

Hand contamination with human rhinovirus in Bangladesh

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Abstract

As one step in developing a measure of hand contamination with respiratory viruses, we assessed if human rhinovirus (HRV) was detectable on hands in a low income non-temperate community where respiratory disease is a leading cause of child death. Research assistants observed residents in a low income community in Dhaka, Bangladesh. When they observed a resident sneeze or touch their nose, they collected a hand rinse and anterior nares sample from the resident. Samples were first tested for HRV RNA by real-time RT-PCR (rRT-PCR). A subset of rRT-PCR positive samples were cultured into MRC-5 and HeLa Ohio cells. Among 177 hand samples tested for HRV by real-time RT-PCR, 52 (29%) were positive. Among 15 RT-PCR positive hand samples that were cultured, 2 grew HRV. HRV was detected in each of the sampling months (January, February, June, July, November and December). This study

demonstrates in the natural setting that at least after sneezing or nasal cleaning, hands were commonly contaminated with potentially infectious HRV RNA. Future research could explore if HRV RNA is consistently present and is sufficiently associated with the incidence of respiratory illness in communities that it may provide a proxy measure of respiratory viral hand contamination.

Introduction

In randomized controlled trials, people who were encouraged to regularly wash their hands with soap or disinfect them with alcohol-based gels had fewer symptomatic respiratory infections compared with groups who did not receive these interventions (1-5).

The efficacy of handwashing interventions to interrupt transmission of enteric pathogens can be assessed by measuring the concentration of fecal indicator bacteria from hand rinse specimens. The concentration of these organisms are associated with the occurrence of diarrhea and so provide a proxy measurement of the efficacy of handwashing promotion interventions (6, 7). There is no analogous assay to assess the efficacy of handwashing interventions on interrupting respiratory virus transmission.

There are limited data on the impact of handwashing on specific respiratory pathogens. In one handwashing promotion trial in Cairo, Egypt, students attending schools that received the handwashing intervention had 50% fewer absences associated with confirmed influenza infections (4).

There is a rich, but inconclusive literature on the role of hand transmission of human rhinovirus (HRV) in wealthy countries in temperate climates. People with symptomatic HRV respiratory infections commonly have culturable HRV on their hands (8-10). Intervention trials to reduce hand transmission of rhinovirus have produced mixed results. Mothers randomized to treat their hands with aqueous iodine after one of their children developed symptoms of an upper respiratory tract infection were 40% less likely to develop symptomatic rhinovirus infection compared with mothers who treated their hands with a similarly colored placebo (11). By contrast adults randomized to receive a hand lotion that contained organic acids with persistent

activity against rhinovirus, were just as likely to develop rhinovirus infection compared with controls (12).

As one step in developing a measure of hand contamination with respiratory viruses, we assessed if HRV would be detectable on hands in a low income non-temperate community where respiratory disease is a leading cause of child death. As part of a randomized controlled trial that encouraged hand washing with soap or a waterless hand sanitizer and evaluated hand washing practices and fecal indicator bacteria on hands (13), we also collected hand rinse samples for HRV detection. The objective of this analysis was to assess if hands were commonly contaminated with viable HRV in these communities.

Methods

The methods of the larger trial have been reported previously (13). Briefly, this study was conducted in the Kamalapur neighbourhood of Dhaka, Bangladesh, a densely populated low income community with a high incidence of childhood respiratory disease (14). Fieldworkers identified 30 eligible housing compounds. Compounds were randomly assigned to different hand hygiene interventions, but for this analysis the data from the different groups were analyzed together.

On the day of structured observation, the research assistant arrived in the compound by 7:00 AM to observe hand-washing behavior. Upon observing a compound resident sneeze, within 10 minutes the research assistant collected one hand rinse and an anterior nares swab sample from the resident. If no compound resident had sneezed by 10:00 AM, then the research assistant collected a hand rinse and anterior nares sample from a compound resident who either picked his/her nose, coughed, had a runny nose or who entered the compound after being outside

for more than 60 minutes. We selected study subjects with symptoms of respiratory illness, because we were concerned that the rate of detection of virus from asymptomatic residents would be too low to assess.

To collect the hand rinse sample, the research assistant instructed the resident to insert his or her hand in a sterile 1 L bag containing 20 mL of viral transport media [VTM - Dulbecco's MEM containing 2.5% bovine serum albumin fraction V, 1% glutamine, 2% HEPES buffer, 1% penicillin-streptomycin (10,000 U penicillin, 10 mg/mL streptomycin) and 1.0% amphotericin B, pH 7.4] and rub his or her fingers against their palm and thumb. After collecting the anterior nares specimen, the assistant broke the swab tips off into vials that contained VTM. The bag and vials were placed on ice and transported to the ICDDR,B laboratory. In the laboratory, a technician concentrated potential HRV in the hand rinse samples by ultracentrifugation. Briefly, 14 mL of VTM wash was centrifuged at 30,000 rpm (67,000 x g) for 2 h at 4°C (Beckman SW 40 Ti), the supernatant discarded and the pellet resuspended in 1 mL of cold VTM. The enriched sample was immediately transferred to a sterile cryovial and stored at -70°C until shipping on dry ice to the U.S. Centers for Disease Control and Prevention for HRV testing.

All participants provided informed consent. The study protocol was reviewed and approved by the Ethics Review Committee of ICDDR,B. Because we added the nasal swab collection as a later amendment to the protocol, field worker did not collect nasal samples at the baseline.

Total nucleic acid was extracted from 200 µL of each swab/hand rinse sample using a NucliSENS® easyMAG® (bioMérieux). Samples were first tested for HRV RNA by an in-house real-time RT-PCR (rRT-PCR) assay (15). rRT-PCR positive samples were inoculated into MRC-5 and HeLa Ohio cells known to be permissive for HRV culture. Prior to inoculation into

cell culture, the hand rinse concentrates were extracted with an equal volume of chloroform to remove potential bacterial and fungal contaminants. Briefly, cells were grown on slanted stationary 15 mL glass tubes in Eagle's MEM growth media supplemented with 10% fetal bovine serum (Hyclone) and 30 mM Mg⁺⁺ (for the HeLa Ohio cells), pH adjusted to 7.0-7.2 (HEPES). When cells reached near confluency (~2 days), the growth media was removed and the cells washed with sterile phosphate buffered saline, pH 7.2 (Gibco). Cells were then inoculated with 200 µL of the sample and incubated on slant at room temperature with gentle rocking for 1 hr. Two mL of maintenance media (as above, but with 2% fetal bovine serum) was then added to the cells and the tubes were loaded onto a roller drum in a 33°C incubator with 5% CO₂ and rolled at low speed. Cultures were observed for cytopathic effect (CPE) at 2 day intervals for up to 10 days. If CPE was observed, cultures were frozen at -70°C and 100 µl of the cell lysate screened for HRV by rRT-PCR. If no CPE was observed, a second passage of the culture lysate was prepared (without chloroform) and inoculated as above. rRT-PCR was performed on the second passage and if negative, the sample was presumed to be culture negative for HRV.

Results

Most samples were collected from children, 53% from children under age 10 years (Table 1). The median level of education among participating adults was x. The median monthly per capita income was y.

We collected and analyzed 177 hand rinse samples and 120 nasal swab samples from 30 compounds (Table 2). Most samples, 140 (79%) hand rinse and 109 (92%) nasal, were collected after sneezing. Overall, among 177 hand rinse samples, 52 (29%) were RT-PCR positive for HRV. HRV was detected in each of the six months when samples were collected (Figure 1).

Among 120 nasal samples, 25 (21%) were rRT-PCR positive for HRV. Of the 24 nasal samples that were rRT-PCR positive and cultured, 6 (25%) grew HRV; of 15 hand rinse samples that were rRT-PCR positive and cultured, 2 (13%) grew HRV.

Among the 110 participants who had both a hand and nasal specimen tested, 20 were rRT-PCR positive for HRV on both their hand rinse and nasal specimen, 5 had HRV RNA detected only on the nasal specimen, and 6 only on the hand specimen (Table 2). Among the 6 residents whose hands were HRV rRT-PCR positive, but whose nasal specimens were rRT-PCR negative, none of their handwash samples grew HRV on culture; two samples grew non-polio enteroviruses.

Discussion

In this densely populated low income urban community where respiratory diseases are a leading cause of child death, hands are frequently contaminated with HRV after sneezing. The extent of viable HRV on the hands of study participants was likely underestimated since HRV RNA was identified more commonly, and recovery by culture is less sensitive (16, 17).

The role of hands in transmission of HRV in this community is unknown. The most common source of HRV in the present study was apparently from the study subjects' own respiratory tract. In two previous studies of patients with culturally confirmed HRV, the virus was rarely recovered from the saliva/mucus ejected by sneezing (8, 18). Although the present study did not collect information on whether study subjects covered their sneeze or cough with their hands, in a different study from Bangladesh 81% of household residents coughed or sneezed into the air, and only 11% coughed or sneezed into their hands (19), so it is likely that

these samples represent the ongoing hand contamination associated with symptomatic respiratory illness.

The concentration of thermotolerant coliforms or *E. coli* on hands provides a measure of bacterial hand contamination that is associated with the risk of diarrhea in settings where diarrhea is a leading cause of death (6, 7). This study demonstrates that at least after sneezing or nasal cleaning, hands were commonly contaminated with HRV RNA. The price of molecular detection will likely continue to decline. Future research could explore if HRV RNA is consistently present and is sufficiently associated with the incidence of respiratory illness in communities that it may serve as a proxy measure of respiratory viral hand contamination.

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Table 1. Participant characteristics

Characteristic	% (n)
Age groups (years) (N=166 [*])	
<5	34 (57)
5 - <10	19 (31)
10 - < 20	18 (30)
20 - 40	22 (36)
>40	7 (12)
Males (N=166 [*])	43 (72)
Education (years) (N=141 [†])	
none	62 (88)
1 – 5	18 (26)
>5 - < 10	18 (25)
≥10	1 (2)
Per capita monthly household income (US\$) [‡]	
< 7.5	5 (7/139)
7.5 - <75	93 (129/139)
>75	3 (3/139) ^{##}

[#]11 samples were not linked to baseline age and gender information

[†]Education analysis restricted to the 141 study subject > age 1 year

[‡]Analysis restricted to 139 households who reported income and could be linked to the sample; 1 US\$ = 66.7 Taka;

Table 2. Collected samples and HRV rRT-PCR results.

Exposure	Hand samples tested by rRT-PCR	Hand samples positive	Nasal samples tested by rRT-PCR	Nasal samples positive n (%)	Paired hand and nasal samples tested by rRT-PCR	Both hand and nasal sample positive n (%)	Nasal sample positive, but hand sample negative n (%)	Hand sample positive, but nasal sample negative n (%)
After sneezing	140	n (%) 36 (26)	110	22 (20)	109	19 (17)	3 (3)	5 (5)
After nose picking	26	14 (54)	3	1 (33)	3	1 (33)	0 (0)	0 (0)
Coughing	6	1 (17)	4	1 (25)	4	0 (0)	1 (25)	1 (25)
Runny Nose	2	1 (50)	1	1 (100)	1	0 (0)	1 (100)	0 (0)
After coming from outside	3	0 (0)	2	0 (0)	2	0 (0)	0 (0)	0 (0)
Total	177	52 (29)	120	25 (21)	119	20 (17)	5 (5)	6 (5)

Figure 1. HRV detections by month.

