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THE STUDY OF QUALITY EVALUATION OF A HAND RUB (HAND SANITIZER) WITH WHO-BASED-STANDARD FORMULATION AND PERCENT REDUCTION OF BACTERIA (PERCENTAGE KILL BACTERIA) IN METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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ABSTRACT

The pandemic that occurred caused both by COVID-19 (currently occurring) as well as the clone of the methicillin resistance staphylococcus aureus (MRSA) in the previous period has really threatened human life. This condition requires materials that can break the chain of transmission from human to human and the environment to human. This study aimed to evaluate the quality of alcohol-based hand rub with WHO-based-standard formulation based on the stability of the formulation, the risk of irritation and the ability to kill bacteria. Assessment on the presence of rancidity, clarity, discoloration, final alcohol content and skin irritation risk was done to know the quality of product formulation. A bacterial Methicillin resistance staphylococcus aureus (MRSA) was used to assess the percentage of bacterial killing power. The selected bacteria were bacteria commonly found in the hospital environment. The results of formulation stability from four variants of level modification showed that MK.IV has good stability compared to other formulation modifications. In terms of irritation risk, twenty-three selected subjects were generally well tolerated in use. The results of the percentage kill test against MRSA showed that the percentage kill is 99.90% at 1st, 2nd, and 5th minute. The selected manufacturer’s product also showed the same percentage kill value at 1st, 2nd, and 5th minute. The results of percentage kill test showed that the effective value for each contact time is ≥ 90%.

Keywords: hand rub, hand sanitizer, ahmad Subhan, percentage kill, WHO

INTRODUCTION

At present, infections widespread in society have truly threatened human life in the world. Corona virus infection or introduced by the World Health Organization (WHO) as COVID-19 (Coronavirus disease 2019) has caused massive death worldwide until today.¹ In Indonesia, Covid-19 infection raised to pandemic events at the 164th day and it has already been 59.394 people were positively infected with 2.987 people died and 26.667 people recovered.²

There was once another pandemic caused by S. aureus happened in the world. This pathogen is resistant to penicillin in phage type 80/81 which is the most numerous and extraordinary clones causing epidemics during 1950s. This clone quickly spreaded and was dominant in Australia, England, America and Canada, which caused severe skin infections, sepsis, and/or pneumonia. Initially, this pandemic was limited to hospital environment. However, gradually this infection was also infected people outside hospital. This pandemic lasted for about 10 years, after a decline of phage type 80/81 was observed. The decline was caused by the introduction of methicillin to market from 2000 to 2006 in Queensland, Eastern Australia. Population studies of antibiotic resistance profile of inpatient MRSA line showed an increase from 71 to 315 cases per million for non-MDR types. This strain is resistant to at least one non-lactam antibiotic and is susceptible to ciprofloxacin. During the same period, parallel increases were seen in outpatient units, from 52 to 490 cases per million. This study proposes the rapid spread of non-MDR MRSA strains. Very high MRSA prevalence rates have also been detected in East Asia. The multinational study center conducted surveillance studies in 2011 and determined prevalence of MRSA in various Asian countries. They concluded that HA-MRSA was responsible for...
for 86.5% in Sri Lanka, 74.1% in Vietnam, 77.6% in South Korea, 65% in Taiwan, 57% in Thailand, and 56.8% in Hong Kong. However, the prevalence is much lower in India and the Philippines, respectively, 22.6% and 38.1%.³

Pathogens transmission can occur during the process of patient care in the hospital. Either through direct or indirect contact, droplets, or contaminated air. Transmission through contaminated hands is the most common pattern in most health services. For this reason, WHO (2009) established 5 (five) conditions that required health workers to wash their hands, namely: (1) after making contact with patients: because the organisms on the patient's skin can move into health workers’ hand whenever there is a direct contact; (2) after being exposed to body fluids: pathogenic organisms in body fluids can contaminate officers' hand, so that they can be transmitted to patients or vice versa; (3) after a direct contact with the patient's immediate environment: this prevention is taken because the patient's immediate environment has been contaminated by patient's droplet or body fluids; (4) before contact with patients: to prevent the transmission of pathogens from the hands of the officer to the patient; (5) before carrying out sterile actions: pathogenic organisms are able to survive for at least a few minutes on officers’ hands. Therefore, before carrying out sterile actions, hand rubbing must be done.⁴

Antiseptics and disinfectants are widely used in hospitals and other health care facilities for various purposes to protect surface areas. In particular, this is an important part of infection control and is a tangible form of prevention of nosocomial infections. The emergence of concerns about the potential for microbial contamination and the risk of infection in food and other consumption materials, has led to an increase in the use of antiseptics and disinfectants by mass public. A variety of active chemical agents (or biocides) is found in these products, many of which have been used for hundreds of years as antisepsis, disinfection, and preservation. However, little is known about how this active ingredient works, compared to antibiotics. In general, biocide has a broader spectrum of activity than antibiotics. Besides, antibiotics tend to have specific intracellular targets, while biocide may have many targets. The widespread use of antiseptic products and disinfectants has led to some speculation about the development of microbial resistance, especially cross-resistance to antibiotics.⁴

In addition, high cost of purchasing hand rub can be a trigger for a lack of meeting a need for hand rub in health care facilities. Based on monthly reports on the use of pharmaceutical supplies in Fatmawati General Hospital, it is known that during the period of May 2016 to September 2018, total hand rub used was 23,129 bottles in volume of 500 ml. The total cost needed was Rp. 2,823,000,000.⁵

For this reason, research needs to be carried out as an effort to control pathogens by conducting analysis studies on quality of WHO hand rub standards. The results of this study may be useful to fulfill health care needs in Indonesia. It also may be used as an input for all health services in Indonesia in making correct, quality and affordable hand rubs in order to improve quality and affordable health services for a community to reach the highest health status. It may increase the repertoire of knowledge which can be applied in health care services in Indonesia.

**Hand Antiseptic**

Hand antiseptics with an alcohol base most often contain ethanol, isopropanol or n-propanol, or a combination of these two types. In general, isopropanol has greater bacterial efficacy and ethanol is more potent against viruses, but it also depends on the concentration of two active substances and microorganism test. For example, isopropanol is more lipophilic than ethanol and has less activity against hydrophilic viruses (e.g. poly viruses).⁴

Ethanol (also called as ethyl alcohol, granular alcohol, drinking alcohol, or just alcohol) is a chemical compound, a simple alcohol with the chemical formula C₂H₅O or C₂H₅OH. The formula can also be written as CH₃-CH₂-OH or C₂H₅OH (ethyl group associated with the hydroxyl group), and is often abbreviated as EtOH. Ethanol is a volatile, flammable, colorless liquid with a slight odor. It is a psychoactive substance and the main active ingredient found in alcoholic drinks.²⁰

60-80% alcohol is the most effective solution in killing microorganisms, with the greater concentration of the alcohol, the smaller the potency of microorganism to keep optimally infecting. There is still little information about specific model of alcohol mechanism, but based on the increased efficacy of the presence of water, in general, alcohol causes membrane damage and protein denaturation, thereby further causing metabolic disorders and protein lysis.⁵

The mechanism of alcohol is damage cytoplasmic membrane thus causing intracellular constituent leakage². Alcohol has excellent germicidal activity in vitro against gram-positive and gram-negative vegetative bacteria (including multidrug-resistant pathogens such as MRSA and VRE), *M. tuberculosis*, and varieties of fungi. However, alcohol has virtually no activity against bacterial spores or protozoan oocytes, and has poor activity against some non-enveloped (non-lipophilic) viruses. Some enveloped (lipophilic) viruses such as the herpes simplex virus (HSV), HIV. Influenza virus, RSV, and vaccinia virus are sensitive to alcohol when tested
in vitro. Other enveloped viruses are less sensitive to alcohol, but some studies show that 60-70% alcohol can be lysed including hepatitis B virus (HBV) and possibly hepatitis C virus. In a carrier model of porcine tissue used to study antiseptic activity, 70% ethanol and 70% isopropanol was found to reduce the titre of enveloped bacteriophages more effectively than antibacterial soap containing 4% CHG.\textsuperscript{4}

Alcohol quickly kills bacteria when applied to skin and it has no persistent activity. However, bacterial regrowth in skin occurs slowly after the use of alcohol-based antiseptic. This is possible because of a sub-lethal effect of alcohol on some skin bacteria.\textsuperscript{4}

A number of studies have documented antimicrobial activity of alcohol in vivo. Early quantitative studies of antiseptic effect of hand rub determined that alcohol effectively reduced the number of bacteria on hands. Typically, reduction in log release of test bacteria from artificially contaminated hands is $3.5 \log_{10}$ on average after 30 minutes of application, and $4.0-5.0 \log_{10}$ after 1 minute of application.\textsuperscript{4}

**RESEARCH METOCHDLOGY**

**Modification of Hand rub Formula**

In this study, the content was modified, by making four levels of concentration ratio. The total volume was 500 ml per preparation, where the ethanol content was made with rising gradation in order to maintain the final alcohol level > 80%. The levels of perhidrol and glycerol were made with a decreasing gradation, while sterile water was kept constant (constant). The following is a comparison of formulation levels between WHO hand rub standards (2009) and modified levels for a total volume of 500 ml.

Table 1. Comparison of WHO formulas and modification of hand rub levels in a total volume of 500 mL

| No | Active substance | WHO   | MK.1    | MK.2   | MK.3   | MK.4   | Unit |
|----|------------------|-------|---------|--------|--------|--------|------|
| 1  | Etanol 96%       | 417   | 433,40  | 437,35 | 439,25 | 439,30 | mL   |
| 2  | H2O2 3%         | 21    | 10      | 7      | 5      | 5      | mL   |
| 3  | Glicerol 98%     | 7     | 0,90    | 0,45   | 0,30   | 0,25   | mL   |
| 4  | Aq. steril ad.500mL | 55   | 55      | 55     | 55     | 55     | mL   |
|    | TOTAL            | 500   | 500     | 500    | 500    | 500    | mL   |

**Tools**

The following are tools used to make an alcohol base hand rub (10 liters).
1. Jerry cans, the tank is closed to a capacity of 10 liters
2. Measuring cup 500 ml
3. Pumpkin measuring 5 liters
4. 500 ml packaging bottle

**Procedure for Making**

The following is a procedure for making hand rub according to WHO recommendations, 9 which in this case was made at Fatmawati General Hospital Jakarta Famasi Installation:

1. Preparation for manufacturing
   a. Preparing tools and materials used for the process of hand rub production.
   b. Documenting production activities of making hand rub.
   c. Labeling and packaging by making stickers/etiquette, writing down the date of production, batch number, and expired date.

2. Manufacturing process
   a. Fill 96% alcohol solution into a tightly closed container (tank/jerry can)
   b. Fill 3% H2O2 using measuring cups
   c. Add 98% glycerol using measuring cups
   d. Rinse the remaining glycerol in a clean measuring cup with a sterile aquadest.
   e. Measure alcohol concentration with an alcohol meter; final grade > 80%
   f. Close tank/jerry can tightly to prevent evaporation.
   g. Stir tank or jerry cans for 10 minutes so that the liquid is mixed evenly (homogeneous).
i. Package the liquid in a 500 mL packaging bottle and tightly closed.

Figure 1. Density measurement on alcohol concentration

3. storage
   a. Place it on shelves/storage cabinets.
   b. Store it for 72 hours at room temperature to kill spores that may grow during formulation process
   c. Label the storage to inform that it is still in process
   d. Measure the alcohol concentration after 72 hours of storage with an alcohol meter; the final alcohol level should not be less than > 80%

RAW MATERIAL SOURCE
The source of raw materials in this study is from PT. Brataco Indonesia. The requirements of these raw materials are pharmaceutical grade or have guaranteed safety if used on humans. Other requirements, these raw materials have been completed with information on the safety data sheet (MSDS)

Testing Methodology

Stage 1: Test of formulation stability
Rancidity testing
In rancidity testing, two tubes for a volume of 100 mL were used. The first tube contained 50 mL of control solution (96% alcohol) while the second tube contained 50 mL of hand rub solution tested. Then, an observation on control solution odor compared to test solution odor. The next step is describing odor from the solution according to a scoring set. Based on rancidity testing, a conclusion of solution concentration of rancid and not rancid was made. Observations were made at week 1 through week 4, month 6 and month 12.

Testing for clarity/turbidity/discoloration
In testing for clarity/turbidity/discoloration, two tubes for a volume of 100 mL were used. The first tube contained 50 mL of control solution (96% alcohol) while the second test tube contained 50 mL of hand rub solution tested. Then, they were carefully observed using a 100 watt tubular lamp (TL) for 5-10 minutes. A comparison of each solution related to its clarity/turbidity/discoloration was made. Results of observations was a standard of clarity, turbidity, and discoloration. Observation was carried out at week 1 through week 4, month 6 and month 12.

Testing for final alcohol levels
In testing the final alcohol content, two tubes for a volume of 250 mL were used. The first tube contained 150 mL of control solution (96% alcohol) while the second tube contained 150 mL of tested hand rub solution. A calibration was carried out to test the final level of alcohol using a calibrated alcohol meter. Then, alcohol control and testing was poured into the first and second tubes, then alcohol meter was done immediately after insertion. Observation was continued for 5-10 minutes by looking at the marking value on the alcohol meter, at the upper limit of the liquid surface tested both control and hand rub. The solution must reach a value of more than 80%. Observation was continued at week 1 through week 4, month 6 and month 12.
Stage 2: Percentage Kill Bacteria Test

Time-kill testing is a method of determining the effectiveness of antimicrobials (hand rub) with plate count techniques and analysis of percent and log reduction. The procedure carried out in this test followed standards of the ASTM (Anti-microbia Susceptibility Testing Method) E-2313. After carrying out bacterial culture preparation, a sufficient number of test samples for testing activities were placed into a sterile petri dish. Then, a number of bacterial cultures were tested (usually 1/10 or less of the volume of the test sample) by inoculating it into petri dish beforehand and then immediately stirred. After determining contact time, a small amount of bacterial mixture and test sample was taken, and put in a cup containing the nutrient agar, and then was incubated at 37 for 24 hours.

A modified formulation of 12 month hand rub/hand sanitizer which was known as the most stable formulation was tested on killing ability of bacteria. It was done to determine the effectiveness of one year hand rub preparation. This is also done to assess expired period of hand rub products. At this stage of testing, hand rub products with an alcohol concentration more than 80% were used as a comparison.

Procedure for testing killing power of bacteria with multi drug resistant organism (MDRO) pathogens is as follows:

1. A test solution was made for the Mc Farland 0.5 equivalence in NaCl, then a 10-1 to 10-5 dilution was carried out. Then the planting of each dilution was carried out into PCA media.
2. Aseptic measurements were taken of a sample of 4.5 cc. Then, control solution was made with Aquadest (without mixed samples) as much as 4.5 cc.
3. About 500 germ were inserted in each sample and control. Then they were homogenized with vortex
4. Samples and controls that had been added by germ – was taken as much as 1000 µl, then put into a tube containing 9 ml Aquadest at the time of 1 minute, 2 minutes and 5 minutes.
5. Planting of 1000 µl into PCA was carried out each time.
6. Incubation was carried out at a temperature of 35 ° C (18 - 24 hours)
7. Calculation was made to interpret results with following formula:

\[
\%\text{Reduction} = \frac{\text{TPC control (without sample)} - \text{TPC sample}}{\text{TPC control (without sample)}} \times 99.9\%
\]

A good percentage kill test is when the results for each contact time are more than or equal to 90% .

Pathogens that became test microbes in this study are MRSA is obtained from the isolation of the patient's wound swab, with an overview of the results of the resistance test as follows:

Table 2: Source of Methicillin Resistance Staphylococcus aureus (MRSA)

(see appendix.1)

| Number         | 201908017267 (155G) |
|----------------|---------------------|
| Check Date     | August 6, 2019      |
| ACC Date       | August 9, 2019      |
| Material       | Wound swab          |
| Culture results| Staphylococcus aureus |

| Jenis Obat       | S/I/R | Jenis Obat       | S/I/R |
|------------------|-------|------------------|-------|
| Gol. Penicillin  |       | Gol. Macrolide   |       |
| Ampicillin (AMP) | R     | Erythromycin E   | >=8   | R   |
| Amoxicillin (AML)| R     | Azithromycin (AZM)|      | R   |
| Amoxiclav/Augmentin (AMC)| R | Clindamycin (DA)  | >=8   | R   |
| Ampicillin Sulbactam (SAM)| R |                  |       |     |
| Oxacillin (OX)  | R     |                  |       |     |
| Cefoxitin screen| POS   |                  |       |     |
| Benzylenicillin  | R     |                  |       |     |
| >=0.5            |       |                  |       |     |
| Gol. Cephalosporin|      |                  |       |     |
| Cefadolin (KF)   | R     |                  |       |     |
| Cefazolin (KZ)   | R     |                  |       |     |
| Cefuroxim (CMX)| R     |                  |       |     |
| Cefoperazone (CFP)| R |                  |       |     |
| Ceftriaxone (CRO)| R     |                  |       |     |
| Cefepime (FEP)   | R     |                  |       |     |
| Gol. Carabenem   | R     |                  |       |     |
| Imipenem (IMP)   | R     |                  |       |     |
|                  |       | Gol. Glicopeptide|       |
|                  |       | Linezolide       | 2     | S   |
|                  |       | Vancomycin (VA)  | >=32  | R   |
|                  |       |                  |       |     |
|                  |       | Gol. Quinolone   |       |
|                  |       | Ciprofloxacin (CIP)| >=8| R |
|                  |       | Levofoxacin (LEV)| 4     | R   |
|                  |       | Moxifloxacin (MXF)| 2     | R   |
|                  |       |                  |       |     |
|                  |       | Gol. Antibiotik Lain|       |
|                  |       | Trimethoprim     | 20    | S   |
|                  |       | /Sulfamethoxazole|       |     |
|                  |       | Tetracycline (TE)| <=1   | S   |
|                  |       | Chloramphenicol (CO)|    |     |
|                  |       | Fosfomycin (POS)*| S     |     |
|                  |       | Tigecycline (TGC)| <=0.12| S   |
Stage 3: User Irritation Risk Test (ethical clearance number: 122/KPP/XII/2018 – see appendix.2)
The next step is to test user irritation risk. This test was carried out using a formula from the best results of product formulation stability test. Observation on user allergic risk using volunteers who had met the inclusion criteria as follows: willing to be a subject, male or female in healthy condition, not a pregnant women or not giving breastfeeding, aged more than 18 years and 65 years old. While the exclusion criteria were: Subject is pregnant or giving breastfeeding, has a significant medical history of a disease or dermatological condition, such as atopy, psoriasis, vitiligo, or a condition known to change the appearance of the skin or physiological response (e.g. diabetes or porphyria), medical history of a condition that will significantly affect the immune response (for example; primary immunodeficiency or acquired diseases such as HIV or AIDS; allergic diseases such as anaphylaxis, asthma, or allergic reactions due to drugs; neoplasms such as lymphoma or leukemia; rheumatoid arthritis; or systemic lupus erythematosus), medical history of significant skin cancer (e.g. melanoma or squamous cell carcinoma), within 72 hours of starting as a subject, using antihistamines or using topical drugs in the hands.

The procedure of collecting observation data using time series method based on two observation techniques, namely: (1) The subject is confirmed (follow-up) in the induction phase; (2) challenge phase (challenge) with general subjects is as follows: 12
(1) Observations on subjects confirmed in the induction phase;
- Subjects had filled in a form; meet the inclusion criteria and is a Hospital employee.
- Induction techniques were performed on five (5) moments of hand washing with hand rub: (a) before contact with the patient; (b) after contact with the patient; (c) before aseptic act; (d) after contact with body fluids; (e) after contact with environment.
- Induction posture and observation were carried out with a six-step technique of washing hands with hand rub, namely (a) on both palms, (b) at the back of both hands; (c) between fingers of both hands; (d) locking fingers of both hands; (e) fingertips and nails of both hands.
- Treat induction: done in normal work cycle, within the duration of observation duration: 70 hours, i.e. 7 hours/shift multiplied by ten (10) working days.
- Treat induction performed step six steps of washing hands with hand rub in five moments with MD4 hand rub products 2-5 mL, with a minimum contact duration of 24 seconds to 60 seconds.
- Reports were confirmed if there was an irritation/allergy done every day during the span of observation either by subject or by researcher.
- If there were reports of irritation/allergy, consult to a dermatologist (genital dermatologist); and researchers gave scoring based on results of assessment, with following criteria:

| No. | Description of allergies on skin | Score |
|-----|---------------------------------|-------|
| 1   | No evidence of irritation        | 0     |
| 2   | No minimal Erythema with almost no evidence of irritation | 1     |
| 3   | Clear Erythema and minimal edema or minimal papula response | 2     |
| 4   | Erythema dan papula             | 3     |
| 5   | Definite edema                  | 4     |
| 6   | Erythema, edema, dan papula     | 5     |
| 7   | Vesicular eruption              | 6     |
| 8   | Strong reactions spread outside the application site | 7     |

- Termination of subject if there was an irritation/allergy due to the treatment of hand rub-MK4 in induction phase; and did treatment based on clinical pathways (CP) and clinical practice guidelines (PPK) that apply in hospitals.

(2) Observation in challenge phase (challenge) with general subjects; 12
- Subjects had been willing to be volunteer subjects; meet the inclusion criteria
- Induction techniques are performed on five moments of hand washing with hand rub: (a) before contact with the patient; (b) after contact with patient; (c) before aseptic act; (d) after contact with body fluids; (e) after contact with environment.
- Induction posture and observation were carried out with a six-step technique of washing hands with hand rub, namely (a) on both palms, (b) at the back of both hands; (c) between fingers of both hands; (d) locking fingers of both hands; (e) fingertips and nails of both hands.
- Treat induction: done in one hand hygiene practice, only when observing the hospital environment.
- Treat induction: did the six steps of washing hands with hand rub; with hand rub-MK4 products 2-5 mL, with a minimum contact duration of 24 seconds to 60 seconds.
- Reports were confirmed if there was an irritation/allergy after hand washing with hand rub, both by subject and by researcher.
- If there were reports of irritation/allergic events, consult to a dermatologist (genital dermatologist); and researchers gave scoring based on results of assessment, with criteria as mentioned above.

**FINDINGS AND DISCUSSION**

**Stage 1: Result and discussion of formulation test**

| Criteria | Observation Parameter |
|----------|-----------------------|
| Formula standard WHO: | |
| Batch: HR-ORG/01-2019/1/3/3/4 modification 1 | |
| clear | clear | clear | clear | clear | clear |
| no | no | no | no | No | |
| 80% | 80% | 80% | 80% | 80% |
| Color change | Rancid odor | Clarity | |
| Formula standard WHO: | |
| Batch: HR-MOZ/01-2019/1/3/3/4 modification 2 | |
| clear | clear | clear | clear | clear | Clear |
| no | no | no | no | no |
| 85% | 85% | 84% | 83% | 83% |
| Color change | Alcohol concentration | |
| Formula standard WHO: | |
| Batch: HR-MOZ/01-2019/1/3/3/4 modification 3 | |
| clear | clear | clear | clear | clear | Clear |
| no | no | no | no | no |
| 87% | 85% | 84% | 84% | 84% |
| Color change | Alcohol concentration | |
| Formula standard WHO: | |
| Batch: HR-MOZ/01-2019/1/3/3/4 modification 4 | |
| clear | clear | clear | clear | clear | Clear |
| no | no | no | no | no |
| 85% | 87% | 87% | 87% | 85% |
| Color change | Alcohol concentration | |

Testing period was conducted from January 2019 - December 2019 or for 12 months. Testing was done by comparing five formulation models. Formulation 1 was WHO standard formula, while formulation 1 - 4 were a modification of concentration. Results of observations on WHO standard formula showed a peculiar rancid odor in week I storage until 12th month. In formulation MK1, rancid odor was identified in week III. In MK 2 it was identified in week IV, while in MK 3, rancidity was identified in 6th month. Whereas in MK 4, no rancid odor was found until storage process in the 12th month. The statements “odorless”, “practically odorless”, “characteristic odor is weak” or otherwise, were determined by observation after material had been exposed to air for 15 minutes which was calculated after container of hand rub solution was opened. The rancid odor mentioned is only descriptive of the material concerned.8

The appearance of this particular rancid odor is probably due to a presence of glycerin (glycerol) in alcohol hydrolyzed. Glycerol in this formulation functions as an emollient to prevent irritation to the skin due to alcohol ingredients. Glycerol according to its molecular formula: C₃H₈O₃, is a simple polyol compound. It is a colorless, odorless, and thick liquid that tastes sweet and non-toxic.13 Change of glycerol to Reuterin (3-hydroxypropionaldehyde) is an organic compound with the formula HOCH₂CH₂CHO is a bi-functional molecule containing hydroxyl and aldehyde functional groups. Whereas Aldehydes have various properties and it depends on the rest of the molecule. Smaller aldehydes are more soluble in water, formaldehyde and acetaldehyde. Aldehydes are volatile and have a pungent odor.14
Based on observation of final alcohol concentration, WHO formula did not experience a decrease in concentration, so that it remained in 80% after 12 months of storage. In MK.1 and MK.2, alcohol concentration decreased from week 3 from 84% to 83% at 12 months of storage. In MK.3, alcohol concentration decreased in week 2 from 85% to 84% at 12 months of storage. In MK.4, alcohol concentration decreased in week 4 from 87% to 86% at 12 months of storage. In general, all formulations met the standards in terms of concentration, which WHO requires. The final alcohol concentration of hand rub preparation was more than 80%.

However, several studies have shown that 60-70% ethanol can lyse bacteria including hepatitis B virus (HBV) and it probably can also lyse hepatitis C virus. In a porcine tissue carrier model used to study antiseptic activity, it was found that 70% ethanol and 70% isopropanol can reduce the titre of enveloped bacteriophages more effectively than antibacterial soap containing 4% CHG. Ethanol actually has bacterial or enveloped (lipophilic) activity such as herpes simplex virus (HSV), HIV. Influenza virus, RSV, and vaccine virus, which are generally sensitive to alcohol when tested in vitro.

Stage 2: Percentage Kill Test Results
Hand rub test with percentage kill method was done for MK.4 formulation which is known from the formulation test. This formulation had the best relative results compared to other modification of concentration. The testing period was carried out after the formulation was kept for 12 months or one year. This was done at the same time with testing effective age of sample and testing its effectiveness in terms of pathogen reduction percentage (%) that is known to have resistance, in this case methicillin-resistant staphylococcus aureus (MRSA). Staphylococcus aureus, gram-positive bacteria, pathogens with coagulase-positive originating from the Staphylococcaceae family, are spherical bacteria with diameters close to 1 µm resembling grape clusters. S. aureus is a commensal that often appears without symptoms on parts of the human body such as skin, skin glands, and mucous membranes, including the healthy nose and intestines of humans. Studies show that about 20% of individuals are persistent carriers of S. aureus and about 30% as intermittent carriers, while the other 50% are not carriers. Therefore, this colonization significantly increases the chance of infection by providing a reservoir of pathogens. In most cases, individuals infected with S. aureus strains are usually carried as commensal.

In this study, the source of MRSA was obtained from the results of the wound swab isolation, from Fatmawati General Hospital laboratory (see appendix.1). The test was conducted at microbiology laboratory of the University of Indonesia (UI), with the following results:

Table 5. Percentage kill test results on formula MK. IV

| No. | Kind of bacteria | Time of Contact* | % reduction of colony numbers |
|-----|------------------|------------------|-----------------------------|
|     |                  | Without sample/control | With sample in Colony Forming Unit (CFU) |                  |
| 1   | methicillin-resistant staphylococcus aureus (MRSA) | 1 Menit 38 | 1 Menit 0 (null) | 99.9% |
|     |                  | 2 Menit 51 | 2 Menit 0 (null) | 99.9% |
| 1   |                  | 5 Menit 52 | 5 Menit 0 (null) | 99.9% |

Based on table above, in sample/control without active substances, in the first minute, the number of colonies was 38 CFU. In the second minute, it was 51 CFU, and at 5th minute, it was 52 CFU. When compared to results of test with active substance of hand rub MK.IV, it is known that in the 1st, 2nd, and 5th minute, it was 0 CFU. So based on the results of percentage reduction calculation in the 1st, 2nd, and 3rd minute, alcohol concentration was 99.9%. This shows that the results of the quality of hand rub products were good, because
based on the results of the percentage kill test, it is declared good if a value is obtained more than or equal to 90% for each contact time.

As a comparison of the results, the manufacturer hand rub was also tested, namely Softaman. The selection of this product is due to having an alcohol content more than 80% as WHO has suggested. The results of the test are presented below.

Table 6. Percentage kill test results on Softaman

| No. | Kind of bacteria                  | Time of contact* |
|-----|-----------------------------------|------------------|
|     |                                   | Without sample/control in Colony Forming Unit (CFU) | With sample in Colony Forming Unit (CFU) |
| 1   | methicillin-resistant staphylococcus aureus (MRSA) | 1 Menit 51 | 1 Menit 0 | 99.9% |
|     |                                   | 2 Menit 55 | 2 Menit 0 | 99.9% |
|     |                                   | 5 Menit 58 | 5 Menit 0 | 99.9% |

Based on table above, in sample/control without active substances, in the first minute, the number of colonies was 51 CFU. In the second minute, it was 55 CFU and in the 5th minute, it was 58 CFU. When it compared to the results of test with the active substance of Softaman hand rub, it is known that in 1st, 2nd, and 5th minute, the total was 0 CFU. So based on the results of percentage reduction calculation in the 1st, 2nd, and 3rd minute, it was 99.9%. This shows that the results of quality of hand rub products are good, because based on the results of the percentage kill test, it is declared good if a value is obtained for each contact time more than or equal to 90%.11

The Percentage kill test was chosen on MRSA, with the aim of assessing the ability of percentage hand rub reduction. This is also due to infection of MRSA strains resulting in higher mortality than infections caused by species that are susceptible to methicillin. This results in longer hospital stays and increased health care costs.10 MRSA strains produce changes in penicillin-binding protein associated with decreased affinity for most semisynthetic penicillin’s. The protein is encoded by the genes obtained, namely mecA.3 This genetic resistance to the methicillin resistant component of the cellular genetic element (MGE) which is characterized by the acquisition of Staphylococcal Cassette Chromosome mec (SCCmec) insertion of genetic elements that move into chromosomes from susceptible strains. The acquisition of antimicrobial resistance is causing new challenges for the medical world in terms of treatment and control of staphylococcal infections. MRSA in many cases accounts for 25 to 50% of S. aureus infections in hospitals.3 This infection is a major concern because of their high morbidity and mortality and resistance to penicillin and most other lactam antibiotics, except ceftaroline and ceftobiprole.

Stage 3. User Irritation Risk Test

This user irritation risk test was conducted on 23 subjects divided into 11 subjects in follow up-induction scheme and 12 subjects in challenge scheme.

Table 7. Analysis of subject demographic data

| Group          | Status          | Number of subjects | Percentage |
|----------------|-----------------|--------------------|------------|
| PROFESSION     | Nurse           | 6                  | 26,1       |
|                | Caregiver       | 11                 | 47,8       |
|                | professional    | 11                 | 47,8       |
|                | miscellaneous  | 6                  | 26,1       |
| Total          | 23              | 100,0              |
| AGE            | 17-25 y.o       | 1                  | 4,3        |
|                | 26-35 y.o       | 8                  | 34,8       |
|                | 36-45 y.o       | 9                  | 39,1       |
|                | 46-55 y.o       | 4                  | 17,4       |
|                | 56-65 y.o       | 1                  | 4,3        |
| Total          | 23              | 100,0              |
| GENDER         | MALE            | 7                  | 30,4       |
|                | FEMALE          | 16                 | 69,6       |
| Total          | 23              | 100,0              |
| LOCATION       | IRNA Terati     | 2                  | 8,7        |
|                | IRNA Anggrek    | 7                  | 30,4       |
IRNA GPS 3 13.0
IGD 1 4.3
Pharmacy 10 43.5
Total 23 100.0

**ALERGIC HISTORY**

|                | YES | 17.4 |
|----------------|-----|------|
| TOTAL          | 19  | 82.6 |

**USER GROUP**

|                | Employee | 69.6 |
|----------------|-----------|------|
| TOTAL          | 16        | 69.6 |

**PUBLIC**

|                | 7  | 30.4 |
|----------------|---|------|
| TOTAL          | 23| 100.0|

Based on demographic data, there were 4 subjects or 17.4% who had an allergic history confirmed since the first day, and 19 subjects or 82.6% with no allergic history, based on subject recognition.

In follow-up (induction) subject group, there were 110 treatments, while in challenge (challenge) subject group, 12 treatments were carried out.

**Table 8. Number of subject and treatment in each subject group**

| GROUP                  | PHASE         | Number of subject | Number of treatment |
|------------------------|---------------|-------------------|--------------------|
| **Subject group**      | **Induction follow-up** | **11**          | **110**            |
| **Induction challenge**|               | **12**           | **12**             |
| **Total**              |               | **23**           | **122**            |

In the induction-follow-up group, there were 11 selected subjects and their adherence was constantly followed/observed in using hand rub at five moments. Observations were made for 10 working days, divided into two periods, namely: the first week of observation on Monday and the second week of observation, which was Monday - Friday. So there was an interval of observation for 2 day off, namely Saturday and Sunday. Observations were only made during working hours or morning service shifts between 08.00 WIB - 15.00 WIB.

During observation period, user irritation risk appeared and it was scored using the following scoring values: Determination of irritation risk grade was done collaboratively with a dermatologist. Based on observational data series, it is known that during 10 days of observation of 11 subjects, a total of 2,435 data were recorded. With an average value per day, there were 244 exposures from the induction follow-up group, where each individual subject on average got 22 exposures.

**Tabel 9: Monitoring Time Series exposure on Induksi-Follow-up group**

| Kod   | Kode Kode Kode Kode Kode | Skor | Skor | Skor | Skor | Skor | Skor |
|-------|---------------------------|------|------|------|------|------|------|
|       |                           | Day1 | Day2 | Day3 | Day4 | Day5 | Total |
| F-01  | Fl                         | 21   | 0    | 0    | 0    | 0    | 24   |
| F-02  | Fl                         | 24   | 0    | 0    | 0    | 0    | 24   |
| F-03  | Fl                         | 25   | 0    | 0    | 0    | 0    | 25   |
| F-04  | Fl                         | 25   | 0    | 0    | 0    | 0    | 25   |
| F-05  | Fl                         | 21   | 0    | 0    | 0    | 0    | 21   |
| F-06  | Fl                         | 23   | 0    | 0    | 0    | 0    | 23   |
| F-07  | Fl                         | 19   | 0    | 0    | 0    | 0    | 19   |
| F-08  | Fl                         | 19   | 0    | 0    | 0    | 0    | 19   |
| F-09  | Fl                         | 21   | 0    | 0    | 0    | 0    | 21   |
| F-10  | Fl                         | 23   | 0    | 0    | 0    | 0    | 23   |
| F-11  | Fl                         | 21   | 0    | 0    | 0    | 0    | 21   |
| **Total** |                         | 21   | 0    | 0    | 0    | 0    | 21   |

**Keterangan:**
- Fl = Follow-up-Induksi
- Σ pap. = jumlah paparan

**Table 2**

**Monitoring Time Series (Tabel 2)**

| Kod   | Kode Kode Kode Kode Kode | Skor | Skor | Skor | Skor | Skor | Skor |
|-------|---------------------------|------|------|------|------|------|------|
|       |                           | Day6 | Day7 | Day8 | Day9 | Day10| Day11|
| F-01  | Fl                         | 21   | 0    | 0    | 0    | 0    | 0    |
| F-02  | Fl                         | 24   | 0    | 0    | 0    | 0    | 0    |
| F-03  | Fl                         | 25   | 0    | 0    | 0    | 0    | 0    |
| F-04  | Fl                         | 25   | 0    | 0    | 0    | 0    | 0    |
| F-05  | Fl                         | 21   | 0    | 0    | 0    | 0    | 0    |
| F-06  | Fl                         | 23   | 0    | 0    | 0    | 0    | 0    |
| F-07  | Fl                         | 19   | 0    | 0    | 0    | 0    | 0    |
| F-08  | Fl                         | 19   | 0    | 0    | 0    | 0    | 0    |
| F-09  | Fl                         | 21   | 0    | 0    | 0    | 0    | 0    |
| F-10  | Fl                         | 23   | 0    | 0    | 0    | 0    | 0    |
| F-11  | Fl                         | 21   | 0    | 0    | 0    | 0    | 0    |
| **Total** |                         | 21   | 0    | 0    | 0    | 0    | 0    |

**Keterangan:**
- Fl = Follow-up-Induksi
- Σ pap. = jumlah paparan
Based on exposure data above, on the 5th day observation, subjects of code F-01 run an irritation risk. After confirmation by a dermatologist, the subject experienced minimal Erythema that was barely visible or in a score of 1, with a 30-minute phasing procedure, the risk could be self-limiting and the subject could continue until the end of observation. Based on demographic data, subjects code F-01 had an allergic history.

In the challenge phase, an open scheme was made, where the chosen subject only got one exposure. Observation of the irritation risk was done before hand hygiene with hand rub up to 30 - 60 minutes after use.

![Image](image1.png)

**Figure 3. Sampling on the use of induction-follow-up hand rub phase**

The next data was in the challenge phase. There were 3 subjects who had an allergic risk, but based on the results of observation, there was no irritation risk in general.

**Table 10. Monitoring Time Series exposure on challenge group**

| Subj ek | Kelomp ok | Profesi | Usia | Sex | Riwayat Alergi | Kelomp ok Pemberi Asuhan | Kejadian Irritasi/Alergi | Monitoring Time Series |
|---------|-----------|---------|------|-----|----------------|--------------------------|------------------------|------------------------|
| C-01 CT | PPA | 26-35 | L | Tidak | Pegawai | Tidak | 0 |
| C-02 CT | PPA | 26-36 | P | Tidak | Pegawai | Tidak | 0 |
| C-03 CT | PPA | 36-45 | P | Tidak | Pegawai | Tidak | 0 |
| C-04 CT | PPA | 36-45 | P | Tidak | Pegawai | Tidak | 0 |
| C-05 CT | PPA | 26-36 | L | Tidak | Umum | Tidak | 0 |
| C-06 CT | Lainnya | 36-45 | P | Tidak | Umum | Tidak | 0 |
| C-07 CT | Lainnya | 36-45 | P | Tidak | Umum | Tidak | 0 |
| C-08 CT | Lainnya | 56-55 | P | Tidak | Umum | Tidak | 0 |
| C-09 CT | Lainnya | 26-35 | L | Tidak | Umum | Tidak | 0 |
| C-10 CT | Lainnya | 17-25 | L | Tidak | Umum | Tidak | 0 |
| C-11 CT | Lainnya | 46-55 | P | Tidak | Umum | Tidak | 0 |
| C-12 CT | PPA | 36-45 | P | Tidak | Pegawai | Tidak | 0 |

Keterangan:
PPA = professional pemberi asuhan; CT = Challenge Tantangan

Based on results of statistical analysis (T-test) and based on correlation between the "exposure amount" based on time series data vs. irritation/allergic event data, it is known as follows:
Based on the data above, Pearson correlation value was 1, so there is a strong relationship between each variable. Based on the value of sig. between variables, it is known that alpha value is equal to 0.00 means that H0 is accepted. So in general, the MK.IV hand rub in this study does not have risk of causing irritation/hypersensitivity.

**CONCLUSION**

Hand rub products show good results:
1. In formulation, MK.IV shows the best value compared to other formulation.
2. Based on the percentage kill test results obtained, value for each contact time was more than or equal to 90%.
3. In general, the formulation used did not pose a significant risk of irritation to the user.

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**REFERENCES**

1. WHO (2020), Coronavirus disease 2019 (COVID-19) Situation Report – 163., https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
2. Kemenkes RI. (2020), Corona Virus Update., https://covid19.kemkes.go.id/
3. Lakhundi, S., & Zhang, K. (2018). Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clinical Microbiology Reviews, 31(4), 1–103.
4. WHO. (2009)., *WHO guidelines in hand hygiene in health care*. WHO/IER/PSP/2009.07, World Health Organisation, Geneva, Switzerland.
5. Instalasi Farmasi, (2018), Laporan Tahunan Pengelolaan Perbekalan Farmasi RSUP Fatmawati, Instalasi Farmasi RSF.,Jakarta.
6. Larson, E. L., Morton H. E., (1991). *Alcohols*. In S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa. p. 422-434
7. Fanning S., (2011), basic medical key – disinfectants and antiseptics modes of action mechanisms of resistance and testing regimens; www.basicmedicalkey.com
8. BPOM RI., (2008), Farmakope Indonesia (FI) edisi V., Badan Pengawas Obat dan Makanan (BPOM) Republik Indonesia, Jakarta.
9. WHO., (2010). WHO guide to local production; WHO-recommended *hand rub* formulations. WHO revise April 2010.
10. Oladosu, P., Isu, N.R., Ibrahim, K., Okolo, P., Oladepo, D.K., (2013). Time kill-kinetics antibacterial study of Acacia nilotica. African Journal of Microbiology Research, 7(46): 5248-5252.

11. ASTM., (2008). Antimicrobial susceptibility testing method. Time – kill Test Protocol 786-071808E. 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959. ASTM E 2315 – 03, p 3.

12. FDA., (2018). Assessing the Irritation and Sensitization Potential of Transdermal and Topical Delivery Systems for ANDAs Guidance for Industry, 10001 New Hampshire Ave., Hillandale Bldg., 4th Floor Silver Spring, MD 20953-0002 301-796-3400; Fax: 301-431-6353 http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

13. Christoph, Ralf; Schmidt, Bernd; Steinberner, Udo; Dilla, Wolfgang; Karinen, Reetta., (2006). "Glycerol". Ullmann's Encyclopedia of Industrial Chemistry. Ullmann's Encyclopedia of Industrial Chemistry. doi:10.1002/14356007.a12_477.pub2. ISBN 3527306730.

14. Katarzyna Leja, Katarzyna Czaczyk, Kamila Myszka, (2011). The use of microorganisms in 1,3-Propanediol production, African Journal of Microbiology Research Vol. 5(26), pp. 4652-4658, 16 November, 2011 Available online at http://www.academicjournals.org/AJMR ISSN 1996-0808 ©2011 Academic Journals DOI: 10.5897/AJMR11.847

15. Gould D, Chamberlaine A. (1995). Staphylococcus aureus: a review of the literature. J Clin Nurs 4:5–12. https://doi.org/10.1111/j.1365-2702.1995.tb00004.x.

16. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. (2005). The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 5:751–762. https://doi.org/10.1016/S14733099(05)70295-4.

17. Williams RE, Jevons MP, Shooter RA, Hunter CJ, Girling JA, Griffiths JD, Taylor GW. (1959). Nasal staphylococci and sepsis in hospital patients. Br Med J 2:658–662. https://doi.org/10.1136/bmj.2.5153.658.

18. Fortuin-de Smidt MC, Singh-Moodley A, Badat R, Quan V, Kularatne R, Nana T, Lekalakala R, Govender NP, Perovic O, for GERMS-SA. (2015). Staphylococcus aureus bacteraemia in Gauteng academic hospitals, South Africa. Int J Infect Dis 30:41– 48. https://doi.org/10.1016/j.ijid.2014.10.011.

19. Katayama Y, Ito T, Hiramatsu K. (2000). A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob Agents Chemother 44: 1549–1555. https://doi.org/10.1128/AAC.44.6.1549-1555.2000.

20. Haynes, William M., ed., 2011. CRC Handbook of Chemistry and Physics (92nd ed.). Boca Raton, FL: CRC Press, p. 3.246. ISBN 1439855310.
Appendix:

1. The source of MRSA: isolation from wound swab
2. Ethical clearance

SURAT PERSETUJUAN ETIK
(Ethical Approval)
Nomor: 122/KPP/XII/2018

Dengan ini Kajetik Komite Penelitian dan Pengembangan Rumah Sakit Umum Pusat Fatmawati dalam upaya melindungi hak asasi manusia dan kesejahteraan subjek penelitian kesehatan/penelitian medik, setelah melakukan pembahasan, perolehan data pengukur secara teliti terhadap proposal penelitian yang berjudul:

“STUDI EVALUASI MUTU FORMULASI HAND RUB (HAND SANITIZER) STANDAR WHO DAN KEMAMPUAN PERSEN DAYA BUNJUH BAKTERIA (PERCENTAGE KILL BACTERIA) PADA METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)”

Peneliti Utama: Ahmad Subhan
Nama Instansi: Program Studi Ilmu Farmasi dan Kebidanan (IF0) – IPB Bogor

Memutuskan bahwa penelitian disetujui untuk diakses. Persetujuan ini bertujuan sejauh mungkin ditunjukkan sampai batas yaktu penelitian yang telah dalam proposal. Sehubungan dengan penelitian ini, peneliti bertanggung jawab untuk:
1. Menjaga kerahasiaan subjek penelitian.
2. Memberi tahu status penelitian apabila:
   a. Selama sampai batas waktu berakhir penelitian masih belum selesai dalam hal ini kerahasiaan etik harus diperpanjang.
   b. Penelitian berhenti ditindaklanjuti/telah mengundurkan diri
3. Melaporkan kejadian serius yang tidak diinginkan (serious adverse event)
4. Peneliti tidak boleh melakukan tindakan apapun pada subjek sebelum penelitian lolos kajetik dan informed consent.
5. Melaporkan hasil penelitian jika pelaksanaan penelitian telah selesai kepada tim Kajetik Komite Penelitian dan Pengembangan RSUP Fatmawati

Demikian atas perhatian dan kerjasama yang baik kami ucapkan terima kasih.

Jakarta, 11 Desember 2018
Kajetik Komite Penelitian dan Pengembangan RSUP Fatmawati

[Signature]

Dir. Bobby Setiadjibrata, Sp.A
NIP: 19801172008011013

Tembusan Yth:
1. Direktur Utama RSUP Fatmawati (sebagai laporan);
2. Ka. Bagian Diklit RSUP Fatmawati
3. Peneliti yang bersangkutan.
3. Percentage kill bacteria result
