Journal of Hospital Infection 94 (2016) 143–149

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Effect of two-step hygiene management on the prevention of nosocomial influenza in a season with high influenza activity

A. Ambrosch [a,*], F. Rockmann [b]

[a] Institute of Laboratory Medicine, Microbiology and Hygiene, Barmherzige Brüder Hospital, Regensburg, Germany
[b] Emergency Department, Barmherzige Brüder Hospital, Regensburg, Germany

ARTICLE INFO

Article history:
Received 15 February 2016
Accepted 10 July 2016
Available online 18 July 2016

Keywords:
Influenza
Hospital setting
Nosocomial infection
Hygiene management

SUMMARY

Background: Rapid identification of patients infected with influenza virus, precise case definition and strict hygiene measures are important for the prevention of nosocomial transmission.

Aim: To prove the usefulness of a case definition for rapid identification of patients with influenza and to investigate the effect of two-step hygiene management, including the continuous use of surgical masks by hospital staff, on the rate of nosocomial infections.

Methods: All patients hospitalized between January and March 2015 with suspected influenza were enrolled. Real-time polymerase chain reaction testing for influenza was performed. Infected patients were managed according to the national hygiene guidelines, including the use of surgical masks by hospital staff during close contact with infected patients. When influenza activity increased, the continuous use of surgical masks by hospital staff was implemented as an add-on measure.

Findings: Most patients enrolled in this study were elderly (N=212, mean age 75 years). Frequency of cough was the only clinical parameter of respiratory infection that differed between influenza-negative and influenza-positive patients. Compared with the targeted use of surgical masks during close contact with infected patients, the continuous use of surgical masks for the entire working shift resulted in a reduction of nosocomial infections from 31% to 16%, respectively (P<0.01).

Conclusion: Discrimination between influenza A and other respiratory infections in elderly hospitalized patients was not possible based on clinical characteristics. With regard to hygiene management, the continuous use of surgical masks by hospital staff seems to be effective for the prevention of nosocomial infections.

Introduction

Seasonality of influenza is influenced by several factors, ranging from virus subtype, demographics, climate, frequency of close contacts in the population, and vaccine effectiveness. In January 2015, the Centers for Disease

* Corresponding author. Address: Institute of Laboratory Medicine, Microbiology and Hygiene, Barmherzige Brüder Hospital, Prüfeningerstrasse 86, 93049 Regensburg, Germany. Tel.: +49 941 36993640.
E-mail address: Andreas.ambrosch@barmherzige-regensburg.de (A. Ambrosch).

http://dx.doi.org/10.1016/j.jhin.2016.07.006

Control and Prevention (CDC) estimated the effectiveness of the seasonal influenza vaccine in terms of the prevention of medical consultations due to acute respiratory illness associated with laboratory-confirmed influenza.[1] A predominance of subtype H3N2 was found in 916 investigated patients infected with influenza A. The adjusted vaccine effectiveness against medical consultations in terms of acute respiratory infections was found to be 22% (95% confidence interval 5–35%) over all age groups. Therefore, it was not surprising that the German national health service reported 88,000 cases of influenza for the 2014/2015 season, which was the highest number of cases since 2001 with the exception of the 2009 influenza pandemic.[2]

Vaccine failure and low vaccine coverage could facilitate increased influenza seasonality, resulting in a higher healthcare and economic burden.[3–5] Although children below 5 years of age are at higher risk of influenza,[6,7] elderly people are at risk of serious complications and hospitalization.[8] In a season with high influenza activity, the capacities of emergency departments and critical care units are rapidly exceeded due to increased hospitalization rates, particularly of patients aged >65 years, and the appearance of the majority of cases within a few days or weeks.[9] In addition, the hospital environment facilitates nosocomial infections.[10,11] Optimal hygiene management is essential for the prevention of nosocomial infections and outbreaks, including rapid and precise identification of patients with influenza. Therefore, a clinical case definition is provided by national and international healthcare services.[8] In addition, rapid, highly sensitive molecular testing is now available.

This prospective, monocentric, observational study investigated the 2014/2015 season with high influenza activity and the management of infected patients at Hospital Barmherzige Brüder. Patient characteristics were evaluated in terms of their usefulness for case definition. Focusing on nosocomial infections, the effect of two-step strict hygiene management, including the continuous use of surgical masks for hospital staff, was evaluated.

Methods

Study characteristics

Data were obtained from a prospective observational study focusing on the hygiene management of hospitalized patients with confirmed influenza over a six-week period of high influenza activity. The study hospital is a 900-bed tertiary care teaching hospital in Regensburg, Germany.

Patients

All patients with a positive influenza A/B test at symptom onset were included in the study. The decision to perform influenza testing was made by the attending physician based on a working case definition given by the CDC, including fever ≥37.8 °C, cough and/or sore throat.[8] During the study period, patients with non-specific symptoms including fatigue, headache, enteritis or pneumonia were also tested, even in the absence of symptoms in the case definition. To validate the clinical symptoms of the influenza A group, polymerase chain reaction (PCR)-negative patients with similar clinical symptoms were recruited as comparators. PCR-negative patients were enrolled continuously for the first five days of the observation period.

First period of infection prevention management

An infection control team was implemented within the first week of increased influenza activity. Daily meetings were held to assess the actual data and rate of influenza infections. Contact precautions were introduced, including strict alcohol-based disinfection of hands and the use of surgical masks by hospital staff during close contact with patients with influenza. Patients infected with influenza were isolated, and isolation management was conducted according to the causative strain. Two separate medical wards with a total of 57 beds were declared as isolation areas for patients with influenza. According to national guidelines, a patient was considered to be non-infectious seven days after the onset of symptoms. General administration of antiviral therapy for patients with influenza, and preventive antiviral therapy for non-influenza patients or hospital staff were not recommended.

Second period of infection prevention management: continuous use of surgical masks

On 9th February 2015, hygiene management was expanded to include the continuous use of surgical masks by hospital staff from the beginning to the end of each working shift. Continuous use of surgical masks was particularly directed at staff working in areas with patients with influenza, in diagnostic areas (i.e. radiology, ultrasound), and for staff responsible for the in-house transportation of patients. Visitors were not restricted, and the use of masks by visitors was optional.

Hygiene management was supervised and controlled by specially trained hygiene staff. The transmission of influenza was classified as nosocomial when infection occurred after two days of hospitalization.[8]

Influenza testing

Nasopharyngeal and throat swabs (UTB-RT kit, Copan, Italy) were taken from symptomatic individuals, particularly when patients arrived at the emergency department. Reverse transcription polymerase chain reaction for influenza A/B was performed with the commercial Xpert Flu test on a GeneXpert platform (Cepheid, Sunnyvale, CA, USA). This assay allows for differentiation between influenza B and A (pandemic 2009 H1N1/seasonal H1N1/H3N2). As results were obtained after 70 min, patients remained in the emergency department until their test results were available.

Statistics

All analyses were performed using Statistical Package for the Social Sciences Version 16.0 (IBM Corp., Armonk, NY, USA). Results were calculated as mean ± standard deviation. Comparisons between means of the groups were performed by analysis of variance and subsequent post-hoc range tests. A two-tailed probability value <0.05 was considered to indicate statistical significance.

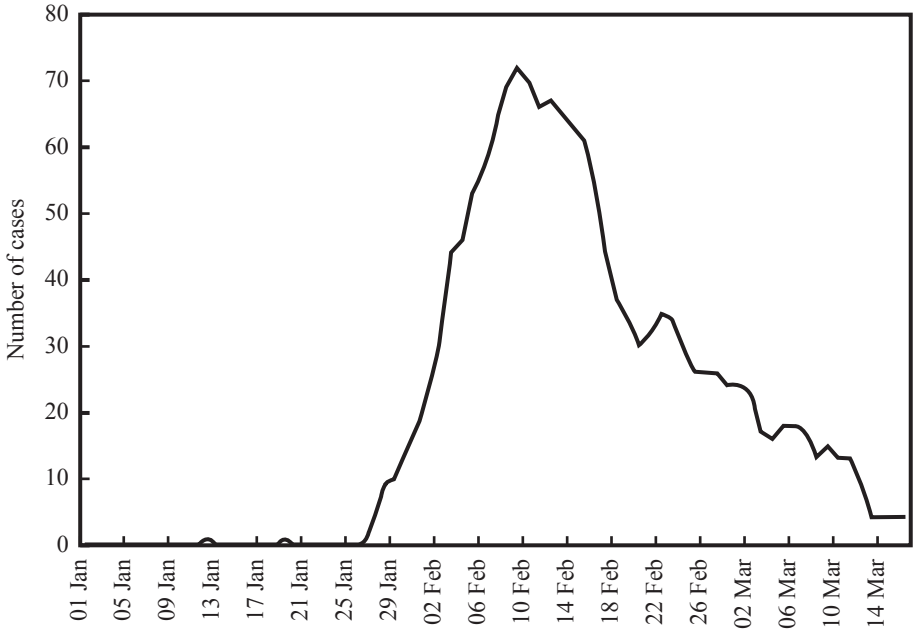


Figure 1. Timeline of hospitalized patients with influenza A/B (N=235). The rapid increase of patients within a few days was characteristic of the 2014/2015 influenza season and challenged the capacity and hygiene management of the emergency department.

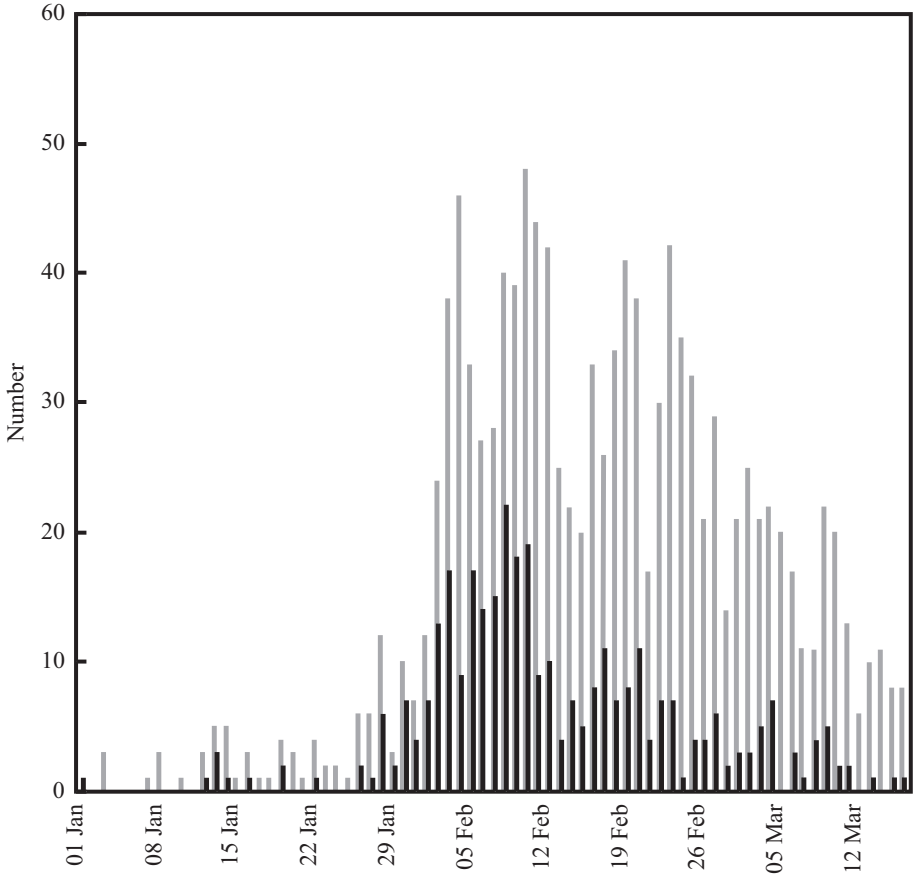


Figure 2. Timeline of influenza polymerase chain reaction testing. The bar chart shows the total number of influenza tests (grey bars) and the number of influenza-A-positive results (black bars) by date. Influenza activity was highest for the first 10 days, reflected by an influenza positivity rate between 50% and 55% (peak 66%), and this subsequently decreased below 50%.

Results

Timeline of hospitalized patients and results of influenza testing

In total, 235 patients with PCR-confirmed influenza A or B were identified between 25th January and 14th March 2015. The timeline for hospitalized patients is given in Figure 1.

Among all positive samples, the frequency of influenza A was 90% (N=212); seasonal H1N1/H3N2 influenza (N=195, 92%) accounted for the majority of cases, and pandemic 2009H1N1 influenza accounted for the rest (N=17, 8%). Influenza B was detected in 10% of positive samples (N=23). Infectious pressure was highest for the first 10 days of increased influenza activity, as reflected by a positivity rate of 66% for PCR tests, and this subsequently decreased below 50% (Figure 2). During this 10-day period, 67 patients were hospitalized until 14th March 2015; in total, 156 patients with influenza required hospitalization.

Clinical characteristics of patients with influenza A in the hospital setting (Table I)

Most patients with influenza A (N=212) were aged >65 years (mean age 75 ± 14 years). The most common symptom was a dry cough, present in nearly half of these patients. Over the observation period, the total mortality rate of patients with confirmed influenza was 10%. When patients with influenza A were compared with the PCR-negative group with similar clinical symptoms (N=87), significant differences were observed in terms of cough frequency and fatigue. The clinical characteristics of patients with influenza A are summarized in Table I. The clinical characteristics of patients with influenza B are not displayed because of the limited number of patients.

Effect of first- and second-step hygiene management

Initially, the hygiene management of patients with influenza was performed in accordance with national guidelines, including isolation of patients in cohorts within an isolation area and use of surgical masks by staff during close contact with infected patients.

However, over the first 10 days of the observation period, 23 of 71 patients (rate 31%) developed clinical symptoms of influenza and tested positive. Most of these patients were outside the isolation area. In the second step, hygiene management was expanded by introducing mandatory continuous use of surgical masks by hospital staff. Compliance with continuous wearing of masks was high and well tolerated by the hospital staff, as reported by supervision protocols of the hygiene staff. After this intervention, the nosocomial rate was nearly halved. Only 24 of 146 patients developed nosocomial influenza until the end of the observation period (16% vs 31% in the first step; P<0.01) (Figure 3).

Table I
Clinical characteristics of hospitalized patients with confirmed influenza A [polymerase chain reaction (PCR)-positive] compared with a PCR-negative control group. The control patients were enrolled continuously during the first five days of the observation period. For all patients, the indication for influenza testing was provided by an attending physician in the case of suspicion of influenza based on clinical symptoms

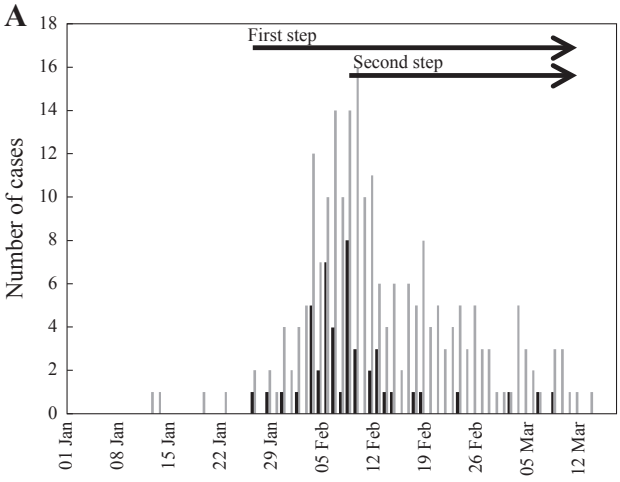
Characteristics	Influenza A/PCR+ adults (N=212)	PCR- adults (N=87)	Significance (P)
Age in years (mean ± SD)	75 ± 15	73 ± 14	n.s.
Sex, male/female	44/56	46/54	n.s.
Clinical symptoms			
Mean temperature in °C (mean ± SD)	37.6 ± 1.1	37.6 ± 1.4	n.s.
Temperature >38 °C	30	32	n.s.
Cough	48	23	<0.05
Headache	9	11	n.s.
Fatigue	17	35	<0.05
Enteritis	9	16	n.s.
Pneumonia	17	14	n.s.
Mortality	10	10	n.s.

SD, standard deviation; n.s., not significant.
All parameters given as % unless otherwise stated.

Discussion

This study demonstrated that a season with high influenza activity can be characterized by rapid accumulation of cases with suspected influenza in a short period of time, a positive rate for influenza testing >50%, and an increasing hospitalization rate of infected patients. Consequently, influenza has a significant effect on emergency department visits, morbidity rates and mortality rates, particularly in the hospital setting.[10,12] As influenza is highly contagious and there are limited isolation ward capacities, it is important to discriminate between patients with true influenza and those with influenza-like illnesses or other respiratory infections very early in the emergency department. For this, real-time testing and a clear case definition, including clinical symptoms such as fever ≥37.8 °C, cough and/or sore throat, may be useful. In this study, a case definition based on clinical symptoms was not effective for the identification of seasonal influenza in the hospital setting. In the internal evaluation of specific clinical symptoms, patients with influenza were compared with a group of PCR-negative patients with respiratory/general clinical symptoms, as shown in Table I. No significant differences in the clinical symptoms of respiratory infections could be found, with the exception of frequency of cough. However, the mild clinical profile demonstrated for the patients with influenza could be attributed to the age of hospitalized patients and the influenza subtype found during the 2014/2015 season. With regard to the age of patients, several studies have investigated the significance of clinical signs in elderly. Falsey *et al.* postulated that clinical decision rules using the presence of cough and fever may be helpful, particularly when a lower threshold value for fever is considered.[13] Their study investigated a total of 2410 subjects. A receiver operating curve (ROC) analysis examining various temperature thresholds combined with cough found the optimal balance between sensitivity and specificity to be at 37.3 °C for older people. In a systematic review of 12 studies using clinical decision rules for the diagnosis of influenza, Ebell and Afonso reported that fever, cough and acute onset had only modest accuracy in adults with an ROC of 0.79, but summary estimates could not be made due to

A



B

Time period	Use of surgical mask	Nosocomial rate (nosocomial/influenza)	Significance (*P*)
Up to 8th February 2015 (first step)	During close contact with patients with influenza	0.31 (23/71)	
9th February to 5th March 2015 (second step)	Continuous use by all hospital staff	0.16 (24/146)	<0.01

Figure 3. (A) Analysis of hospitalized influenza cases. The bar chart shows patients with newly diagnosed influenza A (grey bar) and nosocomial influenza A (black bar) at time of onset. Influenza-positive patients were managed according to national hygiene recommendations, including the use of surgical masks during close contact with infected patients (first step). As a high rate of nosocomial cases was observed during the first 10 days of the observation period, hygiene management was expanded (second step) from 9th February 2015 by introducing continuous use of surgical masks by all hospital staff. (B) Summary of the effects of hygiene management on nosocomial influenza infections. The rate of nosocomial infections decreased significantly from 0.31 to 0.16 ($P<0.01$) after introducing continuous use of surgical masks by hospital staff.

the heterogeneity of the studies.[14] In addition, clinical symptoms may also be influenced by the influenza subtype. In a population-based study by Carrat *et al.*, influenza A/H3N2 was associated with temperature >38.2 °C, myalgia, rhinorrhoea and cough, whereas infections caused by seasonal A/H1N1 were associated with fatigue, lacrimation or conjunctival injection.[15] In a recent study by Petridis *et al.*,[16] no significant differences in symptoms were found between an older study population with influenza A/H3N2 (mean age 62 years) and patients with other respiratory infections. This is in line with the findings of the present study.

However, when a clinical case definition is shown not to be useful due to lack of specificity, a sensitive and specific testing method with a rapid turnaround time should be used to identify patients with influenza. Gene Xpert Flu displayed optimal sensitivity and specificity for the detection of seasonal and pandemic influenza A and B.[17–19] Generally, rapid bedside testing for influenza using antigen assays is also possible and has high specificity.[20] Although these tests are more rapid and less expensive than PCR, sensitivity depends to a greater degree on the quality of the specimen and the viral titre. The latter depends on the duration of illness and the amount of virus being shed, which decreased rapidly three days after the onset of clinical symptoms.[21,22] Therefore, it is imperative to recognize that a negative antigen test does not exclude influenza. For example, in emergency departments, it is recommended that critically ill patients with a syndrome consistent with influenza and a negative rapid antigen test result should receive a confirmatory reverse transcription PCR.[23]

Nosocomial influenza is recognized as an emerging issue with an increasing number of outbreaks. Three reviews have underscored the importance of influenza in the hospital setting.[10,11,24] The present prospective data demonstrate an increasing rate of nosocomial influenza, particularly within the first 10 days of influenza activity, with nearly every third new influenza case being nosocomial. In general, such observations could be due to a high rate of patients at risk of

influenza, the rate of potentially infected hospital staff, the vaccination rate of patients and staff (not documented in the present study), and compliance with hygiene management. With regard to hospital staff, it has been shown that infected staff members with mild influenza can continue working and therefore contribute to transmission of the virus.[25] In two studies, hospital staff were documented as index patients, indicating that they were likely to acquire the influenza virus in the community.[26,27] As the overall sickness rate increased among hospital staff during the first days of February (data not shown), continuous use of surgical masks was introduced for medical staff in wards where patients with influenza were nursed and in wards with a high turnover of patients. Thereafter, the number of nosocomial cases of influenza decreased by nearly 50%. However, this decrease appeared in parallel to the decrease in epidemiological pressure and the number of patients at risk, which may also have influenced the nosocomial rate.[24] Therefore, this effect cannot be attributed to the change in hygiene management, but it is recognized that influenza can be transmitted via droplets.[28–30] Patients with influenza wearing medical masks exhaled fewer infectious droplets compared with those not wearing a mask.[31,32] Consequently, a meta-analysis of interventions to reduce influenza transmission highlighted the potential importance of facemasks, and the modest efficacy of hand hygiene.[33] Although national guidelines recommend that healthcare personnel with clinical respiratory symptoms should not work, there is a period of several hours when an individual is contagious before the development of symptoms during the natural course of infection. Furthermore, a significant rate of PCR-positive healthcare workers can be totally asymptomatic (16.7%), as shown by Esbenshade *et al.*[25] Therefore, the continuous use of surgical masks for medical staff is an easy, well tolerated, effective and easy-to-check hygiene measure in the hospital setting to prevent nosocomial influenza at times of high influenza activity. Further studies are needed to identify and evaluate potential parameters that indicate increased risk for nosocomial infection, and to define the critical point for enhancement of hygiene management.

This study had a few limitations. The activity of the infection control team with daily reporting on the influenza situation led to increased awareness of hospital staff regarding influenza and suspected patients. This may have affected the frequency of testing and staff behaviour in terms of management of patients with infuenza. In addition, it is known that a population becomes more immune during the course of an influenza season due to a boosting effect of cross-reactive antibodies.[34] All these confounders may influence the transmission and the course of influenza in the clinical setting.

Taken together, this prospective observation from the 2014/2015 season demonstrates that a case definition for identification of influenza is poor for the detection and exclusion of influenza in the hospital setting. Therefore, rapid testing using highly specific and sensitive PCR methodology improves active case finding in the emergency department, which is an important factor that contributes to the subsequent optimization of hygiene measures. Furthermore, the probable effect of the continuous use of surgical masks by hospital staff to prevent nosocomial infections was underlined, which supported the potential role of staff in the transmission of transmission.

Conflict of interest
None declared.

References

1. Brendan F, Clippard J, Zimmermann RZ, *et al*. Early estimates of seasonal influenza vaccine effectiveness — United States, January 2015. *MMWR* 2015;**64**:10–15.
2. Robert Koch Institut. Influenzameldungen gem. IfSG in der Saison 2014/2015 unter besonderer Berücksichtigung von Ausbruchsgeschehen in Altenheimen/Pflegeinrichtungen. *Epidemiol Bull* 2015;**28**.
3. Turbelin C, Pelat C, Boelle PY, Levy-Bruhl D, Carrat F. Early estimate of 2009 pandemic influenza A (H1N1) virus activity in general practice in France: incidence of influenza-like illness and age distribution of reported cases. *Euro Surveill* 2009;**14**:pii=19341.
4. Simmermann JM, Chittaganpitch M, Levy J, Chantra S, Maloney S. Incidence, seasonality and mortality associated with influenza pneumonia in Thailand: 2005–2008. *PLoS One* 2009;**4**:e7776.
5. Kiro A, Robinson G, Laks J, Mor Z, Varsano N. Morbidity and the economic burden of influenza in children in Israel: a clinical, virologic and economic review. *Harefuah* 2008;**147**:960–965.
6. Silvennoinen H, Peltola V, Vainionpaa R, Ruuskanen O, Heikkinen T. Incidence of influenza-related hospitalization in different age groups of children in Finland. *Pediatr Inf Dis J* 2011;**30**:e24–e28.
7. Paget WJ, Baklerston C, Casas I, Doker G, Edelman I. Assessing the burden of pediatric influenza in Europa: the European Pediatric Influenza Analysis (EPIA) project. *Eur J Pediatr* 2010;**169**:997–1008.
8. Fiore AE, Ujeki TM, Broder K. Prevention and control of influenza with vaccines: recommendation of the Advisory Committee on Immunization Practices (ACIP) 2010. *MMWR Recommen Rep* 2010;**59**:1–62.
9. Schanzer D, Schwartz B. Impact of seasonal and pandemic influenza on emergency department visits, 2003–2010, Ontario, Canada. *Acad Emerg Med* 2013;**20**:388–397.
10. Salgado CD, Farr BM, Hall KK, Hayden FG. Influenza in the acute hospital setting. *Lancet Infect Dis* 2002;**2**:145–155.
11. Stott DJ, Kerr G, Carman WF. Nosocomial transmission of influenza. *Occup Med* 2002;**52**:249–253.
12. McDonnell WM, Nelson DS, Schunk JE. Should we fear 'flu fear' itself? Effects of H1N1 influenza fear on ED use. *Am J Emerg Med* 2012;**30**:275–282.
13. Falsey AR, Baran A, Walsh EE. Should clinical case definitions of influenza in hospitalized older adults include fever? *Influenza J* 2015;**9**(Suppl. 1):23–29.
14. Ebell MH, Afonso A. A systematic review of clinical decision rules for the diagnosis of influenza. *Ann Fam Med* 2011;**9**:69–77.
15. Carrat F, Tachet A, Rouzioux C, Housset B, Valleron AJ. Evaluation of clinical case definition of influenza: detailed investigation of patients during the 1995–1996 epidemic in France. *Clin Infect Dis* 1999;**28**:283–290.
16. Petridis D, Zarogulidis P, Kallianos A, *et al*. Clinical differences between H3N2 and H1N1 influenza 2012 and lower respiratory tract infection found using a statistical classification approach. *Ther Clin Risk Man* 2014;**10**:77–86.
17. Salez N, Ninove L, Thirion L, *et al*. Evaluation of the Xpert Flu test and comparison with in-house real-time RTPCR assays for detection of influenza virus from 2008 to 2011 in Marseille, France. *CMI* 2012;**16**:e81–e83.
18. Yang JH, Huang PY, Shie S, Huang CG, Tsao KC, Huang CT. Diagnostic capacity of rapid influenza antigen test: reappraisal with

experience from the 2009 H1N1 pandemic. *J Microbiol Immunol Infect* 2012;**45**:102—107.
19. DiMaio M, Sahoo MK, Waggoner J, Pinsky PA. Comparison of Xpert Flu rapid nucleic acid testing with rapid antigen testing for the diagnosis of influenza A and B. *J Virol Meth* 2012;**186**:137—140.
20. Apisarnthanarak A, Mundy LM. Rapid testing for pandemic influenza A (H1N1): diagnostic test utility and specimen source. *Infect Control Hosp Epidemiol* 2010;**31**:663—664.
21. Landry ML. Diagnostic tests for influenza infection. *Curr Opin Pediatr* 2011;**23**:91—97.
22. Randolph AG, Agan AA, Flanagan RF, *et al*. Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) PICFlu Study Group. Optimizing virus identification in critically ill children suspected of having an acute severe viral infection. *Pediatr Crit Care Med* 2016;**17**:279—286.
23. Abraham M, Perkins J, Vilke G, Coyne C. Influenza in the emergency department: vaccination, diagnosis and treatment: clinical practice paper approved by American Academy of Emergency Medicine Clinical Guidelines Committee. *J Emerg Med* 2016;**50**:536—542.
24. Voirin N, Barret B, Metzger MH, Vanhems P. Hospital-acquired influenza: a synthesis using the Outbreak Reports and Intervention Studies of Nosocomial Infection (ORION) statement. *J Hosp Infect* 2012;**186**:137—140.
25. Esbenshade JC, Edwards KM, Esbenshade AJ. Respiratory virus shedding in a cohort of on-duty health care workers undergoing prospective surveillance. *Infect Control Hosp Epidemiol* 2013;**34**:373—378.
26. Horcajada JP, Pumarola T, Martínez JA, *et al*. A nosocomial outbreak of influenza during a period without influenza epidemic activity. *Eur Respir J* 2003;**21**:303—307.
27. Malavaud S, Malavaud B, Sandres K, *et al*. Nosocomial outbreak of influenza virus A (H3N2) infection in a solid organ transplant department. *Transplantation* 2001;**72**:535—537.
28. Hall CB. The spread of influenza and other respiratory viruses: complexities and conjectures. *Clin Infect Dis* 2007;**45**:353—359.
29. Bischoff WE, Swett K, Lang I, Peters TR. Exposure to influenza virus aerosol during routine patient care. *J Infect Dis* 2013;**207**:1037—1046.
30. Zerr DM, Mistone AM, Huskins WC, Bryant KA. The past, present and future of health care associated infection prevention in pediatric viral respiratory infections. *Infect Control Hosp Epidemiol* 2010;**31**(Suppl. 1):S22—S26.
31. Blachere FM, Lindsley WG, Pearce TA. Measurement of airborne influenza virus in a hospital emergency department: virus in a hospital emergency department. *Clin Infect Dis* 2009;**48**:438—440.
32. Milton DK, Fabian MP, Cowling EU. Influenza virus aerosols in human exhaled breath: particle size, culturability, and the effect of surgical masks. *PloS Pathog* 2013;**9**:e1003205.
33. Wong VWY, Cowling BJ, Aiello AE. Hand hygiene and risk of influenza virus infections in the community: a systematic review and meta-analysis. *Epidemiol Infect* 2014;**142**:922—932.
34. Blümel B, Dehnert M, Buda S, *et al*. Age related prevalence of cross reactive antibodies against influenza A (H3N2) variant virus, Germany 2003—2010. *Eurosurveillance* 2015;**20**:16—24.