The yield of nasopharyngeal bacteria from culture compared to polymerase chain reaction in South African children with lower respiratory tract infection

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We compared the yield of five common nasopharyngeal bacteria from culture and multiplex PCR, in South African children with LRTI. Concordance was strong for *S.pneumoniae, S.aureus, H. influenzae* and *K.pneumoniae*, but not for *M.catarrhalis*. PCR is comparable to culture for most common bacteria.

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Abstract

Background

Lower respiratory tract infection (LRTI) is a major cause of morbidity and mortality in children under 5 years of age. Bacterial pathogens contribute significantly to this process. Culture of respiratory tract specimens is labour-intensive and slow. Polymerase chain reaction (PCR) is comparatively, a rapid, sensitive method of detecting low levels of nucleic acid for clinically relevant bacteria. This study compares the yield of bacteria obtained from culture and FTDResp33 multiplex PCR of nasopharyngeal swabs (NPS) during LRTI episodes in children, in the Drakenstein Child Health Study.

Methods

At each episode of LRTI, 2 NPS's were obtained, one for culture and one for PCR testing. Bacterial yields and concordance for the 5 commonest bacteria were compared using frequencies and proportions.

Results

From 13th August 2012 to 23rd November 2020, there were 859 episodes of LRTI in 434 children [median age 9.2 (IQR 3.8; 18.9) months; 0.2% HIV-infected]. *S. pneumoniae, S. aureus, M. catarrhalis, H. influenzae* and *K. pneumoniae* were the predominant bacteria detected by either method. Concordance between culture and PCR for *S. pneumoniae, S. aureus,* and *K. pneumoniae* was 84.9%, 89.7% and 86.3% respectively. Culture and PCR for *H. influenzae* had a concordance of 76.9%. The greatest discordance between culture and PCR was for the detection of M. catarrhalis (34.4%). Median bacterial loads on PCR for all 5 organisms were significantly associated with semi-quantitative culture results ($p<0.001$ for each). Adjusting for age and hospitalization, children on antibiotics at the time of sampling,

had a reduced chance of having a positive culture (OR 0.1; 95% CI 0.1-0.4), but no reduction in PCR yield (OR 0.8; 95% CI 0.4-1.6).

Conclusion

Significant concordance existed between PCR and culture for 4 of the 5 common bacteria, affirming PCR as a comparable method of testing to culture.

Introduction

Lower respiratory tract infection (LRTI) or pneumonia is the leading cause of morbidity and mortality in children under 5 years of age outside of the neonatal period, especially in low-and-middle-income countries (LMIC's). In 2016, it was responsible for an estimated 652 572 deaths with the incidence and severity being highest during the first year of life.[1] Numerous studies have shown that viral as well as bacterial pathogens, contribute to the etiology of LRTI in children. Recent studies done in the era of pneumococcal conjugate vaccine (PCV), including the Pneumonia Etiology Research for Child Health (PERCH) study, a multi-centre case-control study of children with severe or very severe pneumonia conducted across 7 LMIC's, and the Drakenstein Child Health Study (DCHS), a South African birth cohort study confirmed that bacteria such as *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, Mycobacterium tuberculosis, Bordetella pertussis and Klebsiella pneumoniae* contribute to disease in this cohort.[2,3,4]

Historically, culture of respiratory tract specimens has been the mainstay of diagnostic testing to identify bacteria responsible for LRTI. In good quality specimens, a positive result can provide evidence of a causative pathogen but can also reflect a colonizing organism.[5] Using a case-control study design, as in the PERCH and DCHS studies, where controls are matched for age and site, helps to distinguish colonization from disease.

Microbiological culture is a labour-intensive process, reliant on experienced laboratory technicians and is relatively slow.[5,6,7,8] Certain organisms like Legionella pneumophila, Mycoplasma pneumoniae and Chlamydia pneumoniae are particularly difficult to culture.[8,9] Due to it being more laboured and time-consuming[7,8,10], rapid, sensitive and less labour-

intensive methods such as PCR are now widely used.[7,8,9,11,12] PCR allows for detection of multiple targets simultaneously and can therefore detect multiple organisms on a single specimen.[7, 8, 9, 13] The FTDResp33 (FTD, Fast-Track Diagnostics, Luxembourg) multiplex PCR assay in particular, detects low levels of nucleic acid for 33 clinically relevant bacteria and viruses.[5,6] It also less hazardous for laboratory personnel to handle compared to culture.[7,9] However, PCR is unable to distinguish viable from non-viable organisms which may yield false positive results from residual deoxyribonucleic acid (DNA) of prior bacterial infection.[13,14]It is also more costly and requires training of personnel and technical support.[7]

The DCHS is a South African birth cohort study comprising around 1000 mother-child pairs, that investigated the epidemiology and etiology, of LRTI in early childhood. In a nested case-control study both viruses and bacteria were detected by PCR of nasopharyngeal swabs (NPS) using the commercially available FTDResp33 kit, in 87% of LRTI cases and 73% of controls ($p<0.001$).[4] Bacterial pathogens alone, were found in 8% of LRTI cases and 18% of controls ($p<0.001$). The only bacteria associated with LRTI were *B. pertussis* (OR 11.08) and non-typable *H. influenzae* (OR 1.41*).* A median of 5 (IQR 4; 6) organisms were detected in cases or controls, showing the complexity of nasopharyngeal flora and of disease processes.[4]

The PERCH Study used the same commercial kit to detect pathogens in paired nasopharyngeal (NP) specimens amongst children hospitalized with severe or very severe pneumonia. [15] Bacterial pathogens were found in 91% of LRTI case specimens. Bacteria associated with radiographically defined LRTI, included *B. pertussis* (OR 3.0) and

Mycoplasma pneumoniae (OR 3.0). Non-typable *H. influenzae*, was significantly negatively associated with LRTI cases. A median of 4 organisms (IQR 2; 5) were detected in cases and controls. However, both PERCH and DCHS have only reported on bacterial detection by PCR. Culture, which reflects only viable live organisms may provide different results to PCR.

A study performed in Korea, looking at children with acute respiratory disease, compared multiplex PCR and culture for the detection of bacteria on nasopharyngeal aspirates for *S. pneumoniae* and *H. influenzae*. Of the 181 cases of respiratory disease, 81 cases (44.8%) flagged positive on multiplex PCR. Fifty-two cases were positive for *S. pneumoniae* (28.7%) and 47 cases for *H. influenzae* (26.0%). The agreement rates between multiplex PCR and culture for *S. pneumoniae* and *H. influenzae* were 92.9% (kappa index=0.84, *P*<0.001) and 91.1% (kappa index=0.75, *P*<0.001), respectively.[16]

In a smaller study of 75 children conducted in India, looking at the comparison of PCR, culture and serological tests for the diagnosis of *M. pneumoniae* specifically in LRTI, a very small proportion (4/75; 5.33%) were positive on both PCR and culture on NPA.[17]

This study aims to compare the yield and type of bacteria obtained from culture and multiplex PCR on NPS during LRTI in children on the DCHS in order to assess the efficacy of PCR in detecting bacterial organisms during childhood LRTI.

Methods

Study Design and Study Population

This was a secondary analysis of data prospectively collected in the DCHS. The DCHS enrolled pregnant women between 20 – 28 week's gestation from a low socio-economic setting in a peri-urban community outside Cape Town. Children received immunizations at primary health care clinics which included four doses of a 5-vaccine combination (diptheria, tetanus, acellular pertussis, *H. influenzae* b and inactivated polio vaccine) at 6, 10, 14 weeks and 18 months, measles vaccine at 9 and 18 months and the 13-valent pneumococcal conjugate vaccine (PCV 13) at 6 weeks, 14 weeks and 9 months. Study follow-up visits were aligned with routine child health visits with additional study-specific follow-up visits done between 6 – 10 weeks, 6 months and annually, and an intensive cohort, followed-up with NP sampling 2 weekly during infancy.[4] This region also has a strong prevention of maternal to child HIV transmission (PMTCT) program providing combination antiretroviral therapy (ART) to HIV-infected women and testing infants, according to national guidelines.

LRTI Surveillance

Active surveillance for LRTI was done at local clinics and Paarl Hospital. Primary health care nurses as well as study staff were trained to recognize the revised World Health Organization (WHO) criteria for LRTI cases[18] and managed accordingly with hospitalization for severe pneumonia.

Severe cases were managed by hospitalisation at the local regional hospital.[4]

Specimen Collection

At each episode of LRTI, two nasopharyngeal swabs (NPS) were collected by trained clinical nurse practitioners, adhering to Standard Operating Procedures (SOP's). [19]

For each patient, 2 NPS (FLOQSwabs™, Copan Diagnostics, CA) were obtained, one from each nostril. The first swab was placed in a nucleic acid preservation medium (Primestore®, Longhorn Vaccines & Diagnostics, Texas) for PCR testing while the second was placed into 1ml of skim milk-tryptone-glucose-glycerol (STGG) (National Health Laboratory Service) transport medium for culture. These swabs were stored in a fridge while awaiting transport to the laboratory for analysis. Specimens were transported daily on ice, to the laboratory. STGG specimens were immediately cultured, while Primestore specimens were frozen at -80°C until batch PCR testing was performed.

Procedures were terminated if oxygen saturation dropped below 90% for 20 seconds or more or if the child developed respiratory distress.

Children who received antibiotic therapy for more than 48 hours prior to specimen collection, were excluded from the analysis.

Ethics

This study was approved by the Faculty of Health Sciences Research Ethics Committee, University of Cape Town (HREC number:027/2021). Written informed consent was obtained from mothers at enrolment and re-consent was taken annually.

Laboratory Methods[19]

Ten microlitres of STGG was removed and inoculated to appropriate culture media and incubated at 37°C, with and without CO2. The chocolate agar plate was used to culture and

phenotypically identify *H. influenzae* while the 2% horse blood agar plates were used to culture and phenotypically identify *S. pneumoniae, S. aureus, M. catarrhalis* and enteric gram-negative rods while. Bacteria were identified by characteristic colony morphology and organism-specific diagnostic tests using standard microbiological methods.[20] Growth in the presence of factors X (haemin) and V (nicotinamide adenine dinucleotide) and growth observed in the haemolytic zone of *S. aureus* on blood agar plates, were diagnostic of *H. influenzae*. Flooding of the DNA plate with 10% hydrochloric acid followed by a clear zone around the inoculum, is evidence of DNAse activity suggestive of *S. aureus*. Alpha-haemolytic streptococci susceptible to optochin (zone of inhibition >14mm) were identified as *S. pneumoniae*.

Isolates of *S. pneumoniae* were confirmed by whole genome sequencing. A positive push test together with either a positive oxidase, catalase or DNAse response, was diagnostic of *Moraxella* species. Gram-negative rods were sent for further identification using the Vitek 2® (bioMérieux, Marcy l'Etoile, France).

Bacterial load was semi-quantitatively assessed. An aliquot of the 10µl of each sample was streaked for single colonies. Growth in one, two, three or all quadrants of a culture plate was assigned labels scanty, 1, 2 and 3 respectively. Anything less than twenty colonies was reported as scanty.

Forty-five microlitres (5ul x 9 multiplexes) of total nucleic acid was extracted from 60µl of the Primestore® medium using mechanical lysis on a Tissuelyzer LT (Qiagen, Germany) followed by automated extraction with the QIAsymphony® Virus/Bacteria mini-kit (Qiagen, Germany). Quantitative multiplex real-time PCR (qPCR) data were generated via the creation of standard curves using 10-fold serial dilutions of plasmid standards provided by FTDResp33 (Fast Track Diagnostics, Luxembourg), with calculation of pathogen density

(copies/milliliter) from the sample cycle threshold (Ct) values. Quantitative PCR was performed using an Applied Biosystems 7500 (ABI-7500) platform (Applied Biosystems, Foster City, California). Cycling conditions were 50°C for 15 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 8 seconds followed by 60°C for 34 seconds.[6] In order to validate results, each run included a negative, positive and internal control. Any outlier of control values implied that results were not valid and the whole run was repeated. All positive controls and Internal controls showed a positive amplification trace with Ct below 33. All negative controls fell below the threshold of detection. This method allowed for the identification of up to 33 possible organisms including the following bacteria: *M. pneumoniae, Chlamydia pneumoniae, S. pneumoniae, S. aureus, M. catarrhalis, B. pertussis, K. pneumoniae, Legionella species, Salmonella species, H. influenzae* (Supplementary Table 1)

Statistical Analysis

This analysis was restricted to the 5 bacteria commonly associated with LRTI namely *S. pneumoniae, S. aureus, M. catarrhalis, H. influenzae* and *K. pneumoniae*.

Descriptive statistics were used to describe child, maternal and sociodemographic baseline characteristics as well as LRTI episodes and the yield/spectrum of bacterial organisms detected using PCR and culture. Frequency (%) and medians (interquartile range) were used to describe categorical and continuous data respectively. $P<0.05$ was considered statistically significant.

Chi-square or Fisher's exact were used for crude comparison of factors influencing bacterial yield, including antibiotic usage and hospitalization.

Pearson's correlation coefficient (r) was used to determine the most common combinations of organisms found on PCR and culture.

Univariate analysis was performed on an a priori set of confounding factors namely age at LRTI episode, hospitalization and antibiotic use at the time of sampling to determine whether any significant association existed between either of these variables and, PCR and culture.

Forward stepwise logistic regression was then used for the adjusted analysis to assess whether any significant association existed between each confounding factor, under the influence of the others, and, PCR and culture.

Kruskal-Wallis was used to compare levels of bacterial load on PCR with corresponding quantitative culture results, for respective bacterial organisms, to assess whether a significant relationship existed between the two.

Quantitative and qualitative data were analysed using STATA Release 17.0 Statistical Software Package (STATACorp, College Station, USA).

Results

Of the 1143 children enrolled, 51 cases of congenital pneumonia (defined as a LRTI episode before discharge from hospital, after birth) were excluded. Of the remaining children, 498

(43.57%) experienced a LRTI event between 13th August 2012 and 23rd November 2020 with a total of 1065 episodes. Of these NP samples were not collected in 136 episodes, with a further 51 culture and 19 FTD results unavailable due to technical laboratory issues. This analysis therefore included 859 episodes of LRTI in 434 children, providing 2097.8 years of follow-up at 5 years of age. (Figure 1).

Vaccine coverage for the 6, 10, 14 week and 9-month immunizations was 99% but dropped to 83.5% at 18 months. One hundred and ten (25.3) children were HIV exposed; one was HIV-infected while 80 (18.4%) were premature births with associated low birth weight. Exclusive breastfeeding occurred for a median of 27 days (IQR 15; 90) (Table 1). Six children in our cohort died (1.4%) (Table 1).

Four hundred and ninety (57%) LRTI episodes occurred in infants [median age 9.2 (IQR 3.8; 18.9) months] with 199 children (45.9%) experiencing two or more episodes of LRTI (Table 2). One hundred and seventy-one (20%) required hospital admission for a median of 3 days (IQR 2; 5). Two hundred and forty-one (30%) children were on antibiotics (< 48 hours) at the time of NPS sampling (Table 2).

Bacterial positivity on PCR or culture for any of the 5 organisms was 95% (n=815) or 97% (n=833) respectively. Of 833 positive cultures, a median of 2 (IQR 1; 3) organisms were cultured per sample. Similarly of 815 positive PCR results, a median of 2 (IQR 1; 2) organisms were detected per sample. The commonest combinations of organisms on PCR and culture were *S. pneumoniae* and *M. catarrhalis*, *S. pneumoniae* and *H. influenzae,* and *M. catarrhalis* and *H. influenzae* (Figure 2). *S. pneumoniae* and *M. catarrhalis* were more strongly correlated on PCR than on culture (r = 0.31 versus r = 0.16 respectively). *S.*

pneumoniae and *H. influenzae* as well as *M. catarrhalis* and *H. influenzae* were marginally, more strongly correlated on culture than on PCR (r = 0.22 versus r = 0.12 and r = 0.19 versus r = 0.12 respectively).

S. aureus (120/490; 24.5%) and *K. pneumoniae* (90/490; 19.0%) were most prevalent during the first year of life, while *S. pneumoniae* (148/220; 67.3%)*, M. catarrhalis* (111/220; 50.5%) *and non typable H. influenzae* (149/220; 67.7%) were most prevalent during the second year of life. No age-related difference between prevalence of organisms on culture versus PCR was noted (Supplementary Figure 1).

Multivariate analysis looking at factors that could potentially influence bacterial yield on culture or PCR, showed that hospitalized children were less likely to have a positive culture or PCR positive for bacteria than ambulatory cases, adjusting for age and antibiotic use at specimen sampling (OR 0.4; 95% CI 0.2-1.1). Adjusting for age and hospitalization, children on antibiotics at the time of sampling, had a reduced chance of having a positive culture (OR 0.1; 95% CI 0.1-0.4), but no reduction in PCR yield (OR 0.8; 95% CI 0.4-1.6), (Table 3).

Concordance between culture and PCR for *S. pneumoniae, S. aureus,* and *K. pneumoniae* was greater than 80% (84.9%, 89.7% and 86.3% respectively). Culture and PCR for *H. influenzae* had a concordance of 76.9%. The greatest discordance was between culture and PCR for *M. catarrhalis* (34.4%). *S. pneumoniae* and *M. catarrhalis* yielded more positive results on PCR only compared to culture only (8.9% versus 6.2%; p=0.23 and 33% versus 1.4%, p=0.001 respectively) in comparison to *S. aureus, H.* influenzae and *K. pneumoniae* which yielded fewer positive results on PCR only compared to culture only (3.7% versus

6.6%, p=0.1; 9.7% versus 13.4%, p=0.09 and 6.0% versus 7.7%, p=0.55 respectively) (Table 4).

Median bacterial loads on PCR for all 5 bacteria showed a significant relationship with semi-quantitative culture results ($p<0.001$ for each of the 5 bacteria). An increasing median bacterial load on PCR positively correlated with semi-quantitative culture results for *S. pneumoniae, S. aureus, M. catarrhalis and H. influenzae.* This association was less evident for *K. pneumoniae* [table 5].

Discussion

In this birth cohort study, a substantial proportion of children (44%) experienced an episode of LRTI in the first five years of life, highlighting the high burden of disease in LMICs and the need for rapid and reliable etiological testing to optimize treatment. Concordance of bacterial positivity between culture and PCR on NPs specimens for *S. pneumoniae, S. aureus, K. pneumoniae* and *H. influenzae* was greater than 76% with the exception of *M. catarrhalis,* which exhibited a concordance of only 66%. Median bacterial loads from PCR correlated significantly with semi-quantitative culture results. However, due to the high frequency of colonization of the upper respiratory tract (URT) with these common organisms, establishing causality, remains challenging.

S. pneumoniae was the most common bacterial organism isolated on culture (60.6%) and PCR (63.3%) in this analysis, even with high vaccination rates of PCV-13. This probably reflects colonization of the nasopharynx with non-vaccine serotypes causing LRTI. Other colonizing organisms also frequently found in the URT and, also associated with LRTI include

S. aureus, M. catarrhalis and non-typable *H. influenzae*. In this study *M. catarrhalis* and *H. influenzae* were also highly prevalent on culture and PCR (56.8% versus 53.1% and 47.8% versus 79.4% respectively).

M. catarrhalis showed the greatest discordance between PCR and culture, the most likely reason being its' non-specific colony morphology which often goes undetected. Another possibility for discordance, is that PCR may be detecting *Moraxella nonliquefasciens* which has been found in high abundance as a colonising organism in children on previous studies[22] but isn't detected on culture. The increased positivity of *S. pneumoniae* on PCR compared to culture, may reflect a poorer sensitivity of culture because of *S. pneumonia's* characteristic autolyses within the culture medium.[23]

Antibiotic use at the time of sampling played a large role in reducing bacterial positivity, predominantly for culture. This discrepancy is most likely explained by PCR being unable to distinguish viable from non-viable organisms, resulting in discordant results and an overestimation of bacterial positivity while culture of samples after antibiotic use, is more likely to produce discordant results which underestimate bacterial positivity.

Similar relationships between antibiotic use and culture versus PCR bacterial positivity, were demonstrated in the PERCH study. Use of antibiotics at sample collection resulted in a reduction in yield of *S. pneumonia* on NP culture, of 30% in cases and 32% in controls with a more modest reduction in detection by PCR of 5% and 7% respectively.[24] The EPIC study of adults and children under 18 years, hospitalized with community acquired pneumonia similarly showed that bacterial positivity on culture was greater in sputum/endotracheal specimens (50% versus 26.8%; P<0.01) taken before antibiotic use in comparison to PCR on NP/OP specimens (6.7% versus 5.4%; P=0.31) where no significant difference was found in results from specimens taken before or after antibiotic use.[14]

Further reasons for false negative results on culture include inadequate sample volumes. For culture, larger volumes are required to detect target bacteria compared to PCR which requires a much smaller volume and low numbers of target bacteria for DNA isolation.[13] Sensitivity and specificity of PCR and culture assays are highly operator dependent. Inconsistent practices could lead to inaccurate interpretation of results, resulting in a higher proportion of false positives or false negatives. Adhering to strict laboratory standardized operating procedures minimizes this risk.

Limitations of this study include the absence of a control group to compare the yield and spectrum of nasopharyngeal bacteria obtained on cases of LRTI and matched controls, as a way of distinguishing colonization from pathogenic bacteria. However, the aim of this study was primarily to compare yield on PCR and culture. The presence and contribution of non-typable *H. influenzae* to LRTI could also not be established in this analysis.

The findings of this study are generalizable to other LMIC countries, as illustrated by the PERCH study, which obtained results comparable to ours with regards to the presence and spectrum of bacteria detected on nasopharyngeal specimens during episodes of LRTI. However, in LMIC settings with poor resources, access to a more sophisticated technique like PCR, which is more costly and requires training, may be limited. Culture in these settings may be more appropriate to use even though the turn-around time for results is longer.

In summary, the detection and identification of bacterial organisms in LRTI, remains crucial to improving treatment and prevention strategies. This study provides insights into the spectrum and yield of bacteria detected on culture and PCR during LRTI episodes.

Significant concordance existed between PCR and culture for 4 of the 5 bacteria, affirming PCR as a comparable, less laborious method of testing to culture, for common bacterial organisms during episodes of LRTI. Although culture is still the gold standard, PCR serves as a good adjunctive tool to further increase the chance of detecting organisms.

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Competing Interests

All authors have no competing interests to declare.

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Table 1: Baseline characteristics of children with lower respiratory tract infection less than 5 years of age

Child characteristics (N=434)	
Male	254/434 (58.5%)
Age in days at first LRTI; median (IQR)	275 (114 - 569)
HIV exposed	110/433 (25.3%)
Gestational age (weeks)	39 (37 - 40)
Premature births (<37week gestation)	80/434 (18.4%)
Low birth weight	79/434 (18.2%)
Birth weight-for-age z score; median (IQR)	-0.7 (-1.4 - 0.01)
Initiated breastfeeding	399/434 (91.9%)
Time of exclusive breastfeeding in days; median (IQR)	27 (15 - 90)
Vaccination coverage • 6 weeks • 10 weeks • 14 weeks • 9 months • 18 months	 398/400 (99.5%) 397/399 (99.5%) 392/394 (99.5%) 359/363 (98.9%) 274/328 (83.5%)
Recurrent episodes of LRTI	199/434 (45.9%)
Mortality	6/434 (1.4%)
Maternal characteristics (N=434)	
Age in years; median (IQR)	26.5 (22 - 31)
Antenatal smoking (self-reported)	99/430 (23%)
Postnatal smoking (self-reported)	137/425 (32)
Education • Primary • Secondary (some) • Secondary(complete) • Tertiary	 42/434 (9.7%) 238/434 (54.8%) 133/434 (30.6%) 21/434 (4.8%)
Sociodemographic characteristics (N=434)	
Lives in formal home	229/434 (52.8%)
Crowding: individuals per household; median (IQR)	4 (3 - 6)
Household income per month • <R1000 • R1000-5000 • R5000-10000 • >R10 000	 147/427 (34.4%) 223/427 (52.3%) 48/427 (11.2%) 9/427 (2.1%)

Data are reported as number (%) for categorical variables and median (IQR for continuous).

LRTI=lower respiratory tract infection; HIV=Human Immunodeficiency Virus; IQR=interquartile range.

*One child had a negative birth PCR but became HIV positive in the first year of life.

Table 2: Description of lower respiratory tract infection episodes

Overall Episodes LRTI (n=859)	
Age categories at LRTI • 0-1 year • 1-2 years • 2-3 years • 3-4 years • 4-5 years	 490/859 (57%) 220/859 (26%) 106/859 (12%) 30/859 (3%) 13/859 (2%)
Season during LRTI • Autumn • Winter • Spring • Summer	 250/859 (29%) 283/859 (33%) 211/859 (25%) 115/859 (13%)
Hospital Admission	171/859 (20%)
Days in Hospital; median (IQR)	3 (2 - 5)
Antibiotic use at time of 1st sample	241/811 (30%)

Data are reported as number (%) for categorical variables and median (IQR for continuous).

LRTI=lower respiratory tract infection; IQR=Interquartile range.

Table 3: Multivariate analysis of factors associated with positive bacterial culture or polymerase chain Reaction

CULTURE	**Total N=811**	**Culture positive N=786 (96.9)**	**Culture neg N=25 (3.1)**	**OR (95% CI)**	**P value**
Age (days) at LRTI; median (IQR)	329 (132 – 616)	346 (132 – 621)	247 (154 – 337)	1.0 (1.0 – 1.0)	1.000
Hospitalisation	162 (20.0%)	148 (91.4%)	14 (8.6%)	0.4 (0.2 – 1.1)	0.070
Antibiotics at sampling	241 (29.7%)	221 (91.7%)	20 (8.3%)	0.1 (0.1– 0.4)	<0.001
PCR	**Total N=811**	**Positive PCR N=772 (96.6)**	**Negative PCR N=39 (4.8)**	**OR (95% CI)**	**P value**
Age (days) at LRTI Median (IQR)	329 (132-616)	346 (140-616)	213 (54-497)	1.0 (1.0-1.0)	0.183
Hospitalisation	162 (20.0%)	145 (89.5%)	17 (10.5%)	0.4 (0.2-0.8)	0.009
Antibiotics at sampling	241 (29.7%)	223 (92.5%)	18 (7.5%)	0.8 (0.4-1.6)	0.474

Antibiotic use at sampling for 48 LRTI episodes was not reported.

PCR=polymerase chain reaction; OR=odds ratio; 95% CI=95% confidence interval.

Table 4: Concordance/discordance of positivity by culture and polymerase chain reaction, by pathogen

	Total number of samples	Negative on both culture and PCR (%)	Positive on both culture and PCR (%)	Positive on culture only (%)	Positive on PCR only (%)
S. pneumoniae	859	262 (30.5)	467 (54.4)	53 (6.2)	77 (8.9)
S. aureus	859	682 (79.5)	88 (10.2)	57 (6.6)	32 (3.7)
H. influenzae	859	288 (33.5)	373 (43.4)	115 (13.4)	83 (9.7)
K. pneumoniae	859	701 (81.6)	40 (4.7)	66 (7.7)	52 (6.0)
M. catarrhalis	859	165 (19.2)	399 (46.4)	12 (1.4)	283 (33.0)

PCR=polymerase chain reaction; Data are reported as number (%).

Table 5: Comparison of culture results and corresponding polymerase chain reaction bacterial load by pathogen

S. pneumoniae, by culture and PCR positivity with log10 bacterial load (genome copies per ml) from PCR					
Culture	**N (%)**	**PCR positive (%)**	**PCR negative (%)**	**Median log bacterial load (IQR) if positive**	**P-value**
No growth	339 (39.5)	77 (22.7)	262 (77.3)		0.001
<20	47 (5.5)	37 (78.7)	10 (21.3)	6.0 (5.0 – 6.3)	
1+	184 (21.4)	157 (85.3)	27 (14.7)	6.3 (5.7 – 7.0)	
2+	179 (20.8)	169 (94.4)	10 (5.6)	6.8 (6.2 – 7.4)	
3+	110 (12.8)	104 (94.5)	6 (5.5)	6.8 (6.4 – 7.4)	
Total	859	544	315		
S. aureus, by culture and PCR positivity with log10 bacterial load (genome copies per ml) from PCR					
Culture	**N (%)**	**PCR positive (%)**	**PCR negative (%)**	**Median log bacterial load (IQR) if positive**	**P-value**
No growth	714 (83.1)	32 (4.5)	682 (95.5)		0.001
<20	53 (6.2)	20 (37.7)	33 (62.3)	5.2 (4.4 – 6.4)	
1+	44 (5.1)	26 (50.1)	18 (40.9)	5.8 (4.9 – 6.3)	
2+	26 (3.0)	21 (80.8)	5 (19.2)	5.8 (5.2 – 7.5)	
3+	22 (2.6)	21 (95.5)	1 (4.6)	7.0 (6.5 – 8.1)	
Total	859	120 (14.0)	739 (86.0)		
M. catarrhalis, by culture and PCR positivity with log10 bacterial load (genome copies per ml) from PCR					
Culture	**N (%)**	**PCR positive (%)**	**PCR negative (%)**	**Median log bacterial load (IQR) if positive**	**P-value**
No growth	448 (52.2)	282 (63.2)	165 (36.8)		0.001
<20	130 (15.1)	124 (95.4)	6 (4.6)	6.7 (6.1 – 7.2)	
1+	186 (21.6)	181 (97.3)	5 (2.7)	7.0 (6.5 – 7.4)	
2+	69 (8.0)	68 (98.6)	1 (1.5)	7.2 (6.7 – 7.6)	
3+	26 (3.0)	26 (100.0)	0 (100.0)	7.0 (6.4 – 7.6)	
Total	859	682 (79.4)	177 (20.6)		
H. influenzae, by culture and PCR positivity with log10 bacterial load (genome copies per ml) from PCR					
Culture	**N (%)**	**PCR positive (%)**	**PCR negative (%)**	**Median log bacterial load (IQR) if positive**	**P-value**
No growth	371 (43.2)	81 (21.8)	290 (78.2)		0.001
<20	63 (7.3)	31 (49.2)	32 (50.8)	6.4 (6.0 – 7.1)	
1+	188 (21.9)	139 (73.9)	49 (26.1)	6.9 (6.1 – 7.6)	
2+	131 (15.3)	110 (84.0)	21 (16.0)	7.4 (6.6 – 8.1)	
3+	106 (12.3)	92 (86.8)	14 (13.2)	7.6 (7.0 – 8.1)	
Total	859	453 (52.7)	406 (47.3)		
K. pneumoniae, by culture and PCR positivity with log10 bacterial load (genome copies per ml) from PCR					
Culture	**N (%)**	**PCR positive (%)**	**PCR negative (%)**	**Median log bacterial load (IQR) if positive**	**P-value**
No growth	753 (87.7)	52 (6.9)	701 (93.1)		0.001
<20	60 (7.0)	20 (33.3)	40 (66.7)	5.2 (4.8 – 5.8)	
1+	25 (2.9)	10 (40.0)	15 (60.0)	6.1 (4.9 – 7.1)	
2+	8 (0.9)	5 (62.5)	3 (37.5)	4.9 (4.8 – 5.0)	
3+	13 (1.5)	5 (38.5)	8 (61.5)	5.1 (4.8 – 5.3)	
Total	859	92 (10.7)	767 (89.3)		

Data are reported as number (%) for categorical variables and median (IQR for continuous) by culture and PCR positivity with log10 bacterial load (genome copies per ml) from PCR.

PCR=polymerase chain reaction.

Figure 1. Study flow diagram

Figure 2. Venn diagram showing intersection of positive results for *S. pneumoniae, H. influenzae* and *M. catarrhalis* from polymerase chain reaction and culture

Figure 1.

LRTI = lower respiratory tract infection; FTD = Fast track diagnostic respiratory pathogen qPCR panel.

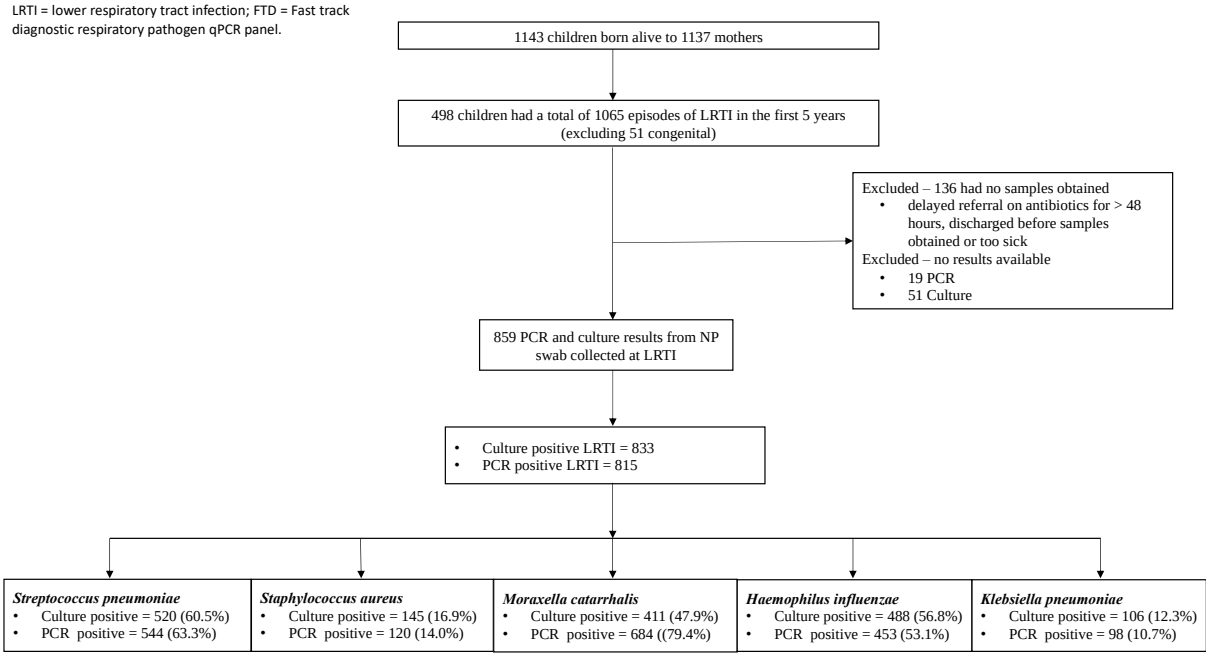
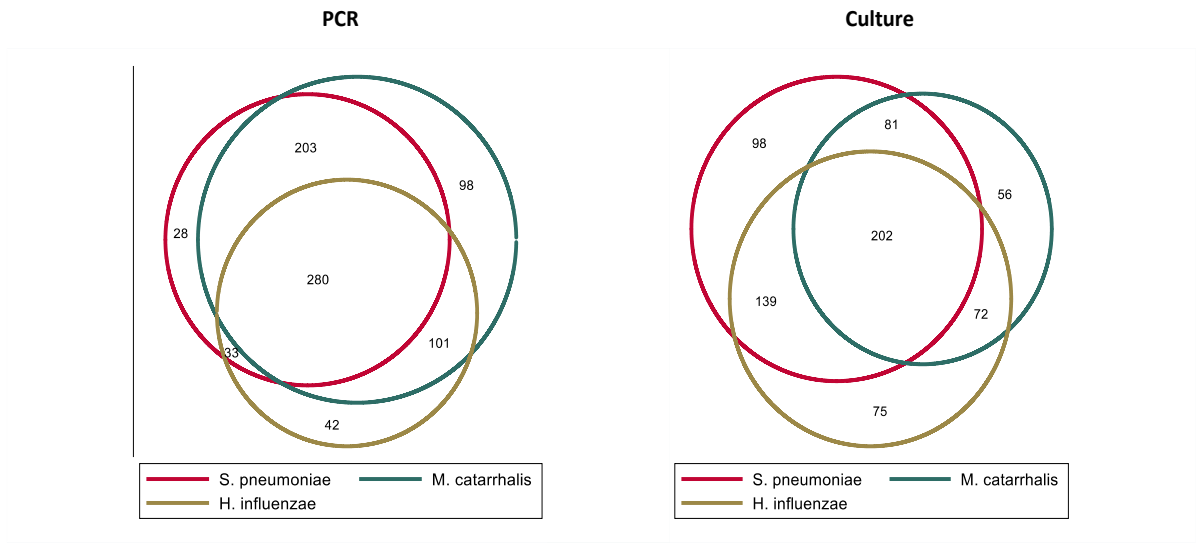


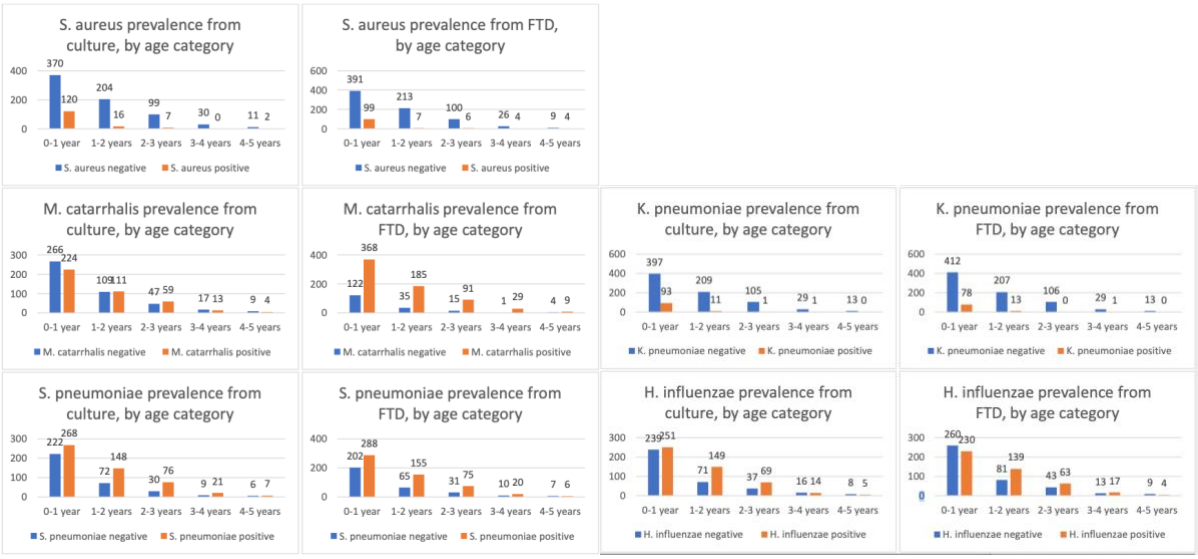
Figure 2.



Intersection of positive results for *S. pneumoniae, H. influenzae* and *M. catarrhalis* from PCR and culture.

Appendices

Figure 1: Spectrum of bacterial organisms across age categories.



S. aureus and *K. pneumoniae* were most prevalent during the first year of life while *S. pneumoniae, M. catarrhalis and non typable H. influenzae* were most prevalent during the second year of life. No obvious age-related difference between prevalence of organisms on culture versus PCR was noted.

Table 1: Gene targets and associated cycle threshold values of bacterial, viral and fungal pathogens

Multiplex (MP)	Pathogen	Cut-off	Multiplex	Pathogen	Cut-off
MP1	Flu A	35	MP6	S.aurues	34.5
	Flu B	33.4		C. pneum	35.5
	Rhino	33.5		S.pneum	34
MP2	Para 2	34		Hib	34
	Para 3	32.5	MP7	PCP	34.5
	Para 4	32		Legio	33.6
MP3	Cor 229	35		K.pneum	34.3
	Cor 63	35		Sal	35.5
	Cor 43	35	MP8	Flu C	34.3
	Cor hku	35		Morax	34
MP4	RSVab	35		Bord	34
	Av	35.6		Hae inf	34.3
	Ev	33	MP9	CMV	35
	Pv	33			
MP5	Para 1	36			
	M pneum	34.8			
	H bov	35			
	Hm pvab	35.5			

Table 2: Association between antibiotic usage and bacterial culture or polymerase chain reaction

		Total	**Antibiotic use at sampling**	**No antibiotic use at sampling**	P-value
Culture	Growth	786 (96.9%)	221(91.7%)	565 (99.1%)	<0.001
	No growth	25 (3.1%)	20 (8.3%)	5 (0.9%)	
	Total	811	241 (29.7%)	570 (70.3%)	
			Antibiotic use at sampling	**No antibiotic use at sampling**	P-value
PCR	Positive	772 (95.2%)	223 (92.5%)	549 (96.3%)	0.021
	Negative	39 (4.8%)	18 (7.5%)	21 (3.7%)	
	Total	811	241 (29.7%)	570 (70.3%)	

Antibiotic use at sampling for 48 LRTI episodes was not reported. Data are reported as number (%). PCR=polymerase chain reaction

Antibiotic use at the time of sampling was negatively associated with culture for bacteria (p=0.001) or detection on PCR (p=0.021), but less so on PCR.

Table 3: Association of hospitalized versus ambulatory lower respiratory tract infection for bacterial culture or polymerase chain reaction.

		Total	**Hospitalised**	**Ambulatory**	P-value
Culture	Growth	833 (97.0%)	157 (91.8%)	676 (98.3%)	<0.001
	No growth	26 (3.0%)	14 (8.2%)	12 (1.7%)	
	Total	859	171	688	
PCR			**Hospitalised**	**Ambulatory**	P-value
	Positive	815 (94.9%)	152 (88.9%)	663 (96.4%)	<0.001
	Negative	44 (5.1%)	19 (11.1%)	25 (3.6%)	
	Total	859	171	688	

Data are reported as number (%); LRTI=lower respiratory tract infection; PCR=polymerase chain reaction.

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- Francis,V. and Bastin,M. (2000) Gene targeting in rat embryo fibroblasts promoted by the polyomavirus large T antigen. *Nucleic Acids Res.* , in press.
- Maniatis T, Fritsch EF, Sambrook J. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, **1982** .

- Huynh TV, Young RA, Davis RW. DNA Cloning. In: Glover DM. DNA Cloning - A Practical Approach. Vol 1. Oxford, UK: IRL Press, **1988** : 49-78.
- Public Health Service Task Force. Recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. Available at: http://www.aidsinfo.nih.org. Accessed 24 April 2002.
- Lyon DJ, Cheng AFB, Norrby SR. Mechanisms of cefotaxime resistance in blood culture isolates of Enterobacter high prevalence of extended-spectrum β-lactamases [abstract C43]. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, **1995** .

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For human genes, use genetic notation and symbols approved by the Human Gene Mapping Workshop (see Wain HM, Bruford EA, Lovering RC, Lush MJ, Wright MW, Povey S. Guidelines for human gene nomenclature. Genomics 2002; 79:464–70). Human gene names and loci should be written in italicized capital letters and Arabic numerals. Human protein product names are not italicized. For human mutation nomenclature, see Antonarakis et al. (Recommendations for a nomenclature system for human gene mutations. Hum Mutat 1998; 11:1–3).

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