Prevalence of atypical bacteria in hospitalized patients with clinical and radiologic findings of atypical pneumonia (2009-2012): Results of the Croatian National Institute of Public Health

Učestalost atipičnih bakterija u hospitaliziranih pacijenata s kliničkom i radiološkom slikom atipične pneumonije (od 2009. do 2012. godine): rezultati Hrvatskoga zavoda za javno zdravstvo

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Abstract. ***Objective:*** Atypical pathogens are a frequent cause of pneumonia all over the world. The etiology varies according to the geographic area and age of the study population. The aim of this study was to determine the prevalence of atypical bacteria in hospitalized patients with pneumonia. ***Patients and Methods:*** From 2009 to 2012, a total of 524 patients hospitalized for pneumonia in twelve Croatian hospitals were tested for the presence of the most common atypical bacteria using ELISA and IFA. ***Results:*** Etiology was documented in 303 (57.8 %) patients. *Mycoplasma (M.) pneumoniae* was the most commonly detected pathogen (145/47.9 %), followed *by Chlamydophila (C.) pneumoniae* (80/26.4 %), *Legionella* spp. (56/18.5 %) and *Coxiella (C.) burnetii* (21/6.9 %). *C. trachomatis* pneumonia was found in one four-month-old baby (0.3 %), while *C. psittaci* was not detected. *M. pneumoniae* affected all age groups, with the highest prevalence in 10-19 age group (50.0 %). Prevalence of *C. pneumoniae* increased with age, starting with 30-39 age group. *Legionella* spp. was detected more commonly in patients older than 40 years. There was no age predilection for *C. burnetii*. Mycoplasmal and chlamydial infections occurred throughout the year. Legionelloses appeared during all seasons with more cases reported in summer and autumn. Q-fever appeared sporadically and in small outbreaks during spring and autumn. ***Conclusions:*** The results of this study showed that in 57.8 % hospitalized patients with pneumonia, atypical bacteria were found with *M. pneumoniae* and *C. pneumoniae* being most common (47.9 % and 26.4 %, respectively).

Key words: atypical pneumonia; Croatia; etiology

Sažetak. ***Cilj:*** Atipične bakterije česti su uzročnici pneumonija diljem svijeta. Etiologija se razlikuje ovisno o zemljopisnom području te dobi ispitivane populacije. Cilj je ovog rada ispitati učestalost atipičnih bakterija u pacijenata hospitaliziranih s kliničkom slikom pneumonije. ***Ispitanici i metode:*** U razdoblju od 2009. do 2012. godine na najčešće uzročnike atipičnih pneumonija metodama ELISA i IFA testirano je ukupno 524 pacijenta hospitaliziranih u dvanaest bolnica u Hrvatskoj. ***Rezultati:*** Etiologija je dokazana u 303 (57,8 %) pacijenata. Najčešći dokazani uzročnik bila je *Mycoplasma* (*M.*) *pneumoniae* (145/47,9 %), zatim *Chlamydophila* (*C.*) *pneumoniae* (80/26,4 %), *Legionella* spp. (56/18,5 %) te *Coxiella* (*C.*) *burnetii* (21/6,9 %). Pneumonija uzrokovana *C. trachomatis* nađena je u samo jednog četveromjesečnog djeteta (0,3 %), dok *C. psittaci* nije dokazana niti u jednom slučaju. *M. pneumoniae* javljala se u svim dobnim skupinama, s najvećom učestalošću u dobnoj skupini od 10 do 19 godina (50,0 %). Prevalencija *C. pneumoniae* rasla je s dobi, počevši od dobne skupine od 30 do 39 godina. *Le-*

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gionella spp. uzrokovala je većinom infekcije u osoba starijih od 40 godina, dok se *C. burnetii* javljala u svim dobnim skupinama. Pneumonije uzrokovane *M. pneumoniae* i *C. pneumoniae* javljale su se tijekom cijele godine. Legionarska bolest javljala se češće u ljetnim i jesenskim mjesecima. Sporadični slučajevi i manje epidemije Q-groznice zabilježeni su tijekom proljeća i jeseni. ***Zaključci:*** Rezultati ovog istraživanja pokazali su da su u 57,8 % pacijenata hospitaliziranih s kliničkom slikom pneumonije uzročnici bile atipične bakterije, s najvećom prevalencijom *M. pneumoniae* (47,9 %) i *C. pneumoniae* (26,4 %).

Ključne riječi: atipične pneumonije; etiologija; Hrvatska

The importance of atypical bacteria in the etiology of community-acquired pneumonia is highlighted in recent years. The etiology varies according to the geographic area and the age of the study population.

INTRODUCTION

The term "atypical pathogens" most commonly refers to *Mycoplasma* (*M.*) *pneumoniae, Chlamydophila* (*C.*) *pneumoniae*, and *Legionella* (*L.*) *pneumophila*. The importance of these pathogens in the etiology of community-acquired pneumonia (CAP) is highlighted in recent years with the development of more sensitive diagnostic techniques[1].

The etiology of atypical pneumonia varies according to the geographic area, age of the study population and diagnostic methods used[2,3]. *M. pneumoniae* and *C. pneumoniae* are the most common atypical pathogens found among children and young adults with CAP.[4] *C. psittaci* and *Coxiella* (*C.*) *burnetii* pneumonia occurs more commonly in adults[5,6].

There are seasonal differences in incidence of many atypical pathogens. Some studies suggest that there is no seasonal variation in mycoplasmal pneumonia, while other data indicate that its incidence is greatest during autumn and winter months[3]. *C. pneumoniae* appears to cause pneumonia year-round[6]. Although there is a summer prevalence of Legionnaires' disease outbreaks, sporadic cases occur during all seasons[7]. Several authors have described a seasonal variation in the incidence of Q fever in spring and early summer, which has been attributed to spring lambing and shearing leading to environmental contamination[8-10].

The aim of this study was to determine the prevalence of atypical bacteria in hospitalized patients with pneumonia.

MATERIALS AND METHODS

The study population consisted of 524 patients aged 4 months to 82 years, hospitalized for pneumonia in twelve hospitals from 6/20 Croatian counties during a four-year period (2009-2012). Two hundred and eighty-two (53.8%) patients were male and 242 (46.2%) patients were female. All patients had clinical features and radiological findings (interstitial infiltrate) consistent with atypical pneumonia.

Etiologic diagnosis was confirmed by detection of specific antibodies: *M. pneumoniae* IgM, IgG and IgA antibodies using enzyme-linked immunoassay (Euroimmun, Lübeck, Germany); *C. pneumoniae*, *C. psittaci* and *C. trachomatis* IgG and IgA antibodies using micro-immunofluorescence assay (Euroimmun, Lübeck, Germany), *Legionella* spp. (Pool I – *L. pneumophila* 1-6, Pool II – *L. pneumophila* 7-14, Pool III – *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. longbeachae* and *L. micdadei)* IgG antibodies using indirect immunofluorescence assay (R-Biopharm, Darmstadt, Germany) and *C. burnetii* IgM and IgG phase II antibodies using indirect immunofluorescence assay (Vircell, Santa Fe, Spain). Criteria for serologic diagnosis of acute infection were presence of IgM or IgA antibodies to *M. pneumoniae*, presence of IgA antibodies to *C. pneumoniae*, *C. psittaci* and *C. trachomatis*, a single IgG titer > 256 to *Legionella* spp. and presence of IgM antibodies to *C. burnetii*.

Statistical analysis

Statistical analyses were performed using MedCalc for Windows version 7.0. (MedCalc Software, Ostend, Belgium). Prevalence of seropositivity is expressed with associated confidence intervals, and the chi-squared test was used to compare differences between groups. $P<0.05$ was considered to be statistically significant.

RESULTS

Atypical bacteria were identified in 303 (57.8 %; 95 %, CI = 53.6–62.0) patients, 160 (52.8 %; 95 %,

CI = 47.2–58.4) males and 142 (47.2 %; 95%, CI = 41.6–52.8) females. The most commonly detected pathogen was *M. pneumoniae* (145/47.9 %), followed by *C. pneumoniae* (80/26.4 %), *Legionella* spp. (56/18.5 %) and *C. burnetii* (21/6.9 %). *C. trachomatis* pneumonia was found in one four-month-old baby (0.3 %), while *C. psittaci* pneumonia was not demonstrated (Table 1).

Table 1. Prevalence of atypical bacteria in patients hospitalized with pneumonia

Etiologic agent	N (%)	95% CI
Mycoplasma pneumoniae	145 (47.9)	42.3 – 53.5
Chlamydophila pneumoniae	80 (26.4)	21.8 – 31.6
Legionella spp.	56 (18.5)	14.4 – 23.2
Chlamydia trachomatis	1 (0.3)	0.06 – 1.9
Chlamydophila psittaci	0 (0)	-
Coxiella burnetii	21 (6.9)	4.6 – 10.4

Table 2. Prevalence of atypical bacteria according to gender and age

Characteristic	Tested N (%)	*M. pneumoniae* N (%)	P value	*C. pneumoniae* N (%)	P value	*Legionella* spp.	P value	*C. burnetii*	P value
Gender			0.930		0.400		0.300		0.420
Male	282 (53.8)	78 (27.6)		47 (16.7)		26 (9.2)		9 (3.2)	
Female	242 (46.2)	67 (27.7)		33 (13.6)		30 (12.4)		12 (4.9)	
Age group (years)			<0.001		<0.001		<0.001		0.120
<10	52 (9.9)	19 (36.5)		3 (5.8)		0 (0)		0 (0)	
10-19	90 (17.2)	45 (50.0)		5 (5.6)		1 (1.1)		1 (1.1)	
20-29	54 (10.3)	22 (40.7)		3 (5.6)		3 (5.6)		2 (3.7)	
30-39	65 (12.4)	14 (21.5)		10 (15.4)		8 (8.4)		4 (4.2)	
40-49	84 (16.0)	17 (20.2)		9 (10.7)		14 (16.7)		7 (8.3)	
50-59	88 (16.8)	15 (17.0)		21 (23.9)		17 (19.3)		2 (2.3)	
60+	91 (17.4)	13 (14.3)		29 (31.9)		13 (14.3)		5 (5.5)	

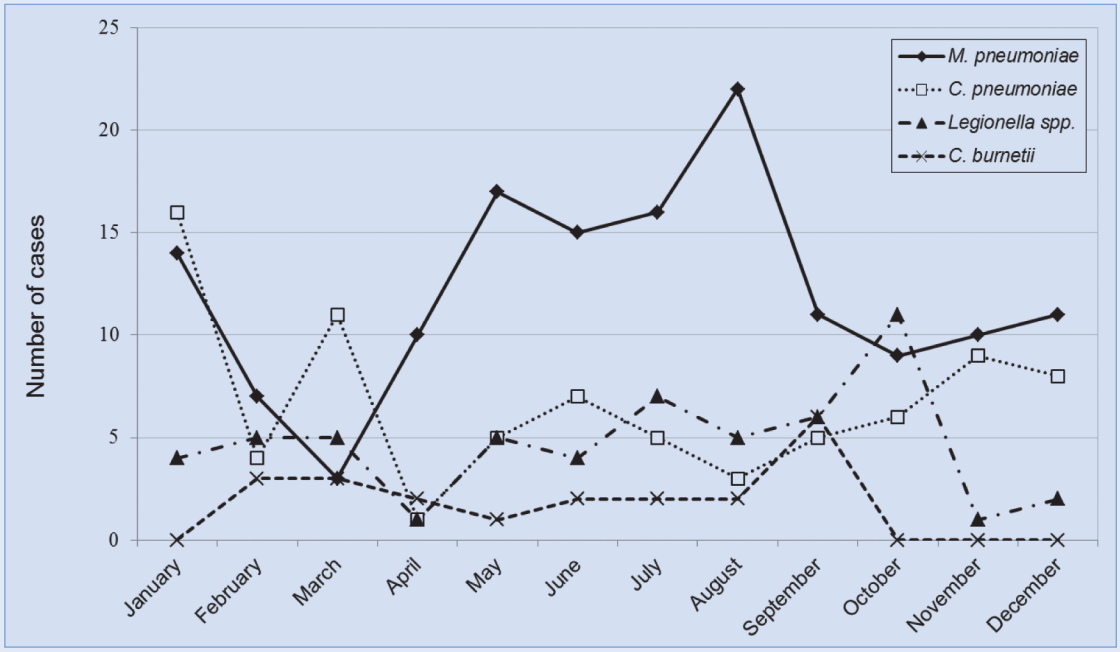

Figure 1. Seasonal distribution of atypical pneumonias (2009-2012)

Prevalence of all tested etiological agents did not differ among males and females. There were significant differences ($p < 0.001$) in the prevalence of *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. among age groups. *M. pneumoniae* affected all age groups but were more common in 10-19 age group (50.0 %). The prevalence of *C. pneumoniae* was low in patients under 30 years of age (5.6%–5.8 %) and increased with age (10.7%–31.9 %). All but one patient with *Legionella* pneumonia were older than 20. Prevalence of *C. burnetii* did not differ among age groups (1.1 %–8.3 %, $p = 0.120$) (Table 2).

M. pneumoniae was the most common in children and adolescents while *C. pneumonia* and *Legionella* spp. occurred more common in adults. There was no age predilection for *C. burnetii.*

Seasonal distribution of atypical pneumonias is presented in Figure 1. *M. pneumoniae* and *C. pneumoniae* infections occurred throughout the year. The most cases of *M. pneumoniae* were registered from May to September. Pneumonias caused by *Legionella* occurred during all seasons with more cases reported in summer and autumn. In October 2011, an outbreak of Legionnaires' disease was notified with 10 reported cases. Q fever appeared sporadically during spring and summer months with two small family outbreaks reported in March and September 2012, respectively.

DISCUSSION

Surveys all over the world suggest that atypical pathogens are a frequent cause of pneumonia, accounting for more than 40 % among patients hospitalized for CAP[1,11-13]. The prevalence of causative pathogens may vary geographically and over time[7,14]. In this study, atypical bacteria were identified in 57.8% patients. In the remaining 42.2 % patients, the possible etiologic agents could be respiratory viruses or typical bacteria. In addition, there is one proportion of patients (up to 50 %) in which etiological agents remains undefined[15].

M. pneumoniae is one of the most common atypical pathogens identified in all regions of the world[16,17]. In the present study, *M. pneumoniae* was the most frequent pathogen detected in 47.9 % hospitalized patients presented with atypical pneumonia. Mycoplasmal infections occur more frequently in children and young adults[3,18]. However, a Korean study showed no age predilection of *M. pneumoniae*[12], while a Chinese study showed a higher incidence of *M. pneumoniae* in older patients[19]. In this study, mycoplasmal pneumonia was detected in all age groups with the highest prevalence (50.0%) in 10-19 age group.

C. pneumoniae is a frequent cause of pneumonia in hospitalized patients. Although prevalence varies from year to year, *C. pneumoniae* causes from 5.2 % to 43 % of CAP[13,20]. Reinfection with *C. pneumoniae* is common, usually in elderly patients with comorbid cardiopulmonary diseases[1]. In this study, *C. pneumoniae* was responsible for 26.4 % cases of atypical pneumonia. The prevalence of *C. pneumoniae* increased with age. The highest prevalence was reported in patients older than 50 (23.9 % in 50-59 age group and 31.9 % in patients older than 60), which is similar to a previous Croatian study[19]. Infections caused by *C. pneumoniae* were notified throughout the year.

The incidence of *Legionella* as a cause of sporadic CAP varies from 0.6% to 12.2% among patients requiring hospitalization[21,22]. In this study, *Legionella* spp. was documented in 18.5 % patients with pneumonia. In October 2011, an outbreak of Legionnaires'disease was reported in South Dalmatia. All but one patients (98.2 %) was older than 20, with the highest prevalence in patients older than 40 years. There was no seasonal variation in occurrence of *Legionella* pneumonias. However, more cases were reported in summer and autumn.

Lower respiratory infections due to *C. trachomatis* have been found mostly in neonates[17]. *C. trachomatis* respiratory tract infections were confirmed in 7 % Dutch[23] and 12.5 % Indian infants[24]. In this study, acute *C. trachomatis* pneumonia was found in only one (0.3 %) baby.

Psittacosis is a zoonosis, classically transmitted from infected birds[25]. *C. psittaci* pneumonia was not found during the tested period. However, a small outbreak of psittacosis in the Split area was responsible for the high incidence of psittacosal pneumonia (19 %) in Croatia in 2002[5].

The rate of *C. burnetii* pneumonia present in this study (6.9 %) was higher than in previous reports (3 %)[6,19]. A higher prevalence could be explained by several small family outbreaks of Q-fever registered during 2012. In contrast to earlier Croatian reports[10,26], cases of Q fever were registered sporadically from spring to autumn. Recent data from the European Union showed a seasonal pattern of Q fever with more cases reported during summer months[27]. Moreover, a recently published Croatian study on a prevalence of *C. burnetii* in febrile patients showed a similar distribution of acute Q fever cases[28]. The modern sheep production technology demands well-organized and systematic sheep breeding which implies lambing every eight months[29], which may affect the transmission of Q fever throughout the year.

CONCLUSIONS

The results of this study showed that in 57.8 % hospitalized patients with clinical and radiologic findings of atypical pneumonia, an atypical bacteria was found with *M. pneumoniae* and *C. pneumoniae* being most common (47.9 % and 26.4 %, respectively). *M. pneumoniae* was more often found in the younger age group (10-19 years) in contrast to *C. pneumoniae,* which was found in age groups above 30. There was no seasonal distribution of *M. pneumoniae* and *C. pneumoniae* cases.

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