Thors, V., Christensen, H., Morales-Aza, B., Oliver, E., Sikora, P., Vipond, I., Muir, P., & Finn, A. (2019). High Density Bacterial Nasal Carriage in Children is Transient and Associated With Respiratory Viral Infections - Implications for Transmission Dynamics. *Pediatric Infectious Disease Journal*, *38*(5), 533-538. https://doi.org/10.1097/INF.0000000000002256

**High density bacterial nasal carriage in children is transient and associated with respiratory viral infections – implications for transmission dynamics**

Valtyr Thors, MD[1,2], Hannah Christensen[3], Begonia Morales-Aza[1], Elizabeth Oliver[1], Paulina Sikora[1], Ian Vipond[4], Peter Muir[4], Adam Finn[1,3]

1: School of Cellular and Molecular Medicine, University of Bristol, Education Centre, Upper Maudlin Street, Bristol BS2 8AE, UK.

2: Children's Hospital, Landspitali University Hospital Iceland. Hringbraut, 101 Reykjavik, Iceland.

3: School of Population Health Sciences, University of Bristol, Canynge Hall, 39 Whatley Road, Bristol BS8 2PS, UK.

4: Public Health Laboratory Bristol, Public Health England, Southmead Hospital, Bristol BS10 5NB, UK.

Running head:

Respiratory viruses and increased bacterial density

Correspondence to:
Prof. Adam Finn
Education Centre, Upper Maudlin Street, Bristol BS2 8AE, UK.
adam.finn@bristol.ac.uk

**Abstract**

**Background:** This longitudinal study describes the associations between respiratory viral infections, rhinitis and the prevalence and density of the common nasopharyngeal bacterial colonisers, *S. pneumoniae*, *M. catarrhalis*, *H. influenzae* and *S. aureus*.

**Methods:** In an observational cohort study, 161 children attending day-care-centres in Bristol, UK were recruited. Monthly nasopharyngeal swabs were taken and stored frozen in STGG broth. qPCR was used for detection of respiratory viruses and four bacterial species. T-tests and logistic regression models were used for analysis.

**Results:** The frequent colonisers, *S. pneumoniae*(Sp), *M. catarrhalis*(Mc) and *H. influenzae*(Hi) were more frequently found at high density in contrast to *S. aureus* although temporally, high density carriage was short lived. Respiratory viral infections and symptoms of rhinitis were both independently and consistently associated with higher bacterial density with an observed twofold increase in density for Sp, Mc and Hi (p= 0.004 - 0.017).

**Conclusion:** For Sp and Hi, the association between young age and higher bacterial DNA density was explained by more frequent viral infection and increased nasal discharge while the associations between some viral species and some bacterial species' density appear to be stronger than others. Increased colonisation density and rhinitis may promote transmission of these commonly carried organisms.

**Introduction**

The prevalence of the most commonly cultured nasopharyngeal (NP) microorganisms, *Streptococcus pneumoniae* (Sp), *Moraxella catarrhalis* (Mc), *Haemophilus influenzae* (Hi) and *Staphylococcus aureus* (Sa) is influenced by several factors including age, day care attendance, social background, geographical location, smoke exposure, season and ethnicity[1-4]. Although these organisms may be associated with disease, usually they form an integral part of the commensal flora of healthy individuals. Many of these bacteria are most frequently found in young children, especially in situations where close contact is common, for instance in day care centres (DCC) where transmission between children occurs easily[5,6]. Several other factors are thought to influence bacterial colonisation directly, including viral infections, host mucosal immune responses and other residing commensal bacteria[1] although interactions between different species in the NP are complex and reports of positive and negative associations between species have been conflicting[7-12].

Viral upper respiratory tract infections (URTI) are common, especially in winter and in young children attending DCCs, leading to a wide range of clinical symptoms, but usually at least some degree of rhinitis[13,14]. We have published observational data on this and found age-independent associations between rhinitis symptoms and the presence of viral nucleic acid (NA), Hi prevalence and density as well as increased Sp density in the presence of viral URTIs[10,15].We and others have in addition contributed to the mounting evidence that viral infections are important in the dynamics of bacterial colonisation[16-20] and animal models have suggested the role of viral URTI, influenza in particular, in the acquisition of new colonising bacteria[21-23].

To explore the associations between viral URTI and the prevalence and density of childhood bacterial NP colonisation over time, we performed a single centre, prospective observational

cohort study, recruiting healthy children attending DCCs in the city of Bristol, UK. The children had nasopharyngeal swabs taken each calendar month at their respective DCCs from November 2011 through March 2012. Based on our previous work, it is plausible that bacteria proliferate in response to viral rhinitis and thus exploit the opportunity for successful transmission[10,15]. The bacteria may perhaps extend or amplify the rhinitis symptoms and increase the chances further. We have published observational data on this and found age-independent associations between rhinitis symptoms and the presence of viral nucleic acid (NA), Hi prevalence and density as well as increased Sp density in the presence of viral URTIs[10]. We have also shown in a randomised interventional study that administration of attenuated influenza viruses as nasal flu vaccine increases carriage density of pneumococci, Hi, Mc and possibly *S. aureus*[15]. Here we report associations between naturally acquired respiratory viral infections in children and bacterial colonisation rates and densities. The aim was to define baseline carriage rates and density distributions of commensal bacteria and the prevalence of the most common viral respiratory infections and to explore associations between these variables. Secondary objectives were to describe the biology of carriage in terms of duration and density.

**Methods**

Children aged up to 5 years old were recruited in DCCs in Bristol, UK for monthly NP sampling in the winter period 2011-2012. All children were attending the DCCs at the time of sampling and were considered healthy. Nine DCCs agreed to participate in the study. After receiving approval from each DCC to conduct the study and to approach parents, information sheets were distributed and signed informed consent for all study procedures obtained. Most children (134/161) were enrolled in November 2011 and each of them had five scheduled visits, one per

calendar month. The remaining 27 children, who were recruited in December 2011 were only offered four visits at their respective DCCs.

The study was approved by the National Research Ethics Service – Central Bristol Committee (Reference number 11/SW/0272) and sponsored by the University of Bristol.

Demographic information and medical history were collected at recruitment. All information was entered into an encrypted and password protected database. At each visit an NP swab was obtained as previously described[10] and a nasal discharge (SNOT) score was recorded (on the scale of 0-3)[10].

Microbiological detection methods:

We used qPCR assays for the detection of Sp, Mc, Hi and Sa and used standard curves for conversion of cycle threshold values to gene copies (GC)/ml as previously described[19]. Lower limits of detection were set at 35 cycles. Nucleic acid (NA) from respiratory viruses were detected using a qPCR panel as previously published[15] with lower limits of detection also set at 35 cycles.

Statistical analysis

Statistical analysis was done using Stata® version 12.1. Univariate chi-squared and t-test analyses comparing bacterial colonisation prevalence and density in children with and without detectable respiratory viruses were done and, where necessary, logarithmic transformation of bacterial density was done to attain normal distribution. Pearson's coefficient and linear regression was used to evaluate linear correlation between age and bacterial density. For multivariable analysis, Generalised Estimating Equations (GEE)[24,25] were used as an analytic

method, taking account of repeated sampling from the same individual and correcting for age, viral infections, nasal discharge and within-subject correlations, expressed as correlation coefficients. Using this approach, the effect of each variable was assessed after correcting for all the others. In the multivariable analysis of the effects of individual viruses on bacterial density each virus was analysed, irrespective of the finding of more than one virus in the same sample.

**Results**

161 children were recruited from the nine participating DCCs (8 – 42 participants from each). The mean age was 32.0 months (SD: 11.5) and 81 (50.3%) were male (Table 1). 137 (85.1%) children had three or more samples taken. Although the majority of children had no significant medical history, 18 reported some underlying condition of whom seven (4.3%) had recurrent wheeze and six (3.7%) recurrent otitis media.

Bacterial and viral prevalence

For the whole sample set, median detection rates (supplementary table 1) were 80.4% (point prevalence range: 74.6-82.7%), 85.2% (82.5-91.3%) and 85.3% (79.1-88.1%) for Sp, Mc and Hi respectively, with few apparent changes throughout the study period. Sa had lower median detection rates (7.0%) with relatively more variation over time (point prevalence range: 4.3-9.5%), with higher rates in older children (figure 1).

Detection of Sp, Mc and Hi was near-universal in this study and only 2, 2 and 3 children, respectively, were consistently negative for each of these species while more than half remained consistently PCR-positive throughout for each of the three. In contrast, the duration of detectable Sa carriage was short, with only five children (of whom three were from the same

DCC) carrying the organism in two consecutive samples. In all other instances, the children either never had Sa detected (126 children, 78.2%), or only had it detected at a single visit (30 children, 18.6%).

Viral NA was found in 284 of 627 samples and rhinovirus was the most frequently identified virus (21% of the samples), most commonly in November and March (in 28.7% and 26.1% of samples, respectively), followed by coronaviruses in 15% of samples with a large peak in December when it was detected in 33.1% of all samples. Among the viruses tested for, others were found in less than 5% of all samples while influenza B was the only virus not detected (data not shown).

In a multivariable regression analysis looking at age, viral NA and SNOT score, Sp NA and Mc NA were more likely to be detected with viral NA, while presence of Hi was associated with rhinitis symptoms (table 2).

Density of colonising bacteria

Overall median (and range) bacterial NA densities in gene copies/ml for the four bacterial species studied over the whole period were 815 (4 - $4.0x10^5$), 2429 (4 - $1.8x10^6$), 2899 (4 - $3.4x10^6$) and 135 (38 - $1.5x10^5$) for Sp, Mc Hi and Sa respectively. Bacterial NA density declined with age for Sp, Mc and Hi while for Sa it increased slightly (figure 1).

The bacterial NA density in individual children of the 3 bacterial species carried at high frequency tended to be very unstable over time. Among all children who had the same bacterial species detected in two or more of their samples, the mean range density was 2.9, 3.3, 3.3 and 2.7 Log10 gene copies/ml for Sp, Mc Hi and Sa respectively. This is further illustrated in figure 2 for 20 children, in each case, who had the highest recorded density samples (generally $>10^5$ gene

copies/ml). In general, samples collected before and after these individual peaks had density values at least one and often several logs lower. In contrast, Sa was rarely detected at high density and few children had this bacterial species detected in consecutive samples.

Regression models for bacterial density

No evidence was found of independent association between colonisation densities of Sp, Mc and Hi with age while they were independently associated with the presence of respiratory viral NA and nasal discharge (table 3) while for Sa there was no evidence of association with age or viral infection and the association with rhinitis score was negative. Associations between the presence of individual viral species and densities of the four bacterial species are shown in table 4. For Sp, Mc and Hi, apparent positive associations were consistently seen although in many cases the strength of the evidence that these were not chance events was weak, possibly due to the relatively low numbers of observations for many of these combinations.

**Discussion**

In this study we report the novel finding of transient peaks in high density bacterial NA in young children along with further evidence of association between high density bacterial NA and respiratory viral infection as previously reported[8,26-28]. Although neither causality nor its direction can be directly inferred from these observations, we have previously described modest increases in colonising bacterial density following administration of live attenuated nasal influenza vaccine[15], and the findings presented here could potentially reflect similar mechanisms

of bacterial behaviour whereby transmission between pre-school aged children is achieved by sensing and responding to a "window of opportunity" provided by the rhinitis symptoms caused by viral infection. However, whether the rises in bacterial NA density we observe are due to previously colonising bacteria, the acquisition of new strains or a mixture of both, remains unclear. Our data suggest that several colonising bacterial species may also contribute to the severity or duration of rhinitis (table 3). High-density carriage episodes seem to be short lived in most cases (Figure 2), which would be compatible with an unstable transmission dynamic in the day care setting with individual children being transiently highly infectious to others for particular bacterial and viral species. This state of constant flux may be further complicated by enhanced susceptibility to acquisition of bacteria in children with intercurrent viral infection[20] and as previously reported in animal models[21].

The introduction of universal pneumococcal conjugate vaccine in children and appreciation of the effects on carriage ecology of Sp has led to collection of significant information about colonisation by this bacterial species[29]. Information about the equally abundant Hi and Mc is scarcer and the colonisation rates we report here for all 3 species in a cohort of young healthy British children attending day care centres are higher than in most previous reports[30-33]. Using detection by qPCR, as described previously in this cohort as well as in other age groups and bacterial species[19,34], permits sensitive, accurate quantification of bacterial genomes in mucosal samples and, although the technique will enumerate both viable and non-viable organisms and therefore may somewhat overestimate carriage rates as detected by culture, this information is relevant to the overall understanding of the microbial biology and life-cycles under study.

Our observations concerning Sa in this study contrast with those for the other bacterial species, suggesting a distinct life style as reported by others[28]. The lower rates of detection may reflect

a distinct anatomical distribution in the human upper respiratory tract[35 36] but alongside the carriage rates[4,10], Sa colonisation density increases with age in pre-school children while density of the other species fall (figure 1). Although the smaller numbers of carriers observed in the study reduced statistical power, in contrast to the other bacterial species, no discernible independent associations between presence of Sa and either detection of viral NA or severity of rhinitis symptoms were evident in this study (table 2) and density was, if anything, inversely correlated with rhinitis and influenza infection (tables 3 and 4). This contrasts with our previously reported data suggestive that Sa density is higher following administration of nasal influenza vaccine[15].

By using a robust statistical approach which corrects for multiple potential confounding factors including repeated sampling from the same individuals, we show that, for both Sp and Hi, the evident downward trends in bacterial detection rates and density with age can be explained largely or entirely by the reduced presence of viral infections and rhinitis. For Mc this is less clearly the case and, with the possible exception of parainfluenza virus, bacterial density associations with viral infections appear to be somewhat weaker (table 4) while for Sa the situation is clearly distinct as discussed above. Thus, our results suggest that gains in immunological experience and/or maturation as children age may be mediating their effects on bacterial colonisation more through reducing viral infections rather than directly by increased mucosal immunity to the bacteria themselves.

Influenza detection rates in this study were in keeping with Public Health England surveillance data on respiratory pathogens in 2011-2012 when flu A was detected predominantly with the peak activity in February and March 2012[37]. A big national rise in the detection of coronaviruses was noted in December[37] as also observed in our cohort affording the opportunity to make the

novel observation that coronavirus infection is associated with increases in colonisation density of Sp and Hi while evidence that several other respiratory viruses are associated with high bacterial colonisation density is presented (table 4). Further, larger studies could confirm or refute and measure the strength of these relationships.

While the dramatic shifts around peaks in bacterial NA density over time observed in this study (figure 2) are likely to be real, since such large and consistent changes are unlikely to occur due to errors and uncontrolled variations in sampling and laboratory methodology, it is much more difficult to draw conclusions about what is going on at the other end of the scale. In this study, about half of the children carrying Sp carried the organism in every sample and few children (21%) had three or more changes in their colonisation status (from positive to negative or vice versa, data not shown). Whether the latter group really represent stable low-density carriage that crosses the lower limit of detection for the qPCR assay or frequent acquisition followed by rapid elimination is unclear. Also, the duration of viral NA carriage is uncertain. We did on occasions observe two consecutive positive samples for one particular virus but due to the small cohort and different viruses, no meaningful analysis could be done regarding these findings. While the design of the study did not permit assessment of transmission of individual microorganisms between children alongside clinical illnesses, we are currently undertaking a follow-on study of bacterial transmission within family units. More detailed genetic analysis of carriage and shorter intervals between samples could help define this in more detail although sensitivity to detect bacterial material or to culture viable strains remains a challenge.

The evident association between respiratory viral infections and rates and density of nasopharyngeal bacterial colonisation raises the possibility that control and prevention of the former might have profound effects upon the prevalence, transmission and ultimately disease

caused by the latter. If onward bacterial transmission occurs more frequently when they are present at high density, then it may be more important and useful to evaluate impact of interventions, including vaccines, upon the size and duration of high density spikes than upon overall prevalence of carriage, much of which, especially when at very low density, may be associated with much lower risk of transmission.

Other determinants of bacterial and host phenotype presumably also affect the capacity to transmit successfully. The interplay between respiratory viral infections, upper respiratory tract-colonising bacteria and host innate and specific anti-viral and anti-bacterial mucosal immune responses therefore needs to be elucidated in more detail. This would help the understanding of this ecosystem, its occasional punctuation by episodes of clinically significant infectious illness and the ways it is affected by both bacterial and viral vaccines. It is important to find ways to answer such questions so that future studies of carriage, conducted to evaluate the indirect effects of vaccines, can be designed and interpreted correctly.

**References**


1. García-Rodríguez JA, Fresnadillo Martínez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother* 2002; **50 Suppl S2**: 59-73.
2. Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; **10**(12): 853-61.
3. Shaikh N, Leonard E, Martin JM. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. *Pediatrics* 2010; **126**(3): e557-64.
4. Bogaert D, van Belkum A, Sluijter M, et al. Colonisation by Streptococcus pneumoniae and Staphylococcus aureus in healthy children. *Lancet* 2004; **363**(9424): 1871-2.
5. Duffy LC, Faden H, Wasielewski R, Wolf J, Krystofik D. Exclusive breastfeeding protects against bacterial colonization and day care exposure to otitis media. *Pediatrics* 1997; **100**(4): E7.
6. Revai K, Mamidi D, Chonmaitree T. Association of nasopharyngeal bacterial colonization during upper respiratory tract infection and the development of acute otitis media. *Clin Infect Dis* 2008; **46**(4): e34-7.
7. van Gils EJ, Hak E, Veenhoven RH, et al. Effect of seven-valent pneumococcal conjugate vaccine on Staphylococcus aureus colonisation in a randomised controlled trial. *PLoS One* 2011; **6**(6): e20229.
8. Chien YW, Vidal JE, Grijalva CG, et al. Density interactions among Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus in the nasopharynx of young Peruvian children. *Pediatr Infect Dis J* 2013; **32**(1): 72-7.
9. van den Bergh MR, Biesbroek G, Rossen JW, et al. Associations between pathogens in the upper respiratory tract of young children: interplay between viruses and bacteria. *PLoS One* 2012; **7**(10): e47711.
10. Rodrigues F, Foster D, Nicoli E, et al. Relationships between rhinitis symptoms, respiratory viral infections and nasopharyngeal colonization with Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus in children attending daycare. *Pediatr Infect Dis J* 2013; **32**(3): 227-32.
11. Ruohola A, Pettigrew MM, Lindholm L, et al. Bacterial and viral interactions within the nasopharynx contribute to the risk of acute otitis media. *J Infect* 2013; **66**(3): 247-54.
12. Pettigrew MM, Gent JF, Pyles RB, Miller AL, Nokso-Koivisto J, Chonmaitree T. Viral-bacterial interactions and risk of acute otitis media complicating upper respiratory tract infection. *J Clin Microbiol* 2011; **49**(11): 3750-5.
13. Rhedin S, Lindstrand A, Rotzén-Östlund M, et al. Clinical utility of PCR for common viruses in acute respiratory illness. *Pediatrics* 2014; **133**(3): e538-45.
14. Jansen RR, Wieringa J, Koekkoek SM, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. *J Clin Microbiol* 2011; **49**(7): 2631-6.
15. Thors V, Christensen H, Morales-Aza B, Vipond I, Muir P, Finn A. The Effects of Live Attenuated Influenza Vaccine on Nasopharyngeal Bacteria in Healthy 2 to 4 Year Olds. A Randomized Controlled Trial. *Am J Respir Crit Care Med* 2016; **193**(12): 1401-9.
16. Syrjänen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK. Nasopharyngeal carriage of Streptococcus pneumoniae in Finnish children younger than 2 years old. *J Infect Dis* 2001; **184**(4): 451-9.

17. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis* 2008; **46**(6): 815-23.
18. Grijalva CG, Griffin MR, Edwards KM, et al. The role of influenza and parainfluenza infections in nasopharyngeal pneumococcal acquisition among young children. *Clin Infect Dis* 2014; **58**(10): 1369-76.
19. Thors V, Morales-Aza B, Pidwill G, Vipond I, Muir P, Finn A. Population density profiles of nasopharyngeal carriage of 5 bacterial species in pre-school children measured using quantitative PCR offer potential insights into the dynamics of transmission. *Hum Vaccin Immunother* 2016; **12**(2): 375-82.
20. Karppinen S, Terasjarvi J, Auranen K, et al. Acquisition and Transmission of Streptococcus pneumoniae Are Facilitated during Rhinovirus Infection in Families with Children. *Am J Respir Crit Care Med* 2017; **196**(9): 1172-80.
21. McCullers JA, McAuley JL, Browall S, Iverson AR, Boyd KL, Henriques Normark B. Influenza enhances susceptibility to natural acquisition of and disease due to Streptococcus pneumoniae in ferrets. *J Infect Dis* 2010; **202**(8): 1287-95.
22. Nakamura S, Davis KM, Weiser JN. Synergistic stimulation of type I interferons during influenza virus coinfection promotes Streptococcus pneumoniae colonization in mice. *J Clin Invest* 2011; **121**(9): 3657-65.
23. Iverson AR, Boyd KL, McAuley JL, Plano LR, Hart ME, McCullers JA. Influenza virus primes mice for pneumonia from Staphylococcus aureus. *J Infect Dis* 2011; **203**(6): 880-8.
24. Hanley JA, Negassa A, Edwardes MD, Forrester JE. Statistical analysis of correlated data using generalized estimating equations: an orientation. *Am J Epidemiol* 2003; **157**(4): 364-75.
25. Burton P, Gurrin L, Sly P. Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling. *Stat Med* 1998; **17**(11): 1261-91.
26. Binks MJ, Cheng AC, Smith-Vaughan H, et al. Viral-bacterial co-infection in Australian Indigenous children with acute otitis media. *BMC Infect Dis* 2011; **11**: 161.
27. Vu HT, Yoshida LM, Suzuki M, et al. Association between nasopharyngeal load of Streptococcus pneumoniae, viral coinfection, and radiologically confirmed pneumonia in Vietnamese children. *Pediatr Infect Dis J* 2011; **30**(1): 11-8.
28. Chochua S, D'Acremont V, Hanke C, et al. Increased Nasopharyngeal Density and Concurrent Carriage of Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis Are Associated with Pneumonia in Febrile Children. *PLoS One* 2016; **11**(12): e0167725.
29. Gladstone RA, Jefferies JM, Tocheva AS, et al. Five winters of pneumococcal serotype replacement in UK carriage following PCV introduction. *Vaccine* 2015; **33**(17): 2015-21.
30. Jourdain S, Smeesters PR, Denis O, et al. Differences in nasopharyngeal bacterial carriage in preschool children from different socio-economic origins. *Clin Microbiol Infect* 2011; **17**(6): 907-14.
31. Shiri T, Nunes MC, Adrian PV, Van Niekerk N, Klugman KP, Madhi SA. Interrelationship of Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus colonization within and between pneumococcal-vaccine naïve mother-child dyads. *BMC Infect Dis* 2013; **13**: 483.
32. van den Bergh MR, Spijkerman J, Swinnen KM, et al. Effects of the 10-valent pneumococcal nontypeable Haemophilus influenzae protein D-conjugate vaccine on

nasopharyngeal bacterial colonization in young children: a randomized controlled trial. *Clin Infect Dis* 2013; **56**(3): e30-9.
33. Kosikowska U, Korona-Głowniak I, Niedzielski A, Malm A. Nasopharyngeal and Adenoid Colonization by Haemophilus influenzae and Haemophilus parainfluenzae in Children Undergoing Adenoidectomy and the Ability of Bacterial Isolates to Biofilm Production. *Medicine (Baltimore)* 2015; **94**(18): e799.
34. Finn A, Morales-Aza B, Sikora P, et al. Density Distribution of Pharyngeal Carriage of Meningococcus in Healthy Young Adults: New Approaches to Studying the Epidemiology of Colonization and Vaccine Indirect Effects. *Pediatr Infect Dis J* 2016.
35. Sollid JU, Furberg AS, Hanssen AM, Johannessen M. Staphylococcus aureus: determinants of human carriage. *Infect Genet Evol* 2014; **21**: 531-41.
36. Mertz D, Frei R, Jaussi B, et al. Throat swabs are necessary to reliably detect carriers of Staphylococcus aureus. *Clin Infect Dis* 2007; **45**(4): 475-7.
37. Surveillance of influenza and other respiratory pathogens in the UK 2011/12. Public Health England, 2012.