Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Available online at www.sciencedirect.com
ScienceDirect
Vaccine 25 (2007) 2458–2464
Vaccine
www.elsevier.com/locate/vaccine

Modelling the co-occurrence of *Streptococcus pneumoniae* with other bacterial and viral pathogens in the upper respiratory tract

Peter Jacoby [a,*], Kelly Watson [a], Jacinta Bowman [b], Amanda Taylor [b], Thomas V. Riley [b,c], David W. Smith [b], Deborah Lehmann [a],

on behalf of the Kalgoorlie Otitis Media Research Project Team [1]

[a] *Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, WA, Australia*
[b] *Division of Microbiology & Infectious Diseases, PathWest Laboratory Medicine, WA, Australia*
[c] *Microbiology & Immunology, The University of Western Australia, WA, Australia*

Available online 22 September 2006

Abstract

Otitis media (OM) is a major burden for all children, particularly for Australian Aboriginal children. *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* and viruses (including rhinovirus and adenovirus) are associated with OM. We investigated nasopharyngeal microbial interactions in 435 samples collected from 79 Aboriginal and 570 samples from 88 non-Aboriginal children in Western Australia. We describe a multivariate random effects model appropriate for analysis of longitudinal data, which enables the identification of two independent levels of correlation between pairs of pathogens. At the microbe level, rhinovirus infection was positively correlated with carriage of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, and adenovirus with *M. catarrhalis*. Generally, there were positive associations between bacterial pathogens at both the host and microbe level. Positive viral–bacterial associations at the microbe level support previous findings indicating that viral infection can predispose an individual to bacterial carriage. Viral vaccines may assist in reducing the burden of bacterial disease.

Keywords: Longitudinal data analysis; Bacterial carriage; Respiratory viruses

1. Introduction

Upper respiratory tract (URT) carriage of respiratory bacterial pathogens is associated with otitis media (OM), which is a major burden of disease in young children, particularly in Australian Aboriginal children [1–4]. Colonisation and survival of an individual microbe in the human nasopharynx are determined by numerous factors, some of which are host-specific, such as environmental exposure to infectious agents and immune function, and some microbial, including interactions between microbial species. It is the extent and mechanisms by which microbes interact and the resulting potential to cause disease that are of particular interest. Several studies have suggested that viral infections of the URT predispose to bacterial respiratory infection and that viruses in the URT enhance bacterial adherence to respiratory tract cells [5–8]. Pathogens can also alter the effects of co-infecting pathogens by destroying or damaging a physical barrier, decreasing mucociliary activity, altering immune cell function or up-regulating gene expression [9]. Data are limited on the co-occurrence of bacteria in the URT [10–12] and on associations between colonising pathogens in the absence of symptoms.

In a cohort study of young children in Western Australia (WA), we noted that age-specific patterns of carriage of *Streptococcus pneumoniae*, *Moraxella catarrhalis* and

* Corresponding author at: Division of Population Sciences, Telethon Institute for Child Health Research, P.O. Box 855, West Perth, WA 6872, Australia. Tel.: +61 8 9489 7747; fax: +61 8 9489 7700.
E-mail address: peterj@ichr.uwa.edu.au (P. Jacoby).
[1] See Appendix A.

0264-410X/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2006.09.020

Haemophilus influenzae were very similar [13], suggesting that the same host factors might influence carriage of all three pathogens. In addition, there may be positive interactions between pathogens in the ecological niche of the URT. Bacterial interference may also occur: there are reports of negative associations between carriage of serotypes included in a 7-valent pneumococcal conjugate vaccine (7vPCV) and *Staphylococcus aureus* [11,12,14].

A major limitation of cross-sectional analyses of longitudinal data is that they do not account for the possibility that samples displaying multiple carriage at different time points may come from the same individuals. Cross-sectional analyses are therefore unable to distinguish between host-level (between subjects) associations and associations at the microbe-level (within subjects), both of which will result in co-occurrence of pathogens within samples.

A positive association between pathogens at the host level suggests that individuals with high susceptibility to infection with one pathogen, as evidenced by higher than average incidence over time, are also susceptible to infection with another pathogen under consideration. Environmental factors and immune function may play a role [15–19].

A positive association at the microbe level is observed when co-occurrence of two pathogens within a particular host occurs more frequently than would be expected given the independent susceptibilities of the host to these pathogens. In the case of nasopharyngeal carriage, this might be the result of a mechanism whereby the presence of one pathogen creates conditions conducive to adherence of another. Conversely, a negative correlation would suggest that the two pathogens of interest are competing for colonisation sites in the nasopharynx.

Statistical modelling of longitudinal data, consisting of repeated sampling of individuals over time, can be used to differentiate between host- and microbe-level correlations and thus assist in understanding the ecology of the URT and identifying appropriate interventions. We have investigated patterns of co-occurrence of pathogens identified in the nasopharynx of Aboriginal and non-Aboriginal children living in a semi-arid area of WA. We describe a multivariate random effects model appropriate for analysis of longitudinal data, which enables the identification of two independent levels of association between pairs of pathogens.

2. Methods

2.1. Study design

This study was conducted in the Kalgoorlie–Boulder area of the Eastern Goldfields located 600 km east of Perth, WA. We used data from the Kalgoorlie Otitis Media Research Project, which aims to investigate causal pathways to OM and the epidemiology of URT micro-organism carriage. The project design has been described elsewhere [13]. Briefly, 100 Aboriginal and 180 non-Aboriginal children were enrolled at birth and followed regularly to age 2 years. Nasopharyngeal aspirates (NPA) were collected at ages 1–3, 6–8 and 4, 6, 12, 18 and 24 months. Primary bacterial isolation of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. aureus* was performed using selective media [13,20]. To identify viruses, nucleic acid extraction for nucleic acid amplification was carried out using QiaAmp Viral RNA kit (QiaGen corp) in accordance to the manufacturer's protocol. In-house nested or semi-nested PCR amplification was performed for rhinoviruses, adenoviruses, respiratory syncytial virus, human metapneumovirus, influenza viruses A and B, coronaviruses and parainfluenza virus types 1–3. However, only adenovirus and rhinovirus were detected in sufficient numbers to be included in this data analysis.

2.2. Statistical analysis

Data from children who contributed four or more samples were included in the analysis. The presence or absence of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, adenovirus and rhinovirus was recorded for every sample. A hierarchical logistic model was constructed with joint binary responses for each of the pathogens. This provided estimates of multivariate normally distributed random effects (under a logit link) at the host level and also residuals at the microbe level which have a multivariate Bernoulli distribution. Modelling of multivariate longitudinal data is frequently performed but in most cases the correlations between dependent variables are regarded as nuisance parameters which must be accounted for in order to estimate fixed effects accurately [21,22]. However, in this study, they are the main focus of interest. One previous study has investigated separate levels of multivariate association in longitudinal binary data with a similar approach to that used here [23]. Estimated covariances of the random effects and their standard errors provide an indication of significance of the association between each pair of pathogens, at both the host and microbe levels. The models incorporated fixed effects to adjust for a quadratic dependence of log odds of carriage on age and also for seasonal effects. Patterns of co-occurrence were analysed separately in Aboriginal and non-Aboriginal children. The analysis was conducted using the MLwiN software package [24] with a penalised quasi-likelihood (PQL) solution method. A detailed model description is presented in Appendix B.

Individual children provided between four and seven samples. Random effects modelling require that data are missing at random (MAR) in order to avoid any bias of parameter estimates [25]. Since data were collected from healthy children during routine follow-up visits, there should be little correspondence between a child's carriage status and attendance for routine follow-up, and therefore the MAR assumption is unlikely to be violated.

Table 1
Numbers (%) of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, adenovirus and rhinovirus identified in NPAs collected from Aboriginal and non-Aboriginal children

	Aboriginal	Non-Aboriginal
Number of samples	435	570
S. pneumoniae	219 (50.3%)	158 (27.7%)
H. influenzae	182 (41.8%)	73 (12.8%)
M. catarrhalis	225 (51.7%)	171 (30.0%)
S. aureus	88 (20.2%)	147 (25.8%)
Adenovirus	37 (8.5%)	20 (3.5%)
Rhinovirus	102 (23.4%)	94 (16.5%)

3. Results

A total of 435 samples from 79 Aboriginal children (median 6, mean 5.5/child) and 570 samples from 88 non-Aboriginal children (median 7, mean 6.5/child) who had at least four specimens examined for all relevant pathogens were included in this analysis. The frequencies of positive samples for each pathogen under investigation are shown in Table 1. The patterns of co-occurrence of *S. pneumoniae* with *M. catarrhalis*, *S. aureus*, rhinovirus and adenovirus are shown in Fig. 1, stratified by Aboriginality and age group. In Aboriginal and non-Aboriginal children of all ages, *S. pneumoniae* was isolated more frequently when *M. catarrhalis* was also isolated than when *M. catarrhalis* was not isolated. A similar pattern was observed for *S. pneumoniae* and *H. influenzae*, and also for *S. pneumoniae* and rhinovirus except in children aged <1 month. On cross-sectional examination, co-occurrence of *S. pneumoniae* with adenovirus and with *S. aureus* varied with age (Fig. 1(b and d)).

Associations between pairs of bacteria at host and microbe levels obtained from longitudinal models are shown in Table 2. There were significant positive host-level associations between *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* in both Aboriginal and non-Aboriginal children. Only the association between *S. pneumoniae* and *H. influenzae* in non-Aboriginal children failed to reach significance. We also observed significant positive associations at the microbe level between *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* in both Aboriginal and non-Aboriginal children; only the association between *S. pneumoniae* and *H. influenzae* in Aboriginal children failed to reach significance. There were no significant bacterial associations involving *S. aureus* with the exception of a negative association with *M. catarrhalis* at the microbe level in non-Aboriginal children.

Associations for each bacterium/virus pair at host and microbe level are shown in Table 3. Generally, there were no significant associations between the viruses under investigation and bacteria at the host level. At the microbe level, we observed significant positive associations between the presence of rhinovirus and the three major respiratory bacteria in all children. There was no consistent pattern of co-occurrence between adenovirus and bacteria but a significant positive association of adenovirus with *M. catarrhalis* in all children and with *H. influenzae* in Aboriginal children.

The covariances computed by our models as indicators of association may perhaps be more easily understood in terms

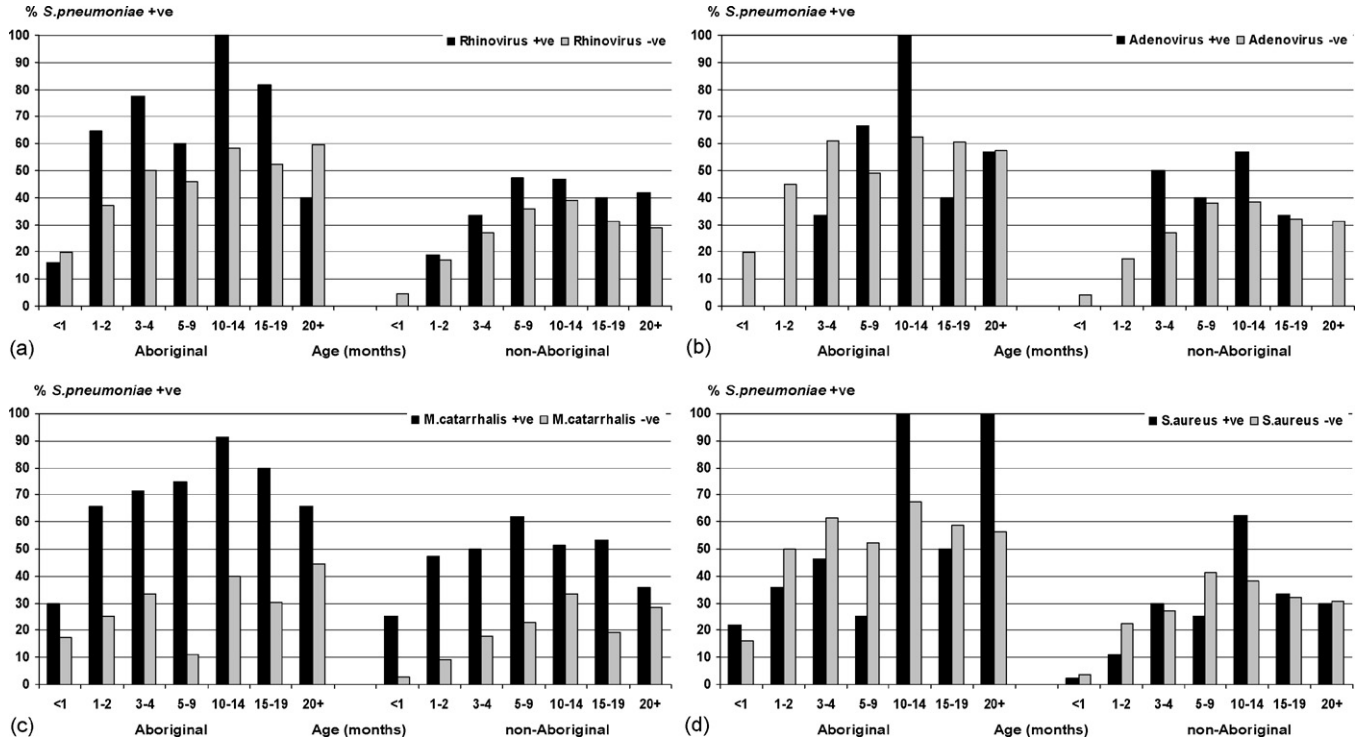

Fig. 1. Proportion of NPAs from which *S. pneumoniae* was isolated in presence or absence of: (a) rhinovirus, (b) adenovirus, (c) *M. catarrhalis* and (d) *S. aureus* by age and Aboriginality.

Table 2
Associations between pairs of bacteria in Aboriginal and non-Aboriginal children

	Host-level covariance		Microbe-level covariance	
	Aboriginal	Non-Aboriginal	Aboriginal	Non-Aboriginal
S. pneumoniae × *H. influenzae*	**1.26 (0.72,1.81)**	0.32 (−0.10,0.75)	0.09 (−0.01,0.19)	**0.20 (0.12,0.28)**
S. pneumoniae × *M. catarrhalis*	**1.34 (0.72,1.96)**	**0.71 (0.31,1.11)**	**0.27 (0.18,0.36)**	**0.25 (0.17,0.33)**
H. influenzae × *M. catarrhalis*	**1.14 (0.57,1.71)**	**0.56 (0.11,1.00)**	**0.19 (0.09,0.29)**	**0.26 (0.18,0.34)**
S. pneumoniae × *S. aureus*	−0.26 (−0.76,0.24)	−0.40 (−0.88,0.08)	0.04 (−0.07,0.14)	0.05 (−0.04,0.14)
H. influenzae × *S. aureus*	0.25 (−0.24,0.74)	−0.24 (−0.79,0.31)	−0.04 (−0.15,0.06)	−0.04 (−0.12,0.05)
M. catarrhalis × *S. aureus*	0.07 (−0.47,0.61)	−0.04 (−0.51,0.42)	−0.05 (−0.15,0.06)	**−0.10 (−0.19,−0.01)**

Associations are statistically significant ($p < 0.05$) where the confidence interval for the covariance does not include zero.
Ninety-five percent confidence limits are shown in parentheses; significant values appear in bold.

Table 3
Associations between bacterium/virus pairs in Aboriginal and non-Aboriginal children

	Host-level covariance		Microbe-level covariance	
	Aboriginal	Non-Aboriginal	Aboriginal	Non-Aboriginal
S. pneumoniae × Rhinovirus	**0.44 (0.05,0.83)**	−0.01 (−0.32,0.31)	**0.14(0.04,0.23)**	**0.14 (0.05,0.23)**
H. influenzae × Rhinovirus	0.39 (0.00,0.77)	−0.11 (−0.47,0.26)	**0.25 (0.16,0.35)**	**0.12 (0.04,0.21)**
M. catarrhalis × Rhinovirus	0.36 (−0.07,0.78)	0.12 (−0.19,0.43)	**0.21 (0.12,0.31)**	**0.14 (0.05,0.22)**
S. aureus × Rhinovirus	0.34 (−0.07,0.74)	0.20 (−0.21,0.61)	0.01 (−0.10,0.11)	**0.12 (0.03,0.20)**
S. pneumoniae × Adenovirus	0.61 (0.00,1.22)	−0.33 (−0.97,0.32)	−0.05 (−0.15,0.05)	**0.21 (0.13,0.29)**
H. influenzae × Adenovirus	0.41 (−0.18,1.00)	0.52 (−0.23,1.26)	**0.15 (0.05,0.25)**	0.07 (−0.02,0.16)
M. catarrhalis × Adenovirus	0.33 (−0.33,0.99)	0.00 (−0.65,0.64)	**0.11 (0.01,0.20)**	**0.33 (0.25,0.40)**
S. aureus × Adenovirus	0.12 (−0.51,0.76)	0.56 (−0.28,1.39)	−0.05 (−0.15,0.05)	0.08 (−0.01,0.17)
Adenovirus × Rhinovirus	0.48 (−0.01,0.96)	−0.02 (−0.59,0.55)	0.09 (−0.01,0.19)	**0.27 (0.19,0.35)**

Associations are statistically significant ($p < 0.05$) where the confidence interval for the covariance does not include zero.
Ninety-five percent confidence limits are shown in parentheses; significant values appear in bold.

of more frequently used measures such as the odds ratio (OR) [26]. A microbe-level covariance of zero for two pathogens describes a situation where the probability of presence of one pathogen is unaffected by whether the other pathogen is also present, i.e. an OR of 1. Similarly, a positive covariance will always equate to an OR of greater than 1. However, there is no simple correspondence between non-zero covariances and ORs when the prevalence of carriage or infection changes over time and individuals have differing susceptibilities. Nevertheless, some indications can be provided: the most significant microbe-level covariances found in this study were in the region of 0.25 which would correspond to an OR of 2.8 if both pathogens were to have a constant prevalence of 50% in all individuals. This rises to an OR of 5.9 for less frequently occurring pathogens with a prevalence of 10%.

4. Discussion

Statistical modelling of longitudinal data has the potential to distinguish two independent levels of association between pairs of pathogens, each of which has a distinct biological interpretation. To our knowledge this approach has not been used before in the area of infectious diseases. A similar method of analysis has, however, been reported for longitudinal data consisting of the incidence of different symptoms in a palliative care trial [23]. This study used an identical modelling approach to that used in the current study and identified associations between symptoms both between and within subjects. The statistical properties of the models were also investigated.

Through statistical modelling we found positive host-level associations between carriage of the three bacterial pathogens causing OM, which is consistent with previous studies showing that environmental factors such as crowding, presence of siblings in the household, day care attendance or exposure to environmental tobacco smoke are associated with high rates of bacterial carriage [15–18,27]. Concurrent multiple bacterial infections may be more pathogenic than single infections. It has been suggested that the more persistent severe OM experienced by Aboriginal children compared with non-Aboriginal children may be related to simultaneous prolonged carriage of multiple OM pathogens in the nasopharynx of Aboriginal children [2]. Hence the host-level social and environmental factors giving a high force of infection must be addressed to reduce the concurrent carriage of pathogens in the URT and the resulting severe outcomes of OM in Aboriginal children. Environmental factors are also likely to contribute to the positive host-level association observed between rhinovirus and *S. pneumoniae* in Aboriginal children. At the host level, mucosal immune function may play an important role in preventing colonisation by potential pathogens [28,29]. Salivary IgA to OM pathogens has been measured as part of the Kalgoorlie OM Research Project and we will

investigate associations between immune status and URT carriage.

We also found positive associations at the microbe level between carriage of all the bacterial OM pathogens, suggesting that these pathogens have an affinity to colonise simultaneously. Our findings are supported by an in vitro study by Budhani and Struthers who showed that *S. pneumoniae* was protected from benzylpenicillin and amoxycillin when grown in the presence of β-lactamase-producing *M. catarrhalis* but susceptible to these antibiotics when grown with non-β-lactamase-producing moraxellae [30]. Thus, β-lactamase-producing *M. catarrhalis* may act as an indirect pathogen. On the other hand, micro-organisms may compete for successful colonisation in the URT [31] and we found negative associations at the microbe level between *S. aureus* and both *M. catarrhalis* and *H. influenzae*. We did not find significant negative associations between all serotypes of *S. pneumoniae* and *S. aureus*. However, significant negative associations have been reported between *S. aureus* and the serotypes included in 7vPCV [11,12,14] and the potential for serotype and species replacement after immunisation with 7vPCV exists [32]. Density of colonisation may also play a role in bacterial interactions and we will examine this further.

The positive viral–bacterial associations observed at the microbe level, particularly those involving rhinovirus, do not necessarily indicate any causal relationship between viral infection and subsequent bacterial colonisation or vice versa. However, our findings support previous studies, which postulate that an existing respiratory viral infection can predispose an individual to bacterial colonisation of the URT by creating conditions for increased adherence [7,8,33,34]. Thus, vaccines targeting URT viruses might have a role in reducing *S. pneumoniae*-related disease. Conversely, a trial in South Africa reported that 7vPCV provided some protection against viral pneumonia, suggesting that *S. pneumoniae* plays an important role in the development of viral pneumonia [35].

We have presented here our initial investigation of host- and microbe-level associations between potential pathogens in the URT. Future analyses of data collected as part of the Kalgoorlie OM Research Project will consider density of bacterial colonisation and specific subtypes of bacteria and we will investigate the impact of social and environmental factors on associations between pathogens. Statistical modelling of longitudinal data is a powerful tool to examine the ecology of the URT and will assist in determining appropriate interventions aimed at reducing the burden of URT microbial carriage and infections.

Acknowledgements

This study was funded through the NHMRC project grant number 212044 and two Healthway grants (numbers 6028 and 10564). PJ, DL and KW are currently funded through NHMRC program grant number 353514.

Appendix A. List of investigators and participating institutions of the Kalgoorlie Otitis Media Research Project 1998–2006

Telethon Institute for Child Health Research (ICHR), Centre for Child Health Research, The University of Western Australia (UWA): J. Aalberse, A. Arumugaswamy, K. Carville, D. Elsbury, J. Evans, J. Finucane, M. Firth, C. Gordon, P. Jacoby, N.H. de Klerk, D. Lehmann, J. Mackenzie, A. Mason, R. Monck, F. Nichols, K. Sivwright, F.J. Stanley, A. Stokes, K. Watson and K. Wood.

Kulunga Research Network: J. Johnston, D. McAullay.

The University of Western Australia: H. Coates, Paediatric Otolaryngologist; J. Spencer, C. Jeffries-Stokes (also ICHR), P. Richmond, pediatricians.

Division of Microbiology and Infectious Diseases, *PathWest Laboratory Medicine*: T.V. Riley (also Microbiology & Immunology, UWA), D. Smith, J. Bowman, A. Taylor, B. Brestovac and G. Harnett.

Audiologists: K. Meiklejohn, Country Audiology, Esperance, WA; S. Weeks, Professional Hearing Services, Perth, WA.

Ngunytju Tjitji Pirni Inc., Kalgoorlie: E. Edwards, J. Carter, A. Forrest, G. Jones, T. Lewis, P. McIntosh, J. Tamwoy and S. Sorian.

Bega Garnbirringu Health Services Aboriginal Corporation, Kalgoorlie: D. Dunn, L. Dorizzi, S. Coleman, R. Bonney and P. Bonney.

University of Canberra: A.W. Cripps (also Griffith University, Qld), J. Kyd, S.M. Kyaw-Myint and R. Foxwell.

Menzies School of Health Research, Darwin, NT: A.J. Leach, B. Harrington and J. Beissbarth.

Public Health Bacteriology Laboratory, Centre for Public Health Sciences, Brisbane, Qld: D. Murphy.

Appendix B. Model details

For pathogen i ($i = 1$–6 representing the four bacterial and two viral pathogens) sampled from subject j on occasion k, the outcome Y_{ijk} is modelled as

$$Y_{ijk} = \pi_{ijk} + e_{ijk}$$

with $Y_{ijk} = 1$ if the pathogen is present in the sample and $Y_{ijk} = 0$ if absent. The $\{\pi\}$ represent the modelled probabilities of a positive sample with

$$\text{logit}(\pi_{ijk}) = b_0 + b_1 t_{ijk} + b_2 t_{ijk}^2 + s_{ijk} + u_{ij}.$$

t_{ijk} is a continuous fixed effect representing the age of subject j on sampling occasion k and s_{ijk} is a four-level categorical fixed effect representing the season of the year when the sample was taken. The $\{u\}$ are subject level random intercepts with

$$u_{ij} = MVN(0, \Omega_{\text{h}})$$

where Ω_h is the between-subjects 6×6 variance/covariance matrix with off-diagonal elements representing the host-level covariances.

The $\{e\}$ are the within-subjects residuals, which are assumed to have a multivariate binomial distribution with variance/covariance matrix, Ω_m, which has variances

$$\text{var}(e_{ijk}) = \sigma^2 \pi_{ijk}(1 - \pi_{ijk})$$

as diagonal elements and off-diagonal elements representing the microbe-level covariances. The dispersion parameter, σ^2, is constrained to equal 1.

References

[1] Gray BM, Converse 3rd GM, Dillon Jr HC. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis 1980;142(6):923–33.

[2] Leach AJ, Boswell JB, Asche V, Nienhuys TG, Mathews JD. Bacterial colonization of the nasopharynx predicts very early onset and persistence of otitis media in Australian Aboriginal infants. Pediatr Infect Dis J 1994;13(11):983–9.

[3] Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children Tonawanda/Williamsville Pediatrics. J Infect Dis 1997;175(6):1440–5.

[4] Morris PS, Leach AJ, Silberberg P, Mellon G, Wilson C, Hamilton E, et al. Otitis media in young Aboriginal children from remote communities in Northern and Central Australia: a cross-sectional survey. BMC Pediatr 2005;5(27).

[5] O'Brien KL, Walters MI, Sellman J, Quinlisk P, Regnery H, Schwartz B, et al. Severe pneumococcal pneumonia in previously healthy children: the role of preceding influenza infection. Clin Infect Dis 2000;30(5):784–9.

[6] Talbot TR, Poehling KA, Hartert TV, Arbogast PG, Halasa NB, Edwards KM, et al. Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. Am J Med 2005;118(3):285–91.

[7] Hament JM, Aerts PC, Fleer A, Van Dijk H, Harmsen T, Kimpen JL, et al. Enhanced adherence of *Streptococcus pneumoniae* to human epithelial cells infected with respiratory syncytial virus. Pediatr Res 2004;55(6):972–8.

[8] Ishizuka S, Yamaya M, Suzuki T, Takahashi H, Ida S, Sasaki T, et al. Effects of rhinovirus infection on the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells. J Infect Dis 2003;188(12):1928–39.

[9] Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. Lancet Infect Dis 2006;6(5):303–12.

[10] Lafontaine ER, Wall D, Vanlerberg SL, Donabedian H, Sledjeski DD. *Moraxella catarrhalis* coaggregates with *Streptococcus pyogenes* and modulates interactions of *S. pyogenes* with human epithelial cells. Infect Immun 2004;72(11):6689–93.

[11] Bogaert D, van Belkum A, Sluijter M, Luijendijk A, de Groot R, Rumke H, et al. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. Lancet 2004;363(9424):1871–2.

[12] Regev-Yochay G, Dagan R, Raz M, Carmeli Y, Shainberg B, Derazne E, et al. Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in children. JAMA 2004;292(6):716–20.

[13] Watson K, Carville K, Bowman J, Jacoby P, Riley TV, Leach AJ, et al. Upper respiratory tract bacterial carriage in Aboriginal and non-Aboriginal children in a semi-arid area of Western Australia. Pediatr Infect Dis J 2006;25(9):782–90.

[14] Watson K, Bowman J, Murphy D, Jacoby P, Riley TV, Lehmann D. Negative association between *Streptococcus pneumoniae* and *Staphylococcus aureus* in the nasopharynx of Aboriginal and non-Aboriginal children in the Kalgoorlie–Boulder area Western Australia. In: In fifth international symposium on pneumococci and pneumococcal diseases. 2006 [Poster presentation P07. 08].

[15] Greenberg D, Givon-Lavi N, Broides A, Blancovich I, Peled N, Dagan R. The contribution of smoking and exposure to tobacco smoke to *Streptococcus pneumoniae* and *Haemophilus influenzae* carriage in children and their mothers. Clin Infect Dis 2006;42(7):897–903.

[16] Regev-Yochay G, Raz M, Dagan R, Porat N, Shainberg B, Pinco E, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* by adults and children in community and family settings. Clin Infect Dis 2004;38(5):632–9.

[17] Aniansson G, Alm B, Andersson B, Larsson P, Nylen O, Peterson H, et al. Nasopharyngeal colonization during the first year of life. J Infect Dis 1992;165(Suppl. 1):S38–42.

[18] Principi N, Marchisio P, Schito GC, Mannelli S. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children Ascanius Project Collaborative Group. Pediatr Infect Dis J 1999;18(6):517–23.

[19] Lindberg K, Freijd A, Rynnel-Dagoo B, Hammarstrom L. Anti pneumococcal antibody activity in nasopharyngeal secretions in healthy adults and children. Acta Otolaryngol 1993;113(5):673–8.

[20] O'Brien KL, Nohynek H, World Health Organization Pneumococcal Vaccine Trials Carriage Working Group. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. Pediatr Infect Dis J 2003;22(2):e1–11.

[21] Das A, Poole WK, Bada HS. A repeated measures approach for simultaneous modeling of multiple neurobehavioral outcomes in newborns exposed to cocaine in utero. Am J Epidemiol 2004;159(9):891–9.

[22] Shelton B, Gilbert G, Liu B, Fisher M. A SAS macro for the analysis of multivariate longitudinal binary outcomes. Comput Methods Prog Biomed 2004;76(2):163–75.

[23] Ribaudo HJ, Thompson SG. The analysis of repeated multivariate binary quality of life data: a hierarchical model approach. Stat Methods Med Res 2002;11(1):69–83.

[24] Rasbash J, Steele F, Browne W, Prosser B. A user's guide to MLwiN Version 2.0. London: Institute of Education; 2004.

[25] Little RJA, Rubin DB. Statistical analysis with missing data. New York: Wiley; 1987.

[26] Lipsitz SR, Laird NM, Harrington DP. Generalized estimating equations for correlated binary data: using the odds ratio as a measure of association. Biometrika 1991;78(1):153–60.

[27] Sulikowska A, Grzesiowski P, Sadowy E, Fiett J, Hryniewicz W. Characteristics of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* isolated from the nasopharynges of asymptomatic children and molecular analysis of *S. pneumoniae* and *H. influenzae* strain replacement in the nasopharynx. J Clin Microbiol 2004;42(9):3942–9.

[28] Zhang Q, Bernatoniene J, Bagrade L, Pollard AJ, Mitchell TJ, Paton JC, et al. Serum and mucosal antibody responses to pneumococcal protein antigens in children: relationships with carriage status. Eur J Immunol 2006;36(1):46–57.

[29] Sun K, Johansen FE, Eckmann L, Metzger DW. An important role for polymeric Ig receptor-mediated transport of IgA in protection against *Streptococcus pneumoniae* nasopharyngeal carriage. J Immunol 2004;173(7):4576–81.

[30] Budhani RK, Struthers JK. Interaction of *Streptococcus pneumoniae* and *Moraxella catarrhalis*: investigation of the indirect pathogenic role of beta-lactamase-producing moraxellae by use of a continuous-culture biofilm system. Antimicrob Agents Chemother 1998;42(10):2521–6.

[31] Lysenko ES, Ratner AJ, Nelson AL, Weiser JN. The role of innate immune responses in the outcome of interspecies competition for colonization of mucosal surfaces. PLoS Pathog 2005;1(1):e1.

[32] Veenhoven R, Bogaert D, Uiterwaal C, Brouwer C, Kiezebrink H, Bruin J, et al. Effect of conjugate pneumococcal vaccine followed by

polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomised study. Lancet 2003;361(9376):2189–95.

[33] Avadhanula V, Rodriguez CA, Devincenzo JP, Wang Y, Webby RJ, Ulett GC, et al. Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner. J Virol 2006;80(4):1629–36.

[34] Peltola VT, Murti KG, McCullers JA. Influenza virus neuraminidase contributes to secondary bacterial pneumonia. J Infect Dis 2005;192(2): 249–57.

[35] Madhi SA, Klugman KP, Vaccine Trialist Group. A role for *Streptococcus pneumoniae* in virus-associated pneumonia. Nat Med 2004;10(8):811–3.