Advances in diagnostics of respiratory viruses and insight in clinical implications of rhinovirus infections

Rijn-Klink, A.L. van

CHAPTER 1

General introduction

RESPIRATORY INFECTIONS

Respiratory infections are among the most common infections in humans and are a major health issue[1,2]. Overall, they are the cause of high morbidity and mortality, and as such associated with high costs due to absence of work and hospitalization, sometimes even in isolation[3-5].

Respiratory infections can be categorized into upper respiratory tract infections and infections of the lower airways. Although upper respiratory tract infections are usually relatively mild, more serious infections occur in specific high-risk groups. The most prevalent and well known upper respiratory tract infection is the common cold. Infections of the lower airways, mainly bronchiolitis and pneumonias, are in general more serious and the leading infectious cause of death world-wide[1,6].

Respiratory infections are caused by a variety of pathogens, but primarily by viruses, with overlapping clinical symptoms. The common cold is a syndrome of upper respiratory tract infections caused by viral pathogens, with rhinovirus being the most prevalent, detected in 40-50% of the cases of the common cold[6-8]. Other common respiratory viruses are respiratory syncytial virus, adenovirus, para-influenza viruses, bocavirus and metapneumovirus[6].

Viruses are the most prevalent pathogens detected in hospitalized adults with community acquired pneumonia (Figure 1). As with upper respiratory tract infections, rhinovirus is the most frequent cause, alone or as co-infection with other viruses or bacteria. Strikingly, in up to 62% of the community-acquired pneumonias no causative agent is found with current diagnostic procedures (Figure 1)[7,8].

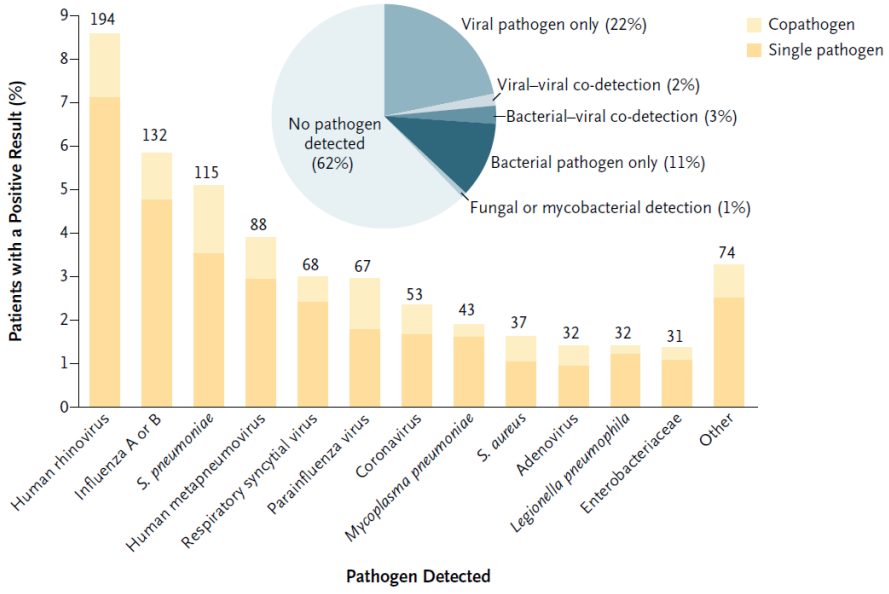

Figure 1. Pathogens detected in adults with community acquired pneumonia requiring hospitalization. (Adapted from Jain et al.[7])

DIAGNOSTICS OF RESPIRATORY INFECTIONS

Respiratory viral causes of infections are hard to distinguish by their clinical presentation, even so from other pathogens such as bacteria. Therefore, a precise diagnosis is important for adequate therapy and isolation measures. Over the years the diagnostic possibilities have expanded and the conventional routine assays, based on viral culture, antigen detection and serology, have been replaced by molecular methods. A routine diagnostic respiratory assay has to be fast, sensitive, specific, with limited hands-on time, cost-effective and has to be able to detect a wide variety of respiratory pathogens.

History of diagnostics of respiratory viruses

In the 1950's, diagnosing viruses was performed by in vitro culture on mono-layer cell lines[9]. Virus culture has been the gold standard for a long time. However, the time to results is considerable and generally too slow for clinical actions. Not only different cell lines and skilled staff are required, but also special safety requirements regarding viable virus[10]. Additionally, some viruses as for example rhinovirus species C, are hard or even impossible to propagate in cell-culture.

Later, new diagnostic options were introduced with antigen detection assays and serology. Antigen detection assays, such as rapid lateral-flow immunoassays and direct fluorescent antibody assays, have been designed for several viruses and are rapid but in general less sensitive and specific[10]. Serology is often limited by the need to detect seroconversion, IgG antibody titer rise or IgM detection, which is less sensitive in case of repeated exposure to for example rhinoviruses. As the induction of a measurable antibody response may take at least a week, the value of serology in the acute phase is limited[10].

In 1985, an *in-vitro* nucleic acid amplification method was designed. The polymerase chain reaction (PCR) relied on exponential amplification and subsequent detection of specific parts of nucleic acid sequences, with much improved sensitivity and specificity. Initially, after the amplification protocol was completed, the amplified PCR products were visualized by gel electrophoreses, southern-blotting or ELISA-like detection systems. In 1992, the real-time PCR concept was launched, which enabled detection of PCR products while they were generated using fluorescent probes. This greatly enhanced the possibilities as it enabled quantitative detection of targets. Real-time PCR is fast, sensitive, specific and has relative low hands-on time. Initially one virus per run was tested, but, using multiple fluorophores that could be differentiated, also duplex and multiplex PCR assays could be designed. However, the amount of targets that could be detected is limited to a maximum of five (in most platforms) by the number of fluorescent probes that could be differentiated and by applying an efficient workflow with batch-wise testing the time to results could be a day[11-13].

Advances in molecular respiratory viral diagnostics

Recently introduced molecular platforms offer the possibility of syndromic testing, as extended multiplex PCRs detect a wide panel of respiratory pathogens implicated in the clinical syndrome. Another benefit is the random and continuous access resulting in very fast and reliable results throughout the day. Whether these super-fast time to results diagnostic tests have clinical implications needs to be determined in more detail[14,15].

Molecular amplification based methods are still limited by the need to pre-define the targets of interest in a diagnostic panel. This is complicated by the high number of possible respiratory pathogens, the genetic diversity of the viruses implicated and the occurrence of new respiratory pathogens. Rare causes are easily overlooked with sometimes great consequences, as encountered for example in the Netherlands during Legionella and Q-fever outbreaks, and MERS-coronavirus in South-Korea[16-18]. The emergence of new clinically relevant respiratory viruses is a real threat, which will be clear to anyone after the devastating appearance of SARS-CoV-2 early in 2020 and indeed, NGS played a crucial role in its initial diagnosis and phylogenetic analysis[19-22]. But in addition, such new viruses may also be present already, without causing a pandemic, like the pigeon paramyxovirus type 1 that was coincidentally found to cause a fatal infection in a stem cell recipient[23]. We can never be informed about the role of such viruses without applying NGS as a broad 'catch all' approach.

While for a large proportion of the pneumonias the causative agent is still unknown, it is suspected that the percentage attributed to viral pathogens is higher than currently is assumed. Therefore there is a need for an unbiased catch-all method (see figure 2).

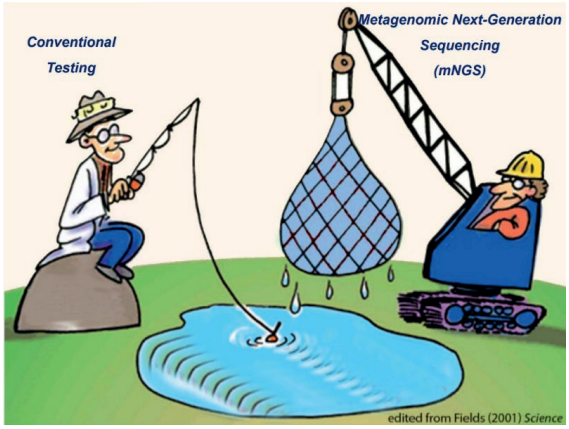

Figure 2. Cartoon representation of selective conventional testing compared to catch-all metagenomic next-generation sequencing.
(adapted from Chiu et al.[24]).

The newest diagnostic development, metagenomic next-generation sequencing (mNGS) is such an unbiased catch-all method, detecting all the genetic material in a sample. Standardized protocols for application of mNGS in the diagnostic field are still lacking and also the time to results and the cost of this approach are reason for concern, although the latter is rapidly declining (figure 3). To use mNGS as routine diagnostic tool, there is a need for fast, automated, combined DNA and RNA pre-treatment and analysis protocols with high sensitivity and specificity.

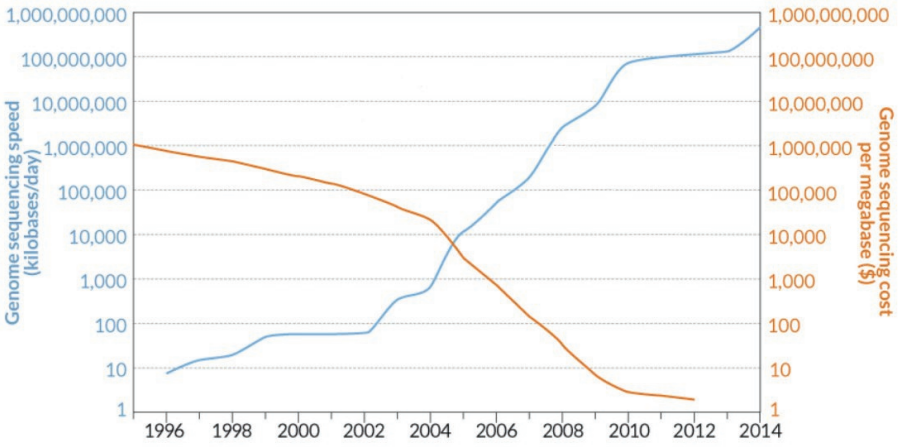

Figure 3. Improvements in costs and speed of genome sequencing.
(adapted from Otwell[25]).

Another advantage of this catch-all technique is that additional information is provided on strain characteristics, genotyping, susceptibility and virulence markers, pathogen evolution and the virome in health and disease. The respiratory virome and its correlation to clinical data is not yet studied extensively.

RHINOVIRUSES

Rhinoviruses are single stranded positive-sense RNA viruses in the family Picornaviridae and the genus *Enterovirus*. Since their discovery in the 1950s over 160 types, subdivided in species A, B and C, have been discovered[26,27] (Figure 4).

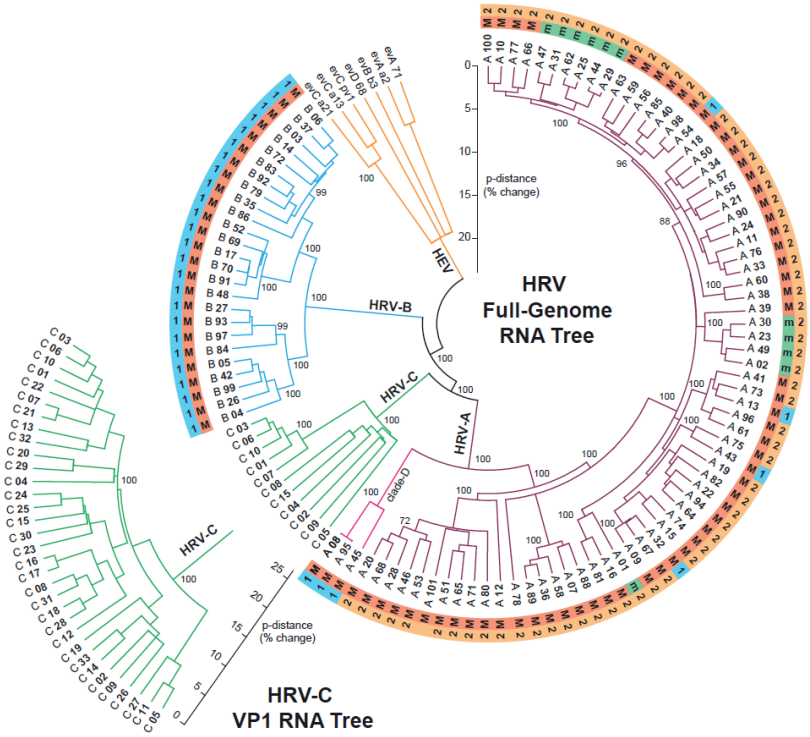

Figure 4. Phylogenetic tree of rhinovirus.
(adapted from Fields et al.[28])

The genome of rhinoviruses is approximately 7,200 bp long and consists of a single open reading-frame that encodes a large polyprotein of nearly 2200 amino acids, which is cleaved to produce 11 viral proteins (VP). VP1, VP2, VP3 and VP4 compose the viral capsid that embeds the RNA, while the non-structural proteins are involved in replication and assembly. At the 5' end of the genome the rhinoviruses have a long untranslated region (UTR), with internal ribosome entry sites (IRES), for initiation of translation. The 3' end untranslated region is much shorter and enables efficient replication[26,29], figure 5.

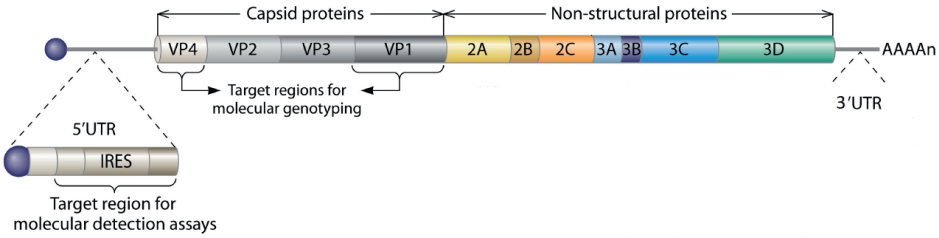

Figure 5. The genome structure of rhinoviruses.
UTR, untranslated region. IRES, internal ribosome entry site (Adapted from Jacobs et al.[26])

Epidemiology of rhinoviruses

Rhinoviruses have a worldwide distribution with a high prevalence, especially in young children. Although rhinovirus infections occur all year round, they have a peak incidence in early fall and spring[30-33].

Rhinoviruses are transmitted by direct contact, droplets or aerosols, but most important experimental evidence supports efficient transmission occurring through (in)direct contact mainly through hands, after which the virus attaches and replicates in the nasal mucosa, with an additional role for aerosol transmission[34-39].

Clinical manifestations of rhinoviruses

Rhinoviruses can cause a wide variety of respiratory symptoms, but asymptomatic shedding does occur as well. Asymptomatic infection tends to be more prevalent in younger patients and was even found in 12-32% of children under the age of four[40-45].

The most frequently encountered symptomatic presentation of rhinovirus infection is the, self-limiting, common cold. This usually starts with a sore throat followed by nasal obstruction and rhinorrhea, but a variety of symptoms has been observed. Rhinoviruses can also cause (rhino)sinusitis and otitis media, but in those cases co-infection with bacteria is common. Although rhinoviruses have long been considered relatively mild viruses, causing benign upper respiratory tract infections, they are now implicated in more serious lower respiratory tract infections in high-risk groups. They have been associated with the development of asthma, asthma and COPD exacerbations, bronchiolitis and life-threatening pneumonia, mainly in children and the elderly[7,26,46-48].

The development of more serious infections by rhinovirus is probably multifactorial and dependent on both host and environmental factors (Figure 6). Though several factors associated with more severe disease are known, much remains unknown.

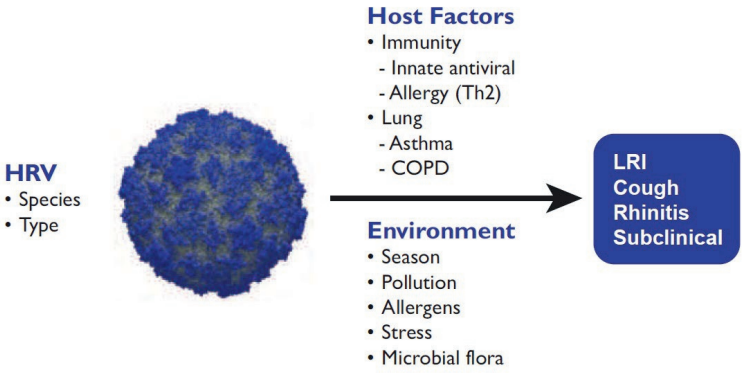

Figure 6. Spectrum of human rhinovirus infections and factors involved.
(adapted from Fields' Virology [28])

More severe rhinovirus infections have been shown in elderly patients, immunocompromised patients and patients with underlying chronic lung disease[49-53]. In children, risk factors for more serious infections are prematurity, congenital heart disease, respiratory syncytial virus co-infections, and non-infectious underlying respiratory disease. Finally, rhinoviruses have been associated with a complicated post-operative course after cardiac surgery[54-56]. In children, higher viral loads and viremia have been found in association to higher disease severity and more extensive clinical symptoms[57-65]. Whether rhinovirus viremia does occur in adults and is associated with more severe disease is still unknown.

Another gap in knowledge exists on whether rhinovirus infection can lead to serious complications in case of extreme stress and special circumstances, for example in children undergoing cardiac surgery.

In addition, the association of specific rhinovirus types to more serious disease is still open for debate. Some studies showed rhinovirus type C to cause more severe illness, while others failed to demonstrate a difference between the rhinovirus types[48,66-70]. A longitudinal study with a large number of patients with a variety of rhinovirus types is needed to determine the influence of rhinovirus species on disease severity.

Although rhinovirus infections have been associated with mortality, the major disease burden is caused by the high frequency of infections, the duration of illness (a median of 7 days) and the associated economic burden[29,46,71].

Treatment and prevention

There is currently no licensed treatment available for rhinovirus infections, although several antiviral treatments have been described or are under development[26,72]. Therefore, at the moment, the best way to prevent rhinovirus infections is adequate hand hygiene measures[73].

SCOPE OF THIS THESIS

The research presented in this thesis aims to determine the implications and performance of new viral respiratory diagnostic methods and the aspects of the disease severity of the most common respiratory virus, rhinovirus.

Part I: Application and added value of advanced respiratory viral diagnostic methods

In this part, advanced diagnostic methods for respiratory infections were studied to determine their clinical implications and their performance, when applied to routine diagnostics.

An in-house developed diagnostic mNGS protocol, with simultaneous RNA and DNA detection, was developed and compared with real-time PCR (chapter two). This protocol was used to study respiratory infections and the respiratory virome in patients with COPD exacerbations and to correlate these results with clinical data and real-time PCR (chapter three).

Rapid molecular syndromic testing by an innovative automated amplification platform was compared to lab-developed multiplex real-time PCR assays, focusing on the difference in time to results and its implications for clinical decision making, regarding isolation, and antimicrobial therapy (chapter four).

Part II: Clinical implications of rhinovirus

In this part, rhinovirus is studied in different patient populations to determine the clinical impact and factors influencing the course of the disease. To determine whether rhinovirus infections, symptomatic or asymptomatic, have a negative effect on the post-operative course of children undergoing cardiac surgery, a large prospective study screening all children at the time of their operation was performed (chapter five).

A retrospective study to detect rhinovirus viremia and its association with disease severity was performed in adult patients with high rhinovirus loads in bronchoalveolar lavage (chapter six). The difference in disease severity between different rhinovirus species and types was studied in a prospective study in an adult population in general practices throughout Europe (chapter seven).

REFERENCES

1. WHO -The top 10 causes of death. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. Last access 17-10-2019. who top 10 death (accessed 31-05-2019.
2. WHO- The global impact of respiratory disease, second edition. https://www.who.int/gard/publications/The_Global_Impact_of_Respiratory_Disease.pdf?ua=1. Last access 17-10-2019. (accessed 19-09-2019.
3. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet Infectious diseases* 2017; **17**(11): 1133-61.
4. Trucchi C, Paganino C, Orsi A, et al. Hospital and economic burden of influenza-like illness and lower respiratory tract infection in adults >/=50 years-old. *BMC health services research* 2019; **19**(1): 585.
5. HCUP- Costs for hospital stays in the United States, 2011.https://www.hcup-us.ahrq.gov/reports/statbriefs/sb168-Hospital-Costs-United-States-2011.jsp. Last access 17-10-2019.
6. Mandell GLB, J.E; Dolin, R. The common cold. Priciples and practice of infectious diseases: 809-14.
7. Jain S, Self WH, Wunderink RG, et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *N Engl J Med* 2015; **373**(5): 415-27.
8. Ieven M, Coenen S, Loens K, et al. Aetiology of lower respiratory tract infection in adults in primary care: a prospective study in 11 European countries. *Clinical microbiology and infection* 2018; **24**(11): 1158-63.
9. Zuckerman B, Schoub, Griffiths, Mortimer. Principles and practices of clinical virology: wiley.
10. Loeffelholz M, Chonmaitree T. Advances in diagnosis of respiratory virus infections. *Int J Microbiol* 2010; **2010**: 126049-.
11. Mackay IM. Real-time PCR in the microbiology laboratory. *Clinical Microbiology and infection* 2004; **10**(3): 190-212.
12. Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science (New York, NY)* 1985; **230**(4732): 1350-4.
13. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology* 1992; **10**(4): 413-7.
14. Nijhuis RH, Guerendiain D, Claas EC, Templeton KE. Comparison of the ePlex(R) Respiratory Pathogen Panel with Laboratory Developed Real-Time PCR for the Detection of Respiratory Pathogens. *Journal of clinical microbiology* 2017; **55**(6): 1938-45.
15. Huang HS, Tsai CL, Chang J, Hsu TC, Lin S, Lee CC. Multiplex PCR system for the rapid diagnosis of respiratory virus infection: systematic review and meta-analysis. *Clinical Microbiology and Infection* 2018; **24**(10): 1055-63.
16. W Den Boer J, Yzerman E, Schellekens J, et al. A Large Outbreak of Legionnaires' Disease at a Flower Show, the Netherlands, 1999. *Emerging infectious diseases* 2002; **8**(1): 37-43.
17. van der Hoek W, Morroy G, Renders NH, et al. Epidemic Q fever in humans in the Netherlands. *Advances in experimental medicine and biology* 2012; **984**: 329-64.
18. Kim KH, Tandi TE, Choi JW, Moon JM, Kim MS. Middle East respiratory syndrome coronavirus (MERS-CoV) outbreak in South Korea, 2015: epidemiology, characteristics and public health implications. *The Journal of hospital infection* 2017; **95**(2): 207-13.
19. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature* 2020.
20. Ji W, Wang W, Zhao X, Zai J, Li X. Cross-species transmission of the newly identified coronavirus 2019-nCoV. *Journal of medical virology* 2020; **92**(4): 433-40.
21. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020.
22. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**(8): 727-33.
23. Kuiken T, Breitbart M, Beer M, et al. Zoonotic Infection With Pigeon Paramyxovirus Type 1 Linked to Fatal Pneumonia. *The Journal of infectious diseases* 2018; **218**(7): 1037-44.
24. Chiu C. UCSF-Metagenomic approaches to diagnosis of infectious diseases. https://impaactnetwork.org/DocFiles/LabCenter/LabRetreat/2017/Chiu_IMPAACT_Lab_Retreat_25JAN17.pdf. Last access 17-10-2019.
25. Science news-The gene sequencing future is here. https://www.sciencenews.org/article/gene-sequencing-future-here. Last access 17-10-2019.
26. Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses. *Clinical microbiology reviews* 2013; **26**(1): 135-62.

27. ICTV. Genus: Enterovirus. https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/picornavirales/w/picornaviridae/681/genus-enterovirus Date last update: 02-2019 . Last access 25-07-2019.
28. J G, A P. Rhinoviruses. Fields virology, 6th edition. Philadelphia, PA.: Wolters Kluwer Health/Lippincott Williams & Wilkins.
29. Mandell GLB, J,E,; Dolin, R. Rhinovirus. Principles and practice of infectious diseases: 2389-98.
30. Galanti M, Birger R, Ud-Dean M, et al. Longitudinal active sampling for respiratory viral infections across age groups. *Influenza and other respiratory viruses* 2019; **13**(3): 226-32.
31. Aydin Koker S, Demirag B, Tahta N, et al. A 3-Year Retrospective Study of the Epidemiology of Acute Respiratory Viral Infections in Pediatric Patients With Cancer Undergoing Chemotherapy. *Journal of pediatric hematology/oncology* 2019; **41**(4): e242-e6.
32. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: Association with symptomatic illness and effect of season. *Journal of medical virology* 2006; **78**(5): 644-50.
33. Miller EK, Lu X, Erdman DD, et al. Rhinovirus-associated hospitalizations in young children. *The Journal of infectious diseases* 2007; **195**(6): 773-81.
34. Dick EC, Jennings LC, Mink KA, Wartgow CD, Inhorn SL. Aerosol transmission of rhinovirus colds. *The Journal of infectious diseases* 1987; **156**(3): 442-8.
35. Hendley JO, Gwaltney JM, Jr. Mechanisms of transmission of rhinovirus infections. *Epidemiologic reviews* 1988; **10**: 243-58.
36. Gwaltney JM, Jr., Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. *Annals of internal medicine* 1978; **88**(4): 463-7.
37. Ikonen N, Savolainen-Kopra C, Enstone JE, et al. Deposition of respiratory virus pathogens on frequently touched surfaces at airports. *BMC infectious diseases* 2018; **18**(1): 437.
38. Rodrigues AF, Santos AM, Ferreira AM, Marino R, Barreira ME, Cabeda JM. Year-Long Rhinovirus Infection is Influenced by Atmospheric Conditions, Outdoor Air Virus Presence, and Immune System-Related Genetic Polymorphisms. *Food and environmental virology* 2019.
39. Kutter JS, Spronken MI, Fraaij PL, Fouchier RA, Herfst S. Transmission routes of respiratory viruses among humans. *Current opinion in virology* 2018; **28**: 142-51.
40. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *Journal of medical virology* 2010; **82**(7): 1282-90.
41. Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkaranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. *Journal of medical virology* 2002; **66**(3): 417-20.
42. van Benten I, Koopman L, Niesters B, et al. Predominance of rhinovirus in the nose of symptomatic and asymptomatic infants. *Pediatric allergy and immunology* 2003; **14**(5): 363-70.
43. Iwane MK, Prill MM, Lu X, et al. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. *The Journal of infectious diseases* 2011; **204**(11): 1702-10.
44. Fry AM, Lu X, Olsen SJ, et al. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. *PloS one* 2011; **6**(3): e17780.
45. Birger R, Morita H, Comito D, et al. Asymptomatic Shedding of Respiratory Virus among an Ambulatory Population across Seasons. *mSphere* 2018; **3**(4).
46. Choi SH, Huh JW, Hong SB, et al. Clinical characteristics and outcomes of severe rhinovirus-associated pneumonia identified by bronchoscopic bronchoalveolar lavage in adults: comparison with severe influenza virus-associated pneumonia. *Journal of clinical Virology* 2015; **62**: 41-7.
47. Bergroth E, Aakula M, Elenius V, et al. Rhinovirus Type in Severe Bronchiolitis and the Development of Asthma. *The journal of allergy and clinical immunology In practice* 2019.
48. Lambert KA, Prendergast LA, Dharmage SC, et al. The role of human rhinovirus (HRV) species on asthma exacerbation severity in children and adolescents. *The journal of asthma* 2018; **55**(6): 596-602.
49. Bruning AHL, Thomas XV, van der Linden L, et al. Clinical, virological and epidemiological characteristics of rhinovirus infections in early childhood: A comparison between non-hospitalised and hospitalised children. *Journal of Clinical Virology* 2015; **73**: 120-6.
50. Louie JK, Yagi S, Nelson FA, et al. Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. *Clinical infectious diseases* 2005; **41**(2): 262-5.
51. Kraft CS, Jacob JT, Sears MH, Burd EM, Caliendo AM, Lyon GM. Severity of human rhinovirus infection in immunocompromised adults is similar to that of 2009 H1N1 influenza. *Journal of clinical microbiology* 2012; **50**(3): 1061-3.

52. Gunawardana N, Finney L, Johnston SL, Mallia P. Experimental rhinovirus infection in COPD: implications for antiviral therapies. *Antiviral research* 2014; **102**: 95-105.
53. Piralla A, Zecca M, Comoli P, Girello A, Maccario R, Baldanti F. Persistent rhinovirus infection in pediatric hematopoietic stem cell transplant recipients with impaired cellular immunity. *Journal of Clinical Virology* 2015; **67**: 38-42.
54. Costa LF, Queiroz DA, Lopes da Silveira H, et al. Human rhinovirus and disease severity in children. *Pediatrics* 2014; **133**(2): e312-21.
55. Simsic J, Phelps C, Yates A, Galantowicz M. Management strategies after cardiac surgery in an infant with human rhinovirus. *Pediatric cardiology* 2013; **34**(8): 1922-4.
56. Delgado-Corcoran C, Witte MK, Ampofo K, Castillo R, Bodily S, Bratton SL. The impact of human rhinovirus infection in pediatric patients undergoing heart surgery. *Pediatric cardiology* 2014; **35**(8): 1387-94.
57. Esposito S, Daleno C, Scala A, et al. Impact of rhinovirus nasopharyngeal viral load and viremia on severity of respiratory infections in children. *European journal of clinical microbiology & infectious diseases* 2014; **33**(1): 41-8.
58. Urquhart GE, Grist NR. Virological studies of sudden, unexplained infant deaths in Glasgow 1967-70. *Journal of clinical pathology* 1972; **25**(5): 443-6.
59. Urquhart GE, Stott EJ. Rhinoviraemia. *British medical journal* 1970; **4**(5726): 28-30.
60. Fuji N, Suzuki A, Lupisan S, et al. Detection of human rhinovirus C viral genome in blood among children with severe respiratory infections in the Philippines. *PloS one* 2011; **6**(11): e27247.
61. Tapparel C, L'Huillier AG, Rougemont AL, Beghetti M, Barazzone-Argiroffo C, Kaiser L. Pneumonia and pericarditis in a child with HRV-C infection: a case report. *Journal of Clinical Virology* 2009; **45**(2): 157-60.
62. Xatzipsalti M, Kyrana S, Tsolia M, et al. Rhinovirus viremia in children with respiratory infections. *American journal of respiratory and critical care medicine* 2005; **172**(8): 1037-40.
63. Lupo J, Schuffenecker I, Morel-Baccard C, et al. Disseminated rhinovirus C8 infection with infectious virus in blood and fatal outcome in a child with repeated episodes of bronchiolitis. *Journal of clinical microbiology* 2015; **53**(5): 1775-7.
64. Las Heras J, Swanson VL. Sudden death of an infant with rhinovirus infection complicating bronchial asthma: case report. *Pediatric pathology* 1983; **1**(3): 319-23.
65. Granados A, Peci A, McGeer A, Gubbay JB. Influenza and rhinovirus viral load and disease severity in upper respiratory tract infections. *Journal of Clinical Virology* 2017; **86**: 14-9.
66. Lauinger IL, Bible JM, Halligan EP, et al. Patient characteristics and severity of human rhinovirus infections in children. *Journal of Clinical Virology* 2013; **58**(1): 216-20.
67. Xiang Z, Gonzalez R, Xie Z, et al. Human rhinovirus C infections mirror those of human rhinovirus A in children with community-acquired pneumonia. *Journal of Clinical Virology* 2010; **49**(2): 94-9.
68. Jacobs SE, Lamson DM, Soave R, et al. Clinical and molecular epidemiology of human rhinovirus infections in patients with hematologic malignancy. *Journal of Clinical Virology* 2015; **71**: 51-8.
69. Choi SH, Hong SB, Kim T, et al. Clinical and molecular characterization of rhinoviruses A, B, and C in adult patients with pneumonia. *Journal of Clinical Virology* 2015; **63**: 70-5.
70. Hasegawa K, Jartti T, Bochkov YA, et al. Rhinovirus Species in Children With Severe Bronchiolitis: Multicenter Cohort Studies in the United States and Finland. *The Pediatric infectious disease journal* 2019; **38**(3): e59-e62.
71. Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza-related viral respiratory tract infection in the United States. *Archives of internal medicine* 2003; **163**(4): 487-94.
72. Casanova V, Sousa FH, Stevens C, Barlow PG. Antiviral therapeutic approaches for human rhinovirus infections. *Future Virol* 2018; **13**(7): 505-18.
73. Jefferson T, Del Mar CB, Dooley L, et al. Physical interventions to interrupt or reduce the spread of respiratory viruses. *The Cochrane database of systematic reviews* 2011; (7): Cd006207.