of chronic wet cough, 107 had radiographically-confirmed bronchiectasis. This study describes the 104 Indigenous children with confirmed bronchiectasis; 64 (62%) were male and the median age was 28.5 (range 5.2-154.6) months. Of these 104 children, 65 had taken antibiotics within 2 weeks of bronchoscopy, including 39 who received macrolides (azithromycin 38, roxithromycin 1) and 26 who were prescribed beta-lactams singly or in combination (benzylpenicillin 3, amoxicillin 19, ampicillin 2, flucloxacillin 1, ceftriaxone 10, cefotaxime 2, cephalexin 1, meropenem 1). Four children received antibiotics from both classes, and nine received other antibiotics (trimethoprim-sulphamethoxazole 3, metronidazole 1, gentamicin 1, vancomycin 3, unknown 3), five in combination with beta-lactams.

Overall, 88/104 (85%) children had received at least two doses of the 7-valent pneumococcal conjugate vaccine (Prevenar®; Pfizer Australia Pty Ltd), and 58/84 (69%) of those aged ≥18 months had been given the 23-valent pneumococcal polysaccharide vaccine (Pneumovax®; Commonwealth Serum Laboratories Ltd) as recommended by the NT immunisation schedule up to October 2009. The 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine (Synflorix®; GlaxoSmithKline Australia Pty Ltd) replaced Prevenar® in the NT schedule in October 2009 and 13 (12.5%) children had received at least two doses of this vaccine.

3.2 Pathogen recovery

The three main respiratory bacterial pathogens detected in the nasopharynx (Table 1) were *H. influenzae* (n=52), *S. pneumoniae* (n=38) and *M. catarrhalis* (n=33). Lower airway infection (>10^4 CFU/mL) was diagnosed in 42 (40%) children who grew *H. influenzae* (n=32), *S. pneumoniae* (n=17) and *M. catarrhalis* (n=12) in their BAL fluid cultures (Table 2). Two of 52 children with *H. influenzae* nasopharyngeal carriage had typeable (non-Hib) strains only. All 32 children with *H. influenzae* lower airway infection had NTHi isolated, and typeable (non-Hib) strains were also found in three. Nasopharyngeal carriage of any of the three respiratory bacteria was significantly lower in children who received antibiotics within 2 weeks of bronchoscopy (Table 3). For individual bacteria, reduced carriage was statistically significant for *S. pneumoniae* in children receiving macrolide or beta-lactam antibiotics. Reduced *M. catarrhalis* carriage in children receiving macrolides just failed to achieve statistical significance. No significant effects were seen for lower airway infection (Table 3).

3.3 Antibiotic resistance

Of 38 children with positive *S. pneumoniae* nasopharyngeal cultures, 13 (34%) carried azithromycin-resistant (MIC >4 mg/L) strains and 11 (29%) had penicillin non-susceptible

(MIC >0.12 mg/L) strains (Table 4). In 17 children with *S. pneumoniae* lower airway infection, 10 (59%) had azithromycin-resistant strains and 6 (35%) had penicillin non-susceptible strains isolated from BAL fluid (Table 5). The azithromycin-resistant pneumococcal serotypes were 6A, 6C, 7C, 7F, 9N, 11A, 15C, 17F, 19F, 22F, 23B, 23F and 31 and penicillin non-susceptible serotypes were 6B, 6C, 11A, 15C, 16F, 19A, 19F, 23B and 35B. Pneumococcal serotypes detected in children with lower airway infection are shown in Table 6. No isolates with high-level penicillin resistance (MIC ≥2 mg/L) or very high-level azithromycin resistance (MIC > 256 mg/L) were detected. Azithromycin MICs had a bimodal distribution and were either <1 mg/L (susceptible) or between 12 and 128 mg/L (resistant).

Amongst 52 children with positive nasopharyngeal cultures for *H. influenzae*, two carried typeable (non-Hib) strains only, which were not tested for antibiotic resistance. MICs were also not determined for NTHi isolates from a further two nasopharyngeal cultures and two BAL fluid cultures. Using EUCAST breakpoints, azithromycin-resistant isolates (MIC >4 mg/L) were detected in 3 of 48 (6%) nasopharyngeal swabs from culture-positive children (Table 4), and in 4 of 30 (13%) BAL specimens from children with NTHi lower airway infection (Table 5). Most NTHi-positive specimens (94% nasopharyngeal swabs and 87% from NTHi lower airway infection) had isolates with intermediate resistance to azithromycin (MIC >0.125 and ≤4 mg/L), while none tested were susceptible by EUCAST criteria (MIC ≤0.125 mg/L).

In the 48 NTHi nasopharyngeal culture positive children tested, 9 (19%) had BL-positive strains and 7 (15%) had ampicillin-resistant (MIC ≥4 mg/L) isolates. In the 30 children tested with NTHi lower airway infection, 10 (33%) harboured BL-positive strains and 9 (30%) had ampicillin-resistant strains. No BL-negative ampicillin-resistant (BLNAR) NTHi

isolates were detected. Most (91%) of the 33 *M. catarrhalis* nasopharyngeal culture-positive children and all 12 children with *M. catarrhalis* lower airway infection had BL-positive strains.

3.4 Impact of antibiotic use on antibiotic resistance

Children who received macrolides were significantly more likely to harbour azithromycin-resistant *S. pneumoniae* isolates in their nasopharynx and to have lower airway infections from these strains than children without a history of recent macrolide antibiotic therapy (Table 7). Similarly, those who received beta-lactam antibiotics were significantly more likely to have lower airway infection with ampicillin-resistant BL-positive NTHi than children who had not been exposed recently to these agents. Finally, those taking macrolides were less likely to have a lower airway infection caused by a BL-positive strain of *M. catarrhalis*.

4. Discussion

To the best of our knowledge this is the first report describing the impact of antibiotics upon nasopharyngeal carriage of *M. catarrhalis* and on lower airway infection with any of the three major respiratory bacterial pathogens in children with non-CF bronchiectasis. Nasopharyngeal carriage by the three respiratory pathogens was lower in children who had received antibiotics in the 2-weeks prior to bronchoscopy. This effect upon carriage appeared to arise principally from the suppression of *S. pneumoniae* by macrolide and beta-lactam antibiotics and probably from a similar effect of macrolides upon *M. catarrhalis*. Studies in Australian Indigenous children treated for acute otitis media have shown similar effects for azithromycin and amoxicillin upon *S. pneumoniae* nasal carriage, while azithromycin has also been reported to have a modest suppressive effect upon NTHi colonisation in this patient

population [14]. Only lower airway infection with *M. catarrhalis* appeared to be reduced in children who received macrolides, but this was not statistically significant. In contrast, beta-lactam antibiotics had little impact on decreasing lower airway infection.

4.1 Impact of antibiotics upon resistance

Although children who received macrolides were less likely to carry *S. pneumoniae*, they were more likely to be colonised by azithromycin-resistant *S. pneumoniae* or to have lower airway infection with azithromycin-resistant strains. This suggests that macrolide use eliminated mainly susceptible strains, consistent with reports of increased carriage of azithromycin-resistant *S. pneumoniae* strains in Indigenous Australian children following azithromycin treatment for acute otitis media [14] and trachoma [15].

Levels of *S. pneumoniae* azithromycin resistance in this study were higher than those found in Indigenous children of a similar age in cross-sectional surveys in remote NT communities in 2003 and 2005 and in both instances involved a diverse range of non-vaccine pneumococcal serotypes [16]. As a proportion of *S. pneumoniae* carriage-positive children, azithromycin resistance was 6% in the community surveys compared with 34% (Table 4, 13/38) in the present study. While higher azithromycin resistance in our study was associated with recent macrolide use, it was also higher than reported previously in children who had not recently received macrolides (Table 4, 4/27 or 15% of carriage-positive children). This may be due to previous azithromycin exposure in these children or to increased azithromycin use in the general NT population, where it is used for treating trachoma and for some respiratory and sexually transmitted infections.

Azithromycin resistance in NTHi has not previously been reported in this population. Most *H. influenzae* strains have an intrinsic macrolide-efflux mechanism and azithromycin MICs of 0.25-4 mg/L [17]. These strains are now defined in the latest EUCAST guidelines as intermediate-resistant [13]. In a study of over 6000 isolates, only 1.3% had high-level macrolide resistance (MIC >4 mg/L) due to ribosomal alterations, while 1.8% were defined as hypersusceptible (MIC <0.25 mg/L) [17]. In our study, high-level azithromycin resistance was detected in 13% of children with NTHi lower airway infection. It seems likely that, as with *S. pneumoniae*, azithromycin-resistant NTHi strains have been selected at the individual or population level by macrolide use.

Children who received beta-lactam antibiotics were also significantly more likely to have lower airway infections with ampicillin-resistant NTHi. Overall, 10% of children in this study carried BL-positive NTHi. While this is lower than reported elsewhere (e.g. 26% in North America [18]), it is higher than the 5% previously reported in this population [14]. As expected, most *M. catarrhalis* isolates in our study were BL-positive. We did not test for azithromycin susceptibility as *M. catarrhalis* is regarded as being almost universally susceptible to azithromycin. A recent study in Western Australia, which included Indigenous children, reported that all 261 *M. catarrhalis* strains tested were susceptible to azithromycin [19]. In our study, azithromycin susceptibility is the most likely explanation for *M. catarrhalis* lower airway infections being less prevalent in children who had received macrolides.

4.2 Clinical relevance of azithromycin resistance

The level of azithromycin resistance observed in our study is concerning. While azithromycin is reported to be effective against susceptible strains of *S. pneumoniae*, the relevant MIC

breakpoint is controversial and depends upon the site of infection. Most treatment failures have been reported for community-acquired pneumonia (CAP) [20] and generally with azithromycin MIC ≥8 mg/L [10]. Treatment failure has also been reported for *S. pneumoniae* lower airway infections with MIC ≥4 mg/L [21] and for otitis media with MIC >1 mg/L [22]. Although guidelines suggest a 25% rate of high-level *S. pneumoniae* resistance (MIC ≥16 mg/L) as a threshold at which macrolides should no longer be used for CAP, some authors believe failure to consider low-level resistance will result in higher rates of morbidity and mortality because of discordant therapy [23]. In our study we found azithromycin-resistant (MIC >4 mg/L) *S. pneumoniae* in 59% of specimens from children with *S. pneumoniae* lower airway infection, and all but one tested isolate had MIC ≥16 mg/L. As a result, treating clinicians may be faced with treatment failure in this setting.

Much less is known about macrolide resistance and its clinical significance in *H. influenzae* infection. An approach utilising pharmacokinetic and pharmacodynamic (PK/PD) modelling reported that none of 148 respiratory NTHi isolates from children attending two United States paediatric hospitals between 2005 and 2007 were susceptible with PK/PD breakpoints compared to 87% using Clinical and Laboratory Standards Institute breakpoints [24]. The EUCAST guidelines have taken into account the intrinsic macrolide efflux mechanism possessed by most NTHi strains and have lowered their clinical breakpoints to include these isolates [13]. Nevertheless, while some experts claim that azithromycin has no clinically useful activity against *H. influenzae* [25], others report that azithromycin is effective for treating chronic lower airway infections, including those caused by *H. influenzae* [5,6,26]. Unfortunately, correlation between macrolide MICs and clinical outcome is weak for *H. influenzae* [13]. As with *S. pneumoniae*, different MIC breakpoints may be needed according

to the site of infection. When assessing the clinical responses to azithromycin it is also important to consider the immunomodulatory properties of this agent [27].

4.3 Limitations

Our study has important limitations. As an observational, point-prevalence study, the impact of previous exposure to macrolide (or other) antibiotics upon respiratory bacterial pathogens could not be analysed. A randomised, placebo-controlled trial of long-term azithromycin in Indigenous children with bronchiectasis (ACTRN1261000038 3066) is currently underway and should help to further address the effects of azithromycin upon respiratory flora.

In addition, BAL fluid cultures may have under-diagnosed lower airway infections in patients with multi-lobar disease through sampling only from a single lobe [28]. Similarly, the proportions of antibiotic-resistant strains identified are likely to have been underestimated by only performing antibiotic susceptibility testing on a limited number of colonies from each culture plate. Small subject numbers may have led to type II errors in some sub-analyses, most notably for *M. catarrhalis*. In this regard the numbers of study participants harbouring *Staphylococcus aureus* in their nasopharynx (n=15) or associated with lower airway infection (n=3) were too small to analyse. Worryingly, macrolide-resistant *S. aureus* isolates were detected in nasopharyngeal and BAL-fluid cultures from 12 (80%) and 2 (67%) children carrying *S. aureus* respectively (data not shown). As azithromycin is associated with rapid emergence of macrolide-resistant strains of *S. aureus* in patients with CF [29], a larger study is being undertaken. Finally, this is a high risk group, and these findings may not be generalisable to other settings.

4.4 Conclusions

Our data show an association between reduced nasopharyngeal carriage of respiratory bacterial pathogens in children who had received macrolide or beta-lactam antibiotics recently, but no such association was observed for lower airway infection. Only infection with *M. catarrhalis* was reduced in children who had received macrolides, suggesting that azithromycin may be effective in clearing *M. catarrhalis* from the lungs of Indigenous children with bronchiectasis, or perhaps in preventing lower airway colonisation. Macrolide use was however associated with increased levels of carriage and infection with azithromycin-resistant *S. pneumoniae*, while the persistence of NTHi infection in the lungs of children receiving azithromycin suggests that azithromycin is ineffective against most NTHi. These findings are consistent with recent reports, including in vitro studies, which found that azithromycin was highly effective against *M. catarrhalis* and susceptible *S. pneumoniae*, while its effect upon *H. influenzae* was limited [30]. A randomised, placebo-controlled trial of long-term azithromycin therapy in our population of Indigenous children with non-CF bronchiectasis will help to determine whether increased levels of resistance in *S. pneumoniae* and NTHi will negate any of the clinical benefits.

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Table 1: Nasopharyngeal carriage by antibiotic use

Antibiotics	None N (%)	Any N (%)	Macrolide N (%)	Beta-lactam N (%)	Both N (%)	Other N (%)	Total N (%)
Children	39	65	35	22	4	4	104
S. pneumoniae	25 (64)	13 (20)	10 (29)	2 (9)	1 (25)	0 (0)	38 (37)
H. influenzae	23 (59)	29 (45)	15 (43)	11 (50)	1 (25)	2 (50)	52[1] (50)
M. catarrhalis	17 (44)	16 (25)	8 (23)	6 (27)	0 (0)	2 (50)	33 (32)
Any pathogen (of the 3)	32 (82)	37 (57)	20 (57)	12 (55)	2 (50)	3 (75)	69 (66)
All three pathogens	11 (28)	5 (8)	4 (11)	1 (5)	0 (0)	0 (0)	16 (15)

[1] Two children carried typeable (non-Hib) strains only.

Table 2: Lower airway infection (>10^4 colony-forming units/mL of bronchoalveolar lavage fluid) by antibiotic use

Antibiotics	None N (%)	Any N (%)	Macrolide N (%)	Beta-lactam N (%)	Both N (%)	Other N (%)	Total N (%)
Children	39	65	35	22	4	4	104
S. pneumoniae	6 (15)	11 (17)	8 (23)	2 (9)	1 (25)	0 (0)	17 (16)
H. influenzae	13 (33)	19 (29)	9 (26)	7 (32)	1 (25)	2 (50)	32[1] (31)
M. catarrhalis	5 (13)	7 (11)	1 (3)	4 (18)	0 (0)	2 (50)	12 (12)
Any pathogen (of the 3)	15 (38)	27 (42)	16 (46)	8 (36)	1 (25)	2 (50)	42 (40)
All three pathogens	3 (8)	1 (2)	0 (0)	1 (5)	0 (0)	0 (0)	4 (4)

[1] All 32 children had nontypeable *Haemophilus influenzae* isolated from their lower airways.

Table 3: Odds Ratio (OR) of antibiotic use for nasopharyngeal carriage and lower airway infection (>10^4 colony-forming units/mL of bronchoalveolar lavage fluid)

	Nasopharyngeal carriage		Lower airway infection	
Pathogen	Macrolides[1]	Beta-lactams[2]	Macrolides[1]	Beta-lactams[2]
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
S. pneumoniae	**0.22**	**0.07**	1.65	0.72
	(0.08, 0.63)	**(0.01, 0.32)**	(0.46, 6.31)	(0.11, 3.80)
	P<0.01	**P<0.01**	P=0.57	P=0.73
H. influenzae	0.48	0.60	0.69	0.89
	(0.18, 1.31)	(0.19, 1.82)	(0.23, 2.05)	(0.26, 2.90)
	P=0.17	P=0.32	P=0.62	P=1.00
M. catarrhalis	0.33	0.39	0.18	1.24
	(0.11, 1.01)	(0.11, 1.31)	(0.00, 1.75)	(0.22, 6.44)
	P=0.05	P=0.12	P=0.20	P=1.00
Any pathogen	**0.28**	**0.26**	1.24	0.85
(of the 3)	**(0.09, 0.88)**	**(0.07, 0.90)**	(0.46, 3.37)	(0.26, 2.67)
	P<0.05	**P<0.05**	P=0.82	P=0.80

[1] 39 children who received macrolides versus 39 who received no antibiotics (N=78).

[2] 26 children who received beta-lactams versus 39 who received no antibiotics (N=65).

Table 4: Resistance in nasopharyngeal carriage by antibiotic use

Antibiotics	None	Any	Macrolide	Beta-lactam	Both	Other	Total
	N	N	N	N	N	N	N
Children	39	65	35	22	4	4	104
S. pneumoniae							
Susceptible	14	2	2	0	0	0	16
PenNS	11	11	8	2	1	0	22
or AziR							
PenNS only	8	1	0	1	0	0	9
AziR only	3	8	6	1	1	0	11
PenNS+AziR	0	2	2	0	0	0	2
Nontypeable *H. influenzae*							
AziIR only	19	18	9	8	0	1	37[1]
BLpos	2	9	5	3	0	1	11
or AziR							
BLpos only	1	7	3	3	0	1	8
AmpR	2	5	2	2	0	1	7
AziR only	0	2	2	0	0	0	2
BLpos+AziR	1	0	0	0	0	0	1
M. catarrhalis							
BLneg	2	1	1	0	0	0	3
BLpos	15	15	7	6	0	2	30

[1] None susceptible by European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria; MIC results not obtained for two nasopharyngeal swabs.

Abbreviations: PenNS, oral penicillin non-susceptible (MIC > 0.12 mg/L); AziR, azithromycin resistant (MIC >4 mg/L); AziIR, azithromycin intermediate resistant (MIC >0.125 and ≤4 mg/L); AmpR, ampicillin-resistant (MIC ≥ 4 mg/L); BLneg, beta-lactamase negative; BLpos, beta-lactamase positive; MIC, minimum inhibitory concentration.

Table 5: Resistance in lower airway infection (>10^4 colony-forming units/mL of bronchoalveolar lavage fluid) by antibiotic use

Antibiotics	None	Any	Macrolide	Beta-lactam	Both	Other	Total
	N	N	N	N	N	N	N
Children	39	65	35	22	4	4	104
S. pneumoniae							
Susceptible	2	1	1	0	0	0	3
PenNS	4	10	7	2	1	0	14
or AziR							
PenNS only	3	1	0	1	0	0	4
AziR only	0	8	6	1	1	0	8
PenNS+AziR	1	1	1	0	0	0	2
Nontypeable *H. influenzae*							
AziIR only	8	8	5	3	0	0	16[1]
BLpos	5	9	3	4	1	1	14
or AziR							
BLpos only	3	7	1	4	1	1	10
AmpR	3	6	0	4	1	1	9
AziR only	2	2	2	0	0	0	4
BLpos+AziR	0	0	0	0	0	0	0
M. catarrhalis							
BLneg	0	0	0	0	0	0	0
BLpos	5	7	1	4	0	2	12

[1] None susceptible by EUCAST criteria; MIC results not obtained for two BAL specimens.

Abbreviations: see Table 4.

Table 6: Serotypes isolated from 17 paired nasopharyngeal swab and bronchoalveolar lavage fluid cultures associated with *Streptococcus pneumoniae* lower airway infection in Indigenous Australian children with bronchiectasis

Pair #	Antibiotics received in 2 weeks prior to bronchoscopy	Serotypes in NP swab	Serotypes in BAL fluid (>10^4 CFU/mL)
1	Azithromycin	21 (sens)	21 (sens), 10A (sens)
2	Azithromycin		31 (AziR)
5	Azithromycin		7F (AziR)
9	Azithromycin		9N (AziR)
10	Azithromycin	22F (AziR)	22F (AziR)
14	Azithromycin	11A (AziR+PenNS)	11A (AziR+PenNS)
15	Azithromycin		22F (AziR)
16	Azithromycin	17F (AziR)	17F (AziR)
4	Azithromycin, amoxicillin	6A (AziR)	6A (AziR)
11	Amoxicillin	23F (AziR)	23F (AziR)
13	Cefotaxime, benzylpenicillin		6C (PenNS), 19F (PenNS)
3	None	6C (AziR)	6C (AziR), 19F (PenNS)
6	None	16F (PenNS)	16F (PenNS)
7	None	16F (PenNS)	16F (PenNS)
8	None	9N (sens)	9N (sens)
12	None	19A (PenNS)	19A (PenNS)
17	None	15A (sens)	15A (sens)

Abbreviations: BAL, bronchoalveolar lavage fluid; CFU, colony forming units; NP, nasopharyngeal; AziR, azithromycin resistant (MIC >4 mg/L); PenNS, oral penicillin non-susceptible (MIC >0.12 mg/L); sens, susceptible to penicillin and azithromycin.

Table 7: Odds Ratio (OR) of antibiotic use for antimicrobial resistance

	Nasopharyngeal carriage		Lower airway infection	
Pathogen	Macrolides[1]	Beta-lactams[2]	Macrolides[1]	Beta-lactams[2]
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Spn AziR	**4.58**	0.51	**8.13**	0.73
	(1.14, 21.7)	(0.05, 2.60)	**(1.47, 81.3)**	(0.07, 4.03)
	P<0.05	P=0.51	**P<0.01**	P=1.00
Spn PenNS	0.34	0.27	0.32	0.58
	(0.03, 1.77)	(0.01, 2.11)	(0.01, 3.01)	(0.01, 5.62)
	P=0.20	P=0.28	P=0.41	P=1.00
NTHi AmpR[3]	0.65	1.22	0.19	**4.40**
	(0.06, 4.23)	(0.11, 8.03)	(0.00, 1.51)	**(0.85, 23.9)**
	P=0.71	P=1.00	P=0.15	**P<0.05**
NTHi AziR	3.66	0.00	1.72	0.00
	(0.18, 219)	(0.00, 3.98)	(0.12, 24.6)	(0.00, 2.79)
	P=0.30	P=1.00	P=0.63	P=0.57
NTHi AziIR	0.47	0.99	0.53	1.43
	(0.18, 1.16)	(0.36, 2.70)	(0.17, 1.54)	(0.48, 4.20)
	P=0.10	P=1.00	P=0.25	P=0.60
Mc BLpos	0.40	0.68	**0.13**	1.59
	(0.13, 1.12)	(0.20, 2.04)	**(0.00, 0.97)**	(0.32, 6.64)
	P=0.07	P=0.62	**P<0.05**	P=0.49

[1] 39 children who received macrolides versus 65 who received no macrolides (N=104)

[2] 26 children who received beta-lactams versus 78 who received no beta-lactams (N=104)

[3] Similar results were obtained for BLpos NTHi (not shown).

Abbreviations: Spn, *Streptococcus pneumoniae;* AziR, azithromycin resistant (MIC >4 mg/L); PenNS, oral penicillin non-susceptible (MIC >0.12 mg/L); NTHi, nontypeable *Haemophilus influenzae*; AziIR, azithromycin intermediate resistant (MIC >0.125 and ≤4 mg/L); AmpR, ampicillin-resistant (MIC ≥4 mg/L); Mc, *Moraxella catarrhalis*; BLneg, beta-lactamase negative; BLpos, beta-lactamase positive; MIC, minimum inhibitory concentration.