

Laboratory Investigation of Trends in Bacterial Pneumonia in Cheonan, Korea, from January 2008 to September 2017

Young-Sam Yook, Jae-Sik Jeon, Ji On Park, and Jae Kyung Kim*

Department of Biomedical Laboratory Science, Dankook University College of Health Sciences, Cheonan 31116, Republic of Korea

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*Corresponding author
Phone: +82-41-550-1451;
Fax: +82-41-550-1451;
E-mail: nerowolf2@dankook.ac.kr

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Bacterial pneumonia is one of the most common causes of mortality in Korea. In 2016, the mortality rate from pneumonia was 16,476 deaths per 100,000, which was an 11% increase from the previous year. The aim of our study was to determine the distribution of the bacterial pathogens causing respiratory symptoms in different age groups over a 10-year period. Between January 2008 and September 2017, 1,861 specimens from 1,664 patients admitted to Dankook University Hospital with respiratory symptoms were examined. We used multiplex polymerase chain reaction (PCR) to detect six bacterial pneumonia pathogens: *Bordetella pertussis*, *Chlamydomphila pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Streptococcus pneumoniae*. We detected bacterial pneumonia pathogens in 1,281 (68.83%) specimens. Of the 1,709 pathogens detected, *S. pneumoniae* was the most common (48.57%; $n = 830$) followed by *H. influenzae* (40.08%; $n = 685$). Most infections were found among children younger than 10 years (92.69%; $n = 1,584$). Although *S. pneumoniae* was the most common pathogen detected in all age groups, *M. pneumoniae* infection increased in prevalence with age ($p < 0.05$). The rate of co-infection was also high among these patients (31.1%; $n = 399$), which peaked in 2015 (54.55%; $n = 42/77$). The prevalence of bacterial pneumonia in Cheonan, along with the proportion of co-infections among patients increased over the 10-year study period. The findings will aid the development of treatment and prevention guidelines.

Keywords: *Bordetella pertussis*, *Haemophilus influenzae*, Korea, *Mycoplasma pneumoniae*, pneumonia, *Streptococcus pneumoniae*

Introduction

Pneumonia is a bacterial infection that is a major cause of mortality worldwide [1, 2]. Importantly, even with improvements in treatment, diagnostics, and prevention, pneumonia remains a public health concern. Globally, pneumonia accounts for 16% of deaths annually among children younger than 5 years [3], but can progress to severe disease in all age groups. The mortality rate due to pneumonia is increasing annually in Korea [4]. In 2016, the number of deaths attributable to pneumonia in Korea increased by 11% compared to the previous year (from 28.9% in 2015 to 32.2%) [4].

The pathogens that cause most bacterial pneumonia infections are *Bordetella pertussis* (BP), *Chlamydomphila pneumoniae*

(CP), *Haemophilus influenzae* (HI), *Legionella pneumophila* (LP), *Mycoplasma pneumoniae* (MP), and *Streptococcus pneumoniae* (SP). These infections predominantly affect people who are very young, old, or immunocompromised. Pertussis, also known as whooping cough, is caused by BP and is one of the most severe respiratory infections [5]. A severe complication of pertussis is pneumonia, which has high rates of morbidity and mortality especially in infants younger than 6 months [6, 7]. HI is a common causative agent of pediatric pneumonia, with 43.8 cases per 100,000 per year of HI-induced pneumonia in Wales, UK [8]. LP accounts for approximately 10% to 20% of atypical pneumonia cases in Europe and the US but is relatively rare in Korea. The major causative agents of community-acquired pneumonia (CAP) are CP, MP, and SP [9]. SP is a major cause of typical

Table 1. Description of target genes for bacterial pneumonia pathogens using multiplex PCR.

Organism	Target gene	Amplicon size (bp)	Accession No.
<i>Bordetella pertussis</i>	Pneumolysin (ply)	350	NC000912
<i>Chlamydia pneumoniae</i>	Outer membrane protein (P6)	257	NC007146
<i>Haemophilus influenzae</i>	16S-23S rRNA intergenic spacer (ITS1)	583	NC000912
<i>Legionella pneumophila</i>	Major outer membrane protein (ompA)	146	NC000922
<i>Mycoplasma pneumonia</i>	Macrophage infectivity potentiator (mip)	472	NC009494
<i>Streptococcus pneumoniae</i>	Outer membrane protein (prn)	200	NC002929

Abbreviations: PCR, polymerase chain reaction

pneumonia, and SP-associated pneumonia accounts for 66% of bacterial pneumonia cases in the US [10]. MP causes atypical pneumonia and accounts for 10–40% of CAP in the US [11].

Understanding the epidemiology and causative organisms of bacterial pneumonia is critical. Since 1990, antibiotic-resistant SP has been rapidly increasing in prevalence [12], and recently, multidrug-resistant SP has been increasing [5]. We investigated the positivity rate and distribution of bacterial pneumonia pathogens from patient respiratory samples in the Cheonan region of Korea over a 10-year period.

Material and Methods

Study Population

From January 2008 to September 2017, 1,861 respiratory specimens were collected from 1,664 patients with respiratory symptoms at Dankook University Hospital. Of 1,664 patients, 1,169 were pathogenic. Patient characteristics at the time of admission, including age, sex, and season were analyzed retrospectively.

Specimen Isolation

Sputum specimens were mixed 1:1 with 4% NaOH, vortexed for 1 min and incubated for 15 min at room temperature. A 1.5 ml aliquot was centrifuged at 16,000 ×g for 10 min, and the supernatant was discarded. We then resuspended the sample with 1 ml of phosphate-buffered saline (PBS), vortexed, and centrifuged at 16,000 ×g for 10 min. The supernatant was discarded, and the sample was resuspended in 150 µl of PBS.

Detection of Bacteria in Respiratory Specimens Using Multiplex PCR

To detect bacterial pneumonia pathogens, deoxyribonucleic acid (DNA) was extracted from 300 µl of the treated samples using the QIAamp DNA Mini Kit (QIAGEN Inc., USA) and polymerase chain reaction (PCR) was performed using the Seeplex Pneumobacter Detection Kit (Seegene Inc., Korea) per the manufacturer's instructions. Briefly, the PCR reaction solution contained 3 µl of

DNA, 4 µl of 5 × Pneumobacter primer, 3 µl of 8-methoxypsoralen, and 10 µl of 2 × Multiplex Master Mix. The PCR reaction was performed at 94°C for 15 min, then 40 cycles at 94°C for 30 sec, 60°C for 1.5 min, and 72°C for 1.5 min, and completed at 72°C for 10 min. The multiplex PCR simultaneously detects gene targets from SP, HI, CP, LP, BP, and MP (Table 1).

Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 20.0 (IBM Corp., USA). *P*-values < 0.05 were considered statistically significant.

Results

Detection of Samples Positive for Pneumonia-Causing Organisms

Overall, 1,861 sputum specimens were collected from 1,664 patients over 10 years, of which 1,281 (68.83%) specimens were positive for a bacterial pneumonia pathogen. Among the positive specimens, 1,709 pathogens were detected. SP was the most commonly detected organism in 48.6% (*n* = 830) of specimens, followed by HI in 40.08% (*n* = 685) of specimens (Table 2). LP was not detected in any specimen.

The percentage of specimens positive for pneumococci

Table 2. Percentage of bacterial pneumonia pathogens detected in sputum specimens obtained from patients at Dankook University Hospital, Cheonan, Korea, January 2008 to September 2017.

Pathogen	Number (%)
<i>Bordetella pertussis</i>	22 (1.29)
<i>Chlamydia pneumoniae</i>	10 (0.59)
<i>Haemophilus influenzae</i>	685 (40.08)
<i>Legionella pneumophila</i>	0 (0)
<i>Mycoplasma pneumonia</i>	162 (9.48)
<i>Streptococcus pneumoniae</i>	830 (48.57)
Total	1,709 (100)

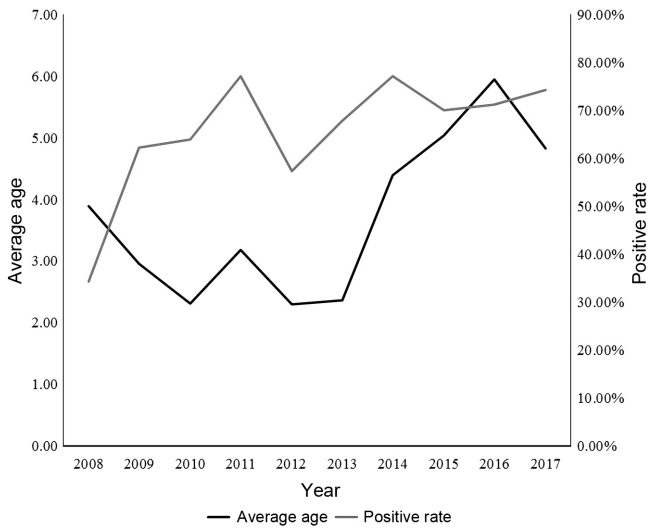


Fig. 1. Annual positivity rate of sputum specimens ($n = 1,709$) containing bacterial pneumonia pathogens, and the mean age of patients ($n = 1,169$) who tested positive at Dankook University Hospital, Cheonan, Korea, January 2008 to September 2017.

were the highest in 2014 (77.24%; $n = 95/123$), followed by 2011 (77.11%; $n = 502/651$) (Fig. 1). In 2015 there was a sharp drop in the number of specimens positive for MP and a dramatic increase in specimens positive for SP (Fig. 2).

The presence of some of the bacterial pneumonia

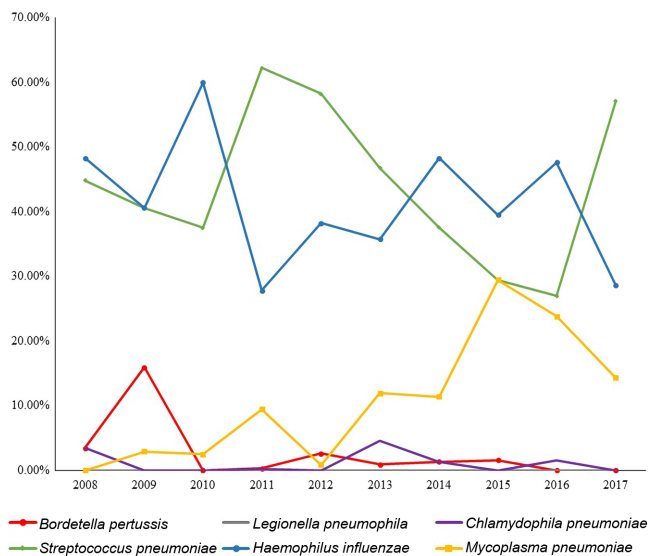


Fig. 2. Annual positivity rates for bacterial pneumonia pathogens from sputum specimens obtained from patients ($n = 1,169$) at Dankook University Hospital, Cheonan, Korea, January 2008 to September 2017.

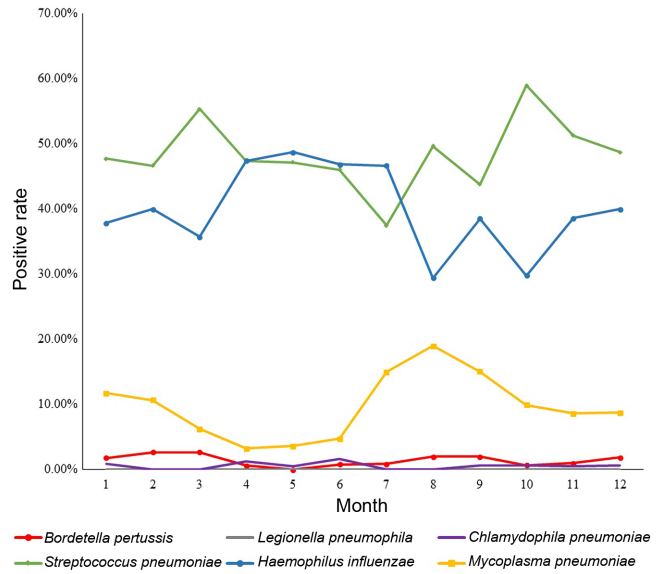


Fig. 3. Positivity rates of bacterial pneumonia pathogens in sputum specimens obtained from patients ($n = 1,169$) at Dankook University Hospital, Cheonan, Korea, January 2008 to September 2017, aggregated by month.

pathogens varied by season (Fig. 3). The detection rate of HI was highest between April and July (47.41%, range 46.67–48.72%), whereas the detection rate of SP was highest between August and February (49.55%, range: 43.79–58.94%). Lastly, the detection rate of MP peaked in August (19.0%, $n = 29$).

Description of Patients Positive for Pneumonia-Causing Bacteria

Similar positivity rates were detected in male (77.0%, $n = 745$) and female (76.9%, $n = 536$) patients. The mean age of patients with respiratory symptoms was 3.20 years (range: 0–82 years, median age = 1.64 years). The mean age increased over time and peaked in 2016 (5.95 years; Fig. 1). Overall, the mean age of patients with SP was 3.06 years (range 0–82.6 years), while that of patients with HI was 2.81 years (range; 0–48.1 years), and that of patients with BP was 1.92 years (range: 0–12.65 years).

Most pathogens (92.69%; $n = 1,584$) were detected in patients younger than 10 years ($n = 1,584$) (Table 3). SP was most commonly detected (49.37%, $n = 782$) in this age group, but was also detected in other age groups. CP was rarely detected, and only in patients younger than 10 years. A total of 629 pathogens (36.81%) were detected in 490 pathogen-positive patients under 1 year of age. SP was the most commonly detected organism (53.6%; $n = 337$) followed

Table 3. Types of bacterial pneumonia pathogens by age group detected in sputum specimens obtained from patients at Dankook University Hospital, Cheonan, Korea, January 2008 to September 2017.

Age	BP	CP	MP	LP	SP	HI	Total	Age	CP	BP	MP	LP	SP	HI	Total
≤10	19	7	130	0	782	646	1584	Under 1-year old infant	2	18	7	0	337	265	629
10-20	3	3	31	0	40	36	113								
≥20	0	0	1	0	8	3	12								
Total	22	7	162	0	830	685	1709								

Abbreviations: BP, *Bordetella pertussis*; CP, *Chlamydophila pneumoniae*; HI, *Haemophilus influenzae*; LP, *Legionella pneumophila*; MP, *Mycoplasma pneumoniae*; SP, *Streptococcus pneumoniae*

by HI (42.13%; $n = 265$) (Table 3). MP was rare in patients younger than 24 months, however, prevalence of infection

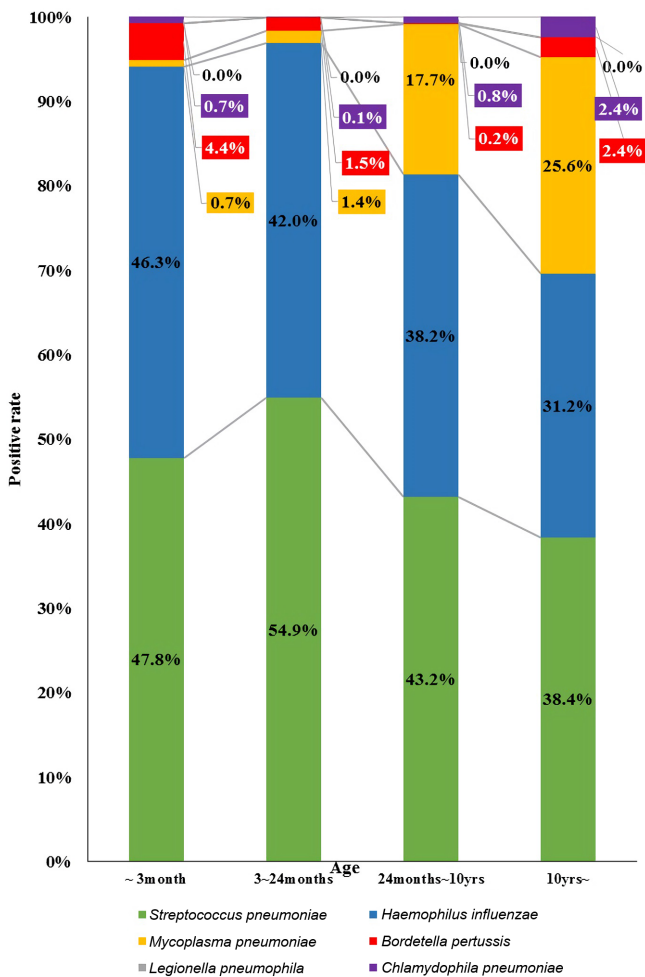


Fig. 4. Percentage of infections caused by each bacterial pneumonia pathogen in patients younger than 3 months ($n = 136$), aged 3–24 months ($n = 783$), 24 months–10 years ($n = 665$), or more than 10 years ($n = 125$) in sputum specimens at Dankook University Hospital, Cheonan, Korea, January 2008 to September 2017.

increased with age (Fig. 4). In contrast, BP was detected most often in infants younger than 3 months (4.4%, $n = 6$). The percentage of specimens positive for BP declined in each age group until patients were 10 years old.

Co-infection with multiple bacterial pneumonia pathogens occurred in 31.1% ($n = 399$) of patients, and the rate of co-infection increased over time (Fig. 5). Most of these patients ($n = 370$) were co-infected with two pathogens, of which HI and SP were most common (81.08%; $n = 300$). A small number of patients ($n = 29$) had three pathogens detected, which were usually HI, SP, and MP (82.75%; $n = 24$). There was no significant difference between male and female patients who had single or co-infection ($p > 0.05$), but the mean age (3.50 years) of co-infected patients was higher.

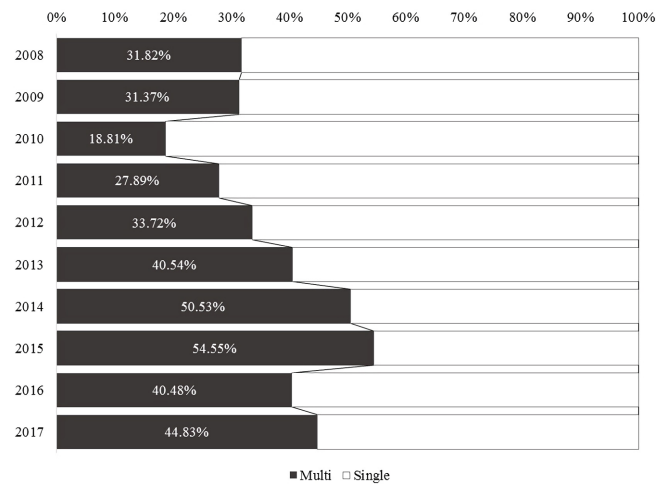


Fig. 5. Percentage of patients co-infected with bacterial pneumonia pathogens (*Bordetella pertussis*, *Chlamydophila pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Streptococcus pneumoniae*) in sputum specimens obtained from patients ($n = 1,169$) at Dankook University Hospital, Cheonan, Korea, January 2008 to September 2017.

Discussion

We investigated the positivity rate and distribution of bacterial pneumonia pathogens causing respiratory symptoms in patients, in the Cheonan region of Korea, from 2008 to 2017. The percentage of specimens positive for bacterial pneumonia pathogens and the distribution of pathogens remained stable over time. CAP is prevalent among children [13], and in this study, most infected children were younger than 10 years. Infants younger than one year were more likely to be infected. Overall, SP was the most common bacterial pneumonia pathogen detected in all age groups, but the percentage of patients with MP increased with age.

A major strength of this study is that specimens were collected from patients in one area over a 10-year period; thus, we were able to measure changes over a long period of time in a similar population. However, since this study was limited to university hospitals in one province in Korea, the results may not be generalizable to other populations. The specimens were analyzed retrospectively, so we were unable to account for underlying diseases or conditions in these patients. Complex co-infections with other respiratory viruses may exist, but we only evaluated bacterial pathogens.

Previous studies conducted in Korea also detected SP as the most common bacterial pneumonia pathogen, with low detection rates of MP and CP [14, 15]. Studies from the US and two hospitals in the UK reported an increase in the prevalence of atypical pneumonia caused by MP and CP [16, 17] and a decline in formal pneumonia caused by SP [18], which is similar to our findings. In our study, the percentage of infections caused by SP decreased and the percentage caused by MP increased until 2015, when the trend reversed. Previous studies also identified similar seasonal patterns associated with MP infection [19, 20]. We detected a decline in the percentage of patients with BP infection after 3 months of age, and a subsequent increase in those 10 years or older. This same trend was also identified in Japan [21]. In Korea, infants and young children are vaccinated with DTaP (a vaccine against diphtheria, tetanus, and *B. pertussis*) at 2, 4, and 6 months, and again at 4–6 years [22]. The length of immunity after vaccination is approximately 4 to 12 years [19]. Therefore, the infection rate is likely to be lowest between 3 months and 10 years of age, but adolescents and adults may be susceptible to infection by BP. In countries such as Canada, Germany, and France, people are vaccinated at three different time points: infancy, between ages 5 to 6 years, and then again

between ages 9 to 17 years [23]. Our data suggest that BP vaccination among adolescents would be beneficial.

Between January 2008 and September 2017, the percentage of co-infected patients in our study increased. The most common co-infections detected were HI and SP, whereas a previous study in Korea detected MP and SP as the most common co-infections [24]. However, the previous study measured asymptomatic carriage among children, which could explain the differences observed.

In conclusion, this study provides information on trends in bacterial pneumonia in one province in Korea. This study was confined to one region of Korea and has the disadvantage of not detecting LP for 9 years; we therefore could not calculate the distribution of LP. However, studies on the distribution of pneumonia in Korea are lacking, so this information will be useful for the development of guidelines for the treatment and prevention of bacterial pneumonia.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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