Molecular Detection and Clinical Characteristics of Bacterial and Viral Main Etiological Agents Causing Respiratory Tract Infections in Tehran, Iran

Taher Azimi Sarikhanbaghloo

Aja University of Medical Sciences

Ramin Hamidi-Farahani

Aja University of Medical Sciences

Ali Asgari

Aja University of Medical Sciences

Jalil Rajabi

Aja University of Medical Sciences

Mousa Ahmadi

Aja University of Medical Sciences

Mohammad Darvishi

Aja University of Medical Sciences

Mohammad Aminianfar

Aja University of Medical Sciences

Saeed Soleiman-Meigooni (✉ Dr.saeed.meigooni@gmail.com)

Aja University of Medical Sciences

Research Article

Keywords: COVID-19, coronavirus disease, respiratory tract infection, viral infection, bacterial infection, military personals

Posted Date: March 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-279011/v1>

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Abstract

Background: Respiratory tract infections are one of the most important infections among military personals, worldwide. The present study aimed to survey the prevalence of bacterial and viral main etiological agents causing respiratory tract infections among the military personnel in Iran. A cross-sectional study was performed from September 2020 to February 2021. Nasopharyngeal swabs were taken from patients, military personnel with respiratory tract infection symptoms. Detection of COVID-19 was performed with one step qRT-PCR method. TaqMan *probe-based real-time PCR* assay was used for the detection of influenza A and B viruses. The prevalence of adenovirus and *M. pneumoniae* were determined using nested PCR. Moreover, *B. pertussis* and *S. pyogenes* were identified by conventional PCR assay. The detection of *H. influenzae* was performed by multiplex PCR method.

Results: Overall, 145 patients were included. Among viral pathogens, COVID-19, influenza A virus, and adenovirus were identified in 85.5%, 4.1%, and 1.4% of patients, respectively. Influenza B virus was not detected among military personnel. The frequency of bacteria etiological agents was as follows: *S. pyogenes* (2%), *M. pneumoniae* (0.7%), *H. influenzae* (0%), and *B. pertussis* (0%). Muscle aches (75.9%), headache (70.3%), lethargy (69%), cough (66.2%), stuffy nose (56.6%), fever (53.8%), and *sore throat* (53.1%) were amongst the most common clinical symptoms.

Conclusions: Results showed that the military personals are the susceptible group to COVID-19 infection. Therefore, the accurate detection and implementation of control strategies such as vaccination are necessary.

Introduction

Globally, respiratory tract infections are one of the most important infections among the most common infectious diseases[1]. Respiratory tract infections were considered life-threatening complications that can cause high morbidity and mortality in both adults and children[2]. Respiratory tract infections can be caused by different classes of etiologic agents and are usually separated into two main groups including lower respiratory tract infections and upper respiratory tract infections[3]. Several groups of pathogens such as bacteria and viruses cause the highest proportion of respiratory tract infections. Among bacterial pathogens, respiratory tract infections can be caused by *Haemophilus influenzae* (*H. influenzae*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Bordetella pertussis* (*B. pertussis*), *Chlamydia pneumoniae* (*C. pneumoniae*), *Streptococcus pyogenes* (*S. pyogenes*), and *Mycoplasma pneumoniae* (*M. pneumoniae*)[4-6]. However, most cases of respiratory tract infections are caused by viruses, and these agents are responsible for a large proportion of human upper respiratory tract infections[7]. Well-diagnosed viral pathogens which can cause a variety of respiratory disease are influenza A and B viruses, adenovirus, respiratory syncytial virus, parainfluenza, metapneumovirus, enterovirus, rhinovirus, and coronavirus[8]. World Health Organization (WHO) considered coronavirus disease 2019 (COVID-19) a public health emergency of international concern[9]. This new virus has caused significant mortality and morbidity, globally[10]. Till 08/February/2021 around 106,696,325 cases of COVID-19 and 2,327,535 deaths have been reported, worldwide (https://www.worldometers.info/coronavirus/?utm_campaign=homeAdUOA?Si). To date, COVID-19 causing >1,466,435 confirmed cases and over >58,469 deaths in Iran and there are no effective antiviral drugs or specific vaccines against COVID-19 (<https://www.worldometers.info/coronavirus/country/iran/>). In general, viruses created similar and nearly indistinguishable clinical signs and symptoms to bacterial pathogens in patients[11]. Moreover, in most cases, physicians prescribe different antibiotics without any laboratory testing for the treatment of respiratory infections[1]. The unnecessarily prescribed antibiotics lead to the emergence of antimicrobial resistance among pathogens. Consequently, in the diagnosis of causative pathogenic agents, clinical findings alone are not reliable and laboratory confirmation is necessary to definitively identify aetiological agents of respiratory infections[12]. Globally, due to several conditions such as insomnia, stressful working environment, demanding physical training programs, and crowded habitation, military populations are more susceptible to respiratory infections, and each year during the autumn and winter seasons, numerous cases of respiratory infections happen among military populations[13,14]. However, only a few data are available on the role of bacterial and viral pathogens in the etiology of respiratory tract infections in military populations in Iran. Hence, this study was performed to investigate the main bacterial and viruses among the military personnel presenting with symptoms and clinical signs of acute respiratory disease in Iran.

Materials And Methods

Study design and sample collection

This cross-sectional study was conducted at AJA University of Medical Sciences from September 2020 to February 2021. Patients presenting different symptoms and clinical signs of respiratory tract infection were included in this study. All participants provided written informed consent before enrolment. After informed consent, sociodemographic data and clinical signs including age, height, weight, blood type, types and times of facemasks used during COVID-19 pandemic, a history of high blood pressure, a history of lung diseases such as chronic obstructive pulmonary disease (COPD), a history of asthma disease, obesity, irritable bowel syndrome (IBS), diabetes, a history of the liver disease (fatty liver disease), contact with a person who has a positive test to COVID-19, alcohol consumption, smoking, fever, chills, cough, muscle aches, lethargy, red eyes, diarrhea, vomiting, nausea, runny or stuffy nose, gastrointestinal bleeding, sore throat, chest pain, headache, dyspnea, changes the sense of smell and taste, loss of consciousness, and joint pain were recorded. The samples were collected by the nasopharyngeal swabs (flock swabs, Copan, Italy) in 10 ml of sterile phosphate-buffered saline (PBS, 0.1M, pH 7.2) and were transported to the Comprehensive Research Laboratory, School of Medicine, AJA University of Medical Sciences. The three nasopharyngeal swab samples were taken from each included patient.

DNA extraction and detection of bacterial respiratory pathogen

The first nasopharyngeal swabs were placed in 1 ml of sterile saline and mixed by vigorous vortex for 30 to 45 s. At the next step, swabs were removed from samples and saline suspensions were transferred to a 1.5 ml Eppendorf tube. The Eppendorf tubes were centrifuged at 13000 rpm for 5 min and pellet suspended in 300 µl 20% Chelex in TE buffer and then heated at 95°C for 5 min to disrupt the cells. All samples were stored at -20°C. For the detection of *B. pertussis*, the specific primers which targeted the *IS481* gene were used. The primer sequence used for the detection of *B. pertussis* were as follows: Pip1-F: 5'-CCCATAAGCATGCCCGATTGAC-3' and Pip2-R: 5'-CGCACAGTCGGCGCGGTGAC-3'. The PCR assay was carried out in a total volume of 50 µl reaction containing 5 mM MgCl₂, 6 µl of 10x PCR buffer, 1 µM of each forward (Pip1) and reverse (Pip2) primers (10mM), 2 units of Taq polymerase (Cinnagene, Iran), 1 µl of 10 mM of each deoxynucleoside triphosphate (dNTPs), 10 ml of the stored clinical sample and sterile distilled water up to 50 µl. The PCR reaction condition and parameters were similar to those described by Brandberg et al[15].

To detection of *M. pneumoniae*, swab samples were placed in microtubes containing phosphate-buffered saline (PBS), and then DNA extraction was conducted using a MagNa Pure LC DNA I isolation kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's guidelines. The semi-nested PCR method was used to screen the presence of *M. pneumoniae* in the swab samples. Briefly, *M. pneumoniae* DNA was amplified and detected using the first-round primer pairs including 5'-TGCCATCAACCCGCGCTTAAC-3' and 5'-CCTTTGCAACTGCTCATAGTA-3' and the second-round primers including 5'- CCTTTGCAACTGCTCATAGTA -3' and 5'-CAAACCGGGCAGATCACCTTT-3'. The semi-nested PCR conditions and parameters were set based on a previously published study by Nilsson et al[16].

The detection of *S. pyogenes* was performed with the PCR method and using specific primer pairs including F: 5'-AAAGACCGCCTTAACCACCT- 3' and R: 5'-TGGCAAGGTAAACTTCTAAAGCA-3' target the *spy1258* gene. PCR was conducted in a total volume of 25 µl reaction including 3 µl of 10x PCR buffer without MgCl₂, 2.5 mmol/l MgCl₂, 0.5 µl of 10mM of each deoxynucleoside triphosphate (dNTPs), 0.5 µM of each primer (10 mM), 1 unit of Taq polymerase (Cinnagene, Iran), 5 µl of template DNA, and 5.5 µl of sterile distilled water. PCR program of *spy1258* gene is followed by initial denaturation for 2 min at 94 °C, 35 cycles of denaturation for 35 s at 94 °C, annealing for the 20s at 55 °C, elongation for 40 s at 72 °C, and final elongation for 2 min at 72 °C.

For *H. influenzae* detection, the multiplex PCR method target genes *HiP6* and *bexA* were used. The primers sequences were as follows: HiP6-F: ACTTTTGGCGTTACTCTGT and HiP6-R: TGTGCCTAATTACCAGCAT, *bexA*-142-F: GGCGAAATGGTGCTGGTAA and *bexA*-241-R: GGCCAAGAGATACTCATAGAACGTT. PCR was performed on Eppendorf Mastercycler EP thermocycler and PCR programs are followed by initial denaturation for 5 min at 94 °C, with 35 cycles of 95 °C for 30 s, annealing for the 60 s at 50 °C, an extension for 1 min at 72 °C, and final extension for 10 min at 72 °C. Finally, all PCR products were electrophoresed and screened on a 1-1.5% agarose gel, and then stained with DNA-safe stain (SinaClon, Tehran, Iran). PCR products were visualized by a UV transilluminator and photographed under UV light.

RNA extraction and complementary DNA synthesis

Total viral RNA was extracted from 200 µl of nasopharyngeal swab samples using the QIAamp Viral RNA Mini kit (Qiagen, Mississauga, Ontario, Canada) according to the supplier's instructions. To remove any genomic DNA carryover, 20 U of RQ1 RNase-free DNase (Promega, Madison, WI, USA) was used and then extraction was suspended in 50 µl of diethyl pyrocarbonate-treated water (0.1% v/v). The complementary DNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, ON, Canada). Synthesis conditions have been described by MacIntyre et al[6].

Detection of viral respiratory pathogens

Nested PCR was used for detecting adenoviruses using specific primer pairs. In brief, adenoviruses DNA was amplified and detected using the first-round primer pairs including 5'-GCCGACAGTGGTCTTACATGCACATC-3' and 5'-CAGCACGCCGCGGATGTCAAAGT -3' and the second-round primers including 5'-GCCACCGAGACGTACTTCAGCCTG-3' and 5'-TTGTACGAGTACGCGGTATCCTCGCGGTC-3'. PCR conditions were set based on a previously published study by Rigotto et al[17]. PCR reactions were conducted under the following condition: initial denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C for 1 min, annealing at 56°C for 1 min, and 72 °C for 45 s with the final incubation at 72 °C for 7 min.

The probe-based real-time PCR was used to detection of influenza A and B viruses. For detection of influenza A and B viruses in nasopharyngeal swab samples, the primes and probes target the matrix protein (M) and hemagglutinin gene segment (HA) genes, respectively. Complementary DNA was amplified using the following primer and probe: M-F: 5'- GGACTGCAGCGTAGACGCTT-3', M-R: 5'- CATCCTGTTGTATATGAGGCCCAT-3', probe: 5'-FAM-CTCAGTTATTCTGCTGGTGCACCTTGCCA-TAMRA-3', and HA-F: 5'-AAATACGGTGGATTAAATAAAAGCAA-3', HA-R: 5'-CCAGCAATAGCTCCGAAGAAA-3', probe: 5'-FAM-ACCCATATTGGGCAATTTCCTATGGC-TAMRA-3'. Real-time PCR conditions were set based on a previously published study by Elden et al[18]. In summary, the reaction was performed with the final volume of 25 µl including 12.5 ml of TaqMan Universal PCR master mix containing ROX as a passive reference (PE Applied Biosystems), 5 µl of cDNA, 1 µl of each influenza virus A and B primers, 100 nM each probe, and sterile distilled water up to 25 µl. The real-time PCR was conducted on a Corbett 6000 Rotor-Gene thermocycler (Corbett Research) under the following conditions: 50°C for 2 min (optimal AmpErase Uracil-*N*-glycosylase activity) followed by of 95 °C for 10 min, 45 cycles of 95 °C for 20 s and of 60 °C for 1 min. During amplification procedures, the fluorescence radiating from the used probes was documented.

Detection of COVID-19 by One-Step Quantitative RT-PCR System

Detection of COVID 19 was performed with one step qRT-PCR method. For this purpose, the primers and probes target the RdRp genes (nCoV_IP2 and nCoV_IP4). The primers and probes sequence used for the detection of viral RNA were as follows: nCoV-IP2-F: 5'-ATGAGCTTAGTCCTGTTG-3', nCoV-IP2-R: 5'-CTCCCTTTGTTGTGTTGT-3', nCoV-IP2-12696bProbe: 5'-Hex-AGATGTCTTGTGCTGCCGTA-3'-BHQ-1 and nCoV-IP4-F-5'-GGTAACTGGTATGATTCG-3', nCoV-IP4-F-5'-CTGGTCAAGGTTAATAGG-3', nCoV-IP4-14084Probe: 5'-Fam-TCATACAAACCACGCCAGG-3'-BHQ-1. Moreover, we used RNase P (C1), labeled with BHQ-1 and HEX fluorescent dye as an internal reference control. Quantitative RT-PCR was performed with a Kit Extraction NucleoSpin Dx Virus (Macherey Nagel ref. 740895.50) and Invitrogen Superscript™ III Platinum® One-Step qRT-PCR system (ref: 11732-088). The optimized concentrations were performed in a total volume of 25 µl reaction including 3.6 µl sterile distilled water, 12.5 µl Reaction mix 2X (3 mM Mg), 0.4 µl MgSO4 (50mM), 1 µl of each primer (10 mM), 0.5 µl of Probes (10µM), 1 µl superscriptIII RT/Platinum Taq Mix, and 5µl of extracted RNA sample. Amplification condition was as follows: reverse transcription for 20 min at 55°C, denaturation for 3 min at 95°C, 50 cycles of denaturation for 15 s at 95°C, annealing for the 30s at 58°C, and cooling for 30s at 40°C. Cycle threshold (CT) value equal to or less than 30 was considered to be positive.

Statistical Analysis

All sociodemographic and clinical data of patients were formatted in an SPSS file, and the data were analyzed by the statistical package SPSS v.23.0 (SPSS Inc., Chicago, IL, USA) using descriptive statistic tests.

Results

Study Population

In the current study, a total of 145 patients who were referred to Imam Reza Hospital, AJA University of Medical Sciences, were selected based on their clinical signs and symptoms of respiratory tract infections. Demographic information and clinical signs of these patients are summarized in Table 1. The age of the patients ranged from 19 to 55 years, with a mean age of 26 ± 0.5 years. Among the studied participants, 55.9% ($n = 81/145$) and 20% ($n = 29/145$) were in the age range of 18 to 25 years and ≥ 36 years, respectively. The mean weight of the patients was 78 ± 0.2 Kg and the weight of the patients ranged from 50 to 125 Kg. The body mass index (BMI) was 18.5 to 24.9 and 25 to 29.9 in 49% and 39.2% of patients, respectively. The frequency of different blood groups among patients were as follows: O⁺ (43.4%; 63/145), O⁻ (2.1%; 3/145), A⁺ (27.6%; 40/145), A⁻ (2.1%; 3/145), B⁺ (14.5%; 21/145), B⁻ (2.8%; 4/145), AB⁺ (7.6%; 11/145), and blood group A⁻ (0%;0/145). Based on the self-declaration, 71.7% ($n = 104/145$) of patients used facemasks at all times during COVID-19 pandemic and 6.2% ($n = 9/145$) of patients rarely used facemasks. Moreover, 84.8% ($n = 123/145$) and 11.7% ($n = 17/145$) of patients were used cloth and surgery facemasks, respectively. 3.4% (5/145) of patients only used N95 or FFP2 facemasks. Out of 145 included patients, 32.4% (47/145) were smokers and 13.1% ($n = 19/145$) have a history of alcohol consumption. The frequency of underlying diseases among patients were as follows: high blood pressure (11.7%; $n = 17/145$), fatty liver disease (13.8%; $n = 20/145$), IBS (6.9%; $n = 10/145$), asthma (6.9%; $n = 10/145$), COPD (4.8%; $n = 7/145$), and diabetes (2.1%; $n = 3/145$). In general, 79.3% ($n = 115/145$) of patients had a history of close contact with COVID positive person in the military medical center, military hospitals, military sanatoria, or community. The main important clinical signs and symptoms among included patients were muscle aches (75.9%; $n = 110/145$), headache (70.3%; $n = 102/145$), lethargy (69%; $n = 100/145$), cough (66.2%; $n = 96/145$), stuffy nose (56.6%; $n = 82/145$), fever (53.8%; $n = 78/145$), and sore throat (53.1%; $n = 77/145$), respectively.

Detection and clinical signs of bacterial pathogens

In total, the frequency of main bacterial pathogens including *H. influenzae* and *B. pertussis*, *M. pneumoniae*, and *S. pyogenes* was surveyed among the military soldiers with acute respiratory disease. The bacterial pathogens detection rates are summarized in Table 1. Overall, respiratory bacterial pathogens were detected in 4 of 145 (2.75%) included patients. *H. influenzae* and *B. pertussis* was not detected among military soldiers with acute respiratory disease. *S. pyogenes* and *M. pneumoniae* were found in 2% ($n = 3/145$) and 0.7% ($n = 1/145$) of the patients. The *M. pneumoniae* was detected in patients 18 to 25 years old ($n = 1/85$; 1.25%). The one patient who was positive for *M. pneumoniae* had a BMI >40 , blood group B- and a history of close contact with a COVID positive person. Moreover, this patient has used a surgical facemask at all times during COVID-19 pandemic and showed several clinical symptoms such as chest pain, muscle aches, and headache. On the other hand, *S. pyogenes* was found among patients 18 to 25 years old ($n = 3/85$; 3.7%). Moreover, 5.6% ($n = 1/18$) of patients with BMI 30 to 34.9 were positive for *S. pyogenes*. All 3 patients who were positive for *S. pyogenes* had the blood group O⁺ and a history of close contact with a COVID positive person. The frequency of *S. pyogenes* among patients that used cloth and surgical facemasks was 5.9% ($n = 1/17$) and 1.6% ($n = 2/123$), respectively. The main clinical symptoms among patients with *S. pyogenes* infection were chest pain (100%; $n = 3/3$), cough (66.6%; $n = 2/3$), lethargy (66.6%; $n = 2/3$), muscle aches (66.6%; $n = 2/3$), and fever (66.6%; $n = 2/3$).

Prevalence and clinical signs of COVID-19

The number of samples found by One-Step Quantitative RT-PCR to be positive for COVID-19 was 124/145 (85.5%). Among different age groups, the highest prevalence of COVID-19 was seen in the patients ≥ 36 years old ($n = 28/29$; 96.5%), 26 to 30 years old ($n = 23/24$; 95.8%), and 31 to 35 years old ($n = 10/11$; 90.1%), respectively. Moreover, the highest frequency of COVID-19 was detected among patients with BMI 30 to 34.9 ($n = 17/18$; 94.5%), 25 to 29.9 (46/51; 88.2%), and 18.5 to 24.9 ($n = 59/71$; 83%), respectively. The frequency of COVID-19 among different blood groups was as follows: O⁺ ($n = 51/63$; 81%), O⁻ ($n = 3/3$; 100%), A⁺ ($n = 35/40$; 87.5%), A⁻ ($n = 3/3$; 100%), B⁺ ($n = 20/21$; 95.2%), B⁻ ($n = 3/4$; 75%), AB⁺ ($n = 9/11$; 82%).

In total, 79% (n = 98/124) of patients with COVID-19 infection had a history of close contact with COVID positive person. Results showed that out of 124 patients with COVID-19 infection, 106 patients (85.5%) used surgical facemasks during COVID-19 pandemic and 90 patients (72.6%) used facemasks at all times. Moreover, out of 124 patients with COVID-19 infection, 33% (n = 41/124) were smokers and 14.5% (n = 18/124) have a history of alcohol consumption. The frequency of underlying disease among patients with COVID-19 infection was as follows: COPD (n = 7/124; 5.6%), asthma (n = 8/124; 6.5%), IBS (n = 8/124; 6.5%), *diabetes* (n = 3/124; 2.4%), fatty liver disease (n = 18/124; 14.5%), and high blood pressure (n = 17/124; 13.7%). The main important clinical signs and symptoms among COVID-19 positive patients were muscle aches (n = 95/124; 76.6%), lethargy (n = 89/124; 71.8%), headache (n = 88/124; 71%), cough (n = 82/124; 66.1%), stuffy nose (n = 71/124; 57.2%), fever (n = 70/124; 56.4%), and *sore throat* (n = 67/124; 54%), respectively.

Detection and clinical signs of viral pathogens

The numbers of samples found by molecular methods to be positive for influenza virus A, influenza virus B, and adenoviruses were 4.1% (n = 6/145), 0% (n = 0/145), and 1.4% (n = 2/145), respectively. The most influenza virus A infection (n = 4/6; 66.7%) was seen among patients 18 to 25 years old. The influenza virus A infection was not detected among patients 26 to 30 years old. Moreover, 4.2% (n = 3/71) of patients with BMI 18.5 to 24.9 and 5.9% (n = 3/51) of patients with BMI 25 to 29.9 were positive for influenza virus A infection. The 66.7% (n = 4/6) of patients who were positive for influenza virus A had blood group O⁺ and all of these patients used surgical facemasks. The only underlying disease among patients who were positive for influenza virus A infection was a fatty liver disease (16.6%; n = 1/6). The main important clinical signs and symptoms among patients who were positive for influenza virus A infection were headache (n = 4/6; 66.7%), cough (n = 4/6; 66.7%), lethargy (n = 3/6; 50%), muscle aches (n = 3/6; 50%), *chest pain* (n = 3/6; 50%), and dyspnea (n = 3/6; 50%), respectively.

Adenovirus infection was detected in 2.5% (n = 2/81) of patients in 18-25 years old. In total, 2.8% (n = 2/71) of patients with BMI 18.5 to 24.9 were positive for adenovirus infection. Among different blood groups, adenovirus infection was seen in 9% (n = 1/11) and 1.6% (n = 1/63) of patients with blood groups AB⁺ and O⁺, respectively. Patients with adenovirus infection are used facemasks at all times. All patients with adenovirus infection (n = 2/2; 100%) showed main clinical manifestations such as cough, muscle aches, *taste loss*, *anosmia*, dyspnea, stuffy nose, and *sore throat*.

Discussion

In general, respiratory tract infections can be caused by different types of bacteria and viruses and presents with overlapping clinical symptoms and signs[3]. Therefore, the etiologic agents of respiratory tract infections cannot be identified by clinical signs and symptoms. Unfortunately, in most cases, physicians prescribe different antibiotics based on the patient's clinical signs. The unnecessarily prescription of antibiotics leads to the emergence of antimicrobial resistance[1]. Therefore, the rapid and accurate identification of etiologic agents of respiratory tract infections is critical for the appropriate prescription of antibiotics and suitable treatment of infection. Respiratory tract infections remain a challenge among military personnel and only limited data are available on their etiology in military soldiers[19]. The purpose of the current study was to access the bacterial (*B. pertussis*, *M. pneumoniae*, *S. pyogenes*, and *H. influenzae*) and viral cause (COVID-19, human *influenza A and B viruses*, and *adenoviruses*) of respiratory tract infections in military personnel in Tehran, Iran. In general, our analyses revealed that out of 145 patients with respiratory tract infections 43.4% and 26.7% had the O⁺ and A⁻ blood group, respectively. Moreover, the main clinical signs and symptoms of respiratory tract infections among all patients were muscle aches and headache, respectively. Results of our study showed that out of 145 patients with respiratory tract infections 124 (85.5%) patients were positive to COVID-19 and 79% of these patients had a history of close contact with COVID positive person. The main underlying disease among patients with COVID-19 infection was fatty liver disease and high blood pressure, respectively. The highest frequency of COVID-19 was seen among patients ≥ 36 years old and BMI 30 to 34.9. In the current study, the main important clinical signs and symptoms among patients with COVID-19 infection were muscle aches (76.6%), lethargy (71.8%), headache (71%), cough (66.1%), stuffy nose (57.2%), fever (56.4%), and *sore throat* (54%), respectively. Numerous studies have surveyed the prevalence, clinical characteristics, and main underlying disease relate to COVID-19, worldwide. Results of a study performed in Italy revealed that the prevalence of COVID-19 among patients with chronic inflammatory rheumatic diseases was 4.5% [20]. Kalantari et al. surveyed the prevalence of COVID-19 with regards to the age range of patients in Tehran. They showed that 70.5% of all patients were positive for COVID-19 infection. Moreover, the results of their study showed that COVID-19 had the highest frequency among patients >80 years old[21]. Shakiba et al. were study the prevalence of asymptomatic COVID-19 infection in the general population. Their finding revealed that more than half (57.2%) of patients with COVID-19 infection had no clinical symptoms[22]. On the other hand, Espinosa et al. from Spain reported that the prevalence of COVID-19 infection in hemodialysis patients was 9.2%[23]. Results of a cross-sectional study conducted by Anand et al. revealed that the seroprevalence of COVID-19 infection was 8% in patients on dialysis in the USA[24]. Gubatan et al. were surveyed the prevalence, risk factors, and clinical outcomes of COVID-19 in patients with a history of pancreatitis. They found that the prevalence of COVID-19 in patients with prior pancreatitis was 7.8%. Moreover, the main clinical symptoms among COVID-19 positive patients were cough, nasal congestion, dyspnea, and fever, respectively[25]. In a study performed by Kashefzadeh et al., hemoptysis, diarrhea, headache, and dry cough were determined as the main clinical features of COVID-19 infection[26]. Karimi et al. revealed that 69.8% and 7% of nasopharyngeal and tear samples were positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), respectively. Moreover, fever was the main clinical signs detected in 95.3% of patients[27]. Among respiratory viral pathogens, the number of samples found by molecular methods to be positive for influenza virus A was 4.1% (n = 6/145). This result was in agreement with studies conducted by MacIntyre et al. from Australia and Malekshahi et al. from Iran[6,7]. They showed that influenza A was detected in 2.7% and 4.9% of included patients, respectively. However, our findings were in contrast with those of Boivin et al. from Canada[28], Knobbe et al. from Senegal[1], Tang et al. from China[5], and Etemadi et al. from Iran[8]. These studies found that the prevalence of influenza A virus in patients with respiratory tract infections was 21.6%, 31.7%, 15.7%, and 9.1%, respectively. Influenza B virus was not detected in our study. Globally, several studies reported that the

frequency of influenza B virus in respiratory tract infections was 0%, 11.9%, 3.2%, and 0.5%, respectively[8,7,5,1]. In the present study, the prevalence of adenovirus infection among *military* personnel was 1.4%. Moreover, among investigated *bacterial pathogens*, the frequency of *S. pyogenes* and *M. pneumoniae* was 2% (n = 3/145) and 0.7% (n = 1/145), respectively. *H. influenzae* and *B. pertussis* were not detected in the present study. In general, several studies have surveyed the prevalence of respiratory agents in military environments, worldwide. Tavakoli et al. were surveyed the molecular epidemiology of respiratory viral pathogens in military trainees. The results of their study revealed that the main clinical symptoms among patients were sore throat, rhinorrhea, cough, fever, and nasal congestion, respectively. Moreover, they showed that human rhinovirus (7.2%), human respiratory syncytial virus A (7.2%), and influenza B virus (6%) bocavirus (3%), influenza A H1N1 (2.2%), influenza A H3N2 (1.5%), human respiratory syncytial virus B (1.5%), adenovirus (1.5%), and human coronavirus 229E (0.7%) were detected among military trainees[14]. Results of a study performed in the Republic of Korea Air Force boot camp in 2011 revealed that respiratory syncytial virus (RSV) was the main causative agent of acute febrile illness in *military* personnel[29]. O'Shea et al. reported that the prevalence of respiratory agents among 54 British military recruits receiving basic training with respiratory symptoms were as follows: adenovirus (35%), influenza viruses (19%), and RSV (14%)[30]. Heo et al. showed that among 622 soldiers with acute lower respiratory tract infections, adenovirus and influenza A viruses were found in 63.2% and 29.9%, respectively[31]. The WHO declared COVID-19 as a pandemic. This virus can infect individuals of all ages[32]. The peoples with underlying medical conditions such as high blood pressure, aneurism or cardiovascular disease, COPD, diabetes, and cancer are considered as susceptible groups to respiratory tract infections, especially COVID-19 [33]. Moreover, due to several conditions such as stressful working environment, crowded habitation, insomnia, and demanding physical training program, military personals are considered a vulnerable group to respiratory tract infections, worldwide[19,34]. In the USA, it is revealed that 25%-30% of the U.S. military population were hospitalized due to respiratory infections annually[31]. Therefore, to proper decisions and control the epidemic, the implementation of several infection control strategies such as rapid diagnosis and isolation of patients, and recognizing the epidemiological features is critical in military environments.

Conclusion

In general, our results showed that among surveyed respiratory tract pathogens, the frequency of COVID-19 is very high among military personals. The low prevalence of other viral and bacterial infections among military personals in our study in comparison with the other studies conducted in different parts of the world can be attributed to the widespread outbreak of the COVID-19 disease. Our finding revealed that clinical signs and symptoms such as headache, cough, lethargy, Somatalgia, fever, and sore throat are similar and common in bacterial and viral respiratory tract infection. Therefore, the accurate detection of respiratory agents is necessary to better treatment of patients.

Declarations

Ethics statements

All **methods and** procedures were performed in accordance with relevant guidelines and regulations and were approved by the Institutional Ethics Committee of the AJA University of Medical Sciences (IR. AJAUMS.REC.1399.202).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in public, commercial and not-for-profit sectors.

Authors' contributions

T.A.S and S.S.M: Conceptualization; Data curation; Formal analysis; and Writing – original draft.

R.H.F, A.A, and J.R: Conceptualization; Methodology; Project administration; and Writing – original draft.

T.A.S, S.S.M, M.D, and M.Ah: Data curation; Formal analysis; Writing – original draft; and Writing – review & editing.

T.A.S and M.A: Language editing.

S.S.M and M.Ah: Prepared Table 1.

Acknowledgement

We would like to thank “AJA University of Medical Sciences, Tehran, Iran” for their kind cooperation.

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Tables

Table 1. Demographic information of *military* personnel with prevalence and clinical signs of respiratory tract infections among these patients.

Characteristic	No.	Total with characteristic N (%)	With COVID-19 N (%)	With Influenza A N (%)	With Influenza B N (%)	With Pertussis N (%)	With Mycoplasma N (%)	With Adeno N (%)	With Pyogenesis	With Haemophilus
Age										
18-25 years	145	81 (55.9%)	63 (77.8%)	4 (4.9%)	0 (0%)	0 (0%)	1 (1.25%)	2 (2.5%)	3 (3.7%)	0 (0%)
26-30 years	145	24 (16.6%)	23 (95.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
31-35 years	145	11 (7.6%)	10 (90.1%)	1 (9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
>=36	145	29 (20%)	28 (96.5%)	1 (3.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
BMI										
<18.5	145	4 (2.8%)	3 (75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
18.5-24.9	145	71 (49%)	59 (83%)	3 (4.2%)	0 (0%)	0 (0%)	0 (0%)	2 (2.8%)	1 (1.4%)	0 (0%)
25-29.9	145	51 (35.2%)	45 (88.2%)	3 (5.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)
30-34.9	145	18 (12.4%)	17 (94.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.6%)	0 (0%)
35-39.9	145	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
>40	145	1 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Blood groups										
O⁺	145	63 (43.4%)	51 (81%)	4 (6.3%)	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)	3 (4.8%)	0 (0%)
O⁻	145	3 (2.1%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
A⁺	145	40 (27.6%)	35 (87.5%)	1 (2.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
A⁻	145	3 (2.1%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
B⁺	145	21 (14.5%)	20 (95.2%)	1 (4.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
B⁻	145	4 (2.8%)	3 (75%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)
AB⁺	145	11 (7.6%)	9 (82%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (9%)	0 (0%)	0 (0%)
AB⁻	145	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
The use of facemasks during COVID-19	145	145 (100%)	124 (85.5%)	6 (4.1%)	0 (0%)	0 (0%)	1 (0.7%)	2 (1.4%)	3 (2%)	0 (0%)
Types of facemasks										
Cloth facemask	145	17 (11.7%)	13 (76.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.9%)	1 (5.9%)	0 (0%)
Surgery facemask	145	123 (84.8%)	106 (86.2%)	6 (4.9%)	0 (0%)	0 (0%)	1 (0.8%)	1 (0.8%)	2 (1.6%)	0 (0%)
N95 or FFP2 facemasks	145	5 (3.4%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Duration of facemask use										
All time	145	104 (71.7%)	90 (86.5%)	3 (2.9%)	0 (0%)	0 (0%)	1 (0.96%)	2 (1.9%)	2 (1.9%)	0 (0%)
Some times	145	32 (22.1%)	25 (78.1%)	3 (9.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.1%)	0 (0%)

Rarely	145	9 (6.2%)	9 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
COPD	145	7 (4.8%)	7 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Asthma	145	10 (6.9%)	8 (80%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Sore throat</i>	145	77 (53.1%)	67 (87%)	2 (2.6%)	0 (0%)	0 (0%)	0 (0%)	2 (2.6%)	2 (2.6%)	0 (0%)
Stuffy nose	145	82 (56.6%)	71 (86.6%)	1 (1.2%)	0 (0%)	0 (0%)	0 (0%)	2 (2.4%)	1 (1.2%)	0 (0%)
IBS	145	10 (6.9%)	8 (80%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nausea	145	19 (13.1%)	17 (89.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Vomiting	145	33 (22.8%)	27 (82%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Diabetes</i>	145	3 (2.1%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Red eyes	145	42 (29%)	38 (90.5%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.4%)	0 (0%)
Headache	145	102 (70.3%)	88 (86.3%)	4 (3.9%)	0 (0%)	0 (0%)	1 (1%)	2 (2%)	1 (1%)	0 (0%)
Fatty liver disease	145	20 (13.8%)	18 (90%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Diarrhea	145	45 (31%)	39 (86.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.2%)	0 (0%)	0 (0%)
Gastrointestinal (GI) bleeding	145	2 (1.4%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Contact with COVID -19 positive person	145	115 (79.3%)	98 (85.2%)	4 (3.5%)	0 (0%)	0 (0%)	0 (0%)	1 (0.9%)	3 (2.6%)	0 (0%)
Dyspnea	145	51 (35.2%)	42 (82.3%)	3 (5.9%)	0 (0%)	0 (0%)	0 (0%)	2 (3.9%)	0 (0%)	0 (0%)
<i>Chest pain</i>	145	57 (39.3%)	48 (84.2%)	1 (1.7%)	0 (0%)	0 (0%)	1 (1.8%)	0 (0%)	2 (3.5%)	0 (0%)
Smoking	145	47 (32.4%)	41 (87.2%)	2 (4.2%)	0 (0%)	0 (0%)	0 (0%)	1 (2.1%)	1 (2.1%)	0 (0%)
Anosmia	145	62 (42.8%)	53 (85.5%)	2 (3.2%)	0 (0%)	0 (0%)	0 (0%)	2 (3.2%)	1 (1.6%)	0 (0%)
<i>Taste loss</i>	145	52 (35.9%)	44 (84.6%)	1 (1.9%)	0 (0%)	0 (0%)	0 (0%)	2 (3.8%)	1 (1.9%)	0 (0%)
<i>Alcohol consumption</i>	145	19 (13.1%)	18 (94.7%)	1 (5.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Loss of consciousness	145	5 (3.4%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Joint pain	145	67 (46.2%)	60 (89.5%)	1 (1.5%)	0 (0%)	0 (0%)	0 (0%)	1 (1.5%)	1 (1.5%)	0 (0%)
Fever	145	78 (53.8%)	70 (89.7%)	2 (2.6%)	0 (0%)	0 (0%)	0 (0%)	1 (1.3%)	2 (2.6%)	0 (0%)
Chills	145	63 (43.4%)	57 (90.5%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)	0 (0%)	0 (0%)
Muscle aches	145	110 (75.9%)	95 (86.4%)	3 (2.7%)	0 (0%)	0 (0%)	1 (0.9%)	2 (1.8%)	2 (1.8%)	0 (0%)
Lethargy	145	100 (69%)	89 (89%)	3 (3%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	2 (2%)	0 (0%)
Cough	145	96 (66.2%)	82 (85.4%)	4 (4.2%)	0 (0%)	0 (0%)	0 (0%)	2 (2.1%)	2 (2.1%)	0 (0%)
High blood	145	17 (11.7%)	17	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)