Original Article

Establishment and application to environment sample test of novel coronavirus

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Background: Since the COVID-19 outbreak, the existence of SARS-CoV-2 virus in the air and objects surface has been proved, how to detect the novel coronavirus effectively and accurately has become the current focus. a detection method for new coronavirus in environmental sample should be established to provide basis for the protection and disinfection of novel coronavirus in the future.

Methods: Environmental samples were collected primarily from isolation rooms for COVID-19 patients. In the environmental samples, the air samples are collected by bioaerosol samplers. For objects surface samples, a cotton swab was moistened with the collection liquid, and then used to wipe the surface of the object once.

Results: In total, we collected 46 air samples, one of these samples' testing results was positive, which from the corridor closed to isolation rooms, and 107 object surface samples, four of these samples' testing results were positive and three were weak positive which from bathroom in the isolation rooms.

Conclusions: The detection method of the novel coronavirus in environmental samples was established successfully, which provides a basis for environmental disinfection and control of the spread of new coronavirus pneumonia. And this indicated that medical staff should strengthen personal protection, strictly implement infection prevention control measures.

Keywords: Novel coronavirus; environment samples; air; objects surface

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Introduction

Since the novel coronavirus pneumonia was discovered in late December 2019 (1), it has been widely spread worldwide, which has become a major global health problem. There were nearly one million confirmed cases and nearly 50,000 deaths recorded by 2 April 2020 (2). In December 2019, World Health Organization (WHO) provisionally named the novel coronavirus caused epidemic of Wuhan as 2019 novel coronavirus (2019-nCov) (3). On January 30, 2020, WHO announced that China's outbreak of COVID-19 has become a public health emergency of international concern, posing a high risk to countries with fragile health system (4). Novel coronavirus pneumonia is not a super spread disease (one patient spreads to many others), statistical analysis of patients diagnosed in the initial laboratory found that some patients had underlying diseases, speculating that these patients may be infected with novel coronavirus due to hospital infection (5). The activities of patients in the hospital caused pollution to the public areas of the hospital, suggesting the importance of virus detection in environmental samples. Backer *et al.* estimated that the average incubation period was 6.4 days, ranging from 2.1 to 11.1 days (2.5% to 97.5%) based on the analysis of clinical data and travel history of confirmed cases of new coronary pneumonia at the beginning of the outbreak (6). The transmission route is mainly the respiratory tract, droplets, contacts and feces, and the possibility of aerosol transmission is high strongly (3). Interpersonal communication is considered to be the main method of propagation. At present, there is no effective treatment and vaccine for COVID-19 (7). In this case, the establishment of an effective detection method for new coronavirus is particularly important for the prevention of novel coronary pneumonia.

Method

Samples and instruments

Samples source

The samples are mainly collected in the air and frequently contacted surfaces of patient wards, corridors and nurse stations on a floor of the designated hospital for New Coronary Pneumonia.

Sampling instruments

An Andersen onstage viable impactor (QuickTake-30, SKC, USA); an AirPort MD8 (Sartorius, Germany); an ASE-100 (Langsi Medical Technology, Shenzhen, China); a WA-15 (Dinglan Technology, Beijing, China); Youkangvirus-sampling kit.

Sampling methods

Sampling location, sampling time and layout principles are all implemented in accordance with DB32/T 3762-2020 *New Coronavirus Detection Technical Specifications*, which is the local standards of Jiangsu Province. Sampling locations are mainly patient wards, nurse stations, corridors, central air conditioning and ventilation system supply and fresh air vents, and outdoor air vents, surface of objects with high frequency contact and easily contaminated in bathroom. Sampling collection should be done before clean and disinfection. The principle of layout of centralized air conditioning and ventilation system shall be implemented according to WS/T 395-2012 *Code for hygienic evaluation of centralized air conditioning and ventilation systems in public places*. Sampling points are distributed uniformly with height of 1.2–1.5 m, distance from wall beyond 1 m and avoiding ventilation.

Air samples

According to the seventh part of DB32/T 3762.7-2020 *New Coronavirus Detection Technical Specifications*: *detection and evaluation of air samples*. Exhaled liquid condensate collectors were used to collect exhaled breath condensate, which principle is that low temperature condensation and strike were adopted to collect biochemical substances and aerosol particles in exhaled breath. Indoor air, air conditioning air supply, air conditioning fresh air and outdoor exhaust air were collected by an aerosol sampler, which principle is that the cyclone separation type and the impact type are combined to directly collect the particulate matter in the air into the liquid.

Samples of surface of objects

According to the eighth part of DB32/T 3762.8-2020 *New Coronavirus Detection Technical Specifications*: *detection and evaluation of samples of surface of objects*. Contamination of surfaces: surfaces may are contaminated by new coronavirus including door handles, armrests, desktops, keyboards, phones, toilet seats, toilet seats, faucets and other surfaces. Sampling coating method is applied for detecting regular surfaces: putting a 5 cm × 5 cm sterilization specification plate on the surface of the objects, applying a cotton swab to the sampling liquid and applying it five times in a horizontal and vertical direction in the specification plate, and then rotating the cotton swab to continuously collect four specifications board (100 cm^2); cotton swabs with sampling solution were applied to collect directly the irregular surfaces, the hand contact part of the cotton swabs were cut off. In order to avoid contamination during the sampling process, the environmental samples should be brought back to the clean areas, and disinfection instruments should also be prepared. After the sampling is finished, the outer surface of the sample box should be sprayed the disinfection solution with 1,000 mg/L of available chlorine, or the surfaces are wiped with an alcohol cotton ball containing 75% ethanol.

Detection methods

Nucleic acid detection methods were applied in this environmental sampling. The whole operation process was carried out in the negative pressure P2 laboratory, and was carried out simultaneously by two persons. The lysate from the sample well (first well) of the nucleic acid extraction reagent tank was aspirated into a 1.5 mL EP tube, and 20 μL of proteinase K for viral DNA/RNA extraction and 200 μL of sample were added to each tube. The EP tube was incubated at room temperature for 10 min before it was thoroughly mixed by vortexing. After the EP tube was centrifuged briefly (12,000 rpm × 10 min), the lysed sample was sucked back into the sample well (first well) of the nucleic acid extraction reagent tank. The reagent tank was put into the automatic nucleic acid extraction instrument for nucleic acid extraction. The extracted nucleic acid was taken out from instrument and was transferred it to a 1.5 mL EP tube, and the sampling location, date, sample type, etc. were attached to the EP tube. The nucleic acid tube was transferred from safety cabinet in the nucleic acid transfer box sterilized both inside and outside. And the nucleic acid was handed to the nucleic acid testing personnel.

Results

The sampling areas are mainly distributed in patient wards, corridors and nurse stations. Sampling in the wards is mainly around the public areas such as patient's beds, the bathrooms, toilets. Sampling in corridors and nurse stations is mainly concentrated in areas of frequent contact.

Distribution of positive sample results

The method of descriptive analysis was used here. A total of 46 air samples were collected from this field sampling, one of which was positive sample came from the corridor near the patient's ward. A total of 107 surface samples, seven of which were positive samples, which from door handles in the ward, toilet seats and toilet seat covers in isolation rooms, bathroom faucets, bathroom door handles.

Discussion

Since the outbreak of the new coronavirus pneumonia in Wuhan, China, in December 2019, China has adopted a series of prevention and control measures and achieved international admiration. Among the samples of this sampling, positive samples exist on the surface samples of objects and air, indicating that the isolation and detection of the SARS-CoV-2 is certainly important, but the detection of viruses in the environment should also be given sufficient attention, which also has important meaning for controlling the spread of the new coronavirus. At present, the main detection methods for SARS-CoV-2 are divided into two categories: nucleic acid detection methods and immune detection methods (8). The transmission route of the SARS-CoV-2 is mainly thought to be spread through droplets generated when patients sneeze or cough. Person-person's transmission route has also been confirmed to exist (9). In addition, aerosol is also considered to be the main source, because coronavirus can survive on inanimate surfaces for up to 9 days (10), and form infectious aerosols when exposed to air. It is verified that the virus may exist as infectious form in aerosols (11). In this sampling survey, most of the positive samples on the surface of the objects came from the toilet, which may be exhaled by patients when using the toilet, or the aerosol generated from feces or urine when the toilet was flushed. The previous detection of virus in stools also proves that virus exists in stools (12-14), as does the fact that stools obtained from the first COVID-19 patient in the United States also tested positive (15). Some diagnosed patients showed gastrointestinal symptoms, mainly vomiting and diarrhea, and infectious viruses were detected in their stools, indicating that there may be a route of fecal transmission (16). There is currently no evidence that the SARS-CoV-2 has an airborne route. An air-positive sample obtained in this sampling came from the corridor close to the isolation room, in contrast to the evidence for the airborne transmission of SARS-CoV in hospital wards that was presented by Li *et al.* in 2005 (17). It is speculated that the possible reason is that the air pressure system between the isolation ward and the corridor is imperfect, causing the air containing virus aerosol particles in the isolation room to escape to the outside. It may also come from the virus aerosol carried on the surface of the protective equipment of medical personnel.

Conclusions

In this environmental sampling survey, a new coronavirus environmental sample detection method was successfully established to provide scientific guidance to control the spread of new coronavirus pneumonia and disinfection measures in the environment. However, no positive control was used in this study, and the sensitivity and specificity of this method have not yet been calculated. Further studies will be conducted in this direction in the future.

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Footnote

Data Sharing Statement: Available at http://dx.doi.org/10.21037/jphe-20-71

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jphe-20-71). Dr. CG serves as an unpaid editorial board member of *Journal of Public Health and Emergency* from Jan 2020 to Dec 2021. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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