UNIVERSITI PUTRA MALAYSIA

THE EFFECTIVENES OF ALCOHOL-BASED HAND SANITIZER IN REDUCING BACTERIA ON HANDS: A SYSTEMATIC REVIEW

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ABSTRACT

The Effectiveness of Alcohol-based Hand Sanitizer in Reducing Bacteria on Hands: A Systematic Review

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Introduction: Hand can harbour bacteria which can be potentially removed by routine hand hygiene. A proper and good compliance of hand hygiene can reduce the rate of bacterial infection. Hand hygiene include traditional handwashing using plain or antibacterial soaps and the use of alcohol-based hand sanitizer (ABHS). The increase usage of ABHS has been seen nowadays, thus scientific and clinical evidence are needed in determining the efficacy. **Objective:** This study generally aims to systematically gather the available evidence in determining the effectiveness of ABHS in reducing bacteria on hands when compared to other hand hygiene methods and to determine the factors involved in influencing effectiveness of ABHS in reducing bacteria on hands. **Methodology:** A systematic literature search in PubMed, ScienceDirect, and Scopus was conducted in June 2021 to retrieve the studies using the predetermined keywords. The title and abstract screening followed by full text screening were done based on eligibility criteria. Then, data extraction of the included studies was done and followed by the quality assessment. **Results:** A total of 41 *in vivo* and clinical studies were included in the systematic review for a descriptive synthesis. The results obtained were 32 studies reported on the effectiveness of ABHS in reducing bacteria when compared to other hand hygiene methods and 9 studies reported on the factors influencing the efficacy of ABHS in reducing bacteria on hands. About 60% (19 out of 32 studies) of the studies reported significant reduction in bacteria when the ABHS is used as compared to traditional handwashing (plain or antibacterial soaps). However, ABHS is reported to be not effective in reducing bacterial spores and when hands are contaminated with soil. The factors affecting the antibacterial efficacy of ABHS includes formats of ABHS, volumes of application, use of hand lotion before using hand sanitizer, and formulation of ABHS. **Discussion:** Poor hand hygiene compliance has been observed due to inaccessible to water or lack of time in handwashing. Alternative method needs to be used to improve hand hygiene compliance in preventing disease transmission. The substantial results obtained in this systematic review suggested ABHS can reduce bacteria present on hands, thus it can be used in replacing the traditional handwashing using soap and water to improve hand hygiene compliance and to prevent bacterial transmission. Nevertheless, when there is soil load on hands, hand hygiene using ABHS alone is not very effective in reducing bacteria and physical or mechanical removal needs to be combined for better efficacy. **Conclusion:** The results from this systematic review suggested that ABHS is effective in reducing bacteria on the hands of humans as a hygienic hand disinfection.

Keywords: Hand hygiene, alcohol-based hand sanitizer, antibacterial, systematic review

ABSTRAK

Keberkesanan Pensanitasi Tangan Berasaskan Alkohol dalam Mengurangkan Bakteria pada Tangan: Kajian Sistematik

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Pengenalan: Tangan dapat menyimpan bakteria yang berpotensi untuk dihapuskan melalui kebersihan tangan secara rutin. Pematuhan kebersihan tangan yang betul dan baik dapat mengurangkan kadar jangkitan bakteria. Kebersihan tangan merangkumi pencucian tangan tradisional menggunakan sabun biasa atau antibakteria dan penggunaan pensanitasi tangan berasaskan alkohol (PTBA). Peningkatan penggunaan PTBA telah dilihat pada masa kini, oleh itu bukti saintifik dan klinikal diperlukan dalam menentukan keberkesanannya. **Objektif:** Kajian ini secara amnya bertujuan untuk mengumpulkan bukti yang ada secara sistematik dalam menentukan keberkesanan PTBA dalam mengurangkan bakteria pada tangan jika dibandingkan dengan kaedah kebersihan tangan yang lain dan untuk menentukan faktor-faktor yang terlibat dalam mempengaruhi keberkesanan PTBA dalam mengurangkan bakteria pada tangan. **Metodologi:** Pencarian kajian secara sistematik di PubMed, ScienceDirect, dan Scopus dilakukan pada 22 Jun 2021 untuk mendapatkan kajian menggunakan kata kunci yang telah ditentukan. Penyaringan berdasrkan tajuk dan abstrak diikuti dengan penyaringan teks lengkap dilakukan berdasarkan kriteria kelayakan. Kemudian, pengekstrakan data dari kajian yang terangkum telah dilakukan dan diikuti dengan penilaian kualiti. **Keputusan:** Sebanyak 41 kajian *in vivo* dan klinikal terlibat dalam kajian sistematik untuk sintesis deskriptif. Hasil yang diperoleh adalah 32 kajian yang dilaporkan mengenai keberkesanan ABHS dalam mengurangkan bakteria jika dibandingkan dengan kaedah kebersihan tangan yang lain dan 9 kajian melaporkan faktor yang mempengaruhi keberkesanan PTBA dalam mengurangkan bakteria pada tangan. Kira-kira 60% (19 dari 32 kajian) kajian melaporkan penurunan bakteria yang ketara ketika ABHS digunakan dibandingkan dengan pencucian tangan tradisional (sabun biasa atau antibakteria). Walau bagaimanapun, ABHS dilaporkan tidak berkesan dalam mengurangkan spora bakteria dan ketika tangan tercemar dengan tanah. Faktor-faktor yang mempengaruhi keberkesanan antibakteria PTBA termasuk format PTBA, isipadu aplikasi, penggunaan losyen tangan sebelum menggunakan pembersih tangan, dan formulasi PTBA. **Perbincangan:** Pematuhan kebersihan tangan yang lemah telah diperhatikan kerana air tidak dapat diakses atau kekurangan masa dalam mencuci tangan. Kaedah alternatif perlu digunakan untuk meningkatkan kepatuhan kebersihan tangan dalam mencegah penularan penyakit. Kebanyakan hasil yang diperoleh dalam kajian sistematik ini menunjukkan bahawa PTBA dapat mengurangkan bakteria yang ada pada tangan, malah dapat digunakan untuk menggantikan pencucian tangan tradisional menggunakan sabun dan air untuk meningkatkan kepatuhan kebersihan tangan dan untuk mencegah penularan bakteria. Walau bagaimanapun, apabila terdapat kekotoran tanah di tangan, kebersihan tangan menggunakan PTBA saja tidak begitu berkesan dalam mengurangkan bakteria dan penyingkiran fizikal atau mekanikal perlu

digabungkan untuk keberkesanan yang lebih baik. **Kesimpulan:** Hasil kajian sistematik ini menunjukkan bahawa PTBA berkesan dalam mengurangkan bakteria pada tangan manusia sebagai pembasmian tangan yang bersih.

Kata kunci: Kebersihan tangan, pensanitasi tangan berasaskan alkohol, antibakteria, kajian sistematik

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LIST OF ABBREVIATIONS

%	Percentage
ABHS	Alcohol-based hand sanitizer
ATCC	American Type Cell Culture
BZK	Benzalkonium chloride
CFU	Colony forming unit
CHG	Chlorhexidine digluconate
DNA	Deoxyribonucleic acid
IPA	Isopropyl alcohol
$\log_{10}$	Logarithm base 10
mL	Millilitre
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
N/A	Not available
PVP-I	Povidone-iodine
PCMX	Parachlorometaxylenol
RCT	Randomized controlled trial
RF	Reduction factor
RNA	Ribonucleic acid
v/v	Volume/volume
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Hand can harbor bacteria and there are two types of bacteria that present which are resident floras and transient floras. The resident floras are the microorganisms that colonize deeper skin layers or below the superficial cells which includes *Enterococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, while the transient floras are the bacteria that colonize superficial or upper layers of skin and it can be potentially removed by routine hand hygiene in which some of the examples are *Escherichia coli*, *S. aureus*, and *Pseudomonas aeruginosa* (Jain et al., 2016). Hand hygiene includes the use of traditional methods such as hand washing using soap and water, hand washing using antiseptic soap, and also waterless techniques such as antiseptic hand rubs or by using hand sanitizers (Singh et al., 2020).

As mentioned before, hand sanitizer is a part of hand hygiene in which it is usually applied when handwashing with soap and water cannot be done. Besides, hand sanitizer is also a part of hand hygiene in which a good hand hygiene is very important as it can be one of the strategies to control the disease transmission especially for the diseases that can be transmitted through indirect contact from surfaces or through air or airborne microorganisms (Jing et al., 2020). There are a variety of hand sanitizers with different modes of delivery and combination of ingredients. Generally, hand sanitizers can be divided into two categories which are alcohol-based hand sanitizers (ABHS) and non-alcohol-based hand sanitizers (NABHS). ABHS are common hand sanitizers used in healthcare and community settings as an alternative to traditional handwashing and the lower cost and better efficacy in reducing transmission of infectious diseases making ABHS commonly used in health care settings compared to NABHS (Golin, Choi & Ghahary, 2020). Besides, the World Health Organization (WHO) has recommended ABHS as it shows benefits that have been reported such as the rapid action and the wide spectrum of microbicidal activity that can defend against viruses and bacteria (Jing et al., 2020). Besides, there is also a guideline published by the WHO that recommends alcohol-based hand sanitizer formulation when there is no access to commercially available hand sanitizers. Thus, as ABHS has been reported to show a good antibacterial activity, there is a need in gathering the *in vivo* and clinical evidence systematically in reporting the effectiveness of using ABHS when applied on hands.

1.2 Problem statements

A proper hand hygiene and a good compliance of it can reduce the rate of nosocomial infections by up to 40% (Kampf, Löffler & Gastmeier, 2009). Hand sanitizer especially the alcohol-based is a part of hand hygiene in which it can remove the pathogenic bacteria on hands. Hand washing is one of the hand hygiene that can be done to remove the pathogens present on hand. However, frequent hand washing can result in skin irritation. Hence, hand sanitizer especially ABHS can be used as an alternative to hand washing. As such, the antibacterial efficacy of ABHS need to be determined as ABHS can be used as a strategy to control bacterial infection.

1.3 Objectives

1.3.1 General Objective

This study generally aims to investigate effectiveness of ABHS in reducing bacterial contamination on hands.

1.3.2 Specific Objectives

The general objective is accomplished through the following specific objectives:

1. To systematically gather the available evidence in determining the effectiveness of ABHS in reducing bacteria on hands.
2. To determine the effectiveness of ABHS in comparing to other hand hygiene methods.
3. To determine the factors involved in influencing effectiveness of ABHS in reducing bacteria on hands.

1.4 Research Hypothesis

It is hypothesized that the ABHS is effective in reducing bacteria on hands by gathering the available evidence through systematic review.

CHAPTER 2

LITERATURE REVIEW

2.1 Hand Sanitizer

Hand hygiene is an important practice to maintain in preventing and reducing the occurrence of infectious diseases in community and hospital settings. However, a stricter hand hygiene is required in clinical settings especially among health-care workers (HCW) as hands that are not clean can lead to the transmission of microorganisms from patient to patient causes higher disease burden, death rate, and costs linked to healthcare-associated infections (HCAI) (Allegranzi & Pittet, 2009). One of the hand hygiene products is hand sanitizers in which hand hygiene is the act of cleansing hands. Hand sanitizers can also be known as hand antiseptics and there are two major categories of hand sanitizer which are alcohol-based hand sanitizer (ABHS) or alcohol hand rubs and non-alcohol-based hand sanitizer (NABHS). According to WHO, ABHS or also known as alcohol-based hand rubs can be defined as a preparation that includes alcohol to be applied on the hands for microorganisms inactivation and/or suppressing their growth temporarily. One or more types of alcohol can be included in the preparations together with other ingredients such as excipients and humectants. While, NABHS does not contain alcohol as its name and benzalkonium chloride is the common disinfectant replacing the alcohol. Moreover, there are various delivery systems of hand sanitizers in which it can be either in the form of gels, foam, cream, spray and wipes as shown in (Jing et al., 2020).

2.1.1 Categories of Hand Sanitizer

As mentioned earlier, there are two main categories of hand sanitizers which can either contain alcohol (alcohol-based) or does not contain alcohol (alcohol-free). In alcohol-based hand sanitizer, the common alcohols used are isopropanol (isopropyl alcohol), ethanol, n-propanol, or a combination of the three alcohols. According to the Centre for Disease Control and Prevention (CDC), the recommended concentration for ethanol in the formulations is 80% (v/v) and for isopropyl alcohol is 75% (v/v). Hence, concentration of 60 to 95% of alcohol is an acceptable range in sanitizers generally. The most common alcohol ingredient is ethanol was found to have better efficacy compared to other alcohols in inactivating viruses, and propanol is the alcohol ingredient that has a better efficacy in inactivating bacteria (Gold, Mirza & Avva, 2020). A better efficacy of sanitizer can be observed by having the synergistic effect of combined alcohols. Although alcohol-based sanitizers are widely used in various settings, there are several concerns regarding this sanitizer such as accidental ingestion of sanitizers in children and the risk of fire as alcohol is a flammable ingredient. Besides, O'Leary and Price (2011) has reported a case of skin burning due to the uses of alcohol-based sanitizers. Hence, application of alcohol-based sanitizers should not be done near to the fire applications.

As for the alcohol-free hand sanitizers, it does not contain alcohol and other disinfectant ingredients will replace the alcohol. The commonly used disinfectant is benzalkonium chloride which is one of the quaternary ammonium compounds. Unlike alcohol, benzalkonium chloride is not flammable and when the sanitizer dries, it is expected to last on the skin. A study conducted by Bondurant, Duley and Harbell (2019) reported that a hand sanitizer containing benzalkonium chloride showed a persistent antibacterial activity that explains the benzalkonium chloride can remain on the skin after application. Besides, the benzalkonium chloride works by targeting the cell membranes of the target organism and disrupting the cell membranes. Hence, alcohol-free sanitizers are safer to be used compared to alcohol-based sanitizers especially among children as there will be no alcohol ingestion to be occurred. However, alcohol-based hand sanitizers are more preferred especially in health care settings due to the low cost and the better effectiveness in reducing the transmission of infectious diseases (La Fleur & Jones, 2017).

2.1.2 Ingredients of Hand Sanitizer

Aside from alcohol and benzalkonium chloride (BZK) as the active ingredients, there are also other ingredients or excipients involved in the formulation of hand sanitizer such as emollients, viscosity enhancers, fragrances, buffers, colourants and preservatives. The choices of the ingredients involved depends on the types of formulation. For example, disinfectant wipes or hand sanitizer that uses wipes formulation contain moisturizers, surfactants, emulsifiers, wetting agents, detergents, and emollients (Todd et al., 2010). As alcohol can cause skin drying, emollients are added to the formulation for better effect on skin moisture as it has been reported that emollients and other skin conditioners can reduce the skin drying caused by alcohol (Ahmed-Lecheheb et al., 2012). Besides, humectants are included ingredients in the hand sanitizer formulation also serve the purpose as skin moisture. One of the commonly used humectants is glycerine and it has been reported to help in hydrating the skin when the glycerine is added in the hand sanitizers. Although the glycerine can give hydration to the skin, Ahmed-Lecheheb et al. (2012) reported that ABHS that contain glycerine were found to be reducing the surface pH and the content of superficial sebum present on the skin, but the effect does not disrupt the function of skin barrier. The next commonly used humectant after glycerine is propylene glycol. Other than propylene glycol and glycerine, *Aloe vera* gel can also act as a humectant as it can provide water retention in the formulation, but glycerine and propylene glycol are better than *Aloe vera* gel as humectants (Berardi et al., 2020).

Other than emollients, viscosity enhancers can also be included in the hand sanitizer formulations specifically in the gel formulations. Viscosity enhancers act as thickening agents to enhance the viscosity of the liquid to ease the users to apply the products. The examples of viscosity enhancers that can be used in gel formulations of sanitizers are carbomer, hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose (HEC) and sodium carboxymethyl cellulose (CMC). Compared to liquid formulations that do not contain viscosity enhancers that will lead the liquid to become runny, gel formulations are more preferable as it is easier to use and has lower chances to spill. This can be further proved as there is a sensory descriptive analysis conducted by Greenaway et al. (2018) resulted that hand sanitizers in gel and foam are more preferable than the liquid due to the better handleability. Although the liquid form of sanitizers is less preferable, the formulations of hand sanitizers that have been recommended by the World Health Organization (WHO) are in the liquid form. Besides, there is no fragrances and colourants to be added in the formulation as it is highly not recommended to add those ingredients to prevent from the allergic reactions to possibly occur to the users, although most of the commercially available hand sanitizers contain fragrances and colourants for a better presentation of the products.

2.1.3 *In Vitro* Study of Alcohol-based Hand Sanitizer (ABHS)

There is a guideline published by the WHO that recommends the use of alcohol-based hand sanitizer for both hygienic and pre-operative hand treatment. The guideline was published in 2009 entitled "Guidelines on Hand Hygiene in Health Care" and there are alcohol-based hand sanitizer formulations recommended in the guideline in which the hand sanitizers are alcohol-based, and it has two different formulations that has different alcohols either ethanol or isopropanol. The ethanol concentration is in 80% (v/v) and the concentration for isopropanol is 75% (v/v). Aside from the alcohols, there are other two ingredients involved in the formulation which are hydrogen peroxide in 0.125% (v/v) to inhibit the potentially contaminating bacterial spores and glycerine in 1.45% (v/v) that act as an emollient in retaining the skin moisture (World Health Organization, 2009).

Aside from the hand sanitizers that are using WHO formulations, there are also other commercially available hand sanitizers that have different formulations and there have been many studies assessing the *in vitro* antibacterial activity of it. Table 2.1 shows several *in vitro* studies that assess the antibacterial activity of commercially available alcohol-based hand sanitizer. As shown in the table, study conducted by Oke at al. (2013) reported that Hygel can inhibit all the test organisms involved in which Hygel is the hand sanitizer that contains alcohol as the main active ingredient which is 62% ethanol. Same goes to the study conducted by Jain et al. (2016) where Sterillium that contains alcohol which is 75% propanol showed the highest inhibition activity against the test organisms. Those two studies used agar well diffusion method to determine the antibacterial activity of the hand sanitizer together with another study

conducted by Otokunefor and Princewill (2017) that was using the same method in assessing the antibacterial activity *in vitro*. Besides, there is a study conducted by Thaddeues et al. (2017) that investigated the effect of the common additives in the formulation of alcohol-based hand sanitizer on the antimicrobial activity. They concluded that the alcohol concentrations used in the hand sanitizers should be in the range of 85% to 95% and the addition of benzalkonium increases the antimicrobial activity. Moreover, Berardi et al. (2020) reported that the hand gels that they tested the antibacterial activity has an ethanolic content ranging of 60 to 95% (v/v) showed a good antibacterial activity especially the products that have the highest ethanolic contents. In short, there are a lot of *in vitro* studies that assessing the antibacterial efficacy of ABHS, but the evidence based on *in vitro* study is not sufficient in determining the bacterial efficacy and *in vivo* or clinical studies are needed to validate the antibacterial efficacy of ABHS.

Table 2.1. *In vitro* studies assessing the hand sanitizers products.

Reference	Objective of study	Methodology	Results
Oke et al. (2013)	To evaluate the antibacterial efficacy of some popular hand sanitizers (Hygel, Dettol, SKP, Samclean) sold in Ilorin.	Well-variant of the agar diffusion test against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and *in vivo* tests of efficacy in reducing bacterial counts.	Hygel was able to inhibit all the test organisms and Dettol can only inhibit *P. aeruginosa*. SKP and Samclean were found to be ineffective against the test organisms. At 100% concentration of MIC, Hygel and Dettol were found bacteriostatic, and none was bactericidal. Mean percentage of cfu reduction in *in vivo* study showed 89.9% for Hygel and 73.8% for Dettol and no significant difference observed in the efficacy of the two products.
Jain et al. (2016)	To evaluate the antimicrobial efficacy of four different hand sanitizers (Dettol, Lifebuoy, PureHands, and Sterillium).	Well variant of agar disk diffusion test.	The maximum zones of inhibition were found for Sterillium against all test organisms (*Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*).
Otokunefor & Princewill (2017)	To evaluate the antibacterial activity of hand sanitizers (Ebecare, Carex, and	Dilution and diffusion susceptibility tests methods.	The products shown a higher inhibitory activity against *Klebsiella pneumoniae* and

	Bactigel) in Nigerian market.		*Staphylococcus aureus* compared to *Escherichia coli* and *Pseudomonas aeruginosa*. Ebecare shown the least inhibitory activity.
Thaddeus et al., (2018)	To observe the antimicrobial activities affected by some common additives in alcohol-based hand sanitizers.	Agar well diffusion method to study *in vitro* antibacterial and antifungal activities.	The isopropyl alcohol ranging of 60%-100% showed a higher inhibition of bacterial and fungal organisms compared to ethanol. The effect of adding glycerine showed a reduces antimicrobial activities of the isopropyl alcohol. While, adding of benzalkonium to isopropyl alcohol showed a better activity of the alcohol.
Berardi et al. (2020)	To analyse the contents of ethanol in the hand sanitisers gels from the Italian market.	Gas chromatography and antimicrobial test using vitality reduction activity.	Three products (Gel 3, Gel 4 and Gel 6) contain less than 60% (v/v) of ethanolic contents and four products (Gel 1, Gel 2, Gel 5, Gel 7) have the contents of ethanol within the recommended range. The products that contain highest ethanolic content generally have a better antibacterial activity observed from the vitality reduction of the organisms.

2.2 Efficacy of Alcohol-based Hand Sanitizers against Microorganisms

The factors that related to the hand sanitizer effectiveness include the proper usage, length of time when the sanitizers are exposed to hands, and the frequency of the usage (Singh et al., 2020). Besides, the infectious agent present on the hand of the host needs to be susceptible to the active or main ingredient in the product to result in effective hand sanitizers. Hence, as mentioned earlier, ABHS are having better efficacy compared to NABHS and this can be further explained as WHO reported that alcohols in the sanitizers are effective against enveloped viruses, non-enveloped viruses, gram-positive and gram-negative bacteria including mycobacteria, and also fungi. Nonetheless, it was found that it is not very effective against some non-enveloped viruses, protozoa and bacterial spores (Gold, Mirza & Avva, 2020). The mechanism of action of alcohol as an antimicrobial agent is believed through the disruption of the lipid membranes and protein denaturation of the microbes (Jing et al., 2020). This will then lead to the protective coatings of the microbes to disappear and become non-functional. However, it is only effective against vegetative forms of bacteria which are bacteria that are still dividing, and not effective against bacterial spores which are in dormant state and commonly found in raw materials. Despite that, according to the WHO, the addition of the hydrogen peroxide might solve this issue.

In comparison to alcohol mechanisms against bacteria, the alcohol mechanism against viruses is not clearly elucidated. Viruses comprise at least two structural components that made them to be the non-complex structural infectious agents. They contain genetic material that can be either DNA or RNA and capsid which is a protein coat that protects and surrounds the genetic materials. Besides, there is also a structure called lipid envelope that categorizes the viruses to either become enveloped or non-enveloped viruses. The antiviral activity of alcohol-based hand sanitizer is believed through disruption of the function or structure of the structural components as the components are crucial in the life cycle of the virus (Golin, Choi & Ghahary, 2020). Despite the exact mechanism is not well defined, it is reported that ethanols have a better and wide spectrum of antiviral activity compared to propanols and a higher concentration of ethanol is reported to be effective against enveloped virus (Kampf & Kramer, 2004).

2.3 Bacteria

Bacteria are living organisms that are single-celled and contain genetic material that is freely floating. The structures of the bacteria typically comprise the inner cell membrane and peptidoglycan which is a part of the outer membrane. There are two categories of bacteria that are differentiated through the composition of the cell wall and the Gram staining procedure which are known as gram-positive and gram-negative bacteria. Gram-positive bacteria have a cell wall that consists of thicker peptidoglycan, while gram-negative bacteria have thinner peptidoglycan and outer membrane consisting of lipopolysaccharide and protein (Gottenbos et al., 2001). Besides, the Gram staining procedure can differentiate gram-positive and gram-

negative bacteria in which the procedure will involve the reaction of the dyes and chemicals to the cell walls or the outer membranes of the bacteria (Thairu et al., 2014). The gram-positive bacteria will result in purple or blue colour staining of the bacteria when viewed under microscope and gram-negative will result in red or pink colour staining (Golin, Choi & Ghahary, 2020). The examples of gram-positive bacteria are *Staphylococcus* species such as *Staphylococcus aureus* and *Staphylococcus epidermidis* and other examples include *Streptococcus* species, *Corynebacterium* species, and *Listeria* species (Sizar & Unakal, 2021; van Haren et al., 2007). While, the gram-negative organisms are *Moraxella* species, *Pseudomonas* species, *Klebsiella* species, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Escherichia coli* (Reubsaet & Ekkelenkamp, 2017; Srinivasan & Evans, 2018).

Moreover, bacteria that present on hands can be generally categorized based on the colonization area of the skin layers which can be called as resident floras and transient floras. The resident floras colonize the deeper layers of skin and are hardly removed by mechanical action in which the examples include *Enterococcus faecalis*, *S. aureus*, and *S. epidermidis* (Jain et al., 2016). The transient floras are the bacteria that colonize superficial or upper layers of skin and it can be potentially removed by routine hand hygiene in which the examples are *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa* (Jain et al., 2016).

The dominant bacterial colonizers of the human skin and mucous membranes are from the genus of staphylococci whereby *S. epidermidis* is the species that is commonly isolated from human epithelia (Otto, 2009). *S. epidermidis* is a gram-positive bacterium that belongs to coagulase-negative staphylococci group and this group is the staphylococci that are not able to produce coagulase contrasting to *S. aureus* that is able to produce coagulase (Otto, 2009; Vuong & Otto, 2002). Besides, *S. epidermidis* is a part of normal flora in human skin, but it can become opportunistic pathogen when there is a predisposed host (Vuong & Otto, 2002). Biofilm formation is one of the virulence factors of the bacteria and it was reported that alcohol such as n-propanol or ethanol can help in reducing the biofilm (Presterl et al., 2007). Besides, hydrogen peroxide in 3% and 5% are reported to be effective in eradicating the inhabitation of *S. epidermidis* from the surface by reducing the biofilms (Prester et al. 2007). Previous study conducted by Gaonkar et al. (2005) reported that hand rub containing ethanol was able to lower the counts of *S. epidermidis* both in *in vitro* tests and in volunteers. Thus, hand hygiene such as hand rub that contains alcohol is presumed to be effective in reducing the counts of *S. epidermidis* present on hand.

Aside from *S. epidermidis*, another important gram-positive organism is *Staphylococcus aureus*. As mentioned earlier, *S. aureus* is contrasting to *S. epidermidis* as it is a coagulase-positive organism and it is a commensal organism that is usually found in human skin, skin glands, and mucous membranes such as noses and guts. Moreover, *S. aureus* causes an abundance of hospital and community-acquired infections and one of the major clinical issues with this organism is their resistance toward multiple antibiotic classes that cause failed treatment (Lakhundi & Zhang, 2018). One of the antibiotic resistances of *S. aureus* strains is methicillin-resistant *S.*

aureus (MRSA) in which this strain has caused infections with high mortality rates and it is very common in communities and hospitals (Chambers, 2001). Hand hygiene can be a way to prevent the incidence of MRSA infections and it is reported that the use of alcohol hand rub can reduce the incidence of MRSA (Matsumoto et al., 2012; Pittet et al., 2000).

E. coli is a gram-negative bacterium and comes from the family of the Enterobacteriaceae. *E. coli* is commonly found in the gastrointestinal tract (GIT), but it can also be found outside GIT which is known as extraintestinal *E. coli*. Although most of the strains of *E. coli* are not harmful or non-pathogenic commensal, there are groups of pathogenic *E. coli* in which the common infections caused are urinary tract infections, meningitis, septicaemia, and diarrheal diseases (Desmarchelier & Fegan, 2014). Hands can be one of the ways for transmission of diarrheal diseases and hand hygiene is important to comply to avoid getting the disease. Besides, it is reported that bacterial counts of *E. coli* can be present on hands approximately from 0.4 to 4.5 $\log_{10}$ per two hands (Mattioli et al., 2014; Pickering, Davis, et al., 2010). Alcohol hand rub can be a part of hand hygiene and there is a study conducted by Nasution et al. (2019) reported that the *E. coli* count was not found after the application of alcohol hand rub on the hand of the participants. Moreover, a study conducted by (Pickering, Boehm, et al., 2010) reported that there are significant reductions of *E. coli* counts on hands after using alcohol hand rub.

2.4 Systematic Review

Literature review is different from systematic review as literature review is a collection and summarization of evidence using informal or subjective methods. In contrast, systematic review can be defined as a tool that applying a systematic approach in summarising, appraising, and communicating the results in which the results from the systematic review can be evaluated by the healthcare providers in identifying the gaps and improvement for a future research (Green, 2005). Another process called a meta-analysis is a quantitative synthesis or a statistical process that might or might not be included in a systematic review as the application of meta-analysis depends on the heterogeneity of the included studies (Ahn & Kang, 2018). As systematic review involves in gathering of the best available evidence, this can help in efficient access to evidence as there is continuous updates of the enormous number of publications day by day in health research has been observed in which over 13 million references and more than 4000 biomedical and health journals available in Medline alone (Gopalakrishnan & Ganeshkumar, 2013).

According to Bartolucci and Hillegass (2010) there are three major components of systematic review in which the first component is the formulation of the research questions and objective and followed by the second component which is the process involve the collection, abstraction, and compilation of the data from the gathered evidence. The third component is meta-analysis whereby it may or may not be included as mentioned earlier. Besides, quality assessment of the included studies needs to also be included in the methodology as the validity of the conclusions from the systematic review may be affected and there are many available tools for quality assessment that can be chosen by the reviewer (Verhagen et al., 2001).

CHAPTER 3

METHODOLOGY

3.1 Protocol

This systematic review is conducted by following guidelines from Muka et al. (2020) and the reporting is done in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2020 statement (Page et al., 2021).

3.2 Eligibility Criteria

3.2.1 Types of Studies

Any human experimental and clinical studies involving hands of humans were included. The human trial can be randomized controlled trials (RCTs), or non-randomized controlled trials. *In vitro* and *ex vivo* studies were excluded as there is no involvement of hands of humans. Observational and pilot studies were also excluded.

3.2.2 Types of Participants

The participants involved in the study should be any human volunteers and the volunteers can be healthy volunteers or health care workers in hospital or health care settings. There are no restrictions on age, gender, or race. The study using animal models in replacing humans are excluded in the review.

3.2.3 Types of Interventions

The interventions involved were the use of waterless hand hygiene by using ABHS as hand hygiene or hygienic hand disinfection. The ABHS used is done following the hand contamination with either artificial contamination or existing hand flora. The comparison groups involved were when the ABHS is compared with other hand hygiene methods such as hand washing using water and soaps that could be either antiseptic, non-antiseptic, or non-medicated soap. The use of waterless hand hygiene for surgical hand disinfection or preparation for surgery or operation is excluded. The use of ABHS as surgical scrub is also excluded.

3.2.4 Types of Outcomes Measures

The outcome measures were the reduction of the bacterial counts observed after the intervention was used. The outcome can be either in forms of log reduction, reduction factor, mean reduction, mean CFU, or mean log reduction.

3.3 Search Strategy and Data Source

The strategy in searching the available evidence was done by using the following search keywords: (efficacy OR effectiveness OR activity) AND alcohol AND (hand sanitizer OR hand disinfectant OR hand antiseptic OR hand gel OR hand liquid) AND (bacteria OR pathogen). There are three databases involved in applying the searching keywords which were Scopus, PubMed, and ScienceDirect. In ScienceDirect, a different search strategy was done as there are limits in the number

of Boolean operators that can be put in the search engine in which it limits up to eight Boolean operators. The search strategy done for ScienceDirect was by using the following keywords: (efficacy OR activity) AND alcohol AND ("hand sanitizer" OR "hand disinfectant" OR "hand antiseptic" OR "hand gel") AND (bacteria OR pathogen). There are no language and publication date restrictions being applied. The only filter used was to filter the document or article types in which the filter applied was to select only the article types. However, the filters only applied on Scopus and ScienceDirect as there is no filter on PubMed that selects only on article document type. The latest date of the search on the databases was done on 22nd June 2021.

3.4 Study Selection

The results obtained from the three databases (Scopus, PubMed, and ScienceDirect), were collected and merged in a single Microsoft Excel file. The details that were included in the file were title of the study, abstract, name of the authors, publication year, Digital Object Identifier (DOI) and link to the article. The duplicates were removed using Microsoft Excel if the identical author and title were detected. Screening of the title and abstract according to the eligibility criteria were done after the removal of the duplicates. Then, the studies that are eligible or potentially eligible after the title and abstract screening were forwarded to the full-text screening for a further determination of the eligibility. Studies that did not meet the eligibility criteria were removed and the reasons for studies that were removed after the full-text screening were recorded. There are two reviewers involved in assessing the eligibility of the studies which are Nur Amirah Fitri Rosli (N.A.F.R.) and Hasni Idayu Saidi (H.I.S.).

3.5 Data Extraction

The data from the studies that were eligible after full-text screening were extracted. For each of the included studies, the following characteristics were acquired: type of study design, settings involved in the study, the number of participants involved, the intervention used, test methods in determining the efficacy of the skin contamination, the types of bacterial contamination, the outcome measures, and the relative efficacy. The extraction of data from the included studies were done by the first reviewer (N.A.F.R.) and verified by a second reviewer (H.I.S.).

3.6 Quality Assessment

The quality of each study was assessed using the Standard quality assessment criteria for evaluating primary research papers from a variety of fields by Alberta Heritage Foundation for Medical Research (AHFMR) (Kmet, Lee & Cook, 2004). There are 14 criteria that need to be rated in the assessment tool. The highest scoring for each criterion was 2 when the study met the criterion, while 1 is for the partial fulfilment and 0 is given when there is no information needed based on the criterion in the tool. The total scores for each study were then converted into percentages.

3.7 Data Analysis

Descriptive analysis was done for the included studies. The effectiveness of ABHS in reducing bacteria on hands were evaluated from the included studies by comparing the effectiveness with other hand hygiene methods and the effectiveness was analysed descriptively. The factors influencing the effectiveness were also described based on the included studies.

CHAPTER 4

RESULTS

4.1 Literature Search

The search keywords used in searching the studies were (efficacy OR effectiveness OR activity) AND alcohol AND (hand sanitizer OR hand disinfectant OR hand antiseptic OR hand gel OR hand liquid) AND (bacteria OR pathogen). The search resulted in a total of 1694 from the three databases in which 391 results from PubMed, 887 results from Scopus, and 416 results from ScienceDirect. From the 1694 results, a total of 116 duplicates were removed whereby 106 duplicates were removed using Excel and 10 duplicates were removed by checking manually. After removal of duplicates, 1578 studies remained and were screened through the abstract and title according to the eligibility criteria. When the studies were screened by the title and abstract, only 113 studies remained, and 1465 studies were excluded as the studies did not meet the eligibility criteria as described in the methodology. The remaining 113 studies were subjected to full-text screening and 41 studies remained and 72 studies were excluded with reasons. The 41 studies were then included in qualitative analysis in the systematic review. The flow of the process was illustrated in Figure 4.1 below.

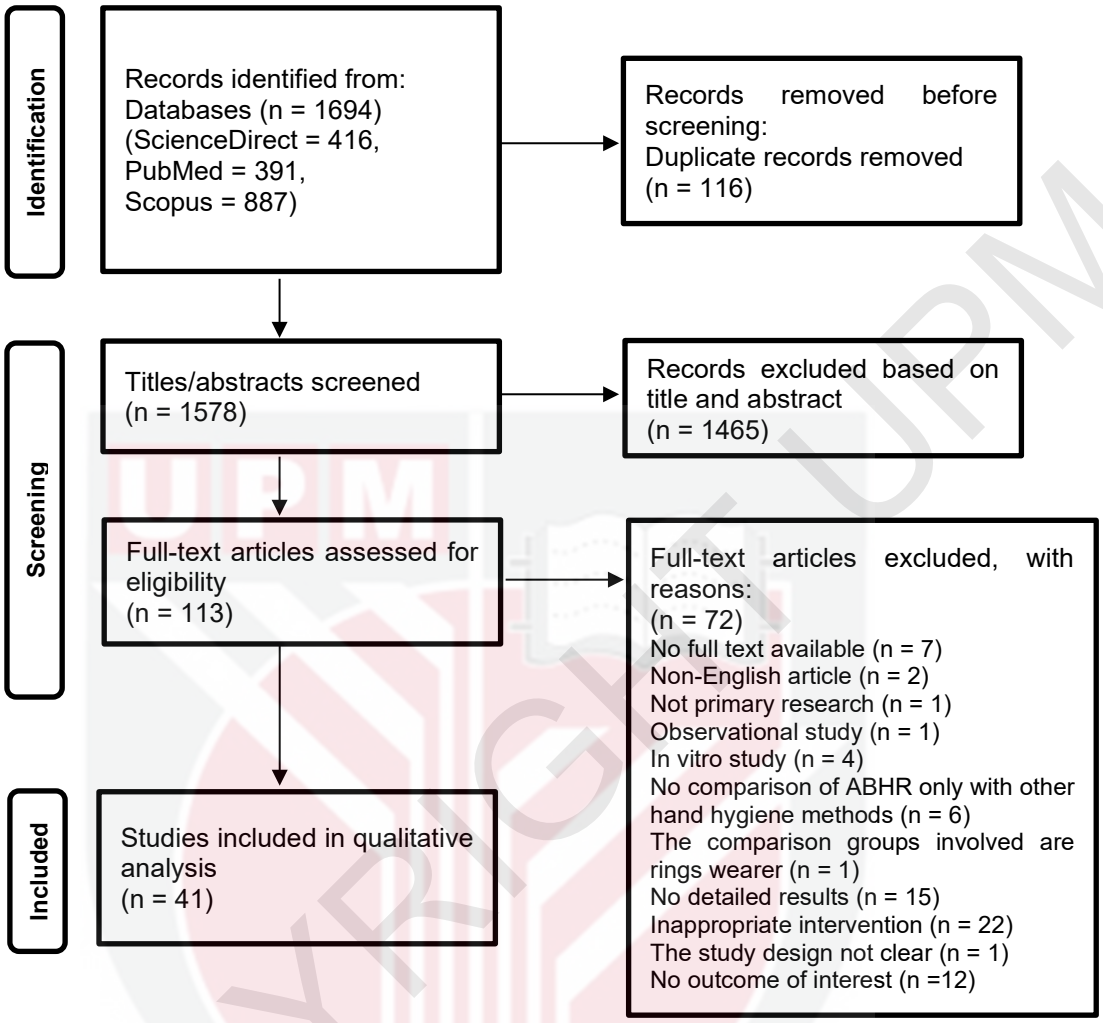

Figure 4.1. Flow chart of the literature search and study selection process according to the PRISMA guidelines.

4.2 Data Extraction

As shown in Table 1 in Appendix A, the characteristics for each included study were illustrated in a table form and the studies were sorted according to the publication date. There are a total of 41 included studies and the detailed characteristics from each of the studies include the number of participants involved, the intervention used, test methods in determining the efficacy of the skin contamination, the types of bacterial contamination, the outcome measures, and the relative efficacy mentioned in the results of the studies.

4.3 Quality Assessment

The quality of the included studies was assessed using Standard quality assessment criteria for evaluating primary research papers from a variety of fields by AHFMR (Kmet et al., 2004). The results for the quality assessment for the included studies were illustrated in a table form as shown in Table 4.1. There are 14 items included in the quality assessment and items 5, 6, and 7 are not available for the non-randomized study as the items are checking for the quality of the randomization method. Table 4.2 is showing the percentages for the results of the quality assessment for each included study. All the included studies (41 studies) are having a good quality of study as the percentages are more than 50%. One study showing the lowest percentage which is 72.73%.

Table 4.1. Results of the quality assessment for the included studies (n=41).

Citation	Item 1 (No N/A)	Item 2 (No N/A)	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Item 13	Item 14	Per 22	Per 28
Larson, Eke & Laughon, 1986	2	2	2	2	1	0	0	2	2	2	2	1	2	2		22
Butz, Laughon, Gullette & Larson, 1990	2	2	2	2	1	2	0	2	2	2	2	1	2	2		24
Larson & Bobo, 1992	2	2	2	2	1	0	0	2	2	2	2	0	2	2		21
Cardoso, Pereira, Zequim & Guilhermetti, 1999	2	2	2	2	2	0	0	2	1	2	2	1	2	2		22
Paulson, Fendler, Dolan & Williams, 1999	2	2	2	2	1	2	0	2	2	2	2	1	2	2		24

Guilhermetti, Hernandes, Fukushigue, Garcia & Cardoso, 2001	2	2	2	2	2	0	0	2	1	2	2	2	2	2		23
Moadab, Rupley & Wadhams, 2001	2	2	2	2	N/A	N/A	N/A	2	2	2	2	1	2	2	21	
Girou, Loyeau, Legrand, Oppein & Brun-Buisson, 2002	2	2	2	2	2	2	0	2	2	2	2	0	2	2		24
Lucet et al., 2002	2	2	2	2	2	1	0	2	2	2	2	0	2	2		23
Dharan, Hugonnet, Sax & Pittet, 2003	2	2	2	1	N/A	N/A	N/A	2	2	2	2	0	2	2	19	
Hernandes et al., 2004	2	2	2	2	2	0	0	2	2	2	2	2	2	2		24
Tvedt & Bukholm, 2005	2	2	1	1	N/A	N/A	N/A	2	2	2	2	0	2	2	18	

Kac et al., 2005	2	2	2	1	2	2	0	2	2	2	2	2	2	2		25
Barbut et al, 2007	2	2	2	1	N/A	N/A	N/A	2	2	2	2	0	2	2	19	
Kampf, 2008	2	2	2	1	1	2	0	2	2	2	2	1	2	2		23
Jabbar et al., 2010	2	2	1	1	N/A	N/A	N/A	2	2	2	2	0	2	2	18	
Abaza, Amine & Hazzah, 2010	2	2	1	1	N/A	N/A	N/A	2	2	2	2	0	2	2	18	
D'Antonio, Rihs, Stout & Yu, 2010	2	2	2	1	N/A	N/A	N/A	2	2	2	2	0	2	2	19	
Goroncy-Bermes, Koburger & Meyer, 2010	2	2	2	1	N/A	N/A	N/A	2	2	2	2	0	2	2	19	
Kawagoe, Graziano, Martino,	2	2	2	2	N/A	N/A	N/A	2	2	2	2	0	2	2	20	

Siqueira & Correa, 2011																
Sharma, Dutta, Taneja & Narang, 2013	2	2	2	2	2	2	0	2	2	2	1	0	2	2		23
Figueiredo, de Siqueira, Poli-de-Figueiredo & d'Avila, 2013	2	2	2	2	N/A	N/A	N/A	2	2	2	2	0	2	2	20	
Racicot, Kocher, Beauchamp, Letellier & Vaillancourt, 2013	2	2	2	1	N/A	N/A	N/A	2	1	2	2	0	2	2	17	
Macinga et al., 2014	2	2	2	2	N/A	N/A	N/A	2	2	1	1	0	1	2	17	
Salmon, Truong, Nguyen, Pittet & McLaws, 2014	2	2	1	1	N/A	N/A	N/A	2	2	2	2	0	2	2	18	
Sasahara, Hayashi,	2	2	2	2	N/A	N/A	N/A	2	2	2	2	0	2	2	20	

Hosoda, Morisawa & Hirai, 2014																
Schaffner et al., 2014	2	2	1	1	N/A	N/A	N/A	2	2	2	2	1	2	2	19	
de Aceituno et al., 2015	2	2	2	2	1	1	0	2	2	2	2	0	2	2		21
Herruzo, Yela & Vizcaino, 2015	2	2	1	1	N/A	N/A	N/A	2	2	2	1	0	2	2	17	
Appelgrein, Hosgood, Dunn & Schaaf, 2016	2	2	1	1	N/A	N/A	N/A	2	2	2	2	0	2	2	18	
de Aceituno et al., 2016	2	2	2	2	1	1	0	2	2	2	2	0	2	2		21
Wolfe et al., 2017	2	2	2	2	1	1	0	2	2	2	2	0	2	2		21
Paula et al., 2017	2	2	2	2	N/A	N/A	N/A	2	2	2	1	1	2	2	20	

Wilkinson, Ormandy, Bradley, Fraise & Hines, 2017	2	2	1	1	N/A	N/A	N/A	2	2	2	2	0	2	2	19	
Alsagher et al., 2018	2	2	2	2	N/A	N/A	N/A	2	2	0	0	0	2	2	16	
Wilkinson, Ormandy, Bradley & Hines, 2018	2	2	2	1	N/A	N/A	N/A	2	2	2	2	0	2	2	18	
Nasution, Yunita, Pasaribu & Ardinata, 2019	2	2	2	0	N/A	N/A	N/A	2	2	2	1	0	2	2	17	
Bondurant, Duley & Harbell, 2019	2	2	2	2	N/A	N/A	N/A	2	2	2	2	0	2	2	20	
Breidablik et al., 2019	2	2	2	2	N/A	N/A	N/A	2	2	2	2	0	2	2	20	

Breidablik et al. 2020	2	1	2	2	N/A	N/A	N/A	2	2	2	2	0	2	2	19	
Khairnar et al., 2020	2	2	2	2	2	0	1	2	2	2	2	0	2	2		23

Note: For column per 22 is the total score for non-RCT study design as items 5, 6 and 7 are not available, while column per 28 is for RCT study design.

Yes = 2, Partial = 1, No = 0

Item 1: Question or objective sufficiently described, Item 2: Study design evident and appropriate to answer study question, Item 3: Method of subject or comparison group selection or source of information is described and appropriate, Item 4: Subject characteristics sufficiently described, Item 5: Description of interventional and random allocation (if possible), Item 6: Report of interventional and blinding of investigators (if possible), Item 7: Report of blinding of subject (if possible), Item 8: Outcome and exposure measure (if applicable) well defined and robust with bias misclassification reported, Item 9: Sample size appropriate, Item 10: Analysis described and appropriate, Item 11: Estimate of variance is reported, Item 12: Controlled for confounding, Item 13: Result reported in sufficient detail, Item 14: Result supported the conclusions

Table 4.2. Percentages of the quality assessment for the included studies.

Citation	<50%	>50%
Larson, Eke & Laughon, 1986		78.57
Butz, Laughon, Gullette & Larson, 1990		85.71
Larson & Bobo, 1992		75.00
Cardoso, Pereira, Zequim & Guilhermetti, 1999		78.57
Paulson, Fendler, Dolan & Williams, 1999		85.71
Guilhermetti, Hernandes, Fukushigue, Garcia & Cardoso, 2001		82.14
Moadab, Rupley & Wadhams, 2001		95.45
Girou, Loyeau, Legrand, Oppein & Brun-Buisson, 2002		85.71
Lucet et al., 2002		82.14
Dharan, Hugonnet, Sax & Pittet, 2003		86.36
Hernandes et al., 2004		85.71
Tvedt & Bukholm, 2005		81.82
Kac et al., 2005		89.29
Barbut et al, 2007		86.36
Kampf, 2008		82.14
Jabbar et al., 2010		81.82
Abaza, Amine & Hazzah, 2010		81.82
D'Antonio, Rihs, Stout & Yu, 2010		86.36
Goroncy-Bermes, Koburger & Meyer, 2010		86.36
Kawagoe, Graziano, Martino, Siqueira & Correa, 2011		90.91
Sharma, Dutta, Taneja & Narang, 2013		82.14
Figueiredo, de Siqueira, Poli-de-Figueiredo & d'Avila, 2013		90.91
Racicot, Kocher, Beauchamp, Letellier & Vaillancourt, 2013		77.27
Macinga et al., 2014		77.27
Salmon, Truong, Nguyen, Pittet & McLaws, 2014		81.82
Sasahara, Hayashi, Hosoda, Morisawa & Hirai, 2014		90.91
Schaffner et al., 2014		86.36
de Aceituno et al., 2015		75.00
Herruzo, Yela & Vizcaino, 2015		77.27
Appelgrein, Hosgood, Dunn & Schaaf, 2016		81.82
de Aceituno et al., 2016		75.00
Wolfe et al., 2017		75.00
Paula et al., 2017		90.91
Wilkinson, Ormandy, Bradley, Fraise & Hines, 2017		86.36
Alsagher et al., 2018		72.73
Wilkinson, Ormandy, Bradley & Hines, 2018		81.82
Nasution, Yunita, Pasaribu & Ardinata, 2019		77.27
Bondurant, Duley & Harbell, 2019		90.91
Breidablik et al., 2019		90.91
Breidablik et al. 2020		86.36
Khairnar et al., 2020		82.14

Note: The value indicated is in percentage.

4.4 Data Analysis

The findings are divided into two parts which are effectiveness of ABHS in reducing bacteria when compared to other hand hygiene methods and the factors influencing the efficacy of ABHS in reducing bacteria on hands. As for the factors influencing the efficacy of ABHS in reducing bacteria on hands, the comparison groups are between the ABHS products.

4.4.1 Description of the Included Studies

From the 41 included studies, there are five types of study design involved as shown in Table 4.3. Most of the study design involved in the included studies was quasi experimental design (20 studies) followed by randomized controlled trial (RCT) (14 studies). The other three study designs of the included studies were controlled clinical trial (CCT) (1 study), cross-over trial (4 studies), and randomized cross-over trial (2 studies). As for the publication year of the included studies, the earliest year was in 1986 and the latest was in 2020. Publication year range between 2016 to 2020 had the highest number of studies (12 studies) followed by year between 2011 to 2015 which is 10 studies. The total of studies for the year before 2011 were 19 studies as shown in Table 4.4. Next, the settings involved in the included studies were laboratory, hospital and field settings as shown in Table 4.6. More than half of the included studies involved laboratory setting (27 studies) and 11 out of 41 studies involving laboratory settings. The least settings involved were field setting whereby only 3 studies used field setting in determining the efficacy of the ABHS. The total studies that using artificial contamination in determining bacterial reduction on hands were 23 studies

and 13 studies were using existing hand flora as shown in Table 4.6. As for the outcome measures, the reduction factor (RF) is referring to the efficacy of the test solution reducing the number of microorganisms present on the hands of the test subjects and it is calculated by using the following formula: $\log_{10}$ of initial/baseline bacterial count - $\log_{10}$ of final/post wash bacterial count. The test methods of the included studies are bag broth technique, glove juice method, drop-plate technique, fingertip sampling, hand sampling technique, EN 1500 standard, prEN 12791, hand swab, cup scrub technique. European norms 1500 (EN 1500) standard is the standard whereby the efficacy of products used for hygienic hand disinfection are tested by comparing with the reference standard (60% n-propanol) and the reduction factor need to be higher than standard for an effective product (Boyce & Pittet, 2002). Another European test method, prEN 12791 is a method that tests the products under practical conditions against the reference product (60% propan-1-ol).

Table 4.3. The number of studies according to the study design (n=41).

Type of Study Design	Number of Studies	Percentage (%)
Randomized controlled trial (RCT)	14	34.15
Randomized cross-over trial	2	4.88
Controlled clinical trial	1	2.44
Cross-over trial	4	9.76
Quasi experimental design	20	48.78

Table 4.4. The number of studies by year (n=41).

Range of Year	Number of Studies	Percentage (%)
1986 – 1990	2	4.88
1991 – 1995	1	2.44
1996 – 2000	2	4.88
2001 – 2005	8	19.51
2006 – 2010	6	14.63
2011 – 2015	10	24.39
2016 – 2020	12	29.27

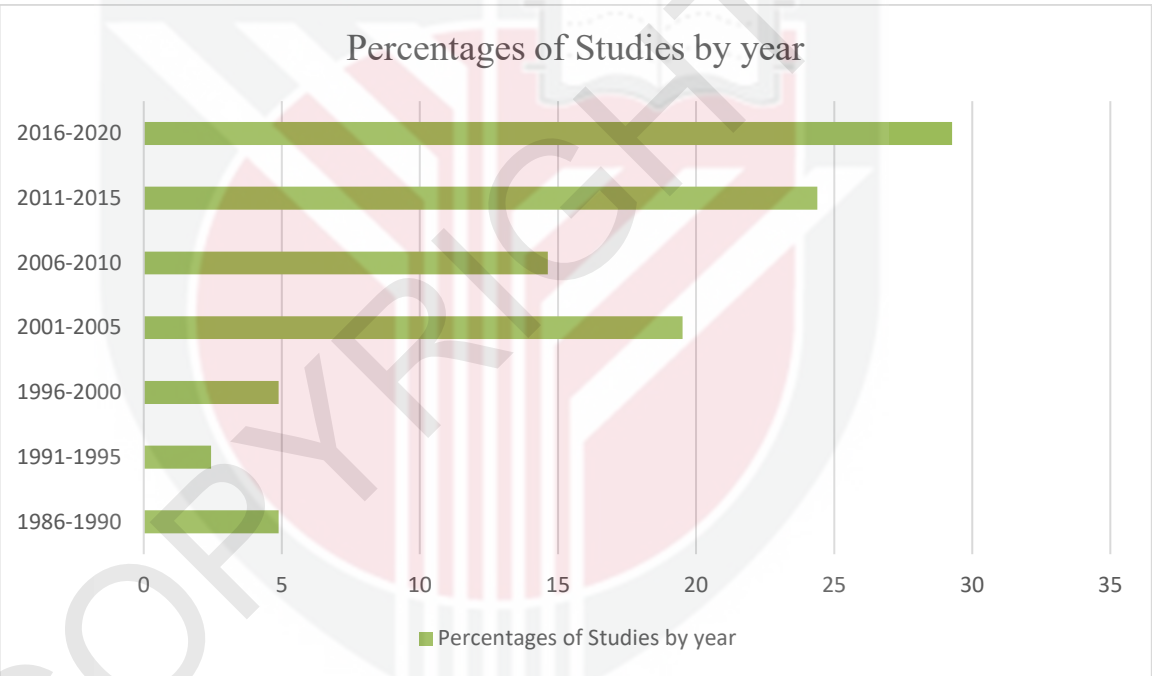

Figure 4.2. The bar graph of percentages of included studies by year (n=41).

Table 4.5. The number of studies according to setting of the study (n=41).

Setting of the Study	Number of Studies	Percentage (%)
Laboratory	27	65.85
Hospital	11	26.83
Field	3	7.32

Table 4.6. The number of studies according to the types of bacterial contamination (n=41).

Types of Bacterial Contamination	Number of Studies	Percentage (%)
Artificial contamination	23	56.10
Existing hand flora	18	43.90

4.4.2 The Comparison of the Alcohol-Based Hand Sanitizer (ABHS) with Other Hand Hygiene Methods and Its Effectiveness in Reducing Bacteria

Out of 41 included studies, 32 studies comparing the use of ABHS with other hand hygiene methods which are handwashing with soap as shown in Table 4.7. The remaining 9 studies reported on the factor influencing the effectiveness of ABHS in reducing bacteria on hands. From the 32 studies, 19 studies reported the ABHS is effective to be used as hand hygiene in reducing bacteria on hands, while the remaining 13 studies reported that ABHS is not effective compared to other hand hygiene methods mentioning in the studies. The significant reduction means the ABHS is showing a significantly greater reduction when compared to other groups, while no significant reduction means the ABHS is showing a significantly lower reduction of bacteria when compared to the other group. In determining the effectiveness of ABHS when compared to the other hand hygiene methods, the comparison groups involved in the included studies include either the comparison of ABHS with nonantibacterial soap and antibacterial soap, comparison of ABHS with nonantibacterial soap and antiseptic detergent, comparison of ABHS with antibacterial soap, or comparison of ABHS with nonantibacterial soap. A study conducted by Abaza, Amine & Hazzah (2010) is considered significant reduction although there are no statistically significant differences observed between groups. This is because the ABHS groups are showing a better reduction of bacteria compared to handwashing.

Three studies (D'Antonio, Rihs, Stout & Yu, 2010; Racicot, Kocher, Beauchamp, Letellier & Vaillancourt, 2013; Sasahara, Hayashi, Hosoda, Morisawa & Hirai, 2014) reported on the ABHS is not effective in reducing bacterial spores. The use of BZK is also showing better efficacy compared to the ABHS in two studies (Herruzo, Yela & Vizcaino, 2015; Bondurant, Duley & Harbell, 2019). As for the other studies that reported on the ABHS is not effective, there are several different key points which are the combination of ABHS and handwashing with soap is having a better efficacy, low concentration of alcohol is being used, and other antiseptic agents such as CHG and ozonated water are having a better efficacy compared to ABHS.

Table 4.7. The number of studies and percentages of the included studies showing the ability of ABHS in reducing bacteria on hands (n=32).

Ability of ABHS to Reduce Bacteria on Hands	Number of Studies	Percentage (%)
Significant reduction	19	59.38
No significant reduction	13	40.63

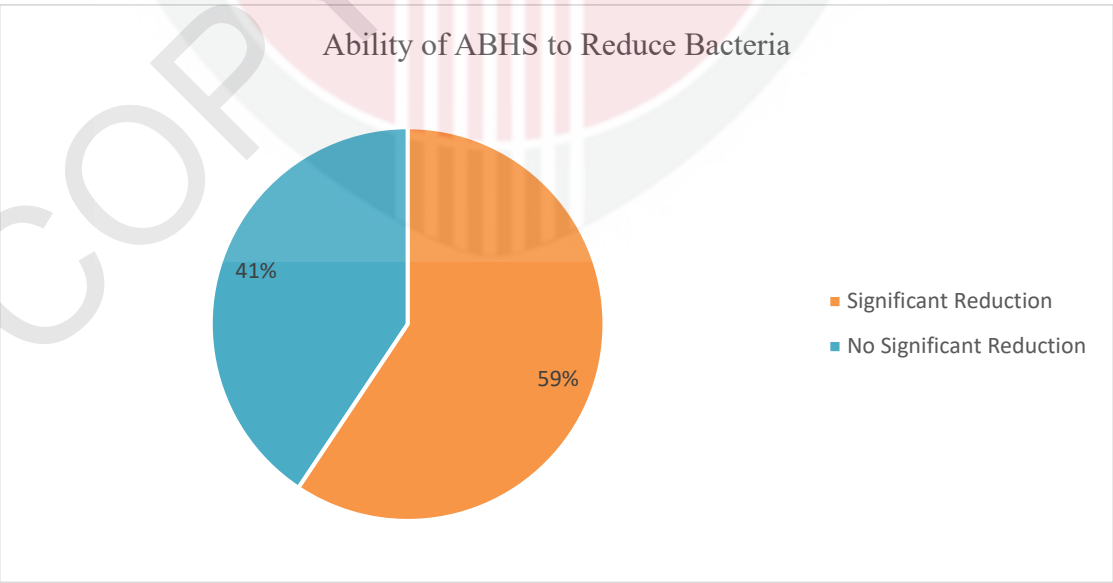

Figure 4.3. The pie chart for the percentages of studies showing the ability to reduce the bacteria (n=32).

Table 4.8. Studies showing ABHS is having greater reduction compared to the other hand hygiene methods (n=19).

Author and Year	Objective of Study	Comparison Groups	Significant Reduction of ABHS
Larson, Eke & Laughon, 1986	To evaluate the antimicrobial efficacy of five hand hygiene products under frequent-use condition (15 hand washes every day for 5 days in a row)	Nonmedicated soap (Safe 'n Sure), (control) Alcohol-based hand rinse containing 60% IPA in emollients (Cal Stat) (Alc A) Alcohol-based hand rinse containing 70% IPA and 0.3% CHG in emollients (Hibistat) (Alc B) Detergent containing 4% CHG and 4% IPA (Hibiclens) (CHG) 70% IPA (IPA)	Alc A, CHG and IPA were showing a significant reduction after first wash compared to the baseline group. Overall, the alcohol-based hand rinses showed a better efficacy compared to the control.
Larson & Bobo, 1992	To investigate the impact of blood on the antibacterial activity of several commonly used hand hygiene products	70% IPA (IPA) Liquid hand rinse containing 70% ethanol and 0.5% CHG (EA) Liquid detergent containing 7.5% PVP-I Liquid detergent-based handwashing product containing 4% CHG (CHG) Nonantimicrobial liquid soap (soap) Control (no degerming product) (All the regimens tested in the presence of blood)	Two alcohol-based products (IPA and EA) have a significant greater reduction of the bacteria in the presence of bloods compared to the other products, but the physical appearance of the blood still exist on hands.
Cardoso, Pereira, Zequim & Guilhermetti, 1999	To examine the effectiveness of hand hygiene products in eliminating a light or heavy contamination of *Acinetobacter*	Plain liquid soap 70% ethanol 10% PVP-I detergent containing 1% active iodine 4% CHG detergent containing 4% IPA	When the light contamination of the bacteria was done, all the four hand cleansing agents were able to reduce it, but no significant differences observed between the groups. When heavy contamination is done,

	baumannii that is artificially applied		there was significant bacterial reduction when using 70% ethanol and 10% PVP-I compared to plain liquid soap and 4% CHG.
Paulson, Fendler, Dolan & Williams, 1999	To evaluate the disinfection efficacy of five hand hygiene products manufactured by GOJO Industries	Nonantimicrobial lotion soap Antimicrobial lotion soap (0.6% PCMX) Alcohol gel sanitizer (62% ethanol) Nonantimicrobial lotion soap and alcohol gel sanitizer (62% ethanol) Antimicrobial lotion soap and alcohol gel sanitizer	Exploratory Data Analysis showed that alcohol gel sanitizer is the only stand-alone product that was able to be effective in reducing the bacteria.
Guilhermetti, Hernandes, Fukushigue, Garcia & Cardoso, 2001	To compare the hand hygiene products in eliminating hospital strain of methicillin-resistant *Staphylococcus* Aureus artificially contaminated to the hands	Plain liquid soap 70% ethanol 10% PVP-I detergent containing 1% active iodine 4% CHG detergent containing 4% IPA	When the light contamination of MRSA was done, 10% PVP-I and 70% ethanol had significantly higher reduction rates than the other two agents. The 10% PVP-I showed the highest reduction factor for both light and heavy contamination of MRSA.
Girou, Loyeau, Legrand, Oppein & Brun-Buisson, 2002	To evaluate the efficacy of alcohol-based hand rub in comparison to conventional handwashing with antiseptic soap in reducing bacteria during patient care	Alcohol-based solution (Sterillium containing 45% 2-propanol and 30% 1-propanol) Antiseptic soap (Hibiscrub containing 4% CHG)	Hand rubbing with ABHS showed a significantly higher median percentage of reduction compared to handwashing method.
Lucet et al., 2002	To evaluate the antibacterial efficacy of hand hygiene methods including using alcohol-based hand rub and	Nonmedicated soap ((1) 10s, (2) 30s) Antiseptic soap, Hibiscrub containing 4% CHG or Betadine containing 10% PVP-I ((3)10s, (4) 30s, (5) 60s)	When handwashing with antiseptic soap (10s, 30s, 60s), or alcohol-based hand rub, there were significant greater bacterial reduction observed

	handwashing with antiseptic agents and with nonmedicated soap	Alcohol-based hand rub (Sterillium containing 45% 2-propanol and 30% 1-propanol)	compared with handwashing with unmedicated soap (10s, 30s). No significant difference between handwashing with antiseptic soap and alcohol-based hand rub.
Hernandes et al., 2004	To compare the efficacy of alcohol gel with traditional hand-cleansing agents in removing bacteria from artificially contaminated hands	Plain liquid soap Alcohol gel (Geef Ltda. containing ethanol and emollient) 70% ethanol 10% PVP-I detergent containing 1% active iodine 4% CHG detergent containing 4% IPA	10% povidone-iodine liquid soap, alcohol gel, 70% ethyl alcohol, and 4% chlorhexidine detergent had significant higher reduction rate compared to plain liquid soap for almost every test bacteria.
Tvedt & Bukholm, 2005	To evaluate the hand hygiene methods used in an intensive care unit (ICU)	Plain liquid soap (Sterisol) Alcohol-based hand sanitizer (Sterisol Hand Hygiene Product containing 70% isopropanol and glycerol) (The effect when using standardized or non-standardized method in intensive care unit)	Non-standardized methods after hand disinfection using alcohol-based hand sanitizer showed a significant reduction and showing the highest mean reduction compared to the other groups. The lowest mean reduction observed was non-standardized method done after handwashing showing that hand washing needs to be use in a proper method, while hand disinfection is a robust method.

Kac et al., 2005	To compare the antimicrobial efficacy of alcohol-based hand rib and handwashing in hospital settings	Nonmedicated soap Alcohol hand rub (Sterillium containing 45% 2-propanol and 30% 1-propanol)	Both handwashing and hand rubbing showed a significant reduction, but when compared the percentage reduction, hand rubbing was significantly higher percentage reduction than handwashing.
Abaza, Amine & Hazzah, 2010	To evaluate efficacy of alcohol-based hand rubs when compared to traditional handwashing with soap and water in reducing contamination on hand present during patient care	Non-antiseptic soap ABHS products found in the Egyptian market ((A) hand liquid containing 80% ethanol, 45% iso-propanol, 30% n-propanol, and 0.2% mecetronium etilsulfate 0.2, (B) hand liquid containing 96% ethanol, (C) hand rub gel containing 60% iso-propanol and 65% ethanol, (D) locally prepared hand rub using WHO formulation containing 80% ethanol)	No statistically significant differences between the comparison groups. All the four products of ABHS showing a better reduction compared to handwashing. The highest reduction observed was when using product B. Product C showed the lowest reduction demonstrating that the types of hand sanitizer may affect the efficacy.
Sharma, Dutta, Taneja & Narang, 2013	To evaluate the hand hygiene methods used in neonatal intensive care unit (NICU)	Plain soap Alcohol hand-rub (Sterillium containing 45% 2-propranol and 30% 1-propranol) PVP-I hand scrub (Hand hygiene used in a neonatal intensive care unit (NICU))	Alcohol hand rub and povidone iodine scrub showed a significantly higher reduction compared to handwashing with plain soap when using it in NICU.
Figueiredo, de Siqueira, Poli-de-Figueiredo & d'Avila, 2013	To evaluate the two hand hygiene methods in reducing bacterial count on hands of the patients experiencing peritoneal dialysis (PD)	Alcohol hand rub (70% ethanol) Glycerine soap followed by alcohol hand rub (Hand hygiene used in preventing peritoneal dialysis (PD)-related infections)	The alcohol hand rub alone had significantly lesser bacterial counts compared to the combination of handwashing with alcohol hand rub when the patients undergoing PD.

Salmon, Truong, Nguyen, Pittet & McLaws, 2014	To evaluate the antibacterial efficacy of different hand hygiene methods used in a tertiary Vietnamese hospital	ABHR (SDS Hand Rub, containing 83% ethanol, 12% IPA, and 0.5% CHG) 4% CHG with filtered or unfiltered water Plain soap and water with filtered or unfiltered water	ABHR showed the greatest reduction compared to the other methods and the difference is significantly different.
Schaffner et al., 2014	To compare the nonbacterial and antibacterial handwashes including ABHS in reducing artificially contaminated *Shigella* on hands	Nonantibacterial hand wash ((1) non-AB (T), (2) non-AB (KMF)) Antibacterial hand wash ((1) chlorhexidine, (2) triclosan) Alcohol hand sanitizer (Purell containing 62% ethanol)	All the three antibacterial methods showed a statistically significant lower reduction compared to nonantibacterial methods and the alcohol hand sanitizer showed the greatest reduction.
de Aceituno et al., 2015	To compare the hand hygiene methods used in reducing microbial loads on farmworker hands after harvesting produce	Label-use ABHS (Purell Advanced Instant Hand Sanitizer containing 70% ethanol) Two-step ABHS (First step using 3 pumps and second step using 1 pump) Traditional soap Pumice soap)	The two-step ABHS group showed a lower log count of coliforms and *Enterococcus* spp. compared to the label-use ABHS and pumice soap groups. As for the *E. coli*, all four interventions showed a significantly lower log count compared to the control group.
Appelgrein, Hosgood, Dunn & Schaaf, 2016	To evaluate the ozonated water as a potential alternative to alcohol-based hand rub in reducing bacteria	Ozonated water (4 ppm) ABHS (60% Propan-1-ol)	The log count for ABHR is significantly lower after using it compared to ozonated water indicated that ABHR is having better efficacy compared to the ozonated water.

Table 4.9. Studies showing ABHS is having lower reduction compared to the other hand hygiene methods (n=13).

Author and Year	Objective of Study	Comparison Groups	No Significant Reduction of ABHS
Butz, Laughon, Gullette & Larson, 1990	To compare antibacterial effectiveness of alcohol-based hand wipes with other hand hygiene methods after using for 5 consecutive days (15 handwashes per day)	30% ethanol-impregnated hand wipes (Microclens) (alcohol wipe) Detergent (1% triclosan) (Tri) Detergent (4% CHG) (Chg) Liquid nonmedicated soap (control)	Control soap showed a greater significant reduction than alcohol wipes. At the end of the test day (total 75 handwashes at day 5), the product that showed the greatest log reduction observed was Chg and alcohol wipe ranked at 3rd from the four products.
Moadab, Rupley & Wadhams, 2001	To compare the antibacterial efficacy of ABHS with alcohol-free formulation of handwash	Alcohol-based hand sanitizer (Purell containing 62% ethanol) SAB hand wash (HandClens containing 0.13% BZK)	The efficacy of alcohol-based hand sanitizer (Purell) was significantly less effective than SAB hand wash.
Jabbar et al., 2010	To compare ABHS with traditional handwashing in removing bacterial spores	CHG soap ABHRs (Isagel, Endure, Purell with minimum concentration of alcohol for the 3 ABHRs is 60%)	When washing with CHG soap, there was a significant reduction of bacterial spores compared to each of the 3 ABHRs. The highest mean reduction observed was chlorhexidine washing.
D'Antonio, Rihs, Stout & Yu, 2010	To reassess the efficacy of alcohol-based rubs and wipes in reducing bacteria and bacterial spores when compared with handwashing	Alcohol hand wipes (Sani-Hands ALC containing 65.9% ethanol) Antibacterial soap (Kindest Kare containing 0.75% triclosan)	The highest log reduction observed for bacteria and spores was when using handwashing with antibacterial soap. However, the 65.9% ethanol hand wipes showed a significant higher log reduction of

	with antibacterial soap	Alcohol-based gel hand rub (Purell containing 62% ethanol)	*S. marcescens* and *G. stearothermophilus* spores than 62% ethanol alcohol rub showing that alcohol-based hand wipe with higher ethanol content is more effective than alcohol-based hand gel rub.
Racicot, Kocher, Beauchamp, Letellier & Vaillancourt, 2013	To compare the hand hygiene methods used in reducing the contamination on hands practically among poultry catching crew members	Alcohol-based hand gel alone (Purell containing 62% ethanol) (Protocol 1) Antibacterial soap and alcohol-based hand gel (protocol 2) Degreasing cream and alcohol-based hand gel (protocol 3) Antimicrobial wipes and alcohol-based hand gel (protocol 4))	Protocol 2 (water, soap, and alcohol-based hand gel) significantly more effective than the protocol 4 (wipes and alcohol-based hand gel) when high to moderate aerobic bacterial contamination was done. For coliforms, there is no statistically significant difference between the protocols when the low to moderate bacterial contamination was done. When the high contamination of coliforms was done, alcohol-based gel alone is significantly less effective than the combination of degreasing cream and the alcohol-based gel. The study concluded that using degreasing cream or water and soap before using alcohol-based gel can increase the effectiveness in reducing bacteria.
Sasahara, Hayashi, Hosoda, Morisawa & Hirai, 2014	To compare the and hygiene methods used in reducing *Bacillus cereus* spores	30-second water-only washing 60-second plain soap washing 30-second plain soap washing	Both alcohol-based products had the lowest log reduction compared to the other methods showing that alcohol-based hand rubbing is not that effective in

		30-second antiseptic soap washing Alcohol-based liquid (76.9-81.4% ethanol) Alcohol-based gel (76.9-81.4%) ethanol	reducing bacterial spores. All the handwashing procedures reduce the number of spores effectively
Herruzo, Yela & Vizcaino, 2015	To compare different hand hygiene methods used in reducing bacteria that is artificially contaminated on hands	2% chlorhexidine 5% chlorhexidine 1% PVP-I 10% PVP-I 60% n-propanol Sterillium containing 45% 2-propranol and 30% 1-propranol Alcoaloe containing 0.6% chlorhexidine, isopropanol and 0.1% BZK	The best products in reducing the bacteria involved were 2% and 5% chlorhexidine and the combination of 0.6% chlorhexidine, isopropanol and 0.1% BZK. The reduction observed for the two products were significant compared to the other four products.
Wolfe et al., 2017	To determine the antibacterial efficacy of different hand hygiene methods from artificially contaminated hands	Soap ABHS Purell Advanced Instant Hand Sanitizer (70% ethanol) 0.05% sodium dichloroisocyanurate (NaDCC) High-test hypochlorite (HTH) Stabilized sodium hypochlorite (NaOCl) solutions Non-stabilized NaOCl solutions (Hand hygiene used with and without soil load)	The six handwashing methods showed an average log reduction of the bacteria compared with the no handwashing (control) when there is soil load or not. The greatest reduction observed when handwashing with NaDCC was done without soil load, while with the soil load, the greatest reduction observed when handwashing with HTH.
Nasution, Yunita, Pasaribu & Ardinata, 2019	To compare alcohol-based hand rub with traditional handwashing in reducing bacteria on hands of nurses in Medan	Soap (0.175% chloroxylenol and 0.3% salicylic acid) Alcohol hand rub (70% ethyl alcohol)	There is no significant difference between handwash and alcohol hand rub. When hand washing with soap, there were higher average reduction compared to hand rub.
Bondurant, Duley &	To evaluate the persistence	Alcohol-free hand sanitizer (commercial	There is statistically significant difference

Harbell, 2019	antibacterial activity after 1, 2, and 4 hours of application of alcohol-based hand sanitizer and alcohol-free hand sanitizer	brand DAB hand sanitizer containing 0.12% BZK) Alcohol-based hand sanitizer containing 63% ethanol)	between alcohol-free hand sanitizer and alcohol-based hand sanitizer. The log reduction for alcohol-based hand sanitizer is lower compared to the alcohol- free hand sanitizer at all the three time points.
Breidablik et al., 2019	To compare antibacterial efficacy of ozonized tap water with alcohol rub	Ozonized tap water (0.8 or 4 ppm) Alcohol-based rub (Antibac containing 85% ethanol, propan-2-ol, and glycerol)	The ozonated water showed a lower mean count of bacteria compared to the alcohol. The study concluded that ozonated water can be an alternative to alcohol hand sanitizer as it showed an average reduction of bacteria.
Breidablik et al. 2020	To compare the different hand hygiene methods in reducing bacteria on hands	Alcohol-based rub (Antibac containing 85% ethanol, <5% propan-2-ol, and glycerine) Non-alcoholic Antibac hand soap 0.8 ppm ozone water	The using of soap and water showed a significantly better lower count of bacteria compared to alcohol hand rub and ozonated water.
Khairnar et al., 2020	To compare the antibacterial efficacy of alcohol-based hand sanitizer, liquid soap, and the combination of the two products	Liquid soap and water (Dettol Original Liquid Handwash containing aqua sodium laureth sulfate, glycol stearate, methyl isothiazolinone, and sodium hydroxide) ABHS (Sterillium containing 45% 2-propranol and 30% 1-propranol) Liquid soap and water followed by application of hand sanitizer (Dettol Liquid Soap followed by Sterillium)	The combination of soap and alcohol-based hand sanitizer showed the highest reduction compared to alcohol-based hand sanitizer or soap alone.

4.4.3 The Factors Influencing the Effectiveness of Alcohol-Based Hand Sanitizer (ABHS) in Reducing Bacteria

As mentioned above, 9 out of 41 included studies reported the factors influencing the effectiveness of ABHS in reducing bacteria on hands and details of the studies were illustrated in Table 4.10. There are several factors that have been studied which are the times of application, formats of ABHS, volumes of application, use of hand lotion before using hand sanitizer, and different formulation of ABHS. The different formats of ABHS are the liquid or also called as hand rinse, gel, and foam form. The different formats of ABHS such as gel and liquid are also being tested when there is presence of blood on hands in which the study reported that both liquid and gel format of ABHS were able to reduce the bacterial contamination in the presence of blood (Kawagoe, Graziano, Martino, Siqueira & Correa, 2011). One study tested the effect of using hand lotion before applying hand hygiene whereby the study reported that there was statistically significant effect of using the hand lotion, but the study recommended hand antisepsis may be applied after 5 minutes of hand lotion application (Paula et al., 2017).

Table 4.10. Studies that comparing different alcohol-based hand sanitizer (ABHS) products (n=9).

Author and Year	Factors	Results
Dharan, Hugonnet, Sax & Pittet, 2003	Shorter application time of ABHS (15 and 30 seconds). The products used are three hand rinse products (A: 80% ethanol, B: 95% ethanol, C: 75% isopropanol) and alcohol-based hand gel (60% isopropanol).	All products except alcohol-based hand gel satisfied the EN 1500 standards after applications for 15 and 30 seconds and alcohol-based hand gel showed the lowest RF compared to other products.
Barbut et al, 2007	Different formats of ABHS which are one alcohol hand rinse (Sterillium rinse: 45% 2-propanol and 30% 1-propanol) and two alcohol-based gel sanitizers (Sterillium gel: 85% ethanol and Manugel Plus: 53% ethanol and 17% isopropanol)	Alcohol hand rinse and Sterillium gel were more effective compared to Manugle Plus that had a lower alcohol content than Sterillium gel.
Kampf, 2008	Different volumes of application (2.4 mL and 3.6 mL for four ethanol-based hand rub products (hand rub A: 85% ethanol, hand rub B: 60% ethanol, hand rub C: 62% ethanol, and hand rub D: 61% ethanol)	Hand rub A with the highest concentration of alcohol was significantly better in reducing bacteria compared to the other 3 hand rubs when the product is applied at 2.4 mL. When 3.6 mL was applied, all of the four products showed a better efficacy.
Goroncy-Bermes, Koburger & Meyer, 2010	Different volumes of application for two different alcohol-based hand rubs (Product A: Spitacid containing 46% ethanol, 27% 2-propanol and 1% benzylalcohol – 2 mL (1), 3 mL (2), 4 mL (3); Product B: desderman N containing 78.2% ethanol and 0.1% 2-biphenylol) – 2 mL (4), 2.5 mL (5), 3 mL (6), 4 mL (7))	Both products when using 4 mL of application showed a significantly higher reduction when compared to the reference solution and when a lower volume (2 mL) was used for both products, the mean reduction observed was lower than the reference solution.
Kawagoe, Graziano, Martino,	Different formats of alcohol-based products when blood is present on hands (Gel-based products: product A, Purell: 62% ethanol,	No significant difference among the three products, but both liquid and gel products showed more

Siqueira & Correa, 2011	product B, Alcool Gel Dr Clean: 70% ethanol; liquid-based: product C, Alcool glicerinadol: 70% ethanol with 2% glycerol)	than 99.9% of bacterial reduction when hands contaminated with bloods.
Macinga et al., 2014	Different formats of ABHS and the different application volume (Foam B: 70% ethanol – 1.6 mL, 3 mL; Rinse E: 80% ethanol – 2 mL, 3 mL; Gel F: 90% ethanol – 2.1 mL, 3 mL)	When the three products were applied according to the efficacy at drying volume in 30s (1.6 ml for Foam B, 2 ml for Rinse F, 2.1 mL for Gel F), the RF did not exceed the standard reference product, thus indicated that the products with the volumes used did not satisfy the EN 1500 standard. However, when all the three products applied at 3 mL application, all products satisfied the EN 1500 standard as the RF exceed the standard reference product.
Paula et al., 2017	The efficacy of hand hygiene methods following hand lotion use (only hand rub (group A), hand lotion for 5 min and hand rub (group B), hand lotion for 1 hour and hand rub (group C), handwashing and hand rub (group D), handwashing, hand lotion (5 min) and hand rub (group E), handwashing, hand lotion (1 hr) and hand rub (group F))	Following the use of hand lotion, the hand hygiene methods did not significantly be affected. The study concluded that hand antisepsis could be applied after 5 minutes of hand lotion application.
Wilkinson, Ormandy, Bradley, Fraise & Hines, 2017	Three different formulations of ABHS with different volumes applied (WHOF1: 80% ethanol, 1.45 % glycerol, 0.125% hydrogen peroxide; WHOF2: 75% IPA, 1.45% glycerol, 0.125% hydrogen peroxide; IPA: 60% IPA). The range of volumes were between 0.5 mL to 3.0 mL.	No significant difference between the different products across all volumes. The highest RF observed was WHOF1 at 3 mL of application.
Wilkinson, Ormandy,	Two different formulations of HS (60% IPA and 80% ethanol	There was no significant difference in the efficacy of two different

Bradley & Hines, 2018	(WHOF1)) with different formats (liquid, gel, and foam)	formulation with different formats. All the products did not meet the EN 1500 standard as the RF did not exceed the standard reference product.

CHAPTER 5

DISCUSSION

ABHS has played an important role nowadays as the increasing usage of ABHS has been observed since 2020 due to the coronavirus disease 2019 (COVID-19) pandemic. Besides, even before COVID-19 pandemic arises, the use of ABHS is also common to be used as hand hygiene, and WHO has recommended the use of it as a hand hygiene in replacing handwashing with soap and water when water is inaccessible (World Health Organization, 2009). The ABHS has been reported in having a good antibacterial activity through *in vitro* study (Oke et al., 2013; Jain et al., 2016; Otokunefor & Princewill, 2017). However, *in vivo* study or clinical trials of antibacterial efficacy of ABHS involving the use of hands of humans are needed to ensure the efficacy and there a lot of studies have been done in determining the effectiveness in reducing bacteria. Hence, this systematic review is done in gathering the best available evidence in determining the effectiveness of alcohol-based hand sanitizer (ABHS) in reducing bacteria on hands. Aside from that, this study also aims to determine the factors influencing the effectiveness of ABHS in reducing bacteria on hands.

In order to achieve the aims of this study, systematic review is implemented in the methodology. Before starting the systematic review, research objectives or research questions need to be developed first. Then, the systematic review started with the formation of search keywords to be used in searching the interested study in the selected databases. After the literature search has been done, the search results from the different databases were collected in a single file and duplicates were removed. Then, screening based on title and abstract followed by the full text screening were done based on the eligibility criteria that had been set before the screening. Data extraction was done afterwards followed by the quality assessment.

After all the screening done, the number of included studies that were selected based on the eligibility criteria are 41 studies. The type of study design is crucial in systematic review as the level of evidence is ranked according to the probability of bias and RCT is on the highest level evidence of studies indicating that the study is unlikely to be biased and have lower risk of systematic errors due to the random allocation (Burns et al., 2011). Among the included studies, only 34% (14 studies) were randomized controlled trials (RCTs) showing that there is not much evidence that use RCT design in their studies. Besides, from the 41 studies, the studies were divided into two parts which are the effectiveness of ABHS in reducing bacteria when compared to the other hand hygiene methods and the factors influencing the effectiveness of ABHS in reducing bacteria on hands. There are 32 studies that compare the ABHS with other hand hygiene methods and 9 studies that compare between ABHS, or it can be categorized under studies that determine the factors influencing the antibacterial efficacy of ABHS.

5.1 The Comparison of The Alcohol-Based Hand Sanitizer (ABHS) with Other Hand Hygiene Methods and Its Effectiveness in Reducing Bacteria

When the ABHS is compared to the other hand hygiene methods, there are more than half of studies that reported on the significant reduction of ABHS in reducing bacteria compared to the other hand hygiene methods. This result is consistent with previous systematic review done by Picheansathian (2004) whereby the study reviewed the effectiveness of ABHS in reducing both bacteria and virus for both surgical hand disinfection and hygienic hand disinfection. The number of studies reported on ABHS is having a greater reduction compared to the other hand hygiene methods were 19 studies from the 32 studies. However, there are also studies that show ABHS is less effective in reducing bacteria in which 13 from 32 studies reported lower reduction of bacteria when using ABHS in comparison to the other hand hygiene methods. Most of the studies (10 out of 19 studies) that reported on the ABHS is effective is comparing the ABHS with non-antibacterial soap and antiseptic detergent in which those studies aim to determine whether the ABHS can replace the use of non-antibacterial soap or antiseptic detergent that contain either chlorhexidine gluconate (CHG) or povidone-iodine (PVP-I). The results from those reported studies suggest that ABHS can be used in replacing the traditional handwashing that uses soap and water. PVP-I and CHG are two important antiseptic or antimicrobial ingredients that have been used in detergent or antiseptic soap for a long time in reducing and preventing infection (Eggers et al., 2018). However, traditional handwashing where water is needed sometimes can cause poor hand hygiene compliance due to inaccessibility to water or lack of time in washing their hands in both healthcare

settings and community settings (Schmidt et al., 2009). Hence, alternative methods such as the use of ABHS is needed in improving hand hygiene compliance to reduce the risk of infection.

Based on Table 4.5 shown in the results, there are 11 studies that done in hospital settings and nine studies reported on the ABHS is effective to be used when compared to the other hand hygiene methods (Larson, Eke & Laughon, 1986; Lucet et al., 2002; Girou et al., 2002; Kac et al., 2005; Tvedt & Bukholm, 2005; Abaza, Amine & Hazzah, 2010; Figueiredo, de Siqueira, Poli-de-Figueiredo & d'Avila, 2013,; Sharma et al., 2013; Salmon et al., 2014). The results suggest that the use of ABHS can be used in hospital settings in reducing bacterial transmission. Two of the studies reported that ABHS can even be used in reducing methicillin-resistant *Staphylococcus aureus* (MRSA), a drug-resistant bacteria that is a common cause in nosocomial infection in which this type of infection usually happens in hospital settings (Guilhermetti et al., 2001; Hernandes et al., 2004). In the presence of blood, Larson and Bobo (1992) reported that the alcohol-based products were having significantly greater bacterial reduction compared to handwashing with soap, but the physical residue of blood was still present on hands. Kawagoe et al. (2011) also reported that the alcohol-based products were able to reduce the bacteria when blood is present on hands. Thus, the results suggested that ABHS can be used when hand is present with blood to reduce bacteria but only when water is inaccessible as physical residue is still present. Besides, the using of two-steps of ABHS is reported to be effective in reducing bacteria on soiled hands when the farmworkers used after harvesting produce in comparison to the use of handwashing with soap and water, and label-use ABHS in which the two-steps ABHS is the uses of ABHS two times where the first step uses

three pumps followed by one pump of ABHS being dispensed on hands (de Aceituno et al., 2015; de Aceituno et al, 2016). The two-steps ABHS include the use of paper towel in removing the excess ABHS produced in the first step, and this might be the reason why two-steps ABHS can be effective in reducing bacteria when hands were contaminated with soil as there is a physical removal from the paper towel. However, Wolfe et al. (2017) reported that ABHS is not very effective in reducing bacteria with the soil load when compared to either handwashing with soap or chlorine solutions. The study suggested that mechanical action might be one of the factors why hand washing with soap or chlorine solutions is seen to be more effective in reducing bacteria when contaminated with soil. Thus, from the results from several studies, it can be suggested that when there is soil load, hand hygiene using ABHS alone is not very effective in reducing bacteria and physical or mechanical removal needs to be combined for a better efficacy. Besides, Racicot et al. (2013) suggested that the use of degreasing cream or handwashing with soap water are recommended before the using of ABHS as the study found that ABHS alone is not very effective to be used compared to other hand hygiene methods when used in reducing the contamination on hands practically among poultry catching crew members.

One study conducted in 1990 showed that lower concentrations of alcohol (30% ethanol) used in alcohol-based hand wipes which is a form of ABHS is not effective when compared to handwashing with soap (Butz et al., 1990). Besides, the latest included study showed an inconsistent result where combination of soap and ABHS is more effective when compared to using only ABHS (Khairnar et al., 2020). Moreover, when bacterial spores are present on hands, hand washing with soap either by using plain soap, antiseptic detergent or antibacterial soap is more effective in

reducing bacterial spores on hands compared to the using of ABHS (Jabbar et al., 2010; D'Antonio et al., 2010; Sasahara et al., 2014). This suggested that in reducing bacterial spores from hand, mechanical action from handwashing with soap and water might be required in reducing bacterial spores. Besides, the use of benzalkonium chloride is used nowadays in replacing alcohol in hand sanitizer as alcohol is volatile. Bondurant et al. (2019) reported that alcohol-free hand sanitizer is showing a better persistence antibacterial activity when compared to the alcohol-based hand sanitizer suggesting that alcohol-free hand sanitizer can be used instead of ABHS. Moreover, there are also studies that studied the use of ozonated water in replacing ABHS and handwashing with soap as these hand hygiene methods can result in adverse effects on skin in sensitive people. Breidablik et al. (2019) reported that ozonated water showed a lower bacterial count compared to alcohol-based hand rub. However, when soap and water is included in the comparison groups, handwashing with soap and water showed a lower count when compared to the ozonated water (Breidablik et al., 2020).

One of the factors influencing the effectiveness of ABHS in reducing bacteria is the concentration of alcohol in which ABHS with a lower than 70% concentration of ethanol showed a lower reduction of bacteria when compared to the other products with higher concentration of ethanol (Barbut et al., 2007; Kampf, 2008). There is a study that tested the effect of hand lotion application before applying hand hygiene methods whereby the study reported that there is a statistically significant effect of using the hand lotion and the study recommended hand antisepsis may be applied after 5 minutes of hand lotion application (Paula et al., 2017). Besides, alcohol-based hand gel is reported to be less effective when compared to alcohol-based hand rinse and the study noted that after 15 seconds of the application of the gel product, the hands were

usually damp (Dharan et al, 2003). Hence, Kramer et al. (2002) suggested that the faster-drying gel formulation and increasing the alcohol content of the product can be done in increasing the efficacy of alcohol-based hand gels. Besides, the application volume of ABHS might affect antibacterial efficacy in which there are studies reported that application volume less than 3 mL is showing less reduction of bacteria when compared to the application volume at 3 m L and 4 mL (Goroncy-Bermes et al., 2010; Macinga et al., 2014). However, no significant differences observed between different formats of ABHS in studies conducted by Wilkinson et al. (2017) and Wilkinson et al. (2018) suggested that ABHS in either liquid, gel or foam format can be used in reducing bacteria, but the specific product need to pass the required efficacy and safety standards.

5.2 Quality Assessment

All of the included studies are showing a good quality of study design as the results of the quality assessment is more than 50% for all of the studies. Both item 6 and 7 which refers to blinding are not possible to be included in most of the RCT study design involved as the test subjects can feel the differences between using the ABHS and handwashing with soap and water. When all the products involved are all ABHS, then the blinding can be possible as the subjects did not know what brands or products they are using. The variables present in the study that affect the same outcome as the variable subjected in the intervention are called confounders (Nijssen et al., 2006). Item 12 which is the control for confounding may also not be feasible to be done in investigating the hand hygiene intervention as the study usually measures pre and post wash which might increase the risk of confounder. However, there are no other issues for the other items present in the quality assessment tool used.

5.3 Limitation of Study

The main limitation of this study is there are not many RCT designs involved, and more quasi-experimental design included in which there is no randomization involved in the experimental design. This might cause the gathered evidence to not be the strongest level of evidence as the study design involved is not involving randomization in the study to prevent the risk of bias. Besides, another limitation is most of the studies assess the hand hygiene intervention in the laboratory setting in which the intervention does not reflect the real situation of using hand hygiene methods and the types of bacterial contamination involved is mostly using the artificial contamination which does not reflect real situation such as in community and hospital settings.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

This systematic review provides evidence on the effectiveness of alcohol-based hand sanitizer in reducing bacteria on hands of humans. There are more studies that reported on greater bacteria reduction on hands compared to the studies that reported on the lower bacterial reduction when the ABHS is compared to the other hand hygiene methods. Hence, the results from this systematic review have gathered the available evidence in showing ABHS is effective in reducing bacteria on hands based on studies involving hands of humans. However, the use of ABHS is not recommended when the hands are contaminated with physical contaminants such as soil. Besides, there are several factors involved in affecting the effectiveness of ABHS in reducing bacterial contamination on hands such as the formats, application volume, and the formulation of ABHS.

RECOMMENDATIONS

More studies using RCTs in investigating the effectiveness of ABHS in community settings are needed as most of the studies have been done in laboratory settings that does not reflect the real situation such as in hospital and community settings. This is important as ABHS is a part of hand hygiene that can be used as a preventive measure in reducing infectious diseases. Besides, alcohol-free hand sanitizer has gained attention nowadays and there is also hand sanitizer that has been made from natural ingredients. Hence, systematic review on the effectiveness of alcohol-free hand sanitizer is recommended. The review involving clinical studies are needed in determining the efficacy of these categories of hand sanitizer as in vitro studies are not sufficient enough in determining the efficacy of the hand sanitizer.

REFERENCES

Abaza, A. F., Amine, A. E., & Hazzah, W. A. (2010). Comparative study on efficacy of different alcohol hand rubs and routine hand wash in a health-care setting, alexandria, egypt. *The Journal of the Egyptian Public Health Association*, *85*(5–6), 273–283. http://www.ncbi.nlm.nih.gov/pubmed/22054102

Ahmed-Lecheheb, D., Cunat, L., Hartemann, P., & Hautemanire, A. (2012). Prospective observational study to assess hand skin condition after application of alcohol-based hand rub solutions. *American Journal of Infection Control*, *40*(2), 160–164. https://doi.org/10.1016/j.ajic.2011.04.323

Ahn, E., & Kang, H. (2018). Introduction to systematic review and meta-analysis. *Korean Journal of Anesthesiology*, *71*(2), 103. https://doi.org/10.4097/KJAE.2018.71.2.103

Allegranzi, B., & Pittet, D. (2009). Role of hand hygiene in healthcare-associated infection prevention. In *Journal of Hospital Infection* (Vol. 73, Issue 4, pp. 305–315). W.B. Saunders. https://doi.org/10.1016/j.jhin.2009.04.019

Alsagher, M. R., Soudah, S. A., Khsheba, A. E., Fadel, S. M., Dadiesh, M. A., Houme, M. A., Eshagroni, A. S., Alosta, F. F., & Almsalaty, S. M. (2018). Hand Washing Before and After Applying Different Hand Hygiene Techniques in Places of Public Concern in Tripoli-Libya. *The Open Microbiology Journal*, *12*(1), 364–375. https://doi.org/10.2174/1874285801812010364

Appelgrein, C., Hosgood, G., Dunn, A. L., & Schaaf, O. (2016). Ozonated water is inferior to propanol-based hand rubs for disinfecting hands. *Journal of Hospital Infection*, *92*(4), 340–343. https://doi.org/10.1016/j.jhin.2015.08.029

Barbut, F., Maury, E., Goldwirt, L., Boëlle, P. Y., Neyme, D., Aman, R., Rossi, B., & Offenstadt, G. (2007). Comparison of the antibacterial efficacy and acceptability of an alcohol-based hand rinse with two alcohol-based hand gels during routine patient care. *Journal of Hospital Infection*, *66*(2), 167–173. https://doi.org/10.1016/j.jhin.2007.03.021

Bartolucci, A. A., & Hillegass, W. B. (2010). Overview, Strengths, and Limitations of Systematic Reviews and Meta-Analyses. *Evidence-Based Practice: Toward Optimizing Clinical Outcomes*, 17–33. https://doi.org/10.1007/978-3-642-05025-1_2

Berardi, A., Perinelli, D. R., Merchant, H. A., Bisharat, L., Basheti, I. A., Bonacucina, G., Cespi, M., & Palmieri, G. F. (2020). *Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID- 19 . The COVID-19 resource centre is hosted on Elsevier Connect , the company ' s public news and information . January*.

Bondurant, S. W., Duley, C. M., & Harbell, J. W. (2019). Demonstrating the persistent

antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application. *American Journal of Infection Control*, *47*(8), 928–932. https://doi.org/10.1016/j.ajic.2019.01.004

Breidablik, H. J., Lysebo, D. E., Johannessen, L., Skare, Andersen, J. R., & Kleiven, O. (2020). Effects of hand disinfection with alcohol hand rub, ozonized water, or soap and water: time for reconsideration? *Journal of Hospital Infection*, *105*(2), 213–215. https://doi.org/10.1016/j.jhin.2020.03.014

Breidablik, H. J., Lysebo, D. E., Johannessen, L., Skare, Andersen, J. R., & Kleiven, O. T. (2019). Ozonized water as an alternative to alcohol-based hand disinfection. *Journal of Hospital Infection*, *102*(4), 419–424. https://doi.org/10.1016/j.jhin.2019.01.026

Burns, P. B., Rohrich, R. J., & Chung, K. C. (2011). The Levels of Evidence and their role in Evidence-Based Medicine. *Plastic and Reconstructive Surgery*, *128*(1), 305. https://doi.org/10.1097/PRS.0B013E318219C171

Butz, A. M., Laughon, B. E., Gullette, D. L., & Larson, E. L. (1990). Alcohol-impregnated wipes as an alternative in hand hygiene. *AJIC: American Journal of Infection Control*, *18*(2), 70–76. https://doi.org/10.1016/0196-6553(90)90084-6

Cardoso, C. L., Pereira, H. H., Zequim, J. C., & Guilhermetti, M. (1999). Effectiveness of hand-cleansing agents for removing Acinetobacter baumannii strain from contaminated hands. *American Journal of Infection Control*, *27*(4), 327–331. https://doi.org/10.1016/S0196-6553(99)70052-0

Chambers, H. F. (2001). The changing epidemiology of staphylococcus aureus? *Emerging Infectious Diseases*, *7*(2), 178–182. https://doi.org/10.3201/eid0702.010204

D'Antonio, N. N., Rihs, J. D., Stout, J. E., & Yu, V. L. (2010). Revisiting the hand wipe versus gel rub debate: Is a higher-ethanol content hand wipe more effective than an ethanol gel rub? *American Journal of Infection Control*, *38*(9), 678–682. https://doi.org/10.1016/j.ajic.2010.07.002

Daniel Hernandes, S. E., De Mello, A. C., Sant'Ana, J. J., Soares, V. S., Cassiolato, V., Garcia, L. B., & Cardoso, C. L. (2004). The effectiveness of alcohol gel and other hand-cleansing agents against important nosocomial pathogens. *Brazilian Journal of Microbiology*, *35*(1–2), 33–39. https://doi.org/10.1590/s1517-83822004000100005

De Aceituno, A. F., Bartz, F. E., Hodge, D. W., Shumaker, D. J., Grubb, J. E., Arbogast, J. W., Dávila-Aviña, J., Venegas, F., Heredia, N., García, S., & Leon, J. S. (2015). Ability of hand hygiene interventions using alcohol-based hand sanitizers and soap to reduce microbial load on farmworker hands soiled during harvest. *Journal of Food Protection*, *78*(11), 2024–2032. https://doi.org/10.4315/0362-028X.JFP-15-102

Desmarchelier, P., & Fegan, N. (2014). Escherichia Coli: Escherichia coli.

Encyclopedia of Food Microbiology: Second Edition, 688–694. https://doi.org/10.1016/B978-0-12-384730-0.00100-2

Dharan, S., Hugonnet, S., Hugo, S., & Pittet, D. (2003). Comparison of waterless hand antisepsis agents at short application times: raising the flag of concern. *Infection Control and Hospital Epidemilogy*, *24*(3), 160–164.

Eggers, M., Koburger-Janssen, T., Ward, L. S., Newby, C., & Müller, S. (2018). Bactericidal and Virucidal Activity of Povidone-Iodine and Chlorhexidine Gluconate Cleansers in an In Vivo Hand Hygiene Clinical Simulation Study. *Infectious Diseases and Therapy 2018 7:2*, *7*(2), 235–247. https://doi.org/10.1007/S40121-018-0202-5

Fabiszewski de Aceituno, A., Heredia, N., Stern, A., Bartz, F. E., Venegas, F., Solís-Soto, L., Gentry-Shields, J., Jaykus, L. A., Leon, J. S., & García, S. (2016). Efficacy of two hygiene methods to reduce soil and microbial contamination on farmworker hands during harvest. *Food Control*, *59*, 787–792. https://doi.org/10.1016/j.foodcont.2015.06.059

Figueiredo, A. E., de Siqueira, S. L., Poli-de-Figueiredo, C. E., & d'Avila, D. O. (2013). Hand hygiene in peritoneal dialysis patients: A comparison of two techniques. *Peritoneal Dialysis International*, *33*(6), 655–661. https://doi.org/10.3747/pdi.2012.00298

Gaonkar, T. A., Geraldo, I., Caraos, L., & Modak, S. M. (2005). An alcohol hand rub containing a synergistic combination of an emollient and preservatives: Prolonged activity against transient pathogens. *Journal of Hospital Infection*, *59*(1), 12–18. https://doi.org/10.1016/j.jhin.2004.06.030

Girou, E., Loyeau, S., Legrand, P., Oppein, F., & Brun-Buisson, C. (2002). Efficacy of handrubbing with alcohol based solution versus standard handwashing with antiseptic soap: Randomised clinical trial. *British Medical Journal*, *325*(7360), 362–365. https://doi.org/10.1136/bmj.325.7360.362

Golin, A. P., Choi, D., & Ghahary, A. (2020). Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. *American Journal of Infection Control*, *48*(9), 1062–1067. https://doi.org/10.1016/j.ajic.2020.06.182

Gopalakrishnan, S., & Ganeshkumar, P. (2013). Systematic Reviews and Meta-analysis: Understanding the Best Evidence in Primary Healthcare. *Journal of Family Medicine and Primary Care*, *2*(1), 9. https://doi.org/10.4103/2249-4863.109934

Goroncy-Bermes, P., Koburger, T., & Meyer, B. (2010). Impact of the amount of hand rub applied in hygienic hand disinfection on the reduction of microbial counts on hands. *Journal of Hospital Infection*, *74*(3), 212–218. https://doi.org/10.1016/j.jhin.2009.09.018

Gottenbos, B., Grijpma, D. W., van der Mei, H. C., Feijen, J., & Busscher, H. J. (2001).

Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. *Journal of Antimicrobial Chemotherapy*, *48*(1), 7–13. https://doi.org/10.1093/JAC/48.1.7

Green, S. (2005). Systematic reviews and meta-analysis. *Singapore Med J*, *46*(6), 270–274.

Greenaway, R. E., Ormandy, K., Fellows, C., & Hollowood, T. (2018). Impact of hand sanitizer format (gel/foam/liquid) and dose amount on its sensory properties and acceptability for improving hand hygiene compliance. *Journal of Hospital Infection*, *100*(2), 195–201. https://doi.org/10.1016/j.jhin.2018.07.011

Guilhermetti, M., Daniel Hernandes, S. E., Fukushigue, Y., Garcia, L. B., & Cardoso, C. L. (2001). Effectiveness of hand-cleansing agents for removing methicillin-resistant staphylococcus aureus from contaminated hands. *Infection Control and Hospital Epidemilogy*, *22*(2), 105–108.

Herruzo, R., Yela, R., & Vizcaino, M. J. (2015). Lasting hand self-disinfection: A backup for hospital hand hygiene? *American Journal of Infection Control*, *43*(7), 697–701. https://doi.org/10.1016/j.ajic.2015.03.014

Jabbar, U., Leischner, J., Kasper, D., Gerber, R., Sambol, S. P., Parada, J. P., Johnson, S., & Gerding, D. N. (2010). Effectiveness of Alcohol-Based Hand Rubs for Removal of Clostridium difficile Spores from Hands . *Infection Control & Hospital Epidemiology*, *31*(6), 565–570. https://doi.org/10.1086/652772

Jain, V. M., Karibasappa, G. N., Dodamani, A. S., Prashanth, V. K., & Mali, G. V. (2016). Comparative assessment of antimicrobial efficacy of different hand sanitizers: An in vitro study. *Dental Research Journal*, *13*(5), 424–431. https://doi.org/10.4103/1735-3327.192283

Jing, J. L. J., Yi, T. P., Bose, R. J. C., Mccarthy, J. R., Tharmalingam, N., & Madheswaran, T. (2020). Hand Sanitizers : A Review on Formulation Aspects , Adverse E ff ects , and Regulations. *International Journal of Environmental Research and Public Health*, *17*, 3326.

Kac, G., Podglajen, I., Gueneret, M., Vaupré, S., Bissery, A., & Meyer, G. (2005). Microbiological evaluation of two hand hygiene procedures achieved by healthcare workers during routine patient care: A randomized study. *Journal of Hospital Infection*, *60*(1), 32–39. https://doi.org/10.1016/j.jhin.2004.10.014

Kampf, G. (2008). How effective are hand antiseptics for the postcontamination treatment of hands when used as recommended? *American Journal of Infection Control*, *36*(5), 356–360. https://doi.org/10.1016/j.ajic.2007.07.017

Kampf, G., & Kramer, A. (2004). Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. In *Clinical Microbiology Reviews* (Vol. 17, Issue 4, pp. 863–893). https://doi.org/10.1128/CMR.17.4.863-893.2004

Kampf, G., Löffler, H., & Gastmeier, P. (2009). Hand Hygiene for the Prevention of Nosocomial Infections. *Deutsches Arzteblatt*, *106*(40), 649–655. https://doi.org/10.3238/arztebl.2009.0649

Kawagoe, J. Y., Graziano, K. U., Valle Martino, M. D., Siqueira, I., & Correa, L. (2011). Bacterial reduction of alcohol-based liquid and gel products on hands soiled with blood. *American Journal of Infection Control*, *39*(9), 785–787. https://doi.org/10.1016/j.ajic.2010.12.018

Khairnar, M. R., Anitha, G., Dalvi, T. M., Kalghatgi, S., Datar, U. V., Wadgave, U., Shah, S., & Preet, L. (2020). Comparative efficacy of hand disinfection potential of and sanitizer and liquid soap among dental students: A randomized controlled trial. *Indian Journal of Critical Care Medicine*, *24*(5), 336–339. https://doi.org/10.5005/jp-journals-10071-23420

Kmet, L. M., Lee, R. C., & Cook, L. S. (2004). Standard quality assessment criteria for evaluating primary research papers from a variety of fields. Alberta. In *Alberta Herit Found Med Res* (Vol. 13, Issue February). https://www.ihe.ca/advanced-search/standard-quality-assessment-criteria-for-evaluating-primary-research-papers-from-a-variety-of-fields

Lakhundi, S., & Zhang, K. (2018). Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. *Clinical Microbiology Reviews*, *31*(4), 1–103. https://doi.org/10.1128/CMR.00020-18

Larson, E., & Bobo, L. (1992). Effective hand degerming in the presence of blood. *Journal of Emergency Medicine*, *10*(1), 7–11. https://doi.org/10.1016/0736-4679(92)90003-C

Larson, E. L., Eke, P. I., & Laughon, B. E. (1986). Efficacy of alcohol-based hand rinses under frequent-use conditions. *Antimicrobial Agents and Chemotherapy*, *30*(4), 542–544. https://doi.org/10.1128/AAC.30.4.542

Lucet, J. C., Rigaud, M. P., Mentre, F., Kassis, N., Deblangy, C., Andremont, A., & Bouvet, E. (2002). Hand contamination before and after different hand hygiene techniques: A randomized clinical trial. *Journal of Hospital Infection*, *50*(4), 276–280. https://doi.org/10.1053/jhin.2002.1202

Macinga, D. R., Shumaker, D. J., Werner, H. P., Edmonds, S. L., Leslie, R. A., Parker, A. E., & Arbogast, J. W. (2014). The relative influences of product volume, delivery format and alcohol concentration on dry-time and efficacy of alcohol-based hand rubs. *BMC Infectious Diseases*, *14*(1), 1–8. https://doi.org/10.1186/1471-2334-14-511

Matsumoto, K., Shigemi, A., Yaji, K., Shimodozono, Y., Takeda, Y., Ikawa, K., Morikawa, N., Miyanohara, H., Kawamura, H., Orita, M., Tokuda, K., Nishi, J., & Yamada, K. (2012). Reduction in the incidence of MRSA with use of alcohol-based hand rub solutions and gloves. *Journal of Infection and Chemotherapy*, *18*(2), 269–271. https://doi.org/10.1007/s10156-011-0291-5

Mattioli, M. C., Boehm, A. B., Davis, J., Harris, A. R., Mrisho, M., & Pickering, A. J. (2014). Enteric pathogens in stored drinking water and on caregiver's hands in Tanzanian households with and without reported cases of child diarrhea. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0084939

Moadab, A., Rupley, K. F., & Wadhams, P. (2001). Effectiveness of a nonrinse, alcohol-free antiseptic hand wash. *Journal of the American Podiatric Medical Association*, *91*(6), 288–293. https://doi.org/10.7547/87507315-91-6-288

Muka, T., Glisic, M., Milic, J., Verhoog, S., Bohlius, J., Bramer, W., Chowdhury, R., & Franco, O. H. (2020). A 24-step guide on how to design, conduct, and successfully publish a systematic review and meta-analysis in medical research. *European Journal of Epidemiology*, *35*(1), 49–60. https://doi.org/10.1007/s10654-019-00576-5

Nasution, T. A., Yunita, R., Pasaribu, A. P., & Ardinata, F. M. (2019). Effectiveness hand washing and hand rub method in reducing total bacteria colony from nurses in Medan. *Open Access Macedonian Journal of Medical Sciences*, *7*(20), 3380–3383. https://doi.org/10.3889/oamjms.2019.427

Nijssen, S., Bootsma, M., & Bonten, M. (2006). Potential Confounding in Evaluating Infection-Control Interventions in Hospital Settings: Changing Antibiotic Prescription. *Clinical Infectious Diseases*, *43*(5), 616–623. https://doi.org/10.1086/506438

O'Leary, F. M., & Price, G. J. (2011). Alcohol hand gel - A potential fire hazard. *Journal of Plastic, Reconstructive and Aesthetic Surgery*, *64*(1), 131–132. https://doi.org/10.1016/j.bjps.2010.03.026

Oke, M. A., Bello, A. B., Odebisi, M. B., Ahmed El-Imam, A. M., & Kazeem, M. O. (2013). Evaluation of antibacterial efficacy of some alcohol-based hand sanitizers sold in Ilorin (North-central Nigeria). *Ife Journal of Science*, *15*(1), 111–117.

Otokunefor, K., & Princewill, I. (2017). Evaluation of antibacterial activity of hand sanitizers – An in vitro study. *Applied Sciences and Environmental Management*, *21*(7), 1276–1280.

Otto, M. (2009). Staphylococcus epidermidis - The "accidental" pathogen. In *Nature Reviews Microbiology* (Vol. 7, Issue 8, pp. 555–567). NIH Public Access. https://doi.org/10.1038/nrmicro2182

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *International Journal of Surgery*, *88*, 1–11. https://doi.org/10.1016/j.ijsu.2021.105906

Paula, H., Hübner, N. O., Assadian, O., Bransmöller, K., Baguhl, R., Löffler, H., & Kramer, A. (2017). Effect of hand lotion on the effectiveness of hygienic hand

antisepsis: Implications for practicing hand hygiene. *American Journal of Infection Control*, *45*(8), 835–838. https://doi.org/10.1016/j.ajic.2017.05.020

Paulson, D. S., Fendler, E. J., Dolan, M. J., & Williams, R. A. (1999). A close look at alcohol gel as an antimicrobial sanitizing agent. *American Journal of Infection Control*, *27*(4), 332–338. https://doi.org/10.1016/S0196-6553(99)70053-2

Picheansathian, W. (2004). A systematic review on the effectiveness of alcoholbased solutions for hand hygiene. *International Journal of Nursing Practice*, *10*(1), 3–9. https://doi.org/10.1111/j.1440-172X.2003.00457.x

Pickering, A. J., Boehm, A. B., Mwanjali, M., & Davis, J. (2010). Efficacy of waterless hand hygiene compared with handwashing with soap: A field study in Dar es Salaam, Tanzania. *American Journal of Tropical Medicine and Hygiene*, *82*(2), 270–278. https://doi.org/10.4269/ajtmh.2010.09-0220

Pickering, A. J., Davis, J., Walters, S. P., Horak, H. M., Keymer, D. P., Mushi, D., Strickfaden, R., Chynoweth, J. S., Liu, J., Blum, A., Rogers, K., & Boehm, A. B. (2010). Hands, water, and health: Fecal contamination in Tanzanian communities with improved, non-networked water supplies. *Environmental Science and Technology*, *44*(9), 3267–3272. https://doi.org/10.1021/es903524m

Pittet, D., Hugonnet, S., Harbarth, S., Mourouga, P., Sauvan, V., Touveneau, S., & Perneger, T. V. (2000). Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. *Lancet*, *356*(9238), 1307–1312. https://doi.org/10.1016/S0140-6736(00)02814-2

Presterl, E., Suchomel, M., Eder, M., Reichmann, S., Lassnigg, A., Graninger, W., & Rotter, M. (2007). Effects of alcohols, povidone-iodine and hydrogen peroxide on biofilms of Staphylococcus epidermidis. *Journal of Antimicrobial Chemotherapy*, *60*(2), 417–420. https://doi.org/10.1093/jac/dkm221

Racicot, M., Kocher, A., Beauchamp, G., Letellier, A., & Vaillancourt, J. P. (2013). Assessing most practical and effective protocols to sanitize hands of poultry catching crew members. *Preventive Veterinary Medicine*, *111*(1–2), 92–99. https://doi.org/10.1016/j.prevetmed.2013.03.014

Reubsaet, L. L., & Ekkelenkamp, M. B. (2017). Pathogen-directed antibiotic therapy. *Management of Periprosthetic Joint Infections (PJIs)*, 231–255. https://doi.org/10.1016/B978-0-08-100205-6.00011-2

Salmon, S., Truong, A. T., Nguyen, V. H., Pittet, D., & McLaws, M. L. (2014). Health care workers' hand contamination levels and antibacterial efficacy of different hand hygiene methods used in a Vietnamese hospital. *American Journal of Infection Control*, *42*(2), 178–181. https://doi.org/10.1016/j.ajic.2013.07.013

Sasahara, T., Hayashi, S., Hosoda, K., Morisawa, Y., & Hirai, Y. (2014). Comparison of hand hygiene procedures for removing Bacillus cereus spores. *Biocontrol Science*, *19*(3), 129–134. https://doi.org/10.4265/bio.19.129

Schaffner, D. W., Bowman, J. P., English, D. J., Fischler, G. E., Fuls, J. L., Krowka, J. F., & Kruszewski, F. H. (2014). Quantitative microbial risk assessment of antibacterial hand hygiene products on risk of shigellosis. *Journal of Food Protection*, *77*(4), 574–582. https://doi.org/10.4315/0362-028X.JFP-13-366

Schmidt, W.-P., Aunger, R., Coombes, Y., Maina, P. M., Matiko, C. N., Biran, A., & Curtis, V. (2009). Determinants of handwashing practices in Kenya: the role of media exposure, poverty and infrastructure. *Tropical Medicine & International Health : TM & IH*, *14*(12), 1534–1541. https://doi.org/10.1111/J.1365-3156.2009.02404.X

Sharma, V. S., Dutta, S., Taneja, N., & Narang, A. (2013). Comparing hand-hygiene measures in a neonatal ICU: A randomized cross-over trial. *Indian Pediatrics*, *50*(10), 917–921. https://doi.org/10.1007/s13312-013-0261-3

Singh, P., Potlia, I., Malhotra, S., Dubey, H., & Chauhan, H. (2020). Hand Sanitizer an Alternative to Hand Washing—A Review of Literature. *Journal of Advanced Oral Research*, *11*(2), 137–142. https://doi.org/10.1177/2320206820939403

Sizar, O., & Unakal, C. G. (2021). Gram Positive Bacteria. *Management of Antimicrobials in Infectious Diseases*, 29–41. https://www.ncbi.nlm.nih.gov/books/NBK470553/

Srinivasan, L., & Evans, J. R. (2018). Health Care-Associated Infections. *Avery's Diseases of the Newborn: Tenth Edition*, 566-580.e6. https://doi.org/10.1016/B978-0-323-40139-5.00040-1

Thaddeus, N., Francis, E., Jane, O., Obumneme, A., & Okechukwu, E. (2018). Effects of some common additives on the antimicrobial activities of alcohol-based hand sanitizers. *Asian Pacific Journal of Tropical Medicine*, *11*(3), 222–226. https://doi.org/10.4103/1995-7645.228437

Thairu, Nasir, I. A., & Usman, Y. (2014). Laboratory perspective of gram staining and its significance in investigations of infectious diseases. *Sub-Saharan African Journal of Medicine*, *1*(4), 168. https://doi.org/10.4103/2384-5147.144725

Tvedt, C., & Bukholm, G. (2005). Alcohol-based hand disinfection: A more robust hand-hygiene method in an intensive care unit. *Journal of Hospital Infection*, *59*(3), 229–234. https://doi.org/10.1016/j.jhin.2004.10.003

van Haren, F. M. P., Foudraine, N., & Gillham, M. (2007). Infection and Sepsis. *Cardiothoracic Critical Care*, 510–536. https://doi.org/10.1016/B978-075067572-7.50038-2

Verhagen, A. P., De Vet, H. C. W., De Bie, R. A., Boers, M., & Van Den Brandt, P. A. (2001). The art of quality assessment of RCTs included in systematic reviews. *Journal of Clinical Epidemiology*, *54*(7), 651–654. https://doi.org/10.1016/S0895-4356(00)00360-7

Vuong, C., & Otto, M. (2002). Staphylococcus epidermidis infections. In *Microbes*

and Infection (Vol. 4, Issue 4, pp. 481–489). Elsevier Masson. https://doi.org/10.1016/S1286-4579(02)01563-0

Wilkinson, M. A. C., Ormandy, K., Bradley, C. R., Fraise, A. P., & Hines, J. (2017). Dose considerations for alcohol-based hand rubs. *Journal of Hospital Infection*, *95*(2), 175–182. https://doi.org/10.1016/j.jhin.2016.12.023

Wilkinson, M. A. C., Ormandy, K., Bradley, C. R., & Hines, J. (2018). Comparison of the efficacy and drying times of liquid, gel and foam formats of alcohol-based hand rubs. *Journal of Hospital Infection*, *98*(4), 359–364. https://doi.org/10.1016/j.jhin.2017.09.024

Wolfe, M. K., Gallandat, K., Daniels, K., Desmarais, A. M., Scheinman, P., & Lantagne, D. (2017). Handwashing and Ebola virus disease outbreaks: A randomized comparison of soap, hand sanitizer, and 0.05% chlorine solutions on the inactivation and removal of model organisms Phi6 and E. coli from hands and persistence in rinse water. *PLoS ONE*, *12*(2), 1–19. https://doi.org/10.1371/journal.pone.0172734

World Health Organization. (2009). *WHO Guidelines on Hand Hygiene in Health Care*.

APPENDICES

Appendix A: Details of The Included Studies

Table 1. Details of the included studies (n=41).

Citation	Study Design, Participant, Setting	Intervention	Test Method	Bacterial Contamination	Outcome Measures	Relative Efficacy
Larson, Eke & Laughon, 1986	RCT, 50 healthy volunteers, hospital	Five hand washing regimens ((1) nonmedicated soap (Safe 'n Sure), (control), (2) alcohol-based hand rinse containing 60% IPA in emollients (Cal Stat) (Alc A), (3) alcohol-based hand rinse containing 70% IPA and 0.3% CHG in emollients (Hibistat) (Alc B), (4) detergent containing 4% CHG and 4% IPA (Hibiclens) (CHG), (5) 70% IPA (IPA)) as hand hygiene.	Bag broth technique	Existing hand flora	Percent reduction	Alc A, CHG and IPA were showing a significant reduction after first wash compared to the baseline group. Overall, the alcohol-based hand rinses showed a better efficacy compared to control and the study concluded that alcohol-based hand rinses are effective and recommended to use as a health care personnel hand wash when water is inaccessible.

Butz, Laughon, Gullette & Larson, 1990	RCT, 48 healthy volunteers, laboratory	Four products ((1)30% ethanol-impregnated hand wipes (Microclens) (alcohol wipe), (2) detergent (1% triclosan) (Tri), (3) detergent (4% CHG) (Chg), (4) liquid nonmedicated soap (control)	Glove juice method	Existing hand flora	Log reduction	There was a significant reduction in all four treatment groups after 15 washes on day 1. Control soap showed a greater significant reduction than alcohol wipes. At the end of the test day (total 75 handwashes at day 5), the product that showed the greatest log reduction observed was Chg and alcohol wipe ranked at 3rd from the four products.
Larson & Bobo, 1992	RCT, 72 healthy volunteers, laboratory	Six hand hygiene products ((1) 70% IPA (IPA), (2) liquid hand rinse containing 70% ethanol and 0.5% CHG (EA), (3) liquid detergent containing 7.5% PVP-I, (4) liquid detergent-based handwashing product containing 4% CHG (CHG), (5) nonantimicrobial liquid	Bag broth technique	Existing hand flora	Log reduction	Two alcohol-based products (IPA and EA) have a significant greater reduction of the bacteria in the presence of bloods compared to the other products, but the physical appearance of the blood still exist on hands.

		soap (soap), (6) control (no degerming product) used in the presence of blood				
Cardoso, Pereira, Zequim & Guilhermetti, 1999	RCT, 5 healthy volunteers, laboratory	Four hand cleansing agents ((1) plain liquid soap, (2) 70% ethanol, (3) 10% PVP-I detergent containing 1% active iodine, (4) 4% CHG detergent containing 4% IPA) in reducing light or heavy contamination of *Acinetobacter baumanniii*	Drop-plate technique	Artificial contamination (*A. baumannii*)	Percent reduction	When the light contamination of *A. baumannii* was done, all the four hand cleansing agents were able to reduce it, but no significant differences observed between the groups. When heavy contamination is done, there were significant reductions observed of bacteria when using 70% ethanol and 10% PVP-I compared to plain liquid soap and 4% CHG.
Paulson, Fendler, Dolan &	RCT, 25 healthy volunteers, laboratory	Five hand cleansing regimens ((1) nonantimicrobial lotion soap, (2) antimicrobial lotion soap (0.6% PCMX), (3) alcohol gel sanitizer (62%	Glove juice method	Existing hand flora (transient)	Mean log reduction	Exploratory Data Analysis showed that alcohol gel sanitizer is the only stand-alone product that was able to be

Williams, 1999		ethanol), (4) nonantimicrobial lotion soap and alcohol gel sanitizer (62% ethanol), (5) antimicrobial lotion soap and alcohol gel sanitizer (0.6% PCMX and 62% ethanol) in reducing microorganisms				effective in reducing the bacteria.
Guilhermetti, Hernandes, Fukushigue, Garcia & Cardoso, 2001	RCT, 5 healthy volunteers, laboratory	four hand cleansing agents ((1) plain liquid soap, (2) 70% ethanol, (3) 10% PVP-I detergent containing 1% active iodine, (4) 4% CHG detergent containing 4% IPA) in reducing light or heavy contamination of MRSA	Drop-plate technique	Artificial contamination (MRSA)	RF	When the light contamination of MRSA was done, 10% PVP-I and 70% ethanol had significantly higher reduction rates than the other two agents. The 10% PVP-I showed the highest reduction factor for both light and heavy contamination of MRSA.
Moadab, Rupley & Wadhams, 2001	Quasi experimental design, 40 healthy	The comparison of alcohol-based hand sanitizer (Purell containing 62% ethanol) and SAB hand wash (HandClens	Glove juice method	Artificial contamination (*S. marcescens*)	Log reduction	The efficacy of alcohol-based hand sanitizer was significantly less effective than the SAB hand wash.

	volunteers, laboratory	containing 0.13% BZK) in reducing *Serratia marcescens*				
Girou, Loyeau, Legrand, Oppein & Brun-Buisson, 2002	RCT, 23 HCW, hospital	Two hand hygiene methods (alcohol-based solution, Sterillium containing 45% 2-propanol and 30% 1-propanol, and handwashing with antiseptic soap, Hibiscrub containing 4% CHG) as hand hygiene before and after patient care	Fingertip sampling	Existing hand flora	Percent reduction	Handrubbing with ABHS showed a significantly higher median percentage of reduction compared to handwashing method.
Lucet et al., 2002	RCT, 43 HCW, hospital	Six hand hygiene methods (handwashing with unmedicated soap ((1) 10s, (2) 30s), handwashing with antiseptic soap, Hibiscrub containing 4% CHG or Betadine containing 10% PVP-I ((3)10s, (4) 30s, (5) 60s), (6) alcohol-based hand rub, Sterillium containing 45% 2-propanol and 30% 1-propanol)	Fingertip sampling	Existing hand flora (transient)	Log reduction	When handwashing with antiseptic soap (10s, 30s, 60s), or alcohol-based hand rub, there were significant greater bacterial reduction observed compared with handwashing with unmedicated soap (10s, 30s). No significant difference between handwashing with

						antiseptic soap and alcohol-based hand rub.
Dharan, Hugonnet, Sax & Pittet, 2003	Quasi experimental design, 12 healthy volunteers, laboratory	Four alcohol-based products ((1) hand rinses A containing 80% ethanol, (2) B containing 95% ethanol (Bode Chemie GmbH), and (3) C containing 75% isopropanol (in-house preparation) and (4) alcohol-based hand gel containing 60% isopropanol (Blue Skin)) when applied in a shorter application time (less than 30 seconds)	Modified EN 1500	Artificial contamination (*Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, and a clinical isolate of *Enterococcus faecali*)	RF	All products except alcohol-based hand gel satisfied the EN 1500 standards after applications for 15 and 30 seconds and alcohol-based hand gel showed the lowest RF compared to other products.
Hernandes et al., 2004	RCT, 6 healthy volunteers, laboratory	Five hand hygiene products (1) plain liquid soap, (2) alcohol gel containing ethanol and emollient (Geef Ltda.), (3) 70% ethanol, (4) 10% PVP-I detergent containing 1% active iodine, (5) 4% CHG detergent containing 4% IPA in reducing the	Drop-plate technique	Artificial contamination (*A. baumannii*, MRSA, *E. coli*, *E. faecalis*, *P. aeruginosa,* and *C. albicans*)	Percent reduction	10% povidone-iodine liquid soap, alcohol gel, 70% ethyl alcohol, and 4% chlorhexidine detergent had significant higher reduction rate compared to plain liquid soap for almost every test bacterium.

		artificial contamination of bacteria on hands				
Tvedt & Bukholm, 2005	Quasi experimental design, 80 HCW, hospital	The comparison of handwashing (plain liquid soap, Sterisol), and alcohol-based hand sanitizer (Sterisol Hand Hygiene Product containing 70% isopropanol and glycerol) and the effect when using standardized or non-standardized method in intensive care unit.	Glove juice method	Existing hand flora	Mean reduction	Non-standardized methods after hand disinfection using alcohol-based hand sanitizer showed a significant reduction and showing the highest mean reduction compared to the other groups. The lowest mean reduction observed was non-standardized method done after handwashing showing that hand washing needs to be use in a proper method, while hand disinfection is a robust method.
Kac et al., 2005	RCT, 50 HCW, hospital	Comparison of two hand hygiene methods (handwashing with unmedicated soap & hand rub	Fingertip sampling	Existing hand flora	Percent reduction	Both handwashing and hand rubbing showed a significant reduction, but when compared the

		using ABHS (Sterillium containing 45% 2-propanol and 30% 1-propanol)) used by HCWs				percentage reduction, hand rubbing was significantly higher percentage reduction than handwashing.
Barbut et al., 2007	Quasi experimental design, 81 HCW, hospital	One alcohol hand rinse (Sterillium rinse containing 45% 2-propanol and 30% 1-propanol) and two alcohol-based gel sanitizers (Sterillium gel containing 85% ethanol and Manugel Plus containing 53% ethanol and isopropanol 17%) after patient care	Glove juice method	Existing hand flora	RF	Alcohol hand rinse and Sterillium gel were more effective compared to Manugle Plus and there is statistically significant differences observed.
Kampf, 2008	RCT, 16 healthy volunteers, laboratory	Four alcohol-based hand rub products (hand rub A (85% ethanol), hand rub B (60% ethanol), hand rub C (62% ethanol), and hand rub D (61% ethanol)) in reducing bacteria when different	Glove juice method	Artificial contamination (*S. marcescens*)	Mean log reduction	Hand rub A with the highest concentration of alcohol was significantly better in reducing bacteria compared to the other 3 hand rubs when the product is applied at 2.4 mL. When 3.6 mL of the four products applied, the

		volumes (2.4 mL and 3.6 mL) of products were applied.				better efficacy was observed.
Jabbar et al., 2010	Quasi experimental design, 10 healthy volunteers, laboratory	One handwashing using CHG soap and three ABHRs (Isagel, Endure, Purell with minimum concentration of alcohol for the 3 ABHRs is 60%) products in removing *Clostridium difficile* spores	Hand stamping technique	Artificial contamination (*C. difficile* spores)	Mean log reduction	When washing with CHG soap, there was a significant reduction of bacterial spores compared to each of the 3 ABHRs. The highest mean reduction observed was chlorhexidine washing.
Abaza, Amine & Hazzah, 2010	Quasi experimental design, 20 HCW, hospital	Handwashing method (handwashing with non-antiseptic soap and water) and four ABHS products found in the Egyptian market ((A) hand liquid containing 80% ethanol, 45% iso-propanol, 30% n-propanol, and 0.2% mecetronium etilsulfate 0.2, (B) hand liquid containing 96% ethanol, (C) hand rub gel containing 60% iso-propanol and 65%	Fingertip sampling	Existing hand flora	Percent reduction	No statistically significant differences between the comparison groups. All the four products of ABHS showing a better reduction compared to handwashing. The highest reduction observed was when using product B. Product C showed the lowest reduction demonstrating that the

		ethanol, (D) locally prepared hand rub using WHO formulation containing 80% ethanol) during routine patient care				types of hand sanitizer may affect the efficacy.
D'Antonio, Rihs, Stout & Yu, 2010	Quasi experimental design, 7 healthy volunteers, laboratory	Comparing the higher ethanol content of alcohol hand wipes (Sani-Hands ALC containing 65.9% ethanol) with handwashing with antibacterial soap (Kindest Kare containing 0.75% triclosan), and alcohol-based gel hand rub (Purell containing 62% ethanol) in reducing bacteria and spores	Glove juice method	Artificial contamination (*S. marcescens*, *Geobacillus stearothermophilus* (test organism for evaluation of spores removal))	Mean log reduction	The highest log reduction observed for both bacteria and spores was when using handwashing with antibacterial soap. However, the 65.9% ethanol hand wipes showed a significant higher log reduction of *S. marcescens* and *G. stearothermophilus* spores than 62% ethanol alcohol rub showing that alcohol-based hand wipe is more effective than alcohol-based hand gel rub.

Goroncy-Bermes, Koburger & Meyer, 2010	Quasi experimental design, 16 healthy volunteers, laboratory	Two different alcohol-based hand rubs and the effect of the volume (Product A (Spitacid containing 46% ethanol, 27% 2-propanol and 1% benzyl alcohol)- 2 mL (1), 3 mL (2), 4 mL (3) and Product B (desderman N containing 78.2% ethanol and 0.1% 2-biphenylol)- 2 mL (4), 2.5 mL (5), 3 mL (6), 4 mL (7))	EN 1500	Artificial contamination	Mean log reduction	Both products when using 4 mL of application showed a significantly higher reduction when compared to the reference solution and when a lower volume (2 mL) was used for both products, the mean reduction observed was lower than the reference solution.
Kawagoe, Graziano, Martino, Siqueira & Correa, 2011	Cross-over trial, 14 healthy volunteers, laboratory	Three alcohol-based products (product A, a gel-based containing 62% ethanol (Purell), product B, a gel-based containing 70% ethanol (Alcool Gel Dr Clean), product C, a liquid-based containing 70% ethanol with 2% glycerol (Alcool glicerinadol)) as hand hygiene when hands with bloods	EN 1500	Artificial contamination (*S. marcescens*)	RF	No significant difference among the three products, but all products showed more than 99.9% of bacterial reduction when hands contaminated with bloods.

Sharma, Dutta, Taneja & Narang, 2013	Randomized cross-over trial, 35 HCW, hospital	Three hand hygiene methods (hand washing with plain soap, alcohol hand-rub (Sterillium containing 45% 2-propranol and 30% 1-propranol) and PVP-I hand scrub) used in a neonatal intensive care unit (NICU)	Fingertip sampling	Existing hand flora	Percent reduction	Alcohol hand rub and povidone iodine scrub showed a significantly higher reduction compared to handwashing with plain soap when using it in NICU.
Figueiredo, de Siqueira, Poli-de-Figueiredo & d'Avila, 2013	Controlled clinical trial, 22 patients, hospital	Two hand hygiene methods (handwashing with glycerine soap followed by alcohol hand rub (70% ethanol), and alcohol hand rub only) in preventing peritoneal dialysis (PD)-related infections	Fingertip sampling	Existing hand flora	Mean CFU	The alcohol hand rub alone had significantly lesser bacterial counts compared to the combination of handwashing with alcohol hand rub when the patients undergoing PD.
Racicot, Kocher, Beauchamp, Letellier & Vaillancourt, 2013	Quasi experimental design, 4 crew members, field	Four hand hygiene methods (alcohol-based hand gel alone (Purell containing 62% ethanol) (Protocol 1), water, antibacterial soap and alcohol-based hand gel	Bag broth technique	Artificial contamination (Aerobic and *E. coli*/Coliform)	Log reduction	Protocol 2 (water, soap, and alcohol-based hand gel) significantly more effective than the protocol 4 (wipes and alcohol-based hand gel) when

		(protocol 2), degreasing cream and alcohol-based hand gel (protocol 3), antimicrobial wipes and alcohol-based hand gel (protocol 4)) in reducing the contamination on hands practically among poultry catching crew members. The four crew members applied each protocol with 32 repetitions.				high to moderate aerobic bacterial contamination was done. For coliforms, there is no statistically significant difference between the protocols when the low to moderate bacterial contamination was done. When the high contamination of coliforms was done, alcohol-based gel alone is significantly less effective than the combination of degreasing cream and the alcohol-based gel. The study concluded that using degreasing cream or water and soap before using alcohol-based gel can increase the effectiveness in reducing bacteria.

Macinga et al., 2014	Quasi experimental design, 13 volunteers, laboratory	Three ABHS with different forms and the influence of the application volume (Foam B containing 70% ethanol (Application volume: 1.6 mL, 3 mL), Rinse E containing 80% ethanol; (2 mL, 3 mL), Gel F containing 90% ethanol (2.1 mL, 3 mL))	EN 1500	Artificial contamination	RF	When the three products were applied according to the efficacy at drying volume in 30s (1.6 ml for Foam B, 2 ml for Rinse F, 2.1 mL for Gel F), the RF did not exceed the standard reference product, thus indicated that the products with the volumes used did not satisfy the EN 1500 standard. However, when all the three products applied at 3 mL application, all products satisfied the EN 1500 standard as the RF exceed the standard reference product.
Salmon, Truong, Nguyen, Pittet &	Quasi experimental design, 134 HCW, hospital	Five hand hygiene methods ((1) ABHR (SDS Hand Rub containing 83% ethanol, 12% IPA, and 0.5% CHG; 4%	Fingertip sampling	Existing hand flora	Mean reduction	ABHR showed the greatest reduction compared to the other methods and the

McLaws, 2014		CHG with (2) filtered or (3) unfiltered water; plain soap and water handwashing with (4) filtered or (5) unfiltered water) used by HCW in Vietnamese hospital				difference is significantly different.
Sasahara, Hayashi, Hosoda, Morisawa & Hirai, 2014	Quasi experimental design, 30 healthy volunteers, laboratory	Six different hand hygiene procedures ((1) 30-second water-only washing, (2) 60-second plain soap washing, (3) 30-second plain soap washing, (4) 30-second antiseptic soap washing, (5) hand-rubbing with an alcohol-based liquid (76.9-81.4% ethanol), (6) hand-rubbing with an alcohol-based gel (76.9-81.4% ethanol)) in reducing *Bacillus cereus* spores	Glove juice method	Artificial contamination (*B. cereus* spores)	Log reduction	Both alcohol-based products had the lowest log reduction compared to the other methods showing that alcohol-based hand rubbing is not that effective in reducing bacterial spores. All the handwashing procedures reduce the number of spores effectively.
Schaffner et al., 2014	163 volunteers	Use of five hand hygiene methods (two nonantibacterial hand wash ((1) non-AB (T), (2) non-AB	Bag broth technique	Artificial contamination (*Shigella*)	Log reduction	All the three antibacterial methods showed a statistically significant lower reduction compared

		(KMF)), two antibacterial hand wash ((3) chlorhexidine, (4) triclosan), and alcohol hand sanitizer ((5) Purell containing 62% ethanol)) in reducing *Shigella*				to nonantibacterial methods and the alcohol hand sanitizer showed the greatest reduction.
de Aceituno et al., 2015	RCT, 181 farmworkers, field	Four hand hygiene methods ((1) label-use ABHS (Purell Advanced Instant Hand Sanitizer containing 70% ethanol), (2) two-step ABHS (First step using 3 pumps and second step using 1 pump), (3) traditional soap, (4) pumice soap) in reducing microbial load after harvesting	Bag broth technique	Artificial contamination (Coliforms, *Enterococcus*, *E. coli*)	Log CFU	The two-step ABHS group showed a lower log count of coliforms and *Enterococcus* spp. compared to the label-use ABHS and pumice soap groups. As for the *E. coli*, all four interventions showed a significantly lower log count compared to the control group.
Herruzo, Yela & Vizcaino, 2015	Quasi experimental design, 6 volunteers, laboratory	Different hand antiseptic products ((1) 2% and (2) 5% chlorhexidine, (3) 1% and (4) 10% PVP-I, (5) 60% n-propanol, (6) Sterillium	Fingertip sampling	Artificial contamination (*S. aureus*, *Pseudomonas*	Log reduction	The best products in reducing the bacteria involved were 2% and 5% chlorhexidine and the combination of 0.6%

		containing 45% 2-propranol and 30% 1-propranol, and (7) Alcoaloe containing 0.6% chlorhexidine, isopropanol and 0.1% BZK) used by health care professional and the residual effect of the methods.		*aeruginosa*, *E. coli*)		chlorhexidine, isopropanol and 0.1% BZK. The reduction observed for the two products were significant compared to the other four products.
Appelgrein, Hosgood, Dunn & Schaaf, 2016	Cross-over trial, 20 volunteers, laboratory	Comparison of ozonated water (4 ppm) and ABHS (60% Propan-1-ol) in reducing bacteria	prEN 12791	Existing hand flora	Log CFU	The log count for ABHR is significantly lower after using it compared to ozonated water indicated that ABHR is having better efficacy compared to the ozonated water.
de Aceituno et al., 2016	RCT, 159 farmworkers, field	Comparison of hand washing with nonantibacterial soap and two-step alcohol-based hand sanitizer (Purell Advanced Instant Hand Sanitizer containing 72% ethanol) when harvesting produce among farmworkers	Bag broth technique	Existing hand flora (*E. coli* and *Enterococcus*)	Log CFU	The two step ABHS had a significantly lower log count for coliforms and *Enterococcus* compared to control. The two-step ABHS might be used as hand hygiene in an agricultural field

						environment when soap and water unavailable.
Wolfe et al., 2017	Randomized cross-over trial, 18 volunteers, laboratory	Six handwashing methods ((1) soap and water, (2) ABHS, Purell Advanced Instant Hand Sanitizer (70% ethanol), and (3) 0.05% sodium dichloroisocyanurate (NaDCC), (4) high-test hypochlorite (HTH), and (5) stabilized and (6) non-stabilized sodium hypochlorite (NaOCl) solutions) with and without soil load	Glove juice method	Artificial contamination (*E. coli*)	Log reduction	The six handwashing methods showed an average log reduction of the bacteria compared with the no handwashing (control) when there is soil load or not. The greatest reduction observed when handwashing with NaDCC was done without soil load, while with the soil load, the greatest reduction observed when handwashing with HTH.
Paula et al., 2017	Quasi experimental design, 20 healthy volunteers, laboratory	The efficacy of hand hygiene methods following hand lotion use (only hand rub (group A), hand lotion for 5 min and hand rub (group B), hand lotion for 1 hour and hand rub (group C),	EN 1500	Artificial contamination	Log reduction	Following the use of hand lotion, the hand hygiene methods did not significantly be affected. The study concluded that hand antisepsis could be

		handwashing and hand rub (group D), handwashing, hand lotion (5 min) and hand rub (group E), handwashing, hand lotion (1 hr) and hand rub (group F))				applied after 5 minutes of hand lotion application.
Wilkinson, Ormandy, Bradley, Fraise & Hines, 2017	Quasi experimental design, 15 volunteers, laboratory	Three different formulations of ABHS with different volumes applied (WHOF1: 80% ethanol, 1.45 % glycerol, 0.125% hydrogen peroxide; WHOF2: 75% IPA, 1.45% glycerol, 0.125% hydrogen peroxide; IPA: 60% IPA). The range of volumes were between 0.5 mL to 3.0 mL.	EN 1500	Artificial contamination	RF	No significant difference between the different products across all volumes. The highest RF observed was WHOF1 at 3 mL of application.
Alsagher et al., 2018	Quasi experimental design, 93 volunteers, laboratory	Six hand hygiene methods (non-antiseptic Soap for 10 (1), and 30 (2) seconds, antiseptic soap for 10 (3), 30 (4), and 60 (5) seconds, alcohol-based hand rub (Dettol) (6)) in places of	Fingertip sampling	Existing hand flora	Percent reduction	The use of alcohol-based hand rub showed a significantly higher percent reduction compared to hand washing with soap.

		public concern in Tripoli, Libya.				
Wilkinson, Ormandy, Bradley & Hines, 2018	Quasi experimental design, 20 volunteers, laboratory	Two different formulations of HS (60% IPA and 80% ethanol (WHOF1)) with different formats (liquid, gel and foam)	EN 1500	Artificial contamination	RF	There was no significant difference in the efficacy of two different formulation with different formats. All the products did not meet the EN 1500 standard as the RF did not exceed the standard reference product.
Nasution, Yunita, Pasaribu & Ardinata, 2019	Quasi experimental design, 16 nurses, hospital	Two hand hygiene methods (handwashing with soap (0.175% chloroxylenol and 0.3% salicylic acid) and alcohol hand rub (70% ethyl alcohol)) in reducing bacteria from hands of nurses in Medan	Hand swab	Existing hand flora	Percent reduction	There is no significant difference between handwash and alcohol hand rub. When hand washing with soap, there were higher average reduction compared to hand rub.
Bondurant, Duley & Harbell, 2019	Quasi experimental design, 24 healthy	Comparison of alcohol-free hand sanitizer (commercial brand DAB hand sanitizer containing 0.12% BZK) and alcohol-based hand sanitizer	Cup scrub technique	Artificial contamination (*S. aureus*)	Mean log reduction	There are statistically significant differences between alcohol-free hand sanitizer and alcohol-based hand

	volunteers, laboratory	(alcohol hand sanitizer containing 63% ethanol) and the persistence antibacterial activity after 1, 2, and 4 hours of application.				sanitizer. The log reduction for alcohol-based hand sanitizer is lower compared to the alcohol- free hand sanitizer at all the three time points.
Breidablik et al., 2019	Cross-over trial, 30 healthy volunteers, laboratory	Two hand hygiene methods (ozonized tap water (0.8 or 4 ppm) and standard alcohol-based rub (Antibac containing 85% ethanol, propan-2-ol, and glycerol))	EN 1500	Artificial contamination (*E. coli* (ATCC 25922))	Mean CFU	The ozonated water showed a lower mean count of bacteria compared to the alcohol. The study concluded that ozonated water can be an alternative to alcohol hand sanitizer as it showed an average reduction of bacteria.
Breidablik et al., 2020	Cross-over trial, 55 healthy volunteers, laboratory	Three hand disinfectant methods (alcohol-based hand rub, Antibac containing 85% ethanol, <5% propan-2-ol, and glycerine), non-alcoholic	EN 1500	Artificial contamination	Mean CFU	The using of soap and water showed a significantly better lower count of bacteria compared to alcohol hand rub and ozonated water.

		Antibac hand soap, and 0.8 ppm ozone water)				
Khairnar et al., 2020	RCT, 90 healthy volunteers, laboratory	Three hand hygiene methods ((1) hand cleaning using liquid soap and water (Dettol Original Liquid Handwash containing aqua sodium laureth sulphate, glycol stearate, methyl isothiazolinone, and sodium hydroxide), (2) hand cleaning using ABHS (Sterillium containing 45% 2-propranol and 30% 1-propranol), (3): hand cleaning using liquid soap and water followed by application of hand sanitizer (Dettol Liquid Soap followed by Sterillium))	Hand swab	Existing hand flora	Percent reduction	The combination of soap and alcohol-based hand sanitizer showed the highest reduction compared to alcohol-based hand sanitizer or soap alone.