Non-typeable *Haemophilus influenzae*, **an under-recognised pathogen**.

A review of the literature.

Johan Van Eldere,* Department of Microbiology and Immunology, Catholic University Leuven, Belgium and Clinical Department of Laboratory Medicine , University Hospital Leuven, Belgium.

Mary P E Slack, WHO Collaborating Centre for *Haemophilus influenzae*, Respiratory and Vaccine Preventable Bacteria Reference Unit, Microbiology Services, Public Health England, Colindale, London, UK.

Shamez Ladhani, Immunisation, Hepatitis and Blood Safety Department, Health Protection Services, Public Health England, Colindale, London, UK

Allan W Cripps, School of Medicine, Griffith Health Institute, Griffith University, Gold Coast, Queensland, Australia.

* Corresponding author

Short title: NTHi: the under-recognised pathogen

Keywords: Non-typeable *Haemophilus influenzae*, mucosal infections, pneumonia, invasive infections, antibiotic resistance, surveillance, typing, pathogen

SUMMARY

Non-typeable *Haemophilus influenzae* (NTHi) is a major cause of mucosal infections such as otitis media, sinusitis, conjunctivitis and exacerbations of chronic obstructive pulmonary disease. In some geographical regions, there is a strong causal relationship between NTHi and infections of the lower respiratory tract. Over the last 20 years, there has been a steady but constant increase in invasive NTHi disease globally with perinatal infants, young children and the elderly being most at risk. Individuals with underlying co-morbidities are most susceptible and infection is associated with significant mortality. Beta-lactamase production is the predominant mechanism of resistance. However, the emergence and spread of beta-lactamase negative ampicillin resistant strains across many regions of the world is of considerable concern, potentially necessitating alterations to current antibiotic treatment guidelines for community acquired upper and lower respiratory tract infection (LRTI) and potentially increasing morbidity associated with invasive NTHi infections. Standardised surveillance protocols and typing methodologies to monitor NTHi as an emerging pathogen across the world should be implemented. International scientific organisations need to take a lead in raising the profile of NTHi in the clinical and research communities, to document the pathobiology of this microbe as it responds to continued antibiotic and vaccination pressure.

INTRODUCTION

When, over 20 years ago, the *Haemophilus influenzae* type b (Hib) conjugate vaccine was introduced, surveillance recorded the rapid reduction of invasive Hib infections and the near absence of replacement with non-type b encapsulated *H. influenzae*. [1] Following this, interest in *H. influenzae* declined , as demonstrated by the fall in the number of peer-reviewed papers on *H. influenzae* from 18·4 per 1000 bacteriological papers per year in 1991, to just 7 in 2009. [2,]

Non-encapsulated (or non-typeable) *H. influenzae* (NTHi) have never enjoyed the 'star pathogen' status of Hib or *Streptococcus pneumoniae*, despite the fact that NTHi are a common cause of respiratory tract infections both in children and adults. Indeed, to our knowledge, there are few scientific fora with sessions devoted to this microbe. HinMax, a group of clinicians and researchers interested in both *Moraxella and H. influenzae,* including NTHi, met in 2008 and in 2011 with 40 and 27 delegates respectively attending and 24 presentations on *H. influenzae*, including NTHi (Hays J. P. personal communication). At the European Congress of Clinical Microbiology and Infectious Diseases in Vienna in 2010, of around 4000 oral and poster presentations, only 11 dealt with NTHi. This apparent lack of interest may be because this pathogen is generally considered to only cause infection in predisposed patients and to be easily treated with beta-lactam antibiotics.

We believe that this lack of focus on NTHi is dangerous, as it facilitates the significant morbidity and increasing mortality associated with NTHi without encouraging the timely development of appropriate interventions. Here, we make the case for heightened surveillance of NTHi because: 1) data are emerging pointing to the increasing importance of NTHi as a pathogen in upper and lower respiratory tract infections,[11] 2) there is evidence of an increase in invasive infections caused by NTHi, and 3) epidemiological data from several countries show an increase in the spread of non-beta -lactamase dependent resistance to beta-lactams in NTHi. In an attempt to raise the profile of NTHi, a website has been recently established: http://nthi-watch.griffith.edu.au/ to provide a single repository of knowledge on the pathogenesis, incidence and treatment of NTHi disease. The European Monitoring Group on Meningococci (EMGM) recently expanded its scope to include *H. influenzae* and in

recognition of the inclusion of *H. influenza*e, the acronym now stands for the European Monitoring Group on Meningitis.

HAEMOPHILUS SPECIES

Haemophilus spp are small fastidious Gram-negative cocco-bacilli that can be speciated by their requirements for X-Factor (haemin) and/or V-Factor (NAD) and other phenotypic characteristics. *H. influenzae* requires both X-Factor and V-Factor. Some strains of *H. influenzae* are encapsulated and can be divided into 6 serotypes (a-f) based on their capsular polysaccharide. [12] NTHi strains cannot be serotyped by conventional type specific anti-serum agglutination. New molecular technologies have allowed a more accurate differentiation between typeable and non-typeable strains by detection of the *cap* locus which encodes the polysaccharide capsule. The *cap* locus consists of 3 regions. [15] Regions 1 and 3 are common to all capsular types and contain highly conserved genes necessary for processing and transport of capsular material to the cell surface.. Region 2 contains type-specific genes involved in capsule biosynthesis. A polymerase chain reaction (PCR)-based method to detect the region 1 *bexB* gene may be used as a proxy for the capsule locus, [19] and can distinguish true NTHi from strains that contain a complete or partial capsule locus. Strains with a partial capsule locus are unable to export polysaccharide to the cell surface and therefore appear to be NTHi as they are non-serotypeable by conventional means.

Serological capsular typing is prone to misinterpretation. Satola et al.[20] reported discrepancies between serological typing and PCR-based capsular typing for 17.5% of 360 invasive isolates of *H. influenzae*: 48 strains reported as encapsulated by serological typing were NTHi by PCR ; 8 NTHi by serotyping were capsulated; 6 capsulated by serotyping were a different type by PCR and 2 capsulated by serotyping were capsule-deficient (Hib-minus) strains. For this reason, molecular typing is the preferred method for accurate identification of NTHi.

There are a number of methods for sub-typing strains of *H. influenzae,* including biotyping, [23] outer membrane protein (OMP) subtyping, [24] and multilocus sequence typing (MLST). [25] The highly clonal nature of Hib strains renders subtyping useful only in epidemiological studies where a strain of an infrequent subtype is involved. In contrast, NTHi exhibit a wider

variety of phenotypes and genotypes and subtyping can be useful when investigating nosocomial transmission, which has been reported in nursing home and respiratory ward settings. [26,28] The preferred method for subtyping is MLST (www.mlst.net). However there are no reliable methods to differentiate commensal and disease-causing NTHi strains, although there is some evidence that bacterial IgA1 protease activity is significantly higher in NTHi strains from symptomatic patients compared to NTHi isolated from throat swabs of asymptomatic individuals.[30] Similarly, there are currently no data to indicate whether different strains of NTHi are involved in mucosal and invasive disease.

Haemophilus influenzae biogroup aegyptius is a distinct NTHi strain. For more than a century, this organism was known to cause seasonal epidemics of acute purulent conjunctivitis, especially in warmer climates.[31, 32] In 1984, a virulent clone of *H. influenzae* biogroup aegyptius emerged in Brazil as the cause of an acute febrile illness in children with high fatality rates. [34] This infection, subsequently known as Brazilian Purpuric Fever, caused a series of outbreaks and sporadic cases almost exclusively in Brazil over the next decade but has virtually disappeared since then.

A second X- and V-factor requiring *Haemophilus* species , *H. haemolyticus*, is frequently found in the upper respiratory tract, and has long been regarded as a commensal. Microbiologists relied on the production of zones of beta-haemolysis on horse blood agar to differentiate *H. haemolyticus* from NTHi. Recent observations have indicated that 10-40% of *H. haemolyticus* strains are non-haemolytic.[35,] PCR–based methods fordetecting protein D and fuculose kinase, which are both positive for NTHi,[37] matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS),[38] and fluorescence in situ hybridisation (FISH),[39] have been reported to facilitate the differentiation of NTHi from non-haemolytic *H. haemolyticus.* However, Binks et al.[40] found that no single gene target could unequivocally differentiate NTHi from *H. haemolyticus.* They recommended the use of an *hpd#3* probe-based real time PCR assay as the best currently available method. A scheme including *hpd*- and *iga*- based PCR assays and standard microbiological methods has been recommended to improve the identification of *H. haemolyticus*.[41]

NTHi AS A CAUSE OF NON-INVASIVE INFECTIONS

The global burden of NTHi non-invasive infections such as otitis media (OM), sinusitis and conjunctivitis, exacerbations of chronic obstructive pulmonary disease (COPD) and non-bacteraemic pneumonia is immense. These infections are by nature mucosal and often poly-microbial. As a consequence, the role of NTHi in the pathogenesis of these infections may be under-reported. The development of new and sensitive PCR technology will be invaluable in understanding the aetiology and pathology of these infections. It may then be possible to determine whether or not "infection strains" of NTHi exist at mucosal surfaces and their relationship to invasive disease.

Otitis Media (OM) is the most common childhood disease for which medical assistance is sought, affecting >75% of children aged <3 years.[42] The predominant bacterial pathogens in OM are *S. pneumoniae*, NTHi and *M. catarrhalis*, with *S. pneumoniae* and NTHi being cultured from the middle ear in 55-95%, depending on geographical location.[43-51] In both the United States (USA) and Australia, pneumococcal conjugate vaccination (Prevenar®, Wyeth Pharmaceuticals, USA) has resulted in aa higher proportion of cases with NTHi isolated from middle ear fluid, from 41% in 1992-1998 to 56% in 2000-2003.[43, 52] With the widespread use of higher-valency pneumococcal conjugate vaccines offering protection against more *S. pneumoniae* serotypes, it is critical to monitor changes in the bacterial aetiology of OM and disease severity. The prevalence of NTHi clearly increases in children with antimicrobial therapy failure,[53,] in those suffering from bilateral OM,[47] and in those with recurrent acute OM (AOM),[43, 55] indicating that NTHi OM may be more resistant to treatment and more chronic in nature. **It has been demonstrated that NTHi is able to form biofilms in the middle ear and exist in a poly-microbial community.[56] This may partly explain, resistance to antibiotic treatment and the recurrent and chronic nature of NTHi OM. A number of options are available for the development of a vaccine against NTHi OM.[9]** Studies with a prototype 11-valent pneumococcal conjugate vaccine in which the pneumococcal polysaccharides were conjugated to the *H. influenzae* derived protein D, demonstrated 35·3% efficacy against AOM caused by NTHi.[49] This vaccine formulation also reduced NTHi carriage in the nasopharynx.[58] However, the descendant licensed 10-valent vaccine does not appear to effect nasopharyngeal NTHi colonisation or bacterial load in healthy young children.[59]

Bacterial conjunctivitis is more common in children than in adults and NTHi is responsible for 44-68% of cases,[60-65] compared with ~25% in adults.[66] Conjunctivitis-otitis syndrome (COS) is often accompanied by AOM and the predominating causative agent in the conjunctiva and the middle ear is NTHi of the same strain.[60, 66, 69] Between 20-70% of children who present with bacterial conjunctivitis also have AOM. Given the high NTHi prevalence in bacterial conjunctivitis and the strong COS association, these children are most likely to also present with NTHi AOM. Conversely, since the introduction of Prevenar® in the USA, an increase in the proportion of NTHi AOM has been observed,[43] , which is also likely to increase the incidence of NTHi conjunctivitis. Surveillance studies should be undertaken to determine the impact of pneumococcal vaccination on COS prevalence.

Bacterial sinusitis is a common complication of childhood viral upper respiratory tract infection, and the fifth most common diagnosis for which antibiotics are prescribed in children in the USA.[71] In children with chronic sinusitis, *S. pneumoniae* and *H. influenzae* predominate.[76] The introduction of Prevenar™ in the USA saw an increase in the proportion of nasopharyngeal NTHi colonisation in children with sinusitis, from 25% to 41% from 1997 to 2005.[77]

Exacerbations of COPD, persistent bacterial bronchitis and cystic fibrosis: COPD is one of the leading causes of death globally with disease burden expected to increase significantly.[78] There is now extensive evidence demonstrating that NTHi colonisation of the airways is a major cause of airway inflammation and tissue damage, particularly during acute exacerbations.[79] Firstly, NTHi is a major pathogen colonising damaged airways in subjects with COPD,[5] and when present, predominates.[6] There is evidence that acute exacerbations of COPD can be attributed to the acquisition of 'exacerbation' isolates of NTHi. [81] During acute exacerbations, NTHi detection in bronchus brush biopsies increases from 33% in subjects with stable COPD to 87% in subjects with exacerbations. [81] Secondly, oral immunisation with an enteric killed whole-cell vaccine of subjects with severe COPD significantly reduces the number and severity of acute exacerbations.[83,84] Thirdly, the detection of specific NTHi IgE antibodies in subjects with COPD suggests that an IgE-mediated hypersensitivity to NTHi antigens contributes to the reversible airway obstruction and bronchial hyper-reactivity often observed in these subjects.[85]

Persistent bacterial bronchitis (PBB) is an underdiagnosed clinical presentation which is most common in <5 year-olds and is characterised by chronic cough.[86, 87] Culture of cough swabs obtained from children with PBB were positive for *H. influenzae* from 81% of samples tested,[88] and most of these were NTHi. Broncho-alveolar lavage (BAL) samples from children with PBB have confirmed NTHi as the major causative agent in Greece and the United Kingdom (UK). [89] Importantly, a recent Australian study of indigenous children with bronchiectasis confirmed by molecular typing that the majority of phenotypic NTHi isolates from both BAL and nasopharynx were PCR-positive for protein D and, therefore, not *H. haemolyticus*.[90] **In young children with cystic fibrosis, NTHi is often isolated from the respiratory tract[91, 92] and in up to 30% of sputum samples.[91] NTHi is thought to set up the inflammatory processes that permit *Pseudomonas aeruginosa* colonisation.[93, 94]** Recent studies have supported this concept and demonstrated the presence of NTHi in biofilms on airway epithelium with increased antimicrobial resistance. [95, 96]

Lower respiratory tract infections (LRTI): The role of NTHi as a causative agent of childhood pneumonia has recently been reviewed.[97] There are substantial technical difficulties in obtaining reliable samples to determine the bacterial aetiology of LRTI. Trans-thoracic fine needle aspiration is the most reliable method but this procedure, which is only performed in the presence of consolidation, is not routinely used. Therefore, other less direct diagnostic approaches have been used, including blood, sputum and BAL cultures. In the early 1980's in Papua New Guinea, NTHi clearly predominated in trans-thoracic fine needle aspiration cultures.[98] Studies considered to be technologically sound and conducted outside of Papua New Guinea have not demonstrated a similar NTHi predominance, but have reported positive lung aspirate cultures and molecular typing for NTHi in 15-40% of cases.[99-102] **In children with bacteraemic pneumonia, positive blood cultues for NTHi have been reported at rates between 2 - 10%.[98, 104-106] However, blood culture detection of *H. influenzae* may vary between isolates and medium. [103]New quantitative real-time PCR technology using serum samples will significantly aid the aetiological diagnosis of pneumonia.[107]** Sputum and BAL cultures from children with LRTI are often criticised because they are contaminated with nasopharyngeal microflora. However, if these samples are collected according to standardised protocols, they provide valuable information on microbial colonisation of the lower airways.[108, 110] Good quality sputum and BAL cultures have reported the presence of

NTHi in 20-94% of samples collected from children with community-acquired pneumonia.[113-115].

NTHi AS A CAUSE OF INVASIVE INFECTIONS

Prior to routine vaccination, Hib was the commonest cause of invasive *H. influenzae* disease, occurring predominantly in healthy <5 year-olds, for whom it was the commonest cause of bacterial meningitis.[116] **In England and Wales, 90% of invasive *H.influenzae* infections were due to Hib, 10% due to NTHi and other serotypes accounted for <1%.[117] Less than a quarter (23%) of NTHi infection occurred in <5 years-olds, 62% in adults, and 39% in >65 year-olds. NTHi incidence varied by age, ranging from 14.9/100,000 in neonates to 0.39/100,000 in >65 year-olds. Bacteraemia without a focus of infection was the predominant clinical presentation (37%), followed by pneumonia (27%) and meningitis (12%).**

The introduction of the Hib conjugate vaccine into national immunisation programmes in the early 1990's led to rapid and sustained reduction in Hib disease across all age groups through a combination of direct and indirect (herd) protection.[116] . **One of the concerns regarding the long-term consequences of Hib vaccination is the possible occurrence of replacement disease caused by other *H. influenzae* strains. As Hib conjugate vaccination reduces pharyngeal carriage, there is a theoretical possibility that other *H. influenzae* strains may take its place and, in turn, cause more invasive disease.**

Most European countries have an established national *H. influenzae* surveillance system, **albeit with varying surveillance and diagnostic methodologies and case definitions. [118]** During 1992-2006, national *H. influenzae* surveillance data from 14 European and other countries (www.eu-ibis.org) that routinely serotyped all *H. influenzae* isolates identified a 3.5% (95% confidence interval (CI), 2.1-5.2%) year-on-year annual increase in invasive non-type b *H. influenzae* disease, mainly due to NTHi, which accounted for 97% of cases.[118] In 2007, surveillance was transferred to the European Centre for Disease Prevention and Control (www.ecdc.eu). In 2008 and 2009, invasive NTHi disease incidence in 29 reporting countries was 0.41/100,000 and 0.36/100,000 respectively, with higher rates in Sweden (1.78/100,000 and 1.58/100,000) and Norway (1.58/100,000 and 1.48/100,000).[119,120] **Not**

all countries, however, have reported an increase in invasive NTHi following routine Hib vaccination. In Italy, for example, even though the proportion of invasive *H. influenzae* cases caused by NTHi increased from 28% of 220 isolates in 1997-2002, to 73% of 78 isolates in 2007- 2009, the incidence remained similar (0·037 and 0·032 per 100,000).[124]

In the US, 80% of invasive *H. influenzae* infections in 1989 were caused by Hib compared with 17% by NTHi, whereas by 2008, Hib was responsible for only 3% compared with 68% for NTHi.[121] During 1999-2008, NTHi incidence was 0.99/100,000, with the highest incidence in <1 –year-olds (5.87/100,000) and ≥65 year-olds (4.09/100,000).[121] . In Canada, Hib incidence in 2011 was 0.11/100,000 and 0.75/100,000 for non-type b disease.[123]

A number of factors may be responsible for the small but steady increase in invasive NTHi infections following routine Hib vaccination and it is likely that they have all contributed to some extent. The evidence for strain replacement following Hib vaccination is limited.[135] In England and Wales, for example, a 3·4% year-on-year increase in invasive NTHi disease was reported in children during 1994-2008, but the resurgence of invasive Hib disease during 2000-2003, eight years after the introduction of routine Hib vaccination, had no impact on invasive NTHi disease epidemiology.[125] On the other hand, it is well-described that invasive NTHI disease has a predilection for vulnerable populations such as premature infants, older adults, the immunosuppressed, individuals with malignancy and those with chronic cardiovascular, respiratory and other conditions.[125] Improvements in survival rates may, therefore, have increased the population of at-risk individuals for invasive NTHi and other infections. Alternatively, the observed increase may be a consequence of any combination of (i) increased recognition by clinicians of the spectrum of disease caused by NTHi, (ii) changes in clinical practice (e.g. number of blood cultures taken), (iii) laboratory testing (e.g. increased sensitivity of modern, automated blood culturing systems), and/or (iv) more complete reporting of laboratory-confirmed infections to national surveillance programmes. In particular, the transition from slide agglutination to PCR-based methods has significantly improved the accuracy of serotyping results. Like many other bacterial infections, the highest incidence of invasive NTHi disease occurs at the extremes of age.Neonatal NTHi infections are well described, with an incidence of 1·6 to 4·9 per 1,000 live births[140-142] and account for about 5% of all neonatal invasive bacterial infections.[140, 141,] The infection develops rapidly (usually

within 24 hours of birth) and follows a fulminant course with a significant case fatality, particularly among premature infants. The organism is known to colonise the female genital tract and it has been proposed that an ascending infection may result in both maternal and foetal infection, resulting in septicaemia, increased complications during labour and preterm delivery. A number of observational studies have resported higher rates of invasive NTHi disease in pregnancy (ref: Gkentzi, Slack, Ladhani, 2012). **Variant strains of NTHi isolated from the genito-urinary tract were first described by Albritton et al.[148] and further studied by Quentin et al. [149] They reported that most of these strains were biotype IV and described them as a "cryptic genospecies of Haemophilus biotype IV". The name *Haemophilus quentini* has been proposed for these strains. These strains are distinguishable from typical strains of NTHi by multilocus enzyme electrophoresis, 16S rRNA gene sequence and DNA hybridisation.[150] Subsequent studies however, have reported more diverse *H. influenzae* biotypes causing female genital tract infections[143, 151-152], highlighting the need for further research on perinatal NTHi infections. "*H. quentini*" has also been isolated from routine urine and urethral cultures in adult males[154], though the significance is unknown.**

After the neonatal period, the incidence of NTHi invasive disease is low and relatively stable. In countries with established Hib vaccination programmes, the median age at onset of invasive *H. influenzae* disease has now shifted from early childhood to late adulthood because most invasive infections are now due to NTHi, which has a median age at disease onset of around 60 years.[118] Most invasive NTHi cases in children (40% to 70%) and adults (60% to 80%) occur in individuals with underlying co-morbidities, particularly chronic respiratory disease and impaired immunity,[137, 141, 155-159] although a recent Swedish study reported co-morbidity in only 38% of cases.[120] Interestingly, this study found disorders of B cell immunity such as chronic lymphatic leukaemia and multiple myeloma to be particularly common among patients with invasive NTHi, suggesting an important role for humoral immunity.[120] The authors also speculated that the age-related decline in B-cell function may also explain the increasing incidence of invasive NTHi with age.[120]

The clinical presentation of invasive NTHi disease varies with age. The vast majority of neonatal cases develop septicaemia without a focus. Clinical presentation with meningitis is more common in older infants and children and then declines with age. The most common

clinical presentation overall is pneumonia, which increases with age and occurs mainly in older adults who often have underlying respiratory tract co-morbidities.[124-, 158] Other clinical presentations such as epiglottitis (characteristic of invasive Hib disease), bone and joint infections, as well as skin and soft tissue infections, have been reported but are uncommon, although cholecystitis appears to be a particular feature of invasive NTHi disease.[124-126, 158] It is important to emphasise that, as in neonates, NTHi can cause serious morbidity in older age groups. The aforementioned Swedish study, for example, reported that 48% of 101 invasive NTHi cases had severe sepsis or septic shock and 20% required intensive care.[120]

Reported case fatality ratio (CFR) for invasive NTHi disease range between 12-22%,[122, 125, 155, 157, 158, 160] and is associated with age, clinical presentation, presence of co-morbidity and duration of follow-up after infection. In Europe, compared with Hib, the age-adjusted odds ratio for death from invasive NTHi disease was 2·4 (95% CI, 1·9–3·1, P < 0·0001) overall, 3·3 (95% CI, 1·5–7·5; P = 0·004) for pneumonia and 3·3 (95% CI, 1·5–7·5; P = 0·004) for bacteraemia, with no significant difference observed for meningitis. CFR also increases with longer follow-up periods after the initial NTHi infection, suggesting that the pathogen may particularly be targeting those with poor health who, even if they survive their infection, will subsequently succumb to their underlying illness. In the Swedish study, for example, the 28-day CFR was 8%, but 1-year CFR increased to 29%.[120]

DEVELOPMENT OF RESISTANCE IN NTHI

In *H. influenzae* and NTHi, beta-lactamase production is still the dominant resistance mechanism against beta-lactams. The beta-lactamase prevalence in NTHi differs widely worldwide. Over the last decade, studies have reported percentages of beta-lactamase -positive NTHi between 10% and 25% in most countries (South-Africa, Europe , USA, Canada, Central and South-America).[161-166] In some regions, beta-lactamase -positive NTHi represent up to 55% of NTHi (Taiwan, Vietnam, Japan, South-Korea).[167-171] The beta-lactamase found in *H. influenzae* is usually a plasmid-encoded class A serine beta-lactamase; most often a TEM-1, rarely ROB-1, although regional differences exist.[172] It is surprising that although mutations have appeared in the promoter region of these genes, no TEM-type extended-spectrum beta-lactamases (ESBLs) have yet been detected in *H. influenzae*, in contrast to

other Gram-negatives. However, the possibility of broader spectrum beta-lactamases emerging in *H. influenzae* cannot be excluded. ESBL-type beta-lactamases have been found in *H. parainfluenzae*, a potential source of DNA for transformation of *H*. influenzae.[173]

More worrying however, are data on the emergence and spread of beta-lactamase independent beta-lactam resistant strains. These strains are called BLNAR, for beta-lactamase negative ampicillin-resistant strains. BLNAR can be identified by their resistance to ampicillin. Different breakpoints for ampicillin resistance have been used (1, 2 or 4 μg/ml, reviewed by Tristram et al [174]) and the term BLNAI is also used, for beta-lactamase negative ampicillin-intermediately-resistant strains. Another way to define these strains is on the basis of resistance-inducing mutations in the *fts*I gene. The *fts*I gene encodes a transpeptidase enzyme called penicillin-binding protein 3 (PBP3), which is involved in septum formation of dividing cells. Based on the type of mutations, BLNAI/R are subdivided into genotypes I, II (low gBLNAR) and III (high gBLNAR).[175-177] These different genotypes are also linked to different minimum inhibitory concentration (MIC) values; genotype I/II strains usually have ampicillin MIC values between 0·5 and 2 μg/ml and genotype III strains have MICs ranging between 1·0 and 16 μg/ml, often combined with reduced susceptibility to cephalosporins (see table 2). There are also BLPACR, for beta-lactamase positive amoxy-clavulanate-resistant strains. These strains combine beta-lactamase production with the presence of PBP3 mutations. Compared to BLNAR with the same PBP3 mutations but without a beta-lactamase, these BLPACR strains have higher ampicillin MICs and similar amoxy-clavulanate and cephalosporin MICs (table 2). BLPACR can again be subdivided based on the specific *fts*I mutations.

In Japan, surveillance studies on respiratory pathogens in children and adults have documented a marked increase of these BLNAI/R. Prior to 1984, no BLNAI/R were isolated; in the 1990's there was a slow increase, but from 2000 onwards, BLNAI/R increased rapidly from 15% to around 50% in 2007 and almost 40% in 2009.[171, 178-183] Notably, type III strains are gradually replacing the type I/II strains. These type III strains can behave as clones that appear to spread among the population.[178, 184] Similar observations were made for BLPACR strains.[185] In a 2007 review on *H. influenzae*,[174] the authors describe BLNAR as genotypically diverse with only a few instances of clonal spread. Since then, more reports of clonal spread have been published. Does this herald the emergence of clones with high beta-lactam MIC

values and epidemic characteristics? The rapid increase in *S. pneumonia*e resistance in the 1990's was also due to the worldwide spread of resistant clones.

A high prevalence of BLNAR strains has also been reported from neighbouring regions such as Taiwan, Vietnam, South-Korea.[167, 169, 170] Most countries in Europe and the USA have reported beta-lactamase in 5% to 25% of NTHi isolates and few or no BLNAR strains. In Europe, an exception is Spain, where higher levels of BLNAI/R and the presence of BLNAR (genotype III-like) exhibiting characteristics of clonality, with increased MIC values for cefuroxime (up to 16 µg/ml) and cefotaxime (up to 4 µg/ml), have been reported.[186, 187] These BLNAR strains were isolated primarily from cases of conjunctivitis. Reports also indicate higher and increasing levels of BLNAI/R in Poland, France and Portugal, with increasing prevalence of genotype III-like strains.[188-192] In Sweden, a significant increase in beta-lactam-resistant invasive *H. influenzae* mainly due to beta-lactamase-negative isolates was observed from 2007 onwards. One cluster of BLNAR genotype IIb isolates was identified and it included isolates from all geographical areas.[193] A preliminary report also suggests and increase in BLNAI in Canada starting in 2000. [194, 195] For many parts of the world, little or no data on BLNAI/R are available. Likely because regular surveillance of NTHi is lacking or microbiological work-up of NTHI is limited to a nitrocefin test for beta-lactamase production. In addition, currently accepted resistance breakpoints of >1 µg/ml or >2 µg/ml as determined by the Clinical and laboratory Standards Institute (CLSI) and EUCAST, respectively, for beta-lactamase negative *H. influenzae* probably led to an underestimation of the prevalence of BLNAR. This is also the case for BLPACR; the CLSI breakpoint of ≥8 µg/ml for co-amoxyclav is likely to miss many strains in which the MIC value is increased to only 2 µg/ml. The EUCAST breakpoint of >1 µg/ml is more likely to pick up these strains. The further spread of non-beta-lactamase dependent beta-lactam resistant strains, and in particular the genotype III BLNAR and BPLACR, has important implications for treatment. This is not only the case for strains with ampicillin MIC values of 4 µg/ml or more. Clinical data suggest that current dosages of aminopenicillins will not guarantee clinical success in certain types of infections, such as chronic or recurrent OM, if MIC values are 1 or 2 µg/ml. Changing to other classes of antibiotics offers only a partial solution. *H. influenzae* is intrinsically resistant to macrolide-lincosamide-streptograminB agents including ketolides.[196-198] Even for strains with low macrolide MIC values, the outcome in AOM for

treatment with macrolides has been shown not to differ from placebo.[199] *H. influenzae* is rarely resistant to quinolones but their use in children is not yet approved. It is also commonly accepted that the use of quinolones in paediatric respiratory infections would significantly enhance selective pressure for the emergence and selection of resistant *S. pneumoniae* and *H. influenzae*. In conclusion, a further spread of these BLNAR and BLPACR could make it necessary to change the current treatment guidelines for community-acquired upper and lower respiratory tract infections.

GENERAL CONCLUSION

Improved surveillance of *H. influenzae* is needed to follow the trends described here. National reference centres could play an important role by ensuring comprehensive and standardised surveillance of all cases of invasive *H. influenz*ae disease; collection of epidemiological data on cases, including demographic details, underlying co-morbidities ,risk factors and outcome of the infection; accurate identification of the organism based on molecular typing methods; antimicrobial resistance rates and mechanisms of resistance of invasive disease isolates. Respiratory isolates could also be monitored to follow trends in antimicrobial resistance. A substantial number of pharyngeal isolates previously identified as NTHi are actually *H. haemolyticus*.[36] Discrimination between these two can be challenging. New PCR technology and MALDI-TOF may offer a solution to this problem.[201] The low rates of NTHi infections observed in some countries probably reflect the low proportion of strains referred to reference laboratories and highlight the potential for improving case ascertainment. At the international level, cooperation and genetic comparison of strains between countries through reference centres would help to document the spread of resistant clones. International scientific organisations should take the lead in establishing networks and study groups. At a more practical level, an international consensus on the breakpoints for ampicillin resistance and the inclusion of ampicillin susceptibility testing in the routine work-up of *H. influenzae* are needed. The novel disk diffusion method to detect beta-lactam resistance in *H. influenzae* issued by the EUCAST (www.eucast.org) in 2011 will hopefully help in harmonising and improving the detection of these strains. Another issue that needs to be resolved is whether sampling from the nasopharynx is a valid alternative for sampling from the middle ear.

The increasing presence of NTHi in difficult-to-treat respiratory infections and the high level of resistance observed in these strains make it necessary to include these isolates in surveillance. Further studies are required to assess whether the recently licensed 10-valent pneumococcal conjugate vaccine (Synflorix,® GSK, Belgium) that includes Protein D from *H. influenzae* as a carrier protein might protect against serious infection in high-risk groups. Ongoing clinical studies will determine the efficacy of this vaccine against NTHi OM. It is also important to define the driving factors underlying the observed trends. Several factors have been put forward. Routine use of the pneumococcal conjugate vaccine has been suggested to increase nasopharyngeal colonisation with NTHi. This may explain the increased prevalence of NTHi in respiratory infections and provides increased opportunity for gene exchange and emergence of resistance. In Japan, the use of oral cephalosporins is thought to be driving the spread of BLNAR and BPLACR. This is in contrast to Europe and the USA, where amoxicillin (or amoxicillin-clavulanate in cases of suspected *H. influenzae* involvement) is used as a first-line therapy for community-acquired respiratory infections. A generalised spread of BLNAR and BLPACR is of considerable concern, as it might threaten the efficacy of our current first line empirical treatment of respiratory tract infections.

ACKNOWLEDGEMENTS

The authors acknowledge Dr Linda Hanssens, GlaxoSmithKline Biologicals, Belgium, for her vision in organising the satellite symposium at the European Congress of Clinical Microbiology and Infectious Diseases 2010 on “NTHi the unrevealed pathogen” and her encouragement to write this manuscript. Also Dr Diana Otczyk and Ms Penny Chapman for proofreading and formatting the paper.

Conflicts of Interest

Professor Allan Cripps received an educational grant from GlaxoSmithKline (GSK) to develop a website on NTHi and the e-journal pneumonia (pneumonia.org.au). Travel support was also received from GSK, to attend a conference on otitis media and other respiratory related meetings, particularly related to NTHi.

Dr Mary Slack has received support to attend and /or present at conferences, scientific meetings and advisory boards from GSK and Pfizer. Her laboratory has received research funding from GSK and Pfizer.

Professor Johan Van Eldere has received support to attend and /or present at conferences, scientific meetings and advisory boards from GSK, Bayer and Abbott.

REFERENCES

1. Gessner B D, Adegbola R A. The impact of vaccines on pneumonia: key lessons from Haemophilus influenzae type b conjugate vaccines. *Vaccine*. 2008; **26 Suppl 2**: B3-8. Epub 2008/09/20.
2. NTHi-Watch. NTHi-Watch. 2013 [cited 2013]; Available from: http://nthi-watch.griffith.edu.au/.

4. Murphy T F, Brauer A L, Schiffmacher A T, Sethi S. Persistent colonization by Haemophilus influenzae in chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*. 2004; **170**(3): 266-72. Epub 2004/05/01.
5. Sethi S, Murphy T F. Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review. *Clinical microbiology reviews*. 2001; **14**(2): 336-63. Epub 2001/04/09.
6. Butt H L, Clancy R L, Cripps A W, Murree-Allen K, Saunders N A, Sutherland D C, et al. Bacterial colonisation of the respiratory tract in chronic bronchitis. *Australian and New Zealand journal of medicine*. 1990; **20**(1): 35-8. Epub 1990/02/01.
7. Foxwell A R, Kyd J M, Cripps A W. Nontypeable Haemophilus influenzae: pathogenesis and prevention. *Microbiology and molecular biology reviews : MMBR*. 1998; **62**(2): 294-308. Epub 1998/06/10.
8. Leibovitz E, Jacobs M R, Dagan R. Haemophilus influenzae: a significant pathogen in acute otitis media. *The Pediatric infectious disease journal*. 2004; **23**(12): 1142-52. Epub 2005/01/01.
9. Cripps A W, Otczyk D C. Prospects for a vaccine against otitis media. *Expert review of vaccines*. 2006; **5**(4): 517-34. Epub 2006/09/23.
10. European Meningococcal Disease Society (EMGM). [cited 2013]; Available from: http://www.emgm2013.com/.
11. Barkai G, Leibovitz E, Givon-Lavi N, Dagan R. Potential contribution by nontypable Haemophilus influenzae in protracted and recurrent acute otitis media. *The Pediatric infectious disease journal*. 2009; **28**(6): 466-71. Epub 2009/06/09.
12. Pittman M. Variation and Type Specificity in the Bacterial Species Hemophilus Influenzae. *The Journal of experimental medicine*. 1931; **53**(4): 471-92. Epub 1931/03/31.
13. Watt J P, Wolfson L J, O'Brien K L, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by Haemophilus influenzae type b in children younger than 5 years: global estimates. *Lancet*. 2009; **374**(9693): 903-11. Epub 2009/09/15.
15. Satola S W, Schirmer P L, Farley M M. Complete sequence of the cap locus of Haemophilus influenzae serotype b and nonencapsulated b capsule-negative variants. *Infection and immunity*. 2003; **71**(6): 3639-44. Epub 2003/05/23.
.
19. Davis G S, Sandstedt S A, Patel M, Marrs C F, Gilsdorf J R. Use of bexB to detect the capsule locus in Haemophilus influenzae. *Journal of clinical microbiology*. 2011; **49**(7): 2594-601. Epub 2011/04/29.
20. Satola S W, Collins J T, Napier R, Farley M M. Capsule gene analysis of invasive Haemophilus influenzae: accuracy of serotyping and prevalence of IS1016 among nontypeable isolates. *Journal of clinical microbiology*. 2007; **45**(10): 3230-8. Epub 2007/08/19.
21. Bokermann S, Zanella R C, Lemos A P, de Andrade A L, Brandileone M C. Evaluation of methodology for serotyping invasive and nasopharyngeal isolates of Haemophilus influenzae in the ongoing surveillance in Brazil. *Journal of clinical microbiology*. 2003; **41**(12): 5546-50. Epub 2003/12/10.
22. LaClaire L L, Tondella M L, Beall D S, Noble C A, Raghunathan P L, Rosenstein N E, et al. Identification of Haemophilus influenzae serotypes by standard slide agglutination serotyping and PCR-based capsule typing. *Journal of clinical microbiology*. 2003; **41**(1): 393-6. Epub 2003/01/09.
23. Killian M A. A taxonomic study of the genus *Haemophilus* with the proposal of a new species. *Journal of General Microbiology*. 1976; **93**: 9-62.

24. van Alphen L. Molecular epidemiology of *Haemophilus influenzae* type b strains. In: Ellis RW, Granoff DM, editors. Development and clinical uses of Haemophilus influenzae type b conjugate vaccines. New York: Marcel Dekker; 1994. p. 129-44.
25. Meats E, Feil E J, Stringer S, Cody A J, Goldstein R, Kroll J S, et al. Characterization of encapsulated and noncapsulated Haemophilus influenzae and determination of phylogenetic relationships by multilocus sequence typing. *Journal of clinical microbiology*. 2003; **41**(4): 1623-36. Epub 2003/04/19.
26. Gough J, Kraak W A, Anderson E C, Nichols W W, Slack M P, McGhie D. Cross-infection by non-encapsulated Haemophilus influenzae. *Lancet*. 1990; **336**(8708): 159-60. Epub 1990/07/21.
28. Goetz M B, O'Brien H, Musser J M, Ward J I. Nosocomial transmission of disease caused by nontypeable strains of Haemophilus influenzae. *The American journal of medicine*. 1994; **96**(4): 342-7. Epub 1994/04/01.
30. Vitovski S, Dunkin K T, Howard A J, Sayers J R. Nontypeable Haemophilus influenzae in carriage and disease: a difference in IgA1 protease activity levels. *JAMA : the journal of the American Medical Association*. 2002; **287**(13): 1699-705. Epub 2002/04/03.
31. Koch R. Report on activities of the German Cholera Commission in Egypt and East India. *[in German] Wien Med Wochenschr*. 1883: 1548-51.
32. Weeks J N. The bacillus of acute catarrhal conjunctivits. *Arch Ophthalmol*. 1886; **15**: 441-51.
34. Centers for Disease Control and Prevention. Brazilian Purpuric Fever: *Haemophilus aegyptius* bacteraemia complicating purulent conjunctivitis: MMWR Morbidity & Mortality Weekly Report, 1986.
35. McCrea K W, Xie J, LaCross N, Patel M, Mukundan D, Murphy T F, et al. Relationships of nontypeable Haemophilus influenzae strains to hemolytic and nonhemolytic Haemophilus haemolyticus strains. *Journal of clinical microbiology*. 2008; **46**(2): 406-16. Epub 2007/11/28.
36
37. Theodore M J, Anderson R D, Wang X, Katz L S, Vuong J T, Bell M E, et al. Evaluation of new biomarker genes for differentiating Haemophilus influenzae from Haemophilus haemolyticus. *Journal of clinical microbiology*. 2012; **50**(4): 1422-4. Epub 2012/02/04.
38. Zhu B, Xiao D, Zhang H, Zhang Y, Gao Y, Xu L, et al. MALDI-TOF MS distinctly differentiates nontypable Haemophilus influenzae from Haemophilus haemolyticus. *PloS one*. 2013; **8**(2): e56139. Epub 2013/03/05.
39. Frickmann H, Christner M, Donat M, Berger A, Essig A, Podbielski A, et al. Rapid discrimination of Haemophilus influenzae, H. parainfluenzae, and H. haemolyticus by fluorescence in situ hybridization (FISH) and two matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) platforms. *PloS one*. 2013; **8**(4): e63222. Epub 2013/05/07.
40. Binks M J, Temple B, Kirkham L A, Wiertsema S P, Dunne E M, Richmond P C, et al. Molecular surveillance of true nontypeable Haemophilus influenzae: an evaluation of PCR screening assays. *PloS one*. 2012; **7**(3): e34083. Epub 2012/04/04.
41. Centers for Disease Control and Prevention. Active Bacterial Core surveillance; [cited 2012]; Available from: www.cdc.gov/abcs/reports-findings.
42. Teele D W, Klein J O, Rosner B. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. *The Journal of infectious diseases*. 1989; **160**(1): 83-94. Epub 1989/07/01.
43. Block S L, Hedrick J, Harrison C J, Tyler R, Smith A, Findlay R, et al. Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. *The Pediatric infectious disease journal*. 2004; **23**(9): 829-33. Epub 2004/09/14.
44. Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *The New England journal of medicine*. 2001; **344**(6): 403-9. Epub 2001/02/15.
45. Grevers G, Wiedemann S, Bohn J C, Blasius R W, Harder T, Kroeniger W, et al. Identification and characterization of the bacterial etiology of clinically problematic acute otitis media after

tympanocentesis or spontaneous otorrhea in German children. *BMC infectious diseases*. 2012; **12**(1): 312. Epub 2012/11/22.
46. Guevara S, Soley C, Arguedas A, Porat N, Dagan R. Seasonal distribution of otitis media pathogens among Costa Rican children. *The Pediatric infectious disease journal*. 2008; **27**(1): 12-6. Epub 2007/12/29.
47. Leibovitz E. The challenge of recalcitrant acute otitis media: pathogens, resistance, and treatment strategy. *The Pediatric infectious disease journal*. 2007; **26**(10 Suppl): S8-S11. Epub 2007/12/06.
48. Naranjo L, Suarez J A, DeAntonio R, Sanchez F, Calvo A, Spadola E, et al. Non-capsulated and capsulated Haemophilus influenzae in children with acute otitis media in Venezuela: a prospective epidemiological study. *BMC infectious diseases*. 2012; **12**: 40. Epub 2012/02/18.
49. Prymula R, Peeters P, Chrobok V, Kriz P, Novakova E, Kaliskova E, et al. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both Streptococcus pneumoniae and non-typable Haemophilus influenzae: a randomised double-blind efficacy study. *Lancet*. 2006; **367**(9512): 740-8. Epub 2006/03/07.
50. Sierra A, Lopez P, Zapata M A, Vanegas B, Castrejon M M, Deantonio R, et al. Non-typeable Haemophilus influenzae and Streptococcus pneumoniae as primary causes of acute otitis media in colombian children: a prospective study. *BMC infectious diseases*. 2011; **11**: 4. Epub 2011/01/07.
51. Suzuki A, Watanabe O, Okamoto M, Endo H, Yano H, Suetake M, et al. Detection of human metapneumovirus from children with acute otitis media. *The Pediatric infectious disease journal*. 2005; **24**(7): 655-7. Epub 2005/07/07.
52. Wiertsema S P, Kirkham L A, Corscadden K J, Mowe E N, Bowman J M, Jacoby P, et al. Predominance of nontypeable Haemophilus influenzae in children with otitis media following introduction of a 3+0 pneumococcal conjugate vaccine schedule. *Vaccine*. 2011; **29**(32): 5163-70. Epub 2011/05/31.
53. Arguedas A, Sher L, Lopez E, Sáez-Llorens X, Hamed K, Skuba K, et al. Open label, multicenter study of gatifloxacin treatment of recurrent otitis media and acute otitis media treatment failure. *The Pediatric infectious disease journal*. 2003; **22**(11): 949-55.
55. Kilpi T, Herva E, Kaijalainen T, Syrjanen R, Takala A K. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *The Pediatric infectious disease journal*. 2001; **20**(7): 654-62. Epub 2001/07/24.
56. Hall-Stoodley L, Hu F Z, Gieseke A, Nistico L, Nguyen D, Hayes J, et al. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. *JAMA : the journal of the American Medical Association*. 2006; **296**(2): 202-11. Epub 2006/07/13.
58. Prymula R, Kriz P, Kaliskova E, Pascal T, Poolman J, Schuerman L. Effect of vaccination with pneumococcal capsular polysaccharides conjugated to Haemophilus influenzae-derived protein D on nasopharyngeal carriage of Streptococcus pneumoniae and H. influenzae in children under 2 years of age. *Vaccine*. 2009; **28**(1): 71-8. Epub 2009/10/13.
59. van den Bergh M R, Spijkerman J, Swinnen K M, Francois N A, Pascal T G, Borys D, 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. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2012. Epub 2012/11/03.
60. Block S L, Hedrick J, Tyler R, Smith A, Findlay R, Keegan E, et al. Increasing bacterial resistance in pediatric acute conjunctivitis (1997-1998). *Antimicrobial agents and chemotherapy*. 2000; **44**(6): 1650-4. Epub 2000/05/19.
61. Buznach N, Dagan R, Greenberg D. Clinical and bacterial characteristics of acute bacterial conjunctivitis in children in the antibiotic resistance era. *The Pediatric infectious disease journal*. 2005; **24**(9): 823-8. Epub 2005/09/09.
62. Cavuoto K, Zutshi D, Karp C L, Miller D, Feuer W. Update on bacterial conjunctivitis in South Florida. *Ophthalmology*. 2008; **115**(1): 51-6. Epub 2007/06/19.

63. Gigliotti F. Acute conjunctivitis. *Pediatrics in review / American Academy of Pediatrics*. 1995; **16**(6): 203-7; quiz 8. Epub 1995/06/01.
64. Harrison C J, Hedrick J A, Block S L, Gilchrist M J. Relation of the outcome of conjunctivitis and the conjunctivitis-otitis syndrome to identifiable risk factors and oral antimicrobial therapy. *The Pediatric infectious disease journal*. 1987; **6**(6): 536-40. Epub 1987/06/01.
65. Weiss A. Acute conjunctivitis in childhood. *Current problems in pediatrics*. 1994; **24**(1): 4-11. Epub 1994/01/01.
66. Bingen E, Cohen R, Jourenkova N, Gehanno P. Epidemiologic study of conjunctivitis-otitis syndrome. *The Pediatric infectious disease journal*. 2005; **24**(8): 731-2. Epub 2005/08/12.
69. Bodor F F, Marchant C D, Shurin P A, Barenkamp S J. Bacterial etiology of conjunctivitis-otitis media syndrome. *Pediatrics*. 1985; **76**(1): 26-8. Epub 1985/07/01.
71. McCaig L F, Besser R E, Hughes J M. Trends in antimicrobial prescribing rates for children and adolescents. *JAMA : the journal of the American Medical Association*. 2002; **287**(23): 3096-102. Epub 2002/06/19.
76. Otten F W, Grote J J. Treatment of chronic maxillary sinusitis in children. *International journal of pediatric otorhinolaryngology*. 1988; **15**(3): 269-78. Epub 1988/09/01.
77. Brook I. Current issues in the management of acute bacterial sinusitis in children. *International journal of pediatric otorhinolaryngology*. 2007; **71**(11): 1653-61. Epub 2007/07/17.
78. Soriano J B, Lamprecht B. Chronic obstructive pulmonary disease: a worldwide problem. *The Medical clinics of North America*. 2012; **96**(4): 671-80. Epub 2012/07/17.
79. Clancy R L, Dunkley M. Acute exacerbations in COPD and their control with oral immunization with non-typeable haemophilus influenzae. *Frontiers in immunology*. 2011; **2**: 7. Epub 2011/01/01.
81. Sethi S, Wrona C, Eschberger K, Lobbins P, Cai X, Murphy T F. Inflammatory profile of new bacterial strain exacerbations of chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*. 2008; **177**(5): 491-7. Epub 2007/12/15.
2006; (4): CD001958. Epub 2006/10/21.
83. Tandon M K, Phillips M, Waterer G, Dunkley M, Comans P, Clancy R. Oral immunotherapy with inactivated nontypeable Haemophilus influenzae reduces severity of acute exacerbations in severe COPD. *Chest*. 2010; **137**(4): 805-11. Epub 2009/12/03.
84. Cripps A W, Kyd J M. A Vaccine for Nontypable Haemophilus influenzae. In: Ellis RW, Brodeur BR, editors. New Bacterial Vaccines. New York: Eurekah.com and Kluwer Academic / Plenum Publishers; 2003. p. 244-59.
85. Otczyk D C, Clancy R L, Cripps A W. Haemophilus influenzae and smoking-related obstructive airways disease. *International journal of chronic obstructive pulmonary disease*. 2011; **6**: 345-51. Epub 2011/07/16.
86. Marchant J M, Masters I B, Taylor S M, Chang A B. Utility of signs and symptoms of chronic cough in predicting specific cause in children. *Thorax*. 2006; **61**(8): 694-8. Epub 2006/05/04.
87. Marchant J M, Masters I B, Taylor S M, Cox N C, Seymour G J, Chang A B. Evaluation and outcome of young children with chronic cough. *Chest*. 2006; **129**(5): 1132-41. Epub 2006/05/11.
88. Donnelly D, Critchlow A, Everard M L. Outcomes in children treated for persistent bacterial bronchitis. *Thorax*. 2007; **62**(1): 80-4. Epub 2006/11/16.
89. Priftis K N, Douros K, Slack M, Everard M L, Litt D, Manglani S, et al. Bacterial bronchitis caused by Streptococcus pneumoniae and nontypable Haemophilus influenzae in children: the impact of vaccination. *Chest*. 2013; **143**(1): 152-7.
90. Hare K M, Binks M J, Grimwood K, Chang A B, Leach A J, Smith-Vaughan H. Culture and PCR detection of Haemophilus influenzae and Haemophilus haemolyticus in Australian Indigenous children with bronchiectasis. *Journal of clinical microbiology*. 2012; **50**(7): 2444-5. Epub 2012/05/04.
91. Bilton D, Pye A, Johnson M M, Mitchell J L, Dodd M, Webb A K, et al. The isolation and characterization of non-typeable Haemophilus influenzae from the sputum of adult cystic fibrosis patients. *European Respiratory Journal*. 1995; **8**(6): 948.

92. Rosenfeld M, Inglis A, Smith A, Martin T R, Ramsey B W, Gibson R L, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. *Pediatric pulmonology*. 2001; **32**(5): 356-66.
93. Abman S H, Ogle J W, Harbeck R J, Butler-Simon N, Hammond K B, Accurso F J. Early bacteriologic, immunologic, and clinical courses of young infants with cystic fibrosis identified by neonatal screening. *The Journal of pediatrics*. 1991; **119**(2): 211-7. Epub 1991/08/01.
94. Smith A. Pathogenesis of bacterial bronchitis in cystic fibrosis. *The Pediatric infectious disease journal*. 1997; **16**(1): 91-5; discussion 5-6, 123-6. Epub 1997/01/01.
95. Cardines R, Giufre M, Pompilio A, Fiscarelli E, Ricciotti G, Di Bonaventura G, et al. Haemophilus influenzae in children with cystic fibrosis: antimicrobial susceptibility, molecular epidemiology, distribution of adhesins and biofilm formation. *International journal of medical microbiology : IJMM*. 2012; **302**(1): 45-52. Epub 2011/10/18.
96. Starner T D, Zhang N, Kim G, Apicella M A, McCray P B, Jr. Haemophilus influenzae forms biofilms on airway epithelia: implications in cystic fibrosis. *American journal of respiratory and critical care medicine*. 2006; **174**(2): 213-20. Epub 2006/05/06.
97. Cripps A W. Nontypeable Haemophilus influenzae and childhood pneumonia. *Papua and New Guinea medical journal*. 2010; **53**(3-4): 147-50. Epub 2010/09/01.
98. Gratten M, Montgomery J. The bacteriology of acute pneumonia and meningitis in children in Papua New Guinea: assumptions, facts and technical strategies. *Papua and New Guinea medical journal*. 1991; **34**(3): 185-98. Epub 1991/09/01.
99. Ikeogu M O. Acute pneumonia in Zimbabwe: bacterial isolates by lung aspiration. *Archives of disease in childhood*. 1988; **63**(10): 1266-7. Epub 1988/10/01.
100. Morris G, Howie S, Ideh R, Ebruke B, Adams N, Jarra E, et al., editors. Pneumococcus and non-typeable Haemophilus influenzae in severe childhood pneumonia in the Gambia, West Africa: implications for vaccine policy. 7th International Symposium on Pneumococci and Pneumococcal Diseases 14-18 Mar 2010; Tel Aviv, Israel,.
101. Prinsloo J G, Cicoria A. [Is lung puncture aspiration a harmless procedure?]. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde*. 1974; **48**(14): 597-8. Epub 1974/03/23. Is longpunksie-aspirasie 'n onskuldige prosedure.
102. Silverman M, Stratton D, Diallo A, Egler L J. Diagnosis of acute bacterial pneumonia in Nigerian children. Value of needle aspiration of lung of countercurrent immunoelectrophoresis. *Archives of disease in childhood*. 1977; **52**(12): 925-31. Epub 1977/12/01.
103. Schell R F, Le Frock J L, Babu J P, Robinson D B. Recovery of Haemophilus influenzae from twenty-three blood culture media. *Journal of clinical microbiology*. 1979; **9**(1): 84-7. Epub 1979/01/01.
104. Ghafoor A, Nomani N K, Ishaq Z, Zaidi S Z, Anwar F, Burney M I, et al. Diagnoses of acute lower respiratory tract infections in children in Rawalpindi and Islamabad, Pakistan. *Reviews of infectious diseases*. 1990; **12 Suppl 8**: S907-14. Epub 1990/11/01.
105. Juven T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. *The Pediatric infectious disease journal*. 2000; **19**(4): 293-8. Epub 2000/04/27.
106. Straus W L, Qazi S A, Kundi Z, Nomani N K, Schwartz B. Antimicrobial resistance and clinical effectiveness of co-trimoxazole versus amoxycillin for pneumonia among children in Pakistan: randomised controlled trial. Pakistan Co-trimoxazole Study Group. *Lancet*. 1998; **352**(9124): 270-4. Epub 1998/08/05.
107. Lai J Y R, Binks M J, Kaestli M, Leach A, Smith-Vaughan H C. Potential use of Quantitative Real-Time PCR for pneumonia diagnostics in a high carriage population. *pneumonia*. 2012; **1**: 7-10.
108. Courcol R J, Damien J M, Ramon P, Voisin C, Martin G R. Presence of alveolar macrophages as a criterion for determining the suitability of sputum specimens for bacterial culture. *European journal of clinical microbiology*. 1984; **3**(2): 122-5. Epub 1984/04/01.

110. Klein D W, Beasley P A, Ilstrup D M, Washington J A, 2nd. Can microscopic screening be used to determine the suitability of sputum for culture of Haemophilus species? *American journal of clinical pathology*. 1986; **86**(6): 771-3. Epub 1986/12/01.
113. Chiang W C, Teoh O H, Chong C Y, Goh A, Tang J P, Chay O M. Epidemiology, clinical characteristics and antimicrobial resistance patterns of community-acquired pneumonia in 1702 hospitalized children in Singapore. *Respirology*. 2007; **12**(2): 254-61. Epub 2007/02/15.
114. De Schutter I, De Wachter E, Crokaert F, Verhaegen J, Soetens O, Piérard D, et al. Microbiology of bronchoalveolar lavage fluid in children with acute nonresponding or recurrent community-acquired pneumonia: identification of nontypeable Haemophilus influenzae as a major pathogen. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2011; **52**(12): 1437-44.
115. Ingarfield S L, Celenza A, Jacobs I G, Riley T V. The bacteriology of pneumonia diagnosed in Western Australian emergency departments. *Epidemiology and infection*. 2007; **135**(8): 1376-83. Epub 2007/02/06.
116. Peltola H. Worldwide Haemophilus influenzae type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. *Clinical microbiology reviews*. 2000; **13**(2): 302-17. Epub 2001/02/07.
117. Anderson E C, Begg N T, Crawshaw S C, Hargreaves R M, Howard A J, Slack M P. Epidemiology of invasive Haemophilus influenzae infections in England and Wales in the pre-vaccination era (1990-2). *Epidemiology and infection*. 1995; **115**(1): 89-100. Epub 1995/08/01.
118. Ladhani S, Slack M P E, Heath P T, von Gottberg A, Chandra M, Ramsay M E, et al. Invasive Haemophilus influenzae Disease, Europe, 1996-2006. *Emerging infectious diseases*. 2010; **16**(3): 455-63.
119. European Centre for Disease Prevention and Control (ECDC). Surveillance report on invasive bacterial diseases in Europe for 2008-2009; [cited 2011 4th October]; Available from: http://ecdc.europa.eu/en/activities/diseaseprogrammes/vpd/Pages/index.aspx.
120. Resman F, Ristovski M, Ahl J, Forsgren A, Gilsdorf J R, Jasir A, et al. Invasive disease caused by Haemophilus influenzae in Sweden 1997–2009; evidence of increasing incidence and clinical burden of non-type b strains. *Clinical Microbiology and Infection*. 2011; **17**(11): 1638-45.
121. MacNeil J R, Cohn A C, Farley M, Mair R, Baumbach J, Bennett N, et al. Current epidemiology and trends in invasive Haemophilus influenzae disease--United States, 1989-2008. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2011; **53**(12): 1230-6. Epub 2011/11/15.

123. Public Agency of Canada. Invasive *Haemophilus Influenzae* Disease 2012; Available from: www.phac-aspc.gc.ca/im/vpd-mev/hib-eng.php.
124. Giufre M, Cardines R, Caporali M G, Accogli M, D'Ancona F, Cerquetti M. Ten years of Hib vaccination in Italy: prevalence of non-encapsulated Haemophilus influenzae among invasive isolates and the possible impact on antibiotic resistance. *Vaccine*. 2011; **29**(22): 3857-62. Epub 2011/04/05.
125. Ladhani S N, Ramsay M, Slack M P. The impact of Haemophilus influenzae serotype B resurgence on the epidemiology of childhood invasive Haemophilus influenzae disease in England and Wales. *The Pediatric infectious disease journal*. 2011; **30**(10): 893-5. Epub 2011/08/26.
127. Blanchard-Rohner G, Pollard A J. Sustaining immunity after immunization against encapsulated bacteria. *Human vaccines*. 2008; **4**(4): 309-12.
128. Tsang R. Capsule switching and capsule replacement in vaccine-preventable bacterial diseases. *The Lancet infectious diseases*. 2007; **7**(9): 569-70. Epub 2007/08/24.
129. Takala A K, Eskola J, Leinonen M, Kayhty H, Nissinen A, Pekkanen E, et al. Reduction of oropharyngeal carriage of Haemophilus influenzae type b (Hib) in children immunized with an Hib conjugate vaccine. *The Journal of infectious diseases*. 1991; **164**(5): 982-6. Epub 1991/11/01.

130. Rotondo J L, Sherrard L, Helferty M, Tsang R, Desai S. The epidemiology of invasive disease due to Haemophilus influenzae serotype a in the Canadian North from 2000 to 2010. *International journal of circumpolar health*. 2013; **72**.
131. Bruce M G, Zulz T, DeByle C, Singleton R, Hurlburt D, Bruden D, et al. Haemophilus influenzae serotype a invasive disease, Alaska, USA, 1983-2011. *Emerg Infect Dis*. 2013; **19**(6): 932-7. Epub 2013/06/06.
132. Menzies R I, Markey P, Boyd R, Koehler A P, McIntyre P B. No evidence of increasing Haemophilus influenzae non-b infection in Australian Aboriginal children. *International journal of circumpolar health*. 2013; **72**. Epub 2013/08/29.
133. Ladhani S N, Collins S, Vickers A, Litt D J, Crawford C, Ramsay M E, et al. Invasive Haemophilus influenzae serotype e and f disease, England and Wales. *Emerg Infect Dis*. 2012; **18**(5): 725-32. Epub 2012/04/21.
134. Urwin G, Krohn J A, Deaver-Robinson K, Wenger J D, Farley M M. Invasive disease due to Haemophilus influenzae serotype f: clinical and epidemiologic characteristics in the H. influenzae serotype b vaccine era. The Haemophilus influenzae Study Group. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1996; **22**(6): 1069-76. Epub 1996/06/01.
135. Ladhani S, Ramsay M E, Chandra M, Slack M P, Eu I. No evidence for Haemophilus influenzae serotype replacement in Europe after introduction of the Hib conjugate vaccine. *The Lancet infectious diseases*. 2008; **8**(5): 275-6. Epub 2008/05/13.
137. O'Neill J M, St Geme J W, 3rd, Cutter D, Adderson E E, Anyanwu J, Jacobs R F, et al. Invasive disease due to nontypeable Haemophilus influenzae among children in Arkansas. *Journal of clinical microbiology*. 2003; **41**(7): 3064-9. Epub 2003/07/05.
138. Fothergill L D, Wright J. Influenzeal meningitis: the relation of age incidence to the bactericidal power of blood against the causal organism. *Journal of Immunology*. 1933; **24**: 273-84.
139. Verani J R, Schrag S J. Group B streptococcal disease in infants: progress in prevention and continued challenges. *Clinics in perinatology*. 2010; **37**(2): 375-92. Epub 2010/06/24.
140. Falla T J, Dobson S R, Crook D W, Kraak W A, Nichols W W, Anderson E C, et al. Population-based study of non-typable Haemophilus influenzae invasive disease in children and neonates. *Lancet*. 1993; **341**(8849): 851-4. Epub 1993/04/03.
141. Heath P T, Booy R, Azzopardi H J, Slack M P, Fogarty J, Moloney A C, et al. Non-type b Haemophilus influenzae disease: clinical and epidemiologic characteristics in the Haemophilus influenzae type b vaccine era. *The Pediatric infectious disease journal*. 2001; **20**(3): 300-5. Epub 2001/04/17.
142. Gkentzi D, Slack M, Ladhani S. The burden of non-encapsulated Haemophilus influenzae infections in children and the potential for prevention. *Current opinion and therapeutic diseases.*2012;25(3): 266-72
146. Friesen C A, Cho C T. Characteristic features of neonatal sepsis due to Haemophilus influenzae. *Reviews of infectious diseases*. 1986; **8**(5): 777-80. Epub 1986/09/01.
147. Horvath B, Yang M, Manning F A. Intrauterine fetal death caused by Haemophilus influenzae infection: a case report. *The Journal of reproductive medicine*. 2008; **53**(1): 55-6. Epub 2008/02/07.
149. Quentin R, Martin C, Musser J M, Pasquier-Picard N, Goudeau A. Genetic characterization of a cryptic genospecies of Haemophilus causing urogenital and neonatal infections. *Journal of clinical microbiology*. 1993; **31**(5): 1111-6. Epub 1993/05/01.
150. Norskov-Lauritsen N. Detection of cryptic genospecies misidentified as Haemophilus influenzae in routine clinical samples by assessment of marker genes fucK, hap, and sodC. *Journal of clinical microbiology*. 2009; **47**(8): 2590-2. Epub 2009/06/19.
151. Cox R A, Slack M P. Clinical and microbiological features of Haemophilus influenzae vulvovaginitis in young girls. *J Clin Pathol*. 2002; **55**(12): 961-4. Epub 2002/12/04.
152. Martinez M A, Ovalle A, Ulloa M T, Vidal R M. Role of Haemophilus influenzae in intra-amniotic infection in patients with preterm rupture of membranes. *European journal of clinical microbiology*

& infectious diseases : official publication of the European Society of Clinical Microbiology. 1999; **18**(12): 890-2. Epub 2000/02/26.
154. Glover W A, Suarez C J, Clarridge J E, 3rd. Genotypic and phenotypic characterization and clinical significance of 'Haemophilus quentini' isolated from the urinary tract of adult men. *Journal of medical microbiology*. 2011; **60**(Pt 11): 1689-92. Epub 2011/07/09.
155. Campos J, Hernando M, Roman F, Perez-Vazquez M, Aracil B, Oteo J, et al. Analysis of invasive Haemophilus influenzae infections after extensive vaccination against H. influenzae type b. *Journal of clinical microbiology*. 2004; **42**(2): 524-9. Epub 2004/02/10.
156. Cardines R, Giufre M, Mastrantonio P, Ciofi degli Atti M L, Cerquetti M. Nontypeable Haemophilus influenzae meningitis in children: phenotypic and genotypic characterization of isolates. *The Pediatric infectious disease journal*. 2007; **26**(7): 577-82. Epub 2007/06/29.
157. Perdue D G, Bulkow L R, Gellin B G, Davidson M, Petersen K M, Singleton R J, et al. Invasive Haemophilus influenzae disease in Alaskan residents aged 10 years and older before and after infant vaccination programs. *JAMA : the journal of the American Medical Association*. 2000; **283**(23): 3089-94. Epub 2000/06/24.
158. Rubach M P, Bender J M, Mottice S, Hanson K, Weng H Y, Korgenski K, et al. Increasing incidence of invasive Haemophilus influenzae disease in adults, Utah, USA. *Emerg Infect Dis*. 2011; **17**(9): 1645-50. Epub 2011/09/06.
159. Sarangi J, Cartwright K, Stuart J, Brookes S, Morris R, Slack M. Invasive Haemophilus influenzae disease in adults. *Epidemiology and infection*. 2000; **124**(3): 441-7. Epub 2000/09/12.
160. Talbot B, Alexander E, Lewis S, Newport M J, Slack M P, Litt D J, et al. Hepatobiliary infections due to non-capsulated Haemophilus influenzae. *Journal of medical microbiology*. 2011; **60**(Pt 9): 1383-6. Epub 2011/04/30.
161. Alpuche C, Garau J, Lim V. Global and local variations in antimicrobial susceptibilities and resistance development in the major respiratory pathogens. *International journal of antimicrobial agents*. 2007; **30 Suppl 2**: S135-8. Epub 2007/10/20.
162. Beekmann S E, Heilmann K P, Richter S S, Garcia-de-Lomas J, Doern G V, Group G S. Antimicrobial resistance in Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and group A beta-haemolytic streptococci in 2002-2003. Results of the multinational GRASP Surveillance Program. *International journal of antimicrobial agents*. 2005; **25**(2): 148-56. Epub 2005/01/25.
163. Heilmann K P, Rice C L, Miller A L, Miller N J, Beekmann S E, Pfaller M A, et al. Decreasing prevalence of beta-lactamase production among respiratory tract isolates of Haemophilus influenzae in the United States. *Antimicrobial agents and chemotherapy*. 2005; **49**(6): 2561-4. Epub 2005/05/27.
164. Jansen W T, Verel A, Beitsma M, Verhoef J, Milatovic D. Surveillance study of the susceptibility of Haemophilus influenzae to various antibacterial agents in Europe and Canada. *Current medical research and opinion*. 2008; **24**(10): 2853-61. Epub 2008/09/02.
165. Liebowitz L D, Slabbert M, Huisamen A. National surveillance programme on susceptibility patterns of respiratory pathogens in South Africa: moxifloxacin compared with eight other antimicrobial agents. *Journal of Clinical Pathology*. 2003; **56**(5): 344-7.
166. Sener B, Tunçkanat F, Ulusoy S, Tünger a, Söyletir G, Mülazimoğlu L, et al. A survey of antibiotic resistance in Streptococcus pneumoniae and Haemophilus influenzae in Turkey, 2004-2005. *Journal of Antimicrobial Chemotherapy*. 2007; **60**(3): 587-93.
167. Bae S, Lee J, Kim E, Lee S, Yu J, Kang Y. Antimicrobial Resistance in Haemophilus influenzae Respiratory Tract Isolates in Korea: Results of a Nationwide Acute Respiratory Infections Surveillance. *Antimicrobial agents and chemotherapy*. 2010; **54**(1): 65-71.
168. Bae S M, Lee J H, Lee S K, Yu J Y, Lee S H, Kang Y H. High prevalence of nasal carriage of beta-lactamase-negative ampicillin-resistant Haemophilus influenzae in healthy children in Korea. *Epidemiology and infection*. 2013; **141**(3): 481-9.

169. Gotoh K, Qin L, Watanabe K, Anh D D, Huong Ple T, Anh N T, et al. Prevalence of Haemophilus influenzae with resistant genes isolated from young children with acute lower respiratory tract infections in Nha Trang, Vietnam. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2008; **14**(5): 349-53. Epub 2008/10/22.
170. Jean S S, Hsueh P R, Lee W S, Chang H T, Chou M Y, Chen I S, et al. Nationwide surveillance of antimicrobial resistance among Haemophilus influenzae and Streptococcus pneumoniae in intensive care units in Taiwan. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*. 2009; **28**(8): 1013-7. Epub 2009/03/13.
171. Niki Y, Hanaki H, Yagisawa M, Kohno S, Aoki N, Watanabe A, et al. The first nationwide surveillance of bacterial respiratory pathogens conducted by the Japanese Society of Chemotherapy. Part 1: a general view of antibacterial susceptibility. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2008; **14**(4): 279-90. Epub 2008/08/19.
172. Farrell D J, Morrissey I, Bakker S, Buckridge S, Felmingham D. Global distribution of TEM-1 and ROB-1 beta-lactamases in Haemophilus influenzae. *The Journal of antimicrobial chemotherapy*. 2005; **56**(4): 773-6. Epub 2005/08/13.
173. Tristram S G, Pitout M J, Forward K, Campbell S, Nichols S, Davidson R J. Characterization of extended-spectrum beta-lactamase-producing isolates of Haemophilus parainfluenzae. *The Journal of antimicrobial chemotherapy*. 2008; **61**(3): 509-14. Epub 2008/02/05.
174. Tristram S, Jacobs M R, Appelbaum P C. Antimicrobial resistance in Haemophilus influenzae. *Clinical microbiology reviews*. 2007; **20**(2): 368-89. Epub 2007/04/13.
175. Dabernat H, Delmas C, Seguy M, Pelissier R, Faucon G, Bennamani S, et al. Diversity of beta-lactam resistance-conferring amino acid substitutions in penicillin-binding protein 3 of Haemophilus influenzae. *Antimicrobial agents and chemotherapy*. 2002; **46**(7): 2208-18. Epub 2002/06/19.
176. Hasegawa K, Chiba N, Kobayashi R, Murayama S Y, Iwata S, Sunakawa K, et al. Rapidly increasing prevalence of beta-lactamase-nonproducing, ampicillin-resistant Haemophilus influenzae type b in patients with meningitis. *Antimicrobial agents and chemotherapy*. 2004; **48**(5): 1509-14. Epub 2004/04/24.
177. Ubukata K, Shibasaki Y, Yamamoto K, Chiba N, Hasegawa K, Takeuchi Y, et al. Association of amino acid substitutions in penicillin-binding protein 3 with beta-lactam resistance in beta-lactamase-negative ampicillin-resistant Haemophilus influenzae. *Antimicrobial agents and chemotherapy*. 2001; **45**(6): 1693-9. Epub 2001/05/17.
178. Hotomi M, Fujihara K, Sakai A, Billal D S, Shimada J, Suzumoto M, et al. Antimicrobial resistance of Haemophilus influenzae isolated from the nasopharynx of Japanese children with acute otitis media. *Acta oto-laryngologica*. 2006; **126**(3): 240-7. Epub 2006/04/19.
179. Kishii K, Chiba N, Morozumi M, Hamano-Hasegawa K, Kurokawa I, Masaki J, et al. Diverse mutations in the ftsI gene in ampicillin-resistant Haemophilus influenzae isolates from pediatric patients with acute otitis media. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2010; **16**(2): 87-93. Epub 2010/01/21.
180. Niki Y, Hanaki H, Matsumoto T, Yagisawa M, Kohno S, Aoki N, et al. Nationwide surveillance of bacterial respiratory pathogens conducted by the Japanese Society of Chemotherapy in 2007: general view of the pathogens' antibacterial susceptibility. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2009; **15**(3): 156-67. Epub 2009/06/26.
181. Seki H, Kasahara Y, Ohta K, Saikawa Y, Sumita R, Yachie A, et al. Increasing prevalence of ampicillin- resistant, non-beta-lactamase-producing strains of Haemophilus influenzae in children in Japan. *Chemotherapy*. 1999; **45**(1): 15-21. Epub 1999/01/07.
182. Sunakawa K, Farrell D J. Mechanisms, molecular and sero-epidemiology of antimicrobial resistance in bacterial respiratory pathogens isolated from Japanese children. *Annals of clinical microbiology and antimicrobials*. 2007; **6**: 7. Epub 2007/08/19.
183. Watanabe A, Ogasawara K, Koashi N, Kozuki T, Komoto A, Takahashi Y, et al. Nationwide surveillance of bacterial respiratory pathogens conducted by the Surveillance Committee of Japanese Society of Chemotherapy, Japanese Association for Infectious Diseases, and Japanese

Society for Clinical Microbiology in 2009: general view of the pathogens' antibacterial susceptibility. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy*. 2012; **18**(5): 609-20.
184. Hotomi M, Fujihara K, Billal D S, Suzuki K, Nishimura T, Baba S, et al. Genetic characteristics and clonal dissemination of beta-lactamase-negative ampicillin-resistant Haemophilus influenzae strains isolated from the upper respiratory tract of patients in Japan. *Antimicrobial agents and chemotherapy*. 2007; **51**(11): 3969-76. Epub 2007/08/19.
185. Ito M, Hotomi M, Maruyama Y, Hatano M, Sugimoto H, Yoshizaki T, et al. Clonal spread of beta-lactamase-producing amoxicillin-clavulanate-resistant (BLPACR) strains of non-typeable Haemophilus influenzae among young children attending a day care in Japan. *International journal of pediatric otorhinolaryngology*. 2010; **74**(8): 901-6. Epub 2010/09/18.
186. Garcia-Cobos S, Campos J, Cercenado E, Roman F, Lazaro E, Perez-Vazquez M, et al. Antibiotic resistance in Haemophilus influenzae decreased, except for beta-lactamase-negative amoxicillin-resistant isolates, in parallel with community antibiotic consumption in Spain from 1997 to 2007. *Antimicrobial agents and chemotherapy*. 2008; **52**(8): 2760-6. Epub 2008/05/29.
187. García-Cobos S, Campos J, Lázaro E, Román F, Cercenado E, García-Rey C, et al. Ampicillin-resistant non-beta-lactamase-producing Haemophilus influenzae in Spain: recent emergence of clonal isolates with increased resistance to cefotaxime and cefixime. *Antimicrobial agents and chemotherapy*. 2007; **51**(7): 2564-73.
188. Barbosa A R, Giufre M, Cerquetti M, Bajanca-Lavado M P. Polymorphism in ftsI gene and {beta}-lactam susceptibility in Portuguese Haemophilus influenzae strains: clonal dissemination of beta-lactamase-positive isolates with decreased susceptibility to amoxicillin/clavulanic acid. *The Journal of antimicrobial chemotherapy*. 2011; **66**(4): 788-96. Epub 2011/03/12.
189. Cohen R, Bingen E, Levy C, Benani M, Thollot F, Klink Z, et al. [Antibiotic resistance of pneumococci and H. influenzae isolated from the nasopharyngeal flora of children with acute otitis media between 2006 and 2010]. *Archives de pediatrie : organe officiel de la Societe francaise de pediatrie*. 2011; **18**(8): 926-31. Epub 2011/06/15. Resistance aux antibiotiques des pneumocoques et H. influenzae isoles de la flore rhinopharyngee d'enfants presentant une otite moyenne aigue entre 2006 et 2010.
190. Dabernat H, Delmas C. Epidemiology and evolution of antibiotic resistance of Haemophilus influenzae in children 5 years of age or less in France, 2001-2008: a retrospective database analysis. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*. 2012; **31**(10): 2745-53. Epub 2012/04/28.
191. Dabernat H, Seguy M, Faucon G, Delmas C. [Epidemiology of Haemophilus influenzae strains collected in 2004 in France and in vitro assessment of their susceptibility to antibiotics]. *Medecine et maladies infectieuses*. 2007; **37**(6): 320-4. Epub 2007/05/29. Epidemiologie et evaluation de la sensibilite aux antibiotiques de souches d'Haemophilus influenzae isolees en 2004 en France.
192. Skoczynska A, Kadlubowski M, Wasko I, Fiett J, Hryniewicz W. Resistance patterns of selected respiratory tract pathogens in Poland. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2007; **13**(4): 377-83. Epub 2007/03/16.
193. Resman F, Ristovski M, Forsgren A, Kaijser B, Kronvall G, Medstrand P, et al. Increase of beta-lactam-resistant invasive Haemophilus influenzae in Sweden, 1997 to 2010. *Antimicrobial agents and chemotherapy*. 2012; **56**(8): 4408-15. Epub 2012/06/13.
194. Sill ML, Tsang RS. Antibiotic susceptibility of invasive Haemophilus influenzae strains in Canada. *Antimicrobial agents and chemotherapy*. 2008; 52:1551-2.
195. Shuel ML, Tsang RS. Canadian beta-lactamase-negative Haemophilus influenzae isolates showing decreased susceptibility toward ampicillin have significant penicillin binding protein 3 mutations. *Diagnostic Microbiology and Infectious Diseases*. 2009; 63:379-83.

196. Bogdanovich T, Bozdogan B, Appelbaum P C. Effect of efflux on telithromycin and macrolide susceptibility in Haemophilus influenzae. *Antimicrobial agents and chemotherapy*. 2006; **50**(3): 893-8. Epub 2006/02/24.
197. Peric M, Bozdogan B, Jacobs M R, Appelbaum P C. Effects of an efflux mechanism and ribosomal mutations on macrolide susceptibility of Haemophilus influenzae clinical isolates. *Antimicrobial agents and chemotherapy*. 2003; **47**(3): 1017-22. Epub 2003/02/27.
198. Sanchez L, Leranoz S, Puig M, Loren J G, Nikaido H, Vinas M. Molecular basis of antimicrobial resistance in non-typable Haemophilus influenzae. *Microbiologia*. 1997; **13**(3): 309-14. Epub 1997/11/14.
199. Dagan R, Leibovitz E. Bacterial eradication in the treatment of otitis media. *The Lancet infectious diseases*. 2002; **2**(10): 593-604. Epub 2002/10/18.
200. The British Society for Antimicrobial Chemotherapy. BSAC Resistance Surveillance Programme; 2013; Available from: http://bsac.org.uk/surveillance/bsac-resistance-surveillance-programme/.
201. Gaillot O. Rapport d'activites du C.N.R Haemophilus influenzae 2009-2010. Available from: http://biologiepathologie.chru-lille.fr/organisation-fbp/Rapport_CNR_Hi_2009-2010.pdf.

Table 1 Predominant diseases caused by NTHi and their burden

	Disease	Burden	Ref
Non-invasive disease			
	Otitis media	55-95% of cases in children	43-51
	Bacterial conjunctivitis	44-68% of cases in children 25% of cases in adults	60-65 66
	Bacterial sinusitis	41% of cases in children	77
	Exacerbations of COPD	>90% during an acute exacerbation	6, 80-81
	Persistent bacterial bronchitis	81% of cases in children	88
	Cystic fibrosis	Up to 30% of sputum samples	91
	Lower respiratory infections	BAL: 20-94%of cases with community acquired pneumonia[1] Lung Aspirate: 15-40% of cases with pneumonia Blood culture 2-10% of cases with bacteriamic pneumonia	113-115 99-102 98, 104-106
Invasive Disease[2]			
	Neonatal infections (septicaemia and meningitis)	1.6 – 4.9 per 100 live births	140-144

[1] Geographically variable

[2] Usually associated with underlying co-morbidity (40-70% in children; 60-80% in adults)

Epiglottis and bone, joint, skin and soft tissue infections have been reported but at a low incidence.[124-126]