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Epidemiological Analysis of Nitric Oxide Nasal Spray (VirX™) Use in Students Exposed to COVID-19 Infected Individuals

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Introduction

On March 11, 2020, Coronavirus disease 19 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was declared a pandemic by the World Health Organization (WHO).[1] By December of 2021, the WHO reported that over 278 million people and just under 5.4 million deaths had been reported globally.[2] This pandemic caused quarantines and closure of many businesses and schools.

In December 2021, the Srinakharinwirot University in Bangkok, Thailand, resumed its health professions' academic operation and required its students to be onsite for classes. Students were housed in university accommodations with 4-5 students sharing private apartments, and depending on the layout of the apartment, most sleeping with 2-5 people sharing a bedroom.

January 2022, saw another wave of COVID-19 in Thailand with a prevalence of Omicron variants reaching more than 99%. The university experienced a cluster outbreak among the university students and staff. In response, the on-campus University Hospital, in collaboration with the university and following the Thai Centre for Disease Control recommendations, organized a "testing and tracing" plan, modified for the university setting to control the outbreak.

The plan called for opening two new quarantine buildings with one for confirmed COVID-19 positive students and another for "high-risk contacts" who were exposed to COVID-19 positive individuals. A high-risk contact (HRC) person was defined as a student who self-reported being in direct contact with a confirmed positive COVID-19 person for more than 5 minutes without wearing a mask, usually when both were either eating or sleeping together in the same room.

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As part of the testing and tracing plan, the university offered the high-risk contacts the option to use a nitric oxide nasal spray (VirX™, Sanotize Research and Development Corp., Canada) supplied by its distributor at no cost to them for added protection against infection. The use of VirX™ was completely voluntary and not mandated by the plan.

VirX™ has been demonstrated to be safe and was approved in Thailand in 2021 by the Ministry of Public Health as a mechanical and chemical barrier against viruses within the nasal cavities. VirX™ is a liquid nasal spray and works to protect the nasal passages with two mechanisms. A mechanical barrier is created by the use of hydroxypropyl methylcellulose (HPMC) in the VirX™ formulation. HPMC is a film forming hydrophilic matrix material that impedes the attachment of viruses to nasal host cells.

The chemical protection comes from both the acidic pH of the citric acid in the solution due to its physical forces on virus' outer envelope and membrane, and the release of nitric oxide gas from the liquid as the liquids from the two chambers mix when sprayed.

Nitric Oxide (NO) is well-known to be an efficient broad-spectrum anti-infective agent. It has been reported to have antimicrobial activity against bacteria, yeast, fungi, and viruses both in vitro and in vivo animal studies.[3-13] NO has been shown to have a direct effect on virions. NO, after nitrosylating cysteine moieties, causes conformational changes on surface glycoproteins and binds to proteases within the virus preventing viral replication.

VirX™ is supplied as a manual pump action nasal spray container with 25mL of solution. Each spray into the nostril dispenses approximately 110-120µL of the nasal solution (Figure 1). The proprietary bottle contains nitric oxide releasing solution (NORS™) that produces the nitric oxide gas, enabling long term stability of the product, so that the gas is created and released only as the spray is dispensed. As an approved product, informed consent was not required for the students to use VirX™.

Sanotize contracted with an independent group of the hospital medical staff to request access to the data for a retrospective epidemiological analysis to evaluate VirX™ safety and efficacy. This was approved by an independent institutional ethics committee.

Figure 1. Pump action of VirX™ in use.

Material and Methods

Inclusion criteria for analysis (based on student forms and self-assessment questionnaire)

- Age 18-24 years.
- Male or female students.
- If female, not pregnant (VirX™ is not approved for use during pregnancy).
- Determined by the hospital to be a high-risk contact based on the student's household and exposure risk.
- Enrolled in the university's quarantine system either at the quarantine building (majority) or home isolation (minority) for 10 days.

Exclusion criteria

- Baseline positive antigen test for SARS-CoV-2 (confirmed with 24 hours by RT-PCR).
- Used concomitant nasal sprays.

Of the 1039 students entering the program, those testing positive by rapid antigen tests had been isolated in the newly established single roomed confirmed COVID-19 student building. Reverse transcription polymerase chain reaction (RT-PCR) was performed to confirm the diagnosis as follow up after a positive rapid antigen test. The students testing negative were housed in a multi-student per room quarantine building or at home if they had their own room. After 7 days of quarantine, all 1039 students were allowed to return to classes, however, they were required to continue wearing protective masks and return to their quarantine rooms after classes for 3 more days.

VirX™ was provided to each faculty head who informed HRC students of the availability of VirX™ and distributed by staff coordinators to students who learned about the therapy and were interested in using it. Students who wanted to use VirX™ were asked to fill out a questionnaire which included demographic information, VirX™ usage, any new symptoms and follow-up information. Compliance was encouraged but not a mandatory requirement for access to VirX™. Questionnaires were approved as part of the hospital plan for recording and tracing.

Results

The investigators performed a retrospective data analysis on 1039 students who reported that they were in contact with a confirmed COVID-19 student between February 8th and April 16th, 2022 (Figure 2). They were housed in either one of the new quarantine buildings or in their own accommodations. Upon data review, 199 students were excluded due to having had low-risk of exposure, while 215 students were excluded due to a positive antigen test for COVID-19 within the first 24 hours.

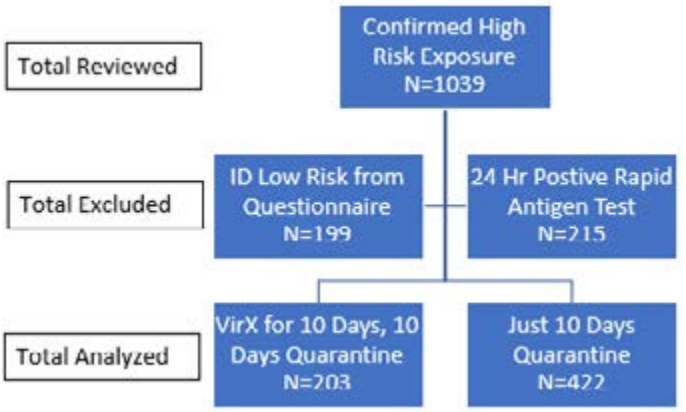

Figure 2. Population in Database

Of the 625 students not in the confirmed COVID-19 student building, 203 students had accepted using VirX™ at the manufacturer's recommended instructions of at least 4 times per day with additional doses ad lib (maximum of six doses daily per the User's Guide), and 422 students did not use VirX™. All students performed a rapid antigen test on the 5th, and 10th day, or if they developed a fever or respiratory symptoms. There was a daily record of symptoms and adverse events as part of the questionnaire.

Table 1. below shows the results of SARS-CoV-2 testing within the 10-day window of the quarantine period.

Table 1. Infection Rate Comparison (SARS-CoV-2 Test Results)

	Positive	Negative	Total
VirX™	13	190	203
Controls	108	314	422
Total	121	504	625

Of the 203 students who used VirX™, 190 tested negative for SARS-CoV-2 on all test days, and 13 tested positive on at least one test day. Of the 422 students who did not use VirX™, 314 students tested negative on all test days and 108 tested positive on at least one test day. These results demonstrated a statistically significant difference at the $p<0.0001$ level in infection rate of 6.40% (13/203) in the VirX™ group versus 25.59% (108/422) in the group that did not use VirX™.

Safety

No severe adverse events were reported. Only 34.5% (70/203) of students receiving VirX™ answered the safety questionnaire. Eight students (11.4%) reported an adverse event. All were mild, most were temporary nasal burning or irritation with VirX™ use. Two students required medical services. Abdominal pain following completion of the quarantine period was diagnosed as a peptic ulcer in a VirX™ treated student and was determined to be unrelated to its use. A second student who did not use VirX™ reported symptoms of an upper respiratory infection with chest tightness (normal SpO_2). The student tested positive for COVID-19 by RT-PCR, without lung pathology by a normal chest X-ray.

Discussion

SARS-CoV-2 is a serious and highly contagious virus that has caused a significant number of deaths and long lasting health sequela. This pandemic has had a major impact on health care

delivery as well as its economic impact worldwide. Therefore, a simple nasal spray that might reduce infection and the spread of this disease could be a major contributor to containing the pandemic. A positive social, leisure, health and economic impact could be realized as a global benefit from this therapy.

This epidemiological report suggests that use of the VirX™ nasal spray can reduce the spread of COVID-19 between individuals who are in close contact. Although the number of participants allowed for identifying a statistically significant differences, limitations of this report include that it was a retrospective analysis, and the students were not randomized. Additionally, as participation was voluntary, returned questionnaires were not mandatory. The study was open-label and not managed by a clinical trial team. The reported 11.4% incidence of adverse events is difficult to interpret. It is possible that only students who experienced the transient nasal discomfort with VirX™ reported it, which would lower the overall percentage of adverse events. However, with a mild adverse events profile and suggestions of statistically significant benefits, future studies should be conducted under a formal clinical trial structure.

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Note: VirX™ is not approved for use in the US. It is sold under the trade name of enovid™ in Israel and Indonesia, VirX™ in Thailand, Singapore, Hong Kong and South Africa, and FabiSpray™ in India.

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