A STUDY TO COMPARE DISINFECTANTS EFFICACY AGAINST *Staphylococcus aureus* IN UNIVERSITI SAINS ISLAM MALAYSIA DENTAL-POLYCLINIC

(STUDI PERBANDINGAN EFIKASI DISINFEKTAN TERHADAP Staphylococcus aureus DI POLIKLINI GIGI UNIVERSITI SAINS ISLAM MALAYSIA (USIM))

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JHDS.unjani.ac.id/jite
Doi: 10.54052/jhds.

Article History
Received:03/11/23
Accepted: 04/11/23

ABSTRACT

Different levels of active ingredients in disinfectants can be classified into three levels of disinfectants. Low-level and low-to-intermediate level disinfectants are commonly used in clinical contact surfaces, while high-level disinfectants are used on submerged inanimate objects that are heat sensitive. This study aimed to compare the disinfection efficacy of different disinfectants used in USIM Dental Polyclinic on rough and smooth surfaces against *Staphylococcus aureus* isolated from USIM Dental Polyclinic. 19 operators were enrolled to get 19 environmental samples from the glove-dominant hand after non-surgical extraction in the Oral Surgery Clinic. *Staphylococcus aureus* was identified, and an antibiotic susceptibility test was done to determine the methicillin-resistant S*taphylococcus aureus* (MRSA) strain. Two different levels of

disinfectants for the disinfection of dental chairs were tested on rough and smooth surfaces that were contaminated experimentally by *Staphylococcus aureus.* The number of colonies without and after disinfection was counted, and the reduction percentage was calculated and analyzed. *Staphylococcus aureus* was detected in 68.42% (n=13) of the samples. 5.26% (n=1) of the samples were *Staphylococcus sp*. 26.32% (n=5) had no bacterial growth. No methicillin-resistant *Staphylococcus aureus* strain was identified. There was no statistically significant difference ($p>0.05$) between the efficacy of the three disinfectants from two different levels used in USIM Dental Polyclinic on rough and smooth surfaces against *Staphylococcus aureus*. The efficacy of different levels of disinfectants used in USIM Dental Polyclinic was comparable to each other on rough and smooth surfaces against *Staphylococcus aureus*.

Keywords: comparative efficacy; disinfectants; *Staphylococcus aureus*

ABSTRAK

Terdapat perbedaan tingkat bahan aktif dalam disinfektan yang dapat diklasifikasikan menjadi tiga tingkatan disinfektan. Disinfektan tingkat rendah dan rendah hingga menengah biasanya digunakan pada permukaan kontak klinis, sedangkan disinfektan tingkat tinggi digunakan pada benda mati terendam yang sensitif terhadap panas. Tujuan dari penelitian ini adalah untuk membandingkan efikasi desinfeksi berbagai disinfektan yang digunakan di Poliklinik Gigi USIM pada permukaan kasar dan permukaan halus terhadap Staphylococcus aureus yang diisolasi dari Poliklinik Gigi USIM. 19 operator dilibatkan untuk mendapatkan 19 sampel lingkungan dari tangan dominan bersarung tangan setelah ekstraksi non-bedah dilakukan di Klinik Bedah Mulut. Staphylococcus aureus diidentifikasi dan uji sensitivitas antibiotik dilakukan untuk mengidentifikasi strain Staphylococcus aureus (MRSA) yang resisten terhadap methisilin. Dua tingkat disinfektan yang berbeda untuk disinfeksi kursi gigi diuji pada

permukaan kasar dan halus yang secara eksperimental terkontaminasi oleh Staphylococcus aureus. Jumlah koloni tanpa disinfeksi dan setelah disinfeksi dihitung dan persentase penurunannya dihitung dan dianalisis. Staphylococcus aureus terdeteksi pada 68.42% (n=13) sampel. 5.26% (n=1) sampel adalah Staphylococcus sp. 26.32% (n=5) sampel tidak memiliki pertumbuhan bakteri. Tidak ada strain Staphylococcus aureus yang resisten terhadap methisilin yang teridentifikasi. Tidak terdapat perbedaan yang bermakna secara statistik (p>0,05) antara efikasi ketiga disinfektan dari dua level berbeda yang digunakan di Poliklinik Gigi USIM pada permukaan kasar dan permukaan halus terhadap Staphylococcus aureus. Efikasi disinfektan dengan level berbeda yang digunakan di Poliklinik Gigi USIM sebanding satu sama lain pada permukaan kasar dan halus terhadap Staphylococcus aureus.

***Kata kunci**: disinfektan; efikasi komparatif; Staphylococcus aureus*

INTRODUCTION

Many microorganisms have been identified to have the potential for transmission in dental health care. In dental practice, the surfaces in the dental operation area are routinely contaminated with fluids such as patient's saliva, blood, and other fluids during dental treatments, which may lead to cross-contamination and cross-infection[15] if the sterilization of the dental instruments or disinfection of the dental unit is inadequate.[1] The disinfection process is essential as it inactivates disease-producing microorganisms[1] that remain on the surface after pre-cleaning but do not destroy bacteria spores. Bacterial spores can only be eradicated by sterilization.[16] Disinfection usually involves chemicals, heat, or ultraviolet light. Antimicrobial chemicals that kill microorganisms on environmental or inanimate surfaces or objects are disinfectants.

Disinfectants are classified into sterilant or high-level, intermediate-level, and low-level disinfectants based on their spectrum of activity. Sterilization or high-

level disinfectants kill all microorganisms on submerged inanimate heat-sensitive objects. In contrast, intermediate-level disinfectants kill vegetative bacteria, most fungi, viruses, and Mycobacterium tuberculosis. In contrast, low-level disinfectants kill most vegetative bacteria, some fungi, and some viruses. Intermediate-level disinfectants are usually used in clinical use for disinfecting clinical contact surfaces and noncritical surfaces with visible blood.[17]

There are different types of active ingredients in disinfectants such as iodophores, quaternary ammonium compounds, water-based phenolics[17], alcohol-based phenolics[17], alcohols, chlorines, alcohol-quaternary ammonium compound[17], aldehydes, peracetic acid, orthophthalaldehyde, hydrogen peroxide, and glucoprotamin.[16] Quartenary ammonium compounds and iodophores are low-level disinfectants. Phenolics are low-to-intermediate-level disinfectants, while chlorines are low-to-high-level disinfectants. On the other hand, aldehydes, peracetic acid, orthophthalaldehyde, hydrogen peroxide, and glucoprotamin are all high-level disinfectants.

Staphylococcus aureus is a gram-positive coccus that commonly colonizes the human anterior nare[6,7,] and the oral cavity serves as its reservoir for infection of the lower respiratory tract and cross-infection to other patients.[8] It has long been recognized as one of the essential human pathogens and has been identified by the International Federation of Infection Control as one of the alert organisms which require continuous monitoring of its incidence isolated in a health care setting.[5]

It has evolved from methicillin-susceptible *Staphylococcus aureus* (MSSA) to methicillin-resistant S*taphylococcus aureus* (MRSA).[9] A problem with MRSA is that it may survive on clinical contact surfaces in the dental environment for up to 6 months.[10] It also has emerged as a public health threat[11] as previously its transmission was healthcare-associated (HA-MRSA). Still, nowadays, community-associated MRSA (CA-MRSA) has also been reported[12,] so dental patients and dental health care personnel are at an increased risk of infection from it.

Petti and Polimeni (2011)[13] reported that transmission of MRSA has been confirmed during surgical interventions, particularly in surgical units. Other than that, some individuals in poor general condition were identified as oral MRSA carriers. However, there is a low risk of infection among patients undergoing conventional therapy.

Kurita et al. (2006)[2] suggested that MRSA contaminates the dental operatory's

surfaces; therefore, the dental operatory should be considered a possible reservoir of MRSA. It is supported by research by Robert et al. (2011)[3,] who stated that MRSA was found in dental clinic surfaces and dental students, suggesting that both may be reservoirs for MRSA. Besides, Messano et al. (2013) stated that staphylococci and MRSA have been detected in samples from the dentists' trays and gloves, suggesting that dominant hands and clinical contact surfaces were frequently contaminated.

Fraise (1999)[18] stated a few methods for disinfectant testing. One of the methods is to assess the in-vitro activity of the disinfectant against relevant pathogens. Other than that, the paper disk method may be employed to test the effect of disinfectants on bacteria.[19] It involves the adsorption of the disinfectants onto a disk of special paper, which is then applied on an inoculated growth medium. The result will be the evidence of clear inhibition zones surrounding the disk. The degree of inhibition may be determined by measuring the diameter of the inhibition ring in millimetres, including the disk. This method is convenient for indicating antibacterial activity, but it does not consider the chemical's diffusion rate and the effect of the growth medium. The bacterial concentration and the chemical concentration are also not standardized.

Singh et al.[20] used a modified quantitative surface disinfection test with two types of surfaces to test the disinfectants available in the market for the hospital. They used rough surface templates, ceramic plaster tiles representing the floors, walls, smooth surfaces, and stainless steel plates depicting the instrument tables and trolleys.

Different levels of disinfectants are used in USIM Dental Polyclinic for disinfecting dental equipment and instruments after dental treatments. Are the different levels of disinfectants effective against the isolated *Staphylococcus aureus*? Is there any difference between the efficacies of these two different levels of disinfectants against the isolated *Staphylococcus aureus*? This research was conducted to test the comparative efficacy of different types of disinfectants used in USIM Dental Polyclinic against *Staphylococcus aureus*.

METHOD

Nineteen operators were enrolled in this study after completing a non-surgical extraction done in the Dental Student Oral Surgery Clinic. A swab sample was taken from the glove of the operator's dominant hand and then streaked on the Mannitol salt agar plate (MSA). Yellow colonies on MSA were assumed to be Staphylococcus aureus.

This was confirmed by gram-positive cocci in clusters that appeared after gram staining. The positive *Staphylococcus sp.* was inoculated on a blood agar plate and then incubated at 37°C for 24-48 hours; the Tube Coagulase test was used to differentiate *Staphylococcus aureus* from other *Staphylococcus sp.* The positive result was indicated by the gelling of the plasma, which remained in place even after inverting the tube. The test result was recorded.

Staphylococcus aureus broth was inoculated into Mueller Hinton agar plate and incubated at 37°C for 30-60 minutes. Antibiotic susceptibility test was done using fusidic acid, gentamicin, mupirocin, cefoxitin, penicillin G, erythromycin, ciprofloxacin, doxycycline, clindamycin, and vancomycin discs. The result was determined by the diameter of the zone of inhibition and interpreted by guidelines from Clinical Laboratory Standard Institutes (CLSI).

Disinfectant test

Two types of low-level disinfectants labelled Disinfectant A and Disinfectant C were used, while low-to-intermediate-level disinfectants labelled Disinfectant B were used. The template for the smooth surface was an 8cmx8cm stainless steel tray, while the template for the rough surface was a 7.5cm-diameter semi-leather. Sterilization of rough and smooth surfaces was done to kill all microbes. These two surfaces were contaminated with *Staphylococcus aureus* broth using a sterile cotton swab and dried at room temperature for 1 hour. Apply disinfectant on one side of the surface for 10 minutes, labelled area B, while the other area was left without disinfection and labelled area A. Each area was swabbed and cultured on blood agar. The blood agar plates were incubated at 37°C for 24-48 hours. The number of colonies of the bacterial growth was counted. The reduction percentage was calculated by using the formula:

$$\text{Reduction percentage (\%)} = \frac{\text{Number of colony before disinfection} - \text{number of colony after disinfection}}{\text{Number of colony before disinfection}} \times 100\%$$

The above steps were repeated three times using disinfectants A, B, and C. The Surface disinfection test represents the practically achieved disinfection done in the clinical setting, as the mechanical forces involved in the disinfection process were included in the test. This is important because applying mechanical action with the effect of resuspending cells in the liquid on the surface is similar to mopping or brushing in the disinfection process. It will result in higher reduction rates in the number of microorganisms.[21]

The Ethical approval was obtained before sample collection. All the instruments were packed in an autoclave packaging and sterilized in an autoclave. Two calibrated examiners conducted all the test

Statistics

The efficacy of the different types of disinfectants against Staphylococcus aureus on rough and smooth surfaces was compared using the Kruskal Wallis test and post-hoc Mann-Whitney test. The specific function of SPSS Version 17.0 is used to analyze the results. Statistical analysis was performed by using the mean values of the results. A p-value ≤ 0.05 was considered statistically significant.

***Staphylococcus aureus* isolation**

Staphylococcus aureus was detected in 68.42% (n=13) of the samples. 5.26% (n=1) of the samples were *Staphylococcus sp.* The samples' remaining 26.32% (n=5) have no bacterial growth, as shown in Diagram 1. No methicillin-resistant *Staphylococcus aureus* strain was identified.

Disinfectant test

The comparative efficacy of different disinfectants on rough and smooth surfaces was tested with the Kruskal Wallis test. The percentage of *Staphylococcus aureus* reduction on each surface with different levels of disinfectants is shown in Table 1 and Table 2.

Table 1. Comparative efficacy of different disinfectants on rough surfaces against *Staphylococcus aureus*

Disinfectant	Number of colonies without disinfection	Number of colonies after disinfection	Reduction percentage (%)
Disinfectant A_1	68	0	100.00
Disinfectant A_2	58	10	82.75
Disinfectant A_3	114	1	99.12
Disinfectant B_1	78	0	100.00
Disinfectant B_2	95	1	98.95
Disinfectant B_3	75	0	100.00
Disinfectant C_1	81	0	100.00
Disinfectant C_2	27	0	100.00
Disinfectant C_3	74	1	98.65

Table 2. Comparative efficacy of different disinfectants on smooth surfaces against *Staphylococcus aureus*

Disinfectant	Number of colonies without disinfection	Number of colonies after disinfection	Reduction percentage (%)
Disinfectant A_1	68	0	100.00
Disinfectant A_2	58	10	82.75
Disinfectant A_3	114	1	99.12
Disinfectant B_1	78	0	100.00
Disinfectant B_2	95	1	98.95
Disinfectant B_3	75	0	100.00
Disinfectant C_1	81	0	100.00
Disinfectant C_2	27	0	100.00
Disinfectant C_3	74	1	98.65

Comparative efficacy between two disinfectants on rough and smooth surfaces against the isolated *Staphylococcus aureus* was tested with a post hoc Mann-Whitney test (Tables 3 and 4).

Table 3. Comparative efficacy between two disinfectants on a rough surface against the isolated *Staphylococcus aureus* tested

Disinfectants		*P*-value with Bonferroni correction
Disinfectant A	Disinfectant B	1.461
	Disinfectant C	1.461
Disinfectant B	Disinfectant A	1.461
	Disinfectant C	2.388
Disinfectant C	Disinfectant A	1.461
	Disinfectant B	2.388

*Post-hoc Mann Whitney test

Table 4. Comparative efficacy between two disinfectants on a smooth surface against *Staphylococcus aureus* tested

Disinfectants		*P*-value with Bonferroni correction
Disinfectant A	Disinfectant B	0.825

	Disinfectant C	1.539
Disinfectant B	Disinfectant A	0.825
	Disinfectant C	1.539
Disinfectant C	Disinfectant A	1.539
	Disinfectant B	1.539

*Post-hoc Mann Whitney test

There was no significant difference ($p>0.05$) between the efficacies of three disinfectants on rough and smooth surfaces against the isolated *Staphylococcus aureus.* Comparative efficacy of the same disinfectant on rough and smooth surfaces against the isolated *Staphylococcus aureus* was tested with Post-hoc Mann Whitney test (Table 5).

Table 5. Comparative efficacy of the same disinfectant on rough and smooth surfaces against *Staphylococcus aureus* tested

Disinfectants	***P*-value with Bonferroni correction**
Disinfectant A	1.539
Disinfectant B	0.363
Disinfectant C	0.363

*Post Hoc Mann Whitney test

DISCUSSION

Among the 19 samples collected from the glove of the operator's dominant hand after non-surgical extraction in the Oral Surgery Clinic in USIM Dental Polyclinic, there was no bacterial growth in 26.32% (n=5) of mannitol salt agar. This is because the mannitol salt agar used is a specific agar used for the detection of bacteria that can survive in environments with high salt concentration (7.5% sodium chloride)[22]; Mannitol salt Agar has 76.5% sensitivity and 99.6% specificity for the detection of *Staphylococcus aureus*[23].

Staphylococcus sp. was detected in 5.25% (n=1), and another 68.42% (n=13) of the samples were identified as *Staphylococcus aureus*. This is because tube coagulase test can identify *Staphylococcus aureus* from Staphylococcus sp. Only *Staphylococcus aureus* can coagulate the blood plasma.[22] This study detected no methicillin-resistant Staphylococcus aureus (MRSA) strain. In contrast, MRSA was detected in 1.5% of the samples in a similar study by Messano et al. in 2013.[14]. The result dissimilarity could be due to the difference in the number of samples collected. The sample collected in that study was 136, compared to only 19 in this study. 1.5% represents only two samples out of 136 samples. Therefore, if more samples are collected, there will be a higher possibility of isolating methicillin-resistant *Staphylococcus aureus.*

Besides, MRSA is transmitted in dental care in many studies[2,3,4,] but evidence for transmission of EBSL- and

carbapenemase-producers does not exist, even though transmission in dental practice is possible.[1]

Disinfectant A contains quaternary ammonium compound and alcohol, which is an intermediate-level disinfectant, while Disinfectant B contains phenolic alcohol, which is a low- to-intermediate-level disinfectant. On the other hand, Disinfectant C includes a quaternary ammonium compound, which is a low-level disinfectant.

When comparing the efficacies of different levels of disinfectants against the isolated *Staphylococcus aureus* on a type of surface, there is no statistically significant difference. Meanwhile, the efficacies of each disinfectant against the isolated *Staphylococcus aureus* on different surface types also have no statistically significant difference. This result is similar to a study done by Singh et al. 2012.[20.] In this study, it was found that despite the different levels of disinfectants used against the isolated *Staphylococcus aureus,* there is no statistical difference, even when used on rough or smooth surfaces. Therefore, either one of the disinfectants in USIM Dental Polyclinic can be used on both rough or smooth surfaces against *Staphylococcus aureus.*

CONCLUSION

In a nutshell, none of the three disinfectants used in USIM Dental Polyclinic for disinfection of clinical contact surfaces is superior to the other on rough and smooth surfaces against the isolated *Staphylococcus aureus.* Therefore, either one of these disinfectants can be chosen based on cost-effectiveness despite the difference in contents and level of the disinfectants to be used to disinfect clinical contact surfaces against *Staphylococcus aureus.* There are a few limitations of this study. One of the limitations is that the sample size is small due to time constraints. Only 19 samples were collected in 2 weeks. The samples were also collected from the operator's glove-dominant hand only. Samples from clinical contact surfaces were not taken due to a limited budget. A larger sample size is needed to study the presence of *Staphylococcus aureus* contamination in USIM Dental Polyclinic. A further study may be done to test the disinfectants against other pathogenic microorganisms such as Mycobacterium tuberculosis, viruses, and fungi.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

Thank you to the Faculty of Dentistry Universiti Sain Malaysia.

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