Persistence of Antimicrobial Effect of Antiseptics in Surgical Hand Hygiene Regimens

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Objectives: *To determine the persistence of antimicrobial effect of antiseptic solutions used in surgical hand hygiene procedure, and the in-house preparation was compared to the commercial solution for its efficacy.*
Material and Method: *The present study was performed in a 150-bed hospital involving 19 staff from general, orthopedics, KUB, and OB-GYN surgical teams in 48 operations. The antimicrobial effects from 4 different solutions were determined and compared.*
Results: *The study showed that the commercial alcohol-based antiseptic solution was equally or more effective than long-time accepted povidone-iodine or chlorhexidine gluconate solutions and had better persistent effect. The in-house preparation was effective comparable to the commercial solution.*
Conclusion: *The commercial alcohol-based antiseptic solution had better persistence of antimicrobial effects compared to the in-house alcohol-based hand rub, povidone iodine and chlorhexidine gluconate.*

Keywords: *Persistence effect, Antimicrobial, Antiseptics, Surgical hand hygiene*

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Hand washing prior to performing surgery by an operating team is to reduce the bacterial normal flora on hands to a minimum to prevent surgical contamination[1]. Good antiseptic solutions used for this purpose should be broad spectrum, rapid, and long-lasting. The long-lasting antiseptic effect is important[2, 3], because perforations of the surgical gloves before or during the operations are found up to 10 %[4, 5]. Chlorhexidine, with lower killing efficacy, has better persistent antiseptic effect compared to iodine or iodophor solutions, but alcohol, with high killing efficacy, has low or no persistent antiseptic effect[6]. Antiseptic solutions in the form of waterless hand cleaning solutions that contain alcohol and chlorhexidine have high killing activity and persistent antibacterial effect[7].

The commonly used method for hand washing prior to surgery is by brushing with 7.5 % povidone-iodine or 4 % chlorhexidine gluconate, followed by rinsing the hands with tap water. There are now concerns whether the traditional hand washing should be changed regarding the safety of using tap water, especially in certain hospitals that may have bacterial contamination in their water reservoirs, and a report of increased bacterial count after hand brushing[8]. The use of waterless hand cleaning antiseptics could be a better alternative to reduce the risk of contamination from the use of contaminated tap water, towels, and brushes. This present study compared the efficacy of alcohol-based hand cleaning antiseptic solutions, an imported preparation and an in-house one, with commonly used antiseptic solutions, povidone-iodine and chlorhexidine gluconate. The present study was performed in clinical practice that would be difficult to reproduce in a laboratory.

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Material and Method

The present study was performed in a 150-bed hospital in Bangkok with about 1,100 admissions/ year. The subjects consisted of 4 male surgeons, 8 as-

sistants, 7 scrub nurses in general, orthopedics, KUB, and OB-GYN surgeries. The age of participants was 26-54 years old. Seventy nine percent were female, 57.9 % and 21 % had 5-9 year and 10-19 year work experience in surgery, respectively. The test results were obtained from the 48 elective surgeries, 12 operations each from four previously mentioned facilities.

The culture specimens were swabs from hands before and after hand washing/cleaning, and at the end of the operation. The antiseptics were neutralized by Letheen broth to prevent antiseptic effect on agar culture plates. The volume of 0.1 ml of the solutions, and those with 1:10 dilution were applied on plate count agar, blood agar, and MacConkey agar. The plates were incubated at 37 C for 24-48 h., and the colonies were counted. The colonies on blood agar were tested for Gram-staining, catalase and coagulase production, and glucose O/F fermentation to identify a Gram-positive species. The colonies on MacConkey agar were tested further in triple sugar iron (TSI) agar for identification of Enterobacteriaceae and non-fermenters, and the oxidase, motility, indole, methyl red, Voges-Proskauer, citrate, urease, decarboxylase, and glucose O/F tests were performed to identify Gram-negative species.

The killing efficacy was calculated from a reduction of colony counts on plate count, blood, and MacConkey agars. The comparison of persistent antiseptic effects and efficacy was performed using Student t-test, ANOVA, and Chi-square for statistic analysis.

Results

Surgical information and bacterial culture:

The duration and types of operations are

Table 1. Duration of an operation according to specialties

Duration of surgery	Cases No	(%)	General	Orthopedics	OBGYN	Urology
<1 h	18	37.5	2	3	8	5
1 – 2 h	22	45.8	8	6	4	4
2 – 3 h	7	14.6	1	3	0	3
> 3 h	1	2.1	1	0	0	0
Total	48	100	12	12	12	12

Table 2. Surgical wound types

Wound types	Total	(%)	Gen. surg	Ortho. Surg.	OB-GYN	Urology
Clean	10	20.8	3	7	0	0
Clean contaminated	28	58.3	5	5	11	7
Contaminated	6	12.5	1	0	1	4
Dirty/Infected	4	8.3	3	0	0	1
Total	48	100	12	12	12	12

Table 3. The bacterial load on the hands of surgical staffs before handwashing procedure

	Plate count agar			**Blood agar**			**MacConkey agar**		
Sex	mean	SD	P	mean	SD	P	mean	SD	p
Male (n = 45)	1.9×10^4	2.8×10^4	0.113	1.8×10^4	2.8×10^4	0.064	2.1×10^2	5.9×10^2	0.029*
Female (n = 99)	1.1×10^4	2.1×10^4		9.3×10^3	1.7×10^4		5.3×10^2	1.2×10^3	

SD = Standard deviation, * statistically significant

shown in Tables 1 and 2. The bacterial loads on hands of the surgical staff were determined by hand swab culturing on three different culture media (Table 3). The species identification was also performed. The bacteria identified in the present study are shown in Table 4.

Comparison of the efficacy of bacterial killing of 4 different antibacterial antiseptic solutions:

The best killing activity, which was 100 % based on an average reduction of total bacterial count, was by the action of commercial alcohol-based hand cleaning antiseptic solution, while the percent reductions from a 4 % chlorhexidine gluconate, an in-house alcohol-based hand cleaning antiseptic solution, and 7.5 % povidone-iodine were 98.3 %, 96.9 %, and 78.9 %, respectively (Table 5).

Persistence of antimicrobial effect by 4 different antibacterial antiseptic solutions:

The persistence of antibacterial effects of antiseptic solutions was tested by swabbing surgeons' hands at the end of the operation. The results are shown in Table 6.

Discussion

Every member of a surgical team performs hand washing to reduce bacterial load on hands to a minimum, because gloves may be perforated during surgery. The surgical site infection was reported to be higher (5.7 %) in cases with glove perforation compared to cases without (1.7 %). In the present study, the rate of glove perforation was 4.1 %, however there were only two cases of surgical site infections and no

Table 4. Micro-organisms isolated from hand (%)

Micro-organisms	Before HW	%	After HW	%	End of surgery	%
Micrococcus spp.	130	90.3	16	11.1	14	9.7
Coagulase negative *Staphylococcus* spp.	124	86.1	28	19.4	25	17.4
Coagulase positive *Staphylococcus* spp.	21	14.6	3	2.1	2	1.4
Streptococcus spp.	21	14.6	3	2.1	2	1.4
Gram-positive rods	59	40.9	2	1.4	0	0
Gram-negative cocci	4	2.8	1	0.7	2	1.4
Yeast	18	12.5	2	1.4	0	0
Pseudomonas spp.	16	11.1	0	0	0	0
Flavobacterium spp.	10	6.9	0	0	0	0
Acinetobacter spp.	18	12.5	1	0.7	0	0
Moraxella spp.	2	1.4	0	0	0	0
Xanthomonas spp.	5	3.5	0	0	0	0
Klebsiella pneumoniae	2	1.4	0	0	0	0
Klebsiella ozanae	1	0.7	0	0	0	0
Klebsiella spp.	1	0.7	0	0	0	0
Enterobacter spp.	4	2.8	0	0	0	0
Escherichia coli	1	0.7	0	0	0	0

HW = Hand washing

Table 5. Average reduction of bacteria after hand washing with 4 different hand leaning solutions (%)

Solutions	Plate count agar			Blood agar			MacConkey agar		
	No.	average reduction	p	No.	average reduction	p	No.	average reduction	p
7.5% PVI	36	78.9	0.274	36	92.3	0.156	19	99.6	0.314
4% CHG	36	98.3		36	92.4		23	100	
Commercial	34	100.0		35	99.9		20	100	
In-house	35	96.9		36	99.8		25	100	

glove perforation had been reported in these two cases. The infections, however, were related to dirty wound operations and bacterial cultures revealed that the bacteria were more likely from endogenous sources. Dirty wounds carry a high rate of surgical site infection as reported in a study from National Nosocomial Infection Surveillance (NNIS) project by the Centers for Disease Control and Prevention (CDC). The data were collected from 84,691 operations, which were categorized into 4 types of surgeries; clean wound, clean contaminated wound, contaminated, and dirty/infected wound surgeries, with the rate of surgical wound infections of 2.1 %, 3.3 %, 6.4 %, and 7.1 %, respectively[9]. There was also a report studied in Thailand by Danchaivijitr et al showing the rate of surgical wound infections of 1.3 %, 1.5 %, 5.1 %, and 9.6 % in clean wound, clean contaminated wound, contaminated wound, and dirty/infected wound, respectively[10].

Bacterial loads on the skin vary and depend on environments, sex, age, nutrition status, personal health, and parts of the body, e.g. the load on the hands is about 1 10^4 CFU/cm^2, and the load on scalp is about 1 10^6 CFU/cm^2[10]. The bacterial loads reported in health care personnel range from 3.9 10^4 CFU/cm^2 to 4.6 10^4 CFU/cm^2[12]. The bacterial loads obtained in the present study were 1.9 10^4 CFU/cm^2 and 1.1 10^4 CFU/cm^2 in male and female personnel, respectively. The present study results were from real clinical practice, no intentional contamination with certain microorganisms was performed to measure the efficacy of hand washing as reported by others[13-17]. The samples were collected by mean of swabbing with sterilized cotton swabs at the areas supposed to have maximum bacterial burden, i.e. thumbs, tips of fingers, palms, back of hands, and finger' webs[1,18]. The recovered bacterial numbers were comparable to 1 10^6 CFU/cm^2 to 2.6 10^6 CFU/cm^2 in the present study reported by Thamlikitkul et al[19], but were different from the results of the study by Larson et al using different collecting methods in which the bacterial numbers prior to handwashing between 5 10^2 CFU/cm^2 to 1.5 10^7 CFU/cm^2 per hand were found[20].

Bacterial count on hands was lower in females who washed their hands more frequently[21]. However, the findings might have been resulted from the fact that female personnel participated in many other operations prior to the operation under investigation, and therefore had washed their hands more often. In contrast, there were more Gram-negative bacteria, according to bacterial growth on MacConkey agar, in females than in males (5.3 10^2 CFU/hand vs. 2.1 10^2 CFU/hand, respectively), ($p = 0.029$, Table 3). The discovery of *Staphylococcus* spp. and yeast on the hands of healthcare personnel was not unusual[12, 22], especially in persons with hand skin lesions[23]. Even though the difference of bacterial load between normal hands and hands with lesions in the present study was not statistically significant (p value = 0.205 - 0.867) this could be due to small sample size. However, strict adherence to hand hygiene practice guideline is essential to reduce the risks of surgical wound infection, as resistant nosocomial pathogens are increasing[24].

Gram-positive bacteria recovered in the study were *Micrococcus* spp. (90.3 %) and coagulase negative staphylococci (86.1 %); they are common normal flora of the skin[4]. For Gram-negative bacteria, the species most recovered were *Acinetobacter* spp. and *Pseudomonas* spp, similar to those reported by Larson et al[25]. However, these transient floras can be effectively reduced by more handwashing than permanent floras[12].

The efficacies in bacterial killing among 4 different antiseptic hand washing solutions were not different significantly, although the alcohol-based solutions were better than water-based antiseptic soaps. Both alcohol-based hand cleaning antiseptic solutions with chlorhexidine have fast killing action from alcohol and persistent action from chlorhexidine[12]. The results

Table 6. Bacterial isolates from hands stratified by types of antiseptics

Solutions	Number of samples	Plate count agar			Blood agar			MacConkey agar		
		Number*	%	p	Number*	%	p	Number*	%	p
7.5% PVI	35	29	82.9	0.526	31	88.6	0.046	35	100	-
4% CHG	33	29	87.9		2	72.7		33	100	
Commercial	32	30	93.8		31	96.9		32	100	
In-house	36	30	83.3		30	83.3		36	100	

* The number of samples that had no bacterial increment at the end of the operations

obtained in the present study were similar to data reported by others that alcohol-based hand cleaning antiseptic solutions can reduce total bacterial counts better than 7.5 % povidone-iodine and 4 % chlorhexidine gluconate[13, 26, 27].

For the persistent killing effects of 4 different solutions, the authors studied only for the total operation time, which in some operations lasted only 20 minutes. The action of povidone-iodine has been reported to last only 30-60 minutes, there was no antimicrobial activity 1 – 4 hours later[4,28, 29,30]. However, there was no statistically different persistent killing effect by all solutions in all operations that differed in operating times. Using total bacterial count, the persistent killing effects were 82.9 % for povidone-iodine, and 93.8 % for commercial alcohol-based solution, but for Gram-negative count, all solutions have persistent killing effect of 100 %.(table 6) The commercial solution had the best persistence activity; this may be due to the effect of chlorhexidine that is an ingredient in this solution[27, 31].

The in-house alcohol-based hand cleaning antiseptic solution showed comparable efficacy in bacterial killing obtained from total bacterial count and Gram-negative count, and insignificant different activity against gram-positive bacteria. However, there were complaints about the stickiness of the solution due to glycerine in the solution and there was a problem related to the stability of the solution in that the chlorhexidine gluconate became crytalized within 4 months of use.

Conclusion

The new alcohol-based hand washing solutions were equally or better in efficacy of killing bacteria and sustaining killing effect compared to long-time accepted antiseptics such as povidone-iodine and chlorhexidine gluconate. Therefore, the in-house preparation would be recommended for use due to its efficacy and lower price.

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ฤทธิ์คงค้างการต้านจุลชีพของน้ำยาฆ่าเชื้อที่ใช้ในการล้างมือสำหรับผ่าตัด

วิไล นพรัตน์, กนกรัตน์ ศริพาณิชกร, ชาญวิทย์ ตรีพุทธรัตน์, สมหวัง ด่านชัยวิจิตร

วัตถุประสงค์ : *ศึกษาฤทธิ์คงค้างการต้านจุลชีพของน้ำยาฆ่าเชื้อที่ใช้ในการล้างมือสำหรับผ่าตัด และศึกษาเปรียบเทียบระหว่างฤทธิ์ของน้ำยาที่ผลิตขึ้นเองกับน้ำยาที่จำหน่าย*

วัสดุและวิธีการ : *ศึกษาในโรงพยาบาลขนาด 150 เตียงแห่งหนึ่ง โดยศึกษาในบุคลากร 19 คนในศัลยกรรมทั่วไป, ศัลยศาสตร์ ออร์โธปิดิกส์, ศัลยศาสตร์ยูโรวิทยา และสูติ-นรีเวชวิทยาในการผ่าตัด 48 ครั้ง โดยศึกษาฤทธิ์ของน้ำยาฆ่าเชื้อ 4 ขนานเปรียบเทียบกัน*

ผลการศึกษา : *น้ำยาฆ่าเชื้อที่มีส่วนผสมแอลกอฮอล์ที่จำหน่ายมีฤทธิ์เท่ากันหรือดีกว่าโพวิโดนไอโอดีนหรือคลอเฮกซิดีนกลูโคเนทและมีฤทธิ์คงค้างนานกว่า น้ำยาฆ่าเชื้อที่ผลิตเองมีฤทธิ์เท่าเทียมกับน้ำยาที่จำหน่าย*

สรุป : *น้ำยาฆ่าเชื้อที่มีส่วนผสมแอลกอฮอล์ที่จำหน่ายมีฤทธิ์คงค้างการทำลายเชื้อมากกว่าโพวิโดนไอโอดีนและคลอเฮกซิดีนกลูโคเนท*