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Nasal swab bacteriology by PCR during the first 24-months of life: A prospective birth cohort study

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Abstract

Background: Most respiratory bacterial carriage studies in children are based on cross-sectional samples or longitudinal studies with infrequent sampling points. The prospective Observational Research in Childhood Infectious Diseases birth cohort study intensively evaluated the community-based epidemiology of respiratory viruses and bacteria during the first 2-years of life. Here we report the bacteriologic findings.

Methods: Pregnant women in Brisbane, Australia were recruited between September 2010 and October 2012, and their healthy newborn children were followed for the first 2-years of life. Parents kept a daily symptom diary for the study child, collected a weekly anterior nose swab and completed an illness burden diary when their child met pre-defined illness criteria. Specimens were tested for respiratory bacteria by real-time polymerase chain reaction (PCR) assays and those containing human genomic DNA, deemed as high-quality, were analyzed.

Results: Altogether 8100 high-quality nasal swab specimens from 158 enrolled children were analyzed. *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae* were detected in 42.4%, 38.9%, and 14.8% of these samples, respectively. Concomitant detection of bacteria was common. In contrast, *Bordetella pertussis*, *B. parapertussis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Simkania negevensis* were rarely identified. The prevalence of the three major bacteria was higher with increasing age and in the winter and spring months. Siblings and childcare attendance were the other risk factors identified.

Conclusions: We confirmed the feasibility of frequent nasal swabbing by parents for studying bacterial colonization. PCR detected the major respiratory tract bacteria with expected high frequencies, but atypical bacteria were found rarely in this cohort.

KEYWORDS
bacteria, carriage, child, colonization, polymerase chain reaction

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Please check the presentation of article footnote for correctness. For cooperative studies, the institution where research was primarily done should be indicated: Menzies Health Institute Queensland, Griffith University, Gold Coast, Queensland.

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1 | INTRODUCTION

Acute respiratory infections (ARI) are responsible for substantial global morbidity and are important causes of mortality in children from low-income countries.[1] Carriage of respiratory bacterial pathogens, such as *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*, is a prerequisite for two important childhood ARIs; acute otitis media, which is the most frequently reported pediatric bacterial infection,[2] and bacterial pneumonia, a leading cause of childhood deaths.[1,3] These bacteria are also carried in the upper airways of healthy young children who are considered the principal reservoirs for maintaining these organisms within populations.[4] Similarly, so-called atypical bacteria, like *Mycoplasma pneumoniae*, can also colonize the upper airways of healthy children and in some cases result in lower respiratory tract infections.[5]

Understanding the nature of upper airway bacterial colonization in young children is critical for determining the impact, including unintended consequences, of antibiotics and vaccines upon this ecological niche and for designing future therapeutic strategies to reduce the burden of ARIs in children. However, colonization is a highly dynamic process and most respiratory carriage studies in children are based upon cross-sectional samples in various patient populations or longitudinal studies of healthy children with infrequent sampling points. We used a novel approach to describe the epidemiology, symptoms, and risk factors associated with carriage of *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* in a longitudinal cohort of young children with nasal samples collected weekly from birth until age 24-months.

2 | MATERIALS AND METHODS

2.1 | Study population and setting

The Observational Research in Childhood Infectious Diseases (ORChID) study was conducted in the subtropical city of Brisbane, Queensland, Australia from September 2010 through October 2014 (clinicaltrials.gov: NCT01304914).[6] In this prospective, community-based birth cohort study, children were enrolled at their mothers' antenatal visits and followed from birth until their second birthday. Recruitment was progressive over 2-years at two metropolitan hospitals (one private and one government-funded). To be eligible to participate, children needed to be healthy, without congenital abnormalities or underlying chronic disorders, and to be born at term (36-42 weeks gestation). Participants were able to leave the study temporarily (such as during family holidays) and re-join at a later date. The primary aim of the ORChID study was to describe the nature and timing of viral respiratory and gastrointestinal infections during the first 2-years of life.[6–8] Here, we undertook a secondary analysis of the ORChID database and sample bank to evaluate bacterial carriage. Parents provided written informed consent for their newborn infants at enrolment. The Children's Health Queensland, Royal Brisbane and Women's Hospital, and The University of Queensland Human Research Ethics Committees approved the study.

2.2 | Data and symptom diary collection

After enrolment, data on demographic, social, and health history, pregnancy, and birth details were collected by parental interview. Subsequently, telephone interviews were conducted every 3-months to update feeding, vaccination, and childcare attendance details. Childcare was categorized as formal (regulated care outside the child's home) or informal (non-regulated care provided by family or friends). Parents received training to recognize respiratory symptoms. Parents used diary cards to record daily, pre-defined respiratory symptoms, or diagnoses in a designed tick-box format. Parents also recorded in a separate illness impact diary any health visits and antibiotic prescriptions for their child's ARI episodes.[9] Both diaries were returned by mail each month to the research team.

2.3 | Nasal swab collection and laboratory methods

Research personnel collected anterior nasal swabs soon after birth and, following training and receiving written instructions, parents obtained swabs weekly thereafter. All swabs were collected using a plastic-shaft, rayon-budded swab in a transport tube with a foam pad reservoir soaked with viral transport medium (Virocult MW950, Medical Wire & Equipment, Wiltshire, England) as one of the primary aims of the ORChID study was to detect respiratory viruses. Swabs were sent by standard surface mail to the laboratory where they were stored at −80°C, at a median of 3 (interquartile range 2-4) days after swabbing.

Since the swab's viral transport medium contained chloramphenicol and amphotericin B, polymerase chain reaction (PCR) assays rather than culture were used to detect the bacterial pathogens of interest. For nucleic acid extraction, each swab was resuspended in 2 mL of phosphate buffered saline from which 200 µL was used for extraction. Samples were extracted on the QIAxtractor automated high-throughput extraction platform using DX reagents following the manufacturer's instructions (Qiagen, Australia). Total DNA and RNA were eluted into 150 µL of elution buffer. Each sample was spiked with whole equine herpesvirus (EHV) to assess nucleic acid extraction quality and presence of potential PCR inhibitors. Any extract having a >3 cycle threshold (Ct) difference to that of the expected value by EHV real-time PCR assay was considered to have failed quality control and the sample was re-extracted. Nasal swab specimen quality was determined by screening for human DNA using an endogenous retrovirus-3 (ERV3) assay as described previously.[10] Swabs with ERV3 Ct values >38 were deemed lower-quality and they, and associated person-time, were excluded from analyses.

Swabs were batch-tested for five bacterial (*S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, *Bordetella pertussis*, *B. parapertussis*) and three atypical bacterial (*M. pneumoniae*, *Chlamydophila pneumoniae*, *Simkania negevensis*) pathogens using validated real-time PCR assays (Supplemental Digital Content 1, Table). Batch testing methods and results for 17 respiratory viruses from the ORChID cohort were available and have been described previously.[6,7] Ct values <40 for individual bacteria were considered positive.[11]

2.4 | Definitions

A new bacterial detection episode (BDE) for each bacterial species occurred when either: (i) a new bacterium was first detected; (ii) the same bacterium was detected after two negative intervening high-quality swabs; or (iii) the same bacterium was detected at least 30-days after the last positive swab. ARIs were categorized hierarchically as either: Lower respiratory tract infection (LRTI) or Upper respiratory tract infection (URTI). An infection was an LRTI when parents reported rattly breathing, moist cough, shortness of breath, wheeze, or doctor-diagnosed pneumonia. An infection was a URTI when parents reported nasal discharge/congestion, dry cough, or a doctor-diagnosed otitis media.[7,9]

2.5 | Analysis

Acquisition rates for bacterial detections were investigated for only high-quality child swabs. Acquisition rate per child-year was estimated based on the definition of BDE using Poisson regression with the logarithm of individual study follow-up time as an offset. The time periods of consecutive positive swabs where the participant was not at risk of a new BDE were removed from the denominator when calculating acquisition rates. The univariable association between potential risk factors and bacterial detection was examined using mixed-effects logistic regression, taking into account the repeated measurements of the subjects through the use of an autoregressive model with lag one, that is, the previous sample result was included as a covariable. Potential risk factors investigated were both fixed (sex, type of delivery, gestational age at birth, family history of atopy [asthma or eczema in parents or siblings], tobacco smoke exposure, presence of siblings in their usual residence, maternal education status) and time-varying (age, breastfeeding, season of swab sampling, type of childcare attendance). A final multivariable model was constructed with age (in 4 categories), sex and the variables with consistent differences in univariable analysis (smoking, siblings, maternal education, childcare, season) included as covariables. The association between potential risk factors and bacterial carriage was estimated rather than acquisition, because no bacterial subtype data were available (serotypes or genotypes). All analyses are exploratory and were not pre-specified in the protocol. Data were analyzed using Stata v12.1 (StataCorp, TX).

3 | RESULTS

The characteristics of the 158 enrolled cohort children, of whom 154 provided daily symptom data, are shown in Table 1. Overall, 11 192 nasal swab samples were submitted, of which 3025 lower-quality swabs were removed, as were 67 swabs submitted after the child's second birthday, leaving 8100 swabs from 157 children (151 with accompanying symptom data) for analysis (Figure 1). The number of high-quality swabs per child ranged from 1 to 102 (median 57, interquartile range 32-74) during a total follow-up time of 245 person-years (median 717-days per subject, interquartile range 449-726). The

TABLE 1 Characteristics of the 158 ORChID study participants

Characteristics (n = 158 unless stated otherwise)	Frequency (%) or mean (±SD)
Sex (male)	75 (47.5)
Gestational age (weeks)	39.8 (±1.3)
Vaginal delivery	107 (67.7)
First born	106 (67.1)
First degree relative with asthma or eczema[a]	83 (52.5)
Maternal education (n = 157)	
Primary/high school	20 (12.8)
Diploma/certificate	38 (24.2)
University degree	99 (63.1)
Tobacco smoke exposure at birth	
Mother (n = 156)	5 (3.2)
Other household member (n = 155)	17 (11.0)
Child vaccination status to age 18-months[b]	
Completed primary PCV series	152 (96.2)
Exclusive breast feeding	
At birth (n = 149)	142 (95.3)
At 3-months (n = 142)	97 (68.3)
At 6-months (n = 133)	5 (3.8)
Childcare	
At 6-months (n = 133)	33 (24.8)
At 9-months (n = 123)	48 (39.0)
At 12-months (n = 115)	72 (62.6)
At 15-months (n = 110)	87 (79.1)

Abbreviation: PCV, pneumococcal conjugate vaccine; SD, standard deviation.

[a]Parent or sibling.

[b]Vaccination status for all cohort subjects at 18-months of age was confirmed by cross checking with the Australian Immunisation Register. The PCV program began in Queensland in 2005 with the 7-valent PCV using 3 primary doses without a booster (3 + 0 schedule, vaccination at ages 2, 4 and 6-months). In mid-2011, the 13-valent PCV replaced the 7-valent PCV. In contrast, influenza vaccines are not part of the standard Australian vaccine schedule and only 7 children received this vaccine during their first 2-years of life.

median interval between successive swabs was 7-days (interquartile range 7-12).

Overall, the prevalence of the major respiratory bacterial pathogens in the 8100 high-quality nasal swabs were: *S. pneumoniae* (42.4%), *M. catarrhalis* (38.9%), and *H. influenzae* (14.8%). Altogether, 58.4% of nasal swabs were positive for at least one of these pathogens. Co-detection of bacterial pathogens was common, occurring in 29.7% of swabs (Table 2 and Figure 2). The prevalence of the major respiratory bacterial pathogens increased with age and during the winter and spring months of June through November (Supplemental Digital Content 2, Figure). The estimated acquisition rates of all new BDEs per 100 child-years were for *S. pneumoniae* 8.9 (95% confidence interval (CI) 8.3-9.6), *M. catarrhalis* 5.9 (5.5-6.5), and *H. influenzae* 2.8 (2.6-3.2), respectively.
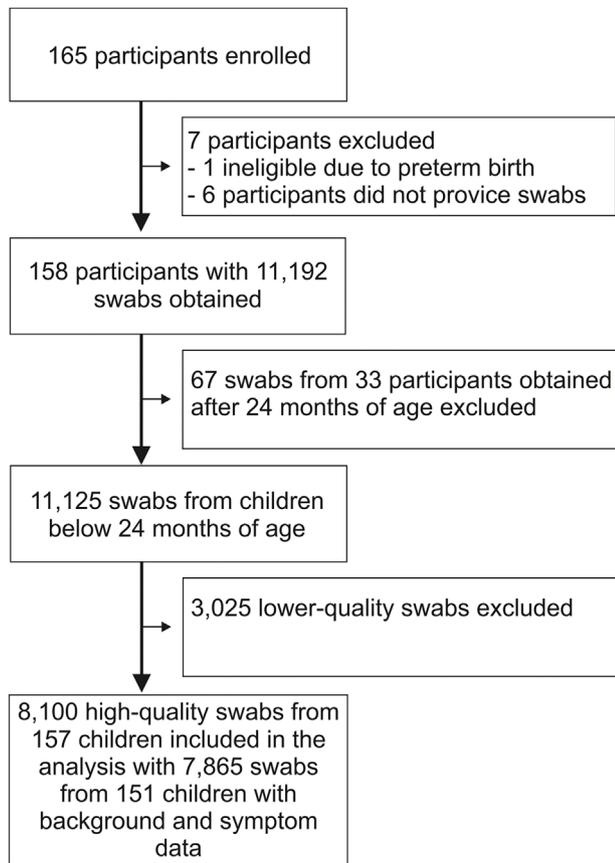
FIGURE 1   Flow chart of the ORChID study

In contrast, *B. pertussis* (*n* = 1), *B. parapertussis* (*n* = 4), and the atypical bacteria *C. pneumoniae* (*n* = 2), *M. pneumoniae* (*n* = 0), and *S. negevensis* (*n* = 0), were detected rarely or not at all. A separate analysis confirmed lower-quality swabs provided significantly lower detections for the three major bacterial pathogens (Supplemental Digital Content 3, Table).

3.1 | Association with clinical indicators

Bacterial prevalence was higher in symptomatic children (Table 2). Higher prevalence of bacteria was detected in swabs taken during respiratory symptoms, especially when acute otitis media was diagnosed. This was observed also in swabs negative for any virus (Supplemental Digital Content 4, Table). Conversely, the prevalence of respiratory symptoms was more common in bacteria-positive (34%) compared to bacteria-negative swabs (12%); this difference was observed both in virus-positive (53% symptomatic in bacteria-positives vs 35% in bacteria-negatives) and virus-negative (21% symptomatic in bacteria-positives vs 7% in bacteria-negatives) swabs. Of the virus-negative, bacteria-positive group, 14.9%, 6.2%, and 0.8% had symptoms of an URTI, LRTI, and acute otitis media, respectively.

A total of 269 antibiotic courses were recorded in 220 illness impact diary cards from 90 children. Exposure to antibiotics did not reduce the high bacterial detection by PCR during the illness episodes.

3.2 | Risk factors for carriage

Table 3 shows the risk factors evaluated, the distributions of risk factors, and the prevalence of the bacterial findings in each stratum. Increasing age, winter and spring seasons, siblings, and attendance in formal childcare were independently associated with each of the bacterial detections. Lower maternal education was also associated with most bacterial detections. Presence of siblings seemed to modify the effect of formal childcare on bacterial detection. Children with siblings experienced high carriage prevalence earlier and the entry into formal childcare had a minor additive risk on bacterial detection (adjusted odds ratio (aOR) 2.0, 95%CI 1.4-2.8 for *S. pneumoniae* carriage). Alternatively, children without siblings had very high carriage proportions associated with formal childcare (aOR 3.8, 95%CI 2.8-5.0 for *S. pneumoniae* carriage) compared to children not entering formal childcare (Supplemental Digital Content 5, Figure). A similar pattern with even higher differences between the aORs was seen for the other bacterial species (data not shown).

4 | DISCUSSION

In this community-based birth cohort we observed the expected prevalence for upper airway carriage by the most common respiratory bacterial pathogens, *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* in children from birth until 24-months of age using weekly nasal swabs collected by parents and sent by mail to the laboratory. Age, having siblings, childcare outside the home and season were the most important risk factors for bacterial carriage. In contrast, *B. pertussis*, *B. parapertussis*, and atypical bacterial pathogens were detected rarely.

Our collection methods vary from the World Health Organization standardized method for evaluating pneumococcal carriage,[12] which recommend nasopharyngeal swabs (NPS), storing samples in tubes containing skim-milk tryptone glucose glycerol transport media and detecting by culture. However, nasal swabs are also reported to be comparable to NPS for detecting *S. pneumoniae*,[13,14] *M. catarrhalis*,[14] and *H. influenzae*[13,14] in children with ARI symptoms. Moreover, parent-collected nasal swabs and healthcare worker-collected NPS had similar detection rates for *S. pneumoniae* in healthy children,[15] although yields for *M. catarrhalis* and *H. influenzae* in parent-collected nasal swabs were slightly lower. Furthermore, compared to healthcare worker-collected NPS from children during ARI episodes, we have observed similar yields by PCR of *H. influenzae*, and higher yields of *S. pneumoniae* and *M. catarrhalis*, from parent-collected anterior nasal swabs mailed into the laboratory (Lambert, unpublished RESPECT data; clinicaltrials.gov: NCT00966069). In addition, a recent 16S rDNA sequencing study in hospitalized infants with bronchiolitis found good within-individual correlations between *Haemophilus* and *Moraxella* in nasopharyngeal aspirate and mailed nasal swab specimens.[16] Finally, while the oropharynx rather than nasopharynx has been suggested as the optimal sampling site for *H. influenzae*, this observation may be biased by inadequate differentiation between *H. influenzae* and *H. haemolyticus*, the latter being common in the oropharynx.[12,17]

TABLE 2 Prevalence of major respiratory bacterial pathogens by clinical status in weekly-collected nasal swabs from 151 children in the ORChID cohort

Pathogen	All nasal swabs %	Swab positive for any respiratory virus[a] %	During LRTI[b] %	During URTI[b] %	During acute otitis media %
	N = 8100	N = 2550	N = 591	N = 1379	N = 60
Streptococcus pneumoniae	42.4	55.7	57.2	56.2	65.0
Moraxella catarrhalis	38.9	55.2	68.7	59.1	60.0
Haemophilus influenzae	14.8	24.7	27.9	27.5	35.0
Positive for any of the 3 bacterial pathogens	58.4	75.4	84.4	77.7	91.7
Multiple pathogens	29.7	46.2	54.7	47.9	50.0

Abbreviations: LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection.

[a]17 respiratory viruses tested by polymerase chain reaction assays (human rhinovirus, respiratory syncytial virus A and B, influenza A and B, parainfluenza 1-3, human metapneumovirus, human coronaviruses (OC43, NL63, 229E, HKU1), adenovirus, human polyomaviruses (KI, WU) and human bocavirus-1).[7]

[b]data based on daily symptom diary cards available for time periods of 7865/8100 high-quality swabs taken.

Another difference with the WHO method was storing nasal swabs in antibiotic-containing viral transport medium and identifying bacteria by real-time PCR instead of culture. However, this method has been used before with high yields of bacterial detection consistent with other studies in similar populations.[11] Indeed, PCR may offer advantages of increased detection sensitivity when target bacterial loads are low or dominated by other bacteria,[18,19] or after antimicrobial exposure as observed in our data.

Despite differences in methods our results agree with earlier prospective studies. Prevalence by age was slightly higher than observed during the 1990s in Finland,[20] but similar to recent studies from the United States,[21] and lower than reported from Peru[18] and Thailand.[22] Although, our study was conducted during an established infant pneumococcal conjugate vaccine (PCV) vaccination program, PCVs affect the vaccine-type carriage, but not the overall carriage of all

serotypes, and therefore, should not impact the current findings despite serotype data being unavailable. Additionally, epidemiologic patterns of seasonality, age-dependent prevalence, and risk factors were consistent with previous studies.[20,22–24]

In concordance with earlier studies, we also observed the association of bacterial detection with respiratory symptoms.[25] Furthermore, although symptoms were more prevalent in virus-positives, the association of bacterial detection with symptoms was also seen in virus-negatives. The longitudinal design of the ORChID study with frequent sampling offers possibilities for detailed analyses on temporal and causal relationships of viruses and bacteria, and their interactions, with symptoms.

An interesting finding was the high proportion of bacterial co-detections, compared to those based on marginal prevalences, in children sharing the same risk factors.[26,27] Bacteria may act synergistically in the nasopharynx, although competition can also occur.[28] PCR detection is probably much more efficient at detecting multiple bacteria than conventional cultures. Nevertheless, the concomitant carriage was especially striking for *H. influenzae*, which was detected alone in only a very small proportion of samples. Meanwhile, *B. pertussis* and *B. parapertussis* are reported uncommonly in infants with ARI symptoms.[29] The incidence of *B. pertussis* and *B. parapertussis* infections in children aged <3-years in a large pertussis vaccine trial with active follow-up was 29 and 2.1 per 1000 person-years, respectively.[30,31] This was higher than the estimated national Australian incidence of 4 per 1000 person-years identified through passive surveillance, despite considerably increased reported cases since 2010 and adopting PCR for diagnosis.[32] Thus, having only 1-2 children with positive *B. pertussis* and *B. parapertussis* swabs, respectively, was not unexpected. Additionally, *M. pneumoniae*, *C. pneumoniae*, and *S. negevensis* occur more commonly in older children,[33,34] and large numbers of positive children in this age group were not anticipated. Due to the low numbers of positives, the suitability of our detection methodology for atypical pathogens remains uncertain.

This study confirms previous findings that the main risk factors for carrying respiratory bacterial pathogens are age, having
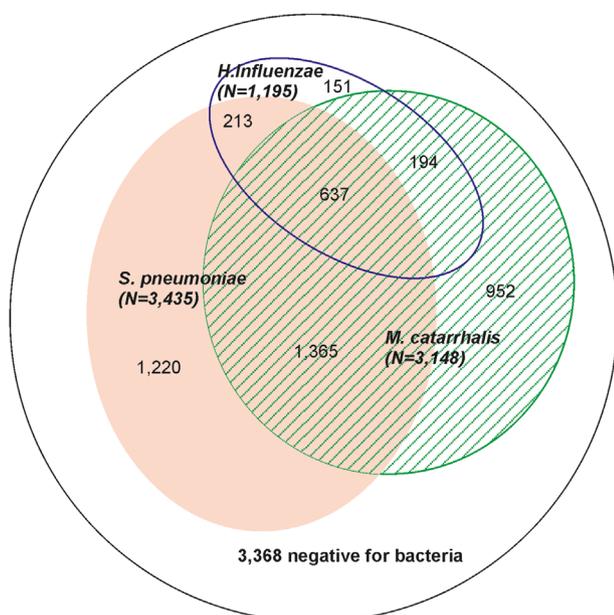

FIGURE 2 Venn diagram on swabs positive for at least one bacterium (N = 4732) in the high-quality swabs (N = 8100)

TABLE 3 Prevalence of bacterial detection in 157 children who returned high-quality swabs, and association with participant characteristics

Factor	Children in the stratum, N (N total = 157)	Number of swabs (N = 8100)	S. pneumoniae (42.4%)	Fully Adjusted[b]	M. catarrhalis (38.9%)	Fully adjusted	H. influenzae (14.8%)	Fully adjusted
Age (months)								
0-<3	157	1176	229 (19.5)	Ref	179 (15.2)	Ref	39 (3.3)	Ref
3-<6	144	1134	317 (27.9)	1.4 (1.1, 1.7)	282 (24.9)	1.6 (1.2, 2.2)	89 (7.8)	2.0 (1.3, 3.2)
6-<12	136	2149	1020 (47.5)	2.3 (1.7, 3.0)	919 (42.8)	3.0 (2.2, 4.0)	271 (12.6)	2.0 (1.3, 3.2)
12-<24	120	3641	1869 (51.3)	2.0 (1.3, 3.0)	1768 (48.6)	3.6 (2.1, 5.3)	796 (21.9)	2.4 (1.3, 4.4)
Sex								
Male	76	3612	1504 (41.6)	Ref	1382 (38.3)	Ref	489 (13.5)	Ref
Female	82	4488	1931 (43.0)	1.0 (0.8, 1.3)	1766 (39.3)	1.1 (0.8, 1.4)	706 (15.7)	1.1 (0.8, 1.6)
Siblings								
None	102	5285	1989 (37.6)	Ref	1811 (34.3)	Ref	660 (12.5)	Ref
At least one	55	2815	1446 (51.4)	1.9 (1.5, 2.4)	1337 (47.5)	2.0 (1.5, 2.7)	535 (19.0)	2.4 (1.6, 3.4)
Maternal education status								
University degree	99	5358	2153 (40.2)	Ref	2039 (38.1)	Ref	715 (13.3)	Ref
Diploma/ Certificate	37	1820	778 (42.8)	1.2 (0.9, 1.6)	664 (36.5)	0.9 (0.7, 1.3)	275 (15.1)	1.3 (0.8, 1.9)
Primary/ high school	20	914	503 (55.0)	1.6 (1.1, 2.3)	444 (48.6)	1.4 (0.9, 2.1)	205 (22.4)	1.6 (1.0, 2.8)
Tobacco smoke exposure								
No exposure	136	7183	2943 (41.0)	Ref	2717 (37.8)	Ref	1001 (13.9)	Ref
Either parent smokes	19	850	445 (52.3)	1.2 (0.8, 1.7)	393 (46.2)	1.0 (0.7, 1.6)	182 (21.4)	1.2 (0.7, 2.0)
Childcare attendance[a]								
No childcare	156	4107	1211 (29.5)	Ref	1058 (25.8)	Ref	272 (6.6)	Ref
Informal childcare	42	998	376 (37.7)	1.2 (0.9, 1.5)	288 (28.9)	0.9 (0.7, 1.2)	84 (8.4)	0.8 (0.5, 1.2)
Formal childcare	89	2995	1848 (61.7)	2.4 (2.0, 3.0)	1802 (60.2)	3.0 (2.4, 3.7)	839 (28.0)	2.8 (2.1, 3.8)
Season								
Summer	145	1811	672 (37.1)	Ref	442 (24.4)	Ref	156 (8.6)	Ref
Autumn	146	1989	785 (39.5)	1.0 (0.9, 1.2)	659 (33.1)	1.6 (1.3, 1.9)	231 (11.6)	1.3 (1.0, 1.7)
Winter	141	2248	1044 (46.4)	1.3 (1.1, 1.5)	1167 (51.9)	3.3 (2.7, 4.0)	435 (19.3)	2.3 (1.8, 2.9)
Spring	141	2052	934 (45.5)	1.2 (1.0, 1.4)	880 (42.9)	2.1 (1.7, 2.5)	373 (18.2)	2.1 (1.6, 2.7)

[a]Childcare was categorized as formal (regulated care outside the child's home) or informal (non-regulated care provided by family or friends).
[b]Adjusted for age, sex, tobacco smoke exposure, older siblings, maternal education, childcare, and season.

siblings, formal childcare attendance, and winter-spring season with frequent viral infections.[20,22–25,34,35] Of these, formal childcare attendance is likely the main modifiable risk factor for reducing the burden of respiratory tract carriage and infections. The interaction of childcare attendance with having siblings suggests that children without siblings might be protected if other

options for formal childcare attendance were available during the most vulnerable age periods.

The strengths of the ORChID study include its prospective design, the comprehensive surveillance provided by daily symptom diaries, and weekly sample collections from birth until age 24-months. Enrolment over two calendar years and follow-up of 4-years allowed

for different seasonal and year-to-year epidemiologic patterns. Considering its intensive nature, cohort retention was good with >70% followed for at least 18-months, and 68% of the maximum expected swabs were collected with a median interval of 1-week as stated in the protocol.[6] Routine laboratory quality assurance included assaying samples for the human DNA marker, ERV3, to ensure human cells were present. While important for viral detection,[10] it seems also relevant for bacteria as we observed considerably higher bacterial detection rates in samples containing ERV-3. Consequently, only high-quality nasal swabs were used to avoid compromising sensitivity in the analysis.

There are, however, important limitations to consider. We did not validate upper airway bacterial detection by nasal swab and PCR against the gold standard of NPS culture. However, our current analyses using previously validated PCR targets resulted in expected bacterial prevalence rates. The specificity of the PCR assays are high, yet the *ply*-PCR may cross-react with oral streptococci.[36] We therefore re-analyzed 34 *ply*-PCR positives with *lytA* PCR, of which 31 (91%) remained positive (data not shown). Differentiating between *H. influenzae* and the closely related respiratory tract commensal, *H. haemolyticus* can also be difficult, although the latter is uncommonly detected in the nasal cavity and the *hpd#3* target gene in our PCR assay is both sensitive and specific for differentiating between the two species.[17,37] Storing nasal swabs in the refrigerator and sending by mail before freezing 4 to 11-days post-sampling results in a 10-13% decrease in sensitivity for detecting bacteria by PCR, with some variation according to the bacterial species.[11] However, in our study transport times were much shorter (median 3-days, interquartile range, 2-4). Another limitation is that the assays lacked serotype/genotype determination for the bacterial detections. Evaluating bacterial species only, underestimates acquisition rates by different bacterial subtypes. Therefore, in the current analysis we focused upon the prevalence when evaluating the overall burden of bacterial carriage in young children. Finally, the ORChID study was conducted in one major subtropical city in Australia and as often occurs with studies of this nature involved smaller, socially-advantaged families. Nonetheless, the findings are valid and prevalence of bacterial detection agrees with cohort studies from other settings[18,20–24] as do the rates of ARI and virus detection.[7,9]

In conclusion, we have used a novel method to evaluate bacterial carriage in children using nasal swabs optimized initially for viral detection. This method, however, is suitable also for bacterial detection and is especially feasible when frequent sampling is required. Future analyses will explore interactions between nasal viruses, bacteria, and ARI symptoms, while subtyping will allow carriage dynamics to be explored in greater detail.

CONFLICTS OF INTEREST

Arto A. Palmu has received research funding from GlaxoSmithKline Vaccines for a nationwide effectiveness trial of 10-valent pneumococcal conjugate vaccine and from Wyeth-Lederle Vaccines and Pediatrics (currently Pfizer Inc.) for the FinOM trial. Michael D. Nissen is currently a full-time employee of GlaxoSmithKline, Vaccines, 23 Rochester Park, Singapore 139234, Singapore. Other authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.