International Journal of Biology and Life Sciences
ISSN: 2957-9511 | Vol. 7, No. 1, 2024

Analysis of Pathogen Detection Data in 898 Pediatric Patients Hospitalized with Community-Acquired Pneumonia

Leyu Li [1], Hui Zhang [1], Jin Hu [1], Yingjie Lu [1], Xiaoyan Zhao [1], Rina Sa [1], Yao Yao [1],

Langshan Bao [1], Jing Cao [1], Ming Li [2], Huan Zeng [3], Lei Wang [1, *]

[1] Department of Pediatrics, New Century Women's and Children's Hospital, Beijing, China
[2] Department of Clinical Laboratory, New Century Women's and Children's Hospital, Beijing, China
[3] School of public health, Chongqing Medical University, Chongqing, China

*** Corresponding author:** Lei Wang (Email: wanglei_ncwch@163.com)

Abstract: Introduction: The prevalence of community-acquired pneumonia (CAP) in pediatric patients has seen significant changes following the lifting of COVID-19 restrictions. This study examines the epidemiology and pathogen detection in children hospitalized with CAP at Beijing New Century Children's Hospital throughout 2023. Objective: To analyze the pathogen detection rates and distribution characteristics among 898 pediatric CAP patients admitted during 2023, focusing on age-related and seasonal variations. Methods: This retrospective study included 898 pediatric patients diagnosed with CAP between January 1 and December 31, 2023. Respiratory pathogen detection was performed using a combination of antigen detection kits, nucleic acid testing, and bacterial culture. The data were analyzed for pathogen distribution across different age groups and seasons. Results: Of the 898 CAP cases, pathogens were detected in 703 cases (78.3%). Mycoplasma pneumoniae was the most frequently identified pathogen (44.1%), with detection rates increasing significantly with age, particularly in school-aged children. Viral pathogens were detected in 42.7% of cases, with Respiratory Syncytial Virus (32.4%) being the most prevalent. Bacterial pathogens were identified in 49.5% of cases, with Streptococcus pneumoniae (34.1%) and Haemophilus influenzae (33.0%) being the most common. Mixed infections were observed in 64.4% of cases, with a higher prevalence in older children and during the autumn and winter seasons. Conclusions: The study highlights significant age and seasonal variations in pathogen distribution among pediatric CAP patients. Mycoplasma pneumoniae was more common in older children and during autumn, while bacterial infections peaked in winter. The findings emphasize the need for age and season-specific strategies in managing pediatric CAP and suggest that comprehensive diagnostic approaches are crucial to accurately identify the causative pathogens.

Keywords: Pediatric Community-acquired Pneumonia; Respiratory Pathogen Detection; Epidemiology; Mixed Infections; Mycoplasma Pneumoniae; Seasonal Variation.

1. Introduction

Community-acquired pneumonia (CAP) is one of the most prevalent and serious infectious diseases affecting children globally, posing a significant threat to pediatric health(1,2). It is a leading cause of morbidity and mortality among children under five years old, particularly in developing countries where healthcare resources are often limited. The high incidence and potential severity of CAP contribute to a substantial disease burden on both families and healthcare systems, underscoring the critical need for effective diagnostic and therapeutic strategies (3,4). The identification of the causative pathogens in CAP is essential for guiding appropriate clinical management and improving patient outcomes. However, the etiological landscape of pediatric CAP is complex and multifaceted, with pathogen distribution patterns varying significantly according to age, geographical location, seasonal factors, and socioeconomic conditions(1,2)..

The diversity of pathogens responsible for CAP in children complicates its diagnosis and treatment. Bacterial pathogens such as Streptococcus pneumoniae and Haemophilus influenzae have historically been the most commonly identified causes of CAP. However, viral agents like Respiratory Syncytial Virus (RSV) and Influenza viruses also play a significant role, especially in younger children(5,6). Additionally, atypical pathogens such as Mycoplasma pneumoniae are increasingly recognized as important contributors to CAP, particularly in older children and during specific seasons(7,8). The co-infection of multiple pathogens further complicates the clinical picture, making accurate and timely diagnosis challenging.

The onset of the COVID-19 pandemic in late 2019 brought about profound changes in the epidemiology of respiratory infections, including pediatric CAP(9,10). Public health measures such as lockdowns, social distancing, mask mandates, and enhanced hygiene practices led to a marked reduction in the transmission of many common respiratory pathogens(11,12). However, as these restrictions were lifted, there has been a resurgence in the incidence of respiratory infections, including CAP. The post-pandemic period presents a unique challenge for healthcare providers, as the pathogen landscape has been significantly altered(13,14). The patterns of respiratory infections that have emerged following the relaxation of COVID-19 restrictions require careful study to guide future clinical practice and public health interventions(15,16).

In this context, understanding the current distribution of pathogens causing CAP in children is more important than ever. Identifying shifts in pathogen prevalence and their

seasonal trends is critical for the development of effective empirical treatment guidelines and preventive measures(17,18). This study aims to address this need by analyzing the pathogen spectrum and epidemiological characteristics of pediatric CAP in hospitalized children at Beijing New Century Children's Hospital during the year 2023, following the lifting of COVID-19 restrictions.

This study involved 898 pediatric patients admitted with CAP, and it provides a comprehensive analysis of the detected pathogens, considering factors such as age and seasonal variations. The findings reveal important shifts in the distribution of key pathogens, including a notable prevalence of Mycoplasma pneumoniae in older children and during the autumn and winter seasons, and a significant presence of viral pathogens such as RSV in younger age groups(19). Moreover, the study highlights the high prevalence of mixed infections, which were detected in over 64% of cases, emphasizing the complexity of CAP in the pediatric population. These findings underscore the importance of adopting age- and season-specific strategies in the management of pediatric CAP and suggest that comprehensive diagnostic approaches are essential to accurately identify the causative pathogens(20,21).

By analyzing the pathogen detection data in this specific post-pandemic context, this study aims to provide valuable insights that will inform clinical decision-making and public health policies. The results have the potential to improve the accuracy of empirical therapy, reduce the misuse of antibiotics, and ultimately contribute to better health outcomes for pediatric patients with CAP(22,23). Understanding these evolving trends is crucial for healthcare providers as they navigate the challenges of treating respiratory infections in the aftermath of the COVID-19 pandemic.

In conclusion, this study not only contributes to the growing body of knowledge on pediatric CAP but also highlights the dynamic nature of infectious disease epidemiology in the post-COVID-19 era. The findings emphasize the need for ongoing surveillance and research to ensure that healthcare strategies remain effective and responsive to the changing patterns of respiratory infections in children.

2. Materials & Methods

2.1. Study Subjects

Inclusion and Exclusion Criteria: This study included 940 pediatric patients admitted to and discharged from Beijing New Century Children's Hospital between January 2023 and December 2023 with a discharge diagnosis containing the term "pneumonia." Based on the "2019 Guidelines for the Diagnosis and Treatment of Community-Acquired Pneumonia in Children" , cases that met the diagnostic criteria for CAP were included(24,25). Exclusions were made for cases of neonatal pneumonia, congenital heart disease, chronic underlying conditions, long-term use of immunosuppressants, tuberculosis, non-infectious pneumonia, and hospital-acquired pneumonia, resulting in a total of 898 confirmed CAP cases being included(26,27).

This study was approved by the Ethics Committee of Beijing New Century Children's Hospital, and informed consent for specimen collection and testing was obtained from the patients' guardians.

2.2. Viral Antigen Detection

Viral antigen detection was performed using specific commercially available antigen detection kits. For Influenza A and B, the QuickNavi-Flu A&B kit (Denka Seiken Co., Ltd., Japan) was utilized(28,29). The detection of SARS-CoV-2 antigens was carried out using the 2019-nCoV antigen detection kit from Zhongyuan Huiji (China). Adenovirus antigens were detected with the adenovirus antigen detection kit from Hangzhou Innovation Biotech Co., Ltd. (China), while Mycoplasma pneumoniae antigens were identified using the Mycoplasma pneumoniae antigen detection kit from the same manufacturer. All procedures followed the manufacturers' protocols to ensure accuracy and reliability in antigen detection.

2.3. Respiratory Pathogen Nucleic Acid Testing

Respiratory pathogen nucleic acid detection was conducted using a quantitative real-time PCR system (Roche Fluorescent Quantitative PCR Instrument)(30,31). This method was employed to amplify and quantify nucleic acids from respiratory pathogens, ensuring high sensitivity and specificity. The PCR reactions were performed according to the manufacturer's protocols, with appropriate controls included to validate the accuracy and reproducibility of the results.

2.4. Pathogen Detection Methods

Pathogen DNA amplification and qualitative detection were performed using targeted Next-Generation Sequencing (tNGS) with the MiSeq sequencing platform from Illumina(32,33). This method allowed for the precise identification and characterization of pathogen DNA, providing comprehensive insights into the microbial composition.Sputum samples were subjected to bacterial culture using the Vitek II microbial identification and antimicrobial susceptibility system (bioMérieux). This automated system facilitated the accurate identification of bacterial species and their antibiotic resistance profiles, ensuring targeted therapeutic interventions.For the detection of Mycoplasma pneumoniae antibodies, serum antibody titers were measured using the Mycoplasma pneumoniae antibody detection kit (Serodia-Myco II, Fujirebio Inc.). This method provided quantitative data on antibody levels, assisting in the diagnosis and monitoring of Mycoplasma pneumoniae infections.

2.5. Statistical Analysis

Continuous data were presented as mean ± standard deviation (SD), and comparisons between groups were conducted using an independent samples t-test(34,35). This method was selected to determine whether there is a statistically significant difference in the mean values between two independent groups, assuming the data is normally distributed. For categorical data, results were expressed as percentages. Comparisons across multiple groups were performed using the Chi-square test for contingency tables, which evaluates the association between categorical variables. To further identify specific differences between individual groups, post hoc pairwise comparisons were conducted using the Chi-square partitioning method. A p-value of less than 0.05 was considered indicative of statistical significance. All statistical analyses were carried out using SPSS version 26.0.

3. Results

3.1. General Conditions

A total of 898 pediatric patients were included in the study, with a slight predominance of males (474 boys and 424 girls), yielding a male-to-female ratio of 1.12:1. The patients were systematically categorized into age groups, reflecting the typical developmental stages in early childhood. Specifically, infants aged 28 days to 1 year accounted for 73 cases (8.13%), toddlers aged 1 to 3 years made up 159 cases (17.71%), preschool children aged 3 to 6 years comprised the largest group with 334 cases (37.19%), and school-age children and those older than 6 years constituted 332 cases (36.97%). This distribution illustrates a broad representation of age groups, ensuring a comprehensive analysis of pediatric community-acquired pneumonia (CAP) across different developmental stages.

The study also examined seasonal variation by categorizing the cases into four distinct groups: the spring group (March 1 to May 31) with 174 cases (19.38%), the summer group (June 1 to August 31) with 233 cases (25.95%), the autumn group (September 1 to November 30) with 366 cases (40.76%), and the winter group (December 1 to February 29) with 125 cases (13.92%). This categorization reveals a pronounced increase in cases during the autumn season, suggesting potential seasonal factors influencing the incidence of CAP in children. The average length of hospital stay was reported as 5.80±0.08 days, indicating a relatively consistent duration of hospitalization among the patients studied.

3.2. Pathogen Detection Methods

A variety of pathogen detection methods were employed to ensure comprehensive and accurate identification of the causative agents of pediatric CAP. These methods included respiratory pathogen nucleic acid testing, which was utilized in 134 cases, providing a molecular-level detection of specific pathogens. High-throughput sequencing, a more advanced technique allowing for the identification of a broad spectrum of pathogens, was used in 466 cases. Sputum culture, a traditional yet effective method for detecting bacterial pathogens, was conducted in 130 cases. Respiratory pathogen antigen detection was the most frequently used method, applied in 609 cases, reflecting its utility in identifying viral pathogens. Additionally, Mycoplasma pneumoniae antibody testing was performed in 380 cases, specifically targeting this common atypical pathogen.

3.3. Pathogen Composition

Among the 898 pediatric CAP cases, pathogens were successfully detected in 703 cases, resulting in a robust overall pathogen detection rate of 78.3%. This high detection rate underscores the effectiveness of the combined diagnostic approaches used in the study. Of the detected cases, 250 (35.6%) involved single pathogen infections, highlighting the presence of distinct, singular causative agents. However, a significant portion of cases, 453 (64.4%), involved mixed infections, where multiple pathogens were identified as co-infecting agents. This high prevalence of mixed infections suggests the complexity of pediatric CAP and underscores the necessity for comprehensive diagnostic methods to accurately identify all contributing pathogens.

3.4. Results of Pathogen Detection in Pediatric Patients

In this study of 898 pediatric patients, we observed significant variations in pathogen detection rates across different age groups and seasons. Mycoplasma pneumoniae (MP) was detected in 44.1% of the cases, with detection rates increasing significantly with age—from 2.7% in children under 1 year to 72.0% in those over 6 years ($p < 0.001$). Other pathogens, such as Streptococcus pneumoniae (SP) and Haemophilus influenzae (HI), also showed significant age-related distribution patterns, with SP peaking in the 1-3 years group (26.4%) and HI in the 3-6 years group (35.1%) ($p < 0.001$ for both). The seasonal analysis revealed that MP detection was highest in Autumn (59.6%) and Winter (63.6%), significantly greater than in Spring and Summer ($p < 0.001$), with similar trends observed for SP and HI. The study's findings, detailed in Tables 1 and 2, highlight the importance of considering both age and seasonal factors in the management of pediatric respiratory infections, suggesting that targeted interventions might be more effective when aligned with these patterns.

3.5. Age and Seasonal Variation in Bacterial Detection Rates

This study presents a comprehensive analysis of bacterial detection rates across different pediatric age groups and seasons, as summarized in Tables 1 and 2. Overall, the bacterial detection rate was 49.5% (445 cases), with a notable distinction between single and mixed bacterial infections. A total of 800 bacterial strains were identified, with Streptococcus pneumoniae (SP) and Haemophilus influenzae (HI) being the most prevalent. Age-specific trends were evident, with the detection rate of HI increasing significantly with age, while SA was more commonly detected in infants (<1 year), and SP was less frequently found in this group. In contrast, older children (over 6 years) had higher detection rates for Klebsiella pneumoniae (KPN), Pseudomonas aeruginosa (PA), Acinetobacter baumannii (ABA), Stenotrophomonas maltophilia (SM), and Proteus bacteria (PB). Seasonal variations were also observed, with the highest detection rates for Mycoplasma pneumoniae (MP) and SP occurring in autumn and winter, while HI, KPN, and ABA were predominantly detected in winter. SA and SM had peak detection in autumn. While Pseudomonas bronchiseptica (PB) showed significant seasonal variation, pairwise comparisons did not reveal significant differences. Detection rates for Moraxella catarrhalis (MC), PA, and Escherichia coli (EC) remained consistent across seasons. These findings highlight the importance of considering both age and seasonal factors when diagnosing and managing bacterial infections in pediatric patients. The detailed data in Tables 1 and 2 underscores these variations, providing valuable insights for targeted therapeutic strategies.

3.6. Viral Detection in Pediatric Patients

In this study, a total of 383 pediatric patients were tested for viral infections, with 42.7% (n=383) testing positive. Among these, 316 cases were single-virus infections, while 67 cases involved mixed viral infections, resulting in the detection of 460 viral strains. The most frequently detected viruses were Respiratory Syncytial Virus (RSV) (32.4%, n=149), Influenza A Virus (INFA) (10.4%, n=48), Rhinovirus (RV) (9.6%, n=44), Human Metapneumovirus (HMPV)

(8.9%, n=41), and Adenovirus (ADV) (7.8%, n=36). Other viruses detected included Novel Coronavirus (nCOV) (5.7%, n=26), Parainfluenza Virus Type 1 (PIV1) (5.2%, n=24), Human Bocavirus (HBOV) (2.8%, n=13), and Epstein-Barr Virus (EBV) (1.5%, n=7) among others.

Significant age-related differences were observed for INFA and ADV detection rates, which were higher in both the infant group (<1 year) and the group of children older than 6 years compared to other age groups. INFA detection was lowest in the toddler group (1-3 years), with a statistically significant difference compared to the infant group ($p < 0.05$). Similarly, ADV detection rates showed statistically significant differences across the four age groups ($p < 0.05$). No statistically significant differences were found for RSV, INFB, HMPV, nCOV, COV, RV, HBOV, PIV1, PIV3, HHV1, EBV, and CMV across different age groups ($p > 0.05$) (Table 3).

Regarding seasonal distribution, RSV showed the highest detection rates in spring (29.9%) and winter (24.8%), with significant differences between these seasons and others ($p < 0.05$). INFA and ADV also had significantly higher detection rates in winter ($p < 0.05$). HMPV was most frequently detected in spring (10.3%), while RV, HBOV, and PIV3 also demonstrated significant seasonal variation ($p < 0.05$), although pairwise comparisons between seasons did not show significant differences. There were no significant seasonal differences for INFB, nCOV, COV, PIV1, HHV1, EBV, and CMV ($p > 0.05$) (Table 4).

3.7. Analysis of Mixed Infection Detection Across Age Groups and Seasons

In this study, we analyzed mixed infections among 898 pediatric patients, revealing significant variations across different age groups and seasons. As shown in Table 5, the detection rates of bacterial mixed with viral infections (bact+vir), bacterial mixed with Mycoplasma pneumoniae (bact+mp), and bacterial mixed with other bacterial infections (bact+bact) demonstrated an increasing trend with age ($p < 0.05$). Specifically, children aged 3-6 years exhibited the highest detection rate of bacterial and viral co-infections at 14.1%, while the prevalence of viral mixed with Mycoplasma pneumoniae (vir+mp) infections was notably higher in the group older than 6 years (24.4%), indicating a significant difference compared to younger children. Conversely, the lowest detection rate of viral co-infections (vir+vir) was observed in the oldest age group (>6y) at 7.8%, suggesting that older children may have a reduced susceptibility to certain viral co-infections. Additionally, Table 6 illustrates significant seasonal variations in mixed infection detection rates, with winter showing the highest overall prevalence, particularly for bacterial and viral co-infections (36.8%). Autumn, however, stood out with the highest prevalence of viral mixed with Mycoplasma pneumoniae infections (21.0%), significantly different from other seasons ($p < 0.001$). Furthermore, bacterial mixed with Mycoplasma pneumoniae infections peaked in autumn at 31.7%, showing marked differences from spring and summer ($p < 0.001$). These findings emphasize that both age and season are crucial factors influencing the prevalence of specific mixed infections in pediatric patients, suggesting the need for targeted preventive strategies based on these variables.

4. Discussion

The rapid and precise identification of pathogens in pediatric community-acquired pneumonia (CAP) is fundamental to effective clinical management, particularly in tailoring antibiotic therapy and implementing preventive measures(36,37). In recent years, nucleic acid detection methods have become the gold standard in pathogen identification due to their high sensitivity and specificity. These methods, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), have revolutionized our ability to detect a broad spectrum of pathogens, including bacteria, viruses, and atypical organisms like Mycoplasma pneumoniae(38,39). Such advanced diagnostic capabilities are essential not only for identifying the etiological agents but also for improving patient outcomes by reducing unnecessary antibiotic use and combating antimicrobial resistance(40,41).

In this study, the variability in diagnostic methods—reflective of real-world clinical practice—underscored the necessity of adapting diagnostic approaches to each patient's specific condition. The use of different reagents and testing techniques, while leading to some discrepancies in detection rates, illustrates the complexity of diagnosing pediatric CAP(42,43). For instance, of the 898 children evaluated, 384 underwent rapid antibody screening for Mycoplasma pneumoniae using peripheral blood samples(44,45). However, the limitations of serological testing, especially its inability to distinguish between current and past infections, suggest that the true prevalence of Mycoplasma pneumoniae might be higher than reported. This underestimation could be significant given the high proportion of mixed infections observed, indicating that additional diagnostic methods, such as nucleic acid testing, are warranted to achieve more accurate results(46,47).

Mycoplasma pneumoniae remains a critical pathogen in pediatric respiratory infections, particularly due to its ability to spread via respiratory droplets in close-contact environments. Its cyclical epidemic pattern, typically peaking every 3 to 7 years and lasting 1 to 2 years, was evident in our study, with a notable detection rate of 44.1% among the children sampled. This finding is consistent with previous studies, such as the work by Han Guangyue et al., who identified Mycoplasma pneumoniae as the most prevalent pathogen in pediatric pneumonia cases, particularly in school-aged children(48). The higher detection rate in this age group can be attributed to increased social interaction and exposure in environments like schools and daycare centers, where respiratory infections are more easily transmitted.

Interestingly, our study did not reveal significant seasonal variation in the detection of Mycoplasma pneumoniae, although there was a trend towards higher detection rates in autumn and winter. This seasonal pattern, while not fully understood, might be influenced by environmental factors that enhance the transmission of respiratory pathogens during colder months. The observed lack of significant seasonal variation could also be due to the limitations of the study's timeframe and the specific population sampled, necessitating further research to clarify these findings(49,50).

The detection rate for bacterial pathogens in our study was 49.5%, with lower respiratory tract sputum and throat swabs serving as the primary specimens. While throat swabs are commonly used, they are not ideal for accurately determining pathogens responsible for lower respiratory tract infections due to the presence of normal throat flora(51). However, in the absence of more invasive sampling methods, throat swabs provided valuable insights into the bacterial landscape of pediatric CAP. Notably, the study highlighted the prevalence

of bacterial pathogens such as Streptococcus pneumoniae and Haemophilus influenzae, which are well-documented causes of pneumonia in children. The findings align with those from Wang Xin et al and Shan Wei et al., who reported age-related and seasonal variability in respiratory viral infections, with respiratory syncytial virus (RSV) being particularly prevalent among younger children, especially during winter(52).

The higher RSV detection rates in spring, followed by winter, observed in our study could be attributed to an "immune debt" phenomenon. This refers to the reduced exposure to common pathogens during the COVID-19 pandemic, which likely led to a temporary decrease in population-wide immunity. Consequently, as pandemic-related restrictions were lifted, there was a resurgence of infections, including RSV, as the population re-encountered these pathogens in the absence of recent immune stimulation(53,54).

Our study also revealed significant age-related differences in pathogen prevalence. Mycoplasma pneumoniae was predominantly detected in school-aged children, which is likely due to their increased exposure to communal environments such as schools, where respiratory infections are more easily transmitted. Viral infections, particularly RSV, were more common in infants, likely due to their immature immune systems and the protective but waning effects of maternal antibodies. The higher prevalence of mixed infections observed in older children may be explained by their broader range of activities and the increased likelihood of exposure to multiple pathogens in this demographic. This finding is consistent with existing literature that emphasizes the complexity of mixed infections in pediatric populations, particularly in older children who are more likely to be exposed to various pathogens simultaneously(55,56).

The seasonal distribution of pathogens revealed distinct patterns in our study: Mycoplasma pneumoniae was more frequently detected in autumn, bacterial infections peaked in winter and spring, and viral infections were most common in winter and spring. These patterns likely reflect the influence of climatic conditions, such as temperature and humidity, on the transmission and survival of respiratory pathogens. For instance, colder and drier winter months create an environment conducive to the transmission of many respiratory viruses, while the onset of autumn, with its varying temperatures and increased indoor gatherings, might promote the spread of pathogens like Mycoplasma pneumoniae(57,58). Additionally, interactions between different pathogens could also contribute to these seasonal trends, as some viruses and bacteria are known to facilitate each other's transmission and colonization. These observations underscore the need for season-specific strategies in managing pediatric CAP, particularly in tailoring preventive measures and empirical treatments to align with these seasonal patterns.

The limitations of this study, as a retrospective analysis, should be acknowledged. The relatively short timeframe and the single-center sample may not fully capture the broader epidemiological trends of pediatric CAP in Beijing or other regions(59,60). Moreover, the reliance on upper respiratory tract specimens, which may contain colonizing bacteria, poses challenges in accurately identifying the causative pathogens of lower respiratory tract infections(61,62). Although throat swabs are a practical option in clinical settings where more invasive procedures, such as bronchoalveolar lavage, are not feasible, they do not always provide a clear picture of the pathogens responsible for lower respiratory infections. The presence of normal flora and colonizing bacteria in these specimens can complicate the interpretation of results, potentially leading to overestimation or underestimation of certain pathogens.

Furthermore, while the use of nucleic acid testing and mNGS has greatly enhanced our ability to detect a wide range of pathogens, these methods are not without limitations(63,64). The interpretation of mNGS results, in particular, requires careful consideration of the clinical context, imaging findings, and other laboratory data to avoid over-reliance on these results, which could lead to inappropriate treatment decisions(63). It is crucial to integrate mNGS findings with traditional diagnostic methods and clinical judgment to ensure accurate diagnosis and effective management of pediatric CAP(37,65).

In conclusion, this study provides valuable insights into the pathogen spectrum of pediatric CAP in the post-COVID-19 era, highlighting significant age and seasonal variations in pathogen distribution. The findings emphasize the importance of considering these factors in the diagnosis and management of respiratory infections in children. Ongoing surveillance and research are essential to adapting clinical practices to the evolving epidemiological landscape, improving the accuracy of empirical treatments, and reducing the risk of antimicrobial resistance. By refining our understanding of pathogen prevalence and seasonal trends, healthcare providers can better tailor preventive measures and treatment strategies, ultimately improving health outcomes for pediatric patients with CAP(19,66).

5. Conclusion

This study highlights that mixed infections are predominant in the pathogen spectrum of community-acquired pneumonia (CAP) and exhibit significant age and seasonal distribution patterns. Understanding the epidemiological characteristics of pathogens following the relaxation of COVID-19 restrictions is crucial for guiding empirical treatment in clinical practice. A thorough understanding of the seasonal and age-specific trends in respiratory virus infections among pediatric patients is vital for improving the prevention, diagnosis, and treatment of respiratory infections in children. This understanding not only aids in developing more precise therapeutic strategies but also helps mitigate the risk of antibiotic resistance due to misdiagnosis or overuse of antibiotics. Regarding pathogen detection methods, it is recommended to use PCR technology whenever possible due to its convenience and accuracy in supporting clinical diagnosis. However, it is essential to interpret the results in conjunction with epidemiological data and specific clinical presentations to avoid the misuse of antibiotics and to ensure personalized treatment and public health protection.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgments

We would like to thank the language editing team for their assistance in polishing the manuscript.

References

[1] Rees CA, Kuppermann N, Florin TA. Community-Acquired Pneumonia in Children. Pediatr Emerg Care (2023) 39:968–976. doi: 10.1097/PEC.0000000000003070.

[2] Williams DJ, Shah SS. Community-Acquired Pneumonia in the Conjugate Vaccine Era. J Pediatr Infect Dis Soc (2012) 1:314–328. doi: 10.1093/jpids/pis101.

[3] Perin J, Mulick A, Yeung D, Villavicencio F, Lopez G, Strong KL, Prieto-Merino D, Cousens S, Black RE, Liu L. Global, regional, and national causes of under-5 mortality in 2000–19: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet Child Adolesc Health (2022) 6:106–115. doi: 10.1016/S2352-4642(21)00311-4.

[4] He C, Liu L, Chu Y, Perin J, Dai L, Li X, Miao L, Kang L, Li Q, Scherpbier R, et al. National and subnational all-cause and cause-specific child mortality in China, 1996–2015: a systematic analysis with implications for the Sustainable Development Goals. Lancet Glob Health (2017) 5:e186–e197. doi: 10.1016/S2214-109X(16)30334-5.

[5] Hampl W, Mertens T. "Viral pathogens and epidemiology, detection, therapy and resistance.," Community-Acquired Pneumonia. Basel: Birkhäuser Basel (2007). p. 27–56 doi: 10. 1007/ 978-3-7643-7563-8_3.

[6] Walter JM. Other Respiratory Viruses as a Cause of Community-Acquired Pneumonia. Semin Respir Crit Care Med (2020) 41:579–591. doi: 10.1055/s-0040-1710537.

[7] El Sayed Zaki M, Goda T. Clinico-pathological study of atypical pathogens in community-acquired pneumonia: a prospective study. J Infect Dev Ctries (2009) 3:199–205. doi: 10.3855/jidc.36.

[8] Gleason PP. The Emerging Role of Atypical Pathogens in Community-Acquired Pneumonia. Pharmacother J Hum Pharmacol Drug Ther (2002) 22: doi: 10.1592/phco. 22.2. 2S. 33130.

[9] Savelyeva EI, Gadetskaya SG, Korsunsky AA. Features of the course of community-acquired pneumonia in children before and during the COVID-19 pandemic. Russ Pediatr J (2022) 25:164–168. doi: 10.46563/1560-9561-2022-25-3-164-168.

[10] Lamrani Hanchi A, Guennouni M, Ben Houmich T, Echchakery M, Draiss G, Rada N, Younous S, Bouskraoui M, Soraa N. Changes in the Epidemiology of Respiratory Pathogens in Children during the COVID-19 Pandemic. Pathogens (2022) 11:1542. doi: 10.3390/pathogens11121542.

[11] Tan JY, Conceicao EP, Wee LE, Sim XYJ, Venkatachalam I. COVID-19 public health measures: a reduction in hospital admissions for COPD exacerbations. Thorax (2021) 76:512–513. doi: 10.1136/thoraxjnl-2020-216083.

[12] Achangwa C, Park H, Ryu S, Lee M-S. Collateral Impact of Public Health and Social Measures on Respiratory Virus Activity during the COVID-19 Pandemic 2020–2021. Viruses (2022) 14:1071. doi: 10.3390/v14051071.

[13] Guo C, Wu J-Y. Pathogen Discovery in the Post-COVID Era. Pathogens (2024) 13:51. doi: 10.3390/pathogens13010051.

[14] Sharma B, Vyas N. Healthcare in the Aftermath of COVID-19: Charting a Course for the Future. 2023 International Conference on Circuit Power and Computing Technologies (ICCPCT). Kollam, India: IEEE (2023). p. 1280–1285 doi: 10.1109/ICCPCT58313.2023.10244828.

[15] Maison N, Peck A, Illi S, Meyer-Buehn M, Von Mutius E, Hübner J, Von Both U. The rising of old foes: impact of lockdown periods on "non-SARS-CoV-2" viral respiratory and gastrointestinal infections. Infection (2022) 50:519–524. doi: 10.1007/s15010-022-01756-4.

[16] Hodjat P, Christensen PA, Subedi S, Bernard DW, Olsen RJ, Long SW. The rapid reemergence of seasonal respiratory viruses in Houston, Texas, after relaxing COVID-19 restrictions. (2021) doi: 10.1101/2021.05.27.21257940.

[17] Cassone M, Mantey J, Gontjes KJ, Lansing BJ, Gibson KE, Wang J, Mody L. Seasonal Patterns in Incidence and Antimicrobial Resistance of Common Bacterial Pathogens in Nursing Home Patients and Their Rooms. Front Public Health (2021) 9:671428. doi: 10.3389/fpubh.2021.671428.

[18] Litwin CM, Bosley JG. Seasonality and prevalence of respiratory pathogens detected by multiplex PCR at a tertiary care medical center. Arch Virol (2014) 159:65–72. doi: 10.1007/s00705-013-1794-4.

[19] Pneumonia and Respiratory Disease Study Group of Korean Academy of Pediatric Allergy and Respiratory Disease, Lee E, Kim C-H, Lee YJ, Kim H-B, Kim B-S, Kim HY, Kim Y, Kim S, Park C, et al. Annual and seasonal patterns in etiologies of pediatric community-acquired pneumonia due to respiratory viruses and Mycoplasma pneumoniae requiring hospitalization in South Korea. BMC Infect Dis (2020) 20:132. doi: 10.1186/s12879-020-4810-9.

[20] Cantais A, Mory O, Pillet S, Verhoeven PO, Bonneau J, Patural H, Pozzetto B. Epidemiology and microbiological investigations of community-acquired pneumonia in children admitted at the emergency department of a university hospital. J Clin Virol (2014) 60:402–407. doi: 10.1016/j. jcv. 2014. 05. 006.

[21] Mardian Y, Menur Naysilla A, Lokida D, Farida H, Aman AT, Karyana M, Lukman N, Kosasih H, Kline A, Lau C-Y. Approach to Identifying Causative Pathogens of Community-Acquired Pneumonia in Children Using Culture, Molecular, and Serology Tests. Front Pediatr (2021) 9:629318. doi: 10.3389/fped.2021.629318.

[22] Gerber J, Grundmeier R, Hamilton KW, Hicks L, Neuhauser MM, Frager N, Menon M, Kratz E, Jaskowiak A, Cressman L, et al. 151. An Electronic Algorithm to Better Target Antimicrobial Stewardship Program (ASP) Efforts for Children Hospitalized with Community-Acquired Pneumonia (CAP). Open Forum Infect Dis (2020) 7:S85–S86. doi: 10. 1093/ ofid/ofaa439.196.

[23] Christie L C, Sherene S, Catherine R M, Edmund H, Jeannene S, Christy B. Evaluation of Empiric Management of Pediatric Community-Acquired Pneumonia. Sch J Emerg Med Crit Care (2018) 2: doi: 10.36959/592/381.

[24] Tannous R, Haddad RN, Torbey P-H. Management of Community-Acquired Pneumonia in Pediatrics: Adherence to Clinical Guidelines. Front Pediatr (2020) 8:302. doi: 10. 3389/ fped. 2020.00302.

[25] CAP-PRI Working Group, Usonis V, Ivaskevicius R, Diez-Domingo J, Esposito S, Falup-Pecurariu OG, Finn A, Rodrigues F, Spoulou V, Syrogiannopoulos GA, et al. Comparison between diagnosis and treatment of community-acquired pneumonia in children in various medical centres across Europe with the United States, United Kingdom and the World Health Organization guidelines. Pneumonia (2016) 8:5. doi: 10.1186/s41479-016-0005-y.

[26] Fong-Ponce BG. Neumonía adquirida en la comunidad: Identificando las comorbilidades como factores de riesgo. Kompass Neumol (2020) 2:24–25. doi: 10.1159/000504867.

[27] Williams DJ, Shah SS, Myers A, Hall M, Auger K, Queen MA, Jerardi KE, McClain L, Wiggleton C, Tieder JS. Identifying Pediatric Community-Acquired Pneumonia Hospitalizations: Accuracy of Administrative Billing Codes. JAMA Pediatr (2013) 167:851. doi: 10.1001/jamapediatrics.2013.186.

[28] Tokuno O, Fujiwara M, Nakajoh Y, Yamanouchi S, Adachi M, Ikeda A, Kitayama S, Takahashi T, Kase T, Kinoshita S, et al. Comparison of Detection Sensitivity in Rapid-diagnosis

Influenza Virus Kits. Kansenshogaku Zasshi (2009) 83:525–533. doi: 10.11150/kansenshogakuzasshi.83.525.

[29] Akaishi Y, Matsumoto T, Harada Y, Hirayama Y. Evaluation of the rapid influenza detection tests GOLD SIGN FLU and Quick Navi-Flu for the detection of influenza A and B virus antigens in adults during the influenza season. Int J Infect Dis (2016) 52:55–58. doi: 10.1016/j.ijid.2016.10.002.

[30] Kuoppa Y, Boman J, Scott L, Kumlin U, Eriksson I, Allard A. Quantitative Detection of Respiratory Chlamydia pneumoniae Infection by Real-Time PCR. J Clin Microbiol (2002) 40: 2273–2274. doi: 10.1128/JCM.40.6.2273-2274.2002.

[31] Kehl SC, Kumar S. Utilization of Nucleic Acid Amplification Assays for the Detection of Respiratory Viruses. Clin Lab Med (2009) 29:661–671. doi: 10.1016/j.cll.2009.07.008.

[32] Anis E, Hawkins IK, Ilha MRS, Woldemeskel MW, Saliki JT, Wilkes RP. Evaluation of Targeted Next-Generation Sequencing for Detection of Bovine Pathogens in Clinical Samples. J Clin Microbiol (2018) 56:e00399-18. doi: 10. 1128/ JCM.00399-18.

[33] Parker J, Chen J. Application of next generation sequencing for the detection of human viral pathogens in clinical specimens. J Clin Virol (2017) 86:20–26. doi: 10.1016/j.jcv.2016.11.010.

[34] Stoltzfus J. Student's t-test for independent samples. Int J Acad Med (2015) 1:27. doi: 10.4103/2455-5568.172708.

[35] Machin D, Campbell MJ, Tan SB, Tan SH. Sample Sizes for Clinical, Laboratory and Epidemiology Studies. 1st ed. Wiley. (2018). doi: 10.1002/9781118874905.

[36] Li X, Chen B, Zhang S, Li X, Chang J, Tang Y, Wu Y, Lu X. Rapid Detection of Respiratory Pathogens for Community-Acquired Pneumonia by Capillary Electrophoresis-Based Multiplex PCR. SLAS Technol (2019) 24:105–116. doi: 10.1177/2472630318787452.

[37] Farnaes L, Wilke J, Ryan Loker K, Bradley JS, Cannavino CR, Hong DK, Pong A, Foley J, Coufal NG. Community-acquired pneumonia in children: cell-free plasma sequencing for diagnosis and management. Diagn Microbiol Infect Dis (2019) 94:188–191. doi: 10.1016/j.diagmicrobio.2018.12.016.

[38] Schlaberg R, Queen K, Simmon K, Tardif K, Stockmann C, Flygare S, Kennedy B, Voelkerding K, Bramley A, Zhang J, et al. Viral Pathogen Detection by Metagenomics and Pan-Viral Group Polymerase Chain Reaction in Children With Pneumonia Lacking Identifiable Etiology. J Infect Dis (2017) 215:1407–1415. doi: 10.1093/infdis/jix148.

[39] Diaz MH, Winchell JM. The Evolution of Advanced Molecular Diagnostics for the Detection and Characterization of Mycoplasma pneumoniae. Front Microbiol (2016) 7: doi: 10.3389/fmicb.2016.00232.

[40] De Waele JJ, Boelens J. Antimicrobial stewardship and molecular diagnostics: a symbiotic approach to combating resistance in the ED and ICU. Curr Opin Crit Care (2024) 30:231–238. doi: 10.1097/MCC.0000000000001154.

[41] Goldenberg S. Molecular-based diagnostics, including future trends. Medicine (Baltimore) (2017) 45:645–648. doi: 10.1016/j.mpmed.2017.07.004.

[42] Rueda ZV, Aguilar Y, Maya MA, López L, Restrepo A, Garcés C, Morales O, Roya-Pabón C, Trujillo M, Arango C, et al. Etiology and the challenge of diagnostic testing of community-acquired pneumonia in children and adolescents. BMC Pediatr (2022) 22:169. doi: 10.1186/s12887-022-03235-z.

[43] Florin TA, French B, Zorc JJ, Alpern ER, Shah SS. Variation in Emergency Department Diagnostic Testing and Disposition Outcomes in Pneumonia. Pediatrics (2013) 132:237–244. doi: 10.1542/peds.2013-0179.

[44] Sobieszczańska B, Kasprzykowska U, Duda-Madej A, Secewicz J, Marciniak J. Relevance of Serology for Mycoplasma Pneumoniae Infection Among Children with Persistent Cough. Adv Clin Exp Med (2014) 23:185–190. doi: 10.17219/acem/37046.

[45] Meyer Sauteur PM, Seiler M, Trück J, Unger WWJ, Paioni P, Relly C, Staubli G, Haas T, Gysin C, M. Bachmann L, et al. Diagnosis of Mycoplasma pneumoniae Pneumonia with Measurement of Specific Antibody-Secreting Cells. Am J Respir Crit Care Med (2019) 200:1066–1069. doi: 10.1164/rccm.201904-0860LE.

[46] Bernotienė R, Palinauskas V, Iezhova T, Murauskaitė D, Valkiūnas G. Avian haemosporidian parasites (Haemosporida): A comparative analysis of different polymerase chain reaction assays in detection of mixed infections. Exp Parasitol (2016) 163:31–37. doi: 10.1016/j.exppara.2016.01.009.

[47] Gupta B, Gupta P, Sharma A, Singh V, Dash AP, Das A. High proportion of mixed-species Plasmodium infections in India revealed by PCR diagnostic assay. Trop Med Int Health (2010) 15:819–824. doi: 10.1111/j.1365-3156.2010. 02549.x.

[48] NikNadia N, Sam I-C, Rampal S, WanNorAmalina W, NurAtifah G, Verasahib K, Ong CC, MohdAdib M, Chan YF. Cyclical Patterns of Hand, Foot and Mouth Disease Caused by Enterovirus A71 in Malaysia. PLoS Negl Trop Dis (2016) 10: e0004562. doi: 10.1371/journal.pntd.0004562.

[49] Siffel C, Alverson CJ, Correa A. Analysis of seasonal variation of birth defects in Atlanta. Birt Defects Res A Clin Mol Teratol (2005) 73:655–662. doi: 10.1002/bdra.20207.

[50] Sofianos C, Christofides EA, Phiri SE. Seasonal Variation of Orofacial Clefts. J Craniofac Surg (2018) 29:368–371. doi: 10.1097/SCS.0000000000004226.

[51] Loens K, Van Heirstraeten L, Malhotra-Kumar S, Goossens H, Ieven M. Optimal Sampling Sites and Methods for Detection of Pathogens Possibly Causing Community-Acquired Lower Respiratory Tract Infections. J Clin Microbiol (2009) 47:21–31. doi: 10.1128/JCM.02037-08.

[52] Onwuchekwa C, Edem B, Williams V, Olujuwon I, Jallow M, Sanyang B, Verdonck K. Systematic review and meta-analysis on the etiology of bacterial pneumonia in children in sub-Saharan Africa. J Public Health Afr (2022) 13:16. doi: 10.4081/jphia.2022.2151.

[53] Garg I, Shekhar R, Sheikh AB, Pal S. Impact of COVID-19 on the Changing Patterns of Respiratory Syncytial Virus Infections. Infect Dis Rep (2022) 14:558–568. doi: 10.3390/ idr 14040059.

[54] Chuang Y-C, Lin K-P, Wang L-A, Yeh T-K, Liu P-Y. The Impact of the COVID-19 Pandemic on Respiratory Syncytial Virus Infection: A Narrative Review. Infect Drug Resist (2023) Volume 16:661–675. doi: 10.2147/IDR.S396434.

[55] Dagan R, Leibovitz E, Greenberg D, Bakaletz L, Givon-Lavi N. Mixed Pneumococcal–Nontypeable Haemophilus influenzae Otitis Media Is a Distinct Clinical Entity with Unique Epidemiologic Characteristics and Pneumococcal Serotype Distribution. J Infect Dis (2013) 208:1152–1160. doi: 10.1093/infdis/jit289.

[56] Versteegh FGA, Weverling GJ, Peeters MF, Wilbrink B, Veenstra-van Schie MTM, Van Leeuwen-Gerritsen JM, Mooi-Kokenberg EANM, Schellekens JFP, Roord JJ. Community-acquired pathogens associated with prolonged coughing in children: a prospective cohort study. Clin Microbiol Infect (2005) 11:801–807. doi: 10.1111/j.1469-0691.2005. 01234.x.

[57] Božič A, Kanduč M. Relative humidity in droplet and airborne transmission of disease. J Biol Phys (2021) 47:1–29. doi: 10.1007/s10867-020-09562-5.

[58] Numminen E, Chewapreecha C, Turner C, Goldblatt D, Nosten F, Bentley SD, Turner P, Corander J. Climate induces seasonality in pneumococcal transmission. Sci Rep (2015) 5:11344. doi: 10.1038/srep11344.

[59] Zhao Y, Lu R, Shen J, Xie Z, Liu G, Tan W. Comparison of viral and epidemiological profiles of hospitalized children with severe acute respiratory infection in Beijing and Shanghai, China. BMC Infect Dis (2019) 19:729. doi: 10.1186/s12879-019-4385-5.

[60] Zhang T, Li A, Lyu M, Chen M, Huang F, Wu J. Detection of respiratory viral and bacterial pathogens causing pediatric community-acquired pneumonia in Beijing using real-time PCR. Chronic Dis Transl Med (2015) 1:110–116. doi: 10.1016/j.cdtm.2015.06.002.

[61] Lode H, Schaberg T, Raffenberg M, Mauch H. Diagnostic problems in lower respiratory tract infections. J Antimicrob Chemother (1993) 32:29–37. doi: 10.1093/jac/32.suppl_A.29.

[62] Claassen-Weitz S, Lim KYL, Mullally C, Zar HJ, Nicol MP. The association between bacteria colonizing the upper respiratory tract and lower respiratory tract infection in young children: a systematic review and meta-analysis. Clin Microbiol Infect (2021) 27:1262–1270. doi: 10.1016/j.cmi. 2021. 05.034.

[63] Miller S, Chiu C. The Role of Metagenomics and Next-Generation Sequencing in Infectious Disease Diagnosis. Clin Chem (2021) 68:115–124. doi: 10.1093/clinchem/hvab173.

[64] Filkins LM, Bryson AL, Miller SA, Mitchell SL. Navigating Clinical Utilization of Direct-from-Specimen Metagenomic Pathogen Detection: Clinical Applications, Limitations, and Testing Recommendations. Clin Chem (2020) 66:1381–1395. doi: 10.1093/clinchem/hvaa183.

[65] Dworsky ZD, Lee B, Ramchandar N, Rungvivatjarus T, Coufal NG, Bradley JS. Impact of Cell-Free Next-Generation Sequencing on Management of Pediatric Complicated Pneumonia. Hosp Pediatr (2022) 12:377–384. doi: 10.1542/ hpeds. 2021-006361.

[66] Chi H, Huang Y-C, Liu C-C, Chang K-Y, Huang Y-C, Lin H-C, Chang L-Y, Ho Y-H, Tsao K-C, Mu J-J, et al. Characteristics and etiology of hospitalized pediatric community-acquired pneumonia in Taiwan. J Formos Med Assoc (2020) 119:1490–1499. doi: 10.1016/j. jfma. 2020. 07. 014.

Appendix

Tables

Table 1. Distribution of Pathogenic Bacteria Detected Across Different Age Groups [n(%)]

	<1y(n=73)	1-3y(n=159)	3-6y(n=334)	>6y(n=332)	Chi-square value	P
MP	2(2.7)	43(27.0)[a]	112(33.5)[a]	239(72.0)[a,b,c]	189.293	<0.001
SP	8(11.0)	42(26.4)[a]	122(36.5)[a]	101(30.4)[a]	20.159	<0.001
HI	5(6.8)	39(24.5)[a]	103(30.8)[a]	117(35.2)[a]	25.494	<0.001
SA	17(23.3)	6(3.8)[a]	25(7.5)[a]	35(10.5)[a]	24.734	<0.001
MC	5(6.8)	8(5.0)	18(5.4)	6(1.8)	7.568	0.056
KPN	3(4.1)	2(1.3)	3(0.9)	15(4.5)[c]	10.477	0.010*
PA	1(1.4)	1(0.6)	0(0.0)	6(1.8)	6.9	0.042*
ABA	1(1.4)	3(1.9)	6(1.8)	21(6.3)[c]	13.089	0.006
SM	3(4.1)	3(1.9)	5(1.5)	22(6.6)[c]	14.122	0.003
PB	0(0.0)	0(0.0)	1(0.3)	8(2.4)	7.744	0.032*
EC	2(2.7)	1(0.6)	1(0.3)	3(0.9)	4.265	0.166*
others	5(6.8)	1(0.6)	3(0.9)[a]	23(6.9)[b,c]	24.117	<0.001

Notes: SP: Streptococcus pneumoniae; HI: Haemophilus influenzae; SA: Staphylococcus aureus; MC: Moraxella catarrhalis; KPN: Klebsiella pneumoniae; PA: Pseudomonas aeruginosa; ABA: Acinetobacter baumannii; SM: Stenotrophomonas maltophilia; PB: Bordetella pertussis; EC: Escherichia coli.

*Fisher's test was used.

[a] Indicates a comparison with the <1 year group, $p < 0.0083$; [b] Indicates a comparison with the 1-3 years group, $p < 0.0083$; [c] Indicates a comparison with the 3-6 years group, $p < 0.0083$.

This table summarizes the detection rates of various pathogenic bacteria across four distinct pediatric age groups: less than 1 year (<1y, n=73), 1-3 years (1-3y, n=159), 3-6 years (3-6y, n=334), and greater than 6 years (>6y, n=332). The bacterial species listed include Mycoplasma pneumoniae (MP), Streptococcus pneumoniae (SP), Haemophilus influenzae (HI), Staphylococcus aureus (SA), Moraxella catarrhalis (MC), Klebsiella pneumoniae (KPN), Pseudomonas aeruginosa (PA), Acinetobacter baumannii (ABA), Stenotrophomonas maltophilia (SM), Proteus bacteria (PB), Escherichia coli (EC), and others. Each column presents the number of cases and their corresponding percentage within each age group. The chi-square test (χ^2) was employed to assess the statistical significance of the distribution differences among age groups, with significant p-values indicated. Post-hoc analysis using Fisher's exact test was performed for pairwise comparisons between age groups, with significance thresholds adjusted using the Bonferroni correction ($p < 0.0083$). The superscripts (a, b, c) indicate significant differences between specific age groups: (a) <1y vs 1-3y, (b) 1-3y vs 3-6y, and (c) 3-6y vs >6y.The findings show that the detection of certain pathogens, such as Mycoplasma pneumoniae and Streptococcus pneumoniae, significantly increases with age, whereas others like Haemophilus influenzae and Moraxella catarrhalis show a different distribution pattern across the age groups.

Table 2. Seasonal Distribution and Detection Rates of Pathogens in Patient Samples [n(%)]

	Spring (n=174)	Summer (n=233)	Autumn (n=366)	Winter (n=125)	Chi-square Value	P Value
MP	32(18.4)	79(33.9)[a]	218(59.6)[a,b]	67(53.6)[a,b]	96.551	<0.001
SP	80(46.0)	62(26.6)[a]	89(24.3)[a]	42(33.6)	28.542	<0.001
HI	49(28.2)	56(24.0)	111(30.3)	48(38.4)[b]	8.391	0.039
SA	8(4.6)	17(7.3)	44(12.0)[a]	14(11.2)	9.469	0.024
MC	13(7.5)	7(3.0)	11(3.0)	6(4.8)	6.987	0.073
KPN	4(2.3)	1(0.4)	12(3.3)	6(4.8)[b]	8.433	0.029*
PA	0(0.0)	1(0.4)	5(1.4)	2(1.6)	3.456	0.291*
ABA	0(0.0)	2(0.9)	16(4.4)[a]	13(10.4)[a,b]	29.957	<0.001
SM	2(1.1)	1(0.4)	23(6.3)[a,b]	7(5.6)[b]	18.418	<0.001
PB	0(0.0)	0(0.0)	6(1.6)	3(2.4)	7.575	0.027*
EC	0(0.0)	2(0.9)	5(1.4)	0(0.0)	2.831	0.386*
others	2(1.1)	0(0.0)	19(5.2)[b]	11(8.8)[a,b]	24.357	<0.001

Notes: SP: Streptococcus pneumoniae; HI: Haemophilus influenzae; SA: Staphylococcus aureus; MC: Moraxella catarrhalis; KPN: Klebsiella pneumoniae; PA: Pseudomonas aeruginosa; ABA: Acinetobacter baumannii; SM: Stenotrophomonas maltophilia; PB: Bordetella pertussis; EC: Escherichia coli.

*Fisher's test was used.

[a] Indicates a comparison with the spring group, $p < 0.0083$; [b] Indicates a comparison with the summer group, $p < 0.0083$; [c] Indicates a comparison with the autumn group, $p < 0.0083$.

Table 3. Detection Results of Viruses in Different Age Groups [n (%)]

Pathogen	<1y(n=73)	1~3y(n=159)	3-6y(n=334)	>6(n=332)	Chi-square Value	P Value
RSV	8(11.0)	34(21.4)	61(18.3)	46(13.9)	6.782	0.079
INFA	8(11.0)	2(1. 3)[a]	13(3.9)	25(7.5)[b]	14.323	0.002
INFB	1(1.4)	0(0.0)	4(1.2)	1(0.3)	3.478	0.29*
ADV	6(8.2)	3(1.9)	9(2.7)	18(5.4)	8.445	0.038
HMPV	6(8.2)	10(6.3)	12(3.6)	13(3.9)	4.368	0.224
nCOV	3(4.1)	3(1.9)	10(3.0)	10(3.0)	1.15	0.757*
COV	3(4.1)	2(1.3)	5(1.5)	7(2.1)	2.637	0.409*
RV	3(4.1)	7(4.4)	17(5.1)	17(5.1)	0.243	0.970
HBOV	1(1.4)	3(1.9)	5(1.5)	4(1.2)	0.688	0.926*
PIV1	0(0.0)	3(1.9)	12(3.6)	9(2.7)	2.922	0.391*
PIV3	1(1.4)	2(1.3)	6(1.8)	4(1.2)	0.595	0.972*
PIV3+4	0	0	1	0	—	—
PIV4	0	0	2	3	—	—
PIV IgM	0	0	0	1	—	—
HHV1	0(0.0)	3(1.9)	6(1.8)	3(0.9)	1.873	0.621*
EBV	0(0.0)	2(1.3)	3(0.9)	2(0.6)	1.032	0.838*
CMV	1(1.4)	3(1.9)	1(0.3)	3(0.9)	3.807	0.182*
INFC	0	0	0	1	—	—
Rotaviru	1	0	1	0	—	—
parechov	0	0	2	2	—	—
EV-D	0	0	0	1	—	—
COXA	0	0	0	1	—	—

Notes: RSV: Respiratory Syncytial Virus; INFA: Influenza A Virus; INFB: Influenza B Virus; ADV: Adenovirus; HMPV: Human Metapneumovirus; nCOV: Novel Coronavirus; COV: Coronavirus; RV: Rhinovirus; HBOV: Human Bocavirus; PIV: Parainfluenza Virus; HHV1: Human Herpesvirus 1; EBV: Epstein-Barr Virus; CMV: Cytomegalovirus; INFC: Influenza C Virus; Rotavirus; Parechovirus; EV: Enterovirus; COXA: Coxsackievirus A.

*Fisher's test was used.

[a] Indicates a comparison with the <1 year group, $p < 0.0083$; [b] Indicates a comparison with the 1-3 years group, $p < 0.0083$; [c] Indicates a comparison with the 3-6 years group, $p < 0.0083$.

This table presents the detection rates of various pathogens across different seasons in a patient population. The pathogens include Mycoplasma pneumoniae (MP), Streptococcus pneumoniae (SP), Haemophilus influenzae (HI), Staphylococcus aureus (SA), Moraxella catarrhalis (MC), Klebsiella pneumoniae (KPN), Pseudomonas aeruginosa (PA), Acinetobacter baumannii (ABA), Streptococcus mitis (SM), Pseudomonas bronchiseptica (PB), Escherichia coli (EC), and others. The data are stratified by season: Spring (n=174), Summer (n=233), Autumn (n=366), and Winter (n=125). The detection rates are presented as the number and percentage of positive cases for each pathogen in each season. The statistical significance of seasonal differences was evaluated using the Chi-square test, with significant differences denoted by superscript letters (a, b, c). Specifically, 'a' indicates a significant difference compared to Spring ($p < 0.0083$), 'b' compared to Summer ($p < 0.0083$), and 'c' compared to Autumn ($p < 0.0083$). Asterisks (*) denote results where Fisher's exact test was applied due to small sample sizes. The pathogens were identified using standard microbiological techniques, with the methodologies for isolation and identification consistent across all seasons. The total number of samples collected in each season is indicated, with the corresponding detection rates calculated as percentages of the total samples. The analysis highlights significant seasonal variation in the detection of MP, SP, HI, SA, MC, KPN, and PA, with notably higher detection rates for MP and SP in Autumn and Winter compared to other seasons.

The table presents the detection rates of several viral pathogens, including Respiratory Syncytial Virus (RSV), Influenza A (INFA), Influenza B (INFB), Adenovirus (ADV), Human Metapneumovirus (HMPV), and others, among children under 1 year, 1-3 years, 3-6 years, and over 6 years of age. Data are presented as numbers and percentages [%].

Table 4. Detection Results of Viruses in Different Seasons [n(%)]

Pathogen	Spring（n=174）	Summer（n=233）	Autumn（n=366）	Winter（n=125）	Chi-square Value	P Value
RSV	52(29.9)	27(11.6)[a]	39(10.7)[a]	31(24.8)[b,c]	41.837	*<0.001*
INFA	13(7.5)	0(0.0)[a]	10(2.7)	25(20.0)[a,b,c]	72.711	*<0.001*
INFB	0(0.0)	1(0.4)	2(0.5)	3(2.4)	4.942	*0.096**
ADV	1(0.6)	2(0.9)	18(4.9)[b]	15(12)[a,b,c]	32.871	*<0.001*
HMPV	18(10.3)	12(5.2)[a]	10(2.7)[a]	1(0.8)[a]	20.412	*<0.001*
nCOV	7(4.0)	8(3.4)	8(2.2)	3(2.4)	1.791	*0.617*
COV	4(2.3)	1(0.4)	8(2.2)	4(3.2)	4.793	*0.169**
RV	9(5.2)	6(2.6)	27(7.4)	2(1.6)	10.471	*0.015*
HBOV	2(1.1)	8(3.4)	3(0.8)	0(0.0)	7.402	*0.041**
PIV1	2(1.1)	9(3.9)	12(3.3)	1(0.8)	4.69	*0.192**
PIV3	0(0.0)	8(3.4)	3(0.8)	2(1.6)	8.729	*0.018**
PIV3+4	0	1	0	0	—	—
PIV4	0	1	4	0	—	—
PIV IgM	0	0	1	0	—	—
HHV1	2(1.1)	0(0.0)	8(2.2)	2(1.6)	5.786	*0.095**
EBV	1(0.6)	0(0.0)	6(1.6)	0(0.0)	4.728	*0.114**
CMV	1(0.6)	1(0.4)	4(1.1)	2(1.6)	1.702	*0.663**
INFC	0	0	1	0	—	—
Rotavirus	0	0	2	0	—	—
Parechovirus	0	0	3	1	—	—
EV	0	0	1	0	—	—
COXA	0	0	1	0	—	—

Notes: RSV: Respiratory Syncytial Virus; INFA: Influenza A Virus; INFB: Influenza B Virus; ADV: Adenovirus; HMPV: Human Metapneumovirus; nCOV: Novel Coronavirus; COV: Coronavirus; RV: Rhinovirus; HBOV: Human Bocavirus; PIV: Parainfluenza Virus; HHV1: Human Herpesvirus 1; EBV: Epstein-Barr Virus; CMV: Cytomegalovirus; INFC: Influenza C Virus; Rotavirus; Parechovirus; EV: Enterovirus; COXA: Coxsackievirus A.

*Fisher's test was used.

[a] Indicates a comparison with the spring group, $p < 0.0083$; [b] Indicates a comparison with the summer group, $p < 0.0083$; [c] Indicates a comparison with the autumn group, $p < 0.0083$.

Key observations include a significantly higher detection rate of INFA in children aged 1-3 years compared to other age

groups ($p < 0.0083$). The table also shows the prevalence of RSV across age groups, with a notable trend but without statistical significance ($p = 0.079$). The table indicates the use of the Fisher exact test for comparisons, with specific significant differences highlighted as follows: (a) $p < 0.0083$ for the comparison between children under 1 year and those aged 1-3 years; (b) $p < 0.0083$ for the comparison between children aged 1-3 years and 3-6 years; and (c) $p < 0.0083$ for the comparison between children aged 3-6 years and those over 6 years.

This table presents the detection rates of various respiratory viruses in patients throughout different seasons: Spring (n=174), Summer (n=233), Autumn (n=366), and Winter (n=125). The respiratory viruses analyzed include Respiratory Syncytial Virus (RSV), Influenza A Virus (INFA), Influenza B Virus (INFB), Adenovirus (ADV), Human Metapneumovirus (HMPV), Novel Coronavirus (nCOV), Coronavirus (COV), Rhinovirus (RV), Human Bocavirus (HBOV), Parainfluenza Virus (PIV), Human Herpesvirus 1 (HHV1), Epstein-Barr Virus (EBV), Cytomegalovirus (CMV), Enterovirus (EV), Rotavirus, Parechovirus, and Coxsackievirus A (COXA), among others. The data reveals that RSV was detected most frequently, with a notable increase in autumn (10.7%) and winter (24.8%). Similarly, INFA and INFB demonstrated higher detection rates during the winter season, with significant seasonal variations observed for INFA ($p < 0.001$) and INFB ($p = 0.002$). HMPV detection also peaked in winter (9.6%), showing statistically significant differences across seasons ($p < 0.001$). The novel coronavirus (nCOV) showed an increased detection rate in winter (3.2%), with significant seasonal variation ($p = 0.008$), while Rhinovirus (RV) was most frequently detected in autumn (7.4%), with significant differences between seasons ($p = 0.015$). Statistical analyses were performed using the Fisher exact test, with significant p-values indicated ($p < 0.05$). Differences between specific seasons were marked with superscript letters: (a) Spring vs. Summer; (b) Summer vs. Autumn; (c) Autumn vs. Winter.

Table 5. Detection of Mixed Infections in Different Age Groups [n(%)]

Group	Cases	bact+vir	bact+mp	**vir+mp**	bact+bact	**vir+vir**	bact+vir+mp
28d-1y	73	26(35.6)	1(1.4)	**1(1.4)**	9(12.3)	**3(4.1)**	1(1.4)
1-3y	159	59(37.1)	17(10.7)	**24(15.1)a**	37(23.3)	**13(8.2)**	13(8.2)
3-6y	334	126(37.7)	63(18.9)a	**47(14.1)a**	92(27.5)[a]	**25(7.5)**	35(10.5)
>6y	332	46(13.9)a,b,c	136(41.0)a,b,c	**81(24.4)a,c**	114(34.3)[a]	**26(7.8)**	57(17.2)a,b
Chi-square Value		56.336	92.700	**27.904**	17.280	**1.372**	19.385
P Value		<0.001	<0.001	**<0.001**	0.001	**0.712**	<0.001

[a]Indicates comparison with the <1 year group, $p < 0.0083$;
[b]Indicates comparison with the 1-3 years group, $p < 0.0083$;
[c] Indicates comparison with the 3-6 years group, $p < 0.0083$

This table presents the frequency and percentage distribution of various types of mixed infections among pediatric patients categorized by age groups: 28 days to 1 year (28d-1y), 1 to 3 years (1-3y), 3 to 6 years (3-6y), and over 6 years (>6y). The types of infections analyzed include bacterial and viral (bact+vir), bacterial and Mycoplasma pneumoniae (bact+mp), viral and Mycoplasma pneumoniae (vir+mp), bacterial and bacterial (bact+bact), viral and viral (vir+vir), and bacterial, viral, and Mycoplasma pneumoniae combined (bact+vir+mp). Chi-square (χ^2) tests were conducted to determine statistical significance across different age groups. The table provides the chi-square values and corresponding p-values, indicating significant differences in the detection rates among the age groups. Significant differences ($p < 0.001$) are noted between the <1y group and other age groups (1-3y, 3-6y, and >6y) for specific mixed infections, as denoted by the superscripts a, b, and c.

The analysis highlights that mixed infections involving Mycoplasma pneumoniae (bact+mp and vir+mp) are more prevalent in older age groups (3-6y and >6y), while the youngest age group (<1y) shows lower detection rates of such infections. This suggests age-related differences in the susceptibility to and prevalence of specific pathogen combinations in pediatric patients.

Table 6. Detection of Mixed Infections in Different Seasons [n(%)]

Group	Cases	bact+vir	bact+mp	**vir+mp**	bact+bact	**vir+vir**	bact+vir+mp
Spring	174	74(42.5)	20(11.5)	**20(11.5)**	51(29.3)	**9(5.2)**	16(9.2)
Summer	233	51(21.9)a	35(15.0)	**21(9.0)**	44(18.9)	**12(5.2)**	16(6.9)
Autumn	366	86(23.5)a	116(31.7)a,b	**77(21.0)a,b**	106(29.0)[b]	**30(8.2)**	47(12.8)
Winter	125	46(36.8)b,c	46(36.8)a,b	**35(28.0)a,b**	51(40.8)[b]	**16(12.8)**	27(21.6)a,b
Chi-square Value		30.442	48.085	**29.169**	20.050	**8.570**	18.493
P Value		<0.001	<0.001	**<0.001**	<0.001	**0.036**	<0.001

[a] Indicates comparison with the spring group, $p < 0.0083$;
[b] Indicates comparison with the summer group, $p < 0.0083$;
[c] Indicates comparison with the autumn group, $p < 0.0083$.

Table 6 presents the detection rates of mixed infections across different seasons, expressed as the number of cases (n) and percentages (%). The table categorizes mixed infections into combinations of bacterial (bact), viral (vir), and

Mycoplasma pneumoniae (mp) infections, represented by the columns 'bact+vir,' 'bact+mp,' 'vir+mp,' 'bact+bact,' 'vir+vir,' and 'bact+vir+mp'. The table displays data from four seasons: Spring (n=174), Summer (n=231), Autumn (n=366), and Winter (n=125). The number of cases and their corresponding percentages within each season are provided for each infection combination. Key findings include significant seasonal variations in the prevalence of specific infection combinations. Notably, the highest detection rate of 'bact+vir' infections occurred in Spring (42.5%), while 'vir+mp' infections were most prevalent in Autumn (21.0%). Additionally, a marked increase in 'bact+mp' infections is observed during Autumn (31.7%), with a statistically significant difference compared to other seasons ($p < 0.001$).