

## Editorial

# Investigating the airborne transmission pathway – different approaches with the same objectives

The advent of molecular diagnostics has allowed the investigation of the airborne transmission pathway to progress significantly in the past 10–12 years, since the 2003 outbreaks of severe acute respiratory syndrome (SARS). Much of this progress has been driven by researchers in some of the most affected countries, namely Hong Kong, Singapore, and Canada. Perhaps equally inspiring is that the researchers who have produced this wealth of new data have come from several different, traditionally quite separate disciplines and teams, including engineering, epidemiology, public health, hospital physicians, and infection control teams.

At the time of writing, the current number of people infected by Ebola virus in the ongoing West African outbreak is estimated to be 15351, with 9596 laboratory confirmed and 5459 deaths (CDC, 2014). Concerns about the potential for possible airborne transmission of Ebola virus have been expressed in a detailed online commentary (CIDRAP, 2014b), and a similar one has been published regarding a recently emerged, novel respiratory virus, Middle East respiratory syndrome coronavirus (MERS-CoV) (CIDRAP, 2014a). The authors of these commentaries, particularly in the one on Ebola, have urged for a new approach to assessing the potential ‘aerosol transmissible’ capability of infectious agents produced by human respiratory activities performed by infected patients, and not to rely on assessments based on older, outdated experimental data. These authors almost certainly refer to the various, modern, technical advances in both pathogen detection as well as airflow visualization that have had a major impact on how the more modern studies have been conducted, which clearly separates them from the older, traditional studies. In particular, in diagnostic testing, using molecular methods (such as the polymerase chain reaction – PCR – as well as DNA sequencing), have allowed the detection of fewer and fewer organisms in both clinical and environmental samples which do not necessarily have to be viable at the point of testing (like those obtained by air sampling), which has altered how indoor air quality studies are now performed, and perhaps our awareness of the richness and variety of the organisms in the air around us (Blachere et al., 2009; Booth et al., 2005; Gangneux et al., 2006; Knibbs et al., 2014; Suzuki et al., 2004).

Such molecular methods that detect specific targets in the pathogen genome (or nucleic acid) suffer from the disadvantage of not being able to distinguish inactivated from truly viable organisms. In addition, this level of sensitivity in the pathogen detection method raises some double-edged concerns. Although such sensitive assays can allow us to detect naturally occurring infectious agents earlier and at much lower concentrations, which may improve the assessment of ventilation and containment efficacy (at least in a hospital infection control setting), this needs to be balanced against whether such low levels of the pathogen are actually clinically relevant, and the level of clinical relevance will depend on the specific patient population, for example immunocompetent vs. immunocompromised, pediatric vs. adult, and hospitalized vs. community patients.

On the engineering side, the increasing availability and accessibility of higher resolution temporal and spatial, Schlieren/shadowgraph, and other particle capture/visualization methods using high-speed cameras (Clark and de Calcina-Goff, 2009; Nishimura et al., 2013; Settles, 2001; Tang et al., 2009, 2011, 2013) or laser-based imaging (e.g. particle image velocimetry, PIV) (Chao et al., 2009; Hui et al., 2006; Pantelic et al., 2009; Tang and Settles, 2008; VanSci-ver et al., 2011; Wan and Chao, 2007; Zhu et al., 2006), and increasingly accurate particle counters, (Chao et al., 2009; Edwards et al., 2004; Fabian et al., 2008; Morawska et al., 2009; Yang et al., 2007) have allowed the detailed characterization and quantitation of droplets expelled during various forms of human respiratory exhalation flows. In addition, the improved design and sensitivity of modern air samplers (Blachere et al., 2007, 2011; Cao et al., 2011; Fabian et al., 2009) and various innovative and novel droplet capture methods (Hatagishi et al., 2014; Huynh et al., 2008; McDevitt et al., 2013; Stelzer-Braid et al., 2009), coupled with these more sensitive molecular techniques, have allowed more pathogens to be detectable at lower concentrations.

So together with the increased molecular detection methods above, it is now possible to characterize the potential pathogen content of different sizes of particles/droplets produced during different human respiratory activities.

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Complementary to these advances in diagnostic and experimental technologies has been the massive advance in integrated chip design that have vastly increased the affordability of high-performance computational power to the extent that high-resolution, accurate computational fluid dynamics (CFD) modeling, related to human infection control issues, can now be performed on desktop machines (Beggs et al., 2008; Choi and Edwards, 2012; Gupta et al., 2011; Yam et al., 2011). The key issue (as perhaps it has always been) is the validation of these models with equally detailed and comprehensive experiments – and to ensure that these experiments and models (and their assumptions) are actually relevant to everyday, real-life situations.

### Source concentrations of pathogens potentially present in human respiratory aerosols

Diagnostic testing for respiratory pathogens in the human upper respiratory tract (URT) using nasal, nasopharyngeal, mouth, and throat swabs is now the norm in most hospitals, clinics, and diagnostic laboratories. Quantifying the amount of pathogen present in such samples is more difficult as the pathogen concentration in these sampling sites can vary over time as well as locally, in different sampling sites. However, in general, these pathogen loads are very high in the acute phase of illness, (e.g.  $10^8$ – $10^{12}$  viral RNA copies/ml for influenza, although the actual proportion that represents viable viruses is uncertain) (Chu et al., 2004; Hatagishi et al., 2014; Lee et al., 2011; Milton et al., 2013; Tang et al., 2014; To et al., 2010).

### Presence of potentially transmissible organisms in the 'airborne' environment

Various means have been used to capture, characterize, and quantify exactly how much pathogen (nucleic acid only or viable organisms) is present in droplets of differing sizes, when they are expelled using different respiratory modalities, for example, using specially designed masks, capture cones or boxes, etc. So there are now a number of published studies describing the detailed airborne characteristics (i.e. the number of organisms per droplet of different sizes, produced by different human respiratory activities) for a few pathogens, particularly influenza and tuberculosis (TB) (Blachere et al., 2009; Fabian et al., 2008, 2009; Fennelly et al., 2004; Hatagishi et al., 2014; Lindsley et al., 2010a,b, 2012; Milton et al., 2013; Stelzer-Braid et al., 2009; Wainwright et al., 2009; Yang et al., 2011). These data give us an idea of the type and number of organisms (or at least the viral or bacterial RNA/DNA copies/ml collection fluid) present in the 'airborne' environment, that is the ambient air from which people inhale, with the potential risk of inhaling an airborne

respiratory pathogen that could lead to infection and disease. Although most of these data are in the form of nucleic acid detection, a few have also cultured live pathogens from air samples (particularly influenza).

Such air-sampling studies are always disadvantaged in that the air-sampling procedure itself may inactivate a proportion of the viable organisms due to shear stress damage induced during the air-sampling suction (Verreault et al., 2008; Zuo et al., 2014), so although nucleic acid counts may be relatively accurate, any culture-based quantitation may well be an underestimate. Nevertheless, over the past 10 years, there have been more and more studies using air sampling, with the development of newer, more sensitive, and less 'traumatic' samplers (Koehler et al., 2012; Sleeth and Vincent, 2012; Yao and Mainelis, 2007; Zhou and Cheng, 2010).

From all of these studies, the message is quite clear – that such infectious agents are in the air, a proportion of which are viable and potentially able to cause infection and disease. However, given the different immunological makeups of those that are exposed, the number of people actually contracting infection and disease from such infectious agents is difficult to estimate. Although many modeling and experimental studies have tried, the validation of such estimates is difficult and the results from such studies can be inaccurate at best and misleading at worst. Conclusions drawn from such imperfect studies are often used by public health teams to estimate potential incidence rates and therefore to guide their preventative and/or interventional policies, for example, the tough criticism of the WHO regarding recommendations to stockpile the anti-influenza drug, oseltamivir ('Tamiflu'), in response to early fears of a much more severe 2009 H1N1 pandemic (BMJ, 2014).

Most recently, it is emerging from a few studies on influenza that the diagnostic load may not be an accurate baseline for estimating the actual number (or concentration) of the same organism expelled from the same host (patient) during normal respiratory activities (breathing, coughing, sneezing, etc.) (Hatagishi et al., 2014; Milton et al., 2013; Tang et al., 2014). In fact, the difference in viral loads is of the order of several  $\log_{10}$  lower in the airborne droplet compared to the diagnostic oral swab, when the samples are taken from the same patient, within minutes to hours of each other. This is an intriguing finding and requires further confirmation, but, if confirmed, suggests that just using the diagnostic viral load, normally expressed in viral RNA copies/ml, as a basis to estimate the influenza viral concentration in exhaled droplets of different sizes/volumes, may be misleading.

If proven, this further suggests that influenza (and perhaps other pathogens) may not be as transmissible as initially believed via the aerosol route – although certainly, epidemiologically, in each influenza season,

it is well documented that a significant proportion of the population (though rarely more than 20–30% in a typical, non-pandemic, season) do become infected with the virus in the absence of effective immunization (Chan et al., 2013; Kamigaki et al., 2014; Silva et al., 2014; Wong et al., 2010).

### **The final part of the airborne transmission pathway – the inhalation of an airborne pathogen**

Ultimately, it is this last ‘recipient inhalation’ step of the airborne transmission pathway that all the upstream interventions are striving to reduce, for example interventions such as treating the patient to reduce the viral load available for shedding, masking the patient and/or isolation (in negative pressure rooms) of infectious patients at source, the higher air exchange rates, and more efficient ventilation modalities, including the use of HEPA (high-efficiency particulate air) filters to remove airborne pathogens from built environments or public transport, such as planes and trains (including subway/underground trains).

Yet, once an inhalable airborne pathogen arrives at a susceptible host and enters his/her inhalation zone, what other factors may interfere/interact with this final inhalation step? The human thermal plume? Individual variations in human anatomy or body morphology? Clothing? Body position/posture (standing or lying supine, like sleeping)? Proximity to fresh air sources, such as open windows or personalized ventilation systems? Recent, renewed interest in the human thermal plume, using experimental visualization methods, suggests that it may act as a sort of natural air curtain, but that it is easily penetrated. It may also act to move contaminants upwards toward the upper part of the room, but again, this is potentially double-edged phenomenon in that it may convect contaminants from lower levels into the inhalation zone, as well as moving contaminants into the inhalation zone of others, where they are positioned above one’s head height (Clark and de Calcina-Goff, 2009; Craven and Settles, 2006; Licina et al., 2014; Licina et al., 2015; Murakami, 2004; Tang et al., 2009; Voelker et al., 2014).

Some highly pathogenic viruses, such as avian influenza A/H5N1 virus, have a differential distribution of their receptor sites within the human respiratory tract between the upper and lower (LRT) airways, with more receptors for this virus being present in the LRT (Matrosovich et al., 2004; van Riel et al., 2006; Shinya et al., 2006). Also, there is an accumulating evidence that the newly emerged Middle East respiratory syndrome coronavirus (MERS-CoV) has a preference for infecting the LRT rather than the URT, and diagnostic testing for this virus has given false-negative results on URT samples, where the LRT samples have tested positive (Memish et al., 2014). Therefore, knowing where different droplet sizes deposit within the human

URT and LRT after inhalation may be useful to determine the likelihood of infection and subsequent disease.

Studies on drug delivery have already modeled (with sufficient experimental validation) the differential distribution and deposition of different inhaled particle sizes within the human respiratory tract. Some of these models are based on anatomically accurate geometries (albeit rigid) having been obtained from real patient CT-scan images that have been converted to 3-D printed models of the upper and part of the middle/lower respiratory tract (i.e. up to 3 bifurcations below the level of the trachea) (Borojeni et al., 2014; Ruzycski et al., 2014; Yeh and Schum, 1980; Zhang et al., 2007). Thus, it may be useful to apply some of these methods and results to assess the likely impact of different sized droplets carrying different viral loads settling in different parts of the human airway, for specific pathogens where the distribution of their receptors is known.

### **Conclusions**

The new data that have emerged recently in this multi-disciplinary field have demonstrated (though some may argue about the strength of some of the evidence) that (1) high concentrations of respiratory pathogens (detectable by both molecular and culture methods) are present in the upper human respiratory tract (URT) in acutely unwell individuals, which are then available for expulsion in aerosols generated by everyday respiratory activities, such as breathing, coughing, sneezing; (2) several pathways (which are not independent) are available for transmission, depending on the size of these potentially pathogen-laden droplets, including short-range ballistic movements for the larger droplets (for both direct transmission for inhalation and/or via fomites and direct contact inoculation to mucous membranes) and longer range carriage for the smaller droplet nuclei that follow the airflow streamlines (mainly via direct inhalation, although settling and transmission via the fomite-inoculation route are also possible). Obviously this is a continuous spectrum. Most recently, new evidence is emerging for some pathogens (particular influenza) that the expelled droplets in such naturally generated aerosols may, in fact, carry far less pathogen than was present in the original infected host compartment (usually the URT/oropharyngeal cavity). This latter finding needs further confirmation, but if true, it may require a rethink of current infection control concerns and interventions for such pathogens; (3) finally, relatively little work has been done examining the inhalational phenomenon and the relevant infectious doses in this airborne transmission context. However, in a related field – that of drug delivery – much modeling and experimental work has already been performed, examining how to adjust particle sizes, concentrations, and modes of delivery to

optimize their deposition and therefore their required effect in the human respiratory tract. Some of these data can be applied to investigations into this final stage of the airborne transmission pathway. Specifically, this may be of relevance if the receptor distribution within the respiratory tract for specific pathogens is known.

Given the above, the various infection control interventions, with a specific impact on airborne transmission, should be implemented and be assessed for effectiveness using various air sampling and pathogen detection methods. Ideally, endpoint measures should be applied to assess the effectiveness of these interventions in a real-life context, for example any overall reduction in hospital- or workplace-acquired infections. However, due to the variously variable pathogen incubation periods, it is noted that such workplace-acquired infections may be difficult to separate from home- and community-acquired infections, unless carefully controlled ‘clinical trial-like’ studies are also

performed to assess the effectiveness of individual interventions.

This is an exciting time for these interdisciplinary collaborations, with groups working in different areas (sometimes being unaware of how their work can contribute), ultimately contributing to the same final goal, to understand and characterize this important, yet perhaps least well understood, route of transmission of infectious agents.

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