A controlled study on the antibacterial efficacy and long-term efficacy of JUC in hand disinfection

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Abstract

Background: Hand hygiene is an important way to prevent infection and transmission of many microorganisms. However, the current alcohol-based hand disinfection products have certain limitations, and the compliance of medical staff with hand hygiene is poor. JUC is a nano-scale long-acting antibacterial dressing, which is composed of 2% silicone double long chain double quaternary ammonium salt and 98% water, and is widely used as an antibacterial agent. Therefore, in order to find a potential alternative, this article investigated the efficacy and persistence of JUC in hand disinfection through a controlled study.

Methods: Experiments designed according to European standard EN1500:1997. Comply with the detection methods and requirements, and use 60% propan-2-ol solution as the control. A total of 15 healthy volunteers were recruited to evaluate the efficacy of JUC, and 8 were tested for antimicrobial persistence. The test strain was *E. coli* K12 NCTC strain 10538.

Results: First, JUC had a similar antibacterial effect compared to 60% propan-2-ol, both reducing bacterial load by approximately 4 log10. Second, there was no significant difference in the antibacterial effect of hand rubbing and hand spraying with JUC. Third, volunteers' hand bacterial counts were still reduced by 2-3 log10 at 8 hours after a single application of JUC compared to untreated volunteers.

Conclusions: JUC can effectively exert the antibacterial effect of hands, and has a long-term antibacterial protection effect of at least 8 hours.

Keywords: JUC; organosilicon double-long-chain diquaternary quaternary ammonium; hand hygiene; long-lasting; nano-antibacterial particl

Background

Prevention of the spread of harmful microorganisms is an important part of infection control, in which hand hygiene plays a key role [1]. The microbial flora on the hand can be divided into resident bacteria and transient bacteria. Resident organisms are stable and generally harmless, whereas transient bacteria usually remain on the hand for a short period of time and are susceptible to infectious disease [1,2]. Healthcare-associated infections (HCAIs) are the most common adverse events in hospital care, which pose a huge threat to patient safety and impose a heavy burden on society [3]. Hands of healthcare workers have a high rate of bacterial infection and are the most common vectors for the transmission of pathogens between patients [4]. Therefore, strategies for good hand hygiene are extremely important in the healthcare sector to reduce the spread and infection of multidrug-resistant bacteria in healthcare settings. Furthermore, due to outbreaks of infectious diseases such as the COVID-19 pandemic, the importance of hand hygiene now extends to the community [5, 6]. Currently, the mainstream recommendation is to use alcohol-based disinfectants for hand hygiene. However, compliance with hand hygiene among health care workers has been suboptimal, with an average of only 40% from 34 studies [7, 8]. A study of factors affecting hand hygiene found the following. First, the time required for frequent hand hygiene, which is also the main limitation[9]. Studies have reported that 100% compliance with hand hygiene using alcohol-based sanitizers in the intensive care unit (ICU) requires approximately 230 minutes/patient/day [10]. Second, skin irritation and dryness. Irritant contact dermatitis is common among health care workers, with a reported incidence of 10-45% [11]. Although allergies may not be caused by alcohol, alcohol-based sanitizers has a promoting effect on skin water loss [12]. Based on these limitations, we believe that it is meaningful and necessary to explore other solutions.

For disinfection products, there are many different types on the market, one of the most common is quaternary ammonium compounds (QACs). QACs are cationic surfactants that replace ammonium hydrogens with alkyl or aryl groups (i.e., benzyl groups), resulting in a strong base and its salts. QACs gained a lot of attention in 1935 when Domagk first described their antibacterial properties [13]. The hydrophilic portion of the cationic surfactant contains positively charged ammonium cations, while the alkyl chains of varying lengths contain the hydrophobic portion. The cationic surface of QACs can adsorb negatively charged bacteria and exert antibacterial activity, which seems to be related to the destruction of microbial lipid membranes [14]. However, due to the shortcomings of common quaternary ammonium salts such as low chemical activity, high irritation, and easy elution. After improvement, siloxane is introduced into the structure of quaternary ammonium salts to form organosilicon quaternary ammonium salts. Organosilicon quaternary ammonium salt is a new type of cationic surfactant, which has high temperature resistance, washing resistance, long-lasting effect, wide antibacterial range, and no irritation and carcinogenic effect on human skin [15].

JUC is a long-acting broad-spectrum antibacterial agent developed and produced by Nanjing Magic Technology Development Co., Ltd. in China in 2002, and is also a registered product of FDA and CE. As a nanomaterial, JUC is composed of 2% organosilicon double long-chain biquaternary ammonium salt and 98% water, and has been widely used in clinical practice [16,17]. Yuen et al. [18] found that the application of JUC as an antibacterial coating could effectively reduce the incidence and bacterial concentration of Staphylococcus contamination at the bedside, and exerted long-lasting antibacterial activity for at least 4 hours after application. They suggest that JUC spray could serve as a potential environmental decontamination strategy to prevent the spread of clinically important pathogens in medical wards. After more than ten years of application, JUC has been proven to be safe and effective, and can even be used on the eyes and mucous membranes [16]. Zeng et al. [19] showed that the application of JUC can shorten the wound healing time in patients with oral cancer, and does not lead to drug resistance, and no obvious adverse reactions. He et al. [20] confirmed by clinical and in vitro experiments that JUC is effective in preventing catheter-related urinary tract infections and bacterial biofilm formation. In addition, Wang et al. [21] reported that the combination of JUC and 5-FU could affect cellular respiratory enzymes by forming a physical film, thereby inhibiting the proliferation of liver cancer cells and inducing apoptosis.

Due to the limitations of alcohol-based hand disinfection products, the compliance of hand hygiene by healthcare workers is poor. Therefore, it makes sense to look for a potential alternative. We investigated the efficacy and persistence of JUC in hand disinfection in a controlled study.

Methods and materials

According to the European standard EN 1500:1997 [22], we evaluated the properties of JUC containing 2% (v/v) organosilicon double-long-chain diquaternary ammonium salt. The JUC was provided by Nanjing Magic Technology Co., Ltd. (Nanjing, China). E. coli K12 NCTC strain 10538 was purchased from Microbiologics (Minnesota, USA). Tryptone Soy Agar (TSA) and Tryptone Soy Broth (TSB) media were purchased from sigma, USA. A total of 15 adult volunteers were recruited and signed informed consent before participating in this study. The research protocol has been ethically reviewed by the Human Ethics Committee of the Hong Kong Polytechnic University and complies with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Before the experiment, volunteers were asked to wash their hands with soft soap for 1 minute. Then, each subject's hands were completely immersed in the E. coli-contaminated solution with a concentration of $2x10^8$ - $2x10^9$ cfu/ml for 5 seconds and rubbed for 1 minute. Subsequent appropriate dilutions in petri dishes containing 10 mL of TSB and spread onto TSA plates for incubation yield pre-treatment results. Use 1.5 ml of JUC to rub hands evenly for 30 seconds, repeat once (i.e. a total of 3 ml of JUC for 1 minute). Rinse with running water for 5 seconds, and obtain the processed results according to the above method. Dilution and stocking procedures were completed within 30 minutes of sample collection. All plates were incubated under aerobic conditions at 36 ± 1° C for 18-24 hours and the number of colony forming units (cfu) per plate was recorded for each dilution. A week later, the same volunteer used 60% (v/v) propan-2-ol to repeat the above steps as a control group. Measurement data are expressed as mean ± standard deviation (SD). Wilcoxon's matched-pairs signed rank test (one-sided) was used for statistical analysis (p=0.01).

To investigate the antimicrobial persistence of JUC, we supplemented the experiment with 8 volunteers. All volunteers were randomly divided into 3 groups: control group (n=2), JUC hand rub group (n=3) and JUC hand spray group (n=3), and the bacterial counts on both hands were obtained as baseline values after handwashing with soft soap. After using JUC, volunteers in the JUC hand rub group (method as described above) and the JUC hand spray group (sprayed 15 cm from the hand on the back of the hand, on both sides of the palm and fingers, three times each, and dried naturally) soaked their hands in the $5.0x10^6$ CFU/ml E. coli K12 contamination solution for 5 seconds, and sampled to obtain the treated hand bacteria value. The control group maintained the same procedure except not using JUC. Volunteers stayed in the laboratory for 8 hours, and at 1-hour intervals, their hands were immersed in the contaminated solution again and the bacterial values of the hands at the time point after JUC treatment were obtained. The contamination solution needs to be re-prepared every 2 hours. The number of colonies was calculated on TSA plates with 1 ml and 0.1 ml of undiluted sample, and then averaged from the two plates and converted to log values for comparison.

Results

The corresponding neutralizers used in the experiments proved to be non bactericidal but were effective in neutralizing the bactericidal activity of the sanitizers (Table 1).

As shown in Table 2, compared with propan-2-ol, the small rank sum after JUC treatment was -36. According to EN1500, it indicate that the antibacterial effect of JUC was not significantly different than that of propan-2-ol. When JUC was used as a hand sanitizer, the actual colony count decreased from $1.33x10^6 \pm 5.6x10^5$ to 260 ± 243 and $1.22x10^6 \pm 5.4x10^5$ to 249 ± 194 in the left and right hands, respectively. Using propan-2-ol as a control, the actual number of colonies in the left and right hands decreased from $1.18x10^6 \pm 5.4x10^5$ to 289 ± 492 and $1.31x10^6 \pm$ 5.8x105 to 290 ± 360, respectively. Colony numbers were reduced by 3.9 ± 0.53 log10 and 4.1 ± 0.67 log10 with JUC and propan-2-ol, respectively (Figure

1). In both groups, the overall log mean before treatment was higher than 5, and the JUC group decreased 3.2-5.3 log10 colonies and propan-2-ol decreased 2.7-5.2 log10 colonies after treatment, indicating the stability of the antibacterial effect of JUC better. Taken together the above results suggest that JUC can be used as an effective antibacterial hand sanitizer.

In a persistence test evaluating the antimicrobial activity of JUC, we compared the effects of two methods of use: hand rubbing (validated to EN1500) and hand spraying (manufacturer's recommendation for hand hygiene practice). The results showed no significant difference in antibacterial activity between hand rubbing and hand spraying (Figure 2A).

As shown in Figure 2B, at different time points of the 8-h experiment, the JUC-treated hands were consistently orders of magnitude (log10 CFU/ml) 2- to 3-fold lower than the untreated hands, indicating that the antibacterial activity of JUC can last for at least 8 hours.

Discussion

The novelty of this study is that it is the first to explore the antibacterial efficacy and persistence of JUC, a nanoantibacterial material, as a hand sanitizer. The results of the study found that JUC has a significant antibacterial effect, and the antibacterial time can reach more than 8 hours, which can effectively reduce the number of hand washing, thereby providing help to improve hand hygiene compliance.

Antibiotics are the foundation of modern medicine and are currently the mainstay of treatment for bacterial infections, and their use reduces mortality and increases life expectancy [23]. However, with the abuse of antibiotics, the problem of drug resistance is becoming more and more serious, especially the emergence of multidrug resistance (MDR). Drug-resistant bacteria that are difficult or impossible to treat are becoming more common, posing a huge global health crisis [24]. The multidrug-resistant bacteria and antibiotic resistance crisis has been described as an urgent global disease [25]. The latest global risk report from the World Economic Forum even listed antibiotic resistance as one of the biggest threats to human health [26]. Even when antibiotics are used correctly to treat pathogens to which they are susceptible, therapy is a double-edged sword. It may clear an ongoing infection, but it may also select for resistant pathogens in a patient's resident microbial population, limiting current and future treatment outcomes [27]. Furthermore, in this day and age, both disinfectants and good biosecurity are essential to control microbial diseases. However, resistance to disinfectants has the potential to change the way we live, from compromising food safety to threatening our healthcare system [28].

Currently, the best available protection against harmful bacterial growth in industrial and medical environments are disinfectants and improved biosecurity [29]. In the European Union, there were an estimated 671,689 infections with drug-resistant bacteria in 2015, of which 63.5% were nosocomial infections [30]. Many hospital-acquired infections are preventable, and good hand hygiene is an important measure. Strict adherence to hand hygiene practices can significantly reduce nosocomial infections and community transmission of disease [31]. In hand hygiene, hand disinfection is the key. However, an increasing number of studies have shown a correlation between antibiotic and disinfectant resistance in some bacteria, that is, exposure to disinfectants may increase antibiotic resistance, creating MDR bacteria [28, 32,33]. If microorganisms are no longer sensitive to the disinfectants used, the food industry, agriculture, and healthcare are all in danger of collapse. Foodborne epidemics threaten food security, and iatrogenic epidemics threaten life and health [34,35]. Therefore, the use of suitable hand sanitizers to avoid resistance is extremely important.

At present, the commonly used hand sanitizers are mainly alcohols, which have the characteristics of non-specific antimicrobial and wide range of action. In the Centers for Disease Control and Prevention (CDC) guidelines, ethanol hand sanitization is the preferred treatment after patient care activities may result in contamination of health care workers' hands [36]. However, as mentioned earlier, alcohol-based disinfectants also have their drawbacks, mainly including alcohol-induced skin dryness and irritation [11,12]. In addition, due to the volatility of alcohol and hypochlorous acid disinfectants, a one-time wipe cannot prevent long-term recontamination of the surface. They can only provide immediate but not persistent antibacterial activity, which is an immutable property that requires frequent hand disinfection to achieve long-lasting antibacterial activity [18,37]. These problems have largely hindered the improvement of hand hygiene compliance.

The antibacterial potency of antimicrobials depends on the dose of QACs. As described in the US patent application document on antimicrobial compositions (US 2009/0042870), in any antimicrobial formulation, QACs are considered active sterilants at a concentration of 0.1% w/v [38]. In EN 1500:1997 it is stated that products containing higher doses of QACs are effective hygienic cleaners that reduce bacterial load to a greater extent [39]. JUC is a polymer surfactant produced by nano-manufacturing technology, and its main component is 2% organic silicon double long chain double quaternary ammonium salt. Combined with the clinical application results, it can be seen that JUC has a strong antibacterial effect [16,17,18]. As a surfactant, JUC can form an antibacterial film on any surface. The thin film formed by nanoparticles is dense and difficult to elute, and can exert long-lasting antibacterial activity [40,41]. In addition, JUC is a water-soluble preparation. Unlike alcohol products, JUC has no abnormal odor, no volatility, no flammability, and no irritation to the skin. It can be used on the skin and mucous membranes including open wounds, and does not affect the skin's normal properties (such as breathability, sweating, etc.), thus exhibiting better applicability. As for the specific antibacterial mechanism of JUC, it is mainly due to the glue layer and the positive charge layer produced by the organic silicon quaternary ammonium salt. The positive charge layer and the cell membrane of anionic microorganisms can generate electrostatic interaction, thereby destroying the cell structure of microorganisms to play a bactericidal effect, while the glue layer can effectively isolate microorganisms for a long time [40,42]. Interestingly, this form of antimicrobial resistance through a physical barrier does not involve biological and chemical processes and can effectively avoid resistance. Undoubtedly, this can provide new research ideas for anti-infection prevention, and help to solve the problem of antibiotic resistance.

Of course, this study has certain limitations. First, the sample size of the study is small, and large-scale, multi-center clinical research data is still needed. Second, more in-depth research and long-term follow-up evaluation are needed for the mechanism of action and drug resistance of JUC.

Conclusion

JUC can effectively exert the antibacterial effect of hands, and has a long-term protective effect of more than 8h. This may help improve hand hygiene compliance as a new option for hand disinfection and hand protection.

List of abbreviations

E. coli: *Escherichia coli*; HCW: healthcare workers; QAC: quaternary ammonium compounds; TSA: tryptone soya agar; TSB: tryptone soya broth.

Competing interests

None.

Authors' contributions

JWMY and CTCL made substantial contributions to conception and design. CTCL made substantial contributions to manage the processes of volunteer recruitment, data collection, and data analysis. JWMY was involved in drafting the manuscript, and both authors gave final approval of the version to be published.

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Table 1. Bactericidal efficacy of test product and activities of the corresponding neutralizer.

	Mean cfu ± SD (n=3)	
	Propan-2-ol	JUC
Antiseptic only	0	0
Antiseptic + neutralizer#	73.67±7.77	83.00±8.89
neutralizer# only	86.33±19.86	79.67±8.51
Pure *E. coli* suspension§	81.67±22.75	

§Concentration of E. coli suspension was $1.5x10^3$ cfu/ml. #Neutralizer used for JUC was TSB containing 3% Tween 80, 3% saponin, 0.1% L-histidine and 0.1% cysteine; Neutralizer used for propan-2-ol was TSB containing 3% Tween 80, 0.3% lecithin and 0.1% L-histidine. The in-vitro experiment was performed by mixing 1 ml of the suspension with 9 ml of undiluted antiseptic/corresponding neutralizer/the mixture of antiseptic and corresponding neutralizer. For culture inoculation, 1 ml of the final mixture was added and spread onto the TSA plate.

Table 2. Wilcoxon's matched-pairs signed-ranks test for statistical comparison of values obtained with the test product (JUC) and Propan-2-ol (control)

Subject	Log reduction factor		Difference of log reduction	Signed rank of difference
	Propan-2-ol	JUC		
1	4.725918	5.269034	-0.54312	-9
2	2.692336	3.22985	-0.53751	-8
3	4.932934	3.710137	1.222797	15
4	3.834748	3.363591	0.471157	6
5	4.061276	3.232957	0.828318	13
6	4.557156	3.908292	0.648864	12
7	4.674546	4.094886	0.57966	11
8	3.894704	4.313114	-0.41841	-5
9	3.909073	3.672817	0.236255	3
10	4.043507	4.000762	0.042745	1
11	3.428545	3.978967	-0.55042	-10
12	4.347685	4.252167	0.095517	2
13	5.243294	4.032192	1.211102	14
14	3.871661	3.391614	0.480047	7
15	3.246851	3.550799	-0.30395	-4

According to the EN1500, the test product has to be rejected as significantly less effective than the Propan-2-ol control if the small sum of ranks ≤ 36, at level of significance = 0.1 with 15 subjects. In the present study, the small sum of ranks was calculated as -36.

Figure legends

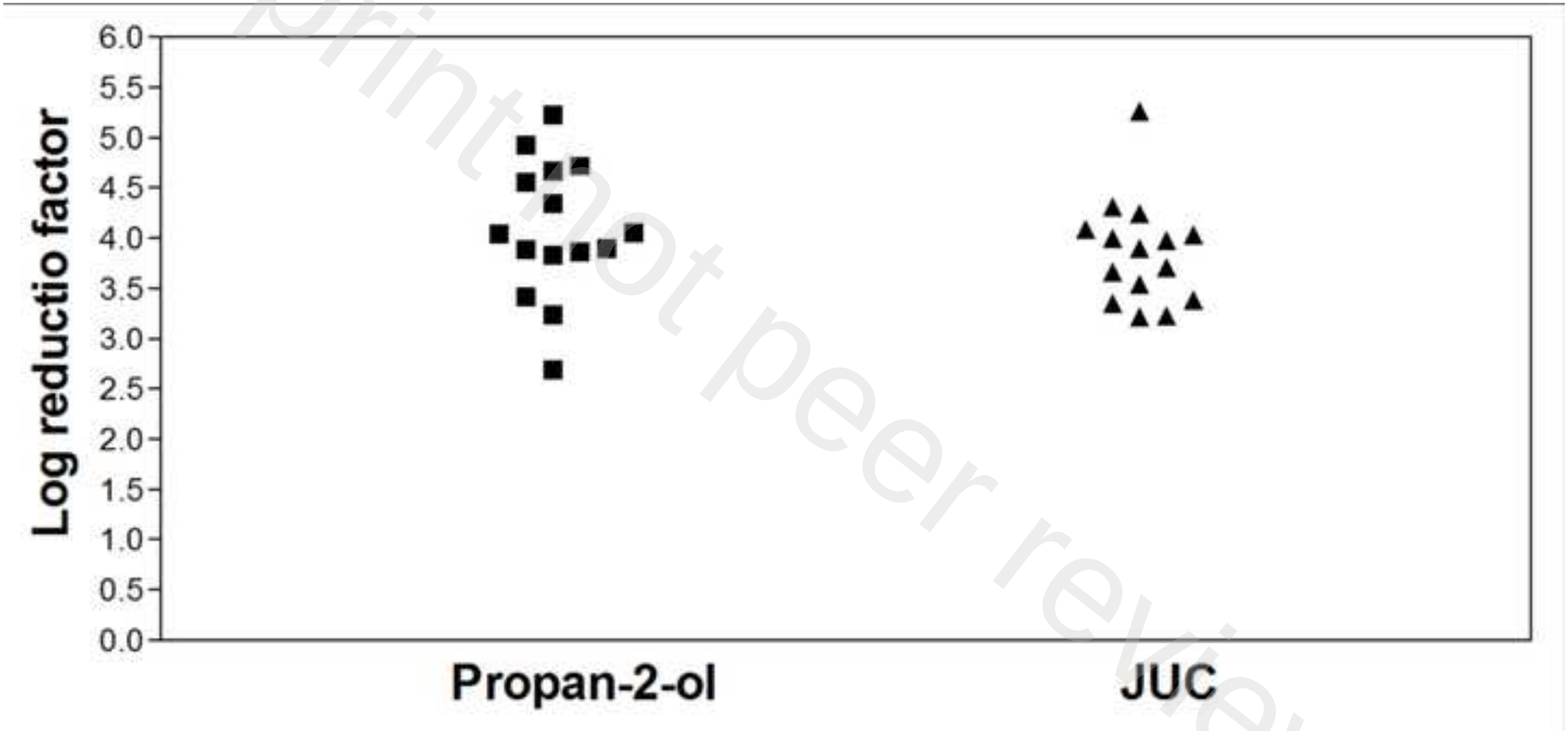
Figure 1

A scatter plot showing the log reduction factors of E. coli K12 amongst 15 subjects receiving hand rub with JUC (mean±SD = 4.10±0.67) and propan-2-ol (mean±SD = 3.87±0.53).

Figure 2

The prolonged effects of JUC applied as hand spray and hand rub, and showing [A] the actual CFU counts per TSA test plate of 10-1 dilution over an eight hour period; [B] the log changes during the course of the 8 hours experiment, representing the logarithmic values of weighted arithmetic mean calculated from viable counts obtained from the TSA plates of 1 ml and 0.1 ml undiluted sample.

Figure 1



Log reductio factor
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Propan-2-ol
JUC

Figure 2A

Click here to access/download;Figure;Figure 2A.tif

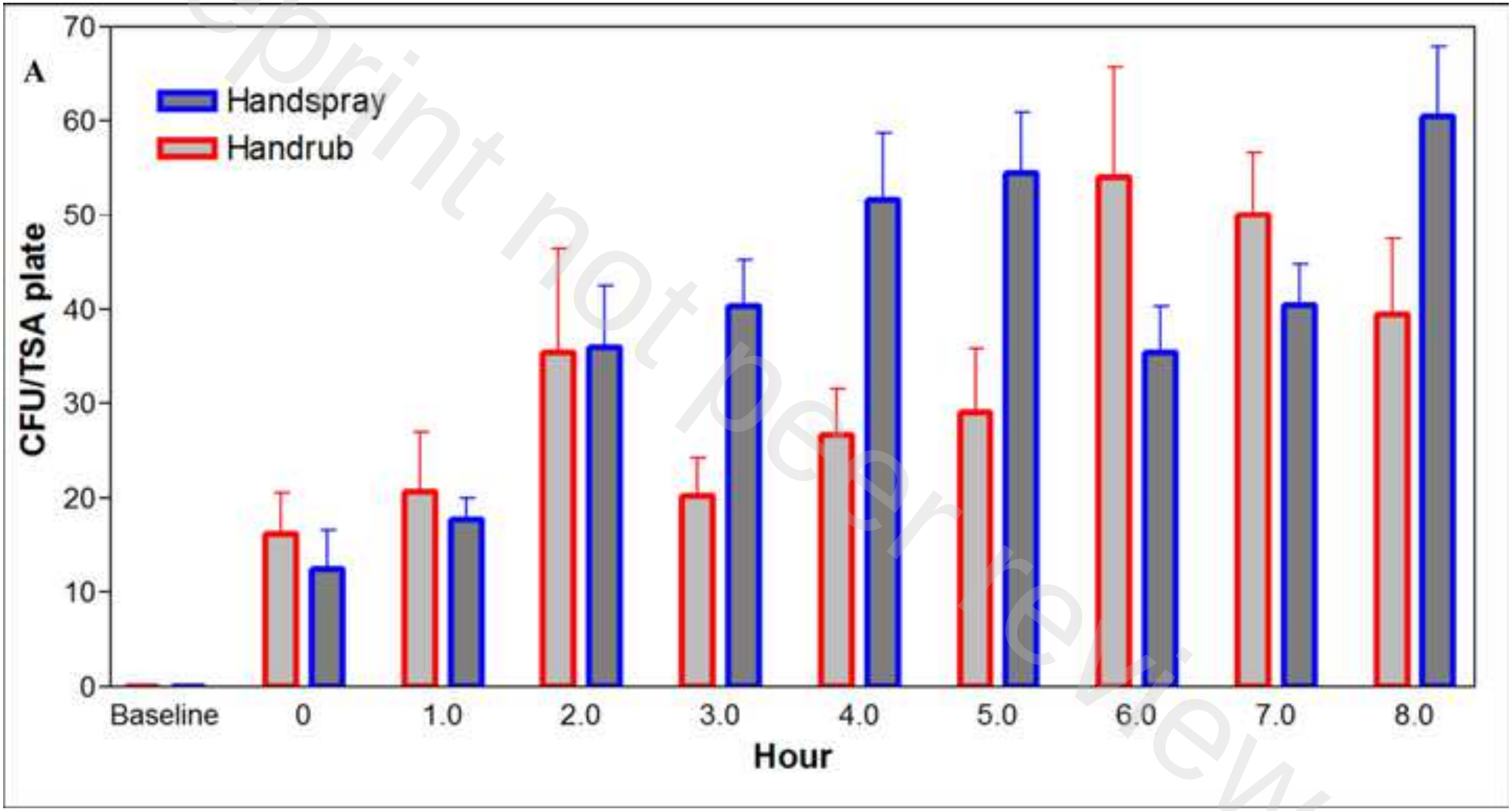


Figure 2B

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