Original Article

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TANAFFOS

Community-Acquired Pneumonia in Adults: A Mono-Center Retrospective Study

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Background: Adult community-acquired pneumonia is the most common cause of hospitalization and a leading cause of death. Identification of microorganisms causing community-acquired pneumonia.

Materials and Methods: A cross-sectional design was used. Information on adults hospitalized due to pneumonia in the cold seasons of 2018 and 2019 was collected. Results of microbiologic tests, other than serology and urinary antigen, were reviewed

Results: 205 patients had eligible criteria. The mean age was 52.4 and 62% were male. Microorganisms were identified in 117 (57%) patients. The most common etiologic agents were Influenza H1N1 2009 (n=39, 33.4%), Tuberculosis (n=21, 17.9%), Non-Albicans Candida Yeast (n=9, 7.8%), Methicillin Resistant Staphylococcus Epidermis (n=7, 6%) and Klebsiella Pneumonia (n=5, 4.3%). Streptococcus Pneumoniae had the 6th rank (n=3, 2.6%). A higher body mass index was associated with superimposed infections. 10 of 18 (56%) patients who died in hospital, got definite microbiologic diagnoses. The maximum mortality was due to staphylococci, with methicillin-resistant strains of *Staphylococcus aureus* (n=2, 66%) and *Staphylococcus epidermis* (n=2, 29%).

Conclusion: H1N1 2009 was the first cause. Tuberculosis with rising incidence could cause acute pneumonia. Pneumococcal incidence had declined. Community-acquired staphylococcal pneumonia is the most dangerous; hence, specific protocol-based treatments should be applied promptly. Community-acquired *Staphylococcus epidermis* and Tuberculosis must be included in differential diagnosis of the disease. Antibiotics need to be individualized in managing the obese patients. A special focus on the epidemiology of virulence factors of *Klebsiella pneumoniae* is needed as it is common, severe, and lethal.

Keywords: Community-Acquired Pneumonia; Hospitalized; Microorganism

INTRODUCTION

Pneumonia is a clinical syndrome resulting from the lung parenchyma infection which causes lower respiratory symptoms and characteristic changes on chest imaging. As noted in the International Classification of Diseases 11th revision, pneumonia is described as a disease of the lungs, frequently but not always caused by an infection with bacteria, virus, fungus, or parasite. This disease presents with fever, chills, productive cough, chest pain, and shortness of breath. Its Confirmation is by chest x-ray (1). The disease is called Community-Acquired Pneumonia (CAP) when the infection is neither from the hospital nor from instruments (e.g. ventilator, tracheostomy, chest tube, etc.). Hospital and instrumental-associated pneumonia respectively occurs in a patient who either did not already have or was incubating pneumonia at the time of admission or if it appears ≥48 hours after admitting a patient to the hospital or application of the instrument. In this study, it is considered developing within 14 days, soon after discharge from the hospital or discontinuation of the instrument. Every other type of pneumonia is referred to as CAP (1).

Adult CAP is universally the most common cause of hospitalization and one of the leading causes of morbidity and mortality (2). However, the basic studies on the epidemiology of the organisms leading to CAP in the developing world are insufficient. Few studies on the epidemiologic and demographic features of CAP have been conducted in Iran. In February 2015, investigators surveyed Mashhad, the 2nd largest city in Iran. Data from 120 patients with CAP were analyzed and microbiologic causes were reported (3). In some studies, the most commonly infected organ in immunocompromised patients was the lower respiratory system (4-6).

Although it is well known that the Coronavirus Disease 2019 (COVID-19) pandemic and health precautions such as lockdowns, wearing masks, and hand washing have changed the epidemiology of CAP microorganisms, it is expected that when the restrictions discontinue, H1N1 2009 and other organisms reemerge while COVID-19 continue infecting people. Centers for Disease Control and Prevention has issued a health advisory to notify clinicians and caregivers about an increase in cases of interseasonal human Respiratory Syncytial Virus (hRSV) across parts of the Southern United States (7).

For the practice of medicine to become more rational, it is essential to identify the exact cause of CAP in patients requiring hospitalization to adjust empirical antibiotic therapies and thereby fight against the emergence of resistance. Annually, a large number of patients with CAP from Isfahan and adjacent provinces are hospitalized at Alzahra tertiary center which results in high costs for the country, health care system, patients, and their families. It can also lead to high rates of morbidity and mortality in Isfahan City and Iran. Our goal is to identify and investigate the underlying causes of acute CAP and their prevalence. This pilot study aims to provide guidance for future research and health policies, as well as serve as a foundation for studies on the emergence of resistance. In this paper, the term acute means less than 2 weeks of the first presenting sign(s)/symptom(s).

The primary objectives are 1) spotting the CAP-causing organisms in patients hospitalized at Alzahra Medical Center and 2) Description of the medical, social, and drug history of the CAP patients in Isfahan Province and nearby. The secondary objective is figuring out whether there is an association between the CAP causative organisms with age; triage status; disease severity; length of hospitalization (LoH); comorbidities such as neoplasm, cardiovascular disease, chronic kidney disease (CKD), liver disorders, neurologic disorders, chronic respiratory disease (CRD), immunosuppression, and diabetes mellitus (DM); anti-acid; tobacco smoking; and illegal drug abuse. To achieve these goals, the hypothesis is that sputum multiplex Polymerase Chain Reactions (PCR), bacterial cultures (BC), sputum cultures (SC), Bacillus de Koch three spot smear microscopy (BK) tests, real-time PCR of Tuberculosis (TB RT-PCR) or pleural fluid cultures (PFC) can detect the microorganisms responsible for CAP.

MATERIALS AND METHODS

We used a cross-sectional design in which the medical documents of every adult, aged ≥18 years, who was hospitalized at Alzahra tertiary care center either by direct admission or referral for acute CAP from September 23, 2018, to March 19, 2019, and from September 23, 2019, to March 19, 2020 (12 months; 2 falls and 2 winters). Patients were selected from the center's medical documents archive by census in 2021 summer in Isfahan, Iran. The International Classification of Diseases 11th revision codes CA40.0, CA40.1, CA40.2, CA43.1, CA40.Y&XB4Q, CA40.Y&XB17, CA40.Y&XB4Q, CA40.Y&XB22, CA40.Z&XB4Q, CA40.Z&XB17 and CA40.Z&XB22 were used to include cases (8).

In this paper, the clinical diagnosis of acute pneumonia was confirmed when at least 2 items listed below were met for less than 2 weeks: cough, dyspnea, sputum, chest pain especially pleuritic, fever, weakness, altered mental status especially in those older than 65 years of age, increased respiratory effort and rate (≥25) and decreased blood O_2 saturation (to <93%, measured by pulse oximetry) in a recently well patient with or without abnormal pulmonary sounds and characteristic changes in Chest X-Ray and/or High-Resolution Computed Tomography scan (HRCT) of lungs. The patients who did not meet these criteria or those with a diagnosis of hospital-acquired instrument-associated pneumonia and/or confirmed COVID-19 were excluded from the study. Thus, the International Classification of Diseases 11th revision codes RA01.0, RA01.1, CA40.Y&XB25, CA40.Z&XB25, CA40.Z&XY69, and CA40.Y&XY69 were used to exclude files from the cases. (8) In addition, some others were excluded from the study because they were discharged from the hospital in ≤3 days (they had no consent to continue the treatment in the hospital). Accordingly, we did not have enough information to include them in the study. Lack of consent to be included in the study was an exclusion criterion. Any case of acute community-acquired upper respiratory tract infection, bronchitis, CRD, and bronchiolitis was included only if it was associated with pneumonia.

Based on clinical and radiographic findings, BK tests were used for patients suspected of having TB. In addition, a specific TB RT PCR test was used for those highly suspicious of having TB despite 3 consecutive negative BKs. We conducted an investigation to determine if specific microbiologic tests, such as Sputum Multiplex PCR, TB RT PCR, SC, BK, BC, or PFC with or without antibiogram, were performed for each case included in the study. If these tests were conducted, we also looked into the results. The Sputum Multiplex PCR kit used in the center's Laboratory (Lab) detects these organisms based on genome equivalents/reaction for each pathogen: 100 copies from each of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pneumoniae* (SPn), *Streptococcus agalactiae*, *Listeria monocytogenes*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Neisseria meningitides*, *Stenotrophomonas maltophilia*, *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (KPn), *Serratia marcescens*, *Enterobacter cloacae*, *Proteus mirabilis*, Candida spp., and *Candida albicans* genome. The kit was also able to detect Adenovirus, Bocavirus, Coronavirus type 229, Enterovirus, Influenza A types H3N2 and H1N1 2009, B and C, Metapneumovirus, Parainfluenza (PIV) 1, 2, 3 and 4, human Rhinovirus (hRV) and hRSV A.

Sputum microscopy is the standard diagnostic method of pulmonary TB in the center. A smear requires 1000 to 10,000 bacilli/mL to be read as positive. Both hot and cold carbol fuchsin methods (Ziehl-Neelsen and Kinyoun) were used. The use of fluorochrome stains such as auramine-rhodamine helped us in more rapid screening of sputum smears. Three specimens, preferably early morning samples of patients suspected of TB were examined to establish the diagnosis.

The combination of the routine BC with aerobic and lytic anaerobic media allows to reduce the time of detection and better recovery of both aerobic and anaerobic organisms (9, 10). The BD BACTEC bottle portfolio provided a full line of BC media developed specifically for the detection of aerobes, anaerobes, yeast, and fungi to help improve the time to detect an organism (11). However, the media in the Lab for BC, SC, and PFC, couldn't detect atypical bacteria such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*. We did not use specific serologic and urinary antigen tests to detect any organism.

We also determined the pneumonia outcome research trial severity index (PSI) (12). PSI Class is II, III, IV, and V when the score is ≤70, 71 to 90, 91 to 130, and >130, respectively. Medical documents and lab data were checked for gender, age, ward or Intensive Care Unit (ICU) admission, LoH, comorbidities (e.g., neoplasms, cirrhosis, chronic hepatitis, cardiovascular disease, CKD, DM, neurologic disease, immunodeficiency, and CRD), drugs the patients were taking before hospitalization (e.g., antibiotics, anti-acids, immunosuppressing medications, systemic and/or inhaled corticosteroids, alcohol consumption, tobacco smoking, and illegal drug abuse), body mass index (BMI), creatinine (Cr), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cell count (WBC), lactate dehydrogenase (LDH), albumin (Alb), and all other parameters contributed to PSI measurement (e.g., nursing home resident, pulse, systolic blood pressure, O_2 saturation, respiratory rate and temperature as vital signs, adjusted arterial pH by measurement of venous pH, blood urea nitrogen, serum sodium, blood glucose, hematocrit, and presence of pleural effusion). Finally, we specified whether the patients expired in the hospital. All data used in this study had been collected on the day of admission by hospital staff (e.g., registry personnel, attending physicians, fellows, residents, nurses, and medical interns), who at the time, did not know the design and purposes of the study. Patient's permission, or their first-degree relative in case of the patient's unconsciousness, was taken for educational and research purposes at the time of admission in the registry paper sheet. Additionally, the study was approved by the Bioethical Research Ethics Committee in the School of Medicine, Isfahan University of Medical Sciences. The data were gathered from the registry, medical intern's history, resident's history, nurse's report, nurse's primary visit, physician's orders, and medical document summary sheets with all other valuable pages. Lab data were collected from the center's computer database. All cases were categorized by PSI, pre-admission medication, comorbidities, BMI, ward or ICU admission, and the identification of any organisms and their types.

The COVID-19 surge in March 2020 in Iran was a potential source of bias. To address this issue, we ended the study in 2020, on March 19th, and excluded all COVID-19 cases suspected earlier by PCR and/or lung HRCT scan. Furthermore, in addition to analyzing all data together, we separately analyzed all hospitalized cases from November 22, 2019, to March 19, 2020, and compared the results with the same time in the previous year.

Statistical Analysis

Descriptive analysis was performed and then reported as frequencies and percentages for qualitative variables and as mean, 25th and 75th percentiles, and confidence interval (CI) for quantitative ones. The Chi-Square test was used to interpret the significance of differences and associations between qualitative variables and reported as a probability value (p). To confirm that the parametric state is present, we used respectively, Kolmogorov-Simonov and Shapiro-Wilk for normality testing, when the sample size was ≥100 or <100. After determining that almost all the data for quantitative variables was nonparametric and didn't follow the normal distribution rules, we used the Mann-Whitney U machine as a nonparametric test to interpret the significance of the difference between means of quantitative data in the two groups of a qualitative variable. When there were more than two groups of qualitative variables, the Kruskal-Wallis machine was used. We have approached $p>0.05$ as statistically non-significant (NS). Data were reported with 95% CI.

RESULTS

Recruitment of the medical documents in the study went through a process shown in Figure 1. The missing data for each variable are as follows: LoH [n=1, (0.48%)]; Cr, complete blood counts and PSI [n=2 for each, (0.97%)]; ESR [n=14, (6.8%)], CRP [n=15 (7.3%)], Alb and BMI [n=24 for each, (11.7%)] and LDH [n=70, (34.1%)]. The lab had not specified non-Candida *albicans* yeast (NCAY). Ultimately, 205 cases were included. The demographic, past medical, and drug history of these cases are summarized in Table 1. Additionally, Tables 2 and 3 report the clinical features and results of Lab tests taken exactly when the patients were admitted. The severity of CAP with which the patients were hospitalized is shown in Table 4.

Despite at least one biological fluid or discharge sample being sent to microbiology for every patient, organisms were identified in 117 (57%) cases. There was a case of hydatid cyst which was diagnosed by direct microscopic examination of the sample taken by bronchoscopy. Three cases of *Brucella melitensis* were suspected by Rose Bengal and Wright Brucella tests and then confirmed by BC. The most commonly reported agents causing >3 days of hospitalization for CAP were Influenza H1N1 2009 (n=39, 33.4%), TB (n=21, 17.9%), NCAY (n=9, 7.8%), methicillin-resistant *Staphylococcus epidermis* (MRSE) (n=7, 6%) and KPn (n=5, 4.3%). Also, superimposed infections were seen in 14 patients. Primary organisms, the most important data related to the study's aims, are summarized in Tables 5, 6, and 7. Data related to MRSE and KPn are discussed further in the text.

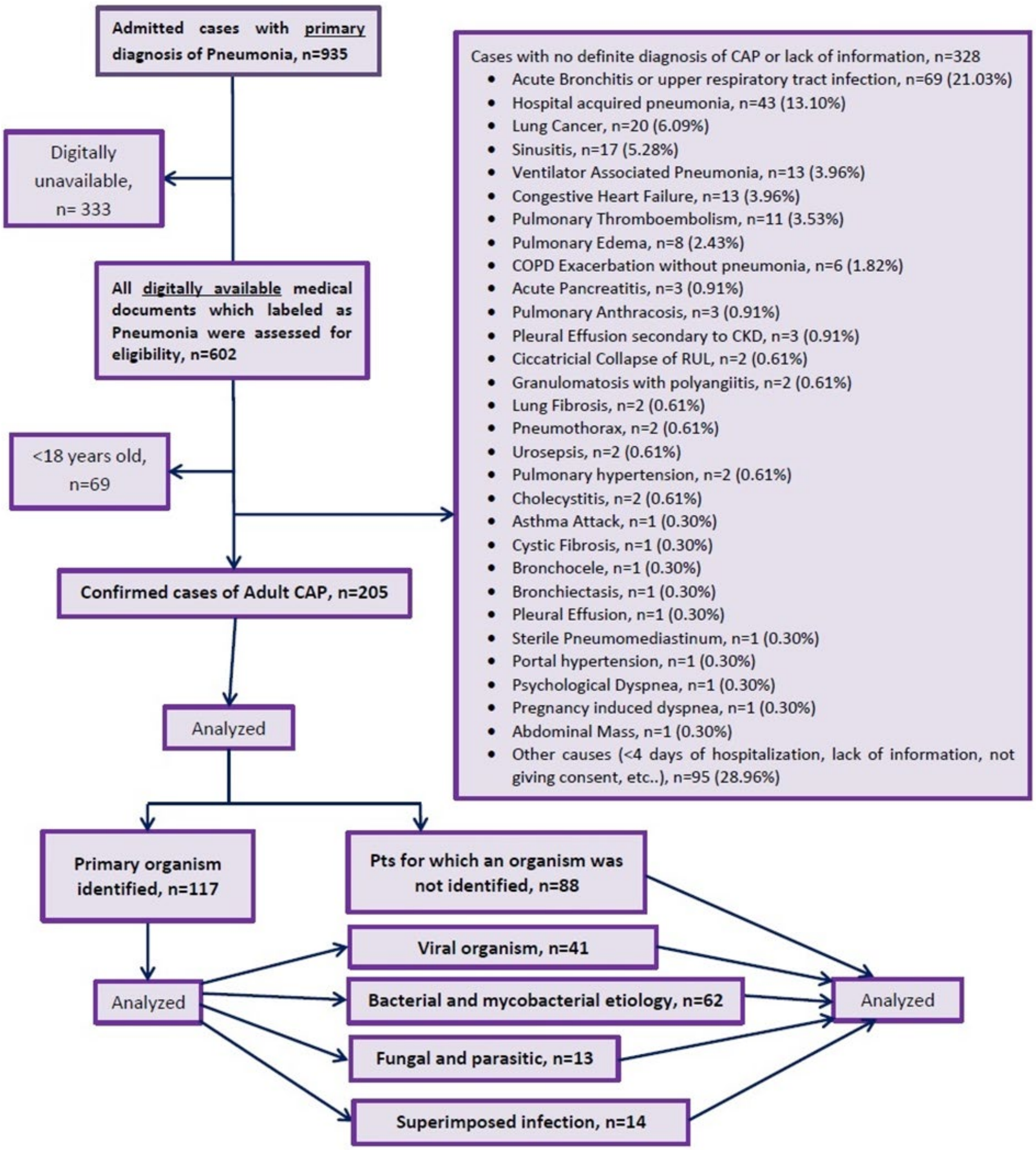

Figure 1. Enrollment process

Table 1. Demographic features and past medical history taken at the time of admission

Feature	Admitted in WARD and survived	Admitted to the ICU and survived	DIED during hospitalization	Total
Total Cases; n (%)	168 (81.95)	19 (9.26)	18 (8.78)	205 (100)
Age; mean years from 205 (25th - 75th percentiles)	50.84 (35 - 64)	50.58 (40 - 63)	69.17 (55 - 84)	52.42 (37 - 65)
Gender male; n (%)	102 (49.75)	12 (5.85)	12 (5.85)	126 (61.46)
Gender female; n (%)	66 (32.19)	7 (3.41)	6 (2.92)	79 (38.53)
LoH; mean days (25th - 75th percentiles)	8.26 (5 - 10)	10.89 (6 - 15)	10.22 (6 - 13)	8.66 (5 - 10)
Prisoner; n (%)	18 (8.78)	3 (1.46)	1 (0.48)	22 (10.73)
At least one comorbidity; n (%)	153 (74.63)	16 (7.80)	17 (8.29)	186 (90.73)
Neoplasm; n (%)	18 (8.78)	2 (0.97)	4 (1.95)	24 (11.70)
Hepatic Disease; n (%)	29 (14.14)	6 (2.92)	4 (1.95)	39 (19.02)
Cardiovascular Disorder; n (%)	70 (34.14)	7 (3.41)	14 (6.82)	91 (44.39)
CKD; n (%)	18 (8.78)	2 (0.97)	4 (1.95)	24 (11.70)
DM; n (%)	32 (15.61)	4 (1.95)	3 (1.46)	39 (19.02)
Neurologic Disorder; n (%)	28 (13.65)	4 (1.95)	5 (2.43)	37 (18.04)
Immunocompromised; n (%)	36 (17.56)	5 (2.43)	5 (2.43)	46 (22.43)
CRD; n (%)	71 (34.63)	11 (5.36)	8 (3.9)	90 (43.90)
BMI; mean kg/m^2 (25th - 75th percentiles)	24.68 (21 - 28)	26.38 (23 – 29)	23.79 (21 - 27)	24.75 (21 - 28)
Previous antibiotic use; n (%)	51 (24.87)	7 (3.41)	5 (2.43)	63 (30.73)
Previous Anti-Acid use; n (%)	31 (15.12)	6 (2.92)	4 (1.95)	41 (20.00)
Previous Immunosuppressive use; n (%)	21 (10.24)	2 (0.97)	4 (1.95)	27 (13.17)
Chronic Systemic Corticosteroid use; n (%)	32 (15.61)	3 (1.46)	4 (1.95)	39 (19.02)
Chronic Inhaled Corticosteroid use; n (%)	38 (18.53)	7 (3.41)	3 (1.46)	48 (23.41)
Tobacco Smokers; n (%)	52 (25.36)	7 (3.41)	5 (2.43)	64 (31.22)
Illegal drug abusers; n (%)	40 (19.51)	5 (2.43)	5 (2.43)	50 (24. 39)
Alcohol abusers; n (%)	8 (3.90)	0 (0)	0 (0)	8 (3.90)

BMI, Body Mass Index; CKD, Chronic Kidney Disease; CRD, Chronic Respiratory Disease; DM, Diabetes Mellitus; ICU, Intensive Care Unit; Kg, Kilograms; m^2, Quarter meters; n, Numbers of Patients; LoH, Length of Hospitalization

Table 2. Symptoms and signs in the different severity groups at the time of admission.

Symptoms and signs		PSI Class				
		II	III	IV	V	Total
Cough†	No; n (%)*	4 (1.95)	4 (1.95)	13 (6.34)	14 (6.82)	35 (17.07)
	Yes; n (%)*	30 (14.63)	29 (14.14)	59 (28.78)	52 (25.36)	170 (82.93)
Sputum†	No; n (%)*	11 (5.36)	14 (6.82)	28 (13.65)	21 (10.24)	74 (36.09)
	Yes; n (%)*	23 (11.21)	19 (9.26)	44 (21.46)	45 (21.95)	131 (63.91)
Dyspnea†	No; n (%)*	9 (4.39)	7 (3.41)	19 (9.26)	13 (6.34)	48 (23.41)
	Yes; n (%)*	25 (12.19)	26 (12.68)	53 (25.85)	53 (25.85)	157 (76.59)
Chest Pain†	No; n (%)*	22 (10.73)	27 (13.17)	56 (27.31)	57 (27.80)	162 (79.02)
	Yes; n (%)*	12 (5.85)	6 (2.92)	16 (7.80)	9 (4.39)	43 (20.98)
Altered Mental Status†	No; n (%)*	29 (14.14)	28 (13.65)	47 (22.92)	23 (11.21)	127 (61.96)
	Yes; n (%)*	5 (2.43)	5 (2.43)	25 (12.19)	43 (20.98)	78 (38.04)
Fever†	No; n (%)*	14 (6.82)	8 (3.90)	23 (11.21)	31 (15.12)	76 (37.07)
	Yes; n (%)*	20 (9.75)	25 (12.19)	49 (23.90)	35 (17.07)	129 (62.93)
Triage condition†	III; n (%)*	17 (8.29)	16 (7.80)	15 (7.31)	9 (4.39)	57 (27.80)
	II; n (%)*	15 (7.31)	14 (6.82)	36 (17.56)	24 (11.70)	89 (43.42)
	I; n (%)*	2 (0.97)	3 (1.46)	21 (10.24)	33 (16.09)	59 (28.78)

* The percentages are described as a ratio of all 205 patients.

† The *p* for all sign and symptoms was non-significant.

PSI, Pneumonia Outcome Research Trial Severity Index; n, Numbers

Table 3. Laboratory parameters at the time of admission

Variable	Cases analyzed	Mean	95% CI	25th and 75th percentiles
Cr (mg/dl)	203	1.35	1.20 - 1.49	0.90, 1.30
CRP (mg/L)	190	56.6	43.5 - 69.7	16.0, 79.0
ESR (mm/hr)	191	47.4	38.7 - 56.1	17.0, 70.0
WBC (x10^3/µl)	203	9.8	9.1 - 10.6	5.9, 12.8
LDH (U/L)	135	707	572 – 843	387, 697
Alb (g/dl)	181	3.67	3.41 - 4.66	3.20, 4.00

Alb, Albumin; CI, Confidence Interval; Cr, Creatinine; CRP, C-Reactive Protein; dl, Deciliter; ESR, Erythrocyte Sedimentation Rate; g, Grams; hr, Hours; L, Liters; LDH, Lactate de-Hydrogenase; mg, Milligrams; mm, Millimeters; U, Units; WBC, White Blood Cell Counts; µl, Microliters

Table 4. Disease severity at the time of admission using PSI

Variable	Mean PSI	95% CI	25th and 75th percentiles
Male	115	107-123	84, 139
Female	107	96-117	76, 134
Prisoners	119	100-138	97, 152
At least one comorbidity	115	108-121	85, 138
Neoplasm	139	119-160	101, 179
Hepatic Disease	132	118-146	107, 147
Cardiovascular Disorder	132	124-141	102, 163
CKD	144	126-172	117, 175
DM	128	115-141	101, 162
Neurologic Disorder	138	121-155	113, 176
Immunocompromised	119	105-132	85, 137
CRD	115	106-125	85, 141
previous Antibiotic use	101	90-112	71, 121
previous Anti-Acid use	114	102- 126	92, 134
previous Immunosuppressive use	113	95-130	83, 137
Chronic Systemic Corticosteroid use	112	97-126	83, 137
Chronic Inhaled Corticosteroid use	114	101- 127	85, 140
Tobacco smoking	116	106-126	[92, 136
Opioid dependents	119	108-130	98, 138
Alcohol Abusers	103	85-121	83, 121
Total Cases	112	106-118	83, 137

CI, Confidence Interval; CKD, Chronic Kidney Disease; CRD, Chronic Respiratory Disease; DM, Diabetes Mellitus PSI, Pneumonia Outcome Research Trial Severity Index

Table 5. Comparison of disease severity and inflammatory markers with common organisms

Variable	Influenza H1N1/2009	TB	MRSE	NCAY	Other Organisms	*p-value*
			Mean (95% CI)			
Age; years	40.8 (36.45-46.07)	57.7 (49.36-66.07)	57.43.0 (42.69-72.17)	63.2 (47.19-79.25)	52.0 (46.10-57.90)	0.001
LoH; days	7.4 (6.5-8.4)	8.3 (6.3-10.3)	16.8 (7.0-26.7)	10.1 (3.3-16.9)	9.2 (7.6-10.7)	NS
BMI; Kg/m^2	25.1 (23.70-26.52)	23.8 (20.36-27.28)	27.3 (21.51-31.09)	24.3 (21.17-27.55)	24.9 (23.35-26.45)	NS
ESR; mm/hr	60 (20-100)	43 (28-58)	43 (2-85)	40 (15-66)	55 (45-66)	NS
CRP; mg/L	50 (38-62)	39 (21-58)	67 (36-98)	47 (22-72)	63 (47-80)	NS
WBC; 103/µl	7.9 (6.69-9.12)	10.3 (7.91-12.71)	11.2 (7.19-16.20)	9.5 (4.54-14.54)	11.4 (9.13-13.70)	NS
PSI Score	90 (76.95-102.90)	113 (92.19-134.00)	170 (111.45-227.69)	132 (94.27-169.96)	116 (101.05-131.97)	0.043

BMI, Body Mass Index; CI, Confidence Interval; CRP, C-Reactive Protein; dl, Deciliter; ESR, Erythrocyte Sedimentation Rate; g, Grams; L, hr, Hours; Liters; LDH, Lactate de-Hydrogenase; LoH, Length of Hospitalization; m^2, Quarter meters; mg, Milligrams; mm, Millimeters; MRSE, Methicillin Resistant Staphylococcus Epidermis; NS, Non-Significant; *p*, Probability; TB, Mycobacterium Tuberculosis; U, Units; WBC, White Blood Cell Counts; µl, Microliters

Table 6. Primary organisms and the tests used to identify them

Microorganism	Distribution	BC (n=136)	SC (n=53)	SMPCR (n=84)	BK (n=82)	TB PCR (n=21)	ME (n=1)	PFC (n=3)
Influenza A (H1N1 2009)	n=39, 33.4%	-	-	39	-	-	-	-
TB	n=21, 17.9%	-	-	-	16	9	-	-
NCAY	n=9, 7.8%	-	9	-	-	-	-	-
MRSE	n=7, 6 %	5	2	-	-	-	-	-
KPn	n=5, 4.3%	4	1	2	-	-	-	-
SPn	n=3, 2.6%	2	2	1	-	-	-	1
Acinetobacter Baumannii	n=3, 2.6%	3	-	2	-	-	-	-
Brucella Melitensis	n=3, 2.6%	3	-	-	-	-	-	-
Pseudomonas Aeruginosa	n=3, 2.6%	3	-	-	-	-	-	-
Candida Albicans	n=3, 2.6%	3	3	1	-	-	-	-
MRSA	n=3, 2.6%	3	1	-	-	-	-	-
Enterobacter Aerogenes	n=3, 2.6%	3	1	-	-	-	-	-
Streptococcus Viridians	n=2, 1.8%	1	1	1	-	-	-	-
Bacillus Cereus	n=2, 1.8%	2	-	-	-	-	-	-
Haemophilus Influenza	n=1, 0.8%	0	1	-	-	-	-	-
Burkholderia Cepacia	n=1, 0.8%	1	1	-	-	-	-	-
Klebsiella Oxytoca	n=1, 0.8%	1	-	-	-	-	-	-
Micrococcus Luteus	n=1, 0.8%	1	-	-	-	-	-	-
Neisseria Meningitides	n=1, 0.8%	1	-	-	-	-	-	-
Enterococcus Faecium	n=1, 0.8%	1	-	-	-	-	-	-
Alcaligenes Faecalis	n=1, 0.8%	1	-	-	-	-	-	-
E. Coli	n=1, 0.8%	1	-	-	-	-	-	-
Echinococcus granulosus	n=1, 0.8%	-	-	-	-	-	1	-
Influenza A (H3N2)	n=1, 0.8%	-	-	1	-	-	-	-
hRV	n=1, 0.8%	-	-	1	-	-	-	-
Total Positive Tests	n=117, 100%	39 (28.6%)	23 (43.4%)	48 (57.1%)	16 (19.5%)	9 (42.8%)	1 (100%)	1 (33%)

BC, Blood Culture by Bac-Tec technology; BK; Bacillus de Koch 3 spot smear microscopic examination; E. Coli, Escherichia Coli; hRV, human Rhinovirus; KPn, Klebsiella Pneumoniae; ME, Direct Microscopic Examination of sample taken by bronchoscopy; MRSA, Methicillin Resistant Staphylococcus Aureus; MRSE, Methicillin Resistant Staphylococcus Epidermis; n, numbers; NCAY, Non Candida Albicans Yeast; PCR, Polymerase Chain Reaction; PFC, Pleural Fluid Culture; SC; Sputum Culture; SMPCR, Sputum Multiplex Polymerase Chain Reaction; SPn Streptococcus Pneumoniae; TB, Mycobacterium Tuberculosis

None of the MRSE cases (n=7) had a history of hospitalization before the episode when they were admitted with CAP. None of the nursing home residents or prisoners used instruments at least 2 months before admission, except for the one who was a prisoner before admission. Two of those with opioid dependence had used to smoke opioids, but neither were IV-drug abuser nor a prisoner. Both latter cases died during hospitalization. We didn't find any association between dying from the disease and being in prison just before the admission.

In total, 5 cases of KPn were found in whom coexisting CRD (n=3), neurologic disease (n=3), cardiovascular disease (n=3), and prior to admission antibiotic therapy (n=2) were detected. The mean age of them was 58 (18-92, 95% CI), a bit higher compared to 50 (46-53, 95% CI) years old of age in others without KPn (p=NS) whilst BMI, CRP, ESR and WBC counts were not so different. Also, 60% of KPn cases had CRD compared to 44% in others. (p=NS). We've found that longer LoH (p=0.025), more severe CAP (p=0.024), and previously diagnosed neurologic disease (p=0.0015) were significantly associated with pneumonia due to primary KPn. The PSI score was 165 (120.3-211.1, 95% CI) in KPn cases compared to 109 (99.9-118.5, 95% CI) in others (n=108). Mean LoH was 17.7 (2.75-32.75, 95% CI) among patients infected with KPn compared to others with 8.6 (7.64-9.57, 95% CI) days. We couldn't find any other associations between primary pulmonary infection due to KPn and other parameters. The superimposed KPn cases (n=4) were not included in the above analyses.

Immunosuppressive drug use (except for systemic corticosteroids) was higher among patients infected with organisms other than H1N1 2009, TB, or NCAY (p=0.031).

Cases taking systemic corticosteroids before hospitalization were in more danger of TB and other organisms than H1N1 2009, NCAY, and MRSE (*p*=0.012).

The causative microorganisms of superimposition and their primary ones were as follows: NCAY (n=4, 27%), KPn (n=4, 27%), *Streptococcus pyogenes* (n=3, n=21%), *Burkholderia cepacia* (n=1, 7%), *Methicillin-resistant Staphylococcus aureus* (MRSA) (n=1, 7%) and *Acinetobacter baumannii* (n=1, 7%). All superimposed infections were post H1N1 2009, except for *Streptococcus pyogenes* with 1 superimposed on H1N1 2009, 1 on TB, and 1 on hRV. In addition, 6 patients were admitted at PSI class V (4 [29%] KPn and 2 [14%] *Streptococcus pyogenes* [p=0.077]). Two (14%) patients with NCAY accounted for all cases who presented at PSI class IV. Two other patients with superimposed KPn were admitted to ICU one of whom didn't survive.

Nobody had a past medical history of neoplasm while 4 cases were reported of having liver disease [*S. pyogenes* (n=2), 1 KPn (n=1), and 1 NCAY (n=1)]; 7 had cardiovascular disease [2 *S. pyogenes* (n=2), 3 KPn (n=3), and NCAY (n=2)]; 1 had CKD (KPn); 4 had DM [*S. pyogenes* (n=2), KPn (n=1), and NCAY (n=1)]; 2 were Immunocompromised [KPn (n=1) and NCAY (n=1)]; 5 had neurologic conditions [*S. pyogenes* (n=1) and KPn (n=4, p=0.050)]; 6 had CRD [*S. pyogenes* (n=2), KPn (n=2), MRSE (n=1), and NCAY (n=1)].

None of these cases with superimposed infections were on immunosuppressive drugs or systemic corticosteroids. Smoking, illegal drug dependence, and alcohol abuse were reported in one patient who had the diagnosis of *Burkholderia cepacia* (*p*=0.016) but others were not cigarette smokers or alcohol abusers (*p*=0.016). Beyond this individual case, opioid dependence was seen in 2 cases with the diagnosis of NCAY infection. The mean PSI Score in the patients with superimposed infection was 120.4 (91.6-147.5, 95% CI) comparing to the rest 109 (99.1-119-1, 95%CI). In addition, higher BMI was associated with the superimposition of infections as a primary cause of CAP. Table 8 summarizes more information about other parameters. Moreover, 10 out of 18 (56%) patients who died due to CAP had definite microbiologic diagnoses. The maximum mortality was due to staphylococci, with Methicillin-resistant strains of *Staphylococcus aureus* (n=2, 66%) and *Staphylococcus epidermis* (n=2, 29%). Others were as follows: TB (n=1), *Candida albicans* (n=1), *Pseudomonas aeruginosa* (n=1), E. coli (n=1), *Brucella melitensis* (n=1), and post-influenza infection caused by KPn (n=1).

Table 7. Comparison of the four most common pathogens using qualitative variables*

Variable		Influenza H1N1/2009	TB	MRSE	NCAY	Other Organisms	*p-value*
Triage	3	14 (35%)	11 (52%)	1 (14%)	1 (11%)	12 (29%)	0.001
	2	21 (53%)	8 (38%)	1 (14%)	4 (44%)	12 (29%)	
	1	4 (10%)	2 (9%)	5 (71%)	4 (44%)	17 (41%)	
Survival during hospitalization		38 (97%)	20 (95%)	5 (71%)	9 (100%)	34 (82%)	0.038
Neoplasm		5 (12%)	2 (9%)	6 (85%)	1 (11%)	5 (12%)	NS
Immunocompromised		9 (23%)	3 (14%)	1 (14%)	1 (11%)	12 (29%)	NS
Liver Disease		9 (23%)	2 (9%)	3 (42%)	1 (11%)	9 (21%)	NS
Immunosuppressive Drugs		3 (7%)	2 (9%)	1 (14%)	0 (0%)	9 (21%)	0.031
Systemic Corticosteroids		3 (7%)	5 (23%)	0 (0%)	0 (0%)	10 (24%)	0.012
Inhaled Corticosteroids		7 (17%)	5 (23%)	1 (14%)	2 (22%)	7 (17%)	NS
Cardiovascular Disease		10 (25%)	10 (47%)	5 (71%)	5 (55%)	17 (41%)	NS
CKD		2 (5%)	2 (9%)	1 (14%)	0 (0%)	6 (14%)	NS
CRD		13 (33%)	14 (66%)	3 (42%)	4 (44%)	13 (31%)	0.084
DM		66 (15%)	8 (38%)	1 (14%)	1 (11%)	6 (14%)	NS
Neurologic Disease		3 (7%)	2 (9%)	1 (14%)	3 (33%)	10 (24%)	NS
Anti-Acid		7 (17%)	4 (19%)	1 (14%)	2 (22%)	8 (19%)	NS
Smoking		9 (23%)	9 (42%)	3 (42%)	2 (22%)	9 (21%)	NS
Illegal Drug Dependence		1 (2%)	0 (0%)	0 (0%)	0 (0%)	9 (21%)	NS
Alcohol Abuse		2 (5%)	1 (4%)	0 (0%)	0 (0%)	3 (7%)	NS

* Numbers are described as frequency and percentages. CRD, Chronic Respiratory Disease; CKD Chronic Kidney Disease; DM, Diabetes Mellitus; MRSE, Methicillin Resistant Staphylococcus Epidermis; NCAY, Non Candida Albicans Yeast; NS, Non-Significant; *p*, Probability; TB, Mycobacterium Tuberculosis

Table 8. Age, BMI, inflammatory markers, and severity by superimposition of infection

Superimposed Infection	Age; years	LoH; days	BMI; Kg/m²	ESR; mm/hr	CRP; mg/L	WBC; 10³/µl	PSI Score
				Mean (95% CI)			
Yes	47 (36-58)	11.1 (7-16)	27.5 (24.6-30.4)	51 (27-74)	44 (24-64)	8.6 (6-11.2)	120 (92-148)
No	52 (48-55)	8.3 (7.4-9.2)	24.5 (23.5-25.5)	53 (39-70)	56 (47-65)	10.1 (8.8-11.3)	109 (99-119)
P	NS	NS	0.027	NS	NS	NS	NS

BMI, Body Mass Index; CI, Confidence Interval; CRP, C-Reactive Protein; Kg, Kilograms; L, Liters; mg, milligrams; mm, Millimeters; NS, non-significant; p, Probability Value; PSI, Pneumonia Outcome Research Trial Severity Index; WBC, White Blood Cell Counts; µl, Microliters

DISCUSSION

It's important to note that the results and sorting of microorganisms by periodic prevalence (fall and winter) could lead to epidemiologic errors for readers interested in studying the annual prevalence of pneumonia-causing microorganisms. This study is a pilot for future research and only reports on microorganisms that caused acute CAP in Isfahan and nearby areas during the fall and winter.

H1N1 2009, the most common organism

In August 2009, a novel H1N1 influenza pandemic virus broke out in Isfahan (13). Influenza type A was the most commonly identified organism with 39 cases of H1N1 2009 and 1 H3N2. Some investigations show a predominant role for Influenza type A as the primary microbiologic cause of CAP. In a study on 304 adults with CAP, a viral diagnosis was made in 88 (29%) with hRV (13%) and influenza type A (10%) being the most common (14). In another study in Indonesia on 148 patients (>14 years**)**, influenza was the most commonly identified organism (18%) followed by KPn (14%) (15). Interestingly, as in our study and unlike most other studies (16, 17), SPn was not the most common pathogen causing CAP. (15) Additionally, it must be considered that the influenza vaccination rate is very low in Iran. (18-21)

Since 2011 when the pandemic was over, the World Health Organization reported that "the virus is spreading around the world on a seasonal pattern mostly during late fall till early spring" (22). All of our cases were admitted in fall and winter, so it is one of or maybe the most important cause of H1N1 2009 being the most common in our study. Our results were also consistent with a study performed on 873 patients of all age groups admitted due to influenza from 2010 to 2016 in Isfahan, in which a strong relation was found between some lunar months (according to the Islamic Calendar; Muharram till Jumada-al-Thani) and influenza epidemics (23). Our cases were mostly chosen in that period (especially Muharram and Safar). It is of great value to notice that many religious ceremonies are held during these lunar months in which numerous people gather and make transmission of the virus easier (24).

H1N1 2009 was more frequently reported in lower ages than other organisms (p=001). Although Influenza A H3N2 resulted in more severe disease in some studies (25) and infected older age groups in some others (26), a study in China on 92 adults with Influenza Type A pneumonia showed a close mean age in those with H1N1 2009 (51 years) compared to those with H3N2 (53 years) (27). It is reported that the mean age of 23 adult New Zealanders with Influenza A CAP was even higher at 66 years (14). Contrary to the latter study, our study consistent with most other studies (13, 27-29), reports that H1N1 infects younger people than other causes of adult CAP do (Table 5). It is a regular age pattern from the 1918 Influenza pandemic to date (29). A study on clinical features of H1N1 2009 in 216 infected patients of all age groups in Isfahan reported a mean age of 26.6 years (13). In another study performed in Isfahan on 26 patients with H1N1 2009 and abnormal lung HRCT, the mean age was 39 years (30). During the H1N1 2009 pandemic, it was observed that the most affected age group comprised of children and young adults. One study on all age groups found that of hundreds of confirmed cases infected by influenza, 40% of patients were aged 10–18 years, while only 5% of cases were adults over 51 years of age (31). A prospective observational study of 168 critically ill patients with H1N1

2009 in Canada found a mean age of 32.3 years (32). In another study on clinical characteristics of H1N1 2009 which caused CAP in 37 adults in Pusan, the mean age was 46.1 years (33) close to another study performed in Isfahan (45.7 years) (25). In 2014, the inverse relationship between age and relative risk of being infected by H1N1 2009 was reported: 1.23, 0.86, and 0.81 for 18-49, 50-64, and 65+ age groups, respectively (34).

The possible explanations for this result are: 1) Although the vaccination rate among high-risk groups including 65+ years old people and health care personnel in Iran is still lower than the ideals (18-21), routine Influenza vaccination of them is on progress. However, vaccination alone is unlikely to explain the higher incidence in younger adults (35).

2) Elderly exposure to the previous waves of Influenza epidemics and the production of natural immunization amongst them (36).

3) Younger age groups are more active in the community and transmission of the disease is much easier among them (11, 36). 4) Although the median age of the Iranian population is increasing, it is still much lower compared to the developed countries (37, 38).

Tuberculosis, the 2nd most common organism

Although routine Bacille Calmette-Guerin (BCG) vaccination in Iran has been in progress since 1952 (39), we reported that TB still can and will cause extra-pulmonary (40) and pulmonary (41) diseases such as acute CAP, both in immunocompromised and immunocompetent individuals (42-45). TB can present as an acute process and mimic classic bacterial pneumonia or masquerade as atypical pneumonia, with nonproductive cough and systemic symptoms (43). Although the code 1B10 represents Tuberculosis as "a progressive or chronic disease resulting from infection with the bacterium *Mycobacterium tuberculosis*" (8), it could also present as an acute process without preliminary sign(s)/symptom(s) (42-45). However, it can be due to inefficient history-taking as well. Interestingly, in this study, patients with TB were at a higher triage status when admitted (p=0.001).

Nowadays, TB is typically associated with socio-economic problems such as war, malnutrition, and Human Immunodeficiency Virus (HIV) prevalence. Despite many progresses carried out to control TB in Iran, different factors such as immigration from neighboring countries especially Afghanistan (46, 47) are effective in the high incidence of TB infection (48). According to the report of the United Nations High Commissioner for Refugees (UNHCR), "in October 2020, 800,000 refugees were living in Iran, of which 780,000 are Afghans. Additionally, it is estimated that approximately 2 million undocumented Afghans and nearly 600,000 Afghan passport holders were living in Iran. These refugees have been given access to education, health, and livelihood opportunities; helping them thrive. It is estimated that 96% of refugees in Iran live in cities, towns, and villages side by side with the Iranian host community" (47, 49). Archives of the Iranian National Center of Statistics reported that Isfahan is both a pathway and the 3rd final destination in Iran for Afghan refugees and immigrants and near 180000 Afghanis are living in Isfahan (50). Isfahan province specially welcomes thousands of Afghan refugees as it is the hub of the country's industry and offers employment opportunities to tens of thousands of workers in Iran, including many Afghans (47, 51). Interestingly, we've found that opposite to the common belief in Isfahan hospitals that "Afghans have TB until proven otherwise", TB was very common among patients of Iranian origin and only 2 of 21 TB cases were Afghan.

In a systematic review, the reported cases of TB in Isfahan were significantly lower compared to the provinces nearer to Afghanistan (52). On the contrary, in a study of 120 adults in Mashhad, which is located very close to Afghanistan, 17.5% of cases presenting with signs and symptoms of acute CAP, had the diagnosis of TB (43), a similar percentage to what we reported (17.9%). In a study on 430 adults in South Africa, TB was the most common agent in both HIV and non-HIV-infected subjects (40% and 35%, respectively) (53).

We have found that TB was strongly associated with chronic use of systemic corticosteroids. Many papers (48, 54) have proved the relationship between TB and immunodeficiency but we couldn't find an association between TB with neither immunosuppressive drugs (other than systemic corticosteroids) nor other immunocompromised conditions. It may probably be due to the low number of cases in our study. The vaccine is the best way to prevent TB. BCG is the only licensed vaccine against TB infection (55). Although BCG can effectively protect infants and young children from TB, its efficacy on pulmonary TB varies, especially among adults (56).

As such, TB should be included in the differential diagnosis of CAP patients. According to the previous studies done in Isfahan (57) and the results of the current study, more invasive methods should be performed to diagnose and control TB in Isfahan including more testing and screening, tracing, and more vaccination, especially among high-risk groups such as health care personnel. Additionally, the antibiotic resistance of TB in Isfahan should be sought in future studies.

NCAY, the 3rd reported result

All cases whose sputum was detected and reported as "Non-Candida Albicans yeast" by microbiology had survived and were discharged. It is not different from the term "Non-Albicans Candida yeast" when a Candida strain other than Albicans causes the infection. Our results tell us when NCAY is reported, it may be due to either contamination of the samples or errors in standard sputum collection (see Table 6; all of the organisms had been extracted of sputum) since there was no mortality due to NCAY. So, if PSI is high or based on clinician judgment, if the patients are in danger, we suggest more aggressive testing and treatment as NCAY strains are rising around the world (58). Otherwise, if the severity is low, routine first-line CAP treatment may suffice due to low numbers of mortality in these patients. Nevertheless, a study with a focus on pneumonia due to NCAY with a larger population than ours is suggested.

Staphylococcus as the most lethal genus

The high mortality due to a Staphylococcal cause was consistent with many other studies that reported staphylococcal pneumonia as a major risk factor for severe disease and death (16, 59-61). So, concerning current data, the isolation of MSSA/MSSE should be taken as seriously as MRSA/MRSE in terms of severity and death.

Some studies were conducted in Isfahan on staphylococcal infections because staphylococcal pathogens have been detected throughout the community, too (62, 63). A cross-sectional study reported the prevalence of staphylococcal nasal carriage as high as 26.6% in 158 healthy adults and suggested eliminating the pathogen to prevent future infections (64). Another study included a total of 25 Community Acquired MRSA isolates from the anterior nares of 410 healthy preschool children to determine its strain (63). In another study, random amplified polymorphic DNA Fingerprinting was used to analyze genetic variation in Community Acquired MRSA (CA-MRSA) isolates (62). It seems that the lack of sensitivity to methicillin in our staphylococcal population did not cause high severity and mortality. This conclusion was drawn from a study involving 133 individuals who had died from staphylococcal pneumonia. The study found no relationship between sensitivity to methicillin and death, indicating that methicillin resistance was not associated with higher mortality rates (65).

Traditionally, isolation of *Staphylococcus epidermis* was associated with health care instruments (e.g., venous catheters) (66) or was considered hospital-acquired (67) or otherwise, the results reported by microbiology were interpreted as due to sample contamination and errors (68). However, 7 MRSE patients in our study, had more severe disease than other cases, stayed longer in the hospital and 2 expired. They did not have any of the exclusion criteria explained respectively in the 1st and 2nd paragraphs of the introduction and method sections. For these reasons, we would better consider their ultimate diagnosis as *Community Acquired* MRSE (CA-MRSE).

So, regarding CAP, the reader needs to understand the severity and mortality of CA-MRSE. The results should not be simply interpreted as hospital-acquired, of instrumental origin, or as sampling errors and contamination. However, since CA-MRSE can be blood-borne in origin, the lungs can be not the primary organ involved, and the sample size in our study was limited, CA-MRSE infection of any organ other than the skin can be the subject of many further studies and special focus should be on medical history and physical examination in the future to eliminate the possibility of sources other than the community.

The increasing importance of *Klebsiella pneumoniae*

KPn incidence and prevalence are different among countries. High prevalence is reported in a multi-central study in Eastern Asia (69-71). In another multi-continental study in the United States, Australia, Turkey, Taiwan, South Africa, Belgium, and Argentina, KPn CAP was reported only in Taiwan and South Africa (72). Although high frequencies were reported in Eastern Asia, KPn was reported as a rare cause of CAP in the United States and Europe (73, 74). Previously, it was rare in Iran too (0.8%) (3), but we claim the incidence of KPn CAP is increasing in Isfahan (4.3% of primary infections). The frequency of KPn CAP can be even higher in Alzahra Center than what we are reporting now because our data were from cases who were hospitalized in fall and winter while KPn can increase through warmer months of the year (75).

Our study is consistent with others who suggested the presence of an association between KPn CAP with more severity (76-80), longer LoH (76), and more incidence in patients with neurologic diseases (70, 81-83). Additionally, in a case-control study in Greece on 162 cases and 161 controls, neurological disease (p=0.007), and older age (p=0.011) were associated with *carbapenem-sensitive* KPn but not with the *carbapenem-resistant* strain (81). COPD exacerbation with KPn was high among some studies but we couldn't detect any association probably because of the low number of cases (84-86).

Future studies should focus on the epidemiology of factors that increase the virulence of KPn in Iran. These factors include: 1) the ratio of capsular serotypes K_1 and K_2 to others because of more lethality (87) and their role in the development of pneumonia (88); 2) Hypermucoviscosity phenotype and lipopolysaccharide O side chain because of more resistance to complement system in the strains having this phenotype (89-94), because of more frequency of hypermucoviscosity among community acquired infections compared to nosocomial ones (95) and more bacteremia and lethal episodes caused by strains having the lipopolysaccharide (96); 3) Aerobactin producers which are 100 times more virulent than others (97, 98); 4) having type 3 fimbrial adhesion protein as it can cause the microorganism to spread in lungs and cause pneumonia (99); and 5) antibiotic resistant strains (100, 101), as in one study on 90 isolates producing extended-spectrum beta-lactamases vs 178 non-producers, the producers not only had gained resistance against antibiotics, but also they resisted the bactericidal features of the human serum too (102). Prevalence of extended-spectrum beta-lactamase genes in KPn isolates could be the subject of future studies as carbapenem resistance was studied in Isfahan, a few years ago (103).

Decrease in isolation of Pneumococci

SPn has traditionally been the most common agent in acute CAP (3, 104). However, either there seems to be a change in its epidemiology in Isfahan, or some sort of selection bias is at play. SPn was the 6th etiological agent isolated here. To complete the data of this study and to evaluate the possibility of a significant change in its frequency, SPn antigen detection methods should be used in both serum and urine samples. Other possibilities for this result are the lack of inclusion of the patients who were hospitalized for ≤3 days and the data were gathered on the patients admitted during fall and winter. Discussion around other organisms including the superimposed ones is beyond the scope of this article.

What had not been detected?

Antigen detection methods, cell cultures, and specific PCR assays (except for TB) were not used to detect atypical organisms so it is not surprising why they aren't isolated in any patient. Despite using multiple methods, some organisms weren't detected: *Streptococcus agalactiae*, *Moraxella catarrhalis*, *Legionella spp*, *Listeria monocytogenes*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Coxiella burnetii*, *Bordetella pertussis*, *Serratia marcescens*, *Enterobacter cloacae*, *Proteus mirabilis*, Adenovirus, Bocavirus, Coronavirus type 229, SARS, MERS, Enterovirus, B and C, Metapneumovirus, PIV 1, 2, 3 and 4, and hRSV.

CONCLUSION

H1N1 2009, TB, MRSE, and KPn had resulted in more than two third of CAP episodes before the COVID-19 pandemic. Incidence of CAP due to SPn had declined according to our results, however, more studies should be conducted to evaluate the prevalence of SPn in CAP. CA-MRSE has been detected and should be included in the differential diagnosis of CAP. TB also must be considered in the case of every acute CAP. Appropriate test to diagnose and control TB should be run based on the clinical situation. H1N1 2009 became the first cause of CAP after its pandemic was over till 2019 February, just before the detection of COVID-19 in Isfahan. So, it raises the question "Will COVID-19 follow the path of H1N1 2009?" Special focus should be on virulence factors and risk factors of KPn as it is rising according to the results. We didn't include the patients' jobs in data and studies are needed to clarify relationship between CAP and high levels of industrial advances and air pollution in Isfahan. Higher BMI was associated with superimposition of infections and prophylactic antibiotics should be considered and individualized in obese patients who present with CAP.

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