CORINA CAZAN

BACTERIAL SUPERINFECTION OF THE RESPIRATORY VIRUSES

Universitatea Lucian Blaga Sibiu (Lucian Blaga University Sibiu, România)
Spital Clinic de Pediatrie Sibiu (Pediatric Clinic Hospital Sibiu, România)
Centrul de Cercetări şi Telemedicină în Bolile Neurologice la copil
(Research and Telemedicine Center of Neurological Diseases in Children)

REZUMAT

SUPRAINFECTIA BACTERIANA A INFECTIILOR RESPIRATORII VIRALE

Actualitatea temei. Suprainfecţia bacteriană a infecţiilor respiratorii virale este un fapt confirmat, atât prin diagnosticul clinic, cât şi cel molecular. *Material şi metod*ă. Au fost luate în considerare studiile care au rectrutat pacienţi de vârstă pediatrică în vederea identificării virale, bacteriene, precum şi coinfecţia viro-bacteriană şi suprainfecţia bacteriană a infecţiilor respiratorii virale premonitorii. *Rezultate*. Studiile au consemnat creşterea incidenţei patogenilor virali odată cu dezvoltarea tehnicilor de detecţie moleculară, modificarea etiologiei bacteriene odată cu introducerea vaccinării conjugate antipneumococice. Coinfecţia viro-bacteriană sau prezenţa concomitentă a mai multor tulpini virale cresc severitatea bolii, cu o staţionare de lungă durată în unităţile de terapie intensivă. *Concluzii*. Suprainfecţia bacteriană a infecţiilor respiratorii virale rămâne un fapt, cu implicare terapeutică şi evolutivă. **Cuvinte cheie**: patogenii respiratori virali şi bacterieni, infecţia respiratorie virală, coinfecţia viro-bacteriană, suprainfecţia bacteriană.

SUMMARY

BACTERIAL SUPERINFECTION OF THE RESPIRATORY VIRUSES

Theme topicality. Bacterial superinfection of the respiratory viruses is a confirm fact, either by clinical diagnosis, and molecular one. *Material and methods*. It were considered some studies which have been recruited pediatric patients for viral and bacterial identification, also for viral and bacterial coinfection and superinfection of the premonitory viral respiratory infection. *Results*. The studies have been recorded an increase incidence of viral pathogens with the improvement of molecular detection techniques, and the bacterial etiology changes with the introduction of conjugate pneumococcal vaccin. Viral and bacterial coinfection or the concomitant presence of a multiple viral strains increase the severity of disease, with a long length-of-stay in pediatric intensive care units. *Conclusions*. Bacterial superinfection of viral respiratory infection remains a fact, with therapeutic and evolutive implication. **Keywords**: viral and bacterial respiratory pathogens, viral and bacterial coinfection, bacterial superinfection.

Introduction

Viral infection-bacterial infection relationship (coinfection, superinfection). In prevaccinal era (conjugate pneumococcal vaccin), S. Pneumoniae was considered bacteriologic leader causing lower airways infection, respectively pneumonia (community-acquired)[4]. S. Pneumoniae has followed, as frequency, by Haemophylus influenzae, Streptoccocus pyogenes, Staphiloccocus aureus and Moraxella catarrhalis. Enteric gram-negative bacteria and those intracellular and atypical (Mycoplasma pneumoniae şi Chlamydophila pneumoniae)[13,15,25] were also reported. With introduction of multi-valent and conjugate haemophilus and pneumococcal vaccine, the frequency of community-acquired pneumonia was significant reduced in countries with a complete national program of vaccination. In developing countries, with an incomplete vaccine program, bacteria remains the main etiologic factor of community-acquired pneumonia.

With the improvement of molecular diagnosis, viruses with respiratory tropism are detected with a higher frequency in children diagnosed with pneumonia, especially those younger than 5 years. The most frequent viruses are respiratory syncytial virus, influenza, parainfluenza, rhinovirus and adenovirus[12]. The newest molecular techniques have allowed identification of metapneumovirus, human coronavirus, and human bocavirus. In industrialized countries up to 81 % children with community-acquired pneumonia claim viral etiology, especially respiratory syncytial virus (up to 48 %).

It exists more and more evidences regarding the important role of respiratory viruses in facilitating bacterial colonization in child. Most of those refer to lower airways infection, respectively pneumonia (community-acquired pneumonia). The mechanism is not totally known, either the faciliting bacterial colonization is producing directly, or viruses contribute of development of severe forms of

disease. Viruses make a vulnerability of host respiratory epithelium, thus favoring the initiation of respiratory bacterial infection. If the bacterial etiology is significant influenced by conjugate pneumococcal vaccine, S. pneumoniae infection being the leader of bacterial infection, the viral etiology being influenced only by improvement of viral techniques detection, the most frecquent viruses have been influenza, respiratory syncytial virus, parainfluenza and adenovirus[30,31]. And the invazive pneumococcal infection has observed mostrly in the context of viruses[19]. The proof of viral and bacterial coinfection is certain, in or not directly relationship with bacterial superinfection of respiratory viral infection. A trial made in South Africa which included children with conjugate pneumococcal vaccination has proven a decrease of pneumonia with S. pneumoniae incidence, also of viral pneumoniae, just in context of respiratory viruses and pneumococcus relationship[26,27]. Much more than that, the Australian[12] statistical data have shown a decrease of viral pneumonia incidence as effect of pneumococcal vaccination. The respiratory syncytial virus has recorded as favorable factor of bacterial colonization with H. influenzae and S. aureus in 25 % of hospitalized patients with severe form of A type influenza infection during 2000 pandemia[2,3]. The patients which have presented viral and bacterial coinfection have presented a severe evolution, with ventilator support and long length-of-stay in intensive care unit[23,24].

Metodology. The relationship between naso-pharyngeal pathogens and the form of severity of lower airways infection has studied in a couple of clinical studies. The naso-pharyngeal pathogens could be considered as predictive factors for pneumonia severity in child. A study made in USA has demonstred a link between respiratory syncytial virus infection and long hospitalization, respiratory failure and intensive treatment. In Vietnam, children with radiologic confirmed pneumonia were detected with pneumococcal infection in a much larger proportion than other etiology, and the pneumococcus presence was 15 times more in patients with viral and bacterial coinfection. Other studies, in Kenya and Holland have reported a nasopharyngeal load of respiratory syncytial virus in children with severe forms of disease.

In comparison with many studies with the same target – the determination of community-acquired pneumonia etiology, a few studies have included healthy children or asymptomatic ones, as control group. The naso-pharyngeal carriage was frequent associated with healthy children or asymptomatic ones. Are required studies which must use supplement investigation to certify that asymptomatic carriage is a fact in healthy population, and its involvement in community-acquired pneumonia become a challenge.

Although the conjugate pneumococcal vaccination has reduced a lot the hospitalization rate of community-acquired pneumonia in industrialized countries, pneumococcus remains an important pathogen regarding non-vaccin serotype with severe and complicate pneumonia potential (inclusively, empiema).

The incidence of viral infection, bacterial infection, ciral and bacterial coinfection and bacterial superinfection varies according to level of income country, patient age and utilized sample (naso-pharyngeal fluid, sputum, broncho-alveolar fluid)[32].

Results. Start with 1993-1995 period, in Finland[22], a study which included children younger than 18 years, diagnosed with community-acquired pneumonia has shown the presence in naso-pharyngeal aspiration of viral pathogen in 62 percent, and of a bacterial pathogen in 53 percent. The pathogens frequency was: S. pneumonie 37 %, respiratory syncytial virus 29 %, human rhinovirus 24 %, parainfluenza 10 %, H. influenzae 9 %, adenovirus and M. pneumoniae each one 7 %, influenza and Moraxella catarrhalis each one 4 %.

The other study[29], performed in 1999-2000 period, made in USA, which included children 6 weeks-17 years old, diagnosed with pneumonia, has showed the following pathogens in naso-pharyngeal aspirate: S. pneumoniae 44 %, influenza 21 %, M. pneumoniae 14 %, respiratory syncytial virus and parainfluenza each 13 %, Chamydophila pneumoniae 9 %, adenovirus 7 %, rhinovirus 3 %.

In 2001-2002 period were performed two studies:

– in Italy[10], children 3 months-16 years old, diagnosed with pneumonia, used naso-pharyndeal swab and blood culture as a detection methods (M. pneumoniae 27 %, S. pneumoniae 18 %, human parainfluenza 12 %, influenza 9 %, human metapneumovirus 5 %);

– in Japan[36], children 1 month-13 years old, diagnosed with pneumonia, used naso-pharyngeal swab and blood culture as a detection methods (respiratory syncytial virus 48%, S. pneumoniae 36 %, H. influenzae 26 %, influenza 22 %, M. pneumoniae 17 %, parainfluenza 14 %).

In 2003-2005 period, în Switzerland[5], was initiated a study which recruited children 2 months-5 years old, diagnosed with pneumonia. The results revealed the presence in the naso-pharyngeal aspirate of the following respiratory pathogens: bacteria, 72 percent (S. pneumoniae, 46 %; M. pneumoniae, 11 %; C. Pneumoniae, 7 %) and viruses, 67 percent (human rhinovirus, 20 %; influenza, 14 %; respiratory syncytial virus and human metapneumovirus, each 13 %; adenovirus, 7 %).

Other study, performed in 2004-2006 period, in Spain[8], recruted children younger than 3 years, diagnosed with pneumonia, use for pathogen detection naso-pharyngeal aspirate. The study showed the following results: viral and bacterial coinfection (30 %), viruses 67 percent (respiratory syncytial virus 20 %, human bocavirus and

rhinovirus each 14 %, human metapneumovirus 12 %, parainfluenza 11 %, influenza 7 %).

A study from USA[34], performed between 2005-2007 period, with patients younger than 3 years, revealed the following:

– for diagnosis of airways infection, with samples from blood and naso-pharyngeal swab, were identified viral pathogens in 90 percent (human rhinovirus 44 %, adenovirus 30 %, respiratory syncytial virus 23 %, parainfluenza 18 %, human metapneumovirus 15 %) and bacteria in 3 percent (S. pneumoniae 2 %);

– for asymptomatics, with samples from naso-pharyngeal swab, were identified viral pathogens in 52 percent (human rhinovirus 33 %, adenovirus 16 %, human metapneumovirus 7 %, respiratory syncytial virus 4 % and parainfluenzae 3 %).

The same differentiation, children with respiratory infection and asymptomatics, was performed in a study from Holland[20], in 2007-2009 period, children younger than 6 years, with samples collected from nasal fluid:

– for airways infection, the viral pathogens were present in 72 percent (respiratory syncytial virus 26 %, human rhinovirus 20 %, adenovirus 9 %, metapneumovirus and influenza each 6 %);

– for asymptomatics, the viral pathogens were present in 26 percent (human rhinovirus 16 %, adenovirus 9 %, human coronavirus 5 %, influenza 3 % and respiratory syncytial virus 1 %).

In 2006-2007 period, in Finland[16], was initiated a study which recruited children 6 months-15 years old, diagnosed with pneumonia. The results revealed the sputum presence of the following pathogens: bacteria, 91 percent (S. pneumoniae, 50 %; H. influenza, 38 %; S. aureus, 13 %) and viruses, 72 percent (human rhinovirus, 30 %; bocavirus, 18 %; metapneumovirus, 14 %, parainfluenza, 8 %; respiratory syncytial virus, 7 %).

A study performed in Holland[35], in 2008-2011 period, with patients 3 months-16 years old, showed the following:

– for airways infection, with samples from blood and naso-pharyngeal swab, were identified bacterial pathogens (S. pneumoniae 28 %, Moraxella catarrhalis 23 %, M. pneumoniae and H. influenzae each 16 %, S. aureus 10 %);

– for asymptomatics, with samples from blood and naso-pharyngeal swab, were identified bacterial pathogens (S. pneumoniae 28 %, M. pneumoniae 21 %, S. aureus 21 %, Moraxella catarrhalis 18 %, H. influenzae 15 %).

Other study performed in USA[18], between 2010-2012, with patients younger than 18 years, evidentiated the following:

– for pneumonia diagnosis, with samples from blood and naso-pharyngeal swab, were identified viral pathogens in 66 percent (respiratory syncytial virus 28 %, human rhinovirus 27 %, human metapneumovirus 13 %, adenovirus 11 % and influenza 7 %), bacterial pathogens in 8 percent (M. pneumoniae 8 % and S. pneumoniae 4 %);

– for asymptomatics, with samples from naso-pharyngeal swab, were identified viral pathogens represented by human rhinovirus in 17 percent (other viruses, less than 3 % percent); viral and bacterial coinfection was identified in 7 percent.

A study performed in Sweden[33], in 2011-2014 period, in children younger than 5 years, has shown the following results:

– in children with pneumonia, from naso-pharyngeal aspirate, were identified viral pathogens in 81 percent (respiratory syncytial virus 32 %, human metapneumovirus and human rhinovirus each 23 % and adenovirus 15 %)

– in asymptomatic, from naso-pharyngeal swab, were identified viral pathogens in 56 percent (human rhinovirus 27 %, human bocavirus 21 %, coronavirus 12 %, adenovirus 7 %, respiratory syncytial virus 6 %).

Disscusions. As can be seen in the presented studies the frequency of respiratory pathogens varies from country to country, according with income level, vaccination program, detection methods used, samples used. Over the decades, with the conjugate pneumococcal vaccine initiation, the S. pneumoniae frequency significant decreased (from 37 % to 4 %). According as, with the improvement of molecular detection techniques, the frequency of viral pathogens increased. The last years studies propose as target the viral and bacterial coinfection detection.

The distribution of identified pathogens in community-acquired pneumonia is variable from country to country[1,11,17,21,28]. Thus:

– for viral pathogens

  • respiratory syncytial virus was detected in a high percent in Kenya (34 %), Nigeria (30,4 %) and USA (28 %) and almost insignificant in Gambia (4 %);

  • rhinovirus was detected in a high percent in USA (27 %) and much less in Great Britain (8,5 %)

  • human metapneumovirus, the most detected in USA (13 %) and insignificant in Great Britain (0,7 %)

  • influenza, in the same percent of detection (between 2 and 7,4 %), knowing the fact that both the virus and the vaccine have the strongest impact in community

  • bocavirus was reported in a less percent and only

in a few countries (3,3 %, in Great Britain and 4 %, in Gambia)

- adenovirus presented the most important detection in USA (11 %)
- parainfluenza, with the big rate of detection in Nigeria (19,5 %)

– for bacterial pathogens

- S. pneumoniae, detected in an extreme percent in Gambia (91 %), and in the opposite percent in USA (4 %)
- H. influenzae, detected in 23 percent in Gambia and 2,3 percent in Great Britain
- A Streptococcus, detected in 10,5 percent in Great Britain and 1 percent in USA
- S. aureus, detected in an important percent only in Nigeria
- M. pneumoniae, (8-9,9 percent)
- Moraxella catarrhalis, in 2,3 percent in Great Britain
- Klebsiella pneumoniae, 15,3 percent in Nigeria and insignificant one in Great Britain (0,8 percent).

The results per se are influenced by sample methods of detection of respiratory pathogens. This stage of diagnosis (molecular diagnosis) become very important, especially in the situation when clinics and radiologic exam are not able to sustain the diagnosis of pneumonia (community-acquired).

The British Thoracic Society and The Infectious Disease Society of America guidelines recommend the PCR virusal tests or immunofluorescence, while for bacterial detection the methods varied according to the forms of gravity of infection.

In Great Britain are used the following tests: blood culture, real-time PCR, naso-pharyngeal fluid, pleural culture, pneumococcal antigen test, endotracheal tube aspirate, broncho-alveolar fluid.

In USA are used the following tests: blood culture, naso-pharyngeal fluis, oro-pharyngeal fluis, pleural culture, broncho-alveolar fluid, culture from endotracheal tube aspirate.

In Gambia are used the following tests: culture from broncho-alveolar and pleural fluid for non-molecular serotypes, uniplex and multiplex PCR, multilocular sequence typing, molecular serotyping.

The virusal diagnosis has been revolutionized in the last 20 years, with ADN detection. With the routinely multiplex PCR technology use in community-acquired pneumonia diagnosis in child, the presence of mutiple virusal pathogens become a fact, with a rate of 30-40 percent of 4 different types at a single case[7].

The significance of this multiple presence remain unclear[6]. For respiratory syncytial virus the coinfection with other viruses creates an increase severity of disease[9]. Recent identified, human bocavirus was associated with a rate of 83 percent of coinfection, when the pathogen represents the etiologic factor per se or an exacerbation factor, or an accidental detection. This context remains unclear. The significance threshold utilization for real-time qPCR[14] for estimative viral load has significance in association with evidence clinics of a viral infection.

The determination of viral etiology represents a challenge in countries where the molecular diagnosis is not routinelly used. There are some studies which demonstrated a high specificity and a negative predictive value in detection of parainfluenza and adenovirus from naso-pharyngeal aspirate, but a discordance between broncho-alveolar fluid and naso-pharyngeal fluid in detection of bacterial infection.

Concluzii. It is widely accepted the idea of bacterial infection that follow a viral one. The ideal bacterial identification is that one which utilized a sample directly obtains by lung without any host flora contamination. It is very interesting the fact that the viral identification from brincho-alveolar aspirate has a lower rate comparing with naso-pharyngeal aspirate. The blood culture has a limited place (because a higher rate of false positive results). Stupum cultures have a limit regarding the modality of collection (after hypersaline solution nebulization or thoracic wall percussion, with the posibility of contamination from upper airways). Conversely, the serologic tests are considerate the diagnosis gold-standard for bacterial pathogens (M. pneumoniae and S. pneumoniae). The last generation is considered the multilocular sequence, and for the future the technology of isothermal amplification.

These molecular detection techniques brought major changes in the etiology of respiratory airways infection. What was believed in the past (bronchiolitis, due to respiratory syncytial virus, does not known the bacterial superinfection) seems to be not longer suoport today (respiratory syncytial virus not only are the favorizant factor for bacterial superinfection, but the coinfection produced a severe form of disease).

LITERATURE LIST

1. Berkley JA, Munywoki P, Ngama M, Kazungu S, Abwao J, Bett A, Lassauniere R, Kresfelder T, Cane PA, Venter M, Scott JA, Nokes DJ. 2010. Viral etiology of severe pneumonia among Kenyan infants and children. JAMA 303:2051–2057. doi:10.1001/jama.2010.675. [PMC free article] [PubMed] [CrossRef]
2. Blyth CC, Webb SA, Kok J, et al. The impact of bacterial and viral co-infection in severe influenza. Influenza Other Respir Viruses 2013;7:168–76.

doi:10.1111/j.1750-2659.2012.00360.x [PMC free article] [PubMed]

3. Bosch AA, Biesbroek G, Trzcinski K, et al. Viral and bacterial interactions in the upper respiratory tract. PLoS Pathog 2013;9:e1003057 doi:10.1371/journal.ppat.1003057 [PMC free article] [PubMed]
4. Cardinale F, Cappiello AR, Mastrototaro MF, et al. Community-acquired pneumonia in children. Early Hum Dev 2013;89 Suppl 3:S49–S52. doi:10.1016/j.earlhumdev.2013.07.023 [PubMed]
5. Cevey-Macherel M, Galetto-Lacour A, Gervaix A, et al. Etiology of community-acquired pneumonia in hospitalized children based on WHO clinical guidelines. Eur J Pediatr 2009;168:1429–36. doi:10.1007/s00431-009-0943-y [PubMed]
6. Chappell KJ, et al. Respiratory Syncytial Virus Infection is Associated with Increased Bacterial Load in the Upper Respiratory Tract in Young Children. J Medical Microbiol Diagnosis 2013;S1:005.
7. Chorazy ML, Lebeck MG, McCarthy TA, et al. Polymicrobial acute respiratory infections in a hospital-based pediatric population. Pediatr Infect Dis J 2013;32:460–6. doi:10.1097/INF.0b013e31828683ce [PMC free article] [PubMed]
8. Cilla G, Oñate E, Perez-Yarza EG, et al. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. J Med Virol 2008;80:1843–9. doi:10.1002/jmv.21271 [PubMed]
9. DeVincenzo JP, El Saleeby CM, Bush AJ. Respiratory syncytial virus load predicts disease severity in previously healthy infants. J Infect Dis 2005;191:1861–8. doi:10.1086/430008 [PubMed]
10. Don M, Fasoli L, Paldanius M, et al. Aetiology of community-acquired pneumonia: serological results of a paediatric survey. Scand J Infect Dis 2005;37:806–12. doi:10.1080/00365540500262435 [PubMed]
11. Elemraid MA, Sails AD, Eltringham GJA, Perry JD, Rushton SP, Spencer DA, Thomas MF, Eastham KM, Hampton F, Gennery AR, Clark JE. 2013. Aetiology of paediatric pneumonia after the introduction of pneumococcal conjugate vaccine. Eur Respir J 42:1595–1603. doi:10.1183/09031936.00199112. [PMC free article] [PubMed] [CrossRef]
12. Fathima P, Blyth CC, Lehmann D, et al. The impact of pneumococcal vaccination on bacterial and viral pneumonia in Western Australian children: record linkage cohort study of 469,589 births (1996-2012). Clin Infect Dis 2017. doi:10.1093/cid/cix923 [PubMed]
13. Foy HM. Infections caused by Mycoplasma pneumoniae and possible carrier state in different populations of patients. Clin Infect Dis 1993;17 Suppl 1(Suppl 1):S37–S46. doi:10.1093/clinids/17.Supplement_1.S37 [PubMed]
14. Fuller JA, Njenga MK, Bigogo G, et al. Association of the CT values of real-time PCR of viral upper respiratory tract infection with clinical severity, Kenya. J Med Virol 2013;85:924–32. doi:10.1002/jmv.23455 [PubMed]
15. Grayston JT, Aldous MB, Easton A, et al. Evidence that Chlamydia pneumoniae causes pneumonia and bronchitis. J Infect Dis 1993;168:1231–5. doi:10.1093/infdis/168.5.1231 [PubMed]
16. Honkinen M, Lahti E, Österback R, et al. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. Clin Microbiol Infect 2012;18:300–7. doi:10.1111/j.1469-0691.2011.03603.x [PubMed]
17. Howie SRC, Morris GAJ, Tokarz R, Ebruke BE, Machuka EM, Ideh RC, Chimah O, Secka O, Townend J, Dione M, Oluwalana C, Njie M, Jallow M, Hill PC, Antonio M, Greenwood B, Briese T, Mulholland K, Corrah T, Lipkin WI, Adegbola RA. 2014. Etiology of severe childhood pneumonia in The Gambia, West Africa, determined by conventional and molecular microbiological analyses of lung and pleural aspirate samples. Clin Infect Dis 59:682–685. doi:10.1093/cid/ciu384. [PMC free article] [PubMed] [CrossRef]
18. Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med 2015;372:835–45. doi:10.1056/NEJMoa1405870 [PMC free article] [PubMed]
19. Jansen AG, Sanders EA, Van Der Ende A, et al. Invasive pneumococcal and meningococcal disease: association with influenza virus and respiratory syncytial virus activity? Epidemiol Infect 2008;136:1448–54. doi:10.1017/S0950268807000271 [PMC free article] [PubMed]
20. Jansen RR, Wieringa J, Koekkoek SM, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. J Clin Microbiol 2011;49:2631–6. doi:10.1128/JCM.02094-10 [PMC free article] [PubMed]
21. Johnson A-WBR, Osinusi K, Aderele WI, Gbadero DA, Olaleye OD, Adeyemi-Doro FAB. 2008. Etiologic agents and outcome determinants of community-acquired pneumonia in urban children: a hospital-based study. J Natl Med A 100:370–385. doi:10.1016/S0027-9684(15)31269-4. [PubMed] [CrossRef]
22. Juvén T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J 2000;19:293–8. doi:10.1097/00006454-200004000-00006 [PubMed]

23. Kash JC, Taubenberger JK. The role of viral, host, and secondary bacterial factors in influenza pathogenesis. Am J Pathol 2015;185:1528–36. doi:10.1016/j.ajpath.2014.08.030 [PMC free article] [PubMed]

24. Kneyber MC, Blussé van Oud-Alblas H, van Vliet M, et al. Concurrent bacterial infection and prolonged mechanical ventilation in infants with respiratory syncytial virus lower respiratory tract disease. Intensive Care Med 2005;31:680–5. doi:10.1007/s00134-005-2614-4 [PubMed]

25. Korppi M, Heiskanen-Kosma T, Kleemola M. Incidence of community-acquired pneumonia in children caused by Mycoplasma pneumoniae: serological results of a prospective, population-based study in primary health care. Respirology 2004;9:109–14. doi:10.1111/j.1440-1843.2003.00522.x [PubMed]

26. Madhi SA, Klugman KP.; Vaccine Trialist Group. A role for Streptococcus pneumoniae in virus-associated pneumonia. Nat Med 2004;10:811–3. doi:10.1038/nm1077 [PubMed]

27. Manikam L, Lakhanpaul M. Epidemiology of community acquired pneumonia. Paediatr Child Health 2012;22:299–306. doi:10.1016/j.paed.2012.05.002

28. Mathew JL, Singhi S, Ray P, Hagel E, Saghafian-Hedengren S, Bansal A, Ygberg S, Sodhi KS, Kumar BV, Nilsson A. 2015. Etiology of community acquired pneumonia among children in India: prospective, cohort study. J Glob Health 5:050418. doi:10.7189/jogh.05.020418. [PMC free article] [PubMed] [CrossRef]

29. Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics 2004;113:701–7. doi:10.1542/peds.113.4.701 [PubMed]

30. Murdoch DR, Jennings LC. Association of respiratory virus activity and environmental factors with the incidence of invasive pneumococcal disease. J Infect 2009;58:37–46. doi:10.1016/j.jinf.2008.10.011 [PubMed]

31. O'Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. Lancet 2009;374:893–902. doi:10.1016/S0140-6736(09)61204-6 [PubMed]

32. Prayle A, Atkinson M, Smyth A. Pneumonia in the developed world. Paediatr Respir Rev 2011;12:60–9. doi:10.1016/j.prrv.2010.09.012 [PubMed]

33. Rhedin S, Lindstrand A, Hjelmgren A, et al. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. Thorax 2015;70:847–53. doi:10.1136/thoraxjnl-2015-206933 [PubMed]

34. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. J Med Virol 2010;82:1282–90. doi:10.1002/jmv.21790 [PubMed]

35. Spuesens EB, Fraaij PL, Visser EG, et al. Carriage of Mycoplasma pneumoniae in the upper respiratory tract of symptomatic and asymptomatic children: an observational study. PLoS Med 2013;10:e1001444 doi:10.1371/journal.pmed.1001444 [PMC free article] [PubMed]

36. Tajima T, Nakayama E, Kondo Y, et al. Etiology and clinical study of community-acquired pneumonia in 157 hospitalized children. J Infect Chemother 2006;12:372–9. doi:10.1007/s10156-006-0476-5 [PubMed]