

# Etiological profile of community acquired pneumonia in hospitalized children in Bulgaria in the era of the pneumococcal vaccine

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## Abstract

**Objectives:** The precise epidemiology of community acquired childhood pneumonia is crucial for timely and adequate treatment. The objective of this study was to determine the current etiological profile of pneumonia in children after introduction of pneumococcal vaccine in Bulgaria.

**Methods:** We evaluated prospectively from December 2015 through November 2018, 285 immunocompetent children hospitalized with radiographically confirmed pneumonia. We specifically looked for prior antibiotic use and immunization status. The laboratory data included - CRP, full blood count, sputum culture examination, PCR and/or serology for respiratory viruses, Chlamydia and Mycoplasma.

**Results:** In 44.9% of the cases we couldn't prove etiological agent, bacteria were confirmed in 41.6% and viruses in 16.2% (in 4.6% - co-infection). For the prior antibiotic use, we couldn't find any significance for etiological confirmation ( $p=0.36$ ) but for *Streptococcus pneumoniae* ( $p=0.015$ ). As expected, *Mycoplasma* was isolated in older children and mainly in summer/autumn season, while *Streptococcus* was mainly isolated in younger ones with no specific seasonal peak. Mean age for children with bacterial pneumonia was 6.54 years, for the ones with viral pneumonia 4.34 yrs., for combined (viral+bacterial) 3.08 yrs. and for fungal one was 10.42 yrs ( $p=0.000$ ). Almost 2/3 of the children have been immunized with pneumococcal vaccine (66%). The immunized patient had higher numbers of viral and lower of bacterial isolates 25.7% and 37.23% vs. non-immunized ones – 4.12% and 48.45% respectively ( $p=0.002$ ). There was no difference in *Streptococcus pneumoniae* isolation and vaccination status, but there was a major drop in *Mycoplasma* isolates in vaccinated ones (6.9% vs. 32.98%,  $p=0.000$ ).

**Conclusion:** In future we could expect more viral pneumonia with increasing of the vaccination coverage and maybe we should reevaluate our treatment guidelines.

**Abbreviations:** CAP: Community-Acquired Pneumonia, RSV: Respiratory Syncytial Virus, RV: Rhinovirus, hMPV: human Metapneumovirus, *M.pneumoniae*: *Mycoplasma pneumoniae*, *S.pneumoniae*: *Streptococcus pneumoniae*, *S. aureus*: *Staphylococcus aureus*, *C. pneumoniae*: *Chlamydia pneumoniae*.

## Introduction

Over the last twenty years, the global incidence of pneumonia in young children (aged <5 years) reduced by 30% and the number of pneumonia deaths in young children almost halved in that time [1]. One big measure in this positive tendency was introduction of pneumococcal vaccine [2]. However, pneumonia remains a leading cause of preventable illness and death in this age group and overcoming this will require considerable effort [3]. The precise epidemiology of childhood pneumonia changes with the patient's age, time of the study, as well as the medical policy and treatment guidelines in different countries [4-6].

Back in 2004 Michelow *et al.* have described the the epidemiology and morbidity of Community-Acquired Pneumonia (CAP) in hospitalized children with identified bacteria in 60% of the cases (73% for *Streptococcus pneumoniae*), viruses in 45%, and mixed bacterial/viral infections in 23%. Atypical bacterial pneumonia was found in 14% for *Mycoplasma pneumoniae* and 9% for *Chlamydia pneumoniae* [7]. Various publications later on however stated drastic drop of CAP

caused by invasive *S. pneumoniae* after introduction of pneumococcal vaccine instead of the rise in non-vaccine serotypes [8,9]. In 2010 10-valent Pneumococcal Vaccine (PCV10) was introduced as an obligatory vaccination in Bulgaria [10].

The accumulated knowledge for the last 15 years, led us to the objective of this study, which was to determine the current etiological profile of pneumonia in children after introduction of pneumococcal vaccine in Bulgaria.

## Material and methods

### Patients

The study material comprised the data from 285 patients. The inclusion criteria that were met by all patients were: children with a clinical and X-ray confirmed pneumonia aged from 1 to 17,9 years,

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requiring in hospital treatment. We included all patients with CAP, hospitalized in the ward for acute respiratory infection during a period of 3 years (December 2015 - November 2018) with the exclusion of the patients with known immunocompromised condition (such as cystic fibrosis, immune-deficiency or bronchiectasis) or patients transferred from other hospitals that were on intravenous antibiotics before admission. All of the tests were performed after obtaining a signed informed consent of the legal guardian. There was no personal patient information in the database. The Hospital's Ethics Committee granted study approval. The therapy prescribed was as all the clinical practice and guidelines approved, according to the isolated pathogen.

### Clinical, imaging and laboratory data methods

Clinical information, including demographic characteristics, immunization record, history of illness, symptoms, initial diagnosis, and comorbid illnesses, was documented on case report forms.

### X-ray confirmation

A senior specialist in imaging diagnosis reviewed all chest X-rays and confirmed presence of pneumonia. The radiologist assigned the type of pneumonia on X-ray as - focal or segmental consolidation with or without pleural effusion or interstitial pneumonia.

### Microbiology

Blood cultures were performed with the BacT/Alert 3D system (Organon Teknika, Boxtel, Netherlands) before initiation of parenteral antibiotic therapy in children in severe clinical condition (24% of all) following the standardized guidelines [11].

At the admission, before we collected sputum samples and deep nasopharyngeal swab (in cases where sputum was not obtained at the admission). All the samples were obtained by a pediatrician and then right away sent to the laboratory in a transport medium. Sputum samples were plated and incubated at 37 °C for 24-48 h. The standard protocol for microbiological diagnostic follow-up and tests was followed in the laboratory. For isolation and detection of the rigorous etiologic bacterial agents we used routine nutrition media such as Blood agar base supplemented with 5% sheep blood, MacConkey agar, Candida chrome agar and developed and implemented by our team selective media chocolate agar with vancomycin for *Moraxellae* and *Haemophilae*, for their identification, as well as a quantitative method for correct evaluation of the clinical significance of isolates [12]. The cultivation of inoculated samples on different media was performed in aerobic and in microaerophilic atmosphere respectively for various pathogens. For identification of the isolated clinical strains we used mainly the products and systems by Crystal BD BBL (BBL; Becton, Dickinson, Germany) and RapID System Remel Thermo Fisher Scientific (Remel, Thermo Fisher Scientific Remel Products; Santa Fe, USA). All bacterial isolates in a microbial number over the critical one for an infectious process (>100 000 CFU/mL), were considered as significant [12]. Susceptibilities to investigated pathogens were determined by Kirby-Bauer disk-diffusion method. Due to the necessity for some peculiar cases and for a representative sample strain, minimal inhibitory concentration according to the criteria of CLSI were measured [13]. Isolated *Streptococcus pneumoniae* strains were preserved for serotyping for a future research in regards immunization status and the type of isolated *S.pneumoniae*.

For viral detection combined nasal and pharyngeal specimens were collected from the enrolled patients by means of commercial polyester collection swabs (Deltalab, Spain). Following collection, swabs were

stored at 4 °C for up to 72 hrs. Specimens were processed immediately or stored at -80 °C prior to analysis. Viral nucleic acids were extracted automatically from 500 µl of each respiratory specimen in a final eluate volume of 75 µl using an ExiPrep Dx Viral DNA/RNA kit (BioNeer, Korea) in accordance with the manufacturer's instructions. Detection and typing/subtyping of influenza viruses were carried out by a real time RT-PCR method and the SuperScript III Platinum<sup>®</sup> One-Step qRT-PCR System (Invitrogen, ThermoFisher Scientific, USA) as previously described [14]. The detection of RSV, hMPV, PIV 1/2/3, RV and AdV was performed using singleplex real time PCR assays and an AgPath-ID One Step RT-PCR kit (Applied Biosystems, ThermoFisher Scientific, USA). The primers, probes and PCR conditions used in the study were identical to those previously described [15].

If indicated some patients were screened for pulmonary tuberculosis with intradermal Mantoux skin tests by using 5TU purified protein derivative.

### Serology

Serum samples were stored at -70°C and subsequently analyzed. Serologic tests for *M. pneumoniae*, *Chlamydia pneumoniae* and *Chlamydia trachomatis* was performed with enzyme-linked immunosorbent assays with ready IgG and IgM kits (Abcam, UK). Additionally to serology for the patients from the 2018 we used nasopharyngeal swabs for Real-Time Amplification test for the qualitative detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* - (Kit *Mycoplasma pneumoniae* / *Chlamydia pneumoniae* Real-TM, Sacace Biotechnologies Srl) following the manufacturer instructions.

### Inflammatory indices

Complete blood count with with manually verified differential count as well as CRP was performed in all children. For CRP detection (positive test 6 mg/L or higher) we used serums collected by standard procedures and slide agglutination test C-Reactive protein (CRP) latex (BioSystems S.A.; Costa Brava, Barcelona, Spain).

### Treatment

All patients were treated with a sequential parenteral and oral antibiotic regimen for presumed bacterial LRI. The antimicrobial agent of choice for uncomplicated pneumonia was 3<sup>rd</sup> generation cephalosporine for a duration of 5 to 14 days. If atypical bacteria was confirmed the therapy was modified with macrolide. Alternative or additional agents were used at the discretion of the attending physician.

### Statistical analysis

The data were analyzed using using Statistical Package for the Social Sciences (SPSS) for Windows, Version 19.0. (SPSS Inc.; Chicago, IL, USA). For nonnormally distributed results, comparisons were made by the Kruskal-Wallis test, Fisher exact test, or Wilcoxon signed-rank test as appropriate. For normally distributed results, comparisons were made by analysis of variance (ANOVA) and the paired t test. Chi Square was used for testing relationships between categorical variables. We considered p values of ≤0.05 to indicate statistical significance.

### Results and discussion

A total of 285 children met the inclusion and had no exclusion criteria for enrollment. Of these patients, 135 (47.3%) were male and 150 females. Ages ranged from 1 year to 17.8 years, 47.0% less than 5 years old. One hundred and five children (36.8%) had received oral antibiotic

therapy prior the hospitalization, most commonly cephalosporin 2<sup>nd</sup> generation or amoxicillin. Forty-one (14.4%) children had additionally asthma and four had neuromuscular disease. The median duration of hospitalization was 5.9 days (from 3 to 14). Only 17 (5.9%) received supplemental oxygen therapy for a median of 3 days, but 126 (44.2%) were with bronchial obstruction, requiring corticosteroid and salbutamol treatment. Thirty-six (12.6%) had pleural effusion, and only 3 of them required surgical intervention. All patients but the mentioned 3 were discharged at home with appropriate therapy. On X-ray studies 138 patients (48.4%) had consolidations while the rest had interstitial changes. Thirty percent (87 children) were hospitalized during winter season, 28.4% during spring, 26.6% during autumn and only 17% during summer. Almost 2/3 of the patients (65.9% or 188 children) had at least 3 shots of PCV10, the rest were not immunized. For this three-year period none of the included children required mechanical ventilation and none died. This also supports the publications regarding the positive impact of PCV10 on hospitalization rates and severity of the pneumonia [2,6,16].

**Etiology**

In 128 of the children (44.9% of the cases) we couldn't prove etiological agent. In 117 (41.6%) children we confirmed bacteria. The most prevalent bacteria expectedly was *S. pneumoniae* (52.4%), followed by *M. pneumoniae* (44.4%), *Staphylococcus aureus* (9.4%), *Haemophilus parainfluenzae* (8.5%), *Haemophilus influenzae* (3.2%). In 3 patients we found *Klebsiela pneumoniae* and in other 3 *Pseudomonas aeruginosa*, in 2 we found *Stenotrophomonas maltophilia* other pathogens found only in single cases included *Moraxella catarrhalis*, *Chlamydia pneumoniae*, *Acinetobacter baumani*, *Streptococcus pyogenes*, *beta hemolytic Streptococcus*, *Mycobacterium tuberculosis*, *Flavobacterium meningosepticum* and *Enterobacter aerogenes*. We found 100% concordance of the results from serology and PCR from nasopharyngeal swabs for the tested with both methods patients with 18 positive on both tests, and no false positive or false negative on the PCR compared with ELISA. In 17 cases we confirmed combined bacterial flora mainly with *S. aureus*, *S.pneumonia* and *M.pneumonia*.

In 46 patients (16.2%) we confirmed viral infection (in 4.6% of the patients there was co-infection of virus and bacteria). The leading viral pathogen was Respiratory Syncytial Virus (RSV) in 28.3%, rhinovirus (RV) in 21.7%, human metapneumovirus (hMPV) and influenza virus A were isolated in 15.21%, adenovirus in 13.0%, influenza virus B and bocavirus were found in 2 patients and parainfluenza, parainfluenza virus in only one. In seven patients we found only *Candida* spp.

Despite PCV10 introduction in Bulgaria for the last 8 years, and relatively high coverage reported by the authorities [17], *S.pneumonia* is still the predominant bacterial pathogen for CAP in Bulgaria. Future studies are planned to confirm the possible seroconversion and the need for evaluation weather more valent pneumococcal vaccine is needed [18]. Surprisingly we couldn't confirm the expected higher rate for *C.pneumoniae*, reported between 9 and 17% for we found only one case [7,19,20].

**Etiology and demographic data**

We couldn't find any significant difference between isolated pathogen and the gender of the patients, there is however higher probability for pleural effusions in male patients (p=0.000). We couldn't identify any correlation between effusion and etiological agent (p=0.35). There was a significant difference in the mean age and distribution of the patients the patients according the type of isolated agent. Mean age

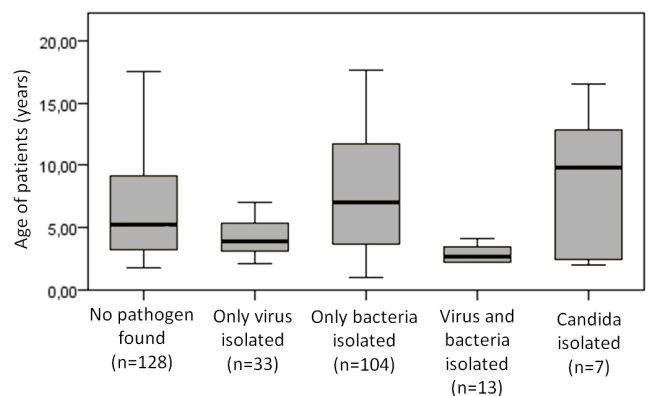
for children with bacterial pneumonia was 6.54 years, for the ones with viral pneumonia 4.34 yrs., for combined (viral + bacterial) 3,08 yrs. and for fungal one was 10.42 yrs (p=0.000) (Figure 1). As expected, *M. pneumoniae* was isolated in older children, while *S. pneumoniae* was mainly isolated in younger ones but the differences were not statistically significant (p=0.14), but the results reflect in the choice of antibiotic therapy – cephalosporin for children under age of 5 (p=0.036) and macrolide for older kids (p=0.000). There was no significant correlation between age and viral isolate. Our results are in concordance with the previously published data [7,19]

**Etiology and seasonal data**

As expected, the lowest number of hospitalized patients with CAP is during summer, and for 3 years we found viral pathogen (adenovirus) only in one patient. RSV is more often isolated during autumn, while influenza and hMPV more during winter (p=0.02). More co-infected patients (bacteria and virus) were found during winter and spring (p=0.01). For *S.pneumoniae* we couldn't find any seasonal prevalence (p=0.5), while for *M.pneumoniae* we found main prevalence during spring and early summer (p=0.000). Maybe these seasonal fluctuations of viruses and *M.pneumoniae* are behind the finding that there is no viral co-infection with *M.pneumonia*. More combined infections were found during winter (p=0.04). Our data is similar to previously published data [7], but different winter percentages are reported this year by Korean group, but the authors there state that there was specific *M.pneumonia* epidemic [6].

**Prior antibiotic use and etiology**

From 285 studied children 105 children have been taken antibiotics prior hospitalization the rest 180 patients were antibiotic naïve. When we divided the patients according this parameter the etiological distribution was: 38.8% no pathogen found, 48.3% bacteria confirmed, 15% virus isolated and 2.2% *Candida* found for antibiotic naïve vs. 55.23%, 28.5%,18.9% and 2.8% for the ones with prior use respectively (p=0.002). When we looked at the bacterial isolates and prior antibiotic use, we couldn't find any significant difference regarding *M.pneumoniae* isolation (p=0.36) but there is one for *S. pneumoniae* (p=0.015), mainly due to the standard guidelines to GPs to start with penicillin antibiotic for pneumonia [21]. There was no significant effect on the prior antibiotic use and type of the isolated virus (p=0.06). Surprisingly the *Candida* isolation was not significantly higher in the group that have used antibiotics prior the tests (p=0.08).



**Figure 1.** Box-plot for age distribution of patients according the microbiology/virology finding

### Etiology and immunization status

Almost 2/3 of the children have been immunized with PCV10 (66%). The immunized patient had higher numbers of viral and lower of bacterial isolates 25.7% and 37.23% vs. non-immunized ones - 4.12% and 48.45% respectively (p=0.002) (Figure 2). Only in vaccinated children there were co-infection found (p=0.000). There was no difference in *S. pneumoniae* isolation and vaccination status, but there is a major drop in *M.pneumoniae* isolates in vaccinated ones (6.9% vs. 32.98%, p=0.000). Our data corroborated findings of other investigators, claiming that PCV10 results in lowering the levels of *S. pneumoniae* and thus creating space for increasing *M. pneumoniae*. [6,7,22]

### Etiology and comorbidities

Due to low number of patients with neuro-muscular diseases we couldn't perform a proper statistic for them. For the patients with asthma only in 14% we couldn't identify microorganism, while in 48.5% and 51.2% we found viruses and bacteria respectively (in 23.26% we found co- infection). The bacterial profile was similar to the whole group with prevalence *S. pneumoniae*, followed by *M. pneumoniae*. In 50% of children without asthma we didn't isolate etiological agent and only in one case there was co-infection, in 10.6% viral and 39.3% bacterial pneumonia was confirmed (p=0.008). We didn't find the expected higher rates of *M.pneumoniae* in asthmatic children, as it was noted previously that *M.pneumoniae* infection is associated with both the initiation and persistence of asthma in children [23]. Since asthma exacerbation due to viral infection can mimic pneumonia, some studies that skip the imaging diagnosis can make improper conclusions. In previously published papers it was confirmed that less than 2% of children with asthma exacerbation have really pneumonic changes on X-ray, thus the routine uses of radiography for children with asthma who are not febrile or hypoxic should be discouraged [24]. To avoid some misunderstandings, all included in our study patients with asthma had X-ray confirmed pneumonia.

### Etiology and inflammatory markers and X-ray changes

The values for CRP were lower in cases with Mycoplasma and in cases with non-compact infiltrate changes on X-rays (p=0.002 and p=0.000). The CRP couldn't be used as a marker for differentiation of atypical from viral pneumonia (p=0.12). The higher CRP couldn't be a marker of only bacterial pneumonia, for some viral cases also had extremely elevated values (p=0.09). We found that children with bronchial obstruction have elevated but not extremely high CRP values, the highest values were in cases without bronchial obstruction (p=0.000). Children with the highest values of CRP required longer in-hospital stay (p=0.020). We couldn't find any correlation between the etiologic agent and the differential blood count (including lymphocytes

and neutrophil number) or wheezing episodes. As other authors have found almost half of the CAP cases with X-ray seen infiltration and confirmed pathogen are caused by *M.pneumoniae* or viruses [25]. We also agree that it is difficult to distinguish clinically between bacterial and viral aetiologies. Fever and tachypnoea are confirmed early features of pneumococcal pneumonia. Cough is not always apparent or required for diagnosis, and may be absent in the early stages of illness. *M. pneumoniae* presents with cough and chest pain and is often associated with wheeze, general malaise, arthralgia, sore throat, and headache [4,5].

### Conclusion

Our study through expanded diagnostic investigations aimed to define the etiological profile of the pathogens that cause pneumonia in hospitalized children in Bulgaria. Our data confirm the frequent occurrence of co-infection with bacteria and viruses in children with pneumonia. Following our findings in future we could expect more viral pneumonia with increasing of the vaccination coverage and maybe we should reevaluate our treatment guidelines. Even the most rigorous efforts to define the epidemiology of pneumonia in children are limited by the paucity of validated data. Therefore, accurate characterization of the causes of CAP is essential to guide appropriate antibiotic utilization.

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### Ethics committee approval

Ethics committee approval for this study was received from the ethics committee of Medical University of Sofia School of Medicine.

### Informed consent

Written informed consent was obtained from parents of the patients who participated in this study.

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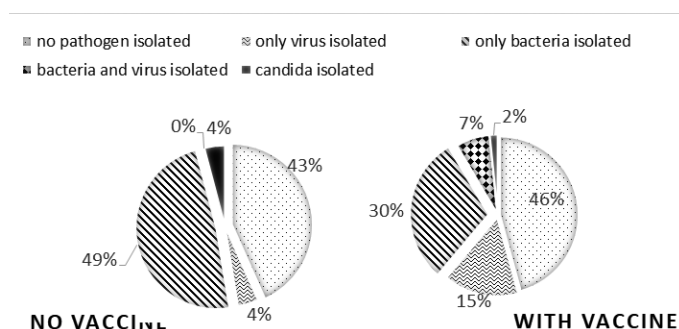


Figure 2. Percentage of isolated pathogen groups (virus, bacteria, combined, candida without isolates) according immunization status with PCV10

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