Original Article

Frequency of Influenza-A-H1N1 in Patients with Community-Acquired Pneumonia Admitted to Loghman Hakim Hospital

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Abstract

Background: Here we assessed the incidence of Influenza-A-H1N1-related pneumonia in community-acquired pneumonia (CAP) at Loghman Hakim Hospital, Tehran, Iran.

Materials and Methods: In this prospective study from November 22, 2016, to June 21, 2017, patients with CAP and suspected to seasonal influenza were included. Rapid Antigen test and quantitative real-time PCR assay were performed on samples. P-value < 0.05 was considered significant. In addition, radiologic patterns of them were evaluated.

Results: a total of 29 admitted CAP patients were suspected of seasonal influenza. Two cases out of them were positive for influenza by real-time PCR, similar to result of influenza rapid test. The most common finding in their chest X ray was consolidation in one lobe. None of them vaccinated against influenza. Only nine patients received empiric Oseltamivir treatment. The amount of irrational antibiotic administration was significant.

Conclusion: Despite low statistical numbers, admitted influenza CAP patients did not have unusual symptoms and radiologic patterns. Other results in this study showed need for antibiotic stewardship program and better training about necessity of vaccination.

Keywords: Influenza A Virus, H1N1 Subtype; Quantitative Real-Time Polymerase Chain Reaction; Pneumonia

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Introduction

Community-acquired pneumonia (CAP) is an acute infection of the pulmonary parenchyma in a patient who has obtained the disease from the community. Community-acquired pneumonia is distinguished by; cough, fever, chills, fatigue, dyspnea, rigors and pleuritic chest pain (with or without new infiltrate on chest radiography), which attained outside of a hospital or long-term care facility[1-3].

Most cases of influenza are diagnosed based on clinical grounds. In some situations, the diagnosis of

influenza will be confirmed by some laboratory tests such as rapid test, PCR or cell culture[4]. Influenza is an acute respiratory disease that affects the upper and/or lower respiratory tracts and is usually associated with systemic signs and symptoms including fever, weakness, myalgia and headache[5]. According to Mandell's principles and practice of infectious disease 2015, pulmonary complications of influenza consist of primary viral pneumonia, secondary bacterial pneumonia, mixed viral and bacterial pneumonia and localized viral pneumonia[4].

Diagnosis of influenza is based on detecting the virus in throat and nasopharyngeal swabs, washes or sputum. The most sensitive and specific test is reverse transcriptase polymerase chain reaction (RT-PCR) that can differentiates influenza subtypes. The virus can be isolated by culture but this method is labored and is no longer used for diagnosis. The most common diagnostic method is rapid tests that detects viral antigens with relative specificity and variable sensitivity. Using nasal swab is easy and painless, and it is feasible everywhere with no additional devices. There are no significant differences in sensitivity for nasal swab and nasopharyngeal aspirates when a sensitive method like PCR is being used for virus detection[6].

The overall rate of CAP in adults was estimated 1.07-1.2/1000 in Europe[7], 24.8/10,000 in America[8] and 780/1000 in Asia[9]. Remarkably, the overall incidence of pneumonia hospitalization with influenza was 1.5/10,000 in the United States. Influenza and bacterial pneumonia combination is the first cause of death from infectious disease in the United States[10]. Last year according to expert opinion, some CAP patients with confirmed influenza had sudden dyspnea and unusual radiological pattern in favour of pulmonary embolism.

We have not found any parallel investigation on Influenza in CAP patients in Iran, and no studies have reported trends that are more recent. Therefore, we designed this research to emphasize on the role of influenza in CAP patients, to introduce the significant use of Real-time PCR assay in such individuals, and to improve the patient's care in Loghman Hakim Hospital, Tehran, Iran. However, the goal of our study was to determine frequency of influenza-A-H1N1 in patients with CAP and in all suspected 29 patients.

Methods

Study Design and Inclusion Criteria: In this cross-sectional study, 61 patients with flu like symptoms (started within three days) were evaluated. Clinical presentation and chest x-ray of 29 patients were compatible with CAP. Patients were over 18 years old.

Collection of Samples: Under the supervision of an infectious specialist, respiratory specimens collected within 12 hours of enrolment. A nasal sample using plastic-shafted Dacron swabs took from selected patients. Transferring to a VTM microtube, the specimens then vortexed to release the particles adhering to the Dacron swab and obtain a final volume of 0.5 to 1 ml. Furthermore, blood, urine and endotracheal aspirates collected before starting antibiotics and antivirals.

Endotracheal aspirates and sputums were sent for semi-quantitative cultures, which were considered positive with moderate to dense growths of bacteria and few epithelial cells seen on Gram stain examination (≤10 per high-power field). Blood cultures were incubated aerobically and anaerobically in three sets. Serum and urine samples were tested in substantial laboratory panels.

Real-Time PCR Amplification Technique for Virus Detection: At the first step, specimens went under RNA extraction using DynaBio™ viral nucleic acid (DNA\RNA) extraction kit. According to the manufacturer's structure, we used cDNA synthesis kit YT4502 M-MLV reverse transcriptase to convert RNA to cDNA. In the last step, we used primer design-swine H1N1 influenza human pandemic strain-genesig advanced kit (England) for performing quantitative Real-time PCR assay. The reaction consisted of 10 µl of Master Mix 1x, one µl of M1-Primer (20 pmol), one µl of endogenous control primer-probe mix, four µl of the template and four µl of RNAse/DNAse free water. The concluding volume was 20 µl, and the cycles were as follows: First-hold at 95ºc for 5 minutes, 40 cycles of 95ºc for 10 seconds and 60ºc for 60 seconds.

Statistical Analysis: Data analysed using the statistical package for social sciences (SPSS) version 22 and the p-value under 0.05 considered statistically significant. Continuous data were analysed by

student's t-test if the data were normally distributed (using Kolmogorov-Smirnov); otherwise, the Mann–Whitney U-test applied. Categorical data compared using Pearson's chi-square test. Baseline demographic variables and screening measure variables compared across groups using chi-square for categorical variables and one-factor ANOVA for continuous variables.

This study was conducted in line with the Helsinki ethical principle and was performed after obtaining the approval of the Ethics Committee for Research in the Faculty of Medicine of Shahid Beheshti University of Medical Sciences (code: IR.SBMU.REC.1395.79). All information gathered from patients is confidential to the researcher. Other personal information of individuals is also kept under the confidentiality law practice process.

Results

From November 22, 2016, to June 21, 2017, 61-suspected cases of seasonal Influenza admitted to the emergency room of Loghman Hakim Hospital in Tehran. Of the 61 patients, 29 of them had the inclusion criteria of this study. None of them had vaccination profile against influenza.

Summarized demographics: The admission peak was in February (48.3%) (Figure 1). Majority of our patients (51.7%) were male. The mean age of patients was 60.07 ± 20.94 years, with a minimum of 25 years and a maximum of 92 years. The highest number of participants related to the age group of 71-80 years and secondarily to the age group of 31-40 years. (Table 1).

Laboratory panels, Clinical manifestations: In this study, five patients required the use of mechanical ventilation and the average number of days needed to use it was 4.57 ± 17.80 days. These five patients became infected due to bacterial pneumonia and had symptoms consistent with CAP. An endotracheal aspirate was requested for them. The bacteriological panel result was two *Acinetobacter spp.* and one *Staphylococcus aureus* cases ($p<0.001$). None of these five patients were positive for influenza. Radiological findings of patients have been demonstrated in Table 2 ($p=.041$). Consolidation was the uppermost sign (44.8%).

Along with our research, head nurse of infection control committee, performed rapid test in 61 suspected cases of influenza admitted to the emergency room (not ward) and seven samples (13.72%) were positive for influenza. This result was positive in two cases out of 29 confirmed CAP patients. In other words, two out of seven positive influenza subjects required hospitalization and

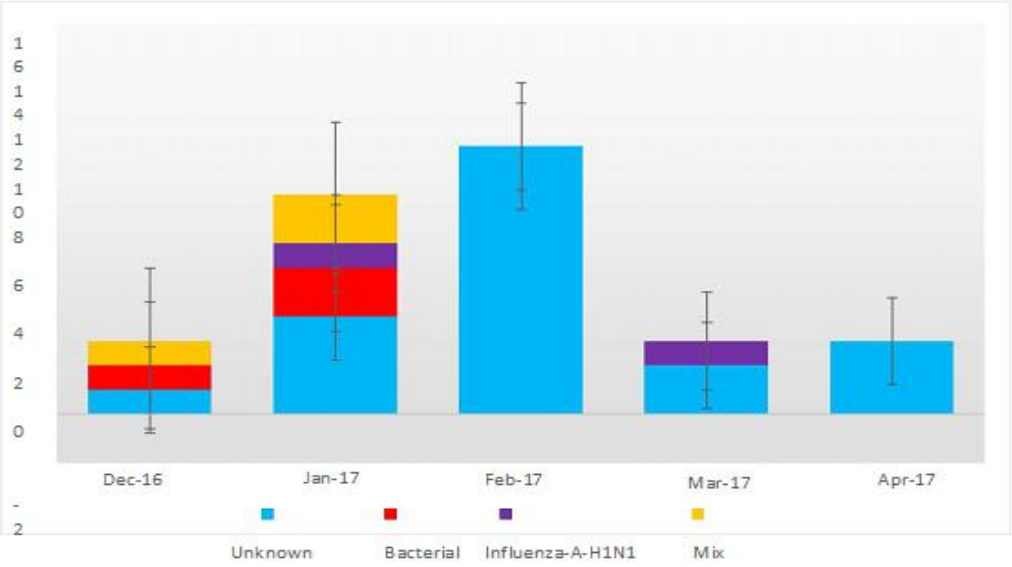

Figure 1. Admission time of this study's participants.

eventually remaining five patients were managed as outpatient.

Based on Quantitative Real-Time PCR testing in patients with CAP, five patients had H1N1-A influenza viral loads. However, according to the kit protocol, samples of more than 30 viral copies/ml are considered positive for the influenza-A-H1N1 virus. For the two samples (6.89%) mentioned in the preceding paragraph, more than 30 copies/ml of the Influenza H1N1 virus was obtained in their respiratory tract (Figure 2).

Management of patients: Out of 61 cases suspected of influenza, 32 (43.13%) patients with flu-like syndrome discharged from the hospital after treatment. In the rest 29 confirmed CAP patients, the most common findings were fever and chills, coughing and confusion (15 cases, 29.41%) (Table 3). No case of admission to the intensive care unit was found.

From the point of empiric treatment, the highest antibiotic combination was Ceftriaxone and Azithromycin (Table 4). A total of 31.3% of patients received empiric oseltamivir therapy. Duration of antibiotic therapy was longer than a CAP antibiotic regimen. According to the CURB65 score results, 89.7 % had a score of two, 6.9% had a score of three, and 3.4% had a score of four. In the two influenza-A-

Table 1: Laboratory panels, vital signs, mortality scores and demographics of CAP patients.

Parameters	Mean ± SD / %			*P-Value*
	Bacterial CAP N=3	**Influenza H1N1 CAP N=2**	**Unknown etiology CAP N=24**	
Laboratory analysis				
White Blood Count(x1000)	20.06 ± 4.45	6.65 ± 3.46	10.2± 4.38	***.002******
C-reactive protein (CRP) (mg/dl)	19.76± 34.23	55.6 ± 73.96	66.05± 51.46	*.353*
Platelet (x1000/mm^3)	250.66± 174.24	312 ± 117.37	210.13 ± 85.04	*.326*
Aspartate aminotransferase (AST)	71.66± 21.12	58.5 ± 50.2	59.50 ± 84.63	*.969*
Ratio of Lymphocytes	17.20± 11.83	32.1 ± 19.56	16.99 ± 9.45	*.156*
Ratio of Neutrophils	79.26± 14.83	61.15 ± 27.22	74.37± 11.33	*.291*
Ratio of Eosinophil/Basophil/Monocytes	3.63 ± 3.69	6.75 ± 7.56	8.63± 5.39	*.318*
Lactate dehydrogenase (LDH)	526 ± 310.98	584 ± 174.07	684 ± 672.5	*.908*
Blood Urea Nitrogen (BUN)	36.91± 29.14	11.91 ± 5.61	17.98 ± 7.5	***.021******
Creatinine	1.6± 0.6	0.9 ± 0.28	1.21± 0.38	*.161*
Total Protein	5.9 ± 1.97	6.26±0.97	6.32± 9.39	*.698*
Albumin	3.56± 0.95	3.73±0.25	3.76± 0.18	*.658*
H1N1 Viral Load (Copy/µl)	0	563 ± 49.49	1.04± 3.95	***.000******
Vital Signs and Mortality scores				
Systolic Blood Pressure	100 ± 10	115 ± 7.07	131.08 ± 21.31	***.046******
Diastolic Blood Pressure	63.33± 15.27	70	79.56± 12.60	*.098*
Respiratory Rate	23.33± 10.21	17 ± 4.24	17.78 ± 2.92	*.096*
CURB65 score*	3± 1	2	2.04 ± 0.2	***.000******
Pneumonia Mortality Rates	0.022			
Demographics				
Age	45.67 ± 25.4	34.50 ± 7.77	64 ± 19.37	*.067*
Males	100 %	100 %	41.66 %	*.060*
Days of Mechanical Ventilation	8 ± 2.64	0	4.52 ± 19.61	*.893*
Prevalence of Influenza	0.06 per 7 months			
Incidence of Influenza	67/1000/year			
Risk of influenza	1.125, CI** 95 %			

*Abbreviation: CURB-65, confusion of new onset, blood urea nitrogen >7 mmol/l, respiratory rate ≥30 breaths per minute, blood pressure <90 mmHg systolic or ≤60 mmHg diastolic, age≥65 years.

**CI= Confidence Interval

***p values are significantly different.

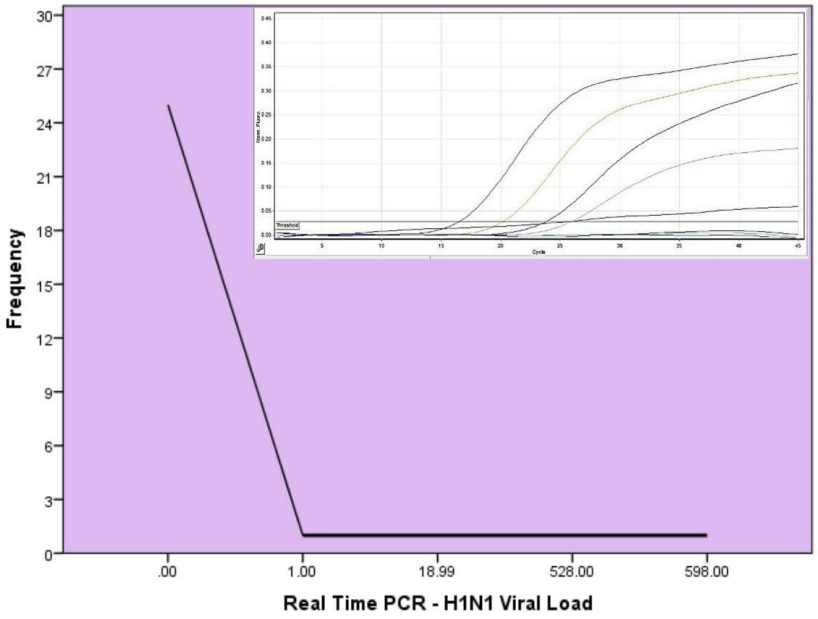

Figure 2. Quantitative Real-time PCR results based on M and N genes.

H1N1 positive patients, the CURB65 score was reported as two and three, respectively. Eventually, 89.7% gained complete recovery, 6.9% deceased, and 3.4% left the hospital with personal responsibility.

Discussion

This study was conducted in Loghman Hakim Hospital, Tehran, Iran for a seven-month period. According to the statistics of previous years in this educational and therapeutic center, the number of clients with influenza-like symptoms in emergency triage were reported as 35 people in 2014, 40 in 2015 and 45 in 2016 years. Finally, in our study 61 patients presented influenza-like illness symptoms started within three days.

Table 2: Radiologic patterns of CAP patients.

Radiography Results	Frequency	Percent
Consolidation	*9*	*31*
Consolidation	*4*	*13.8*
Consolidation+Plural effusion+Cavity Formation	*2*	*6.9*
Peribronchial Nodules	*4*	*13.8*
Peribronchial Nodules+Ground Glass Opacity	*4*	*13.8*
Peribronchial Nodules+Ground Glass Opacity+Random Nodules	*1*	*3.4*
Peribronchial Nodules+Ground Glass Opacity+Infiltration	*1*	*3.4*
Peribronchial Nodules+Ground Glass Opacity+Pleural Effusion	*1*	*3.4*
Random Nodules	*2*	*6.9*
Peribronchial Nodules	*1*	*3.4*
Ground Glass Opacity	*1*	*3.4*
Pleural Effusion+Consolidation	*1*	*3.4*
Total	*29*	*100.0*

Here we studied on 29 suspected cases of influenza presented with community-acquired pneumonia. The majority of our patients were males (51.7%), and this was consistent with a Chinese study (71%)[11]. Nevertheless, in a study published in Argentina in an outbreak season, H1N1 influenza was prevalent among women[12]. According to world health organisation (WHO) reports in the developed countries, the incidence of seasonal flu in all age groups is more prevalent in men (more than 60% in the United States)[13]. The women make robust antibodies in higher titter against influenza viruses, so they are more resistant to influenza than men[14].

In this study, the mean age of patients was 60.07 ± 20.94 years. The highest number of referrals was primarily related to the age group 71-80 years and secondarily to the age group of 31-40 years. Two cases that were positive for the influenza virus had 29 and 40 years of age. One of the characteristics of influenza-A-H1N1 flu is infecting children and adults[15] and only 5% of influenza-A-H1N1 cases around the world had over 65 years[16-18]. This age distribution indicates relative immunity in the adult population. Studies have shown that more than 33% of

Table 3: Presented manifestations of CAP patients at the time of admission in the Loghman Hakim Hospital.

On admission symptoms	Frequency	Percent
Flu-like syndrome	32	52.45
A cough, Phlegm, Fever, and Chills	7	13.72
A cough, Phlegm, Fever and Chills, Weakness and Narcosis, Nausea and Vomiting	1	1.96
A cough, Phlegm, Fever, and Chills, Chest Pain	1	1.96
A cough, Phlegm, Fever, and Chills, Headache, Dizziness	1	1.96
A cough, Phlegm, Fever and Chills, Loss of Consciousness, Sepsis	1	1.96
A cough, Phlegm, Fever, and Chills, Abdominal pain	1	1.96
A cough, Phlegm, Fever, and Chills, Imbalance	1	1.96
A cough, Phlegm, Fever and Chills, Shortness of Breath	1	1.96
A cough, Phlegm, Fever and Chills, Shortness of Breath, Myalgia	1	1.96
Weakness and Narcosis, Loss of Consciousness, Pinpoint pupil, Mouth Bloodshed, Methadone Toxicity	1	1.96
Nausea and Vomiting, Headache, Co toxicity	1	1.96
Nausea and Vomiting, Abdominal pain, Pancreatitis	1	1.96
Nausea and Vomiting, Hypoxic, Cyanosis	1	1.96
Chest Pain	1	1.96
A cough, Phlegm, Fever and Chills, Tachypnea, ARDS, Decreased level of Hemoglobin, Vasculitis	1	1.96
Loss of Consciousness	1	1.96
Seizure and tongue bite with incontinency	1	1.96
Cold symptoms	1	1.96
Spondylodiscitis, Device infection, Edema, Urine incontinency	1	1.96
Craniotomy Postop Infection	1	1.96
ARDS, Feet Erythematous, Sarcoidosis, Pancytopenia	1	1.96
Shortness of Breath, Weakness and Narcosis, Fever and Chills	1	1.96
Shortness of Breath, Fever and Chills, Chest Pain	1	1.96
Total	61	100.0

adults over the age of 60 have cross-reactive antibodies against influenza A (H1N1) 2009 virus[19].

In the study, one of positive influenza-A-H1N1 patients were admitted to the ward and the other one in ICU. Both patients presented with cough, phlegm, fever and chills. Studies have shown that 2-5% of confirmed cases of influenza-A-H1N1 in the United States, Canada and 6% of confirmed cases in Mexico require hospitalization[17], which are consistent with the statistics presented in this study.

Diagnosis of viruses in less than 10% of CAP cases in our study is consistent with the results of many studies[20]. Here, the results of two different assays (Rapid Antigen Test and Quantitative Real-Time PCR test) were similar. As before mentioned, 61 suspected cases of influenza ware temporarily admitted in emergency service. Infection control committee of the hospital checked all of them with rapid test for influenza which seven cases were positive. Five out of them were discharged and two admitted remaining cases were the same two positive influenza cases with PCR in our study. In a meta-analysis study on 159 published articles, the sensitivity and specificity of rapid influenza tests were 98% and 34% respectively[21]. In recent years, the sensitivity and specificity of these tests were 68-79% and 99-100%, respectively[22-24]. In diagnosis of influenza, sensitivities and specificities of RT-PCR and other molecular assays are very high, compared to other FDA-cleared assays. However, false negative results can occur due to improper or poor clinical specimen collection or from poor handling of a specimen after collection and before testing. A negative result can also occur by testing a specimen that was collected when the patient was no longer shedding detectable influenza virus that may also occurred in our study. False positive results, although rare, can occur (due to lab contamination or other factors)[25].

Table 4: Antibiotic regimen of admitted CAP patients.

Antibiotics	Percent
Missing Data	3.4
None	6.9
Clindamycin+ Vancomycin+ Pipractam+ Gentamicin + Ciprofloxacin	3.4
Clindamycine+Vancomycin + Meropenem	3.4
Ceftazidime+ Vancomycin	3.4
Ceftazidime+ Vancomycin+ Pipractam	3.4
Vancomycin+Ciprofloxacin+Ceftriaxone+Meropenem+Levofloxacin+Cotrimoxazole+Colomycin+Amphotericin B	3.4
Vancomycin+ Ciprofloxacin+ Ceftriaxone+ Meropenem+ Cotrimoxazole	3.4
Vancomycin+ Ceftriaxone	6.9
Vancomycin+ Ceftriaxone+ Meropenem+ Levofloxacin+ Cotrimoxazole	3.4
Vancomycin+ Ceftriaxone+ Meropenem+ Levofloxacin	3.4
Vancomycin+ Meropenem+ Levofloxacin	3.4
Ceftriaxone+ Azithromycin	13.8
Ceftriaxone+ Azithromycin+ Levofloxacin	3.4
Ceftriaxone+ Meropenem+ Levofloxacin	3.4
Ceftriaxone+ Levofloxacin	3.4
Ceftriaxone+ Metronidazole+ Imipenem	3.4
Azithromycin	6.9
Azithromycin+ Levofloxacin	3.4
Levofloxacin	13.8
Total	***100.0***

Table 5: Comparative features of pulmonary complications of influenza.

	Primary viral pneumonia	Secondary bacterial pneumonia	Mixed viral and bacterial pneumonia	Localized viral pneumonia
setting	Cardiovascular disease; pregnancy; young adult(pH1N1)	Adults and children	Any associated with A or B	?Normal
Clinical history	Relentless progression from classic 3 day influenza	Improvement, then worsening after 3 day influenza	Features of both primary and secondary pneumonia	Continuation of classic 3 day syndrome
Physical examination	Bilateral findings, no consolidation	Consolidation	consolidation	Area of rales
Sputum bacteriology	Normal flora	Pneumococcus, staphylococcus, Haemophilus influenzae	Pneumococcus, staphylococcus, Haemophilus influenzae	Normal flora
Chest radiography	Bilateral findings	Consolidation	consolidation	Segmental infiltrate
Detection of influenza virus	Yes	Not always	Yes	Yes
Response to antibiotics	No	Yes	Often	No
mortality	High	Variable	variable	Very low

In the following study, nine patients received empiric oseltamivir treatment. In accordance with the guidelines of the CDC, prompt empiric treatment with oseltamivir (oral), zanamivir (inhaled) or peramivir (IV) should be done in persons with suspected or confirmed influenza. They need to admission. Progressive, severe, complicated illness, regardless of previous health status, and/or patients at risk for severe diseasee[26].

In our study, the duration of antibiotic therapy was longer than a CAP antibiotic regimen and the most used antibiotic regimen in patients was Ceftriaxone plus Azithromycin. Between the two influenza-A-H1N1 patients, the first one received the combination of levofloxacin and Tamiflu and the second one treated with the combination of Clindamycin, Vancomycin, Gentamicin and Ciprofloxacin. According to Harrison's principle of medicine, empirical treatment for CAP admitted in ward is different and includes a respiratory fluoroquinolone (e.g., moxifloxacin or levofloxacin) or a β-lactam (e.g., ceftriaxone, ampicillin, cefotaxime, ertapenem) plus a macrolide (e.g., oral clarithromycin or azithromycin or IV azithromycin). Empirical treatment for admitteds in ICU includes an antipneumococcal beta-lactam (cefotaxime, ceftriaxone, or ampicillin-sulbactam) plus azithromycin or an antipneumococcal beta-lactam plus a respiratory fluoroquinolone [27].

In this study, diversity of antibiotic regimens, which was not based on recommended regimens in guidelines, showed the importance and requirement of antibiotic stewardship program in this hospital.

Here, the radiological finding in confirmed cases of H_1N_1 was consolidation in a lobe.

According to Mandell's principles and practice of infectious disease 2015, pulmonary complications of influenza consist of primary viral pneumonia, secondary bacterial pneumonia, mixed viral and bacterial pneumonia and localized viral pneumonia (table 5). It seems that in our study most cases have radiologic patterns other than primary viral pneumonia[4].

In the recent years, Iranian Ministry of health distributed 300,000 to 400,000 influenza vaccines. Unfortunately, none of the patients in our study had been vaccinated before admission.

Considering our research type as a cross-sectional and prospective study, we confronted with two limitations. Some patients did not have cooperation due to bad feeling of nasal swab sampling and we were only able to investigate the influenza A H_1N_1 virus due to limited funding for this project.

Conclusion

There was not any unusual symptoms and

unpredictable radiologic pattern in patients with influenza. Irrational prescription of antibiotics is a strong reason for the need to antibiotic stewardship program. Influenza cases requiring hospitalization, need to be empirically treated with anti-influenza drugs, but in our study low number of cases had been treated then more training about this subject should be given to residents in emergency ward. In addition, according to low number of vaccinated patients, more training and extensive advertise should be done.

Acknowledgment

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