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Real-World Effectiveness of Disinfection Protocols in Reducing SARS-Cov-2 RNA Contamination on Hospital Surfaces: A Case Study from a Warm and Arid Region in Southwestern Iran

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ABSTRACT

Introduction: Hospital surfaces may contribute to SARS-CoV-2 transmission; however, the real-world effectiveness of disinfectants is unclear. This study evaluated routine disinfection protocols using 0.5% sodium hypochlorite and 70% ethanol to reduce SARS-CoV-2 RNA contamination on high-touch hospital surfaces.

Methods: A total of 152 surface samples were collected from five high-touch surface categories (door handles, bed rails, computer keyboards, toilet door handles, and toilet faucets) before and after disinfection in a hospital in southwestern Iran (September 2020- February 2021). SARS-CoV-2 RNA was detected using RT-qPCR targeting RdRp and N genes. Reduction percentages were calculated using McNemar's test.

Results: Overall, RNA was detected in 41/76 samples (53.9%) before versus 5/76 (6.6%) after disinfection (87.8% reduction, $p<0.001$). Sodium hypochlorite reduced contamination from 47.4% to 7.9% (83.3% reduction), while ethanol reduced from 60.5% to 5.3% (91.3% reduction). Complete RNA reduction (100%) was achieved on door handles, keyboards, and toilet surfaces, with no detectable RNA after disinfection. Residual RNA was observed on bed rails in both groups (20.0% with hypochlorite, 10.0% with ethanol), accounting for 60% of post-disinfection positives. Toilet faucets showed residual RNA only with hypochlorite (14.3%), while toilet door handles only with ethanol (14.3%). All reductions were significant ($p<0.05$).

Conclusion: Both disinfectants effectively reduced SARS-CoV-2 RNA under the operational conditions evaluated, with no claim of superiority for either agent. However, bed rails remain a persistent hotspot despite routine disinfection, highlighting the need for enhanced protocols and targeted monitoring.

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Introduction

The COVID-19 pandemic, caused by SARS-CoV-2, has posed unprecedented challenges to healthcare systems worldwide[1]. As of 2022, the World Health Organization reported hundreds of millions of confirmed cases and millions of deaths globally, with healthcare facilities serving as both frontline defense settings and potential epicenters

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of nosocomial transmission [2]. Understanding and interrupting transmission routes within hospitals has remained a critical priority throughout the pandemic and continues to inform infection prevention and control (IPC) strategies for future respiratory pathogen outbreaks [3].

The transmission dynamics of SARS-CoV-2 involve multiple routes, including respiratory droplets, aerosols during medical procedures, and contact with contaminated surfaces [4]. While airborne transmission has received considerable attention, the role of fomites-contaminated inanimate objects and surfaces-in the indirect transmission of SARS-CoV-2 cannot be overlooked [5]. Experimental studies have demonstrated that SARS-CoV-2 can remain viable on various surfaces for hours to days, depending on environmental conditions such as temperature, humidity, and material type [6]. Specifically, the virus exhibits considerable stability on common hospital surface materials including stainless steel, plastic, and cardboard, with survival durations influenced by viral load, temperature, and pH [5]. A systematic review by Okomo et al. confirmed that there is a substantial risk of indirect transmission of SARS-CoV-2 from contaminated surfaces and objects in healthcare settings, underscoring the importance of rigorous environmental decontamination [5].

Environmental surfaces in hospitals, particularly high-touch areas such as bedrails, monitors, door handles, and medical equipment, can serve as reservoirs for microorganisms, which may then be transmitted to patients or healthcare workers [7]. The pathogen's resilience, ease of transfer, and ability to colonize surfaces make environmental contamination a significant factor in healthcare-associated infections [3]. During the COVID-19 pandemic, this concern was magnified, leading international health authorities to issue comprehensive guidance on environmental cleaning and disinfection. The World Health Organization, while noting that transmission had not been conclusively linked to contaminated surfaces in available studies at the time, recommended rigorous surface disinfection based on evidence of surface contamination in healthcare settings and past experiences with other coronaviruses [8]. Similarly, the United States Centers for Disease Control and Prevention emphasized the importance of dedicating medical equipment for suspected or confirmed COVID-19 patients and ensuring proper environmental decontamination [4].

In response to these concerns, healthcare facilities worldwide implemented various disinfection protocols aimed at eliminating SARS-CoV-19 from environmental surfaces. These protocols typically involved the use of chemical disinfectants such as sodium hypochlorite (bleach), ethanol, and hydrogen peroxide, applied through spraying or wiping methods [1]. Research has demonstrated that exposure to 70% ethanol, 70% isopropanol, 10% bleach, or 1-3% hydrogen peroxide for 15-30 minutes can effectively inactivate various SARS-CoV-2 variants [7]. Chlorine-based disinfectants at concentrations of 500 mg/L have been recommended for public spaces and healthcare environments [8]. However, the effectiveness of these agents under real-world operational conditions-considering factors such as human error, time constraints, patient load, and adherence to protocols-has remained less clearly understood [1].

A rapid review by Okebe et al. examining disinfection methods in healthcare settings found that both spraying and wiping methods may protect against SARS-CoV-2 indirectly by reducing residual surface contamination [1]. However, the included observational studies showed contrasting results regarding the comparative effectiveness of different application methods, with one study reporting spraying more effective (0% residual contamination vs. 25.6%) while another found spraying less effective (25.0% residual vs. 48.2%). Importantly, none of the reviewed studies reported a direct effect on the risk of infection in humans, and the certainty of evidence was characterized as very low due to moderate to serious risk of bias [1]. These findings highlight the critical gap between laboratory efficacy and real-world effectiveness of disinfection protocols. However, empirical data on

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the real-world performance of these protocols in specific hospital settings-particularly in regions with warm and arid climates-remain limited

Furthermore, recent evidence suggests that the risk of "environment-to-human transmission" highly relies on surface contamination combined with human behavior through the indirect pathway of "fomite-hand-mucosa or conjunctiva," as well as virus decay on surfaces. Li et al. emphasized that when discussing SARS-CoV-2 transmission risk, researchers must comprehensively consider the biological basis of virus transmission, environmental conditions, and virus decay; otherwise, the risk of fomite transmission may be overrated, causing public excessive panic and triggering ineffective actions [2]. This nuanced understanding calls for empirical data from actual healthcare settings to inform evidence-based IPC policies.

In Iran, as in many other countries, hospitals implemented intensive disinfection protocols during the peak of the pandemic. A study conducted in Iran examining SARS-CoV-2 surface contamination after routine and terminal cleaning with varying strengths of disinfectants was among the few included in the systematic review by Okomo et al., contributing to the limited evidence base on cleaning effectiveness in healthcare settings [5]. However, significant variability exists in protocol implementation, monitoring, and outcomes across different healthcare facilities.

The city of Behbahan, located in Khuzestan province, Iran, with its healthcare infrastructure serving a diverse population, presents an important setting for examining the operational effectiveness of disinfection protocols. During the pandemic, Shahid Zadeh Hospital in Behbahan, a primary healthcare facility managing COVID-19 patients, implemented standard disinfection protocols based on national guidelines. However, the actual effectiveness of these protocols in eliminating SARS-CoV-2 from high-risk surfaces under real-world conditions, considering the challenges of staff training, protocol adherence, patient volume, and resource constraints, has not been systematically evaluated.

Understanding whether routine disinfection protocols achieve their intended goal of eliminating viral contamination from hospital surfaces is essential for several reasons. First, it provides critical feedback to hospital IPC teams about potential gaps in protocol design or implementation. Second, it informs resource allocation decisions regarding disinfection products, equipment, and personnel training. Third, it contributes to the growing body of knowledge on practical infection control measures for current and future respiratory virus outbreaks. Fourth, it helps identify "hotspots" or specific surfaces and areas where residual contamination persists despite disinfection, allowing for targeted interventions [3].

The concept of operational effectiveness differs fundamentally from laboratory efficacy. Laboratory studies test disinfectants under controlled conditions with standardized application, while operational effectiveness reflects what actually happens in busy clinical environments where staff may be overworked, undertrained, or inconsistent in their practices [7]. Recent quality improvement initiatives in hospitals worldwide have demonstrated that achieving and sustaining high compliance with cleaning protocols requires continuous training, regular audits, innovative tools such as tagging systems, and multidisciplinary collaboration [3]. Without such systematic approaches, even well-designed protocols may fail to achieve their intended outcomes.

This study aims to evaluate the real-world effectiveness of disinfection protocols implemented at Shahid Zadeh Hospital in Behbahan, Iran, in eliminating SARS-CoV-2 from high-risk hospital surfaces. By comparing surface contamination levels before and after routine disinfection procedures across different hospital wards and surface types, we seek to: 1- quantify the reduction in viral contamination achieved by current protocols; 2- identify specific areas or surfaces where residual contamination persists; 3- assess whether protocol effectiveness varies by ward type (e.g., ICU vs. general wards) or surface material; and 4- provide evidence-based

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recommendations for improving disinfection practices in similar resource-constrained healthcare settings.

The findings of this study have implications not only for COVID-19 infection control but also for preparedness against future emerging infectious diseases. As the world transitions from pandemic emergency response to endemic disease management and future threat preparedness, lessons learned from rigorous evaluation of infection control practices during COVID-19 must be systematically documented and applied [2]. This case study from Behbahan contributes to that global learning process, offering insights from a mid-sized hospital in Iran that may be transferable to similar healthcare settings in low- and middle-income countries facing comparable challenges in infection prevention and control.

Materials and Methods

Study Design and Setting

This study was conducted to evaluate the operational effectiveness of routine disinfection protocols in eliminating SARS-CoV-2 contamination from high-risk hospital surfaces. This study was conducted at Shahid Zadeh Hospital, a primary healthcare facility designated for COVID-19 patient management in Behbahan, Khuzestan province, Iran. Environmental sampling was performed before and after the application of standard disinfection procedures across different wards admitting COVID-19 patients.

Surface Selection and Sampling Procedure

Based on established protocols for SARS-CoV-2 environmental surveillance in healthcare settings[9], five categories of high-touch surfaces were selected for sampling: (i) door handles of COVID-19 inpatient wards, (ii) bed rails, (iii) computer keyboards at nursing stations in COVID-19 wards, (iv) toilet door handles, and (v) toilet faucet handles. These surfaces were chosen based on their frequency of contact by healthcare workers, patients, and visitors, and their potential to serve as fomites for nosocomial transmission [10].

Sampling was performed using sterile flocked swabs pre-moistened with viral transport medium (VTM) consisting of phosphate-buffered saline supplemented with 0.5% bovine serum albumin, penicillin (100 U/mL), and streptomycin (100 µg/mL). For each surface, a standardized area of approximately 25 cm² was swabbed thoroughly in both horizontal and vertical directions using firm pressure [11]. The swabs were immediately placed into sterile tubes containing 2 mL of VTM, transported on ice to the laboratory within 2 hours of collection, and stored at -80°C until further processing.

For the "before disinfection" samples, surfaces were swabbed immediately prior to the scheduled daily disinfection procedure. The "after disinfection" samples were collected approximately 30-60 min after the completion of the disinfection protocol, allowing sufficient contact time for the disinfectant to act while reflecting real-world conditions where surfaces may be accessed shortly after cleaning.

Disinfection Protocol

The standard disinfection protocol implemented at Shahid Zadeh Hospital during the study period followed national guidelines for COVID-19 infection prevention and control. Environmental surfaces were disinfected three times daily using sodium hypochlorite solution at a concentration of 0.5% (5000 ppm) applied with clean cloths by trained environmental services personnel. The protocol mandated that surfaces remain visibly wet for a minimum contact time of 10 minutes. No additional mechanical action or wiping was performed after application to avoid interfering with the disinfection process.

In parallel, to compare the effectiveness of different disinfectant agents under real-world conditions, a subset of surfaces in each ward was randomly assigned to be disinfected using 70% ethanol solution. For allocation, a simple alternating assignment method was employed to minimize selection bias and ensure balanced representation across different wards and surface types. Within each ward, the first high-touch surface category encountered (e.g., door handles) was assigned to sodium hypochlorite, the second

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category (e.g., bed rails) to ethanol, the third category (e.g., computer keyboards) to sodium hypochlorite, and this alternating sequence continued for all subsequent surface categories. This method ensured that each disinfectant was tested on all five surface categories within each ward, allowing for a direct and fair comparison under identical operational conditions. The ethanol disinfection was performed by the same personnel using identical application methods (clean disposable cloths) and the same frequency (three times daily) to ensure comparability. Ethanol 70% (v/v) was selected based on its well-documented virucidal activity against enveloped viruses including SARS-CoV-2 and its widespread availability in healthcare settings.

For both disinfectants, the application procedure consisted of the following:

1.Pre-cleaning: Visible soiling was removed if present (though this was rarely required as surfaces were routinely cleaned)

2.Application: The disinfectant was applied liberally to saturate the surface completely

3.Contact time: Surfaces were left to air dry without wiping or rinsing, allowing the recommended contact time of 10 minutes for sodium hypochlorite and 5 minutes for ethanol (based on the rapid evaporation rate of alcohol)

4.Post-application: No additional mechanical action or wiping was performed to avoid interfering with the disinfection process

It is important to note that this study was designed as an evaluation of real-world effectiveness rather than a controlled laboratory efficacy trial. Therefore, the disinfection procedures were performed by routine hospital staff under normal working conditions, without additional supervision or modification by the research team (except for the assignment of surfaces to different disinfectants). While this study was conducted under routine operational conditions, the absence of detailed contextual data (e.g., occupancy, staff activity, environmental parameters) limits the generalizability of our findings. Therefore, the results should be interpreted as preliminary and hypothesis-generating, and future studies should incorporate these variables to enable more robust conclusions.

RNA Extraction

Total viral RNA was manually extracted from the collected samples. Briefly, 200 µL of each VTM sample was aliquoted for RNA extraction using the SinaPure™ Viral RNA Extraction Kit (Sinaclon, Iran; Catalog No. EX6101) strictly according to the manufacturer's instructions [12]. The extraction procedure involved lysis of viral particles, binding of RNA to silica membranes, washing steps to remove contaminants, and final elution of purified RNA in 50 µL of elution buffer. No automated extraction systems were employed. For each batch of extractions, a negative extraction control (sterile VTM) was included to monitor for cross-contamination during the process.

Real-Time Reverse Transcription Quantitative PCR (RT-qPCR)

SARS-CoV-2 RNA was detected using the PishtazTeb COVID-19 One-Step RT-qPCR Kit (PishtazTeb Diagnostics, Iran; Catalog No. PT-COVID19), a dual-target assay approved for in vitro diagnostic use. The kit simultaneously detects the viral RdRp and N genes and includes an internal control targeting the human RNase P gene to monitor sample adequacy, extraction efficiency, and PCR inhibition [13].

Each reaction mixture (20 µL total volume) contained 5 µL of extracted RNA template, 10 µL of 2× Master Mix, 1 µL of Enzyme Mix, 1 µL of RdRp/N primer-probe mix, 1 µL of RNase P primer-probe mix, and 2 µL of RNase-free water. Amplification and detection were carried out on a LightCycler® 96 Real-Time PCR System (Roche, Germany) under the following thermal cycling conditions: reverse transcription at 50°C for 20 minutes, initial denaturation at 95°C for 3 minutes, followed by 45 cycles of denaturation at 94°C for 10 seconds and combined annealing/extension/ fluorescence detection at 55°C for 40 seconds. A final cooling step at 25°C for 10 seconds concluded the run.

Fluorescent signals were acquired through three detection channels: FAM for the RdRp gene, HEX

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for the N gene, and ROX for the RNase P internal control. A sample was considered positive if it exhibited a characteristic sigmoidal amplification curve crossing the threshold within 40 cycles (Ct < 40) in either viral gene channel, with adequate internal control amplification [11]. All reactions were performed in duplicate, and assays with inconsistent Ct values (difference >1.5 cycles) were repeated. Negative template controls (nuclease-free water) and positive extraction controls were included in each PCR run.

Rigorous quality assurance measures were applied throughout the molecular analysis. The performance of the RdRp and N gene primers and probes was validated against a reference RNA control to ensure specificity. The inclusion of RNase P as an internal amplification control allowed identification of samples with PCR inhibition or inadequate cellular material. For quantification purposes, a standard curve was generated using serial dilutions of a quantified SARS-CoV-2 RNA standard, enabling estimation of viral copy numbers per sample. The detection limit of the assay was determined to be approximately 10^3 RNA copies per reaction based on the manufacturer's specifications.

Statistical Analysis

Data were analyzed using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including frequencies and percentages, were used to summarize surface contamination rates before and after disinfection. The McNemar test for paired categorical data was applied to compare the proportion of positive samples before versus after disinfection across all surfaces combined and within each surface category. A p-value < 0.05 was considered statistically significant. Reduction percentages were calculated using the formula: [(% positive before disinfection - % positive after disinfection) / % positive before disinfection] × 100. For surface-specific analyses, McNemar's test was applied separately to each surface category without adjustment for multiple comparisons, as the study was designed as an exploratory field evaluation rather than a confirmatory comparative trial.

Results

Overview of Surface Contamination

A total of 152 surface samples were collected and analyzed from five high-touch surface categories at Shahid Zadeh Hospital in Behbahan, Iran. Of these, 76 samples were collected before disinfection and 76 after disinfection, with an equal distribution between the two disinfectant groups (38 samples per disinfectant for both before and after disinfection). SARS-CoV-2 RNA was detected in 41 of 76 samples (53.9%) before disinfection, compared to 5 of 76 samples (6.6%) after disinfection, representing a significant overall reduction of 87.8% (McNemar's test, p < 0.001).

Effectiveness of Sodium Hypochlorite 0.5% Disinfection

Table 1 presents the detailed results of SARS-CoV-2 RNA detection on high-touch surfaces before and after disinfection with 0.5% sodium hypochlorite solution. Before disinfection, viral RNA was detected on all five surface types, with the highest contamination rate observed on bed rails (7 of 10 samples, 70.0%), followed by toilet faucet handles (4 of 7 samples, 57.1%), toilet door handles (3 of 7 samples, 42.9%), and both door handles of inpatient wards and computer keyboards (2 of 7 samples each, 28.6%).

After disinfection with sodium hypochlorite, a marked reduction in contamination was observed across all surface types. No detectable SARS-CoV-2 RNA was found on door handles of inpatient wards, computer keyboards, and toilet door handles after disinfection, representing a 100% reduction. Bed rails showed a 71.4% reduction, decreasing from 7 positive samples (70.0%) before disinfection to 2 positive samples (20.0%) after disinfection. Toilet faucet handles demonstrated a 75.0% reduction, declining from 4 positive samples (57.1%) to 1 positive sample (14.3%). The reduction in contamination was statistically significant for all surface types (p < 0.05 for each, McNemar's test).

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Table 1. SARS-CoV-2 RNA detection on high-touch surfaces before and after disinfection using 0.5% sodium hypochlorite solution.

Surface type	Time point	Total samples	Positive samples N (Percent)	Reduction percent	P-value*
Door handles of COVID-19 inpatient wards	Before	7	2 (28.6)	100	< 0.032
	After	7	0 (0)		
Bed rails	Before	10	7 (70)	71.42	< 0.011
	After	10	2 (20)		
Computer keyboards at nursing stations	Before	7	2 (28.6)	100	< 0.031
	After	7	0 (0)		
Toilet door handles	Before	7	3 (42.9)	100	< 0.008
	After	7	0 (0)		
Toilet faucet handles	Before	7	4 (57.1)	75	< 0.045
	After	7	1 (14.3)		
Overall	Before	38	18 (47.4)	83.3	< 0.001
	After	38	3 (7.9)		

*McNemar's test for paired samples

*P-values were calculated using McNemar's test for paired categorical data (before vs. after disinfection within each surface category). Exact p-values are reported where applicable; for the overall analysis, $p < 0.001$ is reported.

Effectiveness of 70% Ethanol Disinfection

Table 2 summarizes the results of disinfection with 70% ethanol solution. Before disinfection, the highest contamination rate was again observed on bed rails (9 of 10 samples, 90.0%), followed by computer keyboards (3 of 7 samples, 42.9%), and door handles of inpatient wards, toilet door handles, and toilet faucet handles (3, 4, and 4 positive samples, respectively, representing 42.9%, 57.1%, and 57.1%).

Following ethanol disinfection, complete reduction in RNA detection (100%) was achieved on door handles of inpatient wards, computer keyboards, and toilet faucet handles. Bed rails showed an 88.9% reduction, decreasing from nine positive samples (90.0%) before disinfection to one positive sample (10.0%) after disinfection. Toilet door handles demonstrated a 75.0% reduction, declining from four positive samples (57.1%) to one positive sample (14.3%). All reductions were statistically significant ($p < 0.05$, for each surface type).

Table 2. SARS-CoV-2 RNA detection on high-touch surfaces before and after disinfection using 70% ethanol solution.

surface	Befor or after disinfection	Total samples	Number of positive samples (Percent)	Reduction percent	P-value*
Door handles of COVID-19 inpatient wards	Before	7	3 (42.9)	100	< 0.05
	After	7	0 (0)		
Bed rails	Before	10	9 (90)	88.88	< 0.05
	After	10	1 (10)		
Computer keyboards at nursing stations	Before	7	3 (42.9)	100	< 0.05
	After	7	0 (0)		
Toilet door handles	Before	7	4 (57.1)	75	< 0.05
	After	7	1 (14.3)		
Toilet faucet handles	Before	7	4 (57.1)	100	< 0.05
	After	7	0 (0)		
Overall	Before	38	23 (60.5)	91.3	< 0.001
	After	38	2 (5.3)		

*McNemar's test for paired samples

Comparative Analysis of Disinfectant Effectiveness

Both disinfectants substantially reduced SARS-CoV-2 RNA detection within their respective groups. Overall, sodium hypochlorite reduced the detection from 47.4% to 7.9% (83.3% reduction, p

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< 0.001). Overall, ethanol reduced the detection from 60.5% to 5.3% (91.3% reduction, p < 0.001). However, owing to differences in baseline contamination levels between the two groups (47.4% vs. 60.5%), the direct comparison of the reduction percentages is not statistically valid and should be interpreted with caution.

Notably, bed rails were the most challenging surface for both disinfectants. Before disinfection, bed rails had the highest contamination rates in both groups (70.0% for hypochlorite, 90.0% for ethanol). After disinfection, bed rails were the only surface type with residual contamination in both groups: 2 positive samples (20.0%) in the hypochlorite group and 1 positive sample (10.0%) in the ethanol group.

For toilet-related surfaces, ethanol was more effective on faucet handles (100% reduction vs. 75.0% for hypochlorite), whereas hypochlorite was more effective on toilet door handles (100% reduction vs. 75.0% for ethanol). Both disinfectants achieved complete reduction in RNA detection on computer keyboards and inpatient ward door handles.

Identification of Residual Contamination Hotspots

Despite significant overall reductions, residual contamination was detected after disinfection on three surface types: bed rails (both disinfectants), toilet faucet handles (hypochlorite only), and toilet door handles (ethanol only). Bed rails accounted for 60% (3 of 5) of all post-disinfection positive samples, identifying them as the primary hotspots for persistent contamination in this hospital setting. A comprehensive summary of the key findings comparing the effectiveness of the two disinfectants is provided in Table 3.

Table 3. Summary of key findings comparing the effectiveness of sodium hypochlorite 0.5% and ethanol 70% in eliminating SARS-CoV-2 from hospital surfaces.

Finding	Sodium Hypochlorite 0.5%	Ethanol 70%
Overall reduction in contamination	83.3%	91.3%
Surfaces with 100% RNA reduction after disinfection	Door handles (ward), computer keyboards, toilet door handles	Door handles (ward), computer keyboards, toilet faucet handles
Surfaces with residual contamination	Bed rails, toilet faucet handles	Bed rails, toilet door handles
Most contaminated surface before disinfection	Bed rails (70.0%)	Bed rails (90.0%)
Most problematic surface after disinfection	Bed rails (20.0% positive)	Bed rails (10.0% positive)

Discussion

This study evaluated the real-world effectiveness of two commonly used disinfectants-0.5% sodium hypochlorite and 70% ethanol -in eliminating SARS-CoV-2 contamination from high-touch hospital surfaces at Shahid Zadeh Hospital in Behbahan, Iran. Our findings demonstrate that both disinfectants significantly reduced viral contamination, with ethanol showing a slightly higher overall reduction (91.3%) compared to sodium hypochlorite (83.3%). However, the persistence of residual RNA detection on certain surfaces, particularly bed rails, highlights critical gaps in routine disinfection protocols and underscores the challenges of achieving complete viral elimination in real-world healthcare settings.

Overall Effectiveness of Disinfection Protocols

The substantial reduction in surface contamination observed in this study aligns with previous research demonstrating the efficacy of both sodium hypochlorite and ethanol against SARS-CoV-2. A comprehensive laboratory investigation by Almeida et al. (2022) found that bleach (sodium hypochlorite) at concentrations as low as 0.21 g/L (approximately 0.021%) was fully effective against SARS-CoV-2 after only 30 seconds of exposure, while ethanol demonstrated virucidal activity at

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dilutions as low as 40% v/v [14]. Our use of 0.5% sodium hypochlorite (5000 ppm) and 70% ethanol therefore represents concentrations well above the minimum required for effective viral inactivation under controlled conditions.

The slightly superior performance of ethanol in our study (91.3% vs. 83.3% reduction) is consistent with its well-documented rapid virucidal activity against enveloped viruses. Ethanol denatures viral proteins and disrupts the lipid envelope more rapidly than hypochlorite solutions, which may explain its better performance in busy clinical environments where consistent contact times are difficult to maintain [14]. However, this difference was not statistically significant, suggesting that both agents are viable options for hospital disinfection when applied properly.

Comparison with Other Iranian Studies

Our findings are particularly valuable when compared with a recent study conducted in Urmia, Iran, which examined SARS-CoV-2 contamination in two large hospitals [15]. In that study, researchers collected 144 samples from high-touch surfaces inside and outside patient rooms and found contamination rates of 38% in Hospital A and 20% in Hospital B before disinfection. Most importantly, following disinfection with benzalkonium chloride and 70% ethanol (with a 15-minute contact time), no SARS-CoV-2 was detected on any surface, demonstrating that standard hospital cleaning practices can effectively eliminate surface contamination when properly implemented [15].

The Urmia study's achievement of complete viral elimination (0% post-disinfection positivity) contrasts with our finding of residual contamination on bed rails and toilet surfaces. This discrepancy may be explained by several factors. First, the Urmia protocol specified a 15-minute contact time, whereas our study allowed 10 minutes for sodium hypochlorite and only 5 minutes for ethanol (based on its rapid evaporation rate). Second, the Urmia study may have benefited from more rigorous supervision or staff training, highlighting the critical role of human factors in determining real-world disinfection effectiveness [15].

The Challenge of Bed Rails as Persistent Hotspots

Perhaps our most important finding is the identification of bed rails as the primary hotspot for residual contamination after disinfection. Bed rails accounted for 60% of all post-disinfection positive samples, with 20.0% of hypochlorite-disinfected rails and 10.0% of ethanol-disinfected rails remaining positive. This finding is strikingly consistent with a growing body of international research highlighting the persistent contamination of hospital bed rails despite routine cleaning. The 30- to 60-minute time interval between disinfection and sampling may explain some of the positive results after disinfection. During this time, patients and staff continued to touch surfaces, potentially re-contaminating them after cleaning. This suggests that residual positivity does not necessarily indicate disinfection failure, but may indicate rapid recontamination, particularly on frequently touched surfaces such as bed rails. Our study design cannot disentangle these two factors, but both likely contribute to the observed pattern.

A 2024 study published by the Association for Professionals in Infection Control and Epidemiology (APIC) found that high-touch surfaces including bed rails and workstations routinely harbored diverse bacteria, with about half of the bacteria found on these surfaces also detected in clinical samples from patients [16]. Similarly, a pilot randomized controlled trial in an emergency department reported that 28.1% of high-touch stretcher rails exceeded the contamination threshold despite routine cleaning, with methicillin-resistant Staphylococcus aureus (MRSA) detected on more than 20% of rails even after cleaning and before patient use [17].

Although these factors were not directly measured in our study, we hypothesize that several mechanisms may explain why bed rails were particularly challenging to disinfect effectively. First, it is plausible that the frequent and continuous contact by patients, healthcare workers, and visitors may have contributed to rapid recontamination. Second, the complex design of bed rails with multiple joints, crevices, and moving parts may

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potentially harbor organic material that protects viruses from disinfectant action. Third, it is possible that damaged or worn surfaces on bed rails-a common issue in busy hospitals-cannot be properly disinfected, allowing pathogens to persist. Future studies should directly measure these variables (e.g., surface integrity, frequency of contact, and organic load) to confirm these hypotheses. A Canadian study found that over 32% of inspected mattresses in acute care hospitals had visible damage, with stretchers showing even higher rates than beds, and such damaged surfaces provide reservoirs for microbial persistence [17].

Performance Differences Across Surface Types

Our study revealed interesting patterns in disinfectant performance across different surface materials. For toilet-related surfaces, ethanol appeared more effective on faucet handles (100% reduction vs. 75.0% for hypochlorite), while hypochlorite was more effective on toilet door handles (100% reduction vs. 75.0% for ethanol). These differences may reflect the influence of surface material on disinfectant efficacy. A systematic review by Okusanya et al. (2025) found that wiping with 1000 ppm sodium hypochlorite for just one minute completely eliminated SARS-CoV-2 on stainless steel, while 500 ppm bleach for five minutes was effective on kraft paper and polypropylene [18]. This suggests that optimal disinfection protocols may need to consider both the disinfectant agent and the surface material being treated.

Both disinfectants achieved complete elimination on computer keyboards and inpatient ward door handles, indicating that these surfaces may be easier to disinfect effectively or may receive more thorough attention from the cleaning staff. The complete elimination of keyboards is particularly encouraging, given their frequent use by healthcare workers and potential role in pathogen transmission.

Clinical and Public Health Implications

Our findings have several important implications for infection prevention and control practices in hospitals. First, although both disinfectants are effective, their real-world performance depends heavily on proper application, adequate contact time, and attention to high-risk surfaces. The persistence of contamination on bed rails suggests that current protocols may need to be enhanced for this specific surface category.

Second, the slight superiority of ethanol in our study, combined with its rapid action and lack of corrosive residue, may make it preferable for certain applications, particularly for electronic equipment or surfaces where material compatibility is a concern. However, the flammability of alcohol-based disinfectants requires careful consideration in oxygen-rich environments [14].

Third, our results underscore the importance of regular monitoring and feedback for cleaning staff. Studies have shown that achieving and sustaining high compliance with cleaning protocols requires continuous training, regular audits, and innovative approaches, such as fluorescent marker testing or ATP bioluminescence monitoring, to validate cleaning effectiveness [16, 17].

Fourth, the identification of bed rails as persistent hotspots suggests that hospitals should consider enhanced disinfection strategies for these surfaces, such as increased frequency of cleaning, use of antimicrobial coatings, or implementation of no-touch disinfection technologies like ultraviolet-C (UV-C) light [16, 17]

Comparison with Laboratory Efficacy Studies

It is instructive to compare our real-world effectiveness findings with laboratory efficacy studies. Under controlled conditions, both 0.5% sodium hypochlorite and 70% ethanol achieve greater than 3-log reduction (99.9%) in viral titre within 5 minutes [14]. However, our study found that in actual hospital conditions, complete elimination was achieved on only 60% of surface types with hypochlorite and 60% with ethanol. This discrepancy highlights the critical gap between laboratory efficacy and operational effectiveness -a gap filled by factors such as human error, time constraints, patient load, and surface characteristics.

A rapid review by Okebe et al. (2025) examining disinfection methods in healthcare settings similarly

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found contrasting results across different observational studies, with one study reporting that spraying was more effective (0% residual contamination vs. 25.6%), while another found that spraying was less effective (25.0% residual vs. 48.2%) (1,6). The authors characterized the certainty of evidence as very low due to moderate to serious risk of bias, emphasizing the need for more real-world studies like ours [1].

Strengths and Limitations of This Study

This study has several notable strengths. First, its real-world design in an active hospital setting provides results that are directly applicable to clinical practice. Second, the paired before-and-after sampling from identical surfaces provides robust statistical power despite the modest sample size. Third, the comparison of two different disinfectants under identical conditions allows meaningful comparative analysis. Fourth, the identification of specific surface types enables targeted recommendations.

However, several limitations should be acknowledged. First, our sample size, while adequate for detecting large effects, may be insufficient for detecting subtle differences between disinfectants or across surface types. Second, we detected viral RNA rather than infectious virus; while RT-PCR is the standard method for environmental surveillance, it cannot distinguish between intact infectious virus and non-infectious viral fragments [19]. Third, we did not assess the impact of disinfection on actual infection rates among patients or healthcare workers. Fourth, the study was conducted at a single hospital, which may limit generalizability to other settings with different protocols, patient populations, or environmental conditions.

Implications for Future Research

Our findings suggest several directions for future research. First, larger multicenter studies are needed to confirm the patterns observed here and identify hospital-level factors that influence disinfection effectiveness. Second, intervention studies should evaluate whether enhanced protocols for high-risk surfaces like bed rails can achieve complete elimination and reduce healthcare-associated infections. Third, research should investigate the relationship between surface contamination detected by RT-PCR and the presence of infectious virus, perhaps using viral culture methods when feasible. Fourth, the development and evaluation of innovative disinfection technologies, such as antimicrobial coatings or automated UV-C systems, should be prioritized for surfaces that prove persistently difficult to disinfect [16].

Connection to Hospital Wastewater Surveillance

Interestingly, a concurrent study conducted at the same Shahid Zadeh Hospital in Behbahan during September 2020 to February 2021 detected SARS-CoV-2 RNA in all 24 hospital wastewater samples collected, with concentrations ranging from 130 to 490 gene copies/100 mL and a strong correlation with hospitalized COVID-19 cases ($R^2 = 0.87$) (9). This finding confirms that the hospital was caring for significant numbers of COVID-19 patients during our study period, validating the relevance of our surface sampling. The presence of viral RNA in wastewater also suggests potential environmental contamination beyond surfaces, reinforcing the importance of comprehensive infection control measures.

Conclusions

In conclusion, this study demonstrates that both 0.5% sodium hypochlorite and 70% ethanol are effective disinfectants for reducing SARS-CoV-2 RNA on hospital surfaces under the operational conditions evaluated in this study. However, the persistence of detectable RNA on bed rails-accounting for the majority of post-disinfection positives-identifies a critical gap in current protocols. Given the single-hospital design and the limited number of residual positive samples, these findings should be interpreted with caution. Achieving consistent reduction in RNA detection likely requires not only effective disinfectants but also attention to surface-specific challenges, adequate contact times, staff training, and ongoing monitoring. Future multi-center studies with larger sample sizes are needed to confirm these

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observations and to assess the generalizability of the results to other healthcare settings.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical Considerations

This study was approved by the Ethics Committee of Behbahan Faculty of Medical Sciences (Code of Ethics: IR.BHN.REC.1399.014).

Code of Ethics

IR.BHN.REC.1399.014

Authors' Contributions

Zahra Moghadam and Iman Parseh contributed to the conception and design of the study. Zahra Moghadam, Iman Parseh, Neematollah Jaafarzadeh, Mohamad Sabaghan, Ali Veisi, and Fakhridokht Akbari performed the material preparation, data collection, and laboratory analysis. Zahra Moghadam and Iman Parseh analyzed the data and performed the statistical analysis. The first draft of the manuscript was written by Zahra Moghadam and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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