Detection of SARS-CoV-2 on contact surfaces within shared sanitation facilities and assessment of the potential risks for COVID-19 infections

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ABSTRACT:

Contamination of contact surfaces with SARS-CoV-2 has been reported as a potential route for the community 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 present the first report of SARS-CoV-2 contamination on key contact surfaces in shared toilets, in the city of Durban, using droplet digital PCR and assessed the probabilistic risks of COVID-19 infections.

Approximately, 53-69% of the contact surfaces were contaminated, with SARS-CoV-2 viral loads per cm^2 ranging from 25.9 to 132.69 gc/cm^2. Toilet seats had the highest contamination per cm^2. The results suggested that the leading cause of contamination in shared toilets could be the shedding of the viral particles in feces and contaminated hands. 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 of these surfaces. The probabilistic assessment showed a high potential for COVID-19 infections. Touching the internal latch of the toilet cubicle had the highest risk of infections ($4.3 \times 10^{-2}(6.0 \times 10^{-4})$) when a person uses the toilet once in a day, increasing to $1.0 \times 10^{-1}(1.4 \times 10^{-3})$ for three uses in a day. The risks estimated in this study were higher than any of the tolerable/acceptable risk figures proposed for COVID-19 from environmental exposure. This calls for the implementation of risk reduction measures, such as strict adherence to wearing face masks, regular washing of hands with soap, and effective and regular cleaning.

KEYWORDS: SARS-CoV-2; COVID-19; Shared sanitation; Contact surface contamination; digital droplet PCR

INTRODUCTION

The current COVID-19 pandemic has claimed over 943 000 lives and infected another 30 million globally as at 18th September 2020 [1]. The primary mode of transmission of the SARS-CoV-2 virus, the causative agent for COVID-19, is through respiratory droplets[2,3,4,5]. This has led to the implementation of mitigation measures, such as social distancing and the use of face masks[6,7,8,9,10]. Additionally, there are reports of potential transmission through contact with contaminated surfaces[11,12,13]. These are of concern due to the stability/survival of this virus on the surface such as plastic, steel, wood and aluminium [14,15,16]. Their survival on contact surfaces is dependent on the material, for instance, it is reported to persist on plastics for 3-4 days[14,15], aluminium for 2-3 hours[16], stainless steel for four days and on glass for two days[14], all at room temperature. However, Goldman[17] 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[18,19], the risks posed by shared sanitation facilities outside of the hospital environment have been neglected.

The reported shedding of viral particles, by both symptomatic and asymptomatic individuals, highlights the increased risks from the use of shared sanitation. The World Health Organization (WHO) reports that between 2-27% of COVID-19 patients have diarrhoea[20], which results in the frequent 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.,[21], and 6.3×10^6 - 1.26×10^8 gc/g of stool by Lescure et al.,[22]. These results show that in circumstances where fecal 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[23].

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 or urine contaminations. Additionally, several studies have shown strong evidence in support of the indoor airborne transmission of viruses, especially in crowded and poorly ventilated areas[24, 25, 26,27], such as shared toilets. For instance, SARS-CoV-2 is reported to survive in aerosols for up to 3 hours[28], meaning the sharing of toilet facilities could be 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[29,30, 31, 32, 33]. This approach has been used in estimation of the risks for COVID-19 infections for wastewater treatment workers[34] and exposure in a market setting[35].

This research focuses on: (i) the detection of SARS-CoV-2 on key contact surfaces within a shared toilet facility in an informal settlement (slum) in Durban city. This could provide background information on the contamination of such surfaces within similar shared facilities, and (ii) an assessment of the risk of COVID-19 infections due to the use of such shared facilities. The approach presented in this study can be used in developing risks reduction measures aimed at reducing the spread of COVID-19 (and possible other similar outbreaks) via the use of shared toilet facilities.

METHODOLOGY

STUDY AREA AND SAMPLING

Two peri-urban informal settlements located within the eThekwini municipality of South Africa were selected for this study. A total of eight (8) shared toilets, referred to as community ablution blocks (CABs), were investigated, four in each settlement. 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 wash hand basin (Figure 1). These were selected based on recommendations made in previous studies[36, 37, 38]. A total of 68 swab samples were taken, with additional six (6) fecal samples that were intentionally sampled for analysis. Sampling was done twice in September 2020 when the reported active clinical cases were low in South Africa.
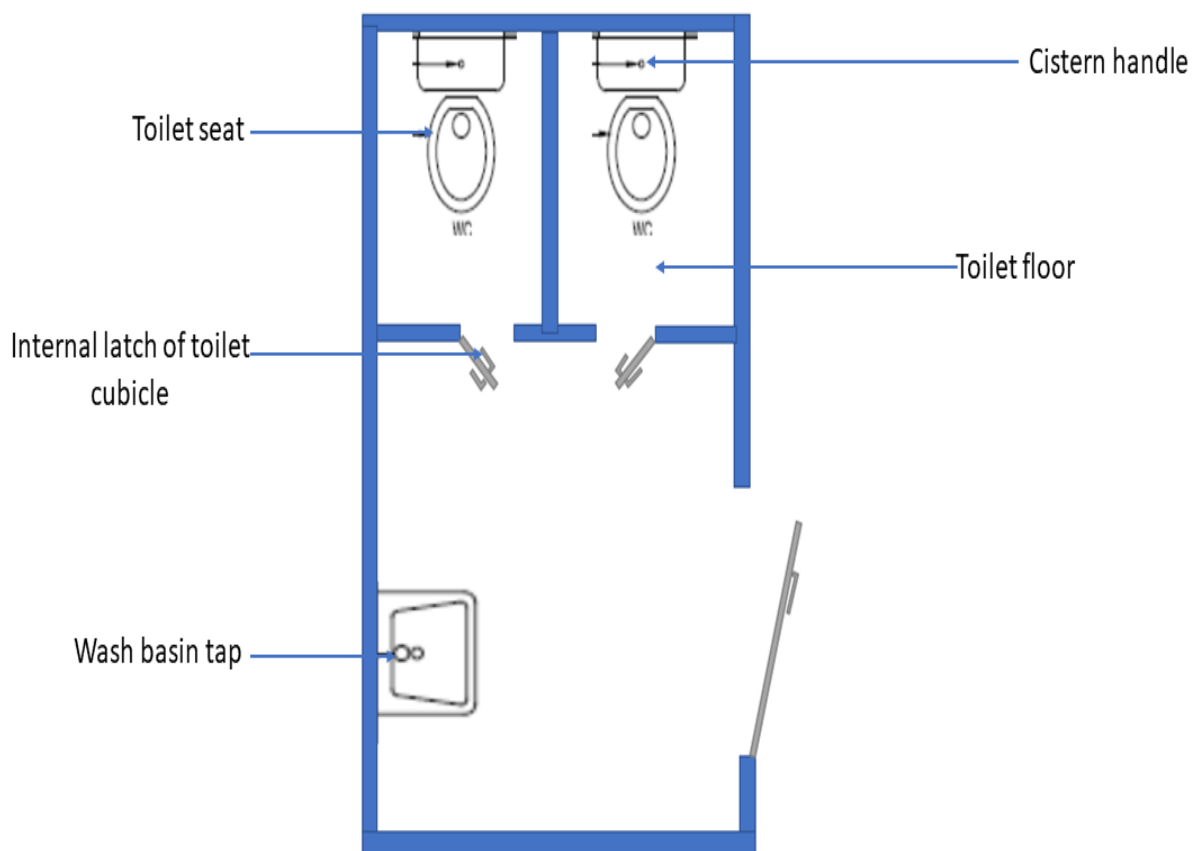
Figure 1: Key contact surface areas within the internal surfaces of CABs that were swabbed.

The swab samples were taken according to the methodology proposed by Park et al.,[37]. Briefly, the swab was moistened with PCR grade nuclease free water moved across the sampling area horizontally, vertically and diagonally. An area of approximately, $50cm^2$ was swabbed for the toilet seat and toilet floors, $20cm^2$ for the cistern handle and internal latch and $30cm^2$ for the tap handle. The swab area was determined based on the available area of these contact surfaces. Swabs were placed in a 400uL PCR-grade nuclease free water and transported to the lab on ice. The personnel carrying out the sampling were fully clothed in personal protective equipment (face masks, shields, lab coats, gloves and face shields).

Molecular detection of SARS-CoV-2

Upon arrival at the laboratory, each tube containing the swab was vortexed for 10s 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. An initial direct detection without RNA extraction was done. This involved using 5 uL of the solution as a template for the molecular analysis. The second approach involved the extraction of the RNA as described below.

RNA extraction:
Nucleic acid 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). In the absence of a viral control strain, the suitability of a concentration method to be used in our study was determined by assessing the

quality and quantity of the extracted RNA. The RNA was then stored at -80 ℃ for further

analysis

Viral detection and quantification using Droplet digital PCR

Detection of SARS-CoV-2 in the samples was carried out using 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. The triplex probe assay also

targets the human RPP30 (HEX labelled) gene for use as an internal extraction control. Briefly,

5 µl of the extracted RNA was used as a template in ddPCR reactions. The ddPCR reaction

mix was prepared according to manufacturer's instructions. 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 ℃ for 1

h, enzyme activation at 95 ℃ for 10 min, 40 cycles of denaturation at 94 ℃ for 30 s and

annealing at 55 ℃ for 60 s. This was followed by enzyme deactivation at 98 ℃ for 10 min and

droplet stabilization at 4 ℃ for 30 min with a ramp rate of 2 ℃/second. The sealed droplet plate

was then transferred to the QX200 Droplet Reader (Biorad, USA). The distribution of positive

and negative droplets in each sample well was read using the QuantaSoft 1.7 software (Biorad,

USA) while further analysis was carried out using the QuantaSoft Analysis Pro 1.0 software

(Biorad, USA).

Quality control with direct detection

Each sample plate contained positive, negative and no template control wells. For ddPCR, the

SARS-CoV-2 positive and negative controls (Exact Diagnostics) were utilized. The positive

control contained synthetic RNA transcripts of 5 gene targets (E, N, ORF1ab, RdRP and S) of

SARS-CoV-2 while the negative control 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. If all controls exhibit the expected performance, a sample is considered positive if it has any or both 2 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.
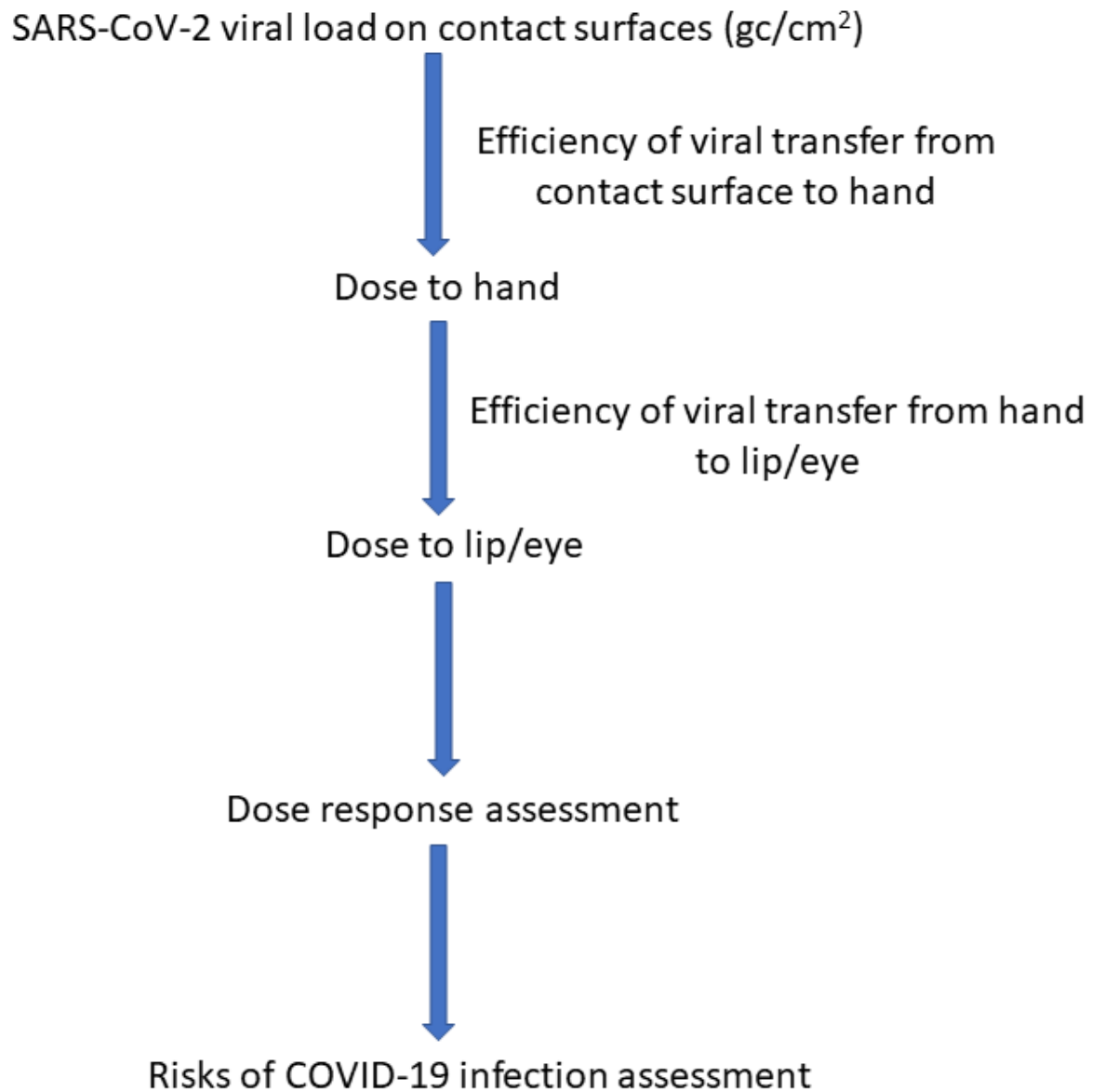
Risk of COVID-19 infection from the use of shared sanitation: A case of the community ablution blocks

The quantitative microbial risk assessment (QMRA) approach was used for the health risk assessment. According to Haas et al.[39] the QMRA approach involves a sequence of interrelated steps: a) hazard identification; b) exposure assessment; c) dose-response assessment and d) risk characterization. This will the first report for assessing risks from the use of sanitation facilities despite the widespread understanding that sanitation facilities may facilitate its spread.

Hazard Identification: The SARS-CoV-2 virus is the hazard of choice for this assessment. To date two papers has reported a risks assessment for SARS-CoV-2 using the quantitative microbial risk assessment approach[34,35]. However, the potential risks of infection with this virus from contact surfaces has been established.

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 outbreaks in cruise ships, restaurants, nursing homes, schools, daycare centres and gyms [40,41,42, 43]. 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 Figure 2 presents the process flow.

SARS-CoV-2 viral load on contact surfaces (gc/cm^2)

Efficiency of viral transfer from contact surface to hand

Dose to hand

Efficiency of viral transfer from hand to lip/eye

Dose to lip/eye

Dose response assessment

Risks of COVID-19 infection assessment

Figure 2: Scenario for assessing the exposure and possible risks associated with contamination of the contact surfaces (Adapted from Ryan et al., [44]).

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

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

Where p(d) is the infection risk at a dose of *d* in units of gc/cm^2 and *k* is a pathogen dependent parameter. 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[45]. 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[46,47]. These dose-response parameters have been used in assessing the risks of COVID-19 infections for workers in wastewater treatment plants[34].

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.

Table 1: Input information 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., [44]
Viral transfer from hands to mouth/lips or eyes	Median value of 0.34	

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). All the QMRA modelling was done with @Risk Version 7.5 (Palisade Corporation, USA) addon to Excel.

RESULTS

Prevalence of contamination

The chance of contamination on the contact surfaces varied over the two sampling events. The highest prevalence of contamination of 68.8% was observed for the tap handle, followed by the toilet floor with the internal latch giving the least prevalence of contamination (53.3%) (Figure 3). Despite the observed difference, there was no statistically significant difference in the prevalence (p value ≥ 0.05). Out of the six (6) faecal samples purposefully taken, five (5) were positive for SARS-CoV-2 representing a prevalence of about 83.3%

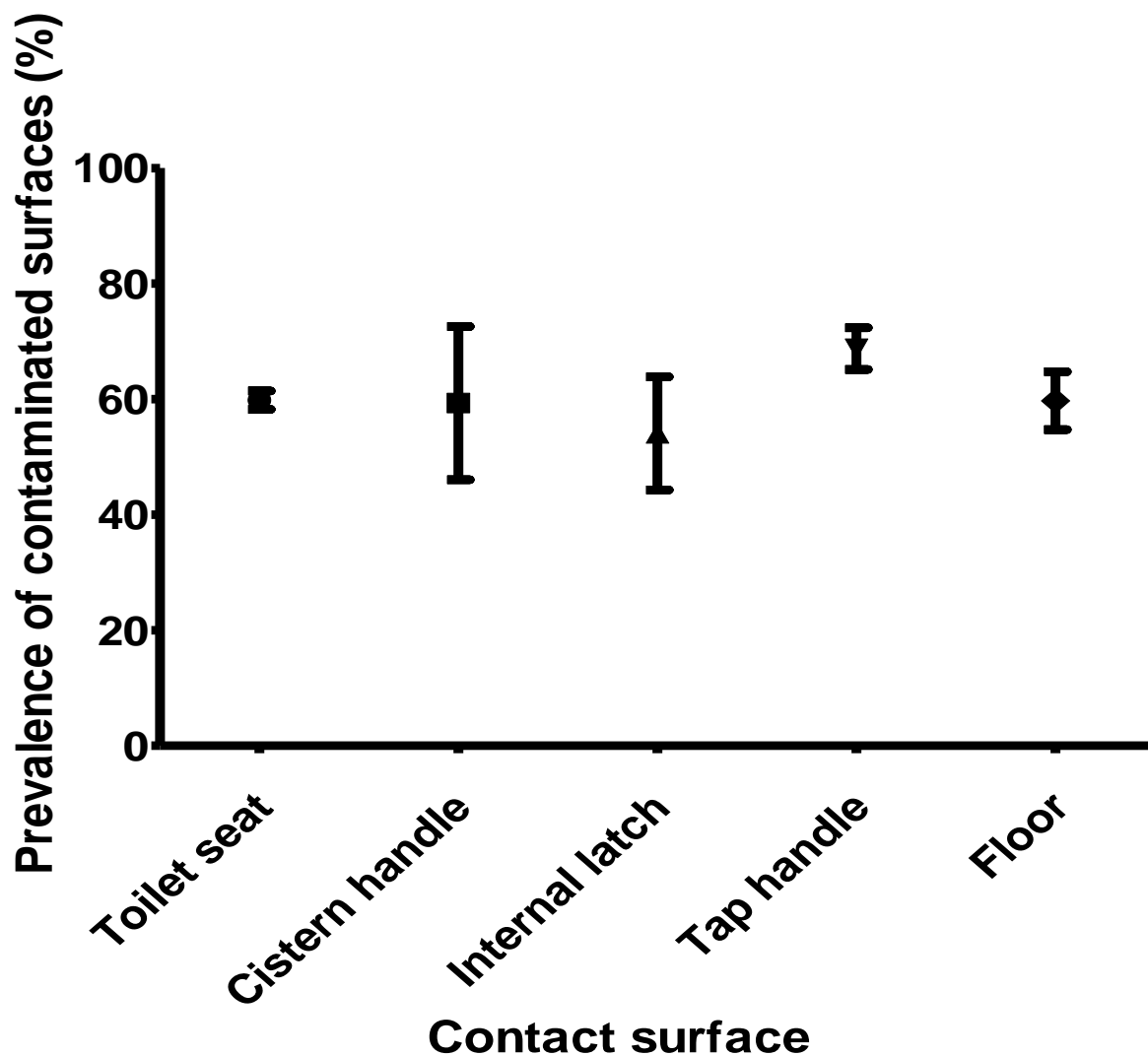Figure 3: Percentage of contact surfaces contaminated with SARS-CoV-2

Concentration of SARS-CoV-2 on contact surfaces before and after cleaning

Per cm^2 swabbed, the concentration of SARS-CoV-2 was highest on the toilet seats (132.7 ($\pm$252.7) gc/cm^2), followed by the cistern handle (72.2 ($\pm$75.3) gc/cm^2) and internal latch (51.5 ($\pm$42.9) gc/cm^2). The differences in the concentration between the different contact surfaces were statistically significant (p value $\leq$ 0.05). Cleaning reduced the concentration on these contact surfaces, with significant (p value $\leq$ 0.05) reduction on the toilet seat, cistern handle, internal latch and toilet floors. For instance, after cleaning, the viral load on the toilet seats was 2.1($\pm$0.6) gc/cm^2 (Figure 4). However, there was no significant reduction in the SARS-CoV-2 viral load on the tap handles after cleaning, as shown in Figure 4. Additionally, the standard deviation in the viral load measured on the cleaned and uncleaned contact surfaces indicated a trend. The variation on the uncleaned surfaces was consistently higher than the mean values calculated; however, on the cleaned surfaces, the standard deviations were lower than the mean values (Table S1: Appendix 1). The mean viral load in the positive faecal samples was 381.3 ($\pm$266.1) gc/g.
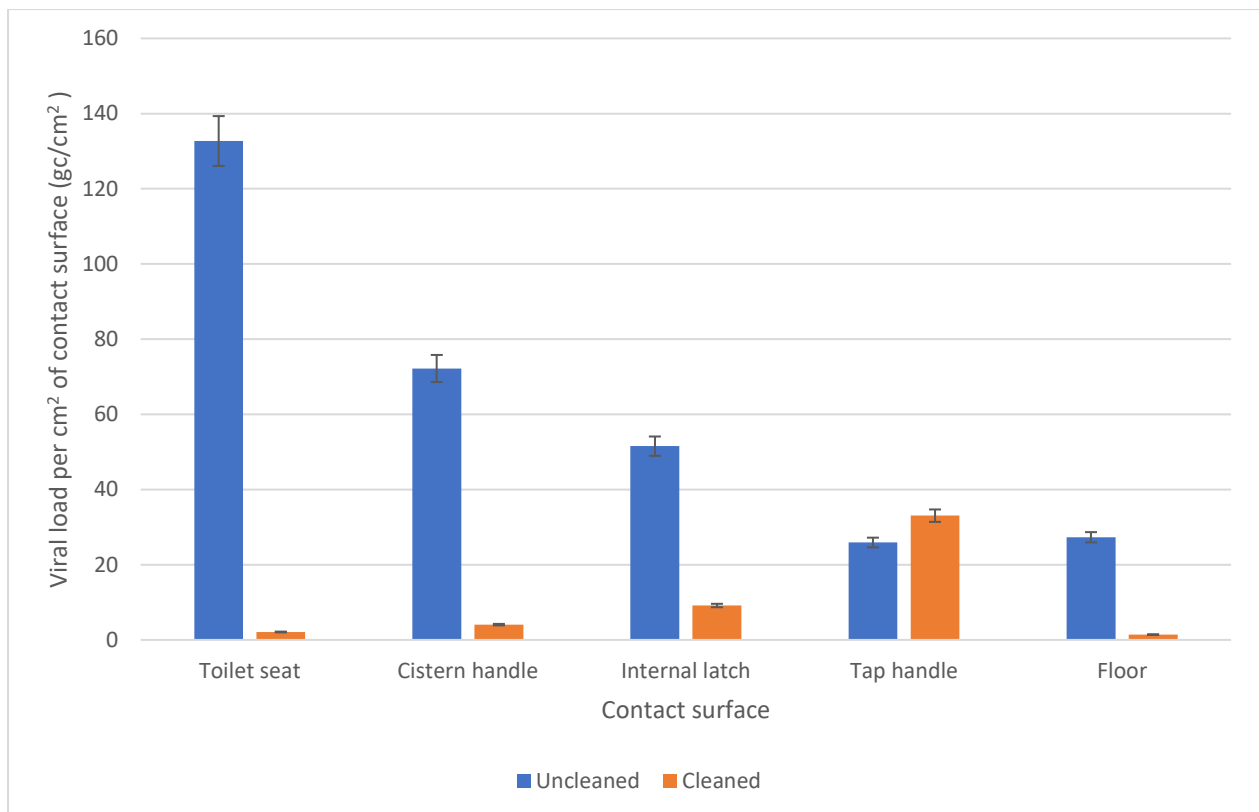
Figure 4: Concentration of SARS-CoV-2 on key contact surfaces in the shared toilets

Comparison of direct quantification and quantification via extracted RNA

Detection of the SARS-CoV-2 on the swab without an 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%) with a corresponding prevalence of 37.5% 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 (66.7%) compared to direct detection (50%) (Figure 5A).

There was a similar trend in the difference in the viral load when these two approaches (direct quantification and quantification via extracted RNA) were used. For instance, via direct quantification without RNA extraction, 244.9 ($\pm$339.6) g/cm^2 was recorded on the toilet seats, however when the RNA was extracted the concentrations were 21 ($\pm$15.8) gc/cm^2. These differences are statistically significant, indicating consistently lower concentrations when the RNA was extracted from the samples prior to analysis. However, as observed with the prevalence, the only exception was the floor swab samples (Figure 5B)
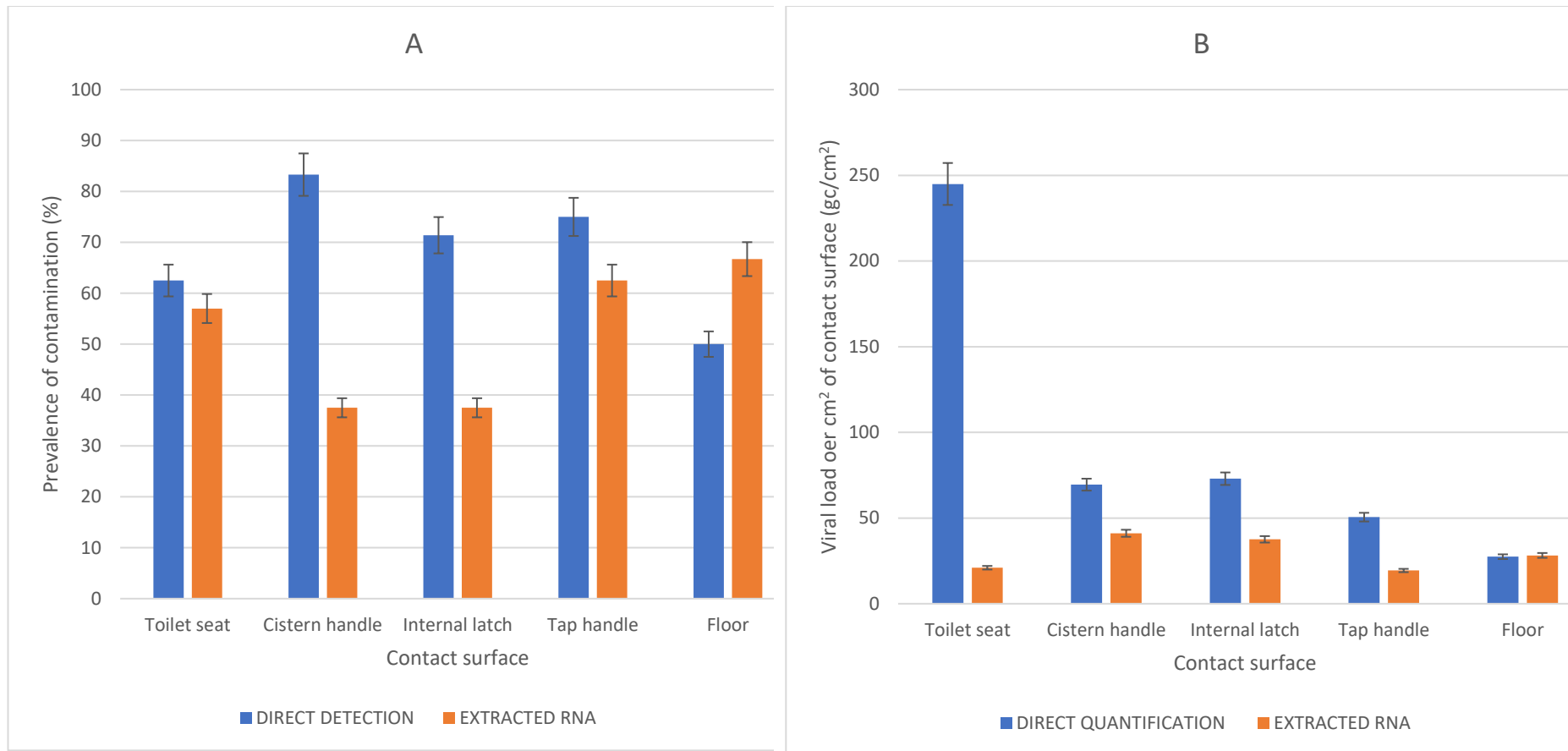
Figure 5: Difference in the detection and quantification of SARS-CoV-2 via direct analysis and RNA extraction

Potential risks 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 on these contact surfaces varied considerably, driven mainly by the difference in the viral loads described above. The magnitude of the risks was similar for contact with all the surfaces (10^{-2}), however the highest risks were observed for contact with the internal latch of the toilet cubicles. It was estimated that approximately four (4) out of every 100 people using the toilet who touch the internal latch could potentially be infected with COVID-19. The lowest risks were determined for contact with the toilet floors, where an estimated eight (8) out of a 1000 people exposed may be infected (Table 2). 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 contact with the internal latch within a day resulted in an increase in the risks from 4.3×10^{-2} (6.0×10^{-4}) for a single exposure to 1.0×10^{-1} (1.4×10^{-3}). This means that for every 10 people who use the toilet facility twice or three times in a day at least one of them will be infected. Similar significantly increased risks were observed for all the other contact surfaces (Table 2).

The risks of infection were reduced, considering exposure after the toilets have been cleaned (Table 3). However, these reductions were not significant for exposure via contact with the toilet seat (1.8×10^{-2}(5.8×10^{-6})) and tap handle (2.7×10^{-2}(8.8×10^{-5})). Most notable reductions were exposure via contact with the cistern handle and internal latch. The probability of infection reduced from three out of a hundred people (3.3×10^{-2}(8.3×10^{-4})) to three out a thousand (3.4×10^{-3}(1.1×10^{-5})) for cistern handle and four out of a hundred (4.3×10^{-2}(6.0×10^{-4}))

to about eight out of thousand (7.6×10^{-3} (2.5×10^{-5})) for internal latch. As observed for multiple exposures to the uncleaned surfaces, multiple exposures to the cleaned surfaces could increase the risks of infection, as reported in Table 3.

Table 2: Median risks (CI) of infection with COVID-19 due to contact with uncleaned shared toilets within one day

	Toilet seat	Cistern Handle	Internal latch	Tap handle	Floor
One exposure event	$2.4 \times 10^{-2}(3.4 \times 10^{-3})$	$3.3 \times 10^{-2}(8.3 \times 10^{-4})$	$4.3 \times 10^{-2}(6.0 \times 10^{-4})$	$1.9 \times 10^{-2}(4.4 \times 10^{-4})$	$8.4 \times 10^{-3}(2.5 \times 10^{-3})$
Multiple exposures	$5.8 \times 10^{-2}(4.2 \times 10^{-3})$	$7.9 \times 10^{-2}(1.8 \times 10^{-3})$	$1.0 \times 10^{-1}(1.4 \times 10^{-3})$	$4.6 \times 10^{-2}(1.0 \times 10^{-3})$	$2.1 \times 10^{-2}(3.2 \times 10^{-3})$

Table 3: Median risk (CI) of infection with COVID-19 due to contact with cleaned shared toilets within one day

	Toilet seat	Cistern Handle	Internal latch	Tap handle	Floor
One exposure event	$1.8 \times 10^{-2}(5.8 \times 10^{-6})$	$3.4 \times 10^{-3}(1.1 \times 10^{-5})$	$7.6 \times 10^{-3}(2.5 \times 10^{-5})$	$2.7 \times 10^{-2}(8.8 \times 10^{-5})$	$1.2 \times 10^{-3}(3.9 \times 10^{-6})$
Multiple exposures	$4.4 \times 10^{-3}(1.7 \times 10^{-5})$	$8.3 \times 10^{-3}(3.2 \times 10^{-5})$	$1.9 \times 10^{-2}(7.2 \times 10^{-5})$	$6.6 \times 10^{-2}(2.5 \times 10^{-4})$	$2.9 \times 10^{-3}(1.3 \times 10^{-5})$

DISCUSSION

Contact surface contamination within the toilet facilities was widespread (Figure 3), despite a higher 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[48,49,50,51,52,53,54]. Notably, Fankem et al[48], 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[51], 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 sing a human adenovirus virus (HAdV), Verani et al.,[52] 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 been reported in hospitals[55,56,57,58] home settings[59,60,61] and public spaces[60].

Contamination of the contact surfaces could be as a result of direct contact with feces or unclean hands. 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 due to direct contact with feces or urine. The frequency of contact has been proposed as the most critical factor in the direct contamination of contact surfaces within public toilets[48]. 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.,[50] 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[49]. These could potentially be a significant source of contamination for other contact surfaces, such as cistern handles. A study by Flores et al., [49] observed that the bacterial community on the toilet floors was similar to those found on the toilet flush/cistern handles. They attributed this to the use of foot in operating these cistern/flush handles by some of the users.

Contamination of the toilet seat and the floors could be contaminated 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[49]. Using modelling approaches, Li et al.[62] postulated that massive upward transport of viral particles is observed with over 40-60% of the particles potentially deposited on the toilet seat. Therefore, shedding of the SARS-CoV-2 viral particles in feces could be deposited on the toilet seat during flushing, this is potentially the main source of the contamination on the toilet seats observed. Contamination of the toilet seat up to 24 flushes after initial shedding in feces could still occur, although the concentrations could reduce with each flush[63]. Using bacterial indicators, Johnson et al.,[63] observed $3\log_{10}$ reduction after the first flush, $1\text{-}2 \log_{10}$ after the second and thereafter less than $1 \log_{10}$ reduction with each flush.

We observed that direct detection and quantification of SARS-CoV-2 in swab solutions gave higher prevalence of contamination and viral load (Figure 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 inhibitors during the RNA extraction. It is worth noting that

the toilet floor was constantly soiled, therefore 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. This is based on the fact that these solids could interfere with the droplet generation step of the ddPCR sample processing, this could be the reason why we detected SARS-CoV-2 in more floor samples that had an RNA extraction step compared to samples that were analysed directly. 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 (Figure 4) could also be attributed to the same factors responsible for the frequency of contamination. However, the viral load on the toilet seats per cm^2 were significantly higher 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 of both symptomatic and asymptomatic patients is well reported[64,65, 66, 67,68], 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[5 69,70,71]. The toilets are cleaned once a day, this resulted in the significant reduction of viral load on almost all the contact surfaces, except for the tap handle (Figure 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 with them. The findings, therefore, show that cleaning of shared sanitation facilities should consider surfaces with high contact frequency such as the toilet seat, tap handle and internal latch.

The viral contamination of key contact surfaces within shared toilets potentially could result in COVID-19 infections. The estimated risks show that the highest risks of infection from a one-time use of the toilets is the contact with the internal latch (Table 2). It is worth noting that the estimated risks from contact with all the contact surfaces were all within the same magnitude (10^{-2}). This shows that despite the significant difference in viral load per cm^2 between the different contact surfaces, the risks did not differ significantly. A manageable risk of 1.17×10^{-3} has been recommended by Zhang et al.,[35], meaning 1 person out of a thousand being infected is acceptable. In contrast Zanetti et al.,[34] 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 both one-time and multiple exposures, the risks estimates from our study are much higher than these recommended tolerable/acceptable risks figure. The risks estimated from this study were higher compared with the risks of COVID-19 infections for customers within a market[35]. However, the estimates were similar to data published by Zanetti et al.,[34] for workers in wastewater treatment plants (2.6×10^{-3} to 1.3×10^{-2}). The comparative risks estimate from our study and for the wastewater treatment workers is largely due to the high viral loads measured on the contact surfaces and wastewater. The most fundamental assumption in these risks estimates is that the viral particles detected are infectious. Reports have shown that SARS-CoV-2 viral particles shed in feces may still be infectious[72,73,74], 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 be survice on surfaces from a few hours [14], to four days [14,15]. 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. The reduction in risks of infections was

not commensurate with the decrease in viral load. However, it must be noted that we assumed a worst-case scenario where a gene copy is considered an infectious viral particle. This could potentially result in over estimation of the associated risks, because the detection and quantification were based on viral RNA and inactivated viruses may still yield positive results. Tuladhar et al.,[75] 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.

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 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. 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. We determined that the use of

the shared toilets could potentially cause COVID-19 infections, with risks estimates higher than any tolerable/acceptable risk figures published.

RECOMMENDATION/ RISK REDUCTION INTERVENTIONS

The observed 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 a large population is relying on community toilets. 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.,[75] 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 is attributed to the shedding of SARS-CoV-2 in feces, these are dispersed unto the toilet seat and floor during flushing. Therefore, by closing the water closet lid, the spread of the droplets or aerosols generated is reduced 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.

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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.

ADDITIONAL INFORMATION

The authors have no competing financial and non-financial interest to declare

APPENDIX 1

Table S1: Concentration of SARS-CoV-2 per cm^2 on the contact surfaces

	Toilet seat	Cistern Handle	Internal latch	Tap handle	Floor
Before cleaning	132.9(252.7)	69.1(75.3)	60.1(42.9)	36.4(45.7)	28.1(26.6)
After cleaning	2.1(0.6)	4.1(0.7)	9.1(2.2)	33.0(31.4)	1.4(0.2)

Table S2: Probability distribution functions for the concentration of SARS-CoV-2 on the contact surfaces

	Toilet seat	Cistern Handle	Internal latch	Tap handle	Floor
Before cleaning	Pareto(0.62426;9.5506)	Expon(65.213)	Extvalue(40.63;34.671)	Expon(32.711)	Pareto(0.63356;3.38)
After cleaning	Mean (4.2)	Mean(8.1)	Mean(18.3)	Mean(66.1)	Mean (2.8)