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Brief report

Performance evaluation of filtering facepiece respirators using virus aerosols

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Physical penetration and infectivity penetration of adenovirus and influenza virus aerosols through respirators were measured to better characterize the effectiveness of filtering facepiece respirators against airborne virus. A physical penetration of 2%-5% was found. However, large sample-to-sample variation made it difficult to quantify the difference in physical penetration caused by the different virus aerosols. Infectivity penetration of adenovirus was much lower than physical penetration, indicating that the latter provides a conservative estimate for respirator performance.

Respiratory protection devices, such as N95 respirators, were widely used as an infection control measure in recent outbreaks of H5N1 and the H1N1 influenza pandemic. However, assessing the efficacy of respirators and other personal protective equipment in reducing the airborne transmission of influenza is a research gap that remains to be filled.[1]

The performance of respirators against virus aerosols has been widely investigated, but most previous studies used a challenge aerosol of MS2 bacteriophage,[2-4] the physical characteristics do not closely represent those of human pathogenic viruses. Two recent studies measured the penetration of influenza virus aerosol through N95 respirators[5,6]; however, data on the particle size of the generated virus aerosol and physical penetration were not reported. In the present study, we compared the physical penetration and virus infectivity penetration through respirators. In addition, we examined whether the physical penetration depended on the type of challenge virus aerosol used.

METHODS

Three models of commercially available filtering facepiece respirators were used for this study. Models A and C were National Institute for Occupational Safety and Health–certified N95 respirators, and model B was not. Before the test, the periphery of each respirator was sealed onto a Plexiglas plate with a circular hole in the center. The Plexiglas plate was then sandwiched between gaskets and held in a test tunnel.[7]

Human adenovirus serotype 1 and swine influenza H3N2 virus (SIV) were obtained from the Veterinary Diagnostic Laboratory at the University of Minnesota. Virus aerosol was generated using a 6-jet Collison nebulizer operated at 10 psi, loaded with 5 mL of thawed virus stock diluted in 45 mL of minimum essential medium with 0.1 mL of antifoam A. The virus aerosol thus generated was passed through a ^{210}Po charge neutralizer, mixed with relative humidity-controlled (40%-50%) and HEPA-filtered room air, and challenged the respirator at a flow rate of 85 L/minute. Virus aerosol was sampled upstream and downstream of the respirator using a scanning mobility particle sizer (TSI, Shoreview, MN). The physical aerosol penetration was then determined as the ratio of downstream to upstream concentration and adjusted by a correction factor to address particle losses in the sampling system.[8]

Because adenovirus aerosol is much more stable than SIV aerosol (data not shown), adenovirus was selected for measurement of infectivity penetration through the respirators. An AGI-30 liquid impinger filled with 20 mL of minimum essential medium was used to collect virus aerosols for 15 min at 12.5 L/minute through the downstream sampling port both with and without a respirator in the tunnel. The sampling with a respirator measured virus concentration penetrating through the respirator, whereas the sampling without a respirator quantified the concentration of the challenge virus. Infectivity penetration was then calculated as the ratio of the 2 values. The collection liquid was analyzed by an infectivity assay, with results expressed as median tissue culture infectious dose ($TCID_{50}$).[7]

All tests were repeated in triplicate using 3 samples of each respirator model at 23-25°C. Data were analyzed by analysis of variance.

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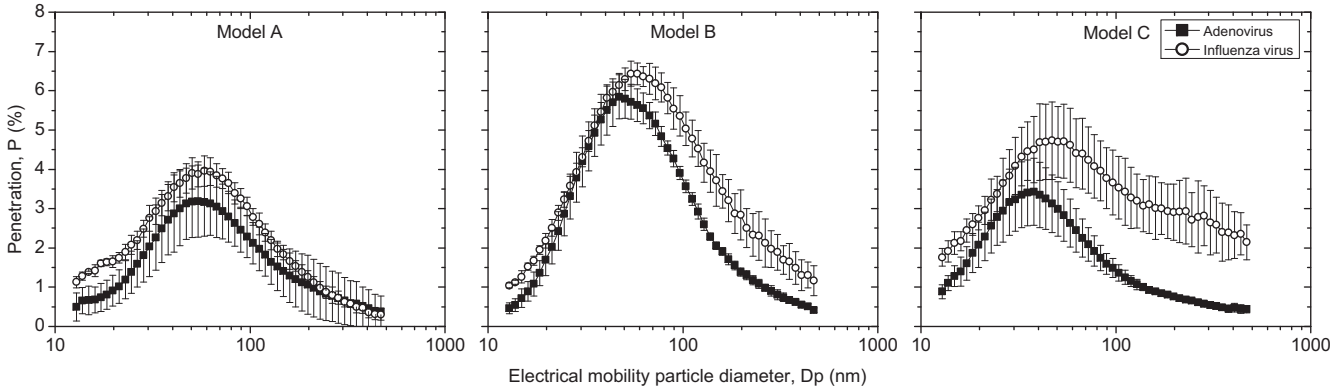

Fig 1. Physical penetration of virus aerosols through 3 models of filtering facepiece respirator as a function of particle size. Values are means with error bars representing ± 1 standard deviation based on the measurement of 3 samples of each model.

Table 1
Penetration of adenovirus and swine influenza virus aerosols

	Penetration at nominal virion size, %			Integrated penetration over the size range tested, %		
Respirator	Adenovirus, particle size 70-90 nm	Influenza virus, particle size 80-120 nm	*P* value	Adenovirus, particle size 70-470 nm	Influenza virus, particle size 80-470 nm	*P* value
Model A	2.71 ± 0.62	2.96 ± 0.24	.564	1.91 ± 0.50	2.22 ± 0.20	.381
Model B	4.73 ± 0.24	5.24 ± 0.46	.166	3.12 ± 0.16	4.19 ± 0.49	.022*
Model C	1.85 ± 0.36	3.63 ± 0.80	.024*	1.32 ± 0.19	3.30 ± 0.76	.012*

NOTE. Data are reported as mean ± standard deviation.
*$P < .05$.

RESULTS

The size distribution of adenovirus and SIV aerosols was similar, generally following a lognormal distribution with a count median diameter of 48-52 nm and a geometric standard deviation of 2.0-2.1. The non-N95 respirator (model B) demonstrated greater penetration than the other 2 models when challenged by different virus aerosols (Fig 1). The penetration curve exhibited an inverted bell shape, with the highest penetration of 3%-6.5% and the particle size that gives the highest penetration of 40-60 nm, which agrees well with results obtained using bacteriophage and inert NaCl aerosols for respirators with electret filter media.[2-4] As indicated by the error bars in Figure 1, large sample-to-sample variation was observed.

Integrated physical penetration at particle sizes representing the nominal virus size (70-90 nm for adenovirus and 80-120 nm for SIV) and larger was determined (Table 1). In all cases, SIV demonstrated greater penetration than adenovirus. The penetration difference was statistically significant ($P < .05$) for respirator model C, but not for model A, however. For model B, the difference was significant only in the larger particle size range.

The challenge adenovirus titer (measured without a respirator in the tunnel) was $10^{4.25}$-$10^{4.75}$ $TCID_{50}$/mL. However, no infectious virus was recovered once the respirators were inserted. Using the lower detection limit of the infectivity assay (10 $TCID_{50}$/mL), the infectivity penetration was measured at <0.0398% ± 0.014% for model A, 0.0480% ± 0.014% for model B, and 0.0224% ± 0.008% for model C, close to values reported elsewhere.[5]

DISCUSSION

In this study, size-dependent penetration curves were determined for the 3 models of respirators and compared using 2 virus aerosols as surrogates of human pathogenic virus. As expected, respirator performance varied among models. SIV demonstrated significantly greater physical penetration than adenovirus in some cases, possibly due in part to the different dielectric constant of viruses.[3] However, the large sample-to-sample variation may overshadow the potential penetration difference related to the type of challenge virus aerosol used. Tests using filter media with better uniformity may help address this issue.

Adenovirus aerosol exhibited an infectivity penetration approximately 2 orders of magnitude lower than the physical penetration, suggesting that the latter is a conservative estimate for evaluating respirator performance against virus aerosols. The large difference between physical penetration and infectivity penetration might be related to the fact that the scanning mobility particle sizer can readily detect particles at the nominal virion size, whereas the liquid impinger has a collection efficiency of only ~10% at 100 nm.[9] Other factors possibly contributing to the low infectivity penetration could be the low virus titer in the nebulizer, the relatively low sensitivity of the virus assay method, and the rapid decay of airborne adenovirus infectivity[10] (eg, a mean decay rate of 2.9%-3.6% per minute). With increased concentrations of challenge virus aerosol, better sampling and assay methods, and improved airborne virus stability, much higher infectivity penetration was found for MS2 aerosol.[4] The foregoing findings also may indicate that not all of the generated particles contain viable virus. Work is currently underway in our laboratory to investigate the association between virus content and its carrier particle size.

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