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Proof of Concept: The Effectiveness of Disinfectant Tunnel as Potential Measure against COVID-19

(Bukti Konsep: Keberkesanan Terowong Sanitasi sebagai Langkah Potensi terhadap COVID-19)

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ABSTRACT

Disinfectant tunnels have attracted attention as a potential measure to prevent the spread of COVID-19, but their safety and effectiveness are questionable. Disinfectants such as sodium hypochlorite were used, yet no scientific evidence is available on its effectiveness to eliminate SARS-CoV-2 on the human body through spraying, although this chemical is effective in the elimination of the virus on inanimate surfaces. Since safety issues are of importance, countries have halted the operation of these tunnels. Available literature has suggested several effective disinfectants against SARS-CoV-2, including iodine-based solution, such as povidone-iodine (PVP-I). This report describes the evaluation of the bactericidal activity of PVP-I in comparison to sodium hypochlorite, both in vitro *and following spraying under a model tunnel. Two bacteria strains* (Pseudomonas aeruginosa *ATCC 27853 and* Staphylococcus aureus *ATCC 12600) were used as model microorganisms. The spraying pattern and droplets distribution from the tunnel are also being described and were correlated with the effectiveness of the disinfectant droplets to eliminate the model bacteria. Results showed that 0.5 % v/v PVP-I and 0.5% v/v sodium hypochlorite are bactericidal (> 5 log_{10} reduction)* in vitro. *However, sprayed disinfectants did not show similar activity. Bacterial growth was seen in all cloth samples for 0.5% v/v PVP-I and all cloth samples except right shoulder for 0.5 % v/v sodium hypochlorite. Hence, the design of any disinfectant tunnel is important, and with an effective disinfectant to justify its efficacy.*

Keywords: Bactericidal; COVID-19; disinfectant; disinfectant tunnel; povidone iodine

ABSTRAK

Terowong sanitasi telah menarik minat sebagai suatu kaedah yang berpotensi untuk mengekang penularan wabak COVID-19, namun keselamatan dan keberkesanannya menjadi satu persoalan. Disinfektan seperti natrium hipoklorit telah digunakan namun tiada bukti saintifik berkaitan keberkesanannya untuk membasmi SARS-CoV-2 pada tubuh manusia melalui semburan, walaupun bahan kimia ini berkesan membasmi virus yang terdapat di permukaan objek tak bernyawa. Isu keselamatan ialah suatu yang penting, maka banyak negara telah menghentikan operasi terowong sebegini. Tinjauan kepustakaan menunjukkan terdapat beberapa disinfektan yang berkesan terhadap SARS-CoV-2 termasuk larutan berasaskan iodin, seperti iodin povidon (PVP-I). Laporan ini akan memperincikan penilaian terhadap aktiviti bakterisid PVP-I berbanding natrium hipoklorit secara in vitro *dan melalui semburan di bawah satu model terowong. Dua strain bakteria* (Pseudomonas aeruginosa *ATCC 27853 dan* Staphylococcus aureus *ATCC 12600) telah digunakan sebagai model mikroorganisma. Corak semburan dan taburan titisan daripada terowong tersebut juga telah dijelaskan dan keputusan ini telah dikaitkan dengan keberkesanan titisan disinfektan tersebut untuk membasmi model bakteria tersebut. Keputusan menunjukkan PVP-I pada kepekatan 0.5 % v/v dan natrium hipoklorit pada kepekatan 0.5 % v/v telah menunjukkan aktiviti bakteriasid (penurunan ≥5 log_{10}) terhadap model bakteria secara* in vitro. *Walau bagaimanapun, disinfektan secara semburan tidak menunjukkan aktiviti yang sama. Pertumbuhan bakteria telah dilihat pada kesemua sampel kain yang digunakan untuk PVP-I dan kesemua sampel kain untuk natrium hipoklorit kecuali di bahu. Reka bentuk sesebuah terowong sanitasi adalah penting dan bersama-sama disinfektan yang berkesan, terowong sebegini mungkin boleh dibuktikan berkesan. Penggunaan terowong ini perlu dilakukan secara berhati-hati dan hanya pada keadaan tertentu, bukan untuk kegunaan harian bagi masyarakat awam.*

Kata kunci: Bakteriasid; COVID-19; disinfektan; iodin povidon; terowong sanitasi

INTRODUCTION

The recent outbreak of coronavirus, officially known as coronavirus disease 2019 (COVID-19), is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Due to the highly infectious nature of this virus, the infectious control procedures such as handwashing and wearing personal protective equipment (PPEs) are very crucial to reduce the transmission of this virus in the community. In addition, cleaning of surfaces is also an important measure to combat its spread. The United States Environmental Protection Agency (US EPA) has compiled an exhaustive list of disinfectants that can be effective in eliminating COVID-19. Among the most common disinfectants used is sodium hypochlorite (bleach) which has shown to be effective at 0.1 % concentration (Kampf 2020). Sodium hypochlorite is also readily available and cheap, which has prompted its use in disinfectant tunnels. Disinfectant tunnels have been developed in many parts of the world to disinfect any virus particles that could be present on the surface of human clothes and body. Although its application has gained interest worldwide, there was no one still suitable disinfectant that could be used safely and effectively as a disinfectant for the tunnel. Sodium hypochlorite has been used in a particular part of the world as a disinfectant in the tunnel. Still, many countries have halted the operation of these tunnels due to safety reasons (Thadani 2020), especially since the use of sodium hypochlorite is recommended on non-porous surfaces and not as a sprayed disinfectant. Though some manufacturers of the disinfectant tunnels may also opt for the use of alcohol-based disinfectants due to the good activity and safety profile on human, these types of disinfectant may not be suitable as alcohol is highly flammable and can easily ignite.

Based on the literature, several main sanitisers and disinfectants are proposed to be effective against SARS-CoV-2 virus. The suggestions were made based on previous activity of the disinfectants against other coronaviruses such as the SARS-CoV and human CoV (hCoV). From the available information (Kampf 2020), the authors would like to highlight the potential of povidone-iodine as a disinfectant.

Povidone-iodine contains iodine complexed in a solubilising agent polymer such as polyvinylpyrrolidone-iodine, hence it is also called as PVP-I. PVP-I is an effective sanitising agent against a wide range of enveloped and non-enveloped viruses, including SARS-CoV (Mendoza 2020). According to Eggers et al. (2018), PVP-I at a concentration of 0.23 % was shown to be effective in eliminating SARS-CoV in 15 s under both clean and dirty environments. The reduction in the virus titre was 4.6 $\log_{10}$ and 4.4 $\log_{10}$ for clean and dirty environment, respectively (Eggers et al. 2018). Kariwa et al. (2006) also highlighted the ability of PVP-I at 0.25 % to eliminate SARS-CoV below detectable level in 60 s, which is equal to the ability of ethanol 70 %.

The application of PVP-I in the last 60 years as external liquid formulation on the body is evidence for its safety. The presence of many PVP-I based products in the market such as gargles, sore-throat spray, eye drops, antiseptic solution for prevention of wound infections and surgical scrubs prove that the substance is relatively safe on skin and mucous membrane (Lachapelle et al. 2013). Recent literature also suggested gargling of PVP-I-based products as a prevention step against COVID-19 infections among the health workers (Kirk-Bayley et al. 2020).

The authors proposed that the safety and efficacy of disinfectant tunnels can be improved by choosing a suitable disinfectant and coupled with an effective tunnel design. Hence, this paper will describe the evaluation of the bactericidal activity of PVP-I in comparison to sodium hypochlorite as a control, which is a common disinfectant used against SARS-CoV. Two bacteria strains will be used as a model to determine the effectiveness of the disinfecting solutions *in vitro* and following spraying under a model tunnel. The spraying pattern and droplets distribution from the tunnel will also be described and this will be correlated with the effectiveness of the disinfectant droplets to eliminate the model bacteria. Model bacteria were used instead of any SARS-CoV due to safety reasons.

MATERIALS AND METHODS

MATERIALS

Trypticase soy agar (TSA) and trypticase soy broth (TSB) were obtained from BD Difco^{TM}, USA. Phosphate buffer saline (PBS), PVP-I and sodium hypochlorite were hospital grade and purchased from local companies.

BACTERIAL STRAINS

The bacterial strains, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 12600, were purchased from American Type Culture Collection, USA. The strains were maintained on TSA medium.

DISINFECTANTS PREPARATION

PVP-I and sodium hypochlorite were freshly prepared at a concentration of 0.5 % v/v in distilled water for the evaluation of the bactericidal activity. Tap water was used to make fresh 0.5 % v/v PVP-I and sodium hypochlorite solutions for the assessment of disinfecting performance of the tunnel.

EVALUATION OF BACTERICIDAL ACTIVITY OF THE DISINFECTANTS *IN VITRO*

The bactericidal activity of the disinfectants on *Pseudomonas aeruginosa* ATCC 27853 and S*taphylococcus aureus* ATCC 12600 was measured according to the EN 1040:2005 guideline (Salvatico et al. 2018; Viroxy Labs 2017).

Briefly, eight parts of the disinfectant were mixed with one part of bacteria (density adjusted to McFarland 3, equivalent to 9×10^8 CFU/mL) in PBS (pH 7.4) and one part of sterile distilled water. The mixture was incubated in a shaking incubator for 5 min ± 10 s at 20 °C. After incubation, one part of the mixture was transferred to eight parts of PBS (pH 7.4) and one part of sterile distilled water. The viable bacterial count was then performed on the mixture using the pour plate method. The plates were incubated for 48 h at 37 °C and CFU/mL were calculated. The experiments were performed in triplicate.

The CFU/mL was expressed in $\log_{10}$ values and subtracted from the CFU/mL of the untreated bacteria inoculum to calculate the log reductions. The disinfectant was classified as bactericidal if more than 5 $\log_{10}$ reductions were observed.

DISINFECTANT TUNNEL MODEL

An in-house disinfectant tunnel was developed following the specifications of tunnels available for public use. Briefly, the tunnel is 202 cm in height, 91.5 cm in width, and 116 cm in length with six nozzles at two opposite corners, three at each corner. The particle has been measured using particle image velocimetry (Dantec Dynamics, Denmark). The tunnel was found emitting droplets with an average size of 250 µm. Hence, it will be safe for the volunteer from inhaling the droplets because they are beyond the inhalable size range of 1-5 µm (Parumasivam et al. 2016). A schematic diagram has been attached in the supplementary data.

Our preliminary study using coloured tap water showed that the tunnel uses approximately 300 mL of disinfectant solution for 10 s of spraying. Gentle 360° rotations would be necessary to get a whole-body exposure (unreported data). Figure 1 shows the deposition profile of the coloured water which was ranked by the intensity of the stain. The shoulder area is highly sprayed onto (A, highly coloured), followed by sleeves (B, moderately coloured) and the least covered area is the waist (C, least coloured). Hence, the right shoulder, left sleeve and lower back of waist were chosen for the evaluation of the tunnel experiment.

It was also found that spraying for more than 10 s which corresponded to using more than 300 mL volume solution would made the volunteer soaked wet, which may not be practical in the application of any disinfectant tunnel.

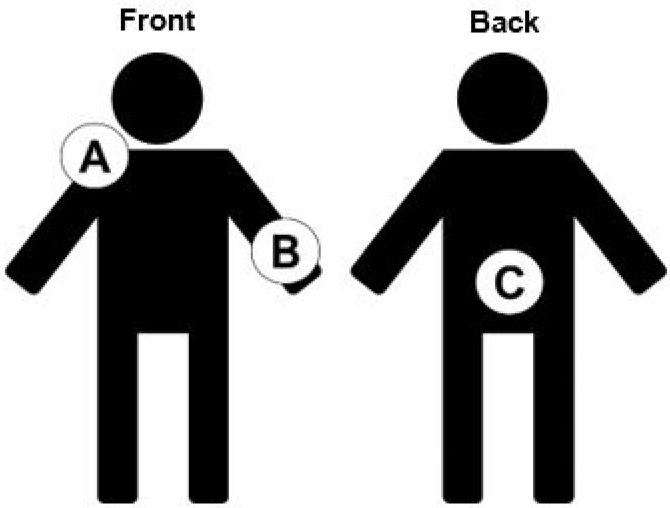

FIGURE 1. Deposition profile of coloured water droplets for 10 s with 360° rotations in the tunnel. (A) Highly, (B) moderately, and (C) least stained area

EVALUATION OF THE DISINFECTING PERFORMANCE OF THE TUNNEL

Sterile cotton clothes (6 × 14 cm) were inoculated with 50 µL of *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 12600, respectively. The density of the inoculum was equivalent to 9×10^8 CFU/mL prepared in PBS (pH7.4). The cloth was aseptically pinned on the right shoulder (Area A), on the lower edge of the left sleeve cuff (Area B), and lower back of waist (Area C) of a full PPE garment worn by a volunteer. The volunteer was then sprayed with the disinfectant solution while moving in circular gentle 360° rotations for 10 s in the disinfectant tunnel. The cloth pieces were then collected, transferred to sterile containers within 20 s, and transported to the lab not longer than 30 min. The experiment was performed in triplicate.

The cloth samples were first soaked in 5 mL neutraliser (PBS, pH7.4) for 5 min. Then, 1 mL of the neutraliser was transferred to 9 mL TSB and incubated for 48 h at 37 °C. If the media turned turbid, it scored as bacterial growth. If the media remain clear, it scored as no bacterial growth. Upon 48 h incubation, the turbid TSB was also streaked onto TSA to confirm that the growth was not due to contamination. If contamination is identified the whole set of experiment was scored not valid.

RESULTS AND DISCUSSION

EVALUATION OF BACTERICIDAL ACTIVITY OF THE DISINFECTANTS *IN VITRO*

Both disinfectants, 0.5 % v/v PVP-I and 0.5 % v/v sodium hypochlorite, showed $\geq 5 \log_{10}$ reduction against *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 12600, which meets the requirements of EN1040 guideline to conclude as bactericidal. Hence, this justifies their suitability for the disinfectant tunnel efficacy study.

EVALUATION OF THE DISINFECTING PERFORMANCE OF THE TUNNEL

The bacterial growth in the cloth samples from the disinfectant tunnel was tabulated in Table 1. Spraying 0.5 % v/v PVP-I did not kill the germs on the clothes as bacterial growth was observed in all cloth samples. However, when sprayed with 0.5 % v/v sodium hypochlorite, bacterial growth was seen in all cloth samples except the shoulder area. These results may be caused by insufficient contact time of the disinfectants. The contact time needed to disinfect human coronavirus ranging from 1 to 10 min for sodium hypochlorite (US EPA 2020) and 15 to 60 s for PVP-I (Eggers et al. 2018; Kariwa et al. 2006). In this study, the spraying time was limited to only 10 s (300 mL solution) as spraying more than the time was deemed impractical due to the soaking of the volunteer after the process. In addition to this, the spraying mechanism may not be optimal to give complete coverage on the body. Spraying of a coloured solution onto the volunteer had also shown a non-uniform distribution of the droplets, with a more concentrated droplets area on the shoulder, followed by sleeve and waist. The high concentration of droplets could explain the bactericidal activity of 0.5 % v/v sodium hypochlorite on the shoulder sample.

TABLE 1. Bactericidal activity of 0.5 % v/v PVP-I and 0.5 % v/v sodium hypochlorite in the tunnel

Replicate	Right shoulder	Left sleeve cuff	Lower back of waist
0.5 % v/v PVP-I			
1	+	+	+
2	+	+	+
3	+	+	+
0.5 % v/v sodium hypochlorite			
1	-	+	+
2	-	+	+
3	-	+	+

+: growth of bacteria following treatment, - no growth of bacteria following treatment

This study did not evaluate the virucidal activity of the disinfectants on the coronaviruses, as their activities have been reported elsewhere (Eggers et al. 2018; Kariwa et al. 2006; US EPA 2020). Bactericidal activity on different parts of the clothes simulates the coverage of the disinfectants during the spraying process.

Based on these findings, it would be useful to highlight the importance of the design of any disinfectant tunnel itself. Despite the effectiveness of sodium hypochlorite to eliminate bacteria and viruses on inanimate surfaces as presented in numerous reports and guidelines, bacterial growth could still be observed on the sleeve and lower back of waist. This could be correlated back to the distribution of spray droplets as presented in Figure 1, in which the distribution of droplets was the highest on the shoulder and less on the sleeve and back waist.

When considering the use of disinfectants on human, PVP-I have been reported a better tolerability and safety profile compared to sodium hypochlorite (Bigliardi et al. 2017; Lachapelle et al. 2013; Mady et al. 2020; Papanikolaou et al. 2011). The use of PVP-I may be hindered by its characteristic smells and potential cloth staining. However, our preliminary study showed that the smell of PVP-I is negligible at the tested concentration. The solution also caused a yellowish stain upon spraying onto a white cotton cloth, but the stain disappeared within 30 s after spraying.

The effectiveness and practicality of the tunnel to disinfect public remains debatable as the disinfection process will only kill the virus on the clothing, not the virus inside the body. Any asymptomatic patients would remain infective and this will create a false sense of security among individuals (Biswal et al. 2020; Ministry of Health 2020). Therefore, a disinfectant tunnel should be carefully introduced and should not replace other behavioral measures (e.g. social distancing, hand hygiene). It may also be used to disinfect the medical front liners after their daily shift before taking off their personal protective equipment (PPEs) as an extra measure to protect them from the virus. This could be safely done using PVP-I as the disinfectant.

CONCLUSION

An ideal disinfectant should be non-volatile, require short contact time, safe to the skin and mucous membrane, with a proven virucidal and bactericidal activity *in vitro* (Biswal et al. 2020). Based on these ideal properties, PVP-I may be one of the suitable candidates for disinfectant tunnels.

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