

# Surveillance of acute respiratory infections among outpatients: A pilot study in Isfahan city

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**Background:** Considering that there was not any regional survey in Isfahan, Iran regarding the epidemiology of acute respiratory tract infections (ARTI) in different age groups of general population, the aim of this study was to determine the epidemiologic feature of ARTIs in Isfahan using multiplex polymerase chain reaction (PCR) method. **Materials and Methods:** In this cross-sectional study, patients aged <80 years with symptoms of ARTI were studied, during 2009-2010 Nasopharyngeal and dry throat swab specimens were collected and pathogens of ARTI was determined using multiplex real-time PCR. **Results:** In this study, 455 cases with ARTI were studied. Mean age of studied population was  $29.9 \pm 18.5$  (range: 0.2-80). Symptoms such as sore throat (86.3%), coryza (68.0%) and dry cough (54.3%) were the most common symptoms in all studied groups, whereas fever was the most clinical presentation of younger patients (<15 years old) and headache and skeletal pain were the most common symptoms of older patients (>15 years old). Rhinovirus was the most common cause of ARTI in patients aged <5 years and those aged >50 years. Influenza virus B was the most common cause of ARTI in patients aged 5-50 years. **Conclusion:** Our study provides baseline information on the epidemiologic and clinical feature of outpatients with ARTIs in Isfahan city. Though our findings in this pilot study could be helpful in diagnosis, treatment, and prevention of ARTI, planning preventive interventional.

**Key words:** Acute respiratory tract infection, multiplex polymerase chain reaction, surveillance

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## INTRODUCTION

Acute respiratory tract infections (ARTIs) considered as the most common acute illness in the general population. Its related serious complication may result in significant morbidity and mortality specially in children, elderly and immunocompromised patients.<sup>[1]</sup> The rate of morbidity and mortality is higher in developing countries.<sup>[2]</sup> The problem is more serious in children under 5 years old.<sup>[3]</sup>

According to the report of World Health Organization and Global Burden of Disease Study, it is estimated that ARTIs are related to 112,900,000 disability adjusted life years and 325 million deaths. It would contain all respiratory tract infections each year.<sup>[4,5]</sup>

The most common causative agents of ARTIs are viruses. Early detection of related causative agents is crucial for preventing the disease, conducting management and its related complications, duration of hospitalization and the cost of the disease in all age groups of the general population. Evidences suggest that regular

surveillance and monitoring studies in community have an important role in this regard. These studies are helpful in determining the pathogens responsible for the infection, the burden of strain subtypes and consequently appropriate clinical interventions for health care providers.<sup>[6]</sup>

Since heterogeneous group of viral and bacterial pathogens are identified as causative pathogens for ARTIs, but the clinical presentations of different pathogens are almost similar, the accurate etiologic diagnosis of ARTIs causes are relies on laboratory investigation.

Different diagnostic methods are available for determining different pathogens related to ARTIs.<sup>[7]</sup> The gold standard method is virus culture. Though this method have advantages such as providing reference strains for seasonal vaccine development, providing baseline data for genetic characterization and determining pathogenesis of the disease, but it is a time consuming test which limit its usefulness in routine surveillance system.<sup>[8]</sup>

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In the last decade, the use of molecular diagnostics such as multiplex polymerase chain reaction (PCR) have gained more popularity due to its higher sensitivity, specificity, rapidity and enhanced efficiency. Using this method we could detect more than one pathogen in a single test.<sup>[9,10]</sup> Several studies confirmed the utility of this method for the detection of a broad spectrum of respiratory infection pathogens, mostly different viruses.<sup>[11-13]</sup>

Considering that there was not any regional survey in our region regarding the most common clinical symptoms and pathogens of ARTIs in different age groups of general population and the fact that understanding these factors could provide us baseline information to develop appropriate management strategies and reducing the disease related morbidity, the aim of the present study was to determine the epidemiologic feature of ARTIs in Isfahan regarding its clinical manifestation and most common pathogens related to the disease.

## MATERIALS AND METHODS

### Study design and participants

This study designed as a pilot, cross-sectional study in Isfahan city during 3 consecutive months in winter in 2009-2010. Patients aged <80 years with symptoms of ARTI diagnosed in two pediatric and 8 general clinics, from different economic and cultural parts of the city, affiliated to Isfahan University of Medical Sciences were enrolled. The clinics were selected from different parts of the city that have at least 40 medical visits per working shifts daily.

Isfahan is the second largest city in Iran, located at the central part of Iran.

The protocol of the study was designed and approved by the Institutional Review Board of Isfahan Infectious Disease and Tropical Medicine Research Center and Regional Bioethics Committee of the Isfahan University of Medical Sciences. The instruction of the study was delivered to health deputy of the Isfahan University of Medical Sciences for providing appropriate collaboration with health centers and clinics selected for the study. The people who were involved in the project were trained.

Considering the age distribution of the population in Isfahan province, participants were selected through nonprobability sampling method.

Written informed consent was obtained from each patient and for pediatric patients from their parents.

Inclusion criteria were as follows; clinical presentation of ARTI, which includes; fever, sore throat, coryza, hoarseness, dry cough, productive cough, dyspnea, red\_eye, purulative discharge, sneeze, headache, otitis, diarrhea, vomiting, abdominal pain, fatigue, skeletal pain and joint pain, first visit (not including those who referred for follow up), ARTI with duration of less than 72 h, lack of chronic respiratory tract disease, lack of having severe form of ARTI, which needs hospitalization.

Patients or parents with inappropriate coordination in sample collection and inadequate or improper samples (unsuitable for laboratory evaluation) were excluded from the study.

Demographic and clinical information, including age, sex and clinical symptoms, was recorded during the medical visit by trained nurses using a structured questionnaire.

Nasopharyngeal and dry throat swab specimens were collected by trained nurses using a standardized protocol and sent to the laboratory of Isfahan Infectious Disease and Tropical Medicine Research Center the same day at 2°C-8°C. The samples analyzed directly or stored at -70°C for later analysis.

### Laboratory measurements

#### *Multiplex real-time polymerase chain reaction*

RNA was extracted from infected cultures using High Pure Viral Nucleic Extraction kit™ (Roche Diagnostics™, Mannheim, Germany). cDNA was synthesized using Expand Reverse Transcriptase (Roche, Mannheim, Germany), according to the manufacturer's instructions.

We developed a real-time PCR procedure, based on automated specimen extraction and multiplex amplification using Corbett Research 6600 Device. Primers and hydrolysis probes were obtained from the literature. Five groups of multiplex nested PCR assays, targeting following respiratory viruses and bacteria, influenza virus A (IFA) and B (IFB), Para-influenza virus B (IFB), Para-influenza virus 1-3 (PIV), human meta pneumovirus (hMPV), respiratory syncytial virus (RSV) rhinovirus (RV), enterovirus (EV). Adenovirus (ADV), human corona viruses (299 E, OC43 and NL 63), *Mycoplasma pneumonia*, *Chlamydomphila pneumonia*, swine H<sub>1</sub>N<sub>1</sub> influenza virus were developed. Each multiplex nested PCR detected four to five viruses and/or bacteria.

Nucleic acid from 100 µL of specimen was extracted into an elution volume of 100 µL by a Magnapure LC robot (Roche Molecular Systems, Mannheim, Germany), using the Total

Nucleic Acid protocol, and was amplified in real-time PCR system (Corbbet Research 6600).

Amplification was carried out in 50 µL reaction volumes. After a reverse transcription step, 45 cycles of two-step PCR was performed (95°C 10 min 45 cycle [95°C 15 s, 58°C 60 s]). The positive cases and the type of viruses and/or bacteria were determined by the software of the real-time PCR device.

**Statistical analysis**

Obtained data were analyzed using SPSS version 15 software and Chi-square test and exact Fisher test.  $P < 0.05$  considered to be statistically significant.

**RESULTS**

In this study, 455 cases with ARTI were studied. Mean age of studied population was  $29.9 \pm 18.5$  (range: 0.2-80). About 43.5% of patients were male and 56.5% were female.

Data regarding to the pathogens isolated from patients with ARTIs are presented in Figure 1.

Data regarding to characteristics of studied population regarding the symptoms of the disease and the pathogens isolated from patients with ARTI in different age groups are presented in Tables 1 and 2.

Regarding the relation between clinical symptoms and related pathogens, there was significant relationship between coryza ( $P = 0.002$ ,  $\chi^2 = 19.53$ ), hoarseness

( $P = 0.003$ ,  $\chi^2 = 17.72$ ) and vomiting ( $P = 0.04$ ,  $\chi^2 = 11.12$ ) with PIV. There was significant relationship between sneeze ( $P = 0.02$ ,  $\chi^2 = 13.2$ ) and skeletal pain ( $P = 0.003$ ,  $\chi^2 = 17.65$ ) with IFA.

Regarding the relation between clinical symptoms and related pathogens in studied age groups the results were as follows;

- There was significant relationship between fever and PIV in children aged <5 years ( $P = 0.03$ ,  $\chi^2 = 8.7$ ).
- There was a significant relationship between sore throat and PIV and RV in adults population aged 15-50 years ( $P = 0.04$ ,  $\chi^2 = 7.8$ ).
- There was significant relationship between coryza and RV in elderly aged >50 years ( $P = 0.009$ ,  $\chi^2 = 11.35$ ).

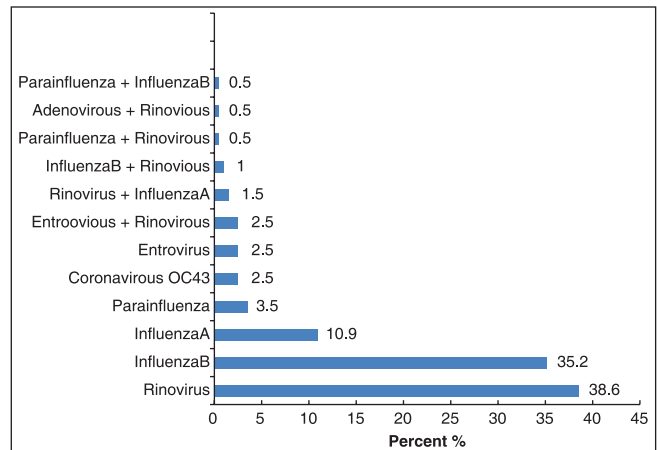


Figure 1: The ratio of pathogens isolated from patients with acute respiratory tract infections

**Table 1: Clinical presentation of ARTI in different studied age groups**

Clinical symptoms	Age (%)		Groups (%)		Total (%)	P
	<5	5-14.99	15-50	>50		
Fever	15 (53.6)	49 (62.8)	94 (35.2)	24 (36.9)	182 (41.6)	<0.001
Sorethroat	23 (82.1)	62 (79.5)	233 (87.3)	60 (92.3)	378 (86.3)	0.13
Coryza	25 (89.3)	49 (62.8)	183 (68.5)	41 (63.1)	298 (68.0)	0.05
Hoarseness	7 (25.0)	23 (29.5)	106 (39.7)	26 (40.0)	162 (37.0)	0.19
Dry cough	17 (60.7)	40 (51.3)	140 (52.4)	41 (63.1)	238 (54.3)	0.37
Productive cough	8 (28.6)	15 (19.2)	63 (23.6)	20 (30.8)	106 (24.2)	0.40
Dyspnea	1 (3.6)	1 (1.3)	33 (12.4)	13 (20.0)	48 (11.0)	0.002
Red_eye	5 (17.9)	7 (9.0)	25 (9.4)	7 (10.8)	44 (10.0)	0.53
Purulative discharge	0 (0.0)	1 (1.3)	1 (0.4)	0 (0.0)	2 (0.5)	0.64
Sneeze	19 (67.9)	37 (47.4)	147 (55.1)	35 (53.8)	238 (54.3)	0.30
Headache	6 (21.4)	39 (50.0)	161 (60.3)	43 (66.2)	249 (56.8)	<0.001
Otitis	1 (3.6)	5 (6.4)	16 (6.0)	6 (9.2)	28 (6.4)	0.72
Diarrhea	2 (7.1)	1 (1.3)	5 (1.9)	0 (0.0)	8 (1.8)	0.12
Vomiting	2 (7.1)	5 (6.4)	5 (1.9)	0 (0.0)	12 (2.7)	0.03
Abdominal pain	5 (17.9)	7 (9.0)	7 (2.6)	1 (1.5)	20 (4.6)	<0.001
Fatigue	6 (21.4)	26 (33.3)	130 (48.7)	33 (50.8)	195 (44.5)	0.005
Skeletal pain	3 (10.7)	20 (25.6)	135 (50.6)	41 (63.1)	199 (45.4)	<0.001
Joint pain	0 (0.0)	1 (1.3)	9 (3.4)	3 (4.6)	13 (3.0)	0.49

ARTI = Acute respiratory tract infections

**Table 2: Viruses isolated from patients with upper respiratory tract infections during the surveillance period in Isfahan according to the age group of studied population**

Isolated viruses	Age (%)		Groups (%)	
	<5	5-14.99	15-50	>50
Negative	11 (39.3)	40 (51.3)	145 (54.3)	36 (55.4)
RV	9 (32.1)	11 (14.1)	42 (15.7)	15 (23.1)
IFB	2 (7.1)	14 (17.9)	47 (17.6)	7 (10.8)
Influenza B + RV	1 (3.6)	1 (1.3)	0	0
Para-influenza + RV	1 (3.6)	0	0	0
ADV + RV	0	1 (1.3)	0	0
Coronavirus OC43	0	0	4 (1.5)	1 (1.5)
EV + RV	0	0	3 (1.1)	1 (1.5)
PIV	2 (7.1)	2 (2.6)	3 (1.10)	0
IFA	0	5 (6.4)	13 (4.9)	4 (6.2)
PIV. IFB. RV	0	0	1 (0.4)	0
PIV + IFB	0	0	1 (0.4)	0
RV + IFA	0	0	3 (1.1)	0
EV	0	1 (1.3)	3 (1.1)	1 (1.5)
RV + EV	1 (3.6)	0	0	0

IFA = Influenza virus A; IFB = Influenza virus B; PIV = Para-influenza virus; RV = Rhinovirus; EV = Enterovirus; ADV = Adenovirus

- There was a significant relationship between skeletal pain and IFA in the adult population aged 15-50 years ( $P = 0.01$ ,  $\chi^2 = 12.3$ ).
- There was not any case with hematuria or rash.

## DISCUSSION

In this pilot study surveillance of ARTI among outpatients in different age groups in Isfahan city was studied. Current study considered the first surveillance study in our region in this field.

The results indicated that symptoms such as sore throat, coryza, and dry cough were the most common symptoms in all studied groups, whereas fever was the most clinical presentation of younger patients and headache and skeletal pain were the most common symptoms of older patients. Regarding microorganism responsible for ARTI in our studied population, using Multiplex real-time PCR method, RV was the most common cause of ARTI in patients aged <5 years and those aged >50 years. IFB was the most common cause of ARTI in patients aged 5-50 years.

As mentioned laboratory investigation are crucial for accurate etiologic diagnosis of ARTIs and surveillance programs in this field should be a laboratory-based study. The findings of surveillance programs with appropriate laboratory methods such as molecular technologies with higher specificity and sensitivity, lower cost and rapidity would help us to improve the ARTIs surveillance networks which consequently result

in timely administration of antiviral drugs to decrease the duration of outbreaks, broad spectrum pathogen detection and lower total costs of the disease. Mentioned benefits in accordance with time saving effects of multiplexed molecular techniques are more critical during epidemics.<sup>[14-17]</sup>

In this study detection of pathogens was assessed using multiplex real-time PCR method which utility is reported in different recent studies. The higher sensitivity and rapidity of the method are the advantages of it which made it an appropriate diagnostic tool for clinical epidemiological and surveillance studies. It has been suggested that a rapid etiologic diagnosis of viral RTI could reduce unnecessary prescription of antibiotics.<sup>[18]</sup>

In this study, the most common pathogens among all studied subjects were RV, IBV, IAV, PIV, EV and coronavirus OC43 respectively. According to age groups RV was common among children aged <5 years and elderly patients aged >50 years and IBV was the most common pathogen among patients aged 5-50 years.

Several studies indicated that RSV and RVs are the most common causes of ARTIs in Asia.<sup>[19-22]</sup> Our results were in line with mentioned studies.

In a similar study in Nikonova *et al.* respiratory viruses detected among 226 patients with ARTI using Multiplex real-time PCR method. They reported that the most common pathogen was IBV with a rate of 58.6%. Other pathogens were as follows; IAV (18.1%), RV in 10.3%, PIV2 in 5.2%, ADV in 3.4%, RSV in 2.6%, EV in 1.7%, PIV4 in 1.7% and PIV1 and 3 in 0.9%. They concluded that Multiplex real-time PCR method is a suitable and effective method for epidemiological studies in this regard due to its low cost and rapidity.<sup>[23]</sup>

Brittain-Long *et al.* evaluated the diagnostic performance and clinical use of multiplex PCR method in adults with ARTIs in Sweden. The most common causes were IFA and RV. They concluded that such information should be used in clinical practice.<sup>[24]</sup>

There were several studies among pediatric groups especially children aged <5 years because it consider one of the most important leading cause of morbidity and mortality in children worldwide. Previous surveys showed that the predominant causes of ARTIs in children <5 years are RSV, hMPV, human RVs and PIVs. Some of them indicated strong seasonal peaks and in 4-33% of cases co infections of mentioned viruses are the cause. Some studies investigated the relation between different pathogens with the severity of ARTIs.<sup>[25-29]</sup> In this study, the relationship

was not investigated. The most common pathogens were RV, IBV and PIV.

Rhinoviruses are reported as more prevalent virus detected in children with ARTIs.<sup>[30]</sup> The results of Miller *et al.* study was similar to ours.<sup>[31]</sup> Similarly, Winther *et al.* have reported same results.<sup>[32]</sup> Thus considering the high proportion of RV infections in young children and long-term of the virus shedding the role of this pathogen in children should be considered in surveillance studies among this age group of the population.<sup>[33]</sup>

Different reports in different age groups of the general population that reported in previous studies may be due to differences in laboratory methods, studied population, and the time the study was performed.

The overall rate of co-infection (dual infection) in our study was 4.4% with a higher rate in children aged <5 years. Reports from patients with ARTIs showed that the rate of co-infection ranging from 5% to 20% with a higher rate among young children.<sup>[24]</sup> Though in our study, the overall rate of co infection was lower than mentioned reported rate, but the rate was higher in pediatric patients aged <5 years (10.6%).

In current study, the detection rate by Multiplex real-time PCR method was 46.2%. Previous studies have reported a detection rate ranging from 43% to 63% for the method.<sup>[24]</sup> Accordingly detection rate is higher in young children and infants. Our results were in accordance with mentioned reports. The detection rate was higher in younger age groups. It seems that inappropriate sample collection or transfer is one of the causes of lower detection rate in our study, so it should be improved in our future surveys.

Different clinical presentation and their rate were reported in this study. Sore throat, coryza and dry cough were the most common symptoms in all studied groups whereas fever was the most clinical presentation of younger patients and headache and skeletal pain were the most common symptoms of older patients. As mentioned the clinical presentation of different pathogens responsible for ARTIs are similar but some studies evaluated the relation between different pathogens with different clinical presentation. In our study there was significant relationship between coryza, hoarseness and vomiting with PIV. Sneezing and skeletal pain was the most common clinical presentation of IFA. Though the relation between clinical symptoms and related pathogens in studied age groups was determined in current study also, but considering that the sample size in age subgroups was small so obtained data would be more conclusive if it evaluate in larger sample size.

According to previous studies headache is associated with PIC, ADV, and HCoV infection, cough is associated with influenza viruses and sore throat, cough, rhinorrhea, nasal symptoms are commonly associated with PIC.<sup>[34,35]</sup>

The current study has some unique aspects such as originality and being the first study in our region using the sensitive method, multiplex real-time PCR for determining causative agents of ARTIs. However, there were some limitations that should be mentioned. The limitations are as follows; not evaluating recently identified viruses in this regard, short duration of the study for determining seasonal variation of the pathogens responsible for ARTIs in our region, not evaluating the effect of duration of symptoms presentation with the responsible pathogens or the relationship between sex and the pathogens due to small sample size in studied age groups.

However some studies suggested that environmental factors such as climatic factors and population determinants such as human crowding could affect the epidemiologic feature of ARTIs.<sup>[36,37]</sup>

Regarding duration of symptoms in patients with ARTIs, Brittain-Long *et al.* have indicated that the duration of symptoms affects the detection rate using Multiplex real-time PCR method. According to their report detection rate was higher in patients referred with duration of symptoms of less than 6 days.<sup>[24]</sup>

In sum, our study provides baseline information on the epidemiologic and clinical feature of outpatients with ARTIs in Isfahan city. Though our findings in this pilot study could be helpful in diagnosis, treatment, and prevention of ARTI, planning preventive interventional programs but it seems that further population-based and hospital-based studies with consideration of mentioned limitation should be established for fully understanding of the epidemiology of the disease, properly providing resources for management of the disease specially among pediatrics and elderly patients with high susceptibility to its related morbidity and mortality.

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## AUTHOR'S CONTRIBUTION

All authors have contributed in designing and conducting the study. ZN and BA collected the data and AJ, BA, and

ZN did the analysis. MY worked experimental test. PA has assisted in preparation of the first draft of the manuscript and revising it critically for important intellectual content. All authors have read and approved the content of the manuscript and are accountable for all aspects of the work.

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