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Synergistic augmentation of EtOH and 4-Watt ultraviolet-C for rapid surface decontamination

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Abstract: Bacterial contamination poses significant health risks, especially in densely populated settings like educational institutions. This study in a Malaysian educational institute examined bacterial deposition on frequently touched surfaces and evaluated the efficacy of 70% ethanol (EtOH) with 10-s of contact time, combined with ultraviolet (UV) light irradiation under varied time exposures durations. Results showed that EtOH-only treatment was least effective on lift-2, with a 20% inactivation rate, while other surfaces revealed efficiencies between 69.19% and 84.4%. However, employment of EtOH-UV treatment achieved highest inactivation across all the samples treated within 60-s requiring 0.15 mJ/cm² of dose. However, swab obtained from lift-1 could sustain 1.41-log₁₀ inactivation under maximum exposure settings. Scanning electron microscopy (SEM) further validated the persistence of *Bacillus* spp, *Staphylococcus* spp, and *E. coli* colonies. This study underscores the need for comprehensive disinfection strategies in educational facilities to reduce bacterial contamination, highlighting the enhanced efficacy of the combined EtOH-UV treatment.

Keywords: bacteria; decontamination; disinfectants; ethanol; EtOH; environment; high-touch surface; low-touch surface; pathogens; ultraviolet-C.

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Sameen Ahmed Malik is an Assistant Professor at the Department of Biomedical Engineering in University of Engineering and Technology, Lahore. His research focuses on the development of novel healthcare technologies that incorporates ideas from life science and engineering. His areas of special interest include nosocomial infections, antimicrobial activities, chronic obstructive pulmonary diseases, artificial intelligence and smart healthcare.

1 Introduction

Preventing the spread of infectious diseases in university settings requires meticulous management of microbes across various surfaces. Invisible to the naked eye, resilient in hostile environments, and microscopic in size, fungi and bacteria can thrive anywhere (Sheikh et al., 2024c). These characteristics enable them to proliferate, flourish, and pose serious health risks to a large number of people (Azhar et al., 2013). Nowadays, ethanol (EtOH) at 70% concentration is recognised for its disinfecting properties. However, using this chemical pose certain limitations, including the need for several minutes of contact time (Maurício et al., 2013) for effective disinfection. The potential of combining UV light in combination with EtOH with 10 s of contact time can potentially enhance the effectiveness of disinfection.

Maintaining hygiene particularly in educational settings necessitate attention to critical areas such as toilets (Abney et al., 2021), as well as food processing facilities like cafeterias (Paramasatiari et al., 2022) and restaurants (Handhal et al., 2020), door knobs (Jaafar et al., 2020; Olaitan et al., 2020; Nikolaevich et al., 2020) and handles (Abougrara et al., 2024), laptop keyboards (Ledwoch, 2021; Ide, 2019; Koscova et al., 2018), and elevator buttons (Kuo et al., 2023; Mulongo et al., 2021), which often receive less attention. Studies have shown that these surfaces can easily become reservoirs for pathogenic bacteria. Similarly, food processing facilities have been identified as critical zones where pathogens can thrive if not properly managed (Saniye et al., 2017).

According to the studies of Bhatta et al. (2018), door knobs and handles are the most frequently touched surfaces that can harbor bacteria, making them potential hotspots for bacterial transmission. Electronic gadgets, particularly keyboards, which are regularly used but seldom cleaned, have also been found to be contaminated with microbes (Ide, 2019; Ledwoch, 2021). Moreover, one study has shown that the elevator buttons, often touched by numerous individuals throughout the day, have been identified as significant vectors for the spread of pathogens (Amir et al., 2016).

In one of the prominent teaching institutes in Iran, Kandel et al. (2014) evaluated the incidence of bacterial colonisation on toilet surfaces and lift buttons. Their study found that lift buttons had a greater incidence of colonisation than toilet surfaces, with the most prevalent organisms on lift buttons being coagulase-negative staphylococci, followed by *Streptococcus* spp. This highlights the importance of maintaining cleanliness in high-touch areas like lift buttons. Barker et al. (2005) further indicated that a single toilet flush can generate hundreds of thousands to millions of aerosols due to the force and turbulence of the water within the bowl, potentially spreading these pathogens into the environment. Nonetheless, the presence of numerous bacteria and viruses in human faeces that can cause gastrointestinal infections has been well-documented. Atmar et al. (2008) identified pathogens such as *Escherichia coli* (*E. coli*), *Enterococcus faecalis* (*E. faecalis*), and *Serratia marcescens* (*S. marcescens*) in fecal matter. Another study by

Nazeri et al. (2019) reported that 76% of 75 computer keyboards and electronic devices were found to be infected with pathogens, where the bulk of such contamination was caused by Gram-positive bacteria, with coagulase-negative *staphylococci* being the most frequently isolated bacterium. These findings underscore the critical need for rigorous cleaning protocols, particularly in high-touch and high-traffic areas, to mitigate the spread of infectious diseases.

In today's time, alcohol-based products are considered state-of-the-art for skin antisepsis (Caldeira et al., 2011). However, achieving complete skin sterility with hand disinfectants and skin antiseptics is not possible (Raedler et al., 1999). According to the Center for Disease Control (CDC), conventional liquid disinfectants like ethanol and isopropanol are not endorsed as high-level disinfectants for spores due to their limited capacity to deactivate bacterial spores (Rutala, 2008). EtOH typically at a concentration of 70% is widely recognised for its strong bactericidal properties (Kampf et al., 2008). However, achieving its maximum effectiveness generally necessitates an exposure time of several minutes (Moufti et al., 2023; Sauerbrei et al., 2020). This prolonged contact ensures thorough microbial eradication, making it a highly reliable disinfectant in various settings. Furthermore, multiple studies have indicated that spores exhibit high survivability rates when exposed to ethanol treatment (Lin et al., 2018).

Universities, as large institutions, encompass a variety of amenities, including teaching buildings, administrative offices, service areas, and gardens. Students and staff often spend more than eight hours a day, five days a week, on campus. This extended exposure increases their risk of encountering various ailments and microorganisms, leading to absences and delays in fulfilling their commitments. One such research by Viegas et al. (2021) found that, despite regular cleaning methods employed in educational institutions, surfaces were commonly contaminated with various pathogens. This highlighted the ongoing challenge of maintaining a hygienic environment in such densely populated settings.

Recent advancements in contactless ultraviolet (UV) irradiation have proven to be highly effective for disinfection, offering a compelling alternative to traditional chemical methods (Sheikh et al., 2023, 2024, 2024b; Alam et al., 2024). Innovations such as quad-lens UV-C LEDs with enhanced power ratings have emerged, yet, their combined use with 70% EtOH for surface disinfection within a 10-second contact time remains unexplored, particularly in university settings. EtOH at a 70% concentration requires more than one minute of contact time for effective disinfection (Maurício et al., 2013) due to its high evaporation rate, which can cause surfaces to dry before the disinfectant achieves full effectiveness. To ensure effective disinfection, it is recommended to keep the surface wet for at least 10 mins. Additionally, while many studies utilise inoculation methods to measure bactericidal rates and treatment effectiveness, there is limited evidence on assessing antibacterial efficacy through direct swabbing of treated surfaces to identify persisting bacteria. The application of scanning electron microscopy (SEM) for morphological validation is also rare. Although gram-staining techniques are used to identify microorganisms swabbed from surfaces (Tripathi et al., 2024), SEM validation is underreported (Haddad et al., 2021).

Therefore, this study aimed to address these gaps by exploring the potential synergistic impact of a combined treatment with 10-s contact time of EtOH for surface disinfection within academic institutions, employing single SMD beaded quad-lens 4W 6565 UV-C LEDs due to its high optical power. The study also evaluated the

effectiveness of EtOH alone and in combination with UV treatment under various UV exposures. Moreover, the microorganisms were validated through SEM samples extracted from various surfaces. This research seeks to provide comprehensive insights into optimising disinfection protocols, particularly in academic settings, by investigating the enhanced efficacy of combined EtOH-UV treatments.

2 Methods and materials

2.1 Sample location and collection

The study was conducted at a higher education institute in Malaysia, targeting areas with significant human interaction to assess microbial load and hygiene levels. Samples were collected from various high-traffic locations frequented by a diverse population, including undergraduate and graduate students, faculty, technical staff, and maintenance personnel. Swab testing focused on high-contact points such as the buttons of lift 1 and lift 2, which are used approximately four times daily by each student and staff member. Additional samples were obtained from toilet 1 and toilet 2, which serve as central washrooms for students, faculty, staff, and technicians. Special attention was given to levels 3 and 4, where the majority of classes are held, resulting in frequent use of these facilities by students. The keyboard of the central library computer, accessible to all students for academic purposes, was also assessed for potential microbial contamination. Swab samples were collected using sterile cotton swabs moistened with a phosphate-buffered saline (PBS) solution and were streaked across the selected surfaces to gather microbial specimens. Each swab was placed in a sterile container and transported to the laboratory for analysis. In the lab, the swabs were cultured on nutrient agar plates and incubated at 37°C for 24–48 hrs to assess microbial load by counting colony-forming units (CFUs). Microbial validation was later performed using SEM. This strategic selection of sampling sites aimed to provide a comprehensive overview of the microbial environment in areas with significant human activity, contributing valuable data for improving sanitation protocols and ensuring a safe educational environment.

2.2 Selection of UV-C LED

UV-C LEDs are available in various package sizes, including 3,535, 3,838, and 6,565, with each variant offering different power ratings and throughput. The utilisation of high-power LED is essential to reduce bacterial burden in less time. Moreover, studies have shown significant antimicrobial effects using 275 nm UV-C LEDs (Song et al., 2022). Therefore, for this study, 4-W UV-C LED variant (KW-6565, OTDiode Co., Ltd.), emitting at a peak wavelength of 275 nm was chosen mainly for its quad-lens design and enhanced power rating [see Figure 1(b)]. This LED, with package dimensions of 6.5 mm × 6.5 mm [as illustrated in Figure 1(a)], was operated at a constant current of 300 mA and a voltage of 12 VDC. Although the UV-LEDs were bought from trusted suppliers and the accompanying datasheets listed their wavelengths with tolerances, the need to accurately evaluate the actual wavelength necessitated the validation of these values prior to research. As a consequence, before starting the investigation, the emission spectra of the KW-6565 LED variation were analysed using a spectrometer (HR4000-Vis-NIR, Ocean Optics, Inc., USA) to validate the actual wavelength.

Figure 1 6,565,275 nm UV-C LED with (a) showing the package size, and (b) showing quad-lens design of LED (see online version for colours)

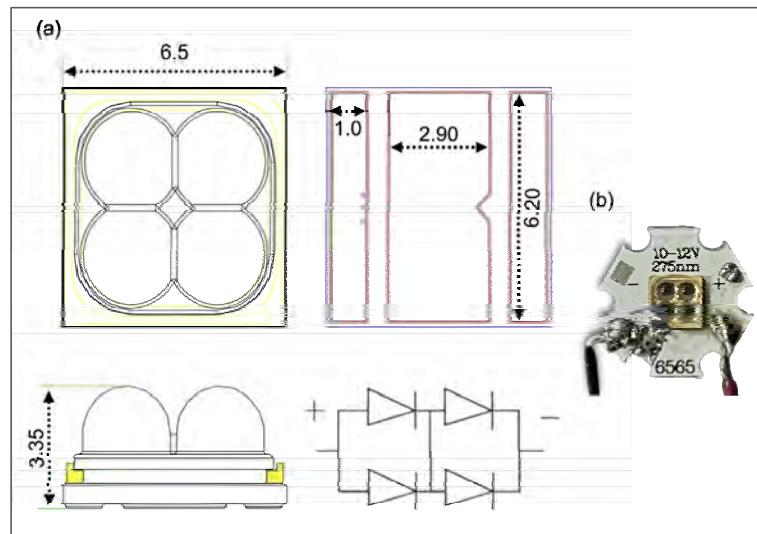
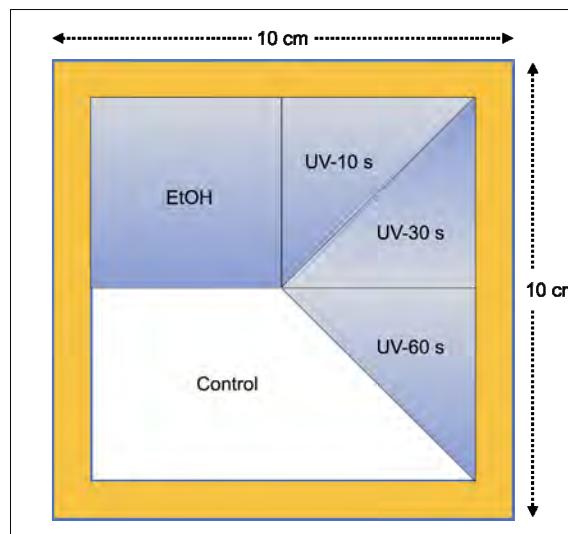


Figure 2 Depicts a 10×10 cm cardboard frame designed for collecting swab samples (see online version for colours)



2.3 Sampling procedure

Each sampling area was demarcated using a 10×10 cm square cardboard frame and divided into five sections (illustrated in Figure 2): one for the control sample, one to be treated with 70% EtOH (HmBG®, Bendosen, Malaysia), and three to be subjected to combined treatments of EtOH and UV light exposure for durations of 10, 30, and

60 seconds. The first section was left untouched and served as the control sample. Nylon swabs (Copan Flock Technologies, Italy) were used for collecting surface samples, with their initial weights precisely measured using a balance (Worku et al., 2018). The investigation began by spraying 70% EtOH over section one, cleaning it with a sterilised cotton cloth, and allowing it to dry for 10 seconds before collecting the sample. Since recent research by Sheikh et al. (2023) showed the effectiveness of inactivation employing treatment duration from 10 to 60 s, the remaining sections received EtOH treatment followed by UV doses of 0.025, 0.075, and 0.15 mJ/cm² for 10, 30, and 60 s, respectively. The swabbing technique involved rotating and rubbing the rayon swab in a zigzag pattern (Hedin, 2010) within the designated surface area of 2 × 2 cm, followed by a second rub at a perpendicular angle. All samples were properly labelled with a unique identification number at the time of collection.

2.4 Sample processing and microbial analysis

Once the swabbing was completed, the tip of each swab was dipped into a sampling solution, then pressed against the tube wall to eliminate excess liquid. The swab was subsequently placed into a tube containing 1 mL of fresh saline solution, pressed against the tube wall again, and agitated to release bacteria. The swab remained in the solution for 5 min before being discarded. The tube was then vortexed for 1 min to ensure thorough mixing. Serial eight-fold dilutions of the sampling solution were made in PBS. A 100 µL sample from each dilution was spread onto nutrient agar plates. These plates were incubated at 37°C for 24 hrs. Colony counts between 25 and 300 CFU were selected for further analysis to ensure the precision and reliability of the bacterial quantification (Ga, 2016). This methodology allowed for precise determination of microbial load on high-contact surfaces within the department, facilitating an assessment of the effectiveness of different disinfection treatments.

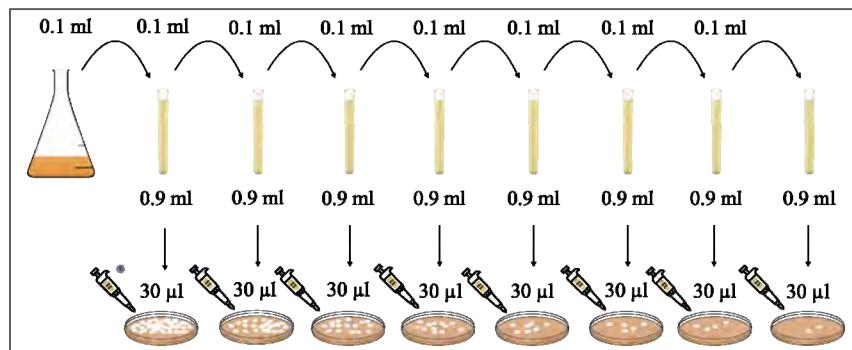
2.5 Quantitative analysis

2.5.1 Colony forming unit

The process (as shown in Figure 3) began with suspending the viable treated colonies in 15 mL of a sterile PBS solution, which was then evenly spread using a spreader while stirring methodically. Next, 1 mL of this suspension was withdrawn using a pipette (Thermofisher Scientific, USA) and transferred into sterilised micro-test tubes. Subsequently, a serial dilution technique was employed by transferring 0.1 mL of the bacterial suspension to successive test tubes, each containing 0.9 mL of fresh sterile saline solution. The contents of these test tubes were vigorously mixed using a vortex mixer, and this process was repeated until an eight-fold dilution of the initial bacterial suspension was achieved. Following this, 30 µL of the diluted suspension was taken using a micropipette and spread onto freshly prepared nutrient agar plates. The plates were then incubated under controlled conditions at 37°C for 25 hours. Quantification was carried out manually by counting colonies falling within the range of 20 to 300. Equation (1) was utilised to determine the number of viable clonogenic bacterial isolates.

$$\text{Number of CFUs / mL} = \frac{\text{Number of colonies counted}}{\text{Volume of suspension plated} \times \text{Dilution factor}} \quad (1)$$

Figure 3 Procedural steps required for the evaluation of colony forming unit (see online version for colours)



2.5.2 Log reduction and inactivation efficiencies

To obtain reduction of bacterial burden, the CFU logarithm of the irradiated samples (final CFU) was divided by the CFU logarithm of the untreated samples (initial CFU), as depicted in equation (2).

$$\text{Log reduction} = \log_{10} \frac{\text{Initial CFU}}{\text{Final CFU}} \quad (2)$$

where

Initial CFU CFU before treatment

Final CFU CFU after treatment.

Consequently, the calculated log inactivation values were translated into inactivation efficiencies using equation (3).

$$\eta = \left[1 - \left(\sum \frac{CFU_{UV-ON}}{CFU_{UV-OFF}} \right) \right] \times 100\% \quad (3)$$

where

CFU_{UV-ON} CFU/mL of irradiated samples

CFU_{UV-OFF} CFU/mL of non-irradiated samples.

2.6 Validation of microorganism

Identification of the microorganism was initially conducted by examining its colony morphology (Amir et al., 2016). This initial identification was then validated through microscopic analysis, employing tabletop SEM (TM3000, Hitachi, Japan). The choice of SEM was based on the findings of a previous study by Haddad et al. (2021), which demonstrated the superiority of using a tabletop SEM over traditional gram staining methods for rapid microbial identification. All examinations were conducted at a

consistent magnification of x7.0k to ensure uniformity and standardisation across analyses.

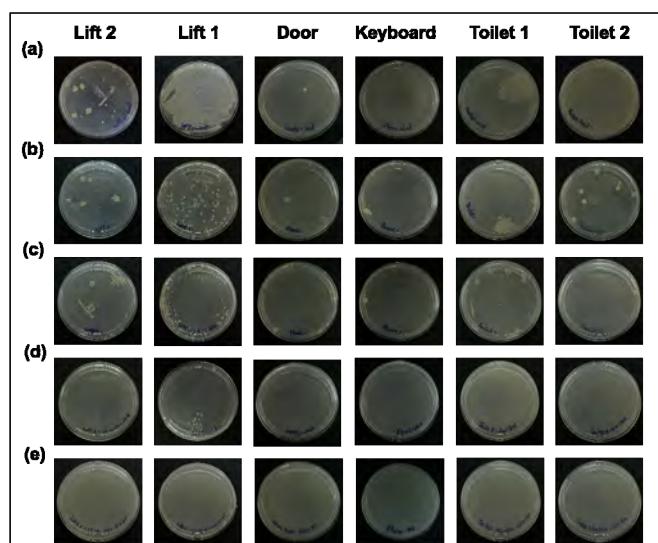
3 Results and discussion

3.1 Solitary and combined treatment synergy

The hygiene of environmental surfaces within educational institute premises, such as laboratories, classrooms, staff rooms, canteens, washrooms, corridors, railings, and miscellaneous sites, plays a crucial role in the spread of various pathogenic bacteria (Sujakhu et al., 2016). In this study, culture results revealed that high-touch surfaces had a median bacterial contamination level of 1.15×10^5 [Figure 4(a)]. The higher levels of bacterial contamination observed on these surfaces could be attributed to ineffective disinfectants used during surface cleaning, inadequate adherence to standard precautions such as hand hygiene and contact precautions, as well as the potential migration of organisms through airflow (Santajit et al., 2016; Mbanga et al., 2018; Weber et al., 2013).

The study by Lee et al. (2021) highlighted that the highest bacterial populations were found within toilets and on lift buttons, emphasising the heightened risk of cross-infection in non-ventilated public restroom environments. Areas surrounding sanitary fixtures like toilet bowls, squat toilets, and urinals were particularly contaminated, corroborating findings from this investigation. Swabs taken from lift 1 and toilet 2 [Figure 4(a)] showed densely packed bacterial growth without isolated colonies, corresponding to high CFU values of 2.24×10^5 CFU/mL and 7.73×10^5 CFU/mL, respectively. Similarly, lift 2 and the main toilet door samples exhibited dense colonies, with CFU values reported as 4.60×10^4 CFU/mL and 4.67×10^4 CFU/mL, respectively.

Figure 4 Petri dishes showing bacterial growth suppression under different treatment conditions, (a) controlled environment (b) treatment with EtOH (c) treatment with EtOH-UV at 10 s exposure (d) treatment with EtOH-UV at 30 s exposure (e) treatment with EtOH-UV at 60 s exposure (see online version for colours)



Additionally, the keyboard samples displayed concentrated micro-colonies, consistent with findings by Nazeri et al. (2019), which documented high contamination rates on computer keyboards and electronic devices used publicly. The study noted that the bacterial population did not significantly decrease when treated with EtOH for 10 s [Figure 4(b)], as this duration is insufficient for effective disinfection according to (Kampf et al., 2018), who recommend approximately 10 mins of contact time for 70% EtOH to effectively eliminate bacteria and viruses. However, the combined treatment of EtOH and UV irradiation demonstrated synergistic effectiveness in reducing bacterial counts. As shown in Figure 4(c) to Figure 4(e), an increase in treatment duration from 10 s to 60 s resulted in a noticeable reduction in bacterial colonies. This underscored the enhanced efficacy of prolonged exposure to combined EtOH-UV treatments in reducing bacterial populations, as supported by Ha (2010).

3.2 Inactivation studies

The experiment assessed the reduction in bacterial growth and the efficiency of inactivation (%) across various tested surfaces. Log reduction values were used to illustrate the maximum inactivation achieved, with a notable 5-log_{10} reduction plotted on a graph for clarity and comprehension. Initially, surfaces without UV exposure showed significantly lower inactivation rates, with Lift 2 exhibiting the lowest efficiency at 20%. This was primarily attributed to the short contact time of only 10 seconds. In contrast, lift 1, door, keyboard, toilet 1, and toilet 2 demonstrated higher inactivation rates ranging from 69.19% to 84.4% [Figure 6(b)]. The variation in inactivation rates can be attributed to different levels of bacterial resistance to disinfectants, influenced by factors such as cellular structure and other inherent properties (Gerasimov et al., 2023).

With combined treatment, as the UV exposure increased to 10 s, the inactivation rates improved notably upon exposure to $0.025 \text{ mJ}\cdot\text{cm}^{-2}$ of UV dose (Figure 5), with lift 2 showcasing a marginal increase to 87.5% and lift 1 escalating to 84.5% [Figure 6(b)], corresponding to the reduction factor of 0.9 and 0.8-log_{10} inactivation. Moreover, door knob and toilet 1 displayed considerable enhancements to 73.00% and 87.15%, reducing the bacterial burden by a factor of 0.56 and 0.89-log_{10} inactivation, respectively, while inactivation at keyboard and toilet 2 likewise exhibited significant improvements to 90.59% and 91.55% respectively.

Subsequently, following a 30-s exposure to UV light and upon exposure to $0.075 \text{ mJ}\cdot\text{cm}^{-2}$ of dose, all surfaces displayed notable improvements in inactivation rates, with the majority reaching 100% efficiency [Figure 6(c)]. However, lift 1 and toilet 2 revealed slightly lower rates of 94.00% and 93.14%, achieving the reduction value of 1.27 and 1.16-log_{10} inactivation, respectively. Despite this, the overall trend indicated near-complete disinfection across all tested surfaces. Notably, for lift 1 and toilet 2, a slightly extended treatment duration was necessary to achieve a complete disinfection, suggesting that residual colonies were present initially but were effectively eliminated with prolonged exposure. This underscored the importance of tailoring treatment durations to ensure thorough disinfection, especially for surfaces where residual microbial contamination may persist despite initial disinfection efforts.

Figure 5 Reduction (\log_{10} CFU-mL $^{-2}$) resulting from the synergistic effects of combined EtOH and UV treatment conducted on various surfaces (see online version for colours)

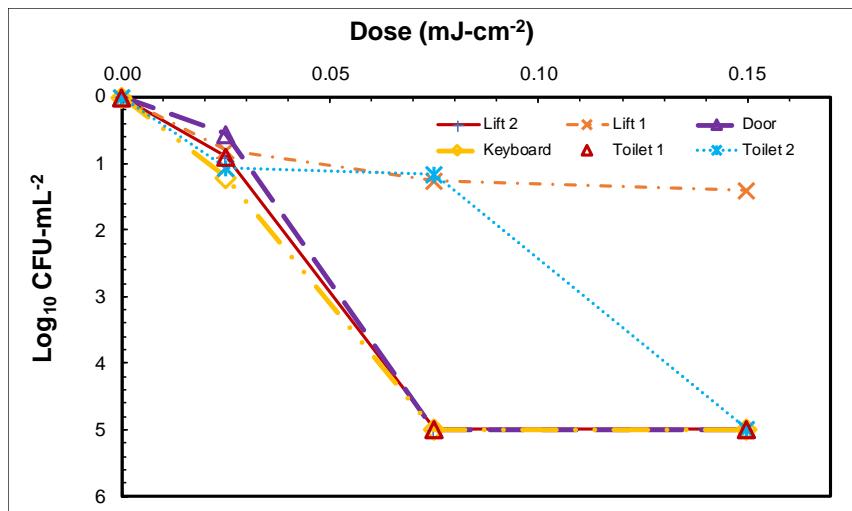
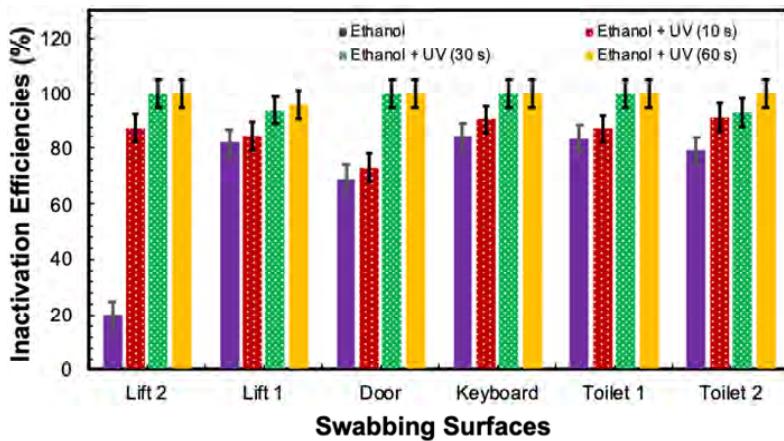


Figure 6 Inactivation efficiencies under various treatment conditions, (a) treatment with EtOH alone (purple bar) (b) treatment with EtOH and UV under 10 s exposure (red bar) (c) treatment with EtOH and UV under 30 s exposure (green bar) (d) treatment with EtOH and UV under 60 s exposure (yellow bar) (see online version for colours)

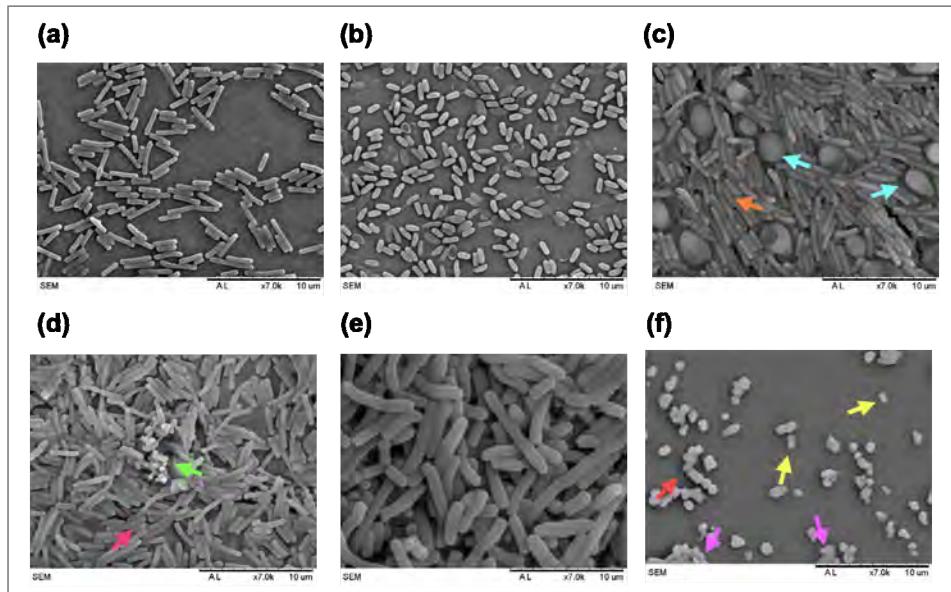


Finally, with 60 s of UV exposure and a dose of 0.15 mJ/cm 2 , slight improvements in inactivation efficiency were observed for lift 1. Meanwhile, door, keyboard, toilet 1, and toilet 2 maintained their maximum efficiency at 100% [Figure 6(d)], ensuring thorough disinfection. Longer exposure times have been noted to enhance disinfection efficacy (Sheikh *et al.*, 2024b). These findings align with the study by Li *et al.* (2021), which demonstrated achieving a 3- \log_{10} reduction with a similar UV dose. In this current study, doses of 0.025, 0.075, and 0.15 mJ/cm 2 were required to achieve nearly the same level of bacterial inactivation, underscoring the effectiveness of UV irradiation in reducing bacterial loads across various surfaces.

3.3 Validation of microorganisms

Prior to analysis, the samples were sputter-coated with platinum, revealing a remarkable variety of microorganisms attached to various surfaces, as depicted in Figures 7(a)–7(f).

Figure 7 SEM images depicting the diversity and morphology of microorganisms present on different surfaces, (a) lift 2: showcasing predominant colonies of *Bacilli* spp. (b) lift 1: predominantly colonised by *E. coli* (c) door knob: mixed community of *Bacilli* spp. (orange arrow) and presence of endospores (cyan arrow) (d) keyboard: mixed colonies of *Bacilli* spp. (pink arrow) and *S. aureus* (green arrow) (e) toilet 1: showcasing abundance of bacilli spp. (f) toilet 2: mix colonies of diplococcus (yellow arrow), *Staphylococcus* (pink arrow) and *Streptococcus* (red arrow) (see online version for colours)



The SEM examination highlighted the dominance of bacilli species across surfaces swabbed at lift 2, door knobs, keyboards, and toilets. Notably, the SEM image revealed the presence of *bacilli* endospores on the door knob [Figure 7(c)], which are generally formed in response to stressful environmental conditions such as nutrient depletion, desiccation or extreme temperatures (Nicholson et al., 2000; Pedraza-Reyes et al., 2024). Furthermore, this sporulation likely resulted from the unfavourable growth conditions typically induced by nutrient limitation (Wohlgemuth, 2014). Furthermore, Figure 6(d) illustrated the coexistence of *S. aureus* with *bacilli* species, with cocci structures clumped together in the centre (Sheikh et al., 2021). Interestingly, Figure 7(b) displayed rod-shape *E. coli* (Sheikh et al., 2024a), measuring between 1 to 2 μm in size (Dahal et al., 2024). Moving towards the Figure 7(f), several cocci species, from diplococcus to streptococcus, along with *S. aureus* was observed on the toilet surface. *S. aureus*, in particular, is commonly found on all surfaces (Maikranz et al., 2020), especially in public areas like hospitals and schools (Timsina et al., 2020), and on frequently touched items such as toilet (Amadi et al., 2023). This bacterium, which typically resides on human skin (Linz et al., 2023) and mucous membranes, exhibits a remarkable ability to persist on surfaces

for extended periods (Gunn et al., 2023), ranging from days to months, depending on environmental conditions.

The resilience of these bacteria is partly attributed to its ability to form biofilms, protecting it from desiccation and disinfectants. In high-traffic areas, where surfaces are frequently touched by multiple individuals, the risk of transmission and infection increases significantly (Nygren et al., 2023). This concern is particularly acute in healthcare settings, where vulnerable populations are at higher risk (Jinadatha et al., 2024). Regular cleaning and disinfection of these surfaces are crucial to controlling the spread of microorganisms and preventing outbreaks of bacterial infections.

4 Conclusions

In conclusion, our study investigated the efficacy of combined EtOH and UV treatment in swiftly reducing microbial contamination on diverse surfaces within university premises, targeting *Bacillus* spp, *E. coli*, and *Staphylococcus* spp in less than a minute. The study found that EtOH alone, with a short contact time of 10-s, did not achieve effective disinfection across all treated surfaces with disinfection efficiencies ranging between 69.19% and 84.4%. However, significant improvements in inactivation rates were observed with combined treatment, particularly with longest UV exposure duration of 60-s, resulting in near-complete disinfection across all treated surfaces except for lift-1, suggesting for prolongation in exposure duration to attain complete inactivation. This underscores the effectiveness of EtOH employment in combination with 60-s UV irradiation. Moreover, different bacteria exhibited varying levels of resistance to the combined treatment, with some surfaces requiring slightly extended treatment durations to achieve promising inactivation rates. Overall, our findings demonstrate effective suppression of microbial growth, with minimal residual colonies observed. The consistency of our results with other studies reinforces the reliability of our protocols and provides valuable insights for developing rapid microbial control strategies in university settings. Future research should focus on refining treatment parameters and exploring additional disinfection methods to further enhance microbial control and reduce contamination risks in diverse environments.

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Data availability

The data that support the findings of this study are available on request from the corresponding author.

References

Abney, S.E., Bright, K.R., McKinney, J., Ijaz, M.K. and Gerba, C.P. (2021) 'Toilet hygiene-review and research needs', *J. Appl. Microbiol.*, December, Vol. 131, No. 6, pp.2705–2714, DOI: 10.1111/jam.15121.

Abougrara, G. et al. (2024) 'Isolation and identification of pathogenic bacteria from hospital door handles/knobs', *Khalij-Libya Journal of Dental and Medical Research*, Vol. 8, No. 1, pp.1–6.

Alam, G.T. et al. (2024) 'Enhancing disinfection and microcontaminant removal by coupling LED driven UVC and UVA/photo-Fenton processes in continuous flow reactors', *Science of the Total Environment*, Vol. 918, p.170655, DOI: 10.1136/bmjopen-2018-026437.

Amadi, L.O. et al. (2023) 'Antimicrobial resistance profile of airborne *Aspergillus flavus* and methicillin-resistant *Staphylococcus aureus* in public toilets within Port Harcourt, Nigeria', *South Asian Journal of Research in Microbiology*, Vol. 16, No. 4, pp.1–13.

Amir, M. et al. (2016) 'Bacterial and fungal contamination of elevator buttons in University Schools of Isfahan University of Medical Sciences, Isfahan, Iran', *Health Scope*, Vol. 5, No. 4, p.e34428.

Atmar, R.L. et al. (2008) 'Norwalk virus shedding after experimental human infection', *Emerg. Infect. Dis.*, Vol. 14, No. 10, pp.1553–1557.

Azhar, M.H. et al. (2013) 'Comparative assessment of microbial contamination from swabs collected within university facilities', *Journal of Health Science*, Vol. 3, No. 2, pp.25–28.

Barker, J. et al. (2005) 'The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet', *J. Appl. Microbiol.*, Vol. 99, No. 2, pp.339–347.

Bhatta, D.R., Hamal, D., Shrestha, R., Hosuru Subramanya, S., Baral, N., Singh, R.K., Nayak, N. and Gokhale, S. (2018) 'Bacterial contamination of frequently touched objects in a tertiary care hospital of Pokhara, Nepal: how safe are our hands?', *Antimicrob. Resist. Infect. Control*, Vol. 7, No. 7, p.97.

Caldeira, D. et al. (2011) 'Skin antiseptics in venous puncture-site disinfection for prevention of blood culture contamination: systematic review with meta-analysis', *J. Hosp. Infect.*, Vol. 77, No. 3, pp.223–232.

Dahal, N. et al. (2024) 'Microwave flow cytometric detection and differentiation of *Escherichia coli*', *Sensors*, Vol. 24, No. 9.

Ga, O.T. (2016) 'Classic spotlight: plate counting you can count on', *J. Bacteriol.*, Vol. 198, No. 23, p.3127.

Gerasimov, V. et al. (2023) 'Inactivating and damaging properties of the disinfectant 'MultiDez' when exposed to bacteria and spores', *Helijon*, Vol. 9, No. 11, p.e21590.

Gunn, N.J. et al. (2023) *Staphylococcus aureus Persistence in Osteocytes: Weathering the Storm of Antibiotics and Autophagy/Xenophagy*, bioRxiv, p.10.

Ha, J-H. (2010) 'Synergistic effects of ethanol and UV radiation to reduce levels of selected foodborne pathogenic bacteria', *Journal of Food Protection*, Vol. 73, No. 3, pp.556–561.

Haddad, G. et al. (2021) 'Scanning electron microscope: a new potential tool to replace gram staining for microbe identification in blood cultures', *Microorganisms*, Vol. 9, No. 6, p.1170.

Handhal, N.A. et al. (2020) 'A survey study to isolate some pathogenic bacteria for cooked rice at Baghdad City', *Journal of Life Science and Applied Research*, Vol. 1, No. 2, pp.54–59.

Hedin, G. (2010) 'Reduction of bacterial surface contamination in the hospital environment by application of a new product with persistent effect', *Journal of Hospital Infection*, No. 2, pp.112–115.

Ide, N.E.A. (2019) 'What's on your keyboard? A systematic review of the contamination of peripheral computer devices in healthcare settings', *BMJ Open*, Vol. 9, p.e026437, doi:10.1136/bmjopen-2018-026437.

Jaafar, Z.S. et al. (2020) 'Hospital door knobs as a source of bacterial contamination: a study in Iraqi hospitals', *Indian Journal of Forensic Medicine & Toxicology*, Vol. 14, No. 3.

Jinadatha, C. et al. (2024) 'Understanding the significance of microbiota recovered from health care surfaces', *American Journal of Infection Control*, Vol. 52, No. 2, pp.220–224.

Kampf, G. et al. (2008) 'Comprehensive bactericidal activity of an ethanol-based hand gel in 15 seconds', *Ann. Clin. Microbiol. Antimicrob.*, Vol. 7, No. 2, pp.1–6.

Kampf, G. et al. (2018) 'Efficacy of ethanol against viruses in hand disinfection', *J. Hosp. Infect.*, Vol. 38, No. 4, pp.331–338.

Kandel, C.E. et al. (2014) 'Elevator buttons as unrecognized sources of bacterial colonization in hospitals', *Open Med.*, Vol. 8, No. 3, pp.e81–e86.

Koscova, J., Hurnikova, Z. and Pistl, J. (2018) 'Degree of bacterial contamination of mobile phone and computer keyboard surfaces and efficacy of disinfection with chlorhexidine digluconate and triclosan to its reduction', *Int. J. Environ. Res. Public Health*, Vol. 15, No. 10, p.2238.

Kuo, S.H. et al. (2023) 'Impact of plastic-wrap properties and cleaning intervals on the disinfection of elevator buttons', *International Journal of Environmental Research and Public Health*, Vol. 20, No. 2, p.1649.

Ledwoch, K. (2021) 'How dirty is your QWERTY? The risk of healthcare pathogen transmission from computer keyboards', *Journal of Hospital Infection*, Vol. 112, No. 1, pp.31–36.

Lee, M. et al. (2021) 'Public toilets with insufficient ventilation present high cross infection risk', *Sci. Rep.*, Vol. 11, p.20623.

Li, P. et al. (2021) 'Mitigation of airborne PRRSV transmission with UV light treatment: proof-of-concept', *Agriculture*, Vol. 11, No. 3, p.259.

Lin, T.H. et al. (2018) 'Relative survival of *Bacillus subtilis* spores loaded on filtering facepiece respirators after five decontamination methods', *Indoor Air*, Vol. 28, No. 5, pp.754–762.

Linz, M.S. et al. (2023) 'Clinical impact of *Staphylococcus aureus* skin and soft tissue infections', *Antibiotics*, Vol. 12, No. 3, p.557.

Maikranz, E. et al. (2020) 'Different binding mechanisms of *Staphylococcus aureus* to hydrophobic and hydrophilic surfaces', *Nanoscale*, Vol. 12, No. 13, pp.19267–19275.

Maurício, U.G. et al. (2013) 'Effectiveness of disinfection with alcohol 70% (w/v) of contaminated surfaces not previously cleaned', *Rev. Latino-Am. Enfermagem*, Vol. 21, No. 2, pp.618–623.

Mbanga, J. et al. (2018) 'Multi-drug resistant (MDR) bacterial isolates on close contact surfaces and health care workers in intensive care units of a tertiary hospital in Bulawayo, Zimbabwe', *J. Adv. Med. Med. Res.*, Vol. 27, No. 2, pp.1–15.

Moufti, M.A. et al. (2023) 'Efficacy and design requirements of UV light cabinets for disinfection of exchangeable non-sterilizable 'dental objects'', *Sci. Rep.*, Vol. 13, p.19755.

Mulongo, T. et al. (2021) 'Elevators and staircase handrails as potential sources of nosocomial pathogens at Ndola Teaching Hospital, Zambia', DOI: 10.21203/rs.3.rs-1066554/v1.

Nazeri, M. et al. (2019) 'Microbial contamination of keyboards and electronic equipment of ICU (Intensive Care Units) in Kashan University of medical sciences and health service hospitals', *MethodsX*, Vol. 6, pp.666–671, <https://doi.org/10.1016/j.mex.2019.03.022>.

Nicholson, W.I. et al. (2000) 'Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments', *Microbiol. Mol. Biol. Rev.*, Vol. 64, pp.548–572.

Nikolaevich, L.I. et al. (2020) 'Surface contamination with antibiotic resistant bacteria', *Process Management and Scientific Developments*, Vol. 113, No. 669, pp.113–191.

Nygren, E. et al. (2023) 'Potential sources of contamination on textiles and hard surfaces identified as high-touch sites near the patient environment', *Plos One*, Vol. 18, No. 7, p.e0287855.

Olaitan, I.A. et al. (2020) 'Bacteriological assessment of door handles and knobs at the federal school of medical laboratory technology offices in Jos.', *Journal: Am. J. Biomed. Sci. & Res.*, Vol. 17, No. 5, pp.40–45, DOI: <http://dx.doi.org/10.34297/AJBSR.2022.17.002387>.

Paramasatiari, A.A.A.L. et al. (2022) 'Identification of pathogenic bacteria in food samples from cafeterias of A University in Denpasar, Indonesia', *Folia Medica Indones.*, Vol. 58, No. 4, pp.313–317.

Pedraza-Reyes, M. et al. (2024) 'Bacillus subtilis stress-associated mutagenesis and developmental DNA repair', *Microbiology and Molecular Biology Reviews*, Vol. 88, No. 2, p.e00158-23.

Raedler, C. et al. (1999) 'Bacterial contamination of needles used for spinal and epidural anaesthesia', *Br. J. Anaesth.*, Vol. 83, No. 4, pp.657-658.

Rutala, W.A. (2008) *Introduction, Methods, Definition of Terms Guideline for Disinfection and Sterilization in Healthcare Facilities*, in Prevention, C.F.D.C.A. (Ed.), CDC, Atlanta, GA.

Saniye, B. et al. (2017) 'Are restaurant menus vectors of bacterial cross-contamination? A pilot study in Turkey', *British Food Journal*, Vol. 119, No. 2, pp.401-410.

Santajit, S. et al. (2016) 'Mechanisms of antimicrobial resistance in ESKAPE pathogens', *BioMed Research International*, Vol. 2016, No. 1.

Sauerbrei, A. et al. (2020) 'Bactericidal and virucidal activity of ethanol and povidone-iodine', *Microbiologyopen*, Vol. 9, No. 9, p.e1097.

Sheikh, J. et al. (2021) 'Bacterial disinfection and cell assessment post ultraviolet-C LED exposure for wound treatment', *Med. Biol. Eng. Comput.*, Vol. 59, No. 5, pp.1055-1063.

Sheikh, J. et al. (2023) 'Surface bacterium disinfection using Everlight 6565 UV-C SMD', *HumEnTec*, Vol. 2, No. 1, pp.11-17.

Sheikh, J. et al. (2024a) 'Bacterial morphology and microscopic advancements: navigating from basics to breakthroughs', *Microbiological & Immunological Communications*, Vol. 3, No. 1, pp.3-41.

Sheikh, J. et al. (2024b) 'Enhanced irradiance levels using synergistically engineered monochromatic wavelength ultraviolet-C arrays configuration', *Journal of Human Centered Technology*, Vol. 3, No. 1, pp.53-60.

Sheikh, J., Swee, T.T., Saidin, S. et al. (2024c) 'Classic and alternative disinfection practices for preventing of hospital-acquired infections: a systemic review', *Int. J. Environ. Sci. Technol.*, Vol. 21, No. 12, pp.8261-8296.

Song, C. et al. (2022) 'UV C Light from a light-emitting diode at 275 nanometers shortens wound healing time in bacterium- and fungus-infected skin in mice', *Microbiol. Spectr.*, Vol. 10, No. 6, p.e0342422.

Sujakhu, C. et al. (2016) *Solation and Identification of Pathogenic Bacteria from College Premises*.

Timsina, R. et al. (2020) 'Inducible clindamycin resistance and ERM genes in *Staphylococcus aureus* in school children in Kathmandu, Nepal', *Future Science*, Vol. OA 7, No. 1, p.FSO361.

Tripathi, N. et al. (2024) 'Gram staining', in *StatPearls*, StatPearls Publishing, Treasure Island (FL).

Viegas, C. et al. (2021) 'Microbiological contamination assessment in higher education institutes', *Atmosphere*, Vol. 12, No. 8, p.1079.

Weber, D. et al. (2013) 'Understanding and preventing transmission of healthcare-associated pathogens due to the contaminated hospital environment', *Infect. Control Hosp. Epidemiol.*, Vol. 34, No. 5, pp.449-452.

Wohlgemuth, S. and Kämpfer, P. (2014) 'BACTERIA | bacterial endospores', *Encyclopedia of Food Microbiology*, 2nd ed., pp.160-168.

Worku, T. et al. (2018) 'Bacterial profile and antimicrobial susceptibility pattern of the isolates from stethoscope, thermometer, and inanimate surfaces of Mizan-Tepi University Teaching Hospital, Southwest Ethiopia', *International Journal of Microbiology*, p.7, <https://doi.org/10.1155/2018/9824251>.