**Microbial epidemiology and carriage studies for the evaluation of vaccines**

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Abstract

Respiratory tract infections (RTI) are responsible for over 2.8 million deaths per year worldwide. Colonisation is the first step in the process of microbes occupying the respiratory tract, which may lead to subsequent infection. Carriage, in contrast, is defined as the occupation of microbial species in the respiratory tract. The duration of carriage may be affected by host immunity, the composition and interactions between members of the microbial community, and the characteristics of colonizing bacteria, including physiology associated with being present in a bacterial biofilm. Numerous vaccines have been implemented to control infections caused by bacteria that can colonise and be subsequently carried. Such vaccines are often species-specific and may target a limited number of strains thereby creating a vacant niche in the upper respiratory tract. Epidemiological changes of bacteria found in both carriage and disease have therefore been widely reported, since the vacant niche is filled by other strains or species. In this review, we discuss the use of carriage prevalence studies in vaccine evaluation and argue that such studies are essential for 1. examining the epidemiology of carriage before and after the introduction of new vaccines, 2. understanding the dynamics of the respiratory tract flora and 3. identifying the disease potential of emerging strains. In an era of increasing antibiotic resistance, bacterial carriage prevalence studies are essential for monitoring the impact of vaccination programmes.

**Key words**

Carriage; Colonisation; Epidemiology; Vaccine; Respiratory

**The Burden of Disease, Colonisation and Carriage**

Respiratory tract infections (RTI) are the most common cause of death in children under the age of one year (1). In 2010, infections of the lower respiratory tract accounted for a total of 2.8 million deaths worldwide in all age groups, with an age-standardised death rate of 41 per 100,000 deaths, and 847,000 deaths worldwide in children under the age of five years (1).

Colonisation is the first step in the process of microbes occupying the respiratory tract. Although colonisation can quickly lead to infection, most infections occur after prolonged carriage, a non-transient state defined as the occupation of microbial species, in this case of the respiratory tract. Carriage is a dynamic process with different species or types being carried for short, prolonged or sequential periods (2). The duration of carriage may be affected by host immunity, environmental factors, the microbiome, their biofilm-associated biology and behaviours within the respiratory airway, and both inter and intra-species interactions (3).

The respiratory tract is host to a multitude of potentially pathogenic organisms, including those that can go on to cause severe respiratory disease(4) (Figure 1). For example, *Streptococcus pneumoniae* is capable of causing serious pneumococcal pneumonia and meningitis but is also a common cause of otitis media, and can be carried asymptomatically (5, 6). *Haemophilus influenzae* is a common cause of otitis media and is associated with exacerbations of respiratory disease, but is also an important cause of invasive infections, and can be carried asymptomatically by healthy individuals (7-9). Other important carried pathogens include *Moraxella catarrhalis* (10), *Neisseria meningitidis* (11) and *Staphylococcus aureus* (12). Duration of carriage is thought to affect the progression towards disease, with the majority of infections taking place shortly after first carriage of a new strain (13).

Potential outcomes of respiratory tract infections are those of sepsis and meningitis when pathogens carried in the upper airways spread to the blood and central nervous system respectively (14, 15). Mortality is considerable with sepsis fatalities in 24.1% of hospital-admitted cases in Europe (16) and meningitis fatality rates in the USA are 14.8% (17).

The Need for Carriage Prevalence Studies

Where colonisation is a prerequisite for disease, and the introduction of vaccines alter population level epidemiology of pathogens, carriage data can provide a number of useful measures. If done prior to or concurrently with vaccine introduction and in combination with studies of disease incidence, carriage studies would add valuable insights into herd immunity. For example, introduction of Pneumococcal Conjugate Vaccine (PCV) 10 in Iceland was shown to result in clear decreases in adult disease incidence due to herd immunity (18) in addition to the revelation that reduced carriage of vaccine-type serotypes was observed in children who had not received PCV10 (19). This later phenomenon was also observed in children >5 years of age and mothers in Malawian households during PCV13 vaccination programmes (20). In addition, carriage studies may give a useful measure of potential disease burden arising due to responses of the pathogen to a potentially significant evolutionary pressure. Finally, the disease risks associated with vaccine escape can be further clarified by understanding the population-level prevalence of those new strains or types that have consequentially become a disease burden. Although some researchers argue that studies measuring the incidence of invasive disease are the only way of truly assessing the effectiveness of vaccination programmes, the importance of undertaking carriage prevalence studies has been highlighted elsewhere (21-23). In this context, carriage prevalence studies involve sampling the upper respiratory tract flora using methods such as swabbing (24), nose blowing (6), nasopharyngeal aspiration (25), nasopharyngeal wash (26) and nasal wash (27). Sampling sites can include the throat (28), nose (6), mouth (29), nasopharynx (29) and oropharynx (25). Carriage is characterised by the presence of a microbial species using culture or culture-independent methods, and can be quantified by counting colony-forming units (CFU), by real-time quantitative polymerase chain reaction (PCR) (30) and by 16S rRNA analysis (31). Existing respiratory tract carriage prevalence studies focus mostly on individual organisms, such as *S. pneumoniae, H. influenzae* or *N. meningitidis* as these species have historically been amongst the common causes of invasive bacterial disease (32-34). Carriage rates of these organisms are highly variable according to age, time, geographical location, vaccine implementation and swabbing methodology (for examples see Table 1). It is therefore vital to undertake multi-centre, longitudinal carriage prevalence studies in different geographical regions in order to develop a clearer understanding of specific microorganisms carried within the human population and the impact of vaccines on carriage patterns. Additionally, as further vaccines are added to national immunisation programmes (NIPs), that target bacteria and viruses carried in the respiratory tract, well-designed carriage studies with the appropriate sample size will support disease incidence studies where case numbers are reduced following vaccine implementation. However, carriage prevalence studies provide the opportunity not only to determine the prevalence of bacteria and viruses targeted by vaccines, but also to determine the prevalence of those not covered by existing vaccines.

Vaccines and their Impact on Respiratory Disease and Carriage

A number of vaccines have been added to NIPs with the aim of reducing morbidity and mortality due to microorganisms that cause RTI and associated meningitis and sepsis. The first conjugate vaccine was developed against *H. influenzae* serotype b (Hib) and introduced in the 1990s, initially in developed countries followed by widespread introduction in developing countries. The conjugation of a protein to the polysaccharide antigen stimulates T-cell responses which are essential for long-term immunity (35) and it is for this reason that conjugate vaccines are thought to reduce carriage within the respiratory tract and boost host immunity by inducing IgA and IgG antibodies (36). In addition to the Hib conjugate vaccine (15) also of note are PCVs 10 (Synflorix™, GSK), 7 and 13 (Prevnar 7® and Prevnar 13®, Wyeth), which target 10, 7 and 13 serotypes of *S. pneumoniae* respectively (37), and the Meningococcal serogroup C (MenC) conjugate vaccine (multiple manufacturers) which protects against serogroup C meningococcal disease. In addition there is the development of conjugate and protein vaccines against *S. pneumoniae* (GSK - Phase II; Merck (PCV15) - Phase III; Pfizer (PCV20) – Phase III), non-typeable *H. influenzae* (GSK - Phase II)*, S. aureus* (Novartis - Phase I; Pfizer - Phase II) and *N. meningitidis* serogroup ABCWY (GSK - Phase III) (37-43). MenC vaccines were introduced in Europe in the late 1990s (38) and then PCVs in the early 2000s, firstly in the United States and then in other developed and developing countries (44, 45). The total number of countries having implemented PCVs now stands at 143 (46), although the formulation and timing varies country to country. For example, PCV7 was introduced into the Gambia, a region with a recognised high burden of pneumonia in 2009 with replacement to PCV13 in 2011 (47). From a developing world perspective, of the 73 countries eligible for Gavi and AMC-supported introduction, 59 have applied and been approved with 58 having implemented a PCV into the national programmes. Of these, 45 have introduced PCV13 (some having switched from PCV7) and the remainder PCV10 (48). Multi-component vaccinesagainst *N. meningitidis*, including serogroup B, have also recently been approved, including 4CMenB (Bexsero®, Novartis), containing antigens factor H binding protein (fHBP), *Neisseria* adhesin A (NadA), Neisserial heparin-binding antigen (NHBA) and outer membrane vesicles (OMV) (39). Furthermore, a meningococcal B vaccine (TRUMENBA®, Pfizer) containing two different variants of fHBP has also been approved (40). A group A meningococcal polysaccharide-tetanus toxoid conjugate vaccine (MenAfriVac) was introduced, in a phased program, into 26 countries of the African meningitis belt, specifically targeting children and young adults from ages 1-29 years (49). This program began in 2010 and is now being implemented as part of expanded immunisation programs targeting infants between 9 and 18 months old (50).

The *H. influenzae* type b conjugate vaccine was found to reduce all cases of invasive disease by 95% (51) and oropharyngeal carriage of *H. influenzae* type b by 6.6% (52) when compared to controls in a Gambian study involving 42,848 children aged 1-2 years old, between 1993 and 1995. PCV was also shown to reduce cases of invasive disease by 77% (53) and carriage of vaccine types by 17.4% in those aged 9-15 months old and 16.2% in those aged 21-27 months old (54) in a Gambian study of 17,437 children aged between 8 and 32 weeks old, between 2000 and 2003. In the UK, invasive disease cases caused by all serotypes of *S. pneumoniae* were reduced by 41% in children less than 5 years of age between 2005/2006 and 2007/2008 following PCV7 introduction (55), and carriage of PCV7 serotypes by 69% within children under 5 years of age within the UK between 2006 and 2009 (32). In South Africa, introduction of PCV13 reduced incidence of invasive disease from 107,600 cases to 41,800 in 2012-13 (56). Prior to the widespread introduction of *N. meningitidis* serogroup C vaccines in the UK, this serogroup represented a substantial burden in meningococcal disease (57). Following its introduction in 1999, meningococcal disease cases reduced by 97% in 15-17 year olds and 92% in 1-2 year olds (58) as well as carriage of serogroup C meningococcus by 66% (34) in the UK. MenAfriVac reduced the incidence of suspected meningitis cases by 57% (59). Significant country to country variation was observed however ranging from a 91% reduction in Chad to 35% in Niger (59). By excluding Benin and Ghana, where increases in meningitis were hypothesised to be a consequence of better surveillance, rather than menA disease specifically, the regional reduction in meningitis was 70% (59).

**The epidemiological evaluation of vaccination strategies**

Selection Pressures Caused by Vaccines

Given that the same bacterial species that can cause disease within the respiratory tract are also capable of being carried asymptomatically, conjugate vaccines targeted against these bacterial species reduce carriage as well as disease. A reduction in carriage within the population is now established as the main mechanism of the widespread effectiveness of conjugate immunisation, rather than individually conferred protection (60). Conjugate vaccines have the potential to impact both disease-related and carried strains. Further, they may also impact co-colonising species indirectly affected as a result of the vaccines’ disruption to the microbial community structure within the respiratory tract. Current conjugate vaccines target specific capsular polysaccharides of *S. pneumoniae, N. meningitidis* and *H. influenzae* (type b) essential for colonisation(61, 62). Intra-species variation in these polysaccharides limits vaccine efficacy as selection pressures imposed by vaccination induce mutation, recombination and antigenic variation as a means of survival. Such variation may select for new genotypes with enhanced immune evasion and virulence properties (63). On-going antigenic variation of colonising respiratory microbes has profound consequences in carriage and disease and requires continuous vaccine development to keep pace with microbial evolution (64, 65). Co-evolution of respiratory microbes and host immune responses, known as Red Queen dynamics, is thought to cause accelerated and divergent intra- and inter-species evolution, leading to evolutionary transformation and even speciation (66, 67). Furthermore biofilms may drive species diversification leading to the emergence of phenotypes which can be linked to specific and maintained mutations such as *rpoE* and small colony variants (SCV) as observed in *S. pneumoniae* (68). However, whether vaccines indirectly drive selection of particular biofilm phenotypes, through actions against particular serotypes of pneumococci for example, has received very little attention.

Bacterial Replacement

After the introduction of PCV7, countries with high uptake of the vaccine reported a shift in the serotypes of *S. pneumoniae* isolated from the respiratory tract which were targeted by the vaccine (vaccine types, VT) towards serotypes not targeted by the vaccine (non-vaccine types, NVT); a process known as serotype replacement. Such replacement was observed in Invasive Pneumococcal Disease (IPD) cases between 1998 and 2004 within eight USA states in individuals aged <5 and ≥65 years with increases in serotypes 3, 15, 19A, 22F and 33F (69). Additionally, similar replacement was observed in the UK within children eligible for pneumococcal vaccination between 2003-2009, with increases in serotypes 17F, 19A and 22F (55). Post-PCV13 saw increases in serotypes 8, 10A, 12F, 15A and 24F in the years 2013-14 compared to pre-PCV13 baseline in those over 5 years of age and 8, 15A, 15B/C, 22F and 24F in the under 5s (70). Colonising serotypes experienced similar replacement, with increases in serotypes 6C, 11A, 14, 19A and 22F in paediatric (≤4 years) outpatients from the UK between 2006 and 2009 (32). By 2013 6C, 11A and 22F were still highly prevalent along with 15A/B, 15C, 21 and 35F (71). Similar patterns were observed in the USA in children aged 3 months to <7 years with 6A, 11A, 15B/C, 19A and 35B colonisation increasing in prevalence post-PCV7 (33), in Canada with serotypes 6A, 15C and 11A in children up to 6 years of age (72), and elsewhere in Europe, South Africa and the Gambia, where in the latter 19A, 35B and 15B increased after the introduction of PCV7 (73). In a recent South African carriage study, PCV-related serotypes remained a significant proportion (22%) of the carried isolates, with 19F, 9V, 19A and 6A being commonly identified in children under the age of 5 years – despite high vaccine coverage and reduction in disease incidence (74). Non-PCV13 serotypes included 15B/15C, 21, 10A, 16F, 35B, 9N and 15A (74). A number of possibilities to explain phenomenon of VT persistence were suggested. These included transmission reservoirs in older, incompletely immunised siblings or adults with HIV, in addition to reduced capsule-specific metabolic load in serogroups 6 and 19 which may aid in avoiding immune-clearance through improved biofilm formation (75). Clearly, identification of pockets of carriage have important implications when determining why, perhaps, disease associated with vaccine types may also persist after successful vaccine implementation. Although most replacement serotypes have been observed to cause disease less commonly than vaccine types, some have a higher probability of leading to disease, including 19A (76). In the UK, IPD incidence rate ratios of serotypes 19A, 22F and 7F in children aged <5 years were 2.22, 3.25 and 4.31 respectively in 2008-2010 compared to 2000-2006 (77). In 2013-14 these had dropped to 0.09, 0.91 and 0.09 respectively (compared to 2008-10) with 24F and 15A the largest burden with IRRs of 10.15 and 6.49 (70). In a USA paediatric population of children aged 3 months to <7 years, molecular sequence types (STs) ST320 and ST695, predominantly associated with serotypes 19F and 4 respectively prior to PCV7, switched to serotype 19A in a vaccine escape capsular recombination event whilst maintaining multi-resistant and virulent properties (78, 79). Novel pneumococcal serotypes 6C and 6D have caused cases of IPD after the introduction of PCV7 in the USA and Europe (80, 81). Meta-analysis of non-PCV13 associated IPD in Europe, North and Latin America, Western pacific and Africa highlighted 22F as the most significant (in terms of incidence) followed by 12F, 15C, 24F and 33F. Interestingly, 24F was not prevalent in North America (82).

Shifts from *H. influenzae* type b to non-b and non-typeable *H. influenzae* (NTHi) have also been observed since the widespread use of the Hib vaccine. In the USA, the incidence of disease caused by *H. influenzae* type a increased by 1.8 cases per 100,000 between 1998 and 2008 in children less than 5 years old (7). Furthermore, increases in NTHi and type f were observed in children under 5 years old in Canada (83). In the UK, *H. influenzae* invasive disease incidence has risen by 7.4% overall and 11% each year in type e and f respectively from 2001-2010 (84). NTHi is now responsible for the majority of *H. influenzae* upper RTI with increases in NTHi-associated recurrent acute otitis media (AOM) being observed in children under 3 years of age in Australia between 2007-2009 (8). In the USA, NTHi-positive AOM samples were found to have increased by 19% in children from 1995 to 2003 (85). Between 2007 and 2014, NTHi accounted for 78 % of invasive disease in 12 EU/EEA countries, predominantly impacting infants and those ≥60 years of age (86).

*N. meningitidis* capsular replacement from serogroup C to B following the meningitis C vaccine introduction has been observed in ST11 isolates collected from two outbreaks in Spain between 1997-2002 (87). Similar replacement was observed in ST11 in the Czech Republic between 1993-1997 (88) and Canada (89). Increases in serogroup Y have also been reported in the UK between 2007 and 2009 (90) as well as in Sweden (91) and the USA (92).

Vaccine Efficacy

The direct relationship between carriage of a pathogenic organism in a target population and incidence of disease enables carriage to be used as a proxy for vaccine-induced protection and can enable the prediction of risk factors for respiratory disease, meningitis and sepsis (93). In terms of carriage, vaccine efficacy (VEacq, acq=acquisition) is the extent of decline in carriage rates of a particular species within a vaccinated population relative to unvaccinated controls (94). The use of such studies in evaluating vaccine efficacy was highlighted by the Joint Committee on Vaccination and Immunisation (JCVI), an expert advisory board for the UK Department of Health, which emphasised a lack of carriage evidence for effectiveness of 4CMenB in preventing MenB disease in the UK (95). Prior to vaccine introduction, levels of carriage are assessed and act as a baseline measure from which any changes resulting from immunisation and herd immunity can be assessed (21). Numerous studies in different countries have undertaken carriage prevalence studies to assess changes in the populations of *H. influenzae*, *N. meningitidi*s and *S. pneumoniae* before and after the implementation of Hib, MenC and pneumococcal conjugate vaccines (32, 34, 69, 96-98)

The ability of respiratory tract microbes to evolve in response to immunisation is a worrying problem, particularly bacterial replacement (99). Although the positive outcomes of vaccinating against VTs are evident, determination of the risks of increased NVT prevalence remains unknown (100). Evolution of bacterial strains is thought to result in changes within the respiratory tract ecological niche (101). Increased incidence of more virulent, invasive or antibiotic-resistant NVT, as more common VT are eliminated from this ecological niche, must not be disregarded (102) and is an occurrence already noted in IPD surveillance in England and Wales in the post-PCV13 period (103). The difficulty of predicting replacement and disease potential of particular serotypes means that carriage prevalence studies are essential for monitoring changes within the population as they can provide real time data which may take time to be detected in disease due to host and environmental factors (104). Moreover, global travel now means that novel strains might emerge in one country but manifest in disease in another due to differences in vaccine policy, antibiotic use, or pre-existing herd immunity in the population.

**What further information can be derived from carriage prevalence studies?**

Differences between Disease and Carriage

Carriage prevalence studies can be used in conjunction with studies measuring disease incidence to ascertain the effect of immunisation on microbes carried within the general population. Phenotypic and genetic differences can be determined between species types causing disease and those that are carried, enabling key determinants of disease to be discerned. For example, in Europe, *N. meningitidis* sequence type ST11 was more commonly present in disease whereas ST23 was more frequently found in carriage (105). This is potentially due to the greater virulence and high fatality rates in cases of meningococcal disease caused by ST11 isolates (106). In 2006, *S. pneumoniae* serotypes frequently associated with IPD, including meningitis and sepsis, were 1, 4, 5, 7F, 14 whereas serotypes commonly associated with colonisation were 6A, 6B, 8, 9V, 15, 18C, 19F, 23F, 33, 38 (107). Clinical trials of vaccine introduction looking at carriage with or without disease incidence are rare. A study of MenACWY-CRM and 4CMenB in the UK found reduced carriage of meningococci 12 months after vaccination, which at the time illustrated the potential benefits of herd protection and the opportunity to use this data to guide implementation decisions (108). More recently, implementation of PCV-10 in Kenya was examined using both the reduced incidence of vaccine targeted serotypes in IPD and carriage (109). Here, continued observations were made of vaccine serotype carriage in children and infants, at a higher level than found elsewhere following PCV use (109). Regardless of the driver of this phenomenon the continuing risk to unvaccinated, under-vaccinated children or adults was noted (109). Likewise, following PCV-10 introduction in Fiji, carriage prevalence suggested both a direct and indirect positive impacts on disease incidence, particularly in those too young to be vaccinated (110). As with other similar studies there was a call to continue monitoring owing to the switch to carriage of non-vaccine type serotypes (110).

Geographical and Temporal Effects in Disease

Knowledge of geographical and temporal distributions of respiratory microbes is important for understanding the dissemination and transmission of circulating strains. Annual carriage prevalence studies can enable the temporal distribution of circulating bacterial species to be tracked (111). Geographical distribution is also essential in determining spatial epidemiology of circulating organisms and how this affects disease. For example, the geographical distribution of carried meningococcal serogroups is heterogenous with serogroup A predominating in central Africa and serogroup Y in the USA (112, 113). In addition, frequency of IPD serotypes after implementation of PCV7 into the childhood immunisation schedule (post-PCV7) were found to be divergent in eight areas of the USA despite similar serotype replacement, possibly due to differing levels of chronic disease and antibiotic resistance (114). Understanding distribution across space and time can allow treatment and prevention strategies to be targeted correctly, improving their effectiveness. Determining patterns of meningococcal or pneumococcal serogroups or serotypes across the UK has enabled an understanding of the relevance of each group and the effect of intervening measures on them (34, 115).

Conclusions

Carriage prevalence studies are becoming increasingly valuable in the epidemiological evaluation of vaccines against respiratory disease, meningitis and sepsis. Vaccines against *S. pneumoniae, H. influenzae* and *N. meningitidis* have brought about evolutionary changes in the microbes carried in the respiratory tract by reducing vaccine type strains, inducing bacterial replacement and modifying diversity. Studies to monitor these changes are essential for the ongoing evaluation of the effectiveness of existing vaccines in disease and carriage, and for the development of new vaccines. Moreover, studies are required in both countries where vaccines have been introduced and those that have yet to do so. For the latter, carriage prevalence studies can act as a rapid and relatively inexpensive means for improving surveillance. This would be ensured through the development and implementation of guidelines for carriage studies, such as those that are available for sentential surveillance from the World Health Organisation (116). Although new vaccines are likely to be licensed in the next few years, and vaccines are currently being developed against important pathogens that are natural commensals of the upper respiratory tract, new vaccine development strategies may be also necessary to control disease caused by upper respiratory pathogens (39). Such strategies include multi-component vaccines (117, 118), vaccines containing whole inactivated cells (119), those containing capsular polysaccharides from all known serotypes or more conserved antigens (67, 120), or by adjusting the components of bacterial conjugate vaccines in a cyclical fashion, although a 4 to 5 year cycle may be required compared to the annual variation of seasonal influenza vaccines. Carriage prevalence studies may also be important for the study of other microbial infections, such as those within the gut or skin, as our understanding of the human microbiome increases. Longitudinal multi-centre studies focusing on the microbiome at various sites within human populations will therefore provide important infectious disease epidemiological data to inform vaccine development and global immunisation strategies.

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Ethics

None required.

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# TABLES

**Table 1.** **Carriage (%) of respiratory tract organisms in children**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Country | Swabbing Year | Age | Swab Type | Carriage (%) | Ref. |
| *S. pneumoniae* | |  |  |  |  |
| Australia | 2002 & 2004 | 2-15 years | Nasopharyngeal | 67 | (121) |
| Australia | N/S | 3-7 years | Nasal  Nose blowing | 93  87 | (6) |
| <4 years | Nasal  Nose blowing | 43  21 |
| Finland | 1993-1994 | <7 years | Nasal  Nasopharyngeal Nasopharyngeal aspirate  Oropharyngeal | 32  30  33  20 | (25) |
| Gambia | N/S | <12 months | Nasopharyngeal | 78 | (122) |
| Israel | 1994 | 12-18 months | Nasopharyngeal | 44 | (123) |
| Israel | 2001-2002 | 0-23 months | Nasopharyngeal Oropharyngeal | 77  2 | (124) |
| 24-59 months | Nasopharyngeal Oropharyngeal | 66  2 |
| Italy | 1996 (Autumn)  1996 (Spring) | 1-7 years | Nasopharyngeal | 3.8  4.7 | (125) |
| Philippines | 1992-1993 | <1 years | Nasal  Oropharyngeal | 47  16.6 | (126) |
| UK | N/S | 0-18 months | Nasopharyngeal | 17.6 | (127) |
| UK | 2006/2007  2007/2008  2008/2009 | 0-4 years | Nasopharyngeal | 32.1  27.9  31.1 | (32) |
| USA | 2001  2004  2007 | 3m-7 years | Nasopharyngeal | 27  23  30 | (33) |
| Taiwan | 2008-2009 | <5 years | Nasopharyngeal | 22.8 | (128) |
| *H. influenzae* | |  |  |  |  |
| Australia  [NTHI only] | 2002 & 2004 | 2-15 years | Nasopharyngeal | 57 | (121) |
| Finland | 1993-1994 | <7 years | Nasal  Nasopharyngeal Nasopharyngeal aspirate  Oropharyngeal | 16  19  31  28 | (25) |
| Gambia | N/S | <12 months | Nasopharyngeal | 71 | (122) |
| Israel | 2001-2002 | 0-23 months  24-59 months | Nasopharyngeal Oropharyngeal  Nasopharyngeal Oropharyngeal | 38  23  29  24 | (124) |
| Italy | 1996 (Autumn)  1996 (Spring) | 1-7 years | Nasopharyngeal | 13.0  18.2 | (125) |
| Nepal  [Hib only] | 2007 | <14 years | Oropharyngeal | 5.0 | (129) |
| Philippines | 1992-1993 | <1 years | Nasal  Oropharyngeal | 36.7  38 | (126) |
| UK | N/S | 0-18 months | Nasopharyngeal | 10.4 | (127) |
| UK  [Hib only] | 1992  1994  1997  2002 | 1-4 years | Throat Swab | 3.98  0.70  0.0  0.0 | (130) |
| Taiwan | 2008-2009 | <5 years | Nasopharyngeal | 5.4 | (128) |
| *M. catarrhalis* | |  |  |  |  |
| Australia | 2002 & 2004 | 2-15 years | Nasopharyngeal | 74 | (121) |
| Belgium | 1988-1990 | <10 years | Throat, Nasal & Nasopharyngeal | 50.8 | (28) |
| Gambia | N/S | <12 months | Nasopharyngeal | 20 | (122) |
| Italy | 1996 (Autumn)  1996 (Spring) | 1-7 years | Nasopharyngeal | 4.5  5.2 | (125) |
| UK | N/S | 0-18 months | Nasopharyngeal | 39.5 | (127) |
| Taiwan | 2008-2009 | <5 years | Nasopharyngeal | 8.5 | (128) |
| *S. aureus* | |  |  |  |  |
| Gambia | N/S | <12 months | Nasopharyngeal | 70 | (122) |
| Greece | 2005-2006 | <15 years | Nasal | 59 | (131) |
| Greece  [MRSA] | 2005-2006 | <15 years | Nasal | 3.3 | (131) |
| Israel | N/S | 1-24 months | Oropharyngeal | 10 | (132) |
| UK | N/S | 0-18 months | Nasopharyngeal | 40.6 | (127) |
| *N. meningitidis* | |  |  |  |  |
| UK  [MenC only] | 1999  2000 | 15-17 years | Oropharyngeal | 0.45  0.15 | (34) |

N/S = not specified; MRSA = methicillin-resistant *S. aureus;* NTHi = Nontypeable *H. influenzae*; Hib = *H. influenzae* type b; MenC = *N. meningitidis* Serogroup C. Note: this list is non-exhaustive and serves to provide examples that demonstrate the variability of bacterial carriage.

# FIGURES

**Figure 1.** **Sites of pathogenic bacterial colonisation in the upper respiratory tract.** Shown are the primary sites of colonisation from the common pathogens of the respiratory tract.Anatomical illustration: © https://www.123rf.com/profile\_terriana.