REVIEW ARTICLE

Viruses and bacteria in acute asthma exacerbations – A GA²LEN-DARE* systematic review

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Abstract

A major part of the burden of asthma is caused by acute exacerbations. Exacerbations have been strongly and consistently associated with respiratory infections. Respiratory viruses and bacteria are therefore possible treatment targets. To have a reasonable estimate of the burden of disease induced by such infectious agents on asthmatic patients, it is necessary to understand their nature and be able to identify them in clinical samples by employing accurate and sensitive methodologies. This systematic review summarizes current knowledge and developments in infection epidemiology of acute asthma in children and adults, describing the known impact for each individual agent and highlighting knowledge gaps. Among infectious agents, human rhinoviruses are the most prevalent in regard to asthma exacerbations. The newly identified type-C rhinoviruses may prove to be particularly relevant. Respiratory syncytial virus and metapneumovirus are important in infants, while influenza viruses seem to induce severe exacerbations mostly in adults. Other agents are relatively less or not clearly associated. *Mycoplasma* and *Chlamydophila pneumoniae* seem to be involved more with asthma persistence rather than with disease exacerbations. Recent data suggest that common bacteria may also be involved, but this should be confirmed. Although current information is considerable, improvements in detection methodologies, as well as the wide variation in respect to location, time and populations, underline the need for additional studies that should also take into account interacting factors.

In recent years, a growing number of observations have highlighted the importance of respiratory infections in acute asthma exacerbations. Respiratory viruses have repeatedly and consistently been associated with asthma exacerbations in different patient groups, with detection frequencies ranging from 40 to 90%. Bacteria, mostly *Mycoplasma* and *Chlamydophila pneumoniae*, are frequently identified in chronic asthma and may also precipitate exacerbations. Taking into account that a major proportion of asthma-related disease burden is caused by exacerbations (1), as well as that infectious agents are potential treatment targets, it becomes evident that the above associations require further attention. To have a reasonable estimate of the burden of disease induced by infectious agents on patients with asthma, it is necessary to understand the nature of these agents and be able to identify them in clinical samples by employing sensitive and accurate methodologies (2). Newer detection techniques are developing at a fast pace and will be the topic of a subsequent review. Newly discovered infectious agents are filling some of the knowledge gaps in epidemiology. Nevertheless, it is still uncertain whether the microbial epidemiology of asthma reflects viral epidemiology in the community, or whether some of the agents, most notably human rhinoviruses (RV), possess specific 'asthmagenic' properties (3). The purpose of this review is to summarize current knowledge and developments in microbial epidemiology of acute asthma in children and adults, describing the known impact for each individual agent.

The strategy to generate the bibliography for this review was the following: the PubMed online bibliographic database was queried for the combinations of keywords: (1) 'x AND wheezing', (2) 'x AND acute asthma' and (3) 'x AND asthma exacerbation' (where 'x' was adenovirus, bocavirus, coronavirus, enterovirus, influenza virus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus, *Chlamydophila* or *Mycoplasma*) and limited to: Humans, English, published in the last 10 years. The reference lists generated from the above searches were merged into one database using Endnote X2 software (Thomson Corp., Carlsbad, CA), giving rise to a total of 654 and 121 unique articles for the viruses and the two bacterial species, respectively. Subsequently, reviews, letters and editorials were removed and the remaining original articles (456 and 85, respectively) were examined for relevance to microbial epidemiology. Finally, a number of articles of historical importance from the 1990s were added to generate the final bibliography of this review.

Abbreviations

AAE, acute asthma exacerbation; *CP, Chlamydophila pneumoniae;* hBoV, human bocavirus; hCoV, human coronavirus; hMPV, human metapneumovirus; LRI, lower respiratory tract infection; *MP, Mycoplasma pneumoniae;* PIV, parainfluenza virus; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; RV, rhinovirus; URI, upper respiratory tract infection.

Association of viral infections with acute asthma

The first studies investigating the specific viral etiology of acute expiratory wheezing in children were already performed over 40 years ago. However, because of the limited sensitivity of the methods used, the frequency of viral detection was low, ranging from 14 to 49% (4). With the introduction of molecular detection methods, the role of viruses in acute asthma exacerbations (AAE) has been increasingly appreciated. Typically, in a French study of children hospitalized for a severe AAE, PCR detected a viral agent in about 82% of the cases, while more traditional methods (virus culture and immunofluorescence) identified only 34% (5). Using molecular detection, between 62% and 95% of children with acute wheezing episodes test positive for respiratory viruses (with most studies reporting a prevalence of over 80%), in both hospital and community settings (6–20). The prevalence in adults is less, but still in the range of 41–78% (21–25). This may be because of real differences or to the fact that adults produce and shed less infective virus and/or that recent studies using PCR are not many.

Rhinoviruses are the most frequently detected virus type at all ages, with the exception of infants hospitalized with bronchiolitis, where respiratory syncytial virus (RSV) prevails. Most other agents have small to moderate involvement. The relative contribution of specific agents is described later. A summary of the data relating infectious agent prevalence and exacerbations of acute asthma is provided in Tables 1 and 2. Although co-infections with more than one infectious agent are not infrequent, and there are suggestions that multiple infections may increase the risk of asthma (26), there is still not enough evidence to evaluate the role of such occurrences.

Rhinoviruses

Pathogen characteristics

Human rhinoviruses are 7.2- to 7.5-kb, single-stranded, positive-sense, nonenveloped RNA viruses. They belong to the *Enterovirus* genus within family *Picornaviridae*. Rhinoviruses exhibit remarkable genetic and antigenic heterogeneity and comprise over 100 serotypes conveying serotype-specific immunity. They have been grouped on the basis of phylogenetic analysis of viral protein VP4/2 coding region, into two groups, HRV-A and HRV-B. Recently, a novel distinct genetic cluster, HRV-C, has been identified (27–30). Although type-C variants have not yet been cultured *in vitro* and therefore cannot be formally assigned into distinctive serotypes, existing genetic sequence data suggest that 16–28% of human RVs belong to this new clade (28, 31).

Pediatric studies

Rhinoviruses are the agents most frequently associated with the common cold as well as asthma exacerbations, with proportions reaching up to 80% (32). In children, most studies have included a wide age range (3–6 months to 13–16 years), and samples are of nasal origin. Rhinoviruses are always

Table 1 Reported prevalence of individual microbial agents in AAE, according to data from the literature reviewed in this manuscript

	Prevalence (%) in AAE			
Pathogen	Infants and pre-school-age children	Children (6–17 years)	Adults	Higher frequency in AAE than control populations
Rhinovirus	17–78 (33)	42–82 (55)	8–65 (29)	Yes
Enterovirus	12–25 (18)	5–16 (7)	?	?
Coronavirus	0–5 (2)	0–13 (1)	4–21 (12)	No
Influenza virus	1–20 (3)	0–7 (2.5)	8–25 (23)	Yes (adults only)
Parainfluenza virus	4–12 (7.5)	0–7 (2)	0–18 (0)	No
Respiratory Syncytial virus	2–68 (19)	1.5–12 (4)	0–39 (3)	Yes (infants only)
Metapneumovirus	1.5–9 (4)	4–7.5 (4.5)	7	?
Adenovirus	1.5–8 (4.5)	0–71 (0)	1–3 (2)	No
Bocavirus	7.5–19 (11)	?	?	?
Chlamydophila Pn	0–45 (4)	4–23 (11)	0–73 (13)	?
Mycoplasma Pn	1–10 (2)	0–50 (14)	0–8 (4)	Yes (children only)

Percentage range and median value (in parentheses) are shown. Percentages referring to viral species are derived predominantly by PCR techniques that are applied in the majority of studies for viral detection. Bacterial (*CP*/*MP*) detection is usually by serology and/or PCR. Remarks in the last column are based on data from case–control studies. ? = Insufficient data.
AAE, acute asthma exacerbation; *CP, Chlamydophila pneumoniae; MP, Mycoplasma pneumoniae*.

Table 2 Reported prevalence of microbial agents in AAE, according to data from the literature reviewed in this manuscript, sorted by geographical region

	Prevalence (%) in AAE (by region)					
	Children			Adults		
Pathogen	Americas	Europe	Australasia	Americas	Europe	Australasia
Rhinovirus	26–77 (57)	17–82 (40)	33–78 (42)	29–36 (33)	35	8–65 (20)
Enterovirus	5	5–25 (9)	?	?	?	3.5
Coronavirus	0–3 (1.5)	0–13 (2.5)	1.5–2 (2)	12–21 (17)	?	4
Influenza virus	0–20 (10)	0–7 (3)	1–12 (6.5)	8–13 (11)	?	12–25 (23)
Parainfluenza virus	2–6 (4)	0–7 (4)	7.5–8 (8)	0–18 (9)	?	0
Respiratory syncytial virus	8–68 (40)	1.5–61 (12)	7.5–17 (16)	3	?	0–39 (2)
Metapneumovirus	7.5	2–8.5 (4)	1.5–8 (2.5)	7	?	0
Adenovirus	0–4 (2)	0–5 (4)	1.5	1	?	2.5–3 (3)
Bocavirus	?	7.5–19 (12.5)	11.5	?	?	?
Chlamydophila Pn	4	0–45 (6.5)	3–45 (24)	?	0–73 (18)	9–18 (14)
Mycoplasma Pn	0	1–50 (5)	3	?	0–4 (3.5)	8

Percentage range and median value (in parentheses) are shown. Percentages referring to viral species are derived predominantly by PCR techniques that are applied in the majority of studies for viral detection. Bacterial (*CP*/*MP*) detection is usually by serology and/or PCR.
? = Insufficient data.
AAE, acute asthma exacerbation; *CP, Chlamydophila pneumoniae; MP, Mycoplasma pneumoniae*.

dominant in emergency/hospital settings (28% in Sweden (12), 25% (10) or 52% (including enteroviruses) (10) in Finland, 47% in France (5), 65% in Greece (33), 58% in Korea (13), 71% in the USA (7), 82% in South Africa (34)). In the community, 46–50% of wheezing episodes in 9- to 11-year-old children during a 1-year period could be attributed to RV (6, 35). In studies where a control group has been included in the design, RVs are most frequently the only pathogen significantly higher in cases, possibly because of the small number of other pathogens, whether compared with normal subjects where odds ratios range from 2 to 10 (7, 11, 19), controlled asthmatics (18), or patients with upper respiratory tract infection (URI) (36). Variability within the above studies can be explained by differences in methodologies, sampling, seasonality, duration, age distribution and more. It should be pointed out that in temperate climates RV induces disease mostly in the fall and spring, in association with return from school holidays and is the major pathogen behind the September asthma epidemic (18). Seasonality may be different in tropical climates (37).

Rhinovirus in early life and its consequences

In recent years, the importance of RV in wheezing episodes in infants and its association with subsequent asthma has been revisited. It appears that RV, rather than RSV, are the agents more frequently associated with wheezing, even in infants. In a birth cohort from Australia intensively monitoring for respiratory infections, 39% of all wheezing episodes in the first year of life were associated with RV, three times as many as those associated with RSV (14). A similar proportion has been reported before (9). In a noteworthy study, Malstrom et al. used bronchial biopsies of 47 infants (4–26 months old) with recurrent asthma-like respiratory symptoms to conduct *in situ* hybridization for RV. Rhinoviruses were detected in 45% of specimens. Abnormal lung function (decreased airway conductance) was significantly more common among RV-positive infants (86%) than in RV-negative ones (58%). Occurrence of a respiratory infection in the 6 weeks preceding bronchoscopy significantly correlated with RV positivity (38).

Furthermore, recent evidence suggests that early RV infection is strongly associated with asthma symptoms and/or persistence later in childhood, once again more than RSV (9, 16, 17). The duration of such associations awaits further follow-up of these cohorts.

The time course of RV infection in AAE is still not completely understood. It seems that RV may induce early viremia, probably in more severe cases (33) and may persist for several weeks (34, 39), although this has been challenged with the possibility of frequent re-infections (40).

Studies in adults

In adults the pattern is similar, although the literature is less extensive. In South African adults with acute severe asthma who required hospital admission, RVs were detected in nasal aspirates by PCR in 35% on admission, in 16% after 7 days, in one patient after 28 and 56 days and were absent in controls (41). In a North American study, 29% of asthma exacerbations in adults were associated with RV in a longitudinal arm and 36% in a cross-sectional one (22). Somehow lower prevalence has been reported from Australia (8% and 11% in hospitalized patients or in the emergency room, respectively) (24, 42). A study from Singapore on adult patients with near-fatal asthma requiring mechanical ventilation and patients with acute asthma, found RVs (picornaviruses) in lower respiratory samples in 47% and 28%, respectively (23). In one study, detection in 65% of cases has been reported (25).

RV-C: a newly identified pathogen

A considerable number of studies have recently focused on the role of the newly identified RV-C group and suggest that a major proportion of all RV infections are RV-C infections (27, 43–46). Wheezing or AAE are common clinical manifestations of RV-C infections (27, 44–46). In 142 asthmatic children over 2 years of age, RV-C comprised one-third of RV-positive cases and was found exclusively in children with an AAE (31). Interestingly, a strong interfering effect of RV-C infections on susceptibility to co-infection with other RNA respiratory viruses, especially RSV, has been reported (47).

Enteroviruses

Enteroviruses also belong to the family of *Picornaviridae* and share many of their viral features with RVs (48). Often, the differentiation between enteroviruses and RVs is difficult, as many studies use methods that detect picornaviruses without differentiating between RVs and enteroviruses. Direct data are still lacking in adults. In cohort studies of exacerbating children, the reported prevalence is 9.8–18%, mostly evaluated by PCR (5, 8, 9, 39). Higher prevalence has been reported in one study that assessed 293 hospitalized children over 2 years (25%) (10) and in another study that evaluated samples only during the summer (29%) (36).

In two case–control studies, prevalence was lower ($< 5\%$) and not significantly different from controls, although the studies were probably not powered adequately for such comparison (11, 19). More studies are needed to evaluate the contribution of enteroviruses in AAE.

Coronaviruses

Human coronaviruses (hCoV) are single-stranded positive-sense RNA viruses. Historically two different groups were distinguished, represented by hCoV-229E and hCoV-OC43 (48). Recently, two more strains relevant to AAE, hCoV-NL63, (49) and hCoV-HKU1 were discovered (50). Coronaviruses are regarded as important common cold agents. Overall, reported respiratory infection rates for hCoV among children usually range from $< 1\%$ to 9% with varied distribution between subtypes (13, 20, 51–55).

Early findings suggested an association of hCoV with AAE in 9- to 11-year-old children (13%) (6) and adults (12–21%) (22). However, more recent studies, all using PCR with or without additional methods, have yielded rather low frequencies. In children, some studies were unable to identify hCoV at all (9, 18), while others did so at a level of 1–4.5% (5, 10, 20). Frequently, hCoV infections are co-infections with other viruses (20). Similar numbers were found for the newer strain hCoV-NL63 in pre-school children from South Africa (2.4%) and Korea (1.3%) (13, 56). In case–control studies, hCoV was not present more frequently in wheezing children in comparison with controls (11, 14, 19). In a study of 49 adults presenting to the emergencies with AAE, hCoV was found in 4% of induced sputum samples (24). Overall, hCoV seem to have a minor, if any, contribution to AAE.

Influenza viruses

Influenza viruses belong to the orthomyxoviruses. Influenza produces yearly epidemics during the winter months; this should be taken into account in the interpretation of the studies on AAE. For example, when sampling AAE during

September, no influenza cases were found (controls 1%) (18), while the highest reported proportion comes from infants during the flu season (20%) (11). Nevertheless, most studies in children report an overall low prevalence, ranging from close to zero (13, 19), up to 7% in a 1-year cohort in the community (6), with a median of around 4% (5, 8, 10, 20). However, among children with asthma, exacerbations caused by influenza may lead to hospital admission, and healthcare utilization attributable to influenza is high (57–59). The 2009 pandemic H1N1 influenza virus has also been reported to induce severe exacerbations in patients with asthma, mostly children (60); however, the detailed kinetics of this epidemic in relation to asthma is outside the scope of this review.

In contrast, prevalence in adults with AAE appears to be higher. Several studies from patients in the emergency department or hospitalized with AAE have identified influenza viruses in around 20–25% of cases (23, 24, 42, 61), while less have been reported in severe patients (6–8%) (22, 23). The differential response between pediatric and adult asthmatic patients to influenza viruses is interesting, it needs however confirmation through case–control studies, before it can be helpful in designing public health strategies with respect to influenza protection.

Parainfluenza viruses

Parainfluenza viruses (PIV) belong to the *Paramyxoviridae* family. In studies evaluating viral involvement in AAE in children, PIV have been found in 0–7.4% of cases (median 4%) (5, 6, 8, 10, 13, 19). However, case–control designs have failed to show significantly increased prevalence in comparison with either nonwheezy lower respiratory tract infection (LRI) or URI (14, 15), healthy controls (11), or stable asthmatics (18).

In adults, while one longitudinal cohort found PIVs in 18% of AAE in the community, it failed to identify any in patients at the emergency department (22). Similarly, no PIV was found in near-fatal asthma or AAE in Singapore (23). In summary, PIVs are detected at low frequency in AAE in children; an association is doubtful. No conclusion can be drawn yet in adults.

Respiratory syncytial virus

Respiratory syncytial virus belongs to the genus *Pneumovirus* within the family *Paramyxoviridae*. There are two known serotypes A and B. Most infections occur from December to February, and it is the main pathogen associated with severe bronchiolitis in infants (48, 62, 63). Prevalence of RSV in acute wheezing can be dramatically different depending on age, but also between studies. RSV has been associated with at least four different wheezing phenotypes: acute bronchiolitis, postbronchiolitis, the inception of asthma, and AAE (64). In infancy and early childhood differentiation between bronchiolitis, acute wheeze and AAE is difficult, making studies with a wide age range equally difficult to interpret.

In infants, prevalence can vary from as low as 16% (9), to as high as 54–68% (7, 10, 20). It should be noted however that the majority of these infants were or could be diagnosed as bronchiolitis. In a birth cohort study from Australia, RSV was the second most frequently detected virus type (after RV) in wheezy LRI cases (16.8%) (14). Similar results in a community setting have been reported from the United Kingdom (27%) (65).

In pre-school children hospitalized for wheezing, prevalence can remain as high as 30% (66), but can be lower (7.4%) (13). In older children, RSV has been associated with 4–12% of AAE (6, 18, 34).

In studies including a wide age range, results are not consistent and difficult to interpret with some studies finding very low prevalence (1.5–3.6%) (8, 19) and other at the range of 20–40% (5, 10, 11).

In adults with acute asthma early studies have reported a low proportion (1.3–3%) (22, 42). In contrast, two more recent studies from Australia found RSV in 37% and 39% of AAE in the emergency department using induced sputum samples (24, 61). However, in older (>65 years) adults, where RSV is believed to reappear as an important infectious pathogen, RSV accounted for 7.2% of hospitalizations for asthma (67), while no RSV could be detected in near-fatal asthma in Singapore (23).

In summary, RSV is a major viral pathogen inducing wheezing episodes in infants and pre-school children with a frequency of up to 70%. RSV may also be important in older children and adults; however, we cannot yet explain the high variability reported in the literature.

Human metapneumovirus

Human metapneumovirus (hMPV) is a recently discovered respiratory virus belonging, like RSV, to the genus *Pneumovirus* within *Paramyxoviridae* (68). Frequencies of detection of hMPV in respiratory tract infections (RTI) varies from 2 to 3% to just over 20%, with most studies reporting 5–9% and virus activity peaking in winter/early spring, similarly to the seasonal distribution of RSV. Symptoms reported are also similar to RSV and include URI, bronchiolitis and pneumonia. In a large Australian study conducted over four consecutive years, over 10 000 nasopharyngeal aspirate (NPA) specimens from patients with acute lower RTI were tested. Of these, 7.1% were hMPV positive, with 92% of hMPV-positive children being <5 years of age (69).

Infection with hMPV has been associated with AAE and wheezing, mostly in young children. The majority of studies have been performed in hospitalized pediatric populations: when inclusion was because of an asthma exacerbation, hMPV has been detected in around 4–8% (10, 13, 19, 62); however, this was not necessarily higher than the control population (19). Increased impact has been observed in younger children [1.9% *vs* 0% in infants younger than 3 months *vs* those 3–12 months old (70) or 8.9% in children younger than 3 years in comparison with 7.5% in the whole population of 133 children (71)]. When study inclusion was based on specific infection rather than clinical presentation, the

proportion who wheezed with hMPV was 25% in a French study and 55% in an Israeli one, significantly higher in comparison with RSV or influenza, respectively (63, 72). Slightly lower prevalence rates have been reported in children presenting to emergency departments (hMPV positive in 2.2–2.8%) (36, 73). Exacerbations were more frequent in children with hMPV, in comparison with those with RSV or influenza infection (73). In South Africa, among the 'novel' respiratory viruses [hMPV, hCoV-NL63 and human bocavirus (hBoV)], hMPV was found to be the most prevalent (8.3%), with a frequency peaking for children aged 7–9 months (56).

In a birth cohort study from Australia, wheezing was present in 29% of the 329 episodes of LRI within the first year. hMPV was detected in 3% of LRI in general and was 10 times more likely to be associated with nonwheezy LRI (n-wLRI), than wheezy (wLRI) (14). In a later report from the same group describing a subpopulation of the original report, hMPV was identified in 4.7% of n-wLRI and in 1.4% of wLRI cases in children at high atopic risk (15).

In adults, in a large group of hospitalized patients in the USA (1386 adults, mean age: 75 years), hMPV incidence was 8.5% over four consecutive winters, with wheezing being a common clinical characteristic (80%) in infected patients (74). In another North American study, Williams et al. have ascribed an etiologic role of hMPV infection in triggering AAE, because none of the 6.9% of patients hospitalized for AAE who were hMPV positive (by PCR) on admission, tested positive 3 months after discharge (75). In a study of 88 Egyptian adults presenting, among others, with AAE (67%), hMPV was significantly associated only with pneumonia (76).

In summary, hMPV is detected mostly in young children with wheeze, at the age where AAE is difficult to distinguish from acute bronchiolitis, with a frequency $<10\%$. Its association with asthma later on in life is less clear.

Adenoviruses

Adenoviruses are nonenveloped, double-stranded DNA viruses. Forty-seven human adenovirus types are classified and subdivided into six subgenera A to F. Infection may be productive, abortive, or latent (chronic). In latent infections as well as in transformed and/or tumor cells, viral DNA is integrated into the host genome (48). Virus–host DNA recombinants are also found in productive infections. Adenoviruses bind to a receptor called CAR (coxsackie and adenovirus receptor) (77).

In wheezing children, adenovirus detection rates are 1.3–5% (5, 10, 13, 20), with slightly higher frequencies (7–8%) in general diagnoses of acute RTI (51, 78).

In a case–control study of 133 children hospitalized for wheezing, no significant difference was observed in adenovirus frequencies compared to controls (PCR) (11). In two other case–control studies of children hospitalized for asthma exacerbations the investigators failed to detect adenovirus at all using PCR (18, 19).

In contrast to the above, in a study from Turkey, adenovirus DNA was detected in 20/28 (71.4%) of children with acute asthma exacerbation, while it was 33–37% in asymptomatic controls or healthy subjects (79). Taking into account, the quite high proposition of positives in the control groups, these results should be interpreted with caution.

In adults, the frequency is low ranging from 0.7 to 2.5% of asthma exacerbations (22, 42). In contrast, in a study from Singapore on adult patients with near-fatal asthma requiring mechanical ventilation and patients with acute asthma, adenoviruses were found in lower respiratory samples in 24% and 3%, respectively (23). These results suggest a minor role of adenoviruses in AAE in adults with the exception of near-fatal asthma, where a special role should be further investigated.

Human bocavirus

Human bocavirus belongs to the *Parvoviridae*, which are nonenveloped, single-stranded DNA viruses (80). Several studies in children have used PCR to identify hBoV in respiratory tract infections worldwide, with reported detection rates between 1.5% and 8% (19% in one case) (12, 51, 54, 81, 82). Co-infection with other viruses is fairly frequent (14–60%), while in the small number of case–control studies the detection rate in healthy individuals is almost zero.

In a study of 231 Korean children (1 month–5 years) hospitalized with acute wheezing, hBoV was the third most frequently detected virus, after RV and RSV, with an incidence of 5.6% (13). Similarly, Allander and colleagues identified hBoV as the sole viral agent in 5% of 259 children (3 months–15 years; median age 1.6 years) hospitalized for acute expiratory wheezing; mixed hBoV infection with other viruses was detected in 19% (12). In 710 Spanish children hospitalized with respiratory infection, hBoV was found in 13.9% (mostly infants), with one-third of these being single-positives. The virus was associated with recurrent wheezing in half of the cases (83). A similar frequency in acute wheezing (7.4%) was reported from South Africa (56). Reports so far indicate that hBoV may be among the agents associated with AAE, but more studies are needed. It has been suggested that serological analysis and serum PCR, in addition to nasopharyngeal material PCR, may be needed to optimize detection rates (84).

Bacteria

Atypical bacteria such as *Chlamydophila pneumoniae* (*CP*) and *Mycoplasma pneumoniae* (*MP*) represent an important cause of human respiratory tract disease, involved in URI, acute bronchitis, exacerbations of chronic bronchitis, and pneumonia. Clinical diagnosis is based on the micro-immunofluorescence test for *CP*, and specific IgM/IgG EIA for *MP*. Molecular diagnosis (PCR, RT-PCR) can also be used; however, these assays are not yet optimized and cannot readily differentiate between acute and latent (chronic) infection.

Pediatric studies

A longitudinal study of 108 children (9–11 years old) with asthma symptoms showed no difference in the frequency of

CP detection when sampling symptomatic (23%) or asymptomatic periods (28%) (85). Nevertheless, *CP*-specific secretory IgA was much higher in subjects with four or more exacerbations, compared to those with one, suggesting that chronic *CP* infection is associated with the frequency of AAE. In a French study of 132 nasal aspirates from 75 children hospitalized for a severe attack of asthma, *CP* and *MP* were identified by PCR in 4.5% and 2.2%, respectively (5). In another population of children hospitalized for severe asthma, *MP* was identified in 20% of 119 children with known asthma and in 50% of 51 children hospitalized because of their first asthma attack. The frequency of *CP* was lower (3.4% and 6%, respectively). Asthma recurred among 62% of those in whom an atypical pathogen was diagnosed, but in only 27% of patients not infected with an atypical pathogen, suggesting an effect of *MP* in initiation and recurrence of asthma (86). Lehtinen et al. determined bacterial co-infections in 220 children hospitalized for acute wheezing with defined viral etiology. Bacterial co-infections were more frequent (22%) for patients positive for RV, compared to children positive for other respiratory viruses; *MP* was the second most frequent bacterial species identified, with an incidence of 5% (87).

In a recent case–control study from Japan, 103 infants (3–23 months), hospitalized for an acute episode of wheezing, and 64 healthy matched controls were tested for the presence of *CP* by serology and reevaluated after 1 year. Wheezing infants had significantly higher *CP*-IgM levels and were more frequently classified as *CP* infected (44.6%), compared to controls (17%), while asthma developed significantly more frequently in wheezing infants with than without *CP* infection (70% *vs* 20.7%, respectively) (88). In a case–control study from Italy, 71 children (2–14 years) presenting to the pediatric emergency department with an acute episode of wheezing had a significantly higher incidence of acute infection with *MP* (22.5%) and *CP* (15.5%), than healthy controls (89). During a 3-month follow-up, recurrent wheezing was significantly more frequent in nonantibiotic-treated children with acute *MP* and/or *CP* infection, than in children without. In another study of 82 acute asthmatic children in northern France, 5% were infected with *CP* and an additional 5% with *MP*. Persistent symptoms and reduced recovery after 3 weeks were more frequently associated with atypical bacterial infections compared to viral infections (8). In a study from Turkey including 79 asthmatic children (37 with acute asthma attacks, 42 with stable asthma) and 36 healthy controls, significantly higher *MP*- and *CP*-specific IgM was found between the acute asthma and the other two groups (90).

In contrast, a number of epidemiological studies of pediatric AAE report low or no detection of atypical bacteria. An Italian, 6-month study of 85 infants hospitalized for a first episode of wheezing detected *MP* as a single pathogen in only one child; *CP* was not detected at all (20). Low infections rates (≤4%) and no significant difference between controls and asthmatic children during the September epidemic in Canada were reported by Johnston et al. (18). A birth cohort study in Australia also reported low frequency of detection for CP and *MP* (< 3%) with no significant difference between controls and patients (14, 15).

Adult studies

In adults, an early British study did not provide evidence for a significant association between *CP* infection and acute asthma serologically comparing 123 patients hospitalized with acute asthma *vs* 1518 controls (91). In contrast, *CP* infection was confirmed in 168 Japanese adults with AAE by three different methods and found to be significantly higher (1.2–8.9%) than matched controls (n = 108; 0–2.8% positive) (92). Wark et al. observed a fourfold increase in *CP* IgG or IgA titers in 38% of patients with acute asthma (93). In the *Telicast* study, IgM for CP was positive in around 60% of cases (94). In a case–control study from Italy, *CP* was identified as a single agent in 19 of 58 patients referring to the Emergency Department with AAE, while *MP* was detected in only 2/58. The severity of exacerbations was higher in patients with acute atypical infections at admission and up until 2 weeks later (95). In an study from Israel, screening for 12 respiratory tract pathogens, including five bacterial species, by serology, *CP* was the only bacterial agent significantly more frequent (18%) in 100 hospitalized asthmatic adults than in matched controls; *MP* prevalence in the former group was lower (8%) (96).

Nevertheless, as in the case of children, a number of studies in adults report no association between *CP*/*MP* infection and AAE. Out of 30 adult asthmatics hospitalized for an exacerbation over a 12-month period in Italy, 6.6% were identified with *CP* and *MP* infection (3.3% each pathogen), by both serology and PCR. However, no significant difference was observed compared to matched controls with no signs of respiratory illness (n = 40) (97). In a British study, 60 adult patients (aged 17–50) admitted to hospital with acute asthma were each matched for sex, age, and smoking status with two control subjects: patients with stable asthma and inpatients with nonrespiratory conditions. *CP* and *MP* were not detected in any of the study groups by PCR (98).

In conclusion, atypical bacteria seem to be inconsistently associated with AAE. This could be explained by suboptimal detection and/or it could be because of significant temporal variability. Nevertheless, more information is needed, especially in regard to the persistent nature of these infectious agents; methodological improvements in diagnosis are also needed.

Conclusions

In recent years, the development of sensitive, robust, and rapid diagnostic methodologies such as PCR has aided the design and implementation of epidemiological studies that have highlighted the importance of viral infections as precipitants of asthma exacerbations in both children and adults. The involvement and preponderance of RV is clear, and the plethora of data/studies associating this virus type with AAE leaves little doubt about the importance of this pathogen, although the kinetics of RV infection in the community still

requires attention, especially considering the newly identified RV-C. The relationship with different wheezing illnesses is also clear for RSV, which is mostly prevalent in infants. The association with other respiratory pathogens is not as clear with sometimes inconsistent results, possibly because of the relatively small proportion of cases for each, in addition to geographic and temporal variability. This is particularly true in regard to any possible involvement of bacteria in exacerbations, on which very little information is available.

Several important questions should be addressed in further research studies. Taking into account that respiratory pathogens may interact between themselves and with other factors, such as pollution, allergens, stress, nutrition, etc., in the induction of AAE, large studies with comprehensive analysis of infectious agents and other possible precipitants are among research priorities to evaluate the relative contribution of respiratory pathogens in asthma exacerbations. As breakthroughs in microorganism detection become available for epidemiological use, in parallel to bioinformatics tools for their analysis, extensive virology and bacteriology should become a vital part of longitudinal cohort studies, aiming at better understanding the kinetics and natural history of microbial exposures, and reveal new targets for therapeutic intervention. Such interventions will also be needed to confirm causality behind the described associations.

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