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Major Article

Viral and bacterial coinfection among hospitalized children with respiratory tract infections

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Key Words:
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Background: The epidemiology of *Mycoplasma pneumoniae* (MP) and local dominant etiologies of pathogens that cause respiratory tract infections (RTIs) among central China children (≤14 years old) hospitalized are poorly understood.

Methods: A total of 10,429 specimens were analyzed, and IgM antibodies against 9 respiratory pathogens including MP were detected using indirect immunofluorescence assay from serum.

Results: It showed that 59.3% of the enrolled children were positive for at least 1 pathogen; highest detection rates included those between 3 and <6 years of age (70.4%), female (63.2%), and who were hospitalized in 2014 (80.9%). The most predominant pathogen was MP (45.6%), followed by Parainfluenza viruses (PIVs) (22.6%) and influenza B viruses (IFVB) (14.7%). Coinfection was observed in 2,907 specimens (27.9%); the coinfection combination containing MP and PIVs had the highest detection rate of 15%, followed by MP and IFVB as well as IFVB and PIVs.

Conclusions: MP was the most commonly detected bacteria among hospitalized children, which should be included in the differential diagnosis for hospitalized children with RTI. These findings will contribute to the effective prevention and therapeutic approaches of pathogens among local children suffering from RTI.

Respiratory tract infections (RTIs) represent an important health issue, as they are a leading cause of mortality in children worldwide, particularly in developing countries, causing nearly 19% of all deaths among children under 5 years and 8.2% of all disability and premature mortality.[1-4] From the severe acute respiratory syndrome outbreak in 2003, the influenza A (H1N1) pandemic in 2009, to the influenza A (H7N9) pandemic in 2013, new respiratory infections have become increasingly important in modern infectious diseases, especially H1N1. The US Centers for Disease Control and Prevention first reported 2 cases of H1N1 in April, 2009. And the outbreak spread rapidly around the world, the WHO announced that it had raised its alert level to phase VI marking the start of a pandemic on June 11, 2009. The first imported case in mainland China was reported on 11 May, 2009. Then the H1N1 epidemic in mainland China has increased rapidly and spread widely, with the outbreak of H1N1 mainly in schools. WHO declared the epidemic was ended on August 10, 2010. Over time, however, the virus continues to mutate. It is clear that respiratory infections caused by virus are a serious threat to children health, we need to be constantly vigilant against it.

Besides viruses, there are also many other pathogens causing respiratory infections. RTIs are also caused by other pathogens in addition to viruses, it is estimated that 3%-10% of the children with respiratory infection due to *Mycoplasma pneumoniae* (MP) will develop community-acquired pneumonia, up to 5% of whom will require hospitalization, and up to 10% of the hospitalized children will be admitted to the intensive care unit.[5,6] MP is one of the predominant causative agents of pneumonia in children, in addition to the respiratory infection system, MP contributes to a broad array of extrapulmonary diseases, some of which can be even life-threatening.[7,8] A better understanding of the prevalence of RTIs in children is essential for implementing an effective approach for prevention,

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control, and treatment, because of lacking vaccines for most of these respiratory pathogens, especially MP.

The distribution of RTI-causing respiratory viruses varies with population, climate, and socioeconomic conditions.[9,10] We found that the epidemiology of respiratory viruses, studying with different detection methods and virus types, has been performed in big cities such as Beijing,[11] Shanghai,[12] Shenzhen,[13] Guangzhou,[14] and Hong Kong.[15] Luoyang city (34° 32′-45′N, 112° 16′-37′E) is a major city in central China with a population of 6.8 million people in 2016. It belongs to a transitional climate zone, featuring weather between subtropical to warm temperate. Climate is with 4 distinct seasons: spring is dry and windy; summer is hot, rainy, and humid; autumn is long and sunny; and winter is cold with little rain or snow. Limited data exist on the epidemiology of respiratory pathogens, MP in particular, causing RTIs from children in the region of Luoyang. Therefore, we recruited the hospitalized children in pediatric ward at the Luoyang Central Hospital, to evaluate the epidemiologic characteristics of 9 respiratory pathogens including MP among hospitalized children with RTI from June 2014 to May 2019. We investigated MP, Legionella pneumophila (LP), Coxiella burnetiid (CB), and Chlamydophila pneumoniae (CP), and 5 respiratory viruses: respiratory syncytial virus (RSV), adenovirus (ADV), influenza A viruses (IFVA), influenza B viruses (IFVB), and Parainfluenza 1, 2, and 3 viruses (PIVs). Although respiratory pathogens have different epidemic characteristics in different geographic regions and populations, respiratory diseases spread quickly and widely. Even now we only deal with the population in Luoyang, which also has high value and contribute to a further understanding of the prevalence.

METHODS

Participants

The study protocol was approved by the Institutional Review Board of Luoyang Central Hospital, and the study was conducted in accordance with guidelines for the protection of human subjects. Our study is a retrospectively observational case control study. A total of 10,429 specimens have taken from children (≤14 years old) and tested between June 1, 2014 and May 31, 2019. In this study, we analyzed IgM antibodies against 9 respiratory pathogens using indirect immunofluorescence assay from serum. Sometimes it is not possible to obtain a respiratory sample in some patients, due to his/her age, symptomatology or any special circumstance; in these cases, blood samples are needed for serologic analyses. Health care workers are well trained to obtain blood samples but not all of them are properly trained for obtaining high quality respiratory samples. When a quick and secure transport condition shipment of the respiratory samples cannot be guaranteed, it may be advisable to obtain blood samples rather than respiratory ones. IgG and IgM antibodies show a different behavior during the primoinfections and reinfections. In a primoinfection, IgG and IgM appear in almost all cases (IgM appears earlier than IgG). In reinfections IgM antibodies do not appear in all cases; therefore, IgG detection is the only method that is useful to perform the diagnosis. High titers of IgG can exist in many diseases during the whole patient life, while IgM, generally, only is measurable in sera during 2 or 3 months after the infection, and therefore is an ideal marker of recent infections.

We reference to the inclusion criteria to diagnose respiratory infection of the Centers for Disease Control and Prevention criteria for symptoms. Selected patients with RTI admitted to the pediatric wards were enrolled. The inclusion criteria were as follows: cough, hoarseness of voice, a sore throat, and a body temperature above 38° C. The following underlying conditions in medical records were recorded: bronchitis, pneumonia, asthmatic bronchitis, and other respiratory diseases. The patients were divided into 4 age groups: 0-<1, 1-<3, 3-<6, and 6-14 years old. The seasons were defined according to the seasonal division method in the northern hemisphere: March to May was considered as spring, June to August as summer, September to November as autumn, and December to February as winter.

Blood sample collection

Venipuncture blood samples obtained from each child were collected in a 3 mL vacuum tube without anticoagulant via sterile or aseptic technique, and transported to the lab. Following standard blood processing protocol, coagulation factors were added and samples were centrifuged at 3,000 *g* for 15 min to separate the serum. Serum samples were refrigerated upon collection (2-8°C). If it was not possible to perform the assay within 7 days, serum samples were frozen (−20°C) avoiding repeated rounds of thawing and freezing.

Pathogen-related antibodies analysis

Indirect immunofluorescence assays (IFA) tested for IgM antibodies against 9 respiratory pathogens: MP, LP, CB, CP, ADV, RSV, IFVA, IFVB, and PIVs. Antipathogen antibodies were detected using the commercial kit (Diagnostic Hybrids, Vircell, S. L., Spain) by trained personnel according to the manufacturer's protocol as serum samples were became available.[16] Fluorescent microscope was performed using Zeiss (Primo Star with iLED, Germany).

Positive and negative controls were included in each test run. In the observed fluorescence pattern, positive control: apple green nuclear, cytoplasmatic, and/or peripheral fluorescence could be observed; negative control: no fluorescence for the red cellular pattern. The reaction was positive when apple green nuclear, cytoplasmatic and/or peripheral fluorescence in 1%-15% of the cells for positive controls to ADV, influenza, RSV, or PIVs (peripheral pattern is the most frequent with weak sera; in PIVs and RSV dyed syncytia together with the previous pattern could be observed); apple green fluorescence was observed in all the bacteria in cases of LP, CP, or CB; apple green fluorescence in the periphery of the cell for positive to MP can be observed. The reaction is negative when no fluorescence for LP, CP, and CB and red cellular pattern for MP, ADV, IFVA, IFVB, and PIVs could be observed.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL). Pathogen prevalence was compared using the χ^2 test for categorical variables. $P < .05$ was considered to indicate a statistically significant difference.

RESULTS

Positive rate of the study population

From June 2014 to May 2019, a total of 10,429 children of less than 14 years old who presented with RTIs were enrolled in the study. In all, 6,183 (59.3%) samples tested positive for the pathogens in question. A total of 6,072 and 3,427 positively testing specimens (56.4%) were from males, and 4,357 total and 2,754 positively testing specimens (63.2%) were from females. By age groups: patients less than 1 year old had a 46.6% (2,216 of 4,754) positive detection rate; patients 1-<3 years old had a 69.9% (1,931 of 2,759) positive detection rate, patients 3-<6 years old had a 70.4% (1,278 of 1,816) positive detection rate, and patients 6-14 years old had a 68.9% (758 of 1,100) positive detection rate. Our data showed significant differences in the detection rate by age and sex (all $P < .001$). No evidence for an association between the detection rate and sex (male or female) were

found in 2014 ($P > .05$), but we found significant differences in other years (all $P < .001$). We also found that the detection rates were associated with age in years 2015 to 2019 (all $P < .001$) except 2014 ($P > .05$). The demographics of the study population are summarized in Table 1.

Annual distribution of pathogens

A total of 163 children tested positive for at least 1 of 9 respiratory pathogens from June to December 2014, and 1,158 children tested positive from January to May 2019. The overall detection rates in each year are as follows: 80.9% in 2014, 75.3% in 2015, 67.7% in 2016, 63.5% in 2017, 44.7% in 2018, and 67.2% in 2019. The most predominant pathogen was MP, with a detection rate of 45.6% (4,752 of 10,429), followed by PIVs (22.6%, 2,352 of 10,429), IFVB (14.7%, 1,528 of 10,429), LP (9.4%, 982 of 10,429), RSV (4.9%, 515 of 10,429), ADV (2.5%, 261 of 10,429), and IFVA (1.9%, 193 of 10,429) with lower detection rate. The detection rates of 7 pathogens including LP, MP, ADV, RSV, IFVA, IFVB, and PIVs, were associated with differences between years from 2014 to 2019 (all $P < .001$).

Age and gender distribution of pathogens

All of the children were grouped by age into 4 groups or by sex (Table 2). The total detection rate increased with increasing age of the enrolled children, 46.6% (2,216 of 4,753) in patients 0-<1 year old, 69.9% (1,931 of 2,760) in patients aged 1-<3 years old, 70.4% (1,278 of 1,816) in patients aged 3-<6 years old, and 68.9% (758 of 1,100) in patients aged 6-14 years old (Table 1). The specific detection rate of MP, PIVs, and IFVB mirrored patterns of total rate, wherein higher rates were detected in patients aged 3-<6 years old. The detection rate of RSV decreased with age, and detection rates of LP and CP were detected most frequently in patients aged 6-14 years and least frequently in children less than 1 year of age. The predominant pathogens among different age groups varied. Trend in predominant pathogens between genders followed the same trends across age groups (Table 2).

The detection rates of 7 pathogens were found to be associated with age ($P < .05$ or $P < .001$) (Table 2). The highest detection rates of MP in male and female were 42.1% and 50.4%, respectively. The detection rate of MP in females was significantly higher than males ($\chi^2 = 71.041$, $P < .001$). The detection rates of 3 pathogens including PIVs, IFVB, and LP, were significantly different between male and female groups (all $P < .001$) (Table 2).

Seasonal variation of pathogens

The seasonal distribution of patients sampled was 2,285 in spring, 1,923 in summer, 2,628 in autumn, and 3,593 in winter. Among the 6 major pathogens, the detection rates of MP, PIVs, IFVB, RSV, IFVA, and CB were found to be associated with seasons (all $P < .001$ or $P < .05$). The detected rates of MP (47.9%), CB (0.9%), IFVA (2.5%), and PIVs (31.2%) in spring were the highest, and the detected rates of RSV (6.7%), IFVA (2.5%), and IFVB (17.9%) were more common in winter. MP and PIVs were mostly frequently detected throughout the 4 seasons. MP, PIVs, IFVB, RSV, and IFVA were the most prevalent pathogens detected in the context of the 9 selected respiratory pathogens. MP was detected almost throughout the whole year.

Coinfection of multiple pathogens

In general, the detection rate was 31.4% (3,274 of 10,429) for cases of 1 sole positively-testing pathogen. The coinfection of multiple pathogens (2 or more positively-testing pathogens) occurred in 2,907 specimens, or 27.9% (2,907 of 10,429) of all specimens. Among the 2,907 coinfected specimens, 1,776 tested positive for 2 pathogens, 816 tested positive for 3 pathogens, and 315 tested positive for 4 pathogens and more. The rate of coinfected detection was higher in 2014 (47.9%, 78 of 163) than from 2015 to 2019 ($\chi^2 = 201.296$, $P < .0001$). The detection rate of coinfection was the lowest in 2018 (18.2%, 654 of 3,596). Children aged 1-<3 years had the highest detection rate (36.2%, 998 of 2,760) of singular infection. Rates of detected coinfection are as follows: 18.5% (881 of 4,753) in children 0-<1 year old, 33.8% (933 of 2,760) in patients 1-<3 years old, 37.3% (678 of 1,816) in patients 3-<6 years old, and 37.7% (415 of 1,100) in

Table 1
Detection respiratory pathogens positive and detection rates in children from June 2014 to May 2019

	Year N (%)								
Case characteristic	2014 N = 163	2015 N = 909	2016 N = 1,422	2017 N = 3,181	2018 N = 3,596	2019 N = 1,158	Total N = 10,429	χ^2	*P*
Sex									
Male	74 (79.6)[1]	378 (71.1)[2]	535 (62.9)[3]	1,133 (61.0)[4]	887 (43.2)[5]	420 (61.1)[6]	3,427 (56.4)[7]	250.282	<.001
Female	58 (82.9)	306 (81.2)	427 (74.8)	885 (66.8)	720 (46.7)	358 (76.0)	2,754 (63.2)	318.940	<.001
Age, year									
0∼< 1	41 (80.4)	223 (58.7) [b]	345 (53.9) [c]	744 (51.9) [d]	580 (33.9) [e]	283 (52.3) [f]	2,216 (46.6) [g]	194.748	<.001
1∼< 3	32 (72.7) [a]	199 (86.9)	305 (78.6)	647 (73.9)	506 (54.6)	242 (81.8)	1,931 (69.9)	174.285	<.001
3∼< 6	33 (86.8)	128 (87.7)	191 (79.3)	402 (72.8)	359 (57.3)	165 (77.8)	1,278 (70.4)	93.998	<.001
6∼14	26 (86.7)	134 (87.0)	121 (79.1)	226 (70.6)	163 (48.8)	88 (80.7)	758 (68.9)	105.949	<0.001

Comparison of detection rates between male and female:
[1] $\chi^2 = 0.280$, $P = .597$;
[2] $\chi^2 = 11.818$, $P = .001$;
[3] $\chi^2 = 21.898$, $P < .001$;
[4] $\chi^2 = 11.010$, $P = .001$;
[5] $\chi^2 = 4.260$, $P = .039$;
[6] $\chi^2 = 28.037$, $P < .001$;
[7] $\chi^2 = 49.357$, $P < .001$.
Comparison of detection rates in 4 different age-groups:
[a] $\chi^2 = 3.435$, $P = .329$;
[b] $\chi^2 = 95.233$, $P < .001$;
[c] $\chi^2 = 99.813$, $P < .001$;
[d] $\chi^2 = 150.497$, $P < .001$;
[e] $\chi^2 = 160.263$, $P < .001$;
[f] $\chi^2 = 102.773$, $P < .001$;
[g] $\chi^2 = 581.296$, $P < .001$.

Table 2
Detection 9 respiratory pathogens positive and detection rates in children of different age-group and sex-group

Pathogens	0~<1 (%) N = 4,753	1~<3 (%) N = 2,760	3~<6 (%) N = 1,816	6~14 (%) N = 1,100	Male (%) N = 6,072	Female (%) N = 4,357
Legionella pneumophila	165 (3.5)**	332 (12.0)	256 (14.1)	229 (20.8)	502 (8.3)##	480 (11.0)
Mycoplasma pneumoniae	1,473 (31.0)**	1,621 (58.7)	1,071 (59.0)	587 (53.4)	2,555 (42.1)##	2,196 (50.4)
Coxiella burnetiid	20 (0.4)*	27 (1.0)	13 (0.7)	7 (0.6)	43 (10.7)	24 (0.6)
Chlamydophila pneumoniae	6 (0.1)**	7 (0.3)	4 (0.2)	25 (2.3)	21 (0.5)	21 (0.5)
Adenovirus	135 (2.8)	64 (2.3)	41 (2.3)	21 (1.9)	151 (2.5)	110 (2.5)
Respiratory syncytial virus	369 (7.8)	73 (2.6)	39 (2.1)**	34 (3.1)	310 (5.1)	205 (4.7)
Influenza A viruses	81 (1.7)	57 (2.1)	35 (1.9)	20 (1.8)	110 (1.8)	83 (1.9)
Influenza B viruses	496 (10.4)**	509 (18.4)	336 (18.5)	187 (17.0)	820 (13.5)##	707 (16.2)
Parainfluenza 1, 2 and 3 viruses	809 (17.0)**	743 (27.0)	522 (28.7)	277 (25.2)	1,266 (20.8)##	1,085 (24.9)
One positive	1,333 (28.1)**	998 (36.2)	600 (33.0)	343 (31.2)	1,880 (31.0)	1,396 (32.0)
Multiple positives	881 (18.5)**	933 (33.8)	678 (37.3)	415 (37.7)	1,546 (25.5)##	1,360 (31.2)

Comparison of detection rates in 4 different age-groups:
*$P < .05$,
**$P < .001$.
Comparison of detection rates between male and female:
# $P < .05$,
##$P < .001$.

patients 6-<14 years old ($\chi^2 = 130.561$, $P < .0001$). Rates of detected coinfection were more common in girls (31.2%, 1,360 of 4,357) than in boys (25.5%, 1,546 of 6,072) ($\chi^2 = 76.452$, $P < .0001$; Table 2).

The rates of detected coinfection were 32.2% (735 of 2,285), 29.4% (566 of 1,923), 22.1% (580 of 2,628), and 28.6% (1,026 of 3,593), in 4 seasons respectively ($\chi^2 = 197.306$, $P < .0001$). Coinfection rates were lowest in autumn, and obvious seasonal peaks were observed during those months with peak strength varying from 1 year to another. Among the 4 seasons, the highest detection rate was 19.9% (454 of 2,285) in the coinfection combination containing MP and PIVs in spring, then, 18.5% (356 of 1,923) in summer. The highest detection rate of 13.1% in winter was the coinfection combination containing MP and IFVB.

The coinfection combination containing MP and PIVs had the highest detection rate of 15% (1,564 of 10,429), followed by MP and IFVB (10.9%), IFVB and PIVs (8.3%). The coinfection combination containing MP and IFVB had the highest detection rate of 39.9% (65 of 163) in 2014, then 25.5% (232 of 909) in 2015 in the study. The coinfection combination containing MP, IFVB, and PIVs has the highest detection rate of 6.2% (649 of 10,429) in the combination of 3 or more pathogens, with the highest detection rate of 14.7% in 2014 during the 6 years of the study. The detection rate of coinfection combination containing MP, LP, IFVB, and PIVs was 1.3% (131 of 10,429), which was the highest among the combinations of 4 or more pathogens, among which the highest detection rate was 3.1% in 2015 (Table 3).

DISCUSSION

In this study, the most predominant pathogen was MP, with a detection rate of 45.6% in the study. This suggests that MP is the leading pathogen of RTI in the children we investigated. MP was responsible for 26.6% of the cases and was frequent in children under 6 years of age.[6] In another study designed to evaluate the incidence of MP infection in children with community-acquired lower RTIs, 59.2% children <5 years and 40.7% children ≥5 years age group were positive for MP infection.[17] And we found that MP, PIVs, IFVB, LP, and RSV were the most important pathogens of RTI in children. In previous study, RSV, ADV, PIVs, IFVA, and IFVB are the most important viruses in infants and young children in Beijing, Shenzhen, Guangzhou, which have been detected by RT-PCR or PCR methods.[18-20] However, as those data were in different climate zones or based on different kinds of pathogens and that most previous studies have focused solely on viruses, we conducted the study from 2014 to 2019 to assess the regional common pathogens infection pattern, especially MP, including 5 viruses and 4 other pathogens in children in Luoyang, China.

We evaluated the 9 prevalence of the most frequent respiratory pathogens based on prospective analysis of 6 consecutive years' data from hospitalized children with RTI in the study. The detection rate for MP was 45.6% (4,752 of 10,429), and the total detection rate for at least 1 of the 9 respiratory pathogens by IFA was 59.3% (6,183 of 10,429) in hospitalized children with RTI in Luoyang. Generally, the detected rate of MP was declining year by year from 2015 to 2018. The reported frequency of community-acquired pneumonia due to MP in pediatric patients worldwide varies from 1.5% to 17.6%.[21-23] Studies conducted in Latin America report frequencies ranging from 0.74% to 43.8%.[24-26] Most of the reports are from a previously healthy pediatric population.

We attribute this as, likely a result of employing IFA as opposed to PCR methodology and detection of additional virus strains, the different types of pathogens tested, or of variation in sample collection time or geographic differences in these studies. PCR is more sensitive, but IFA represents an alternative and reliable point-of-care detection method, and that IFA can independently and simultaneously detect a variety of different pathogens without cross-interference, especially during early phase of illness.[27]

Of the children who had MP, 59% were 1-6 years, 53.4% were over 6 years, and 31% were 0-1 year ($P < .001$). Previous studies from Mexican have reported that 53.7% of the infections occurred in children under 6 years,[6] from Korea that 70.9% occurred in children between 1 and 6 years.[28] In China, a frequency of 80% was reported in children under 7 years.[29] While a study conducted in the United States reported that 73.3% of the pediatric patients with community-acquired pneumonia due to MP were over 5 years.[30] Children of age with 1 year younger may be not easy susceptible to infection with MP, as infants not favors overcrowding and decreases the risk of infection.

The detection rate of MP, PIVs, and IFVB were the predominant pathogens. The total detection rate increased with increasing age of the enrolled children, 46.6% in patients 0-<1 year old, which was the lowest in the 4 age groups. Among the 6 major pathogens, the detection rates of MP, PIVs, IFVB, RSV, IFVA, and CB were found to be associated with seasons. The seasonal detection characteristics of PIVs, IFVB, RSV, and ADV reported here were different from other studies, such as reports from other city of China, Japan and the United States.[31-34] The detection rate of MP peaked twice each year of our study, and seasons with high detection rates alternate years.

A lot of studies have shown that some pediatric patients with acute lower RTI infected simultaneously with multiple respiratory

Table 3
Detection rates of coinfection with 2 or more pathogens in children from June 2014 to May 2019

Pathogens of coinfection	Year N (%)						Total N = 10,429
	2014 N = 163	2015 N = 909	2016 N = 1,422	2017 N = 3,181	2018 N = 3,596	2019 N = 1,158	
MP, LP	6 (3.7)	116 (12.8)	175 (12.3)	178 (5.6)	166 (4.6)	77 (6.6)	718 (6.9)
MP, IFVB	65 (39.9)	232 (25.5)	146 (10.3)	202 (6.4)	326 (9.1)	169 (14.6)	1,140 (10.9)
MP, PIVs	28 (17.2)	229 (25.2)	281 (19.8)	573 (18.0)	319 (8.9)	134 (11.6)	1,564 (15.0)
LP, PIVs	3 (1.8)	50 (5.5)	96 (6.8)	113 (3.6)	54 (1.5)	25 (2.2)	341 (3.3)
IFVB, PIVs	30 (18.4)	135 (14.9)	125 (8.8)	245 (7.7)	234 (6.5)	94 (8.1)	863 (8.3)
MP, IFVB, PIVs	24 (14.7)	123 (13.5)	89 (6.3)	146 (4.6)	182 (5.1)	85 (7.3)	649 (6.2)
MP, PIVs, RSV	3 (1.8)	13 (1.4)	12 (0.8)	36 (1.1)	26 (0.7)	12 (1.0)	102 (1.0)
MP, PIVs, ADV	7 (4.3)	11 (1.2)	16 (1.1)	34 (1.1)	29 (0.8)	1 (0.1)	98 (0.9)
LP, IFVB, PIVs	3 (1.8)	29 (3.2)	32 (2.3)	41 (1.3)	29 (0.8)	14 (1.2)	148 (1.4)
IFVB, PIVs, RSV	3 (1.8)	9 (1.0)	13 (0.9)	36 (1.1)	24 (0.7)	8 (0.7)	93 (0.9)
MP, LP, IFVB, PIVs,	3 (1.8)	28 (3.1)	26 (1.8)	33 (1.0)	27 (0.8)	14 (1.2)	131 (1.3)
MP, IFVB, PIVs, ADV	7 (4.3)	9 (1.0)	8 (0.5)	10 (0.3)	22 (0.6)	1 (0.1)	57 (0.5)
MP, IFVB, PIVs, RSV	3 (1.8)	9 (1.0)	7 (0.5)	14 (0.4)	20 (0.6)	8 (0.7)	61 (0.6)
IFVB, PIVs, RSV, ADV	1 (0.6)	1 (0.1)	5 (0.4)	8 (0.3)	6 (0.2)	0 (0)	21 (0.2)
MP, IFVB, PIVs, RSV, ADV	1 (0.6)	1 (0.1)	3 (0.2)	4 (0.1)	5 (0.1)	0 (0)	14 (0.1)

NOTE. We have only listed the coinfection combinations that account for a relatively large proportion, not all of them. The N in the table refers to the total number of cases where coinfection with these pathogens is counted. In addition to the 2, 3, 4, or 5 pathogens listed, there are other pathogens that are coinfected.
ADV, adenovirus; *IFVB*, influenza B viruses; *LP*, Legionella pneumophila; *MP*, Mycoplasma pneumoniae; *PIVs*, Parainfluenza 1, 2 and 3 viruses; *RSV*, respiratory syncytial virus.

pathogens. In our study, the ratio of coinfection of 2 or more pathogens to the total number of positive cases is 27.9%, this mostly included MP codetection. Moreover, MP, PIVs, IFVB, LP, RSV, and ADV are pathogens with high positive detection rates. Therefore, we summarized some coinfection combinations containing these pathogens. In the years we investigated from 2014 to 2019, the coinfection combinations containing MP and PIVs, MP and IFVB, IFVB and PIVS, MP and LP all had high detection rates, and the coinfection containing MP and PIVs had the highest detection rate of 15%, followed by MP and IFVB, IFVB and PIVs. These pathogens were found to be commonly coinfected in patients with RTI, which is different from the coinfected viruses in previous studies.[13] There is no consensus on the effect of coinfection on disease severity, which may depend on which pathogens are coinfected.[35,36]

CONCLUSIONS

In this study, a total of 10,429 specimens were analyzed. We evaluated MP and other 8 pathogens in hospitalized pediatric patients with RTI, using indirect IFA from serum, from June 2014 to May 2019 in Luoyang, central China. MP was the most commonly detected bacteria among hospitalized children, although usually considered as causing a mild pneumonia, 27.9% of whom had multiple pathogens codetections. MP should be included in the differential diagnosis for hospitalized children with RTI.

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