Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Journal of Hygiene and Environmental Health 236 (2021) 113807

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Detection of SARS-CoV-2 RNA on contact surfaces within shared sanitation facilities

Isaac Dennis Amoah [a], Leanne Pillay [a], Nashia Deepnarian [a], Oluyemi Awolusi [a], Kriveshin Pillay [a], Preshod Ramlal [b,c], Sheena Kumari [a], Faizal Bux [a,*]

[a] Institute for Water and Wastewater Technology, Durban University of Technology, P.O. Box 1334, Durban, 4000, South Africa
[b] School of Life Sciences, University of KwaZulu-Natal, Durban, KwaZulu-Natal, 4001, South Africa
[c] eThekwini Health Department, eThekwini Municipality, KwaZulu-Natal, 4001, South Africa

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Shared sanitation
Contact surface contamination
Digital droplet PCR
Risk assessment

ABSTRACT

Contamination of contact surfaces with SARS-CoV-2 has been reported as a potential route for the transmission of COVID-19. This could be a major issue in developing countries where access to basic sanitation is poor, leading to the sharing of toilet facilities. In this study, we report SARS-CoV-2 contamination of key contact surfaces in shared toilets and the probabilistic risks of COVID-19 infections based on detection and quantification of the nucleic acid on the surfaces. We observed that 54–69% of the contact surfaces were contaminated, with SARS-CoV-2 loads ranging from 28.1 to 132.7 gene copies per cm^2. Toilet seats had the highest contamination, which could be attributed to shedding of the virus in feces and urine. We observed a significant reduction in viral loads on the contaminated surfaces after cleaning, showing the potential of effective cleaning on the reduction of contamination. The pattern of contamination indicates that the most contaminated surfaces are those that are either commonly touched by users of the shared toilets or easily contaminated with feces and urine. These surfaces were the toilet seats, cistern handles and tap handles. The likelihood (probability) of infection with COVID-19 on these surfaces was highest on the toilet seat (1.76×10^{-4}(1.58×10^{-6})) for one time use of the toilet. These findings highlight the potential risks for COVID-19 infections in the event that intact infectious viral particles are deposited on these contact surfaces. Therefore, this study shows that shared toilet facilities in densely populated areas could lead to an increase in risks of COVID-19 infections. This calls for the implementation of risk reduction measures, such as regular washing of hands with soap, strict adherence to wearing face masks, and effective and regular cleaning of shared facilities.

1. Introduction

The current COVID-19 pandemic has claimed over 3.9 million lives and infected another 184 million globally, as at 7th July 2021 (WHO, 2021). The primary mode of transmission of the SARS-CoV-2 virus, the causative agent for COVID-19, is through respiratory droplets (Chan et al., 2020; Cai et al., 2020; Bahl et al., 2020; Morawska and Milton, 2020). This has led to the implementation of mitigation measures, such as social distancing and the use of face masks (Liu and Zhang, 2020; WHO, 2020; Howard et al., 2020; Dalton et al., 2020; Viner et al., 2020). Additionally, transmission of the virus through contaminated contact surfaces has been postulated (Qu et al., 2020; Zoran et al., 2020; Jones, 2020). These are of concern due to the stability/survival of this virus on surfaces such as plastic, steel, wood and aluminium (Van Doremalen et al., 2020; Pastorino et al., 2020). Their survival on contact surfaces is dependent on the material and environmental conditions, for instance, it is reported to persist on plastics for 3–4 days at 65% relative humidity (RH) and 21–23 °C (Van Doremalen et al., 2020), aluminium for 2–3 h at 19 °C−21 °C temperature range (Pastorino et al., 2020), stainless steel for four days and on glass for two days (Chin et al., 2020), all at room temperature. However, Goldman (2020) posited that most of these studies reporting on the survival of SARS-CoV-2 or surrogate viruses on fomites exaggerate the potential risks due to the use of unrealistic viral titre. Despite in-depth information on the potential transmission routes of the virus, there is a lack of data on the role of shared sanitation facilities as a possible route of transmission. Although this has been studied within hospital settings (Ye et al., 2020; Ong et al., 2020), the risks posed by shared sanitation facilities outside of the hospital

* Corresponding author.
E-mail address: faizalb@dut.ac.za (F. Bux).

https://doi.org/10.1016/j.ijheh.2021.113807
Received 29 March 2021; Received in revised form 7 July 2021; Accepted 8 July 2021
Available online 10 July 2021

environment has been neglected.

The reported shedding of viral particles in feces and urine, by both symptomatic and asymptomatic individuals, highlights the increased risks from the use of shared sanitation. The World Health Organization (WHO) reports that between 2 and 27% of COVID-19 patients have diarrhoea (WHO, 2020b), which may result in the shedding of this virus in feces. SARS-CoV-2 viral loads of 1.7×10^6–4.1×10^7 gc/mL have been reported by Han et al. (2020), and 6.3×10^6–1.26×10^8 gc/g of stool by Lescure et al. (2020). Additionally, although not common, the detection of SARS-CoV-2 concentrations of 3.2×10^2 gc/ml (Peng et al., 2020) and 6.1×10^5 gc/ml (Yoon et al., 2020) have been reported in urine. These results show that in circumstances where fecal and urine contamination of surfaces could occur, such as shared sanitation facilities, the risks of COVID-19 infections could be high. This is especially important in slums or informal settlements in developing countries such as South Africa, where a lack of basic sanitation facilities is a significant concern. The World Bank reported that living in cramped conditions within cities has a significant contribution to a high risk of infections with COVID-19 (WBG, 2020).

The risks associated with shared sanitation could be due to the contamination of contact surfaces by infected individuals either via deposition of aerosols or faecal matter contaminations. Additionally, several studies have shown strong evidence in support of the indoor airborne transmission of viruses, especially in crowded and poorly ventilated areas (Nishiura et al., 2020; Coleman et al., 2018; Knibbs et al., 2012), such as shared toilets. For instance, SARS-CoV-2 is reported to survive in aerosols for up to 3 h (Kumar et al., 2020), meaning the sharing of toilet facilities could be a major risk factor.

Therefore, by detecting and quantifying the concentration of SARS-CoV-2 on key contact surfaces within these shared sanitation facilities, the risks of infection could be estimated. The quantitative microbial risks assessment (QMRA) approach has been encouraged as a tool to assess risks associated with bioaerosols, drinking water, reclaimed water and irrigation water (Carducci et al., 2016; Petterson and Ashbolt, 2016; Girardi et al., 2019; Gularte et al., 2019; Ezzat, 2020). This approach has been used in estimation of the risks for COVID-19 infections for wastewater treatment workers (Zaneti et al., 2020; Dada & Gyawali, 2020), exposure in a market setting (Zhang et al., 2020) and most recently via contact surfaces (Pitol and Julian, 2021). According to Haas et al. (2014) the QMRA approach involves a sequence of four interrelated steps: a) hazard identification; b) exposure assessment; c) dose-response assessment and d) risk characterization. This is the first study using QMRA for assessing risks from the use of shared sanitation facilities outside the clinical setting, focusing on contact surfaces despite the widespread understanding that sanitation facilities may facilitate its spread. This could therefore provide background information on the contamination of such surfaces and could be used in developing risk reduction measures aimed at reducing the potential spread of COVID-19 (and possibly other similar outbreaks) via the use of shared toilet facilities.

2. Methodology

2.1. Study area and sampling

Two peri-urban informal settlements located within the eThekwini Municipality (Durban) of South Africa were selected for this study. These two settlements are located approximately 1.5 km apart, with an approximate total population of 16 500. This study was done at a time the reported active clinical cases were low in South Africa, with about 600 000 active cases of COVID-19 in South Africa, specifically the KwaZulu-Natal province had over 100 000 active cases.

A total of eight (8) shared toilets, referred to as community ablution blocks (CABs), were investigated, four in each settlement. It is worth noting that the CABs are categorized into males and females, however, this study focused on the difference in contamination within the vaiorus CABs irrespective of gender. The contact surfaces selected included the following: cistern handle, toilet seat, floor surface in front of the toilet, internal pull latch of cubicle door and tap in handwash basin (Fig. 1). These were selected based on recommendations made in previous studies (Park et al., 2017; Bohnert et al., 2016; Mpotane et al., 2013). A total of 68 swab samples were taken. Sampling was done twice (two weeks apart) in September 2020. On each sampling event, samples were taken in the morning before the toilets are cleaned and approximately 30 min after cleaning by trained caretakers. Cleaning was done with antiseptic detergents and water. The swab samples were taken according to the methodology proposed by Park et al. (2017). Briefly, the swab was moistened with PCR grade nuclease free water moved across the sampling area horizontally, vertically and diagonally. An area of approximately, 50 cm^2 was swabbed for the toilet seat and toilet floors, 20 cm^2 for the cistern handle and internal latch and 30 cm^2 for the tap handle. The swab area was determined based on the available area of these contact surfaces. Swabs were placed in a 400 µL PCR-grade nuclease free water and transported to the laboratory on ice. The personnel carrying out the sampling were fully clothed in personal protective equipment (face masks, shields, lab coats, gloves and face shields).

2.2. Molecular detection of SARS-CoV-2

Upon arrival at the laboratory, each tube containing the swab was vortexed for 10 s and the swab carefully removed from the tube, pressing gently against the side of the tube to remove excess water. The swab was then discarded and disposed of as biohazard waste. Two approaches were used in the detection of the viral RNA in the samples. This include direct quantification without the RNA extraction step, using 5 µL of the initial sample as a template for the molecular analysis. The second approach involved extraction of RNA from the swab samples using the extraction kit followed by quantification of the RNA copy numbers as described below.

2.3. RNA extraction

Nucleic acid (RNA) was extracted directly from 140 µl of swab solution using the QiAmp Viral RNA MiniKit (Qiagen, Hilden, Germany), according to manufacturer's instructions. RNA was eluted in 80 µl of sterile nuclease free water and then quantified using the Implen Nanophotometer® NP 80 (Implen GmbH, Munich, Germany). The quality of the extracted RNA was determined based on the Nanophotometer® NP 80 results prior to amplification. The extracted RNA was then stored at −80 °C for further analysis. The second detection and quantification approach did not require RNA extraction, therefore the swab samples were vortexed vigourously and these samples were used for droplet

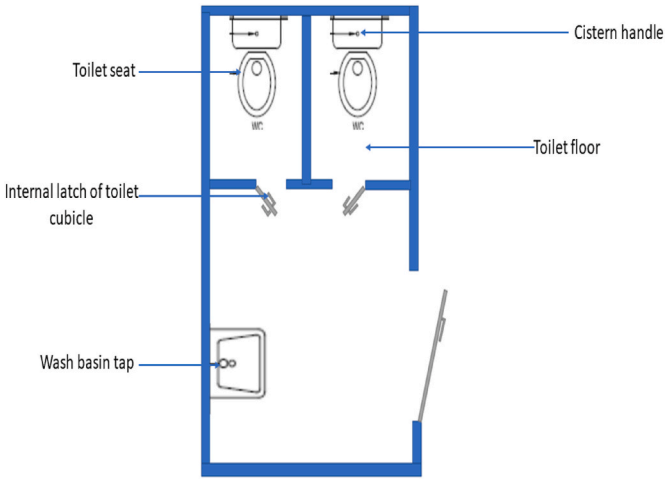

Fig. 1. Key contact surface areas within the internal surfaces of CABs that were considered in this study.

digital Polymerase Chain Reaction (ddPCR) amplification using the protocol described below (Section 2.4).

2.4. Viral detection and quantification using droplet digital PCR

RNA, which was stored for not more than 24 h at −80 °C, was thawed at room temperature and quantitified using the Implen Nanophotometer® NP 80 (Implen GmbH, Munich, Germany). All RNA samples were then diluted and standardized to 1 ng using sterile nuclease free water. Additoinally, direct detection without RNA extraction was also done to determine the suitability of this approach in detection and quantification of viral loads on the surfaces. For the detection of SARS-CoV-2, the 2019-nCoV CDC ddPCR Triplex Probe Assay (Biorad, USA), which simultaneously targets the N1 (FAM labelled) and N2 (FAM and HEX labelled) region of the SARS-CoV-2 genome was used. The assay also targets the human RPP30 (HEX labelled) gene for use as an internal control. Amplification was achieved using the One-Step RT-ddPCR Advanced Kit for Probes Supermix (Biorad, USA), which contains reverse transcriptase and 300 mM Dithiothreitol (DTT). Each ddPCR reaction mix contained 5.5 µl supermix, 2.2 µl reverse transcriptase, 1.1 µl of 300 mM DTT, 1.1 µl of 20X 2019-nCoV CDC ddPCR Triplex Probe Assay, 6.6 µl of sterile DNase free water and 5 µl of the standardized RNA template to get a final volume of 22 µl. All sample plates contained positive, negative and no template control wells. The SARS-CoV-2 positive control (Exact Diagnostics) contained synthetic RNA transcripts of 5 gene targets (E, N, ORF1ab, RdRP and S) while the negative control (Exact Diagnostics) contained human genomic DNA and RNA spiked into a synthetic matrix. Sterile nuclease-free water was used in place of RNA for the no template control. Sample plates were sealed and vortexed for 20 s. Thereafter, droplet generation was carried out using the QXDx Automated Droplet Generator (Biorad, USA), and the plates were then heat sealed with a pierceable foil. A C1000 Touch Thermal Cycler (Biorad, USA) was then used to perform PCR under the following conditions: Reverse transcription at 50 °C for 1 h, enzyme activation at 95 °C for 10 min, 40 cycles of denaturation at 94 °C for 30 s and annealing at 55 °C for 60 s. This was followed by enzyme deactivation at 98 °C for 10 min and droplet stabilization at 4 °C for 30 min with a ramp rate of 2 °C/second. The sealed droplet plate was then transferred to the QX200 Droplet Reader (Biorad, USA). The distribution of positive and negative droplets in each well was read using the QuantaSoft 1.7 software (Biorad, USA) while data analysis was carried out using the QuantaSoft Analysis Pro 1.0 software (Biorad, USA). The results were interpreted as follows: a sample is considered positive if it has any or both of the SARS-CoV-2 markers even in the absence of the RPP30 gene. Similarly, a sample is considered negative if it does not contain any of the SARS-CoV-2 markers even if it contains RPP30. Presence of the RPP30 gene is not mandatory for the presence of SARS-CoV-2. A sample run was considered invalid if there are positives in the negative and no template control wells.

2.5. Probability of COVID-19 infection from the use of shared sanitation: A case of the community ablution blocks

The four interrelated steps used in assessing the potential risks of COVID-19 infections are described below:

***Hazard Identification*:** The SARS-CoV-2 virus is the hazard of choice for this assessment. The concentration of this virus determined based on the extracted RNA was used for the risk assessment.

***Exposure assessment*:** Contact surfaces are recognized as important routes for the spread of infectious diseases, mainly through surface-hand interactions. These surfaces sometimes referred to as fomites, have been associated with different outbreaks in cruise ships, restaurants, nursing homes, schools, daycare centres and gyms (Bures et al., 2000; Aitken and Jeffries, 2001; Barker et al., 2004; Boone and Gerba, 2005). Therefore, the main exposure scenario considered in this study is hand contamination as a result of contact with the surfaces monitored. To assess the dose of the SARS-CoV-2 virus ingested via this route Fig. 2 presents the process flow.

Dose−Response Model: The dose-response relation adopted for this study is the exponential model expressed as;

$$p(d) = 1 - \exp\left(-\frac{d}{k}\right) \quad (1)$$

Where p(d) is the infection risk at a dose of d in units of PFU and k is a pathogen dependent parameter, referred to as the infectivity constant. The k was taken as 4.1×10^2 PFU for SARS-CoV. The dose response model and k were determined based on data for the infection of transgenic mice susceptible to SARS-CoV (Watanabe et al., 2010). These are adopted for the SARS-CoV-2 because SARS-CoV-2 and SARS-CoV have the same cell receptor (angiotensin-converting enzyme 2 (ACE2)) and a similar cellular tropism (Chu et al., 2020; Hoffmann et al., 2020). These dose-response parameters have been used in assessing the risks of COVID-19 infections for workers in wastewater treatment plants (Zaneti et al., 2020).

The dose d was based on the concentration of the viral RNA detected by the ddPCR analysis. This accounted for the fraction of the viral particles that are transferred from the contact surfaces to the mouth/lips or eyes. A two-step process was used to calculate the dose;

1. The efficiency of viral transfer from the contact surface to the hand was accounted for by assuming that 2 cm^2 of the surface will be touched with a transfer efficiency as presented in Table 1.
2. The potential of transfer of the viral particle on the hands to the mouth/lips or eyes.

Table 1 presents the information used to ascertain the concentration of the SARS-CoV-2 virus transferred from the contact surface to the hands and subsequently from the hands to the mouth/lips or eyes.The dose (d) also took into account the ratio of genome copies to viable SARS-CoV-2 viral particles. For this study we assumed a uniform distribution ratio between 1:100 to 1:1000 for genome copy to viable SARS-CoV-2 viral particle (Pitol and Julian, 2021). Additionally, we

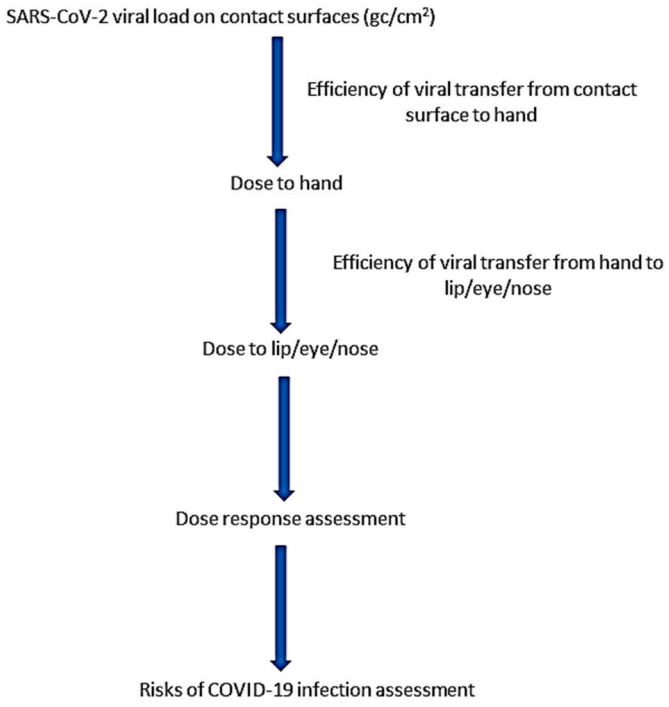

Fig. 2. Scenario for assessing the exposure and possible risks associated with contamination of the contact surfaces (Adapted from Ryan et al., 2014).

Table 1
Transfer efficiencies for determination of dose of SARS-CoV-2 transferred from contact surfaces to mouth/lips or eyes.

Parameter	Input value	Reference
Viral transfer from contact surface to hands	Uniform distribution (0.33; 0.68)	Ryan et al. (2014)
Viral transfer from hands to mouth/lips or eyes	Median value of 0.34	

factored the prevalence of contamination of the various contact surfaces into the risk assessment. This accounts for the likelihood that the contact surfaces will be contaminated at the time when a user comes into contact.

***Risk characterization*:** The outcome of the previous steps were combined to determine the risks of infection for users of the shared toilet facilities. Risk of infection from multiple exposures within a day were assessed by assuming that inhabitants use the toilet facilities between two to three times daily. Therefore the number of times of exposure per day was assumed to be uniformly distributed between 2 and 3. This was used in assessing the daily risks as well as yearly risks based on exposures for everyday in the year. This was determined due to the fact that these shared toilet facilities are the only source of sanitation access in the study area. The risks from multiple exposures was therefore determined using the following formula:

$$p(n) = 1 - (1 - p(d))^n \quad (2)$$

Where *p(n)* is the risks of infection after *n* times of exposure; and *p(d)* is the risk of infection from a single exposure. To determine the annual risks of infection, *p(d)* refers to the daily risks of infection.

2.6. Sensitivity analysis of QMRA inputs

To determine the impact of the various inputs in the QMRA analysis, the following parameters were considered, concentration of the SARS-CoV-2 (gc/cm^2), gene copy to infective viral particle ratio, the transfer efficiency of the viral particles from the surface to the hand and the number of times of exposure within a day. These parameters were varied from their minimum to maximum values. For the purpose of the sensitivity analysis only the risk of infection from exposure to the uncleaned toilet seats was considered. To determine the impact of these parameters, the calculated median infection risks were averaged and used to calculate the factor sensitivity coefficients (*FSi*), using the equation:

$$FSi = P_{i,x}/P_{baseline} \quad (3)$$

Where $P_{i,x}$ is the calculated averaged median risks per parameter, after varying the input values *x*, and $P_{baseline}$ is the baseline median infection risks.

2.7. Statistical analysis

Descriptive statistics to represent the mean and standard deviation were performed with Excel (Microsoft Corporation, USA). Comparison of viral load between the different contact surfaces was performed using the Kruskal-Wallis Test, comparison between two data categories (such as comparing viral load on cleaned and uncleaned surfaces) was done using the Mann Whitney Test. Comparative statistical analysis were all performed with GraphPad Prism Version 7 (GraphPad Software, CA, USA).

3. Results

3.1. Prevalence of contamination using extracted RNA

The chance/likelihood of contamination on the contact surfaces varied over the two sampling events. The highest prevalence of contamination of 68.8 (±20.6) % was observed for the tap handle, followed by the toilet floor with the internal latch giving the lowest prevalence of contamination (54.1 (±16.2) %) among the studied contact surfaces (Fig. 3). Despite the observed difference, there was no statistically significant difference in the prevalence (p value ≥ 0.05). This information on likelihood of contamination was used in estimating the probable risk of infection due to contact with these surfaces.

3.2. Concentration of SARS-CoV-2 on contact surfaces before and after cleaning based on extracted RNA

Per cm^2 swabbed, the mean concentration of SARS-CoV-2 was highest on the toilet seats (132.9(±39.8) gc/cm^2), followed by the cistern handle (69.1(±21.6) gc/cm^2) and internal latch (60.1(±14.5) gc/cm^2). The differences in the concentration between the different contact surfaces were statistically significant (p value ≤ 0.05).

Cleaning reduced the concentration of SARS-CoV-2 RNA on these contact surfaces, with significant (p value ≤ 0.05) reduction on the toilet seat, cistern handle, internal latch and toilet floors. For instance, after cleaning, the mean viral load on the toilet seats was reduced to 2.1 (±0.21) gc/cm^2 from the initial 132.9(±39.8) gc/cm^2 (Fig. 4). However, there was no significant reduction observed on the tap handles after cleaning (p value ≥ 0.05), as shown in Fig. 4.

3.3. Comparison of direct quantification vs quantification via extracted RNA

Detection of the SARS-CoV-2 on the swab without the initial RNA extraction step presented higher prevalence compared with the prevalence observed using the extracted RNA. For instance, via direct sample analysis, the highest prevalence was observed for cistern handle (83.3 (±29.2) %) with a corresponding prevalence of 59.3(±17.8) % when the viral RNA was extracted first before analysis. Similar trends were observed, where prevalence was consistently lower when the RNA was extracted. The only exception were swab samples from the floor, where prevalence via analysis of extracted RNA was higher (59.7(±19.3) %) compared to direct detection (50(±17.5) %) (Fig. 5A).

There was a similar trend in the viral load difference when these two approaches (direct quantification and quantification via extracted RNA) were used. For instance, via direct quantification without RNA extraction, 244.9(±85.7) g/cm^2 was recorded on the toilet seats, however when the RNA was extracted, the concentrations was reduced to 132.7 (±39.8) gc/cm^2. These differences are statistically significant (p value ≤ 0.05), indicating consistently lower concentrations when the RNA was extracted from the samples prior to analysis. However, as observed with the prevalence, the only exceptions were the floor and cistern handle

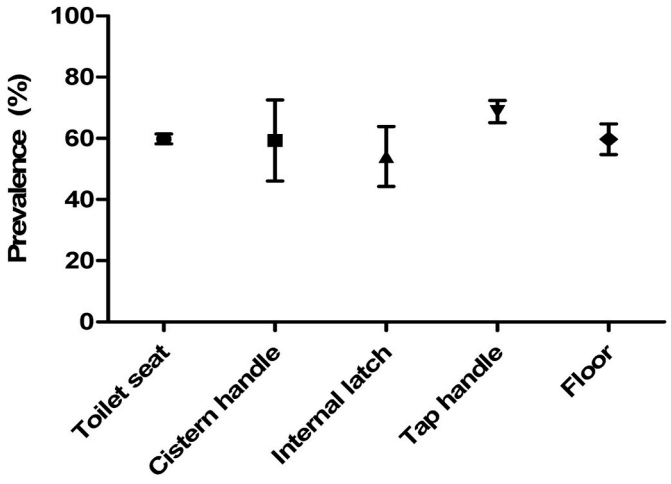

Fig. 3. Percentage of contact surfaces contaminated with SARS-CoV-2 (n = 16).

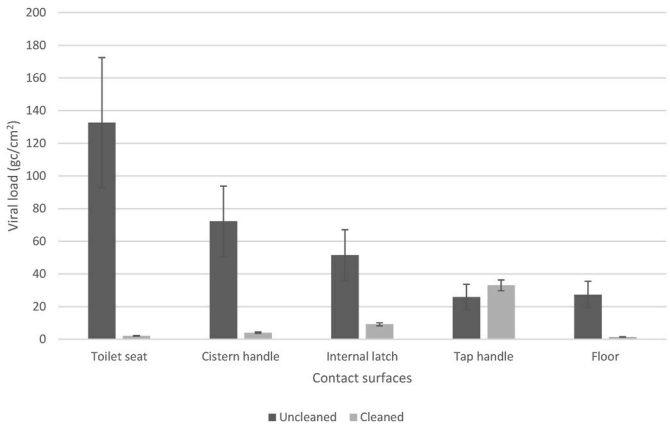

Fig. 4. Concentration of SARS-CoV-2 on key contact surfaces in the shared toilets (n = 16). *Error bars representing standard deviation.

swab samples (Fig. 5B). The RPP30 gene was present in all extracted and unextracted RNA samples regardless of whether or not they contained any of the SARS-CoV-2 genetic markers. Presence of the RPP30 gene is indicative of sufficient cellular material and proper nucleic acid extraction.

3.4. Probability of infection with COVID-19 from use of the shared toilets

The probability of infection with COVID-19 as a result of exposure to the SARS-CoV-2 virus particles on the contact surfaces varied considerably, driven mainly by the difference in the viral loads described above and the prevalence/likelihood of contamination of these surfaces. The magnitude of the risks after single exposure was similar for contact with almost all the surfaces (10^{-5}), however the highest median risks were observed for contact with the uncleaned toilet seats. It was estimated that approximately two people out of every 10 000 people using the toilet who touch the toilet seat could potentially be infected with COVID-19 (1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$) per person). These estimates were made based on a single exposure event. However, considering that these toilet facilities are the only source of sanitation services within the communities studied, providing both access to potable water and sanitation, multiple exposures within a day were considered. Use of the toilet facilities twice or three times in a day was observed to increase the risks of infections with COVID-19. For instance, multiple contacts with the toilet seat within a day (daily risks) resulted in an increase in the median risks from 1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$) per person for a single exposure to 4.33×10^{-4} (4.03×10^{-6}) per person for daily risks (multiple exposures in a day). This means that for every 10 000 people who use the toilet facility between two or three times in a day, about four of them may be infected. Similar significantly increased risks (p value ≤ 0.05) were observed for all the other contact surfaces (Table 2). We further observed an increase in the risks of infection with COVID-19 when exposure over the course of a year (yearly risk) is considered (Table 2), relying on the fact that these shared sanitation facilities are the only source of sanitation in the studied areas.

The risks of infection were reduced, considering exposure after the toilets have been cleaned, although not statistically significant for most of the surfaces (Table 2). Most notable reductions were exposure via contact with the toilet seat, internal latch and toilet floor. For instance, the probability of infection reduced from about two people out of 10 000 exposed people potentially been infected to about two people out of one million being infected (2.34×10^{-6} ($\pm 2.09 \times 10^{-8}$)). Similar significant reduction in probable risks were recorded after cleaning for contact with the other contact surfaces mentioned previously (p value ≤ 0.05), except the tap handle and internal latch (Table 2). As observed for multiple exposures to the uncleaned surfaces, multiple exposures to the cleaned surfaces could also increase the risks of infection, as reported in Table 2.

3.5. Parameter sensitivity in the infection risk calculation

The sensitivity analysis for the various input parameters based on their minimum and maximum input ranges showed that these values had an impact on the risk estimates calculated. However, their impact varied depending on the parameter. The gene copies of SARS-CoV-2 measured on the various contact surfaces was determined to have the highest impact on the risks estimates, with an *FSi* of 2.88 (Fig. 6). Among the four parameters chosen for the sensitivity analysis, varying the number of times of exposure within a day between twice or three times had the least impact on the risk estimates, with an *FSi* of 1.01. These results therefore, shows that the concentration of the viral particles measured could be the main parameter affecting the risks estimates.

4. Discussion

Contact surface contamination within the toilet facilities was widespread (Fig. 3), with a high prevalence of contamination on the tap handles, the floor of these toilets and the internal latch of the toilet cubicles. Several studies have reported similar findings in relation to the most contaminated surfaces in toilet facilities (McGinnis et al., 2019; Abiose, 2019; Verani et al., 2014; Sabra, 2013; De Alwis et al., 2012; Flores et al., 2011; Fankem et al., 2006). Notably, Fankem et al. (2006)

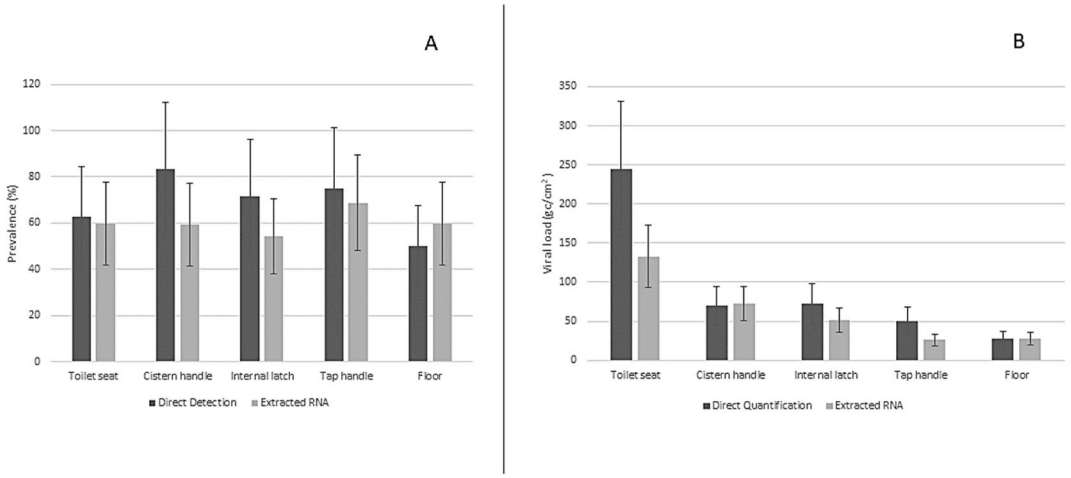

Fig. 5. Difference in the detection and quantification of SARS-CoV-2 via direct analysis and RNA extraction (n = 16): (A) comparison on prevalence and (B) viral loads. *Error bars representing standard deviation.

Table 2
Median risks (±90% CI) of infection with COVID-19 due to contact with surfaces within shared toilets.

Exposure frequency	Toilet seat		Cistern handle		Internal Latch		Tap handle		Floor	
	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned
One-time risk	1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$)	2.34×10^{-6} ($\pm 2.09 \times 10^{-8}$)	9.16×10^{-5} ($\pm 8.20 \times 10^{-7}$)	9.00×10^{-6} ($\pm 8.07 \times 10^{-8}$)	6.10×10^{-5} ($\pm 5.47 \times 10^{-7}$)	2.03×10^{-5} ($\pm 1.82 \times 10^{-7}$)	3.95×10^{-5} ($\pm 3.54 \times 10^{-7}$)	3.67×10^{-5} ($\pm 3.29 \times 10^{-7}$)	3.79×10^{-5} ($\pm 3.39 \times 10^{-7}$)	3.13×10^{-6} ($\pm 2.80 \times 10^{-8}$)
Daily risk	4.33×10^{-4} ($\pm 4.03 \times 10^{-6}$)	5.73×10^{-6} ($\pm 5.33 \times 10^{-8}$)	2.24×10^{-4} ($\pm 2.09 \times 10^{-6}$)	2.21×10^{-5} ($\pm 2.06 \times 10^{-7}$)	1.49×10^{-4} ($\pm 1.58 \times 10^{-6}$)	4.98×10^{-5} ($\pm 4.63 \times 10^{-7}$)	9.69×10^{-5} ($\pm 9.02 \times 10^{-7}$)	8.99×10^{-5} ($\pm 8.37 \times 10^{-7}$)	9.29×10^{-5} ($\pm 8.65 \times 10^{-7}$)	7.67×10^{-6} ($\pm 7.14 \times 10^{-8}$)
Annual risks	6.03×10^{-2} ($\pm 5.22 \times 10^{-4}$)	8.22×10^{-4} ($\pm 7.41 \times 10^{-6}$)	3.17×10^{-2} ($\pm 2.80 \times 10^{-4}$)	3.16×10^{-3} ($\pm 2.84 \times 10^{-5}$)	2.12×10^{-2} ($\pm 1.89 \times 10^{-4}$)	7.12×10^{-3} ($\pm 6.39 \times 10^{-5}$)	1.38×10^{-2} ($\pm 1.23 \times 10^{-4}$)	1.28×10^{-2} ($\pm 1.15 \times 10^{-4}$)	1.32×10^{-2} ($\pm 1.18 \times 10^{-4}$)	1.10×10^{-3} ($\pm 9.91 \times 10^{-6}$)

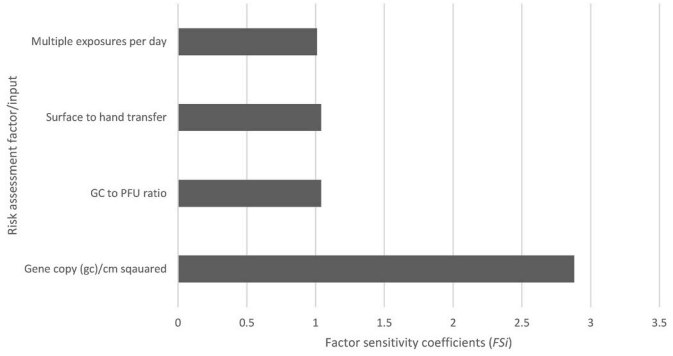

Fig. 6. Sensitivity ranking of the infection risks calculation input parameters for exposure to the uncleaned toilet seat.

observed that the most contaminated surfaces in public toilets found in airports, bus terminals, and universities were the sanitary napkin dispensers, toilet seats, sinks, and floors. However, in those studies, the frequency of contamination on these surfaces was much lower (3–21%) compared to the frequency observed in our study. Our prevalence of contamination was in accordance with the observations reported by Sabra (2013), where 91.3% toilet handles, 73% of toilet doors, 53% of toilet sink and 50% of tap handles were reportedly contaminated with bacteria. It must be noted that these findings were observed for bacterial contamination; therefore, the difference could further be due to the difference in organisms. When using a human adenovirus virus (HAdV), Verani et al. (2014) found 135 out of 172 surfaces within toilet facilities in a health care setting to be contaminated. Contamination of contact surfaces outside the sanitation setting has also been reported in hospitals (Chia et al., 2020; Ryu et al., 2020; Peyrony et al., 2020; Lei et al., 2020), home settings (Xie et al., 2020a,b; Fernández-de-Mera et al., 2020; Döhla et al., 2020) and public spaces (Fernández-de-Mera et al., 2020).

Contamination of the contact surfaces could be as a result of direct contact with feces or urine, unclean hands or even through cough or sneeze. For instance, the high frequency of contamination on the cistern handle, the tap handle and internal latch could be as a result of this direct contact with uncleaned hands. Contamination of the toilet seat and the toilet floor could also be from contaminated fecal matter and urine. The frequency of contact has been proposed as the most critical factor in the direct contamination of contact surfaces within public toilets (Fankem et al., 2006). The higher frequency of contact could therefore be responsible for the high prevalence of contamination on these contact surfaces. In addition to the frequency of use, the contamination of these contact surfaces could be an indication of hygiene. De Alwis et al. (2012) reported a high bacterial contamination on door handles used by males, whereby 50% of the users of these toilets did not wash their hands with soap. The contamination of toilet floors has been attributed to a high frequency of contact with the bottom of shoes (Flores et al., 2011). This could potentially be a significant source of contamination for other contact surfaces, such as cistern handles. A study by Flores et al. (2011) observed that the bacterial community on toilet floors was similar to those found on toilet flush/cistern handles. They attributed this to the use of foot in operating these cistern/flush handles by some of the users. This is a common practice in shared sanitation facilities.

Contamination of the toilet seat and the floors could be via indirect contact. For instance, flushing of toilets could be a significant source of contamination. Flushing results in the generation of droplets and aerosols that could be deposited on theses surfaces (Flores et al., 2011). Using modelling approaches, Li et al. (2020) postulated that massive upward transport of viral particles is observed with over 40–60% of the particles potentially deposited on the toilet seat. Contamination of the toilet seat up to 24 flushes after initial shedding in feces and urine could still occur, although the concentrations could reduce with each flush (Johnson et al., 2017). Using bacterial indicators, Johnson et al. (2017) observed 3log$_{10}$ reduction after the first flush, 1–2 log$_{10}$ after the second and thereafter less than 1 log$_{10}$ reduction with each flush. Therefore, SARS-CoV-2 viral particles shed in feces and urine could be deposited on the toilet seat during flushing, this could potentially be the main source of the contamination of the toilet seats. Additionally, contamination of the floor could be due to accidental urination on the floor, which could be a common phenomenon in the male toilets, although this study did not specifically measure the difference in contamination within the male and female toilets.

We observed that direct detection and quantification of SARS-CoV-2 in swab solutions gave higher prevalence of contamination and viral load (Fig. 5). The lower numbers recorded for analysis done using the RNA extraction approach, could be attributed to losses during the RNA extraction process. The higher frequency of contamination and viral load on the floor swabs determined via the RNA extraction approach as compared with the direct estimation approach could be due to the elimination of PCR inhibitors during RNA extraction as compared to other surfaces. It is worth noting that the toilet floor was constantly soiled, as a result without RNA extraction, several PCR inhibitors inherent in soil could be transferred to the amplification stage resulting in interferences. Therefore, although direct quantification of SARS-CoV-2 on contact surfaces without RNA extraction is possible and gives higher concentrations, we do not recommend it for surfaces with high solid contents, such as floors. However, direct quantification is an important approach to consider for the estimation of risks from contact with contaminated surfaces with less solids.

The difference in concentration of SARS-CoV-2 observed in this study (Fig. 4) could also be attributed to the same factors responsible for the frequency of contamination, which are fecal matter contamination, unclean hands and cough or sneeze. However, the viral load on the toilet seats per cm^2 were significantly higher (p value ≤ 0.05) than any of the

other contact surfaces. This could be attributed to the phenomenon of droplet and aerosol generation during flushing. Shedding of SARS-CoV-2 in feces and urine of both symptomatic and asymptomatic patients is well reported (Jones et al., 2020; Amirian, 2020; Bowser, 2020; Pan et al., 2020; Xie et al., 2020a,b; Peng et al., 2020; Yoon et al., 2020), therefore higher viral load on the toilet seats is to be expected. The concentrations on the other contact surfaces points towards direct contamination via uncleaned hands. Hand transmission of COVID-19 is one of the main routes of transmission, leading to hand washing as a major intervention to reduce infections (Gupta and Lipner, 2020; Lin et al., 2020; Beiu et al., 2020). The toilets are cleaned once a day, which resulted in a significant reduction of viral load on almost all the contact surfaces, except for the tap handle (Fig. 4). The viral loads detected on the internal latch and tap handle indicates that cleaning does not usually focus on these surfaces, despite a high contact frequency. The findings, therefore, show that cleaning of shared sanitation facilities should consider surfaces with high contact frequency and small crevices, such as the toilet seat, tap handle and internal latch.

The viral contamination of key contact surfaces within shared toilets could potentially result in COVID-19 infections. The estimated risks show that the highest probability of infection from a one-time use of the toilets is the contact with the toilet seat (Table 2). A manageable risk of 1.17×10^{-3} has been recommended by Zhang et al. (2020), meaning 1 person out of a thousand being infected is acceptable. In contrast Zaneti et al. (2020) derived a tolerable risk of infection for SARS-CoV-2 to be 5.5×10^{-4} per person per year (pppy), setting a very high tolerable/acceptable risk figure. Considering one-time exposures, the risks estimates from our study are lower than these recommended tolerable/acceptable risks figures. However, with multiple exposure within a day or over a year, the risks of infection with COVID-19 within our study area were higher than these tolerable or acceptable risks estimates published (Table 2). Comparatively, the risks estimated from this study are lower compared to the risks published by Zaneti et al. (2020) for workers in wastewater treatment plants (2.6×10^{-3} to 1.3×10^{-2}) per exposure. Furthermore, Pitol and Julian (2021) reported median risks of 1.6×10^{-4} to 5.6×10^{-9} when they modelled the risks of infection with COVID-19 based on surface contamination, similar to our findings. The application of QMRA to measure the potential risks of infection via surfaces, therefore shows that this may not be a significant route of infection. This could be due to the conversion ratio of the gc/cm^2 to PFU/cm^{2}of 1:100 and 1:1000 of gc/cm^2 to PFU/cm^2 which was used both in our study and the study by Pitol and Julian (2021). Reports have shown that SARS-CoV-2 viral particles shed in feces may still be infectious (Zhang et al., 2020; Wang et al., 2020; Xiao et al., 2020), however this is inconclusive due to the varying reports on their survival in the environment. It is also important to consider that the potential risk can be high due to the frequent use of these facilities by the communities. The contact time is very short due to a high population that rely on these facilities and the SARS-CoV-2 virus is reported to survive on surfaces from a few hours (Chin et al., 2020), to four days (Chin et al., 2020; Van Doremalen et al., 2020).

Cleaning could potentially reduce the risks of infection, however, in our study, we observed that despite the significant reduction in viral load after cleaning on almost all the surfaces, the potential of infections with COVID-19 was still high. Tuladhar et al.,(2012) found residual bacterial and viral contamination on surfaces after cleaning, which means the detection of the SARS-CoV-2 on the contact surfaces after cleaning could be residual viral particles. Therefore, the estimated risks on the contact surfaces after cleaning could be much lower. However, to ensure maximum protection for users of these shared toilets and other facilities with similar characteristics, other risks reduction interventions should be considered.

5. Limitation of the study

The risk or probability of infection with COVID-19 was based on the assumption of a worst-case scenario where a gene copy is considered an infectious viral particle. By using the ratio of genome copies to viable SARS-CoV-2 viral particles of 1:100 to 1:1000 (Pitol and Julian, 2021), this was addressed. However, the risk assessment based on SARS-CoV-2 viral RNA concentration could potentially result in over estimation of the associated risks, because the detection and quantification of viral RNA and inactivated viruses may still yield positive results.

6. Conclusions

We established in this study that key contact surfaces within shared toilets investigated in this study were contaminated with SARS-CoV-2, with the highest prevalence of contamination on the floor, tap and cistern handles. This shows areas of high hand contact had the highest possibility of being contaminated, indicating that uncleaned hands may be the main source of contamination. However, based on viral load per cm^2, the most contaminated surface is the toilet seat, the shedding of SARS-CoV-2 virus in feces and urine could be the main reason for this high concentration. We also showed that the presence and quantity of SARS-CoV-2 on contact surfaces could be determined directly without an RNA extraction step using ddPCR, which can potentially reduce the cost associated with such analysis. However, this is not recommended for surfaces with high solid contents, such as floors. Cleaned contact surfaces had significantly lower viral load compared to the uncleaned surfaces except for the tap handle, this shows that the potential risks of infection with COVID-19 due to contact with these surfaces could be reduced with effective and regular cleaning.

7. Recommendation/risk reduction interventions

The calculated risks of infections associated with the use of the shared toilets call for the introduction of additional measures to protect public health, especially in developing countries where large proportion of the population may rely on shared toilet facilities. Some of these risk reduction measures are:

1. **Frequent and effective cleaning:** Cleaning of the shared toiles is currently done once a day, due to the high contamination found on the key contact surfaces we recommend that cleaning be carried out at least twice. For instance, Tuladhar et al. (2012) observed that a second wipe of a contaminated surface with chlorine resulted in an extra 1–3 $\log_{10}$ reduction in concentration of various pathogens including influenza virus.
2. **Close of water closet lid during flushing:** The viral concentration on the toilet seats was the highest, this could be attributed to the shedding of SARS-CoV-2 in feces and urine. These could have been dispersed unto the toilet seat and possibly the floor during flushing. Therefore, by closing the water closet lid, the spread of the droplets or aerosols generated could be reduced, therefore limiting exposure.
3. **Hand washing with soap:** To reduce the possibility of transmission and contamination of the contact surfaces, frequent washing of hands with soap, as recommended, should be encouraged. This provide a two-way protection, firstly limits contamination of contact surfaces and secondly, reduces the possibility of infection from contaminated hands.
4. **Face masks:** Aerosols are easily generated during flushing and these may remain suspended for a while, therefore the use of face masks could provide an additional layer of protection.

Author contributions statement

All authors were involved in the conceptualization of the manuscript, data collection was performed by I.D.Amoah, L. Pillay, N. Deepnarian, O. Awolusi, K. Pillay and P. Ramlal. Writing of the original draft manuscript was done by I.D.Amoah, L. Pillay, N. Deepnarian, O. Awolusi and K. Pillay under the supervision of S. Kumari and F. Bux. Initial

reviewing and editing of the manuscript was done by I.D.Amoah, S. Kumari and F. Bux. Final revision of the and approval was done by all authors.

Acknowledgement

We acknowledge the financial support from the South African Research Chair Initiative (SARChI) of the Department of Science and Technology, the National Research Foundation of South Africa and Umgeni Water. We are also grateful for the support from our institution, the Durban University of Technology, specifically the Institute for Water and Wastewater Technology and the caretakers of the shared toilet facilities for providing access during the study. We have no conflict of interest to declare.

References

Abiose, O.F., 2019. Bacterial contamination of selected public toilet door handles within adekunle ajasin university campus, akungba-akoko, ondo state, Nigeria. Int. j. sci.: basic appl. 43, 76–86.

Aitken, C., Jeffries, D.J., 2001. Nosocomial spread of viral disease. Clin. Microbiol. Rev. 14, 528–546.

Amirian, E.S., 2020. Potential fecal transmission of SARS-CoV-2: current evidence and implications for public health. Int. J. Infect. Dis. 95, 363–370.

Bahl, P., Doolan, C., de Silva, C., Chughtai, A.A., Bourouiba, L. & MacIntyre, C.R. Airborne or droplet precautions for health workers treating COVID-19?. J. Infect. Dis. DOI: 10.1093/infdis/jiaa189.

Barker, J., Vipond, I.B., Bloomfield, S.F., 2004. Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. J. Hosp. Infect. 58, 42–49.

Beiu, C., Mihai, M., Popa, L., Cima, L., Popescu, M.N., 2020. Frequent hand washing for COVID-19 prevention can cause hand dermatitis: management tips. Cureus 12, e7506.

Bohnert, K., Chard, A.N., Mwaki, A., Kirby, A.E., Muga, R., Nagel, C.L., Thomas, E.A., Freeman, M.C., 2016. Comparing sanitation delivery modalities in urban informal settlement schools: a randomized trial in Nairobi, Kenya. Int. J. Environ. Res. Publ. Health 13, 1189.

Boone, S.A., Gerba, C.P., 2005. The occurrence of influenza A virus on household and day care center fomites. J. Infect. 51, 103–109.

Bowser, A.D., 2020. Coronavirus may cause environmental contamination through fecal shedding. Medscape medical news. Accessed on 28th September, 2020 at. https://www.medscape.com/viewarticle/926390.

Bures, S., Fishbain, J.T., Uyehara, C.F., Parker, J.M., Berg, B.W., 2000. Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit. Am. J. Infect. Contr. 28, 465–471.

Cai, J., Sun, W., Huang, J., Gamber, M., Wu, J., He, G., 2020. Indirect virus transmission in cluster of COVID-19 cases, Wenzhou, China, 2020. Emerg. Infect. Dis. 26, 1343–1345.

Carducci, A., Donzelli, G., Cioni, L., Verani, M., 2016. Quantitative microbial risk assessment in occupational settings applied to the airborne human adenovirus infection. Int. J. Environ. Res. Publ. Health 13, 733.

Chan, J.F.W., Yuan, S., Kok, K.H., To, K.K.W., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C.C. Y., Poon, R.W.S., Tsoi, H.W., 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395, 514–523.

Chia, P.Y., Coleman, K.K., Tan, Y.K., Ong, S.W.X., Gum, M., Lau, S.K., Lim, X.F., Lim, A. S., Sutjipto, S., Lee, P.H., Son, T.T., 2020. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. Nat. Commun. 11, 1–7.

Chin, A.W.H., Chu, J.T.S., Perera, M.R.A., 2020. Correspondence. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 1, E10.

Chu, H., Chan, J.F.W., Yuen, T.T.T., Shuai, H., Yuan, S., Wang, Y., Hu, B., Yip, C.C.Y., Tsang, J.O.L., Huang, X., Chai, Y., 2020. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. The Lancet Microbe 1, e14.

Coleman, K.K., Nguyen, T.T., Yadana, S., Hansen-Estruch, C., Lindsley, W.G., Gray, G.C., 2018. Bioaerosol sampling for respiratory viruses in Singapore's mass rapid transit network. Sci. Rep. 8, 1–7.

Dalton, C.B., Corbett, S.J., Katelaris, A.L., 2020. Pre-emptive low cost social distancing and enhanced hygiene implemented before local COVID-19 transmission could decrease the number and severity of cases. Med. J. Aust. 212, 1.

De Alwis, W.R., Pakirisamy, P., Wai San, L., Xiaofen, E.C., 2012. A study on hand contamination and hand washing practices among medical students. ISRN public Health. https://doi.org/10.5402/2012/251483.

Döhla, M., Wilbring, G., Schulte, B., Kümmerer, B.M., Diegmann, C., Sib, E., Richter, E., Haag, A., Engelhart, S., Eis-Hübinger, A.M., Exner, M., 2020. SARS-CoV-2 in environmental samples of quarantined households. Preprint available at doi: https://doi.org/10.1101/2020.05.28.20114041. medRxiv.

Ezzat, S.M., 2020. Applying quantitative microbial risk assessment model in developing appropriate standards for irrigation water. Integrated Environ. Assess. Manag. 16, 353–361.

Fankem, S., Kennedy, D., Enriquez, C., Gerba, C., 2006. Assessment of enteric pathogen exposure in public toilets. Epidemiology 17, S457.

Fernández-de-Mera, I.G., Rodríguez del-Río, F.J., Fuente, J.D.L., Pérez-Sancho, M., Hervás, D., Moreno, I., Domínguez, M., Domínguez, L., Gortázar, C., 2020. Detection of environmental SARS-CoV-2 RNA in a high prevalence setting in Spain. Transbound Emerg Dis. https://doi.org/10.1111/tbed.13817.

Flores, G.E., Bates, S.T., Knights, D., Lauber, C.L., Stombaugh, J., Knight, R., Fierer, N., 2011. Microbial biogeography of public restroom surfaces. PloS One 6, e28132.

Girardi, V., Mena, K.D., Albino, S.M., Demoliner, M., Gularte, J.S., de Souza, F.G., Rigotto, C., Quevedo, D.M., Schneider, V.E., Paesi, S.O., Tarwater, P.M., 2019. Microbial risk assessment in recreational freshwaters from southern Brazil. Sci. Total Environ. 651, 298–308.

Goldman, E., 2020. Exaggerated risk of transmission of COVID-19 by fomites. Lancet Infect. Dis. 20, 892–893.

Gularte, J.S., Girardi, V., Demoliner, M., de Souza, F.G., Filippi, M., Eisen, A.K.A., Mena, K.D., de Quevedo, D.M., Rigotto, C., de Barros, M.P., Spilki, F.R., 2019. Human mastadenovirus in water, sediment, sea surface microlayer, and bivalve mollusk from southern Brazilian beaches. Mar. Pollut. Bull. 142, 335–349.

Gupta, M.K., Lipner, S.R., 2020. Personal protective equipment recommendations based on COVID-19 route of transmission. J. Am. Acad. Dermatol. 83, e45–e46.

Haas, C.N., Rose, J.B., Gerba, C.P., 2014. Quantitative Microbial Risk Assessment. John Wiley & Sons.

Han, M.S., Seong, M.W., Heo, E.Y., Park, J.H., Kim, N., Shin, S., Cho, S.I., Park, S.S., Choi, E.H., 2020. Sequential analysis of viral load in a neonate and her mother infected with SARS-CoV-2. Clin. Infect. Dis. https://doi.org/10.1093/cid/ciaa447 ciaa447.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., Müller, M.A., 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181, 271–280.

Howard, J., Huang, A., Li, Z., Tufekci, Z., Zdimal, V., van der Westhuizen, H.M., von Delft, A., Price, A., Fridman, L., Tang, L.H.& Tang, V. Face masks against COVID-19: an evidence review. Preprints 2020, 2020040203 (doi: 10.20944/preprints202004.0203.v1).

Johnson, D.L., Lynch, R.A., Villanella, S.M., Jones, J.F., Fang, H., Mead, K.R., Hirst, D.V., 2017. Persistence of bowl water contamination during sequential flushes of contaminated toilets. J. Environ. Health 80, 34.

Jones, D.L., Baluja, M.Q., Graham, D.W., Corbishley, A., McDonald, J.E., Malham, S.K., Hillary, L.S., Connor, T.R., Gaze, W.H., Moura, I.B., Wilcox, M.H., 2020. Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19. Sci. Total Environ. 749, 141364.

Jones, R.M., 2020. Relative contributions of transmission routes for COVID-19 among healthcare personnel providing patient care. J. Occup. Environ. Hyg. 17, 1–8.

Knibbs, L.D., Morawska, L., Bell, S.C., 2012. The risk of airborne influenza transmission in passenger cars. Epidemiol. Infect. 140, 474–478.

Kumar, S.S., Shao, S., Li, J., He, Z., Hong, J., 2020. Droplet Evaporation Residue Indicating SARS-COV-2 Survivability on Surfaces arXiv preprint arXiv:2005.12262.

Lei, H., Ye, F., Liu, X., Huang, Z., Ling, S., Jiang, Z., Cheng, J., Huang, X., Wu, Q., Wu, S., Xie, Y., 2020. SARS-CoV-2 environmental contamination associated with persistently infected COVID-19 patients. Influenza Other Respir Viruses. https://doi.org/10.1111/irv.12783.

Lescure, F.X., Bouadma, L., Nguyen, D., Parisey, M., Wicky, P.H., Behillil, S., Gaymard, A., Bouscambert-Duchamp, M., Donati, F., Le Hingrat, Q., Enouf, V., 2020. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. *Lancet Infect. Dis.* 20, 697–706.

Li, Y.Y., Wang, J.X., Chen, X., 2020. Can a toilet promote virus transmission? From a fluid dynamics perspective. Phys. Fluids 32, 065107.

Lin, Y.H., Liu, C.H., Chiu, Y.C., 2020. Google searches for the keywords of "wash hands" predict the speed of national spread of COVID-19 outbreak among 21 countries. Brain Behav. Immun. 87, 30–32.

Liu, X., Zhang, S., 2020. COVID-19: face masks and human-to-human transmission. *Influenza and Other Respiratory Viruses*., S., 2020. COVID-19: face masks and human-to-human transmission. Influenza Other Respir Viruses 14, 472–473.

McGinnis, S., Marini, D., Amatya, P., Murphy, H.M., 2019. Bacterial contamination on latrine surfaces in community and household latrines in Kathmandu, Nepal. Int. J. Environ. Res. Publ. Health 16, 257.

Morawska, L., Milton, D.K., 2020. It is time to address airborne transmission of COVID-19. Clin. Infect. Dis. 6, ciaa939.

Mpotane, T., Ntswabule, V., Mcpherson, C., Botes, E., 2013. The role of toilet hygiene in transmission of vaginal and urinary tract infections in Huis Welgemoed, CUT Campus. Interim: Interdisciplinary Journal 12, 26–31.

Nishiura, H., Oshitani, H., Kobayashi, T., Saito, T., Sunagawa, T., Matsui, T., Wakita, T., Covid, M., Suzuki, M., 2020. Closed environments facilitate secondary transmission of coronavirus disease 2019 (COVID-19). medRxiv. https://doi.org/10.1101/2020.02.28.20029272.

Ong, S.W.X., Tan, Y.K., Chia, P.Y., Lee, T.H., Ng, O.T., Wong, M.S.Y., Marimuthu, K., 2020. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. Jama 323, 1610–1612.

Pan, Y., Zhang, D., Yang, P., Poon, L.L., Wang, Q., 2020. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect. Dis. 20, 411–412.

Park, G.W., Chhabra, P., Vinjé, J., 2017. Swab sampling method for the detection of human norovirus on surfaces. JoVE 120, e55205. https://doi.org/10.3791/55205.

Pastorino, B., Touret, F., Gilles, M., de Lamballerie, X., Charrel, R., 2020. Prolonged Viability of SARS-CoV-2 in Fomites.

Peng, L., Liu, J., Xu, W., Luo, Q., Chen, D., Lei, Z., Huang, Z., Li, X., Deng, K., Lin, B., Gao, Z., 2020. SARS-CoV-2 can be detected in urine, blood, anal swabs, and oropharyngeal swabs specimens. J. Med. Virol. 92 (9), 1676–1680.

Petterson, S.R., Ashbolt, N.J., 2016. QMRA and water safety management: review of application in drinking water systems. J. Water Health 14, 571–589.

Peyrony, O., Ellouze, S., Fontaine, J.P., Thegat-Le Cam, M., Salmona, M., Feghoul, L., Mahjoub, N., Mercier-Delarue, S., Gabassi, A., Delaugerre, C., Le Goff, J., 2020. Surfaces and equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the emergency department at a university hospital. Int. J. Hyg Environ. Health 230, 113600.

Pitol, A.K. and Julian, T.R., Community transmission of SARS-CoV-2 by surfaces: risks and risk reduction strategies. Environ. Sci. Technol. Lett.. DOI: 10.1021/acs.estlett.0c00966.

Qu, G., Li, X., Hu, L., Jiang, G., 2020. An imperative need for research on the role of environmental factors in transmission of novel coronavirus (COVID-19). Environ. Sci. Technol. 54, 3730–3732.

Ryan, M.O., Haas, C.N., Gurian, P.L., Gerba, C.P., Panzl, B.M., Rose, J.B., 2014. Application of quantitative microbial risk assessment for selection of microbial reduction targets for hard surface disinfectants. Am. J. Infect. Contr. 42, 1165–1172.

Ryu, B.H., Cho, Y., Cho, O.H., Hong, S.I., Kim, S., Lee, S., 2020. Environmental contamination of SARS-CoV-2 during the COVID-19 outbreak in South Korea. Am. J. Infect. Contr. 48, 875–879.

Sabra, S.M.M., 2013. Bacterial public health hazard in the public female restrooms at Taif, KSA. *Middle East.* J. Sci. Res. 14, 63–68.

Tuladhar, E., Hazeleger, W.C., Koopmans, M., Zwietering, M.H., Beumer, R.R., Duizer, E., 2012. Residual viral and bacterial contamination of surfaces after cleaning and disinfection. Appl. Environ. Microbiol. 78, 7769–7775.

Van Doremalen, N., Bushmaker, T., Morris, D.H., Holbrook, M.G., Gamble, A., Williamson, B.N., Tamin, A., Harcourt, J.L., Thornburg, N.J., Gerber, S.I., Lloyd-Smith, J.O., 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N. Engl. J. Med. 382, 1564–1567.

Verani, M., Bigazzi, R., Carducci, A., 2014. Viral contamination of aerosol and surfaces through toilet use in health care and other settings. Am. J. Infect. Contr. 42, 758–762.

Viner, R.M., Russell, S.J., Croker, H., Packer, J., Ward, J., Stansfield, C., Mytton, O., Bonell, C., Booy, R., 2020. School closure and management practices during coronavirus outbreaks including COVID-19: a rapid systematic review. Lancet Child Adolesc. Health. 4, 397–404.

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., Tan, W., 2020. Detection of SARS-CoV-2 in different types of clinical specimens. Jama 323, 1843–1844.

Watanabe, T., Bartrand, T.A., Weir, M.H., Omura, T., Haas, C.N., 2010. Development of a dose-response model for SARS coronavirus. Risk Anal. 30, 1129–1138.

Wbg-World Bank Group, 2020. The impact of COVID-19 (Coronavirus) on global poverty: why Sub-Saharan Africa might be the region hardest hit. viewed 27th September 2020, from. https://blogs.worldbank.org/opendata/impact-covid-19-coronavirus-global-poverty-why-sub-saharan-africa-might-be-region-hardest.

HO-World Health Organization. *Water, Sanitation, Hygiene and Waste Management for COVID-19: Technical Brief, 03 March 2020* (No. WHO/2019-NcOV/IPC_WASH/2020.1). World Health Organization.

WHO-World Health Organization, 21 August 2020. Advice on the use of masks for children in the community in the context of COVID-19: annex to the Advice on the use of masks in the context of COVID-19. (No. WHO/2019-nCoV/IPC_Masks/Children/2020.1).

WHO-World Health Organization, 2020. Coronavirus disease (COVID-19) situation report. Available at. https://covid19.who.int/. Accessed on 7th July 2021.

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., Shan, H., 2020. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology 158, 1831–1833.

Xie, C., Jiang, L., Huang, G., Pu, H., Gong, B., Lin, H., Ma, S., Chen, X., Long, B., Si, G., Yu, H., 2020a. Comparison of different samples for 2019 novel coronavirus detection by nucleic acid amplification tests. Int. J. Infect. Dis. 93, 264–267.

Xie, C., Zhao, H., Li, K., Zhang, Z., Lu, X., Peng, H., Wang, D., Chen, J., Zhang, X., Wu, D., Gu, Y., 2020b. The evidence of indirect transmission of SARS-CoV-2 reported in Guangzhou, China. BMC Publ. Health 20, 1–9.

Ye, G., Lin, H., Chen, L., Wang, S., Zeng, Z., Wang, W., Zhang, S., Rebmann, T., Li, Y., Pan, Z., Yang, Z., 2020. Environmental contamination of SARS-CoV-2 in healthcare premises. J. Infect. 81, e1–e5.

Yoon, J.G., Yoon, J., Song, J.Y., Yoon, S.Y., Lim, C.S., Seong, H., Noh, J.Y., Cheong, H.J., Kim, W.J., 2020. Clinical significance of a high SARS-CoV-2 viral load in the saliva. J. Kor. Med. Sci. 35 (20).

Zaneti, R.N., Girardi, V., Spilki, F.R., Mena, K., Westphalen, A.P.C., da Costa Colares, E. R., Pozzebon, A.G., Etchepare, R.G., 2020. Quantitative microbial risk assessment of SARS-CoV-2 for workers in wastewater treatment plants. Sci. Total Environ. 754, 142163.

Zhang, X., Ji, Z., Yue, Y., Liu, H., Wang, 2020. J. Infection risk assessment of COVID-19 through aerosol transmission: a case study of South China Seafood Market. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.0c02895.

Zhang, Y., Chen, C., Zhu, S., Shu, C., Wang, D., Song, J., Song, Y., Zhen, W., Zijian, F., Wu, G., Xu, J., 2020. Isolation of 2019-nCoV from a stool specimen of a laboratory-confirmed case of the coronavirus disease 2019 (COVID-19). China CDC Weekly 2, 123–124.

Zoran, M.A., Savastru, R.S., Savastru, D.M., Tautan, M.N., 2020. Assessing the relationship between surface levels of PM2. 5 and PM10 particulate matter impact on Covid-19 in Milan. Italy. *Sci. Total Environ.* 738, 139825.