Journal of Infection (2017) 74, 377–384

BIA
British Infection Association

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Haemagglutinin and neuraminidase sequencing delineate nosocomial influenza outbreaks with accuracy equivalent to whole genome sequencing

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Accepted 19 December 2016
Available online 16 January 2017

KEYWORDS
Influenza;
Genomics;
Outbreak;
Healthcare associated infection;
Renal transplantation

Summary *Objectives:* We describe haemagglutinin (HA) and neuraminidase (NA) sequencing in an apparent cross-site influenza A(H1N1) outbreak in renal transplant and haemodialysis patients, confirmed with whole genome sequencing (WGS).
Methods: Isolates were sequenced from influenza positive individuals. Phylogenetic trees were constructed using HA and NA sequencing and subsequently WGS. Sequence data was analysed to determine genetic relatedness of viruses obtained from inpatient and outpatient cohorts and compared with epidemiological outbreak information.
Results: There were 6 patient cases of influenza in the inpatient renal ward cohort (associated with 3 deaths) and 9 patient cases in the outpatient haemodialysis unit cohort (no deaths). WGS confirmed clustered transmission of two genetically different influenza A(H1N1)pdm09 strains initially identified by analysis of HA and NA genes. WGS took longer, and in this case was not required to determine whether or not the two seemingly linked outbreaks were related.
Conclusion: Rapid sequencing of HA and NA genes may be sufficient to aid early influenza outbreak investigation making it appealing for future outbreak investigation. However, as next generation sequencing becomes cheaper and more widely available and bioinformatics

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http://dx.doi.org/10.1016/j.jinf.2016.12.015

software is now freely accessible next generation whole genome analysis may increasingly become a valuable tool for real-time Influenza outbreak investigation.

Background

Influenza A(H1N1)pdm09 has been associated with severe disease and high mortality in young adults, including those who lack major co-morbidities.[1] Transplant recipients have an increased risk of severe disease and death from influenza.[2,3] In addition, prolonged viral shedding in immunosuppressed individuals may increase the risk of viral transmission and the development of resistance to antiviral agents such as oseltamivir.[3,4] This highlights the need for rapid identification and control of healthcare associated influenza outbreaks. Similarly to multi-locus sequence typing (MLST) and variable number tandem repeat (VNTR) analysis in bacteriology, real-time polymerase chain reaction (RT-PCR) lacks the resolution to accurately determine transmission chains, rule out non-outbreak cases and associate clusters; all crucial information for timely outbreak control. WGS of healthcare associated infections has advanced our understanding of hospital transmission and acquisition of *Staphylococcus aureus*, delineated MRSA outbreaks and demonstrated the importance of multiple hospital introductions and asymptomatic carriage in Clostridium difficile infection.[5–7] WGS techniques have shown great promise for outbreak investigation of an ever-increasing number of infectious pathogens[8–10] and additional deep sequencing has further demonstrated transmission dynamics in healthcare associated Norovirus infection.[11] WGS of Influenza virus has largely been used for epidemiological studies[12–14] with smaller outbreaks employing focal HA and NA sequencing.[15–18]

HA and NA sequencing has been employed to determine evolutionary characteristics of the 2009 Influenza A(H1N1) pandemic,[19] attempt to link genotypic data to disease severity[20] and to understand smaller outbreaks and local epidemiology.[4,15,16,21,22] It has been helpful to understand a Influenza B outbreak on a cruise ship with one fatal case,[23] and investigate a Influenza A(H1N1) outbreak on a children's haematology ward.[4] Notably within the context of Influenza A(H1N1), haemagglutinin (HA) and neuraminidase (NA) gene sequencing was utilized to identify a single site, nosocomial influenza outbreak in HIV infected patients, unexpectedly linking an immunocompetent individual previously thought to be unrelated to the outbreak.[17] HA and NA sequencing also identified five distinct clusters caused by separate imported strains of Influenza A(H3N2) within an apparent outbreak in a geriatric hospital in Switzerland.[18] Whole genome sequencing of influenza A(H1N1)pdm09 during the 2009 pandemic enabled better understanding of the genetic epidemiology in the UK and USA[13,14] it has also been used to identify an outbreak of variant H3N2 in association with probable swine-human transmission at an agricultural fair,[24] but it has not been widely used to investigate smaller scale outbreaks. No studies have directly compared the clinical utility of WGS to the more focal sequencing of HA and NA genes in Influenza outbreaks.

Relatively low levels of influenza activity occurred in the UK during the 2013–14 season. Increased hospital outbreaks and intensive care admissions were seen with the predominant circulating strain, A(H1N1)pdm09.[25] Early in 2014 we experienced simultaneous influenza A(H1N1) pdm09 outbreaks in a regional renal transplant unit (inpatient setting) and a community haemodialysis centre (outpatient setting). We report the utility of partial (HA and NA) and whole genome sequencing, where results were available after the clinical outbreak, but in sufficient time to aid the on-going public health investigation. The outbreak triggered a regional targeted vaccination program of renal transplant and haemodialysis patients to increase vaccine uptake in this vulnerable group facilitated by Public Health England (PHE).

Methods & outbreak summary

The first outbreak case of influenza A(H1N1)pdm09 was confirmed in week 08 2014 on a nasopharyngeal sample from an inpatient on a renal transplant unit in the South of England, using RT-PCR (Qiagen Artus® Infl./H1 LC RT-PCR on the Qiagen EZ1, Qiagen, Hilden, Germany) (Fig. 1, Inpatient 1). The patient was moved from a four-bedded bay into a side room after developing an undifferentiated febrile illness in the preceding 24 h. Unfortunately the patient was non-adherent and frequently left isolation without respiratory precautions. Contact tracing ensued and a total of twenty-nine patient and staff contacts were identified. Of those contacts, five additional symptomatic inpatients tested positive by RT-PCR for influenza A(H1N1)pdm09 virus (Fig. 1, Inpatients 2–6). Two members of staff had confirmed influenza A (H1N1)pdm09, one having developed symptoms shortly after nursing the index case and the second following social contact with the first staff member (Fig. 1 Ward Staff 1 & 2). A community contact of the first staff member also developed symptoms and had confirmed influenza A(H1N1)pdm09 infection (Table 1A case 9).

Influenza positive individuals were treated with oseltamivir or zanamivir depending on their level of immunocompromise and vulnerable inpatients and staff contacts received antiviral prophylaxis according to UK Public Health England (PHE) guidance. As the majority of patients were moderately or severely immunocompromised, whole ward screening was carried out, with no additional cases identified. Only two of the six infected inpatients, neither staff member nor the community contact had received the 2013–14 influenza vaccine. Despite pro-active outbreak management and escalation of clinical care, three of the six influenza infected inpatients subsequently died. All inpatients remained isolated throughout their admission