J Infect Chemother 25 (2019) 129–136

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Community-acquired bacterial co-infection predicts severity and mortality in influenza-associated pneumonia admitted patients☆

Fei Teng , Xin Liu , Shu-Bin Guo *, Zhuo Li , Wen-Qing Ji , Fang Zhang , Xiao-Mei Zhu

Emergency Department, Beijing Chao-Yang Hospital, Capital Medical University, Chaoyang District, Beijing 100020, China

ARTICLE INFO

Article history:
Received 4 August 2018
Received in revised form
17 October 2018
Accepted 25 October 2018
Available online 15 November 2018

Keywords:
Influenza
Bacterial co-infection
Community-acquired pneumonia
Mortality
Staphylococcus aureus

ABSTRACT

Background: Influenza is frequently complicated by bacterial co-infection, causing additional hospitalization and mortality. We determined the incidence, risk factors and outcomes of patients with influenza-associated community-acquired bacterial co-infection.

Method: This was a retrospective, observational study. Influenza was diagnosed using the polymerase chain reaction. Co-infection had to be confirmed using standard bacteriological tests. The primary endpoint was presence of community-acquired co-infection, and the secondary endpoint was in-hospital mortality.

Results: During the 8 influenza seasons from 2010 to 2018, of the 209 influenza-associated pneumonia admitted patients, 41 (19.6%) were identified with community-acquired bacterial co-infections and *Staphylococcus aureus* was the predominant strain. Compared with patients without co-infection, patients with co-infection had similar demographic characteristics and co-morbidities, obtained a higher APACHE II score and a higher SOFA score, and had higher ratio of sepsis shock, invasive mechanical ventilation, and ICU requirement. In-hospital mortality independently associated with bacterial co-infection (adjusted hazard ratio (aHR) 2.619; 95%CI 1.252–5.480; $p = 0.011$); in subgroup *S. aureus* (aHR 6.267; 95%CI 2.679–14.662; $p < 0.001$) and other pathogens (aHR 2.964; 95%CI 1.160–7.577; $p = 0.023$); and in subgroup positive findings in bloodstream (aHR 7.420; 95%CI 2.712–20.302; $p < 0.001$) and positive findings in other site (aHR 3.427; 95%CI 1.514–7.757; $p = 0.003$).

Conclusion: Community-acquired bacterial co-infection was frequent in influenza-associated pneumonia, without risk factor identified yet. Bacterial co-infection was likely to predict severity, and was an independent risk factor for in-hospital mortality. Co-infection of *Staphylococcus aureus* with influenza was identified as a lethal synergism, and should be targeted when developing clinical antibiotic strategies.

1. Introduction

Influenza has been recognised since 1580 and spreads rapidly through communities in outbreaks [1]. Two forms of influenza occur around the world: epidemic (seasonal or interpandemic) influenza caused by influenza A and B viruses, and novel pandemics caused by influenza A viruses [2]. Annual influenza epidemics result in substantial mortality, and Danielle Iuliano et al. [3] estimated that seasonal influenza accounts for 291,243-645,832 respiratory deaths (4.0–8.8 per 100,000 individuals) annually.

Seasonal and pandemic influenza are frequently complicated by bacterial co-infection, causing additional hospitalization and mortality [4,5]. Studies analysing the frequency of influenza and bacterial co-infection have reported high heterogeneity.

Abbreviations: ICU, intensive care unit; MSSA, methicillin-sensitive *S. aureus*; MRSA, meticillin-resistant *S. aureus*; CAP, community-acquired pneumonia; RT-PCR, reverse transcription polymerase chain reaction; Ig, immunoglobulin; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; SD, standard deviation; IQR, inter-quartile range; CI, confidence interval; aHR, adjusted hazard ratio.

☆ ICMJE Statement: All authors meet the ICMJE authorship criteria.

* Corresponding author. No. 8, South Road of Worker's Stadium, Chaoyang District, Beijing 100020, China.

E-mail addresses: tengfei.vip@live.cn (F. Teng), 474181972@qq.com (X. Liu), shubinguo@126.com (S.-B. Guo), lizhuo03@126.com (Z. Li), jiwenqing86@sina.com (W.-Q. Ji), allice1990ff@163.com (F. Zhang), zhuxm0722@163.com (X.-M. Zhu).

https://doi.org/10.1016/j.jiac.2018.10.014
1341-321X/

A recent systematic review and meta-analysis involving 27 studies demonstrated that the results from these studies were highly variable, ranging from 2 to 65%, although the majority of studies ranged between 11 and 35% [6]. Particularly notable is the increase in the number of patients with influenza who are admitted to hospitals with bacterial co-infections. As a large multicenter study with 2901 ICU patients admission recently showed a significant increase in occurrence from 11.4% in 2009 to 23.4% in 2015 [7].

During the 2009 influenza A H1N1 pandemic, bacterial co-infection was frequently reported in fatal cases with *Streptococcus pneumoniae* as the most predominant pathogen identified [8,9]. In other studies, the presence of bacterial co-infection, mainly caused by *Staphylococcus aureus*, seemed to result in a higher mortality rate [10,11]. Taken together, the most common causative pathogens of bacterial co-infection in influenza are *S. pneumoniae*, *S. aureus* (including community-acquired methicillin-sensitive *S. aureus* (MSSA) and meticillin-resistant *S. aureus* (MRSA)), *Haemophilus influenzae*, other *Streptococcus* species, and other Gram-negative rods [2].

In August 10, 2010 the WHO announced that the 2009 H1N1 pandemic had moved into the post-pandemic period [12]. We therefore determined the incidence, risk factors and outcomes of patients with influenza-associated community-acquired bacterial co-infection in the subsequent seasonal influenza period.

2. Patients and methods

2.1. Study design

We conducted a retrospective monocentric observational study in Beijing Chao-Yang Hospital, Capital Medical University (Beijing, China), which is an urban university hospital with 1300 beds and an annual census of approximately 250,000 Emergency Department visits. The steps of enrolling subjects were as follows: i) during the study period from 2010 to 2018, including 8 epidemic influenza seasons, all consecutive patients hospitalized with diagnosis of influenza were abstracted and screened; ii) all enrolled patients should be laboratory confirmed influenza A or/and influenza B infection by reverse transcription polymerase chain reaction (RT-PCR) test in accordance with the published guidelines from the Centers for Disease Control and Prevention [13], and patients with insufficient evidence were excluded; iii) patients who developed fever or flu-like symptoms during their hospitalization for treating other diseases and were diagnosed with influenza were excluded; iv) patients who didnot meet the community-acquired pneumonia (CAP) criteria [14] were excluded; v) referrals from other wards to our hospital were excluded, but outpatients and emergency patients from other hospitals were also included. Medical records were independently reviewed by two physicians. This study was approved by the Institutional Review Board and Medical Ethics Committee of Beijing Chao-Yang Hospital. The primary endpoint of this analysis was the presence of community-acquired co-infection, and the secondary endpoint was in-hospital mortality.

2.2. Definitions

CAP was defined by acute symptoms and presence of signs of lower respiratory tract infection initiated in the community without other obvious cause, whereas new pulmonary infiltrate on chest radiograph [14]. Influenza-associated CAP was defined in CAP patients with confirmed influenza infection. Bacterial co-infection was defined in patients with one or more isolates obtained from a blood culture and/or a pleural fluid, sputum, tracheal or bronchoscopic sample if the isolate was a pathogen considered to be causing a true infection in the opinion of the treating expert team (including a respiratory specialist and an infectious disease and microbiological specialist). Community-acquired bacterial co-infection was diagnosed in patients whose sputum and blood (when indicated) samples were collected for bacterial culture preparation within 48 h of hospital admission. Individual influenza group was defined as non-positive detection of community-acquired bacterial co-infected pathogen group, including patients with negative bacterial investigations, failure of valid specimens and without investigations. A causative co-pathogen of the pneumonia should fulfill at least one criterion among the following: i) *S. pneumoniae* or *S. aureus* or *L. pneumophila*, whatever type of testing and level of positivity; ii) identified in pleural fluid or blood, and blood cultures should be obtained from 2 or 3 sets [15]; iii) *Chlamydiae pneumoniae* or *Mycoplasma pneumoniae* identified with immunoglobulin (Ig) antibodies testing positive if IgM antibodies were identified or if a significant increase in IgG antibodies was observed between paired serum samples; iv) *C. pneumoniae* or *M. pneumoniae* identified by multiplex real-time fluorescence quantitative PCR; v) identified within a valid sputum specimen (leucocyte >25/field and epithelial cells <10/field) and $\geq 10^6$ colony-forming units/mL; vi) identified within bronchoalveolar lavage fluid $\geq 10^4$ colony-forming units/mL. vii) identified by urine antigen within 24 h after admission. Septic shock should meet the following criteria: i) subsequent organ dysfunction caused by a dysregulated host response to infection; ii) persisting hypotension requiring vasopressors to maintain MAP ≥65 mm Hg and having a serum lactate level >2 mmol/L despite adequate volume resuscitation [16]. Obese patients were defined as those with a body mass index >30 kg/m^2.

2.3. Data collection

At admission and during hospitalization stay, data regarding medical identification, demographic characteristics, co-morbidities, clinical symptoms and examinations, laboratory and radiological findings, microbiologic investigations, and therapeutic management were collected. Invasive mechanical ventilation and vasopressor drugs requirements were also recorded within 24 h of hospitalization. Acute Physiology and Chronic Health Evaluation (APACHE) II score [17] and Sequential Organ Failure Assessment (SOFA) score [18] were calculated for each patient using data obtained within 24 h from admission.

2.4. Data presentation and statistical analysis

Continuous variables were expressed as means ± standard deviation (SD) or median (inter-quartile range, IQR) and were compared using the Student *t*-test or Mann-Whitney *U* test. Discrete variables were expressed as counts (percentages) and were evaluated using the Chi-square test or Fisher's exact test. Missing blood routine examination was found in one case, biochemical examination in five cases, and arterial blood gas analysis in four cases. The corresponding items of these missing data in the APACHE II score and SOFA score were assumed to be zero. A multivariable logistic regression with clinically relevant variables was used to identify risk factors for co-infection. A Cox regression was used to identify variables independently associated with in-hospital mortality. Analyses were performed using the SPSS 21.0 software (IBM SPSS Statistics for Windows, version 21.0; Armonk, NY, USA). A difference was considered statistically significant when p value less than 0.05.

3. Results

3.1. Patient characteristics and risk factors for Co-infection

During the study period, 279 patients with the diagnosis of influenza were screened. Of these, 70 were excluded: 9 unconfirmed by RT-PCR; 26 had hospital-acquired influenza; 23 was not radiologically diagnosed with CAP; and 12 referrals from other wards with exacerbation. The final cohort consisted of 209 patients with influenza-associated CAP. No patients had been documented with influenza vaccination in their medical history. In multivariable analyses, none of the following variables in age, sex, influenza subtype, co-morbidities, and clinical manifestations was identified as an independent risk factor for bacterial co-infection (Table 1).

3.2. Pre-admission treatment

The median (IQR) time from onset of symptoms to hospitalization was 6 (4, 8) days, 151 (72.2%) patients transferred from outpatient department or emergency room of inferior hospitals or community medical service centers. Almost all patients are treated with cold medicine and/or acetaminophen at the beginning of their illness, 149 (71.3%) patients had received previous neuraminidase inhibitors based on rapid antigen testing positive or experience, 28 (13.4%) patients were administrated within 48 h. Before being hospitalized, 192 (91.9%) patients had received broad-spectrum antibiotics. The antibiotic regimens were quinolone monotherapy (45, 21.5%), beta-lactam monotherapy (62, 29.7%), macrolide monotherapy (6, 2.9%), quinolone plus beta-lactam (56, 26.8%), macrolide plus beta-lactam (20, 9.6%), and beta-lactam plus vancomycin (3, 1.4%). In multivariable analyses, none of the above pre-admission treatments was identified to be associated with bacterial co-infection (Table 1).

3.3. Severity assessment

Within 24 h of hospital admission, all of the patients were risk-stratified according to APACHE II score, with a mean of 12.1 ± 6.5, and SOFA score, with a mean of 3.7 ± 3.2. Patients with bacterial co-infection obtained a higher APACHE II score and a higher SOFA score than those with individual influenza infection ($p = 0.018$; $p = 0.006$). Meanwhile, at the first 24 h after admission, sepsis shock occurred in 16 (7.7%) cases and invasive mechanical ventilation was performed in 31 (14.8%) cases. The rates of sepsis shock and invasive mechanical ventilation requirement in the bacterial co-infected patients was significantly higher than that in the

Table 1
Characteristics and risk factors for bacterial co-infections in hospitalized patients with influenza-associated CAP.

Variables	Total n = 209	Individual influenza n = 168	Bacterial co-infection n = 41	*p* value
Age, median (IQR)	59 (47,73)	59 (49,75)	56 (38,71)	0.145
Age group, n (%)				
age <45	46 (22.0)	34 (20.2)	12 (29.3)	0.211
45 ≤ age <65	90 (43.1)	75 (44.6)	15 (36.6)	0.350
age ≥65	73 (34.9)	59 (35.1)	14 (34.1)	0.907
Sex (male), n (%)	136 (65.1)	109 (64.9)	27 (65.9)	0.636
Influenza type and subtype, n (%)				
A/H1N1	105 (50.2)	88 (52.4)	17 (41.5)	0.391
A/unclassified	70 (33.5)	55 (32.7)	15 (36.6)	0.719
B	29 (13.9)	21 (12.5)	8 (19.5)	0.099
A and B	5 (2.4)	4 (2.4)	1 (2.4)	0.560
Co-morbidities, n (%)				
Solid organ transplantation	2 (1.0)	1 (0.6)	1 (2.4)	0.125
Steroid therapy	8 (3.8)	7 (4.2)	1 (2.4)	0.998
Active cancer	6 (2.9)	5 (3.0)	1 (2.4)	0.678
Hemopathy	3 (1.4)	3 (1.8)	0 (0)	0.998
Chronic respiratory disease[a]	35 (16.7)	28 (16.7)	7 (17.1)	0.526
Coronary artery disease	20 (9.6)	17 (10.1)	3 (7.3)	0.878
Chronic heart failure	9 (4.3)	9 (5.4)	0 (0)	0.997
Chronic hepatopathy	7 (3.3)	7 (4.2)	0 (0)	0.998
Chronic kidney disease	5 (2.4)	4 (2.4)	1 (2.4)	0.998
Diabetes mellitus	31 (14.8)	27 (16.1)	4 (9.8)	0.377
Obesity, n (%)	16 (7.7)	11 (6.5)	5 (12.2)	0.394
Pregnancy, n (%)	3 (1.4)	3 (1.8)	0 (0)	0.999
Clinical manifestations				
Fever	199 (95.2)	160 (95.2)	39 (95.1)	0.486
Pharyngalgia	56 (26.8)	40 (23.8)	16 (39.0)	0.209
Cough	199 (95.2)	162 (96.4)	37 (90.2)	0.401
Dyspnea	136 (65.1)	108 (64.3)	28 (68.3)	0.177
Myalgia or arthralgia	59 (28.2)	49 (29.2)	10 (24.4)	0.237
Gastrointestinal symptoms[b]	32 (15.3)	25 (14.9)	7 (17.1)	0.859
Neurological symptoms[c]	42 (20.1)	31 (18.5)	11 (26.8)	0.613
Days from onset to hospitalization,median (IQR)	6 (4,8)	6 (4,7)	6 (4,8)	0.484
Received previous neuraminidase inhibitors, n (%)	149 (71.3)	117 (69.6)	32 (78.0)	0.155
Neuraminidase inhibitor administration within 48 h, n (%)	28 (13.4)	22 (13.1)	6 (14.6)	0.795
Received previous antibiotics	192 (91.9)	156 (92.9)	36 (87.8)	0.079
Received previous antibiotics days, n (%)				
1–2 days	70 (33.5)	55 (32.7)	15 (36.6)	0.640
3–5 days	83 (39.7)	69 (41.1)	14 (34.1)	0.417
6 or more days	39 (18.7)	32 (19.0)	7 (17.1)	0.771

[a] Chronic respiratory disease: asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, bronchiectasis.
[b] Gastrointestinal symptoms: vomiting, abdominal pain, diarrhea.
[c] Neurological symptoms: headache, dizziness, altered mental status.

influenza individual infected patients ($p = 0.028$; $p = 0.004$). During the hospital stay, 63 (30.1%) required ICU admission. Patients with bacterial co-infection were more likely to ICU requirement than patients without co-infection ($p = 0.001$) (Table 2).

3.4. Bacterial Co-infection investigation

The number (percentage) of cases where each bacteriological examination was performed was bronchial lavage cultures of 15 (7%), sputum cultures of 172 (82%), qualified sputum cultures of 109 (52%), *Streptococcus* urine antigen tests of 34 (16%), blood cultures of 52 (25%), throat swab PCR for *L. pneumophila*, *C. pneumoniae* and *M. pneumoniae* of 9 (4%), sputum PCR for *L. pneumophila*, *C. pneumoniae* and *M. pneumoniae* of 85 (41%). Overall, 41 (19.6%) were identified with community-acquired bacterial co-infections and the bacterial pathogen spectrums isolated from the cohort were *Staphylococcus aureus* (including community-acquired methicillin-susceptible and meticillin-resistant *S. aureus*), *Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa,* and *Mycoplasma pneumoniae* (Table 3). None of them were identified by pleural fluid culture or IgG/IgM antibodies against *C. pneumoniae* or *M. pneumoniae*. Of the 41 patients co-infected with bacterial pathogens, *S. aureus* was the predominant strain and was responsible for all the 10 positive blood cultures. For the 18 patients with *S. aureus* co-infection, 2 cases (all MSSA) were with no pre-admission antibiotic administration, 7 cases (all MSSA) with short course (1–2 days), 5 cases (3 MSSA, 2 MRSA) with intermediate course (3–5 days), and 4 cases (all MRSA) with long course (6 or more days).

3.5. Clinical outcomes

In-hospital mortality was not significantly different among age, sex, influenza types, co-morbidities and the pre-admission treatments (Table 4). In this cohort, 30 (14.4%) patients died in the hospital. Within 24 h of hospital admission, non-survival patients, with a higher APACHE II score ($p < 0.001$) and a higher SOFA score ($p < 0.001$), more frequently needed for mechanical ventilation ($p < 0.001$) and more likely to present sepsis shock ($p < 0.001$). Patients with ICU admission was associated with a higher in-hospital mortality ($p < 0.001$). The rates of bacterial co-infection ($p < 0.001$), especially *S. aureus* ($p = 0.001$), were higher in the non-survivors than those in the survivors (Table 4).

In-hospital mortality was associated in bivariate analyses with bacterial co-infection, sepsis shock, invasive mechanical ventilation, ICU requirement, APACHE II score and SOFA score. After Cox regression analysis, in-hospital mortality remained independently associated with bacterial co-infection, together with age, ICU requirement and SOFA score, and the adjusted hazard ratio (aHR) values were summarized in Table 5. Survival curves were described in Fig. 1. A subgroup analysis for pathogen species of the 41 bacterial co-infected patients showed that positive cultures in *S. aureus* (aHR 6.267; 95%CI 2.679–14.662; $p < 0.001$) and other pathogens (aHR 2.964; 95%CI 1.160–7.577; $p = 0.023$) were independent risk factors for in-hospital mortality. A subgroup analysis for infection site of the 41 bacterial co-infected patients showed that positive findings in bloodstream (aHR 7.420; 95%CI 2.712–20.302; $p < 0.001$) and positive findings in other site (aHR 3.427; 95%CI 1.514–7.757; $p = 0.003$) were independent risk factors for in-hospital mortality.

4. Discussion

During post-pandemic seasons, influenza A/H1N1 was the predominant circulating influenza strain, and also was the most common subtype in our study cohort. Patients infected with influenza A/H1N1 were more likely to develop severe disease, compared with those infected with other influenza A subtypes and influenza B [19,20]. Our results showed that more virulent influenza A/H1N1 accounted for a higher mortality rate, but not a higher bacterial co-infection rate. Of the total 209 patients with influenza-associated CAP, 19.6% had community-acquired bacterial co-infections. Our rate was in line with the rates of 11–35% for bacterial co-infections although the criteria for defining a co-infected patient and the criteria for setting study enrollment varied by study [6]. Most of the previous studies have been done on critically ill patients in ICU. Unlike these, we included both critically and non-critically ill patients.

Some studies measured and reported on co-morbidities of patients with co-infection and attempted to demonstrate that older age [21–23], obesity [7], immunosuppression [7], chronic obstructive pulmonary disease (COPD) [24] and diabetes [25] were risk factors for bacterial co-infection. Risk factors varied widely even contradicted reciprocally in these studies. On the contrary, Blyth et al. [5] demonstrated that patients with co-infection were younger and had fewer co-morbidities normally associated with an increased need for ICU admission. These inconsistent conclusions may be due to the heterogeneity of the enrolled patients, and detailed subgrouping, such as taking into account corticosteroid dose or COPD severity, may be an alternative way to remove confounders. Recently, Martin-Loeches et al. [26] summarized this phenomenon that although many critical determinants of susceptibility to secondary infections have been identified, most of the underlying mechanisms and interactions have not been delineated, and studies so far have had conflicting results. In our study, no differences in demographic characteristics and co-morbidities were observed between patients with and without co-infection in multivariable analysis. As enrolment including many younger people without co-morbidities unexpectedly underwent the worst outcome, which could attribute to bacterial co-infection. Analysis according to our findings suggests that determining the interaction mechanism among severe influenza infection, bacterial co-infection, human susceptibility and the immune response in combating is not easy, although understanding it is strategic for developing clinical management.

Bacterial co-infection likely predisposed patients to have a higher admission APACHE II score and SOFA score, more frequently present sepsis shock, and require invasive mechanical ventilation and ICU admission. But none of the variables was independently associated in multivariate analyses with bacterial co-infection.

Table 2
Severity assessment in influenza-associated CAP patients.

Variables	Individual influenza n = 168	Bacterial co-infection n = 41	*p* value
APACHE II score, mean ± SD	11.6 ± 6.3	14.2 ± 7.0	0.018
SOFA score, mean ± SD	3.4 ± 3.0	5.1 ± 3.8	0.006
Sepsis shock, n (%)	9 (5.4)	7 (17.1)	0.028
Invasive mechanical ventilation, n (%)	19 (11.3)	12 (29.3)	0.004
ICU requirement, n (%)	42 (25.0)	21 (51.3)	0.001

Table 3
Frequency and proportions of the pathogens isolated in hospitalized influenza-associated CAP patients with bacterial co-infections.

Pathogen	Frequency n (%) n = 41	Blood isolates n = 10	Sputum isolates n = 25	Urinary antigen n = 4	Sputum PCR n = 2	Multiple isolates[a] n = 4
Staphylococcus aureus	18 (43.9)	10 (55.6)	8 (44.4)	–	–	–
MSSA[b]	12 (29.3)	3 (25.0)	6 (50.0)	–	–	3 (25.0)
MRSA[c]	6 (14.6)	5 (83.3)	1 (16.7)	–	–	–
Streptococcus pneumoniae	11 (26.8)	–	7 (63.7)	4 (36.3)	–	–
Haemophilus influenza	3 (7.3)	–	3 (100)	–	–	–
Klebsiella pneumoniae	3 (7.3)	–	3 (100)	–	–	–
Escherichia coli	2 (4.9)	–	1 (50)	–	–	1 (50)
Pseudomonas aeruginosa	2 (4.9)	–	2 (100)	–	–	–
Mycoplasma pneumoniae	2 (4.9)	–	–	–	2 (100)	–

[a] Of the 3 MSSA cases by using multiple detective methods, one case were isolated from blood culture, valid sputum and bronchoalveolar lavage fluid; one case from blood culture and valid sputum; one case from valid sputum and bronchoalveolar lavage fluid. One *E. coli* case were isolated from valid sputum and bronchoalveolar lavage fluid.
[b] MSSA: Methicillin susceptible *Staphylococcus aureus*.
[c] MRSA: Methicillin resistant *Staphylococcus aureus*.

Table 4
Comparison of the clinical characteristics between survival and non-survival influenza-associated CAP patients.

Variables	Survivors n = 179	Non-survivors n = 30	*p* value
Age, median (IQR)	59 (47,73)	62 (50,73)	0.530
Age group, n (%)			
age <45	40 (22.3)	6 (20.0)	0.774
45 ≤ age <65	79 (44.1)	11 (36.7)	0.445
age ≥65	60 (33.5)	13 (43.3)	0.297
Sex (male), n (%)	117 (65.4)	19 (63.3)	0.829
Influenza type and subtype, n (%)			
A/H1N1	86 (48.0)	19 (63.3)	0.438
A/unclassified	63 (35.2)	7 (23.3)	0.146
B	26 (14.5)	3 (10.0)	0.325
A and B	4 (2.2)	1 (3.3)	0.914
Co-morbidities, n (%)			
Solid organ transplantation	1 (0.6)	1 (3.3)	0.267
Steroid therapy	7 (3.9)	1 (3.3)	0.999
Active cancer	6 (3.4)	0 (0)	0.579
Hemopathy	3 (1.7)	0 (0)	0.999
Chronic respiratory disease[a]	31 (17.3)	4 (13.3)	0.589
Coronary artery disease	18 (10.1)	2 (6.7)	0.804
Chronic heart failure	9 (5.0)	0 (0)	0.442
Chronic hepatopathy	6 (3.4)	1 (3.3)	0.999
Chronic kidney disease	5 (2.8)	0 (0)	0.999
Diabetes mellitus	26 (14.5)	5 (16.7)	0.978
Obesity, n (%)	13 (7.3)	3 (10.0)	0.880
Pregnancy, n (%)	3 (1.7)	0 (0)	0.999
Bacterial co-infection, n (%)	27 (15.1)	14 (46.7)	<0.001
Staphylococcus aureus	10 (5.6)	8 (26.7)	<0.001
Other pathogens	17 (9.5)	6 (20.0)	0.026
Bloodstream detected	5 (2.8)	5 (16.7)	0.001
Other site detected	22 (12.3)	9 (30.0)	0.004
Days from onset to hospitalization, median (IQR)	6 (4,7)	7 (4,9)	0.650
APACHE II score, mean ± SD	11.0 ± 5.6	18.9 ± 7.2	<0.001
SOFA score, mean ± SD	3.1 ± 2.7	7.3 ± 3.8	<0.001
Sepsis shock, n (%)	7 (3.9)	9 (30.0)	<0.001
Invasive mechanical ventilation, n (%)	17 (9.5)	14 (46.7)	<0.001
ICU requirement, n (%)	39 (21.8)	24 (80.0)	<0.001
Received previous neuraminidase inhibitors, n (%)	127 (70.9)	22 (73.3)	0.789
Neuraminidase inhibitor administration within 48 h, n (%)	26 (14.5)	2 (6.7)	0.379
Received previous antibiotics, n (%)	164 (91.6)	28 (93.3)	0.999
Received previous antibiotics days, n (%)			
1–2 days	59 (33.0)	11 (36.7)	0.691
3–5 days	74 (41.3)	9 (30.0)	0.240
6 or more days	31 (17.3)	8 (26.7)	0.224

[a] Chronic respiratory disease: asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, bronchiectasis.

Despite the failure to distinguish differences in severity of illness in subjects with and without co-infection, it is still possible that co-infection would contribute to severe disease. This opinion coincided with the findings by Martin-Loesches et al. [23] that patients with bacterial co-infection were more likely to require invasive ventilation and vasopressors. Besides, the delayed hospitalization was not independently associated with bacterial co-infection and mortality, but the patients with delayed hospitalization seemed to need more invasive mechanical ventilation at the first 24 h after hospitalization ($Z = 2.064$; $p = 0.039$) and ICU admission ($Z = 2.823$; $p = 0.005$). The timing of hospitalization in our study closely match that of peak illness (at day 6 post-influenza infection)

Table 5
Independent predictors for in-hospital mortality in hospitalized patients with influenza-associated CAP.

Variable	aHR	95% CI	*p* value
age <45	Ref	–	0.004
45 ≤ age <65	2.080	0.737–5.867	0.166
age ≥65	5.161	1.855–14.363	0.002
SOFA score	1.177	1.069–1.295	0.001
ICU requirement	6.172	2.153–17.690	0.001
Bacterial co-infection	3.037	1.401–6.583	0.005

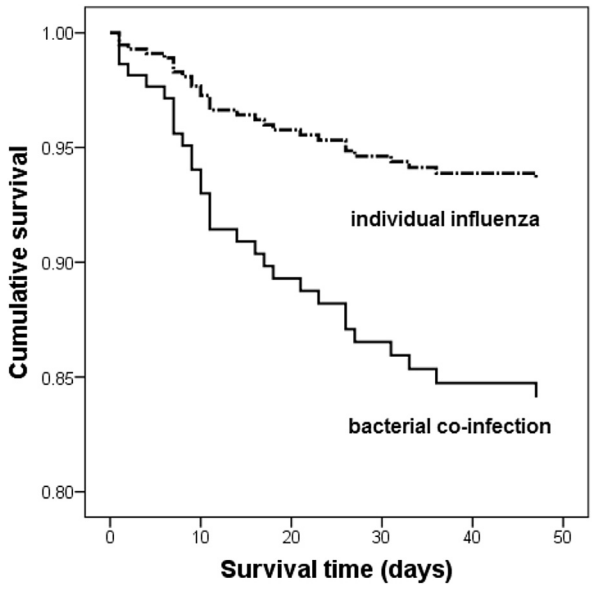

Fig. 1. Survival curves for influenza-associated pneumonia patients with and without community-acquired bacterial co-infection. (Censored at 50 days).

observed in rhesus macaques model [27]. It is also interesting to note that the frequency of males enrolled in the analyzed cohort was higher than that of females. The time interval from symptom onset to hospitalization of males (7 (5,8) days) was longer than that of females (5 (4,7) days), although there was no significant difference of the time interval between males and females ($Z = 1.895$; $p = 0.058$). We speculated that males customarily experience a longer delay to hospitalization because of their tolerance for disease, which account for more severe cases. This may be a possible epidemiological explanation for differences in sex constituent ratio. The ratio of male patients in our cohort (65.1%) was similar to that in a multi-center study including 2901 ICU patients (59.1%) [7]. Whether male patients are more likely to develop severe cases and the pathophysiological mechanisms need further exploration.

Of the 30 dead patients, we found that the mortality rate was higher in the elder age group than it was in the younger age group. Although no differences were found between survivors and non-survivors, age was identified as an independent risk factor for mortality. Besides, 14/30 (46.7%) non-survivors had community-acquired bacterial co-infection, and it was identified as an independent predictor for in-hospital mortality. Nevertheless, previous studies have provided conflicting results regarding the impact of co-infection on patient outcomes. Martin-Loesches et al. [23] found no significant association between co-infection and ICU mortality after adjustment for confounding factors. Subsequently, Cillóniz C et al. [24] demonstrated that bacterial co-infection did not influence mortality of these patients. Blyth et al. [5] later demonstrated that no significant difference in outcomes was observed. Recent researches seem to have reversed that view. A retrospective study analysing 507 U.S. patients found that bacterial co-infection, especially with *S. aureus*, was associated with significantly higher mortality [11]. Another prospective multicentre study involving 2901 ICU patients performed in Europe showed that co-infection in critically ill patients with influenza was an independent risk factor for ICU, 28-day and in-hospital mortality [7]. The heterogeneity among these studies may attribute to the diversity of co-pathogens, and different bacterial strains result in different outcomes. The changes in epidemiology over the years and the local differences, lead to different proportions of co-pathogens detected in each epidemic or pandemic influenza monitoring data. The predominant bacterial strain will drive the outcome consistent with its own pathogenic characteristics. Further intensive studies on co-infection should be deep into the level of correlation between bacterial strains and prognosis.

S. pneumoniae was not detected by blood cultures in our study. A multi-center study focusing on utility of blood culture among children hospitalized with CAP showed that among the 2568 children in whom a blood culture was obtained, 51 (1.99%) demonstrated growth of *S. pneumoniae*, 46 (92%) of which were susceptible to penicillin. This study suggests that the positive rate in blood cultures of pneumococcal bacteremia is very low, and recommends that blood cultures may not be needed for most children hospitalized with CAP [28]. In our cohort, 192/209 (91.9%) patients had received previous broad-spectrum antibiotics before admission, and it might be the main reason that *S. pneumoniae* were not detected by blood cultures. Another reason might be that a blood culture was not obtained when *S. pneumoniae* was the suspected co-infected pathogen, and instead of a blood culture, a sputum culture and a urine antigen test were frequently acquired. *S. aureus* was the most prevalent causing pathogen for community-acquired bacterial co-infection, and was prone to result in bacteremia. When analysing results for each pathogen individually, we found that co-infection with *S. aureus*, especially MRSA, was associated with significant mortality. Patients with bacterial co-infected in bloodstream had an adverse outcome than those with other co-infecting sites. All the 10 positive blood cultures in our cohort attributed to *S. aureus*, half of which were MRSA. Of these 10 patients, 5 died, and 3 of them due to MRSA. Our findings supported the views that *S. aureus* has a synergistic relationship with influenza, even for people in the community [11], and there have been significant increases in the incidence of MRSA infections in the last decade, particularly community-associated MRSA [29]. Another explanation for the increased detection rate of MRSA is the redistribution of pathogenic bacteria resulted from exposure of prehospital broad-spectrum antibiotics, which was supported by our findings. These findings may explain why, as shown in our study, some young adults co-infected bacteria without any risk factor underwent the worst outcome. However, it is not very clear why young adults without risk factors are also vulnerable to bacterial co-pathogen attacks. These cases underscore the need for healthcare providers to be vigilant, especially during the influenza season, for severe cases of CAP that might be caused by MRSA [30]. A multicenter, prospective active surveillance study at 5 US hospitals with systematic detection of influenza-*S. aureus* co-infection in 2259 pneumonia patients and seasonal variation indicated that the highest levels of detection for influenza and *S. aureus* tended to cluster together in the same months [31]. Aslam N, et al. demonstrated that post-influenza pneumonia is one of the major causes of *S. aureus* bacteremia [32]. Persistent nasal carriers of *S. aureus* are predisposed to invasive disease, including secondary staphylococcal respiratory infection [33–36]; *S. aureus* may be aspirated from the nose into the lung, with the potential to cause respiratory infection and bacteremia through damaged bronchial epithelium in

a host made susceptible by the presence of influenza [37]. In addition, influenza virus primes mice for pneumonia due to *S. aureus* co-infection, which impaired the anti-influenza immune response and increased the mortality [38]. Both viral and bacterial titers increase due to alveolar macrophage impairment [39]. Synergistic mechanism of influenza virus and *S. aureus* in host deserve further exploration in translational research.

Although there is a high proportion of neuraminidase inhibitors administration pre-admission, only a few patients were administrated within 48 h of the illness onset. The constituent ratio of patients with delayed administration (>48 h) of neuraminidase inhibitor was similar between the individual influenza group and the bacterial co-infection group, and was higher in the non-survivors than that in the survivors. Although it was not been identified as an independent risk factor for mortality, this finding was consistent with the report that the delayed administration of antiviral treatment was a risk factor for in-hospital mortality [40]. There were no differences in the antiviral treatment within 48 h given to patients presenting with or without co-infection that could explain why co-infection patients experienced a worse outcome. Thus, recognition and treatment of potential bacterial co-infections is important, particularly CAP in which pathogens are difficult to detect [41]. With the increase of antibiotic time course, the positive detection rate of pathogens decreased. This was in line with the conclusion of a multi-center study, which demonstrated that bacteria were less frequently detected in culture-based tests collected after antibiotic exposure [42]. However, in subgroups of short, intermediate and long course pre-admission antibiotics, differences were neither found between the individual influenza group and the bacterial co-infection group, nor between the survivors and the non-survivors. Antibiotic exposure appears to have a little impact on the detection rate of *S. aureus*, and the significant impact is that the MSSA strains drift towards MRSA strains with the extension of antibiotic exposure time course. When bacterial co-infection occurs, it usually manifests as deterioration of the patient's condition, but is not specific enough to fully identify the progression of influenza infection itself. Therefore, influenza-associated CAP patients who manifest or have manifested signs and symptoms of exacerbation incompatible with individual influenza should be empirically treated with antibiotics targeting *S. aureus* (including MRSA), *S. pneumoniae, H. influenzae* and other Gram-negative bacterium. Our study supports Infectious Diseases Society of America (IDSA) recommendations for empiric antibiotic coverage for influenza-associated CAP [14]. To avoid overuse of antibiotics, routine cultures are advisable in patients hospitalized with influenza, particularly those started on antibiotic therapy empirically. Antibiotic therapy may then be de-escalated as necessary based on microbiological results.

Several limitations need to be addressed. First, the relatively small number of patients may limit the generalisability of our results for external validity, and further limit subgroup analysis, in particular, the co-infection strains may be a crucial factor affecting mortality. Second, bacterial detection rate in patients with CAP is generally reported to be approximately 50% [43,44], while in patients with influenza-associated CAP ranged between 11 and 35% [6]. Our rate (19.6%) is on the low side, which could possibly be underestimated since numerous referrals had a pre-admission exposure to antibiotics. Another reason is that patients in the individual influenza group with failure of valid sputum sampling or without sputum sampling would be left with limited chance to be detected with bacterial co-infection. Third, the influenza vaccination history was usually not available in medical records, preventing any conclusion on this point. Fourth, co-infection was defined as positive detection of bacterial pathogens in different ways, which have varying sensitivity for different organisms and potential coinfecting sites. Some difficult-to-detect bacteria may be underdiagnosed. Hence, these results may underrepresent the actual distribution of bacterial co-infected pathogens.

In conclusion, our results indicated that community-acquired bacterial co-infection was frequent in influenza-associated pneumonia, without risk factor identified yet. Bacterial co-infection was likely to predict severity, and was an independent risk factor for in-hospital mortality. Co-infection of *S. aureus* with influenza was identified as a lethal synergism, and should be targeted under consideration when developing clinical antibiotic strategies.

Declarations

Ethics approval and consent to participate

The Institutional Review Board and Medical Ethics Committee of Beijing Chao-Yang Hospital approved the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest statement

All of the authors declare that they have no competing interests.

Funding

Not applicable.

CRediT authorship contribution statement

Fei Teng: Formal analysis, Data curation, Writing - review & editing. **Xin Liu:** Data curation, Writing - review & editing. **Shu-Bin Guo:** Formal analysis, Writing - review & editing. **Zhuo Li:** Data curation, Writing - review & editing. **Wen-Qing Ji:** Data curation, Writing - review & editing. **Fang Zhang:** Writing - review & editing. **Xiao-Mei Zhu:** Writing - review & editing.

Acknowledgments

The authors would like to thank the staff of the Microbiology Laboratory, the Analysis Department and the Medical Records Department of Beijing Chaoyang Hospital for their helpful contributions to the study.

References

[1] Woźniak-Kosek A, Kempińska-Mirosławska B, Hoser G. Detection of the influenza virus yesterday and now. Acta Biochim Pol 2014;61(3):465–70.
[2] Paules C, Subbarao K. Influenza. Lancet 2017;390(10095):697–708. https://doi.org/10.1016/S0140-6736(17)301290.
[3] Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet 2018;391(10127):1285–300. https://doi.org/10.1016/S0140-6736(17)33293-2.
[4] van der Sluijs KF, van der Poll T, Lutter R, Juffermans NP, Schultz MJ. Bench-to-bedside review: bacterial pneumonia with influenza – pathogenesis and clinical implications. Crit Care 2010;14(2):219. https://doi.org/10.1186/cc8893.
[5] Blyth CC, Webb SA, Kok J, Dwyer DE, van Hal SJ, Foo H, et al. The impact of bacterial and viral co-infection in severe influenza. Influenza Other Respir Viruses 2013;7(2):168–76. https://doi.org/10.1111/j.1750-2659.2012.00360.x.

[6] Klein EY, Monteforte B, Gupta A, Jiang W, May L, Hsieh YH, et al. The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. Influenza Other Respir Viruses 2016;10(5):394–403. https://doi.org/10.1111/irv.12398.
[7] Martin-Loeches I, Schultz J, Vincent JL, Alvarez-Lerma F, Bos LD, Solé-Violán J, et al. Increased incidence of co-infection in critically ill patients with influenza. Intensive Care Med 2017;43(1):48–58. https://doi.org/10.1007/s00134-016-4578-y.
[8] Lee EH, Wu C, Lee EU, Stoute A, Hanson H, Cook HA, et al. Fatalities associated with the 2009 H1N1 influenza A virus in New York city. Clin Infect Dis 2010;50(11):1498–504. https://doi.org/10.1086/652446.
[9] Centers for Disease Control and Prevention (CDC). Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) – United States, May-August 2009. MMWR Morb Mortal Wkly Rep 2009;58(38):1071–4.
[10] Rice TW, Rubinson L, Uyeki TM, Vaughn FL, John BB, Miller 3rd RR, et al. Critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. Crit Care Med 2012;40(5):1487–98. https://doi.org/10.1097/CCM.0b013e3182416f23.
[11] Shah NS, Greenberg JA, McNulty MC, Gregg KS, Riddell 4th J, Mangino JE, et al. Bacterial and viral co-infections complicating severe influenza: incidence and impact among 507 U.S. patients, 2013-14. J Clin Virol 2016;80:12–9. https://doi.org/10.1016/j.jcv.2016.04.008.
[12] Recommendations for the post-pandemic period Pandemic (H1N1) 2009 briefing note 23. World Health Organization; 2010. http://www.who.int/csr/disease/swineflu/notes/briefing_20100810/en/index.html. [Accessed 10 August 2010].
[13] World Health Organization. CDC protocol of realtime RTPCR for influenza A(H1N1). 2009. http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html. [Accessed 10 August 2009].
[14] Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;44(Suppl 2):S27–72.
[15] Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson Jr RB, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)(a). Clin Infect Dis 2013;57(4):e22–121. https://doi.org/10.1093/cid/cit278. PMID: 23845951.
[16] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third International consensus definitions for sepsis and septic shock (Sepsis-3). JAMA 2016 Feb 23;315(8):801–10. https://doi.org/10.1001/jama.2016.0287. PMID: 26903338.
[17] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. Apache II: a severity of disease classification system. Crit Care Med 1985;13(10):818–29.
[18] Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on sepsis-related problems of the European society of intensive care medicine. Intensive Care Med 1996;22(7):707–10.
[19] Chaves SS, Aragon D, Bennett N, Cooper T, D'Mello T, Farley M, et al. Patients hospitalized with laboratory-confirmed influenza during the 2010-2011 influenza season: exploring disease severity by virus type and subtype. J Infect Dis 2013;208(8):1305–14. https://doi.org/10.1093/infdis/jit316.
[20] Cummings MJ, Bakamutumaho B, Kayiwa J, Byaruhanga T, Owor N, Namagambo B, et al. Epidemiologic and spatiotemporal characterization of influenza and severe acute respiratory infection in Uganda, 2010-2015. Ann Am Thorac Soc 2016;13(12):2159–68.
[21] Ahn S, Kim WY, Kim SH, Hong S, Lim CM, Koh Y, et al. Role of procalcitonin and C-reactive protein in differentiation of mixed bacterial infection from 2009 H1N1 viral pneumonia. Influenza Other Respir Viruses 2011;5(6):398–403. https://doi.org/10.1111/j.1750-2659.2011.00244.x.
[22] Dimopoulos G, Tsiodras S, Lerikou M, Chranioti Aik, Perros E, Anagnostopoulou U, et al. Viral profile of COPD exacerbations according to patients. Open Respir Med J 2015;9:1–8. https://doi.org/10.2174/1874306401509010001.
[23] Martín-Loeches I, Sanchez-Corral A, Diaz E, Granada RM, Zaragoza R, Villavicencio C, et al. Community-acquired respiratory coinfection in critically ill patients with pandemic 2009 influenza A(H1N1) virus. Chest 2011;139(3):555–62. https://doi.org/10.1378/chest.10-1396.
[24] Cillóniz C, Ewig S, Menéndez R, Ferrer M, Polverino E, Reyes S, et al. Bacterial co-infection with H1N1 infection in patients admitted with community acquired pneumonia. J Infect 2012;65(3):223–30. https://doi.org/10.1016/j.jinf.2012.04.009.
[25] Cordero E, Pérez-Romero P, Moreno A, Len O, Montejo M, Vidal E, et al. Pandemic influenza A(H1N1) virus infection in solid organ transplant recipients: impact of viral and non-viral co-infection. Clin Microbiol Infect 2012;18(1):67–73. https://doi.org/10.1111/j.1469-0691.2011.03537.x.
[26] Martin-Loeches I, van Someren Gréve F, Schultz MJ. Bacterial pneumonia as an influenza complication. Curr Opin Infect Dis 2017;30(2):201–7. https://doi.org/10.1097/QCO.0000000000000347.
[27] Chertow DS, Kindrachuk J, Sheng ZM, Pujanauski LM, Cooper K, Nogee D, et al. Influenza A and methicillin-resistant Staphylococcus aureus co-infection in rhesus macaques - a model of severe pneumonia. Antiviral Res 2016;129:120–9. https://doi.org/10.1016/j.antiviral.2016.02.013.
[28] Neuman MI, Hall M, Lipsett SC, Hersh AL, Williams DJ, Gerber JS, et al. Utility of blood culture among children hospitalized with community-acquired pneumonia. Pediatrics 2017;140(3). https://doi.org/10.1542/peds.2017-1013. pii: e20171013.
[29] Klein EY, Sun L, Smith DL, Laxminarayan R. The changing epidemiology of methicillin-resistant Staphylococcus aureus in the United States: a national observational study. Am J Epidemiol 2013;177(7):666–74. https://doi.org/10.1093/aje/kws273.
[30] Centers for Disease Control and Prevention (CDC). Severe methicillin-resistant Staphylococcus aureus community-acquired pneumonia associated with influenza-Louisiana and Georgia, December 2006-January 2007. MMWR Morb Mortal Wkly Rep 2007;56(14):325–9.
[31] Self WH, Wunderink RG, Williams DJ, Zhu Y, Anderson EJ, Balk RA, et al. Staphylococcus aureus community-acquired pneumonia: prevalence, clinical characteristics, and outcomes. Clin Infect Dis 2016;63(3):300–9. https://doi.org/10.1093/cid/ciw300.
[32] Aslam N, Mehdi N, Izhar M. Root cause analysis of methicillin resistant Staphylococcus aureus bacteraemia. J Pak Med Assoc 2015;65(10):1089–93.
[33] von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteremia. N Engl J Med 2001;344(1):11–6.
[34] Tilahun B, Faust AC, McCorstin P, Ortegon A. Nasal colonization and lower respiratory tract infections with methicillin-resistant Staphylococcus aureus. Am J Crit Care 2015;24(1):8–12. https://doi.org/10.4037/ajcc2015102.
[35] Corne P, Marchandin H, Jonquet O, Campos J, Bañuls AL. Molecular evidence that nasal carriage of Staphylococcus aureus plays a role in respiratory tract infections of critically ill patients. J Clin Microbiol 2005;43(7):3491–3.
[36] Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet 2004;364(9435):703–5.
[37] Kash JC, Taubenberger JK. The role of viral, host, and secondary bacterial factors in influenza pathogenesis. Am J Pathol 2015;185(6):1528–36. https://doi.org/10.1016/j.ajpath.2014.08.030.
[38] Iverson AR, Boyd KL, McAuley JL, Plano LR, Hart ME, McCullers JA. Influenza virus primes mice for pneumonia from Staphylococcus aureus. J Infect Dis 2011;203(6):880–8. https://doi.org/10.1093/infdis/jiq113.
[39] Smith AM, Adler FR, Ribeiro RM, Gutenkunst RN, McAuley JL, McCullers JA, et al. Kinetics of coinfection with influenza A virus and Streptococcus pneumoniae. PLoS Pathog 2013;9(3):e1003238. https://doi.org/10.1371/journal.ppat.1003238.
[40] Muthuri SG, Venkatesan S, Myles PR, Leonardi-Bee J, Al Khuwaitir TS, Al Mamun A, et al. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. Lancet Respir Med 2014;2(5):395–404. https://doi.org/10.1016/S2213-2600(14)70041-4.
[41] Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, et al. Community-acquired pneumonia requiring hospitalization among U.S. Adults. N Engl J Med 2015;373(5):415–27. https://doi.org/10.1056/NEJMoa1500245.
[42] Harris AM, Bramley AM, Jain S, Arnold SR, Ampofo K, Self WH, et al. Influence of antibiotics on the detection of bacteria by culture-based and culture-independent diagnostic tests in patients hospitalized with community-acquired pneumonia. Open Forum Infect Dis 2017;4(1). https://doi.org/10.1093/ofid/ofx014. ofx014.
[43] Brar NK, Niederman MS. Management of community-acquired pneumonia: a review and update. Ther Adv Respir Dis 2011;5(1):61–78. https://doi.org/10.1177/1753465810381518.
[44] Bartlett JG. Diagnostic tests for agents of community-acquired pneumonia. Clin Infect Dis 2011;52(Suppl 4):S296–304. https://doi.org/10.1093/cid/cir045.