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Persistence of influenza on surfaces

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SUMMARY

Background: Close contact transmission (either direct or large droplet/droplet nuclei) is considered the main driver of influenza outbreaks but there is limited information regarding the role of fomites in transmission.

Aim: To investigate the surface stability of influenza strains and thereby the role of fomites in transmission.

Methods: The viability and quantitative reverse transcription–polymerase chain reaction (qt-RT–PCR) signal of five influenza strains (A/PR/8/34/H1N1, A/Cal/7/09/H1N1, A/Cal/4/09/H1N1, A/Sol/54/06/H1N1, and A/Bris/59/07/H1N1) seeded on to three surfaces (cotton, microfibre, and stainless steel) were assessed over time. Coupons of material were seeded with 10 μL of a 10^6–10^8 pfu/mL suspension of cell culture-derived virus stock supplemented with 0.3% bovine serum albumin. Coupons were assayed by plaque assay and qt-RT–PCR at 1, 24 h, and weekly for seven weeks using a vortex-mixing elution method.

Findings: Viable virus was detected from coupons for up to two weeks (stainless steel) and one week (cotton and microfibre), whereas detection of viruses by PCR was made for the entire seven-week study period. No strain differences were found.

Ninety-nine percent reduction values (as a function of the seeding stock) were determined to be 17.7 h for cotton ($R^2 = 0.86$), 34.3 h for microfibre ($R^2 = 0.80$), and 174.9 h for stainless steel ($R^2 = 0.98$).

Conclusion: Viable influenza was recovered from surfaces for up to two weeks. By contrast, influenza could be detected by PCR for more than seven weeks. These results have important implications for determining infection control protocols, cleaning regimes and sampling methods in healthcare settings.

Introduction

Transmission of influenza by indirect contact may occur when infectious particles are deposited on to hand-touch surfaces which act as a vector for transfer of infectious particles to the mucous membranes of a susceptible individual. However, the evidence for transmission via this route is limited, with the vast majority of evidence supporting close contact transmission, either by the direct route, or by airborne transmission of large respiratory droplets or droplet nuclei.[1] The transmissibility of infectious droplets which land on surfaces has primarily been investigated by researchers sampling various environments for traces of influenza. Influenza virus RNA has been found on numerous surfaces, although how this relates to infectious virus deposition is unknown.[2,3] The

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detection of infectious virus from surfaces has been more limited and is potentially constrained by low collection efficiencies associated with swab sampling and relatively high detection limits of conventional methods to enumerate influenza viruses.[2]

In this study, surface-dried suspensions of influenza were assessed for persistence by plaque assay (viability) and quantitative reverse transcription–polymerase chain reaction (qt-RT–PCR) (copy number) without using environmental swabs. Three test surfaces were chosen; stainless steel, microfibre cloth, and cotton bedsheets. Stainless steel was chosen to simulate a non-porous hospital surface. The porous cotton bedsheet was chosen to investigate the potential persistence of influenza respiratory secretions in a hospital or home environment, and the microfibre cloth was chosen as it is frequently used for cleaning in both hospital and home environments.

Several virus strains (influenza A virus PR/8/34/H1N1, Cal/7/09/H1N1, Cal/4/09/H1N1, Sol/54/06/H1N1, or Bris/59/07/H1N1) were tested to establish whether there were strain differences in survival on surfaces.

Methods

Preparation of surfaces

Stainless steel coupons (grade 316) were prepared by cleaning with Decon 90 (SLS, Hove, UK), washing three times with demineralized water and autoclaving at 126°C for 21 min. Microfibre cloth sections (Black & Decker, Slough, UK), were cut into 1 cm^2 portions and autoclaved at 121°C for 21 min. Cotton bedsheets (Value Range, Boots plc, Nottingham, UK) were cut up into 1 cm^2 portions and autoclaved at 121°C for 21 min.

Standard curve

RNA from cell culture-grown stock cultures of influenza A/Bris/59/07/H1N1 (NIBSC), influenza A/Cal/4/09/H1N1 (NIBSC), influenza A/Cal/7/09/H1N1 (NIBSC), influenza A/PR/8/34/H1N1, influenza A/Sol/3/06/H1N1 (NIBSC), and influenza A/Wis/67/05/H3N2 (supplied by Retroscreen, London, UK) were extracted using the QIAmp Viral RNA Extraction Kit (Qiagen, Manchester, UK). The full-length influenza segment 7 was amplified by RT–PCR using primer Uni12 (5′AGCAAAAGCAGG3′) with the SuperScript III reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions. cDNA was produced using the PCR primers GRAM-1Fw (5′AGCAAAAGCAGGTAGATATATTGA3′) and GRAM-1027Rvw/T7 (5′*GAAATTAATACGACTCACTATAGGG*AGTAGAAACAAGGTAGTTTTTTACTC3′) using the Phusion high-fidelity DNA Polymerase kit (New England Biolabs, Inc., Ipswich, MA, USA) as per the manufacturer's instructions.

Correct band size (~1027 bp) was confirmed by gel electrophoresis after which the Ambion MEGAscript kit (Life Technologies, Carlsbad, CA, USA) was used to synthesize large amounts of RNA as per the manufacturer's instructions. The RNA transcript was purified using an RNeasy mini kit (Qiagen) RNA clean-up protocol into 50 μL of RNase free H_2O (Qiagen) and the copy number calculated following determination of the RNA transcript concentration with a Nanodrop ND100 spectrophotometer.

All qt-RT–PCR reactions were performed on 96-well plates using the superscript III platinum one-step qt-RT–PCR kit (Invitrogen) and run on the Applied Biosystems 7900 real-time PCR system as previously described.[4] Each 96-well plate consisted of the relevant virus standard curve (a 10-fold dilution series of the RNA transcript in H_2O), a negative control (molecular grade water) and samples run in duplicate. Each of the standard curves quantified RNA concentrations down to 40 copies per 25 μL reaction.

Cells and viruses

Madin Darby Canine Kidney cells (MDCKs) (Cat. No.: 85011435, European Collection of Cell Cultures) were maintained, and used to grow and titrate the human influenza virus strains as previously described.[5,6] Upon harvesting, the viral suspension was aliquotted into 2 mL working stocks and stored at −80°C.

Surface survival study

The stainless steel, cotton, and microfibre coupons were inoculated with 10 μL of either influenza A virus PR/8/34/H1N1 (1.3×10^8 pfu/mL), Cal/7/09/H1N1 (3.25×10^6 pfu/mL), Cal/4/09/H1N1 (4.5×10^6 pfu/mL), Sol/54/06/H1N1 (5.0×10^6 pfu/mL), or Bris/59/07/H1N1 (1.3×10^7 pfu/mL) supplemented with 0.3% bovine serum albumin (BSA) (Sigma–Aldrich, Gillingham, UK), and dried in a class 2 cabinet for 1 h. Half of the coupons were placed in the dark (within a sealed, opaque container) and the remainder left in the light in a position sealed loosely in a plastic Petri dish. A USB temperature and humidity probe was placed inside the sealed container and alongside the samples left in the light to monitor environmental conditions every 24 h over the course of the experiment. Two of each set of coupons were assayed for virus viability at 1 h, three coupons were assayed at 24 h and then each week until week 7. Coupons were assayed by immersion in 2 mL of serum-free media (Dulbecco's modified Eagle's medium) in a 5 mL bijoux and then vortex mixing, to elute virus from the material into the media for 30 min. This vortex-mixing elution method was chosen to avoid the use of environmental sampling swabs which typically have low recovery efficiencies.[2] The samples were then immediately frozen at −80°C until processing. Prior to processing, samples were removed from the −80°C, defrosted rapidly at 37°C and vortex-mixed for 20 min. Samples were then assayed using plaque assay methodology as previously described, and RNA samples were extracted and stored at −80°C for analysis by influenza real-time quantitative PCR.[6]

Calculations

Log_{10} reductions between the starting titre and the titre at 1 h were calculated using the following equation:

$$Log_{10}\ \text{reduction} = \log(\text{seeding titre}/\text{titre at 1 h}).$$

Statistical analysis

Statistical analysis was performed with Minitab 16 and Sigma plot using general linear model and linear regression analysis tools.