EFFICACY OF HAND SANITIZERS AGAINST STANDARD BACTERIAL CULTURES

A PROJECT WORK SUBMITTED TO THE

DEPARTMENT OF MICROBIOLOGY

AMRIT CAMPUS

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE AWARD OF

BACHELOR OF SCIENCE (B. Sc.) IN MICROBIOLOGY

BY

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This is to recommend that **Ms. Shyron Sherpa,** Symbol No: 500330084, T.U. Registration No. 5-2-33-156-2017, has carried out project work entitled **"Efficacy of Hand Sanitizers against Standard Bacterial Cultures"** for the requirement to the project work in Bachelor of Science (B.Sc.) degree in Microbiology under my supervision in the Department of Microbiology, Amrit Campus, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

To my knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the submission of the project work for the partial fulfillment of Bachelor of Science (B.Sc.) degree.

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DECLARATION

This project work entitled **"Efficacy of Hand Sanitizers against Standard Bacterial Cultures"** is being submitted to the Department of Microbiology, Amrit Campus, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the partial fulfillment of the requirement to the project work in Bachelor of Science (B.Sc.) degree in Microbiology. This project work is carried out by me under the supervision of Assistant Professor Suman Rai in the Department of Microbiology, Amrit Campus, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

This work is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

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On the recommendation of **Assistant Prof. Suman Rai,** this project work is submitted by **Ms. Shyron Sherpa,** Symbol No: 500330084, T.U. Registration No: 5-2-33-156-2017, entitled **"Efficacy of Hand Sanitizers against Standard Bacterial Cultures"** is forwarded by the Department of Microbiology, Amrit Campus, for the Approval to the Evaluation Committee, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the project work.

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This project work (PRO-406) entitled **"Efficacy of Hand Sanitizers against Standard Bacterial Cultures"** by Ms. Shyron Sherpa (Symbol No: 500330084 and T.U. Registration No: 5-2-33-156-2017) under the supervision ofAssistant Professor Suman Rai in the Department of Microbiology, Amrit Campus, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), is hereby submitted for the partial fulfillment of the Bachelor of Science (B.Sc.) degree in Microbiology. This report has been accepted and forwarded to the Controller of Examination, Institute of Science and Technology, Tribhuvan University, Nepal for the legal procedure.

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ABSTRACT

Hand sanitizers are used to maintain hand hygiene. Corona pandemic had gain the attention; many companies are producing it and are marketed but their efficacy have not been well established. Thus, the main aim of this study was to evaluate the antibacterial efficacy of the hand sanitizers sold in Kathmandu. This study was carried out from March to April, 2022. In this study, 31 alcohol based hand sanitizers were purchased from the markets of Kathmandu. Among them, 15 liquid based hand sanitizers (9 contain alcohol and 6 contain alcohol with additional ayurvedic ingredients) and among 16 gel based hand sanitizers, (10 contain alcohol and 6 contain alcohol and additional ayurvedic ingredients). Efficacy of hand sanitizers were evaluated using standard ATCC cultures: *Escherichia coli* 35218, *Escherichia coli* 25922, *Staphyloccoccus aureus* 43300 and *Klebsiella pneumoniae* 700603 by agar well diffusion method. In the volume of 50 µl hand sanitizers, (46.67%, 60%, 26.67% and 33.33%) liquid based, (20%, 40%, 33.33% and 26.67%) liquid with ayurvedic ingredients based, (12.5%, 18.75%, 18.75% and 12.5%) gel based and (12.5%, 12.5%, 12.5% and 6.25%) gel with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. In the volume of 100 µl hand sanitizers, (53.33%, 60%, 46.67% and 46.67%) liquid based, (40%, 40%, 40% and 40%) liquid with ayurvedic ingredients based, (50%, 62.5%, 62.5% and 62.5%) gel based and (25%, 31.25%, 25% and 25%) gel with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. In the volume of 150 µl hand sanitizers, (60%, 60%, 60% and 60%) liquid based, (40%, 40%, 40% and 40%) liquid with ayurvedic ingredients based, (62.5%, 62.5%, 62.5% and 62.5%) gel based and (31.25%, 31.25%, 31.25% and 31.25%) gel with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. Comparatively, liquid with ayurvedic ingredients based sanitizers were more effective than gel with ayurvedic ingredients based sanitizers.

Keywords: Agar well diffusion method, Antibacterial effect, ATCC Cultures, Hand sanitizers.

शोधसार

ह्याण्ड स्यानिटाइजरहरु हातको स्वच्छता कायम राख्न प्रयोग गरिन्छ । कोरोना महामारीले यसको प्रयोग बारे ध्यान खिचेको थियो । धेरै कम्पनीहरुले उत्पादन गरिरहेका छन् तर प्रभावकारीता राम्रोसँग स्थापित हुन सकेको छैन । यस अध्ययनको मुख्य उद्देश्य काठमाण्डौमा बिक्री हुने ह्याण्ड स्यानिटाइजरहरुको व्याक्टेरियालाई मार्ने क्षमताको प्रभावकारीता मूल्याङ्कन गर्नु थियो । यस अध्ययन मार्च देखि अप्रिल २०२२ सम्म गरिएको थियो । यस अध्ययनमा काठमाण्डौका बजारबाट ३१ वटा अल्कोहल आधारित ह्याण्ड स्यानिटाइजर किनिएको थियो । १५ (९ अल्कोहल र ६ आयुर्वेदिक सहित) तरल आधारित ह्याण्ड स्यानिटाइजरहरु तथा १६ (१० अल्कोहल र ६ आयुर्वेदिक सहित) जेल आधारित ह्याण्ड स्यानिटाइजरहरु रहेको छ । ह्याण्ड स्यानिटाइजरहरुको प्रभावकारीता मानक ATCC cultures: *Escherichia coli* 35218, *Escherichia coli* 25922, *Staphylococcus aureus* 43300 , र *Klebsiella pneumoniae* 700603 को प्रयोग गरि एगार ह्वेल डिफ्यूजन विधिद्धारा मूल्याङ्कन गरिएको थियो । ५० µl ह्यान्ड स्यानिटाइजरको मात्रामा (४६.६७%, ६०%, २६.६७% र ३३.३३%) तरल आधारित, (२०%, ४०%, ३३.३३% र २६.६७%) तरल आयुर्वेदिक आधारित, (१२.५%, १८.७५%, १८.७५% र १२.५%) जेल आधारित र (१२.५%, १२.५%, १२.५% र ६.२५%) आयुर्वेदिक आधारित जेल ह्यान्ड सेनिटाइजरले *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 विरुद्ध जीवाणुरोधी प्रभाव देखायो । १०० µl ह्यान्ड सेनिटाइजरको मात्रामा (५३.३३%, ६०%, ४६.६७% र ४६.६७%) तरल आधारित, (४०%, ४०%, ४०% र ४०%) तरल आयुर्वेदिक आधारित, (५०%, ६२.५%, ६२.५% र ६२.५%) जेल आधारित र (२५%, ३१.२५%, २५% र २५%) आयुर्वेदिक आधारित जेल ह्यान्ड सेनिटाइजरले *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 विरुद्ध जीवाणुरोधी प्रभाव देखायो । १५० µl ह्यान्ड सेनिटाइजरको मात्रामा (६०%, ६०%, ६०% र ६०%) तरल आधारित, (४०%, ४०%, ४०% र ४०%) तरल आयुर्वेदिक सामग्रीमा आधारित, (६२.५%, ६२.५%, ६२.५% र ६२.५%) जेल आधारित र (३१.२५%, ३१.२५%, ३१.२५% र ३१.२५%) आयुर्वेदिक आधारित जेल ह्यान्ड सेनिटाइजरले *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 विरुद्ध जीवाणुरोधी प्रभाव देखायो । तुलनात्मक रुपमा आयुर्वेदिक सहित जेल आधारित भन्दा आयुर्वेदिक सहित तरलमा आधारित ह्याण्ड स्यानिटाइजरहरु बढी प्रभावकारि थिए ।

Keywords: Agar well diffusion method, Antibacterial effect, ATCC Cultures, Hand sanitizers.

LIST OF ACRONYMS AND ABBREVIATIONS

ABHRs:	Alcohol Based Hand Rubs
ABHS:	Alcohol Based Hand Sanitizer
ANOVA:	Analysis of Variance
ATCC:	American type Culture Collection
BAC/ BC:	Benzalkonium Chloride
BP:	Boiling Point
CDC:	Centers for Disease Control
CDCP:	Centers for Disease Control and Prevention
CFU:	Colony Forming Unit
DIN:	Deutsches Institut fur Normung
EDTA:	Ethylene Diamine Tetraacetic Acid
EN:	European Norm
FDA:	Food and Drug Administration
H_2SO_4:	Sulfuric Acid
L.S.D:	Least Significant Difference
MHA:	Mueller Hinton Agar
MIC:	Minimum Inhibitory Concentration
MPS:	Methylprednisolone
N/A:	Not Available
NABHS:	Non-alcohol Based Hand Sanitizer
PPS:	Polyphenylene Sulphide
QAC:	Quaternary Ammonium Compounds
Q.S:	Quantity Sufficient
WHO:	World Health Organization
ZOI:	Zone of inhibition

LIST OF SYMBOLS

µl	Microlitre
cm^2	Square centimeter
%	Percentage
mm	Millimeter
nm	Nanometer
gm	Gram
ml	Milliliter
°C	Degree Centrigrade/Celsius
mg	Miligram
L	Litre
v/v	Volume/Volume
w/w	Weight/Weight
lbs	Pound

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CHAPTER 1

1. INTRODUCTION

1.1 General Introduction

A hand sanitizer or hand antiseptic is a supplement or alternative to hand washing with soap and water. Keeping hand clean is one of the most essential actions for the reduction of transmission of infectious diseases in the community and hospitals environment (Pittet 2006; Zapka, 2017). Cold viruses, flu viruses, and pathogenic bacteria are easily spread through public meeting places such as hospital, school, bus, office, etc (Boone, 2007). One gram of human feces which is about the weight of a paper clip can comprises one trillion of microorganisms (Franks, 1998).

Hands are primary mode of transmission of microbes and infections. Hand hygiene is therefore the most important measure to avoid the transmission of harmful germs and prevent the infections. Hand hygiene is the single most important, simplest, and least expensive means of preventing nosocomial infections (Ravi, 2005). Contaminated hands can serve as vectors for the transmission of microorganisms. Pathogenic microorganisms accountable for outbreaks are spread from the hands of the food handler to others when the food handler contaminates his/her hands and then passes these microorganisms to consumers via hand contact with food or drinks. The consumer is exposed following the ingestion of these microorganisms, which may cause gastrointestinal illness. Hand contact with ready-to-eat foods represents a very important mechanism by which pathogens may enter the food supply. Food handlers whose work involves touching unwrapped foods to be consumed raw or without further cooking or other forms of treatment have been identified as a particular risk group (National Disease Surveillance Centre, 2004). To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases, hand washing is absolutely an important precaution. Food production workers and food service personnel must be taught to use correct hand and fingertip washing by management in preparation for work (Snyder, 1988).

A decent hand hygiene practice have been shown to be effective in various situations such as the reduction of gastrointestinal infection and diarrhea (Aiello, 2008; Ejemot, 2015; Meadows, 2004) alleviate the outbreaks of the Ebola-Virus Disease (Wolfe, 2017), lowers the rate of the respiratory illnesses, like common colds (Aiello, 2008), and finally overcome the global morbidity and minimize health care cost (Haque, 2018). In a health

care setting hand washing is mandatory procedure according to Centers for Disease Control and Prevention (CDCP) and it may protect us from thousands of microbes (Aiello, 2008).

It is well recognized that hand hygiene is essential to reducing microbial burden, transmission, and infection. The density and species of bacteria that colonize the hands of individuals are highly variable and can be influenced by a number of factors including age, sex, ethnicity and profession (Rosenthal, 2011). Health care workers have been of particular interest, as they may provide a reservoir for the circulation and transmission of drug- resistant bacteria within the hospital setting (Aiello, 2003). Indeed, studies have revealed that 10.5% to 78.3% of health care workers are colonized with up to 2.4×10^7 per hand of the bacterial pathogen *Staphylococcus aureus* and may be a source of nosocomial *S. aureus* infections (Kampf, 2004). Fortunately, conventional hand washing using water, soap and friction is an effective means of reducing microbial burden, which when combined with other infection control practices (i.e., glove usage, compliance, and education) has significantly reduced microbial transmission, hospital-acquired infections, reduced gastrointestinal and respiratory illness, and improved overall health (Aiello, 2008; Burton, 2011; Sickbert, 2016).

Unfortunately, due to lack of knowledge and awareness about risks and non-availability of hand hygiene facilities, poor hand hygiene practices have been observed (Larson, 1999). To overcome the negative impact of microbial contamination in health-care settings, hand sanitizers are recommended. Use of hand sanitizers has gained popularity in the recent past years which has led to the development, production of several hand sanitizers by various companies (Ochwoto, 2017). With huge amounts spent for advertisements and false claims made by manufacturers, clinicians and common man do not have any clue regarding the effectiveness of these commercially available hand sanitizers (Kotia, 2019).

Hospital-associated and community-acquired infections being a serious public health problem all over the world and have become a major concern (Hassan, 2012). Hospital-acquired infections (nosocomial infections) are infections developing in hospitalized patients, (Jain, 2007) whereas community-acquired infection on the other hand is an infection acquired anywhere other than a healthcare facility (Hassan, 2012). These infections have considerable impacts on individuals such as prolonged hospitalization,

disability, increased risk of antimicrobial resistance, huge financial burden and deaths (WHO, 2009).

Alcohol-Based Hand Rubs (ABHRs) are the most widely used hand sanitizers (White, 2003). They may contain additional active ingredients such as quaternary ammonium compounds (QAC), povidone-iodine, triclosan or chlorhexidine that mainly serve to contribute to the efficacy of formulations (Ayliffe, 1988; Larson, 1986; Rotter, 1999). In the use of alcohol rubs, ethanol destroys bacteria by causing damage to it's cell membrane and denaturation of proteins. Ethanol also prevents the spread of microbes by interfering with cell metabolism and cell division. Although found effective, the mode of action of other antimicrobial agents is not known (Power, 1995; Russell, 1994).

Alcohols act by denaturing proteins, and are most effective at concentrations of 60–80%. Concentrations higher than 80% alcohol are less potent because proteins are not easily denatured in the absence of water (Larson, 1991). The reference standard against which ABHRs are compared is 60% Isopropanol (European standard DIN EN 1500, 2013). In most cases, the efficacy of ethanol and isopropanol are comparable, though ethanol has been found to have better efficacy profile against viruses (Kampf, 2004). Some studies have demonstrated that ethanol gel formulations, unless they have been specially formulated and tested, are less efficacious than ethanol solution formulations (Dharan, 2003; Kramer, 2002).

According to the Centers for Disease Control and Prevention (CDC), alcohols have excellent in vitro germicidal activity against gram-positive and gram-negative vegetative bacteria, including multidrug-resistant pathogens (MRSA, VRE), Mycobacterium tuberculosis, HIV, influenza virus, RSV, vaccinia, and hepatitis B and C viruses. Alcohols manifest a good in vitro germicidal activity against Gram-positive and Gram-negative vegetative bacteria as well as various strains of fungi. However, they have minimal activity against bacterial spores, protozoan oocytes and some non-enveloped (non-lipophilic) viruses (Larson, 1991)

The hand sanitizers available in the market are both alcohol based and non-alcohol. The alcohol based hand sanitizer claims to kills 99.99% microorganisms including the most resistant form. The alcohol free hand sanitizer viz. povidone-iodine, benzalkonium chloride (BAC) or triclosan have persistent antimicrobial activity for a prolonged period and claim to be effective in killing microorganism. The hand sanitizers are

available in the form of liquid, foam or easy flowing gel formulations which can be applied on palm of the hand, rub the product over all surfaces of hands and fingers until hands are dry. The product is widely used by the doctors, surgeons before and after the surgery, pathologists, and researchers and is also used at restaurants, toiletries etc. The medical and applied medical science colleges in their laboratories also have hand sanitizer which the students use after every practical class (Yousaf, 2015). The present study aims to determine the efficacy of locally available hand sanitizers against the standard ATCC cultures namely *E. coli* 35218, *E. coli* 25922, *K. pneumoniae* 700603 and *S. aureus* 43300.

1.2 Rationale

Since, hand sanitizers had become an essential part of hand hygiene, the efficacies of these hand sanitizers are less unknown. So, the findings of this study will be useful in gaining the information regarding the antibacterial effect of hand sanitizers against the standard American Type Culture Collection (ATCC) cultures named *E. coli* 35218, *E. coli* 25922, *K. pneumoniae* 700603 and *S. aureus* 43300. Not only that, it will help the consumers to know about the effectiveness of hand sanitizers. As a result, this could also help manufacturers to know about their product efficacy and do improvements in their products. In today's context, it can be one of the important information for the people in different sectors like hospitals, offices, schools, colleges etc.

1.3 Objectives

1.3.1 General objective

1. To assess the efficacy of hand sanitizers against the standard bacterial cultures.

1.3.2 Specific objectives

1. To determine the zone of inhibition of hand sanitizers in their different volumes.
2. To determine the antibacterial effect of hand sanitizers according to their consistency and ingredients wise.

CHAPTER 2

2. LITERATURE REVIEW

Now, in the era of COVID-19, prevention is pricy more than ever, considering the events still taking place due to the worldwide spread of the various organisms and its ferocity, the virus lives for several hours to days depending on the environment according to the WHO (WHO, 2020). However, not all sanitizers work against all pathogens, in other words, one sanitizer is effective against one type of germs but not the other (Ochwoto, 2017). This effectiveness is determined by several factors including the type and concentration of alcohol, formulation and the nature of product, presence of excipients, applied volume, contact time and viral contamination load (Singh, 2020). Hand cleanliness will be broadly perceived as a large portion vital in keeping the transmission of contamination especially in the case of disease (Haas, 2007). The CDC guideline reported that, about two million people get hospitalized each year due to infections and that around ninety thousands of these patients die as a result of their infections (Zerr, 2005). Improved hand hygiene practice by health care workers and better cleaning of common hospital equipment could reduce the probability of patients becoming colonized and lead to subsequent reductions in infectious diseases. Thus it was calculated that, routine hand hygiene could save one million lives per year (Curtis, 2003).

2.1 Liquid and gel based hand sanitizers

Currently different types of hand sanitizers, cleanser or disinfectants are available on the market in various forms such as gels, quick-drying materials, foams and wipes which are sometimes mixed with moisturizing lotions (FDA, 2009). Liquid based hand sanitizers are those type of hand sanitizers which have liquid consistency whereas gel based hand sanitizers are those type of hand sanitizers which have gel based consistency. Liquids act more rapidly (~15 s) and leave less residual substance on hands. Gels require about 30 seconds to act, and time loss can reduce compliance (Voss, 1997). Some studies reported high efficacy of cleanser in the reduction of microbial flora while others showed counter effect (Blaney *et al*., 2011; Liu *et al*., 2010; CDC, 2003; Boyce and Pittet, 2002; Kampf *et al*., 1999; Sakuragi *et al*., 1995). Generally hand sanitizers are available as alcohol and non-alcohol based cleansers and their use in liquid, foam, gel and cosmetics is common (Boyce and Pittet, 2002).

2.2 Alcohol based hand sanitizers

Alcohol-based hand antiseptics mostly contain isopropanol, ethanol, n-propanol, or a mixture of these as their active ingredients. The antimicrobial activity of alcohols is attributed to their ability to denature and coagulate proteins. This causes microbes to lose their protective coatings and become non-functional. The Center for Disease Control and Prevention recommends formulations containing 80% (percent volume/volume) ethanol or 75% isopropyl alcohol; however, generally speaking, sanitizers containing 60 to 95% alcohol are acceptable. The recommended percentages of ethanol and isopropyl alcohol are kept as 80% and 75% because these values lie in the middle of the acceptable range (Kampf, 2004). Alcohol based hand sanitizers are proved to be the best for gastrointestinal and respiratory infections caused by viruses and gram negative bacteria. The side effect of alcohol based hand sanitizer is its dryness of the skin. However it can be prevented by addition of humectants and skin conditioning agents. Ethanol, the most common alcohol ingredient, appears to be the most effective alcohol against viruses, whereas propanol is considered a better bactericidal alcohol. The combination of alcohols may also have a synergistic effect. The alcohol concentration in hand sanitizers also changes its efficacy, with one study demonstrating that a hand rub with 85% ethanol content was significantly better at reducing bacterial populations than preparations of 60% to 62% ethanol. ABHS also often contain humectants, like glycerin, which helps prevent skin dryness, and emollients or moisturizers, like aloe vera, which help replace some of the water stripped off during use. None of the above-mentioned alcohols have shown a potential for acquired bacterial resistance and are therefore considered highly effective for repeated use in medical settings (Deshpande, 2018; Rai, 2017).

2.3 Non-alcohol based hand sanitizers

Similar to alcohol-based hand sanitizers, benzalkonium chloride (BC), the primary ingredient of NABHS, is generally not effective against non-enveloped viruses (Resnick, 1986) though a study demonstrating its efficacy against the non-enveloped human coxsackie virus suggest exceptions exist (Wood, 1998). Despite this exception, it appears that the lipid envelope of either bacteria or viruses are critical structures for BC's effectiveness. The cationic “headgroup” of BC is progressively adsorbed to the negatively charged phosphate heads of phospholipids in the lipid bilayer, and as a result, increase in concentration. The consistent increase of BC concentration results in reduced fluidity of the membrane and thus the creation of hydrophilic gaps in the membrane. In

addition, the alkyl chain “tail” component of BC further perturbs and disrupts the membrane bilayer by permeating the barrier and disrupting its physical and biochemical properties. Protein function is subsequently disturbed and the combination of the aforementioned effects results in the solubilization of the bilayer constituents into BC/phospholipid micelles (Wessels, 2013). Non-alcohol based hand sanitizer viz. benzalkonium chloride is known to have weak activity against gram negative bacteria as compared to alcohol and is prone to contamination by these bacteria. Hand sanitizer is better option than the soap and water. It is safe and easy to handle and can be used in the community (Yousaf, 2015).

2.4 Use of hand sanitizers

The Centers for Disease Control and Prevention, the World Health Organization, and many other health experts promote hand hygiene as the single most important measure in the prevention of hospital-acquired infections. A study done by Reena Rajkumari (2015) showed the importance of proper hand hygiene in reducing the incidence of nosocomial infections. The use of hand sanitizers has become a cornerstone in clinical practice for the prevention of disease transmission between practitioners and patients (Rajkumari, 2015).

Use of waterless hand sanitizers as an alternative to conventional hand washing has long been debated. Despite some potential advantages over conventional water and soap (quicker and easier usage), instant hand products are generally considered to more effectively meet needs in hospital and health care settings rather than food preparation settings. ABHRs containing 60 to 95% alcohol are recommended as an alternative to hand washing in hospital and health care settings when hands are not visibly soiled (CDC, 2002). In contrast, use of these alternatives has not been recommended in food establishments because of the inability of these products to remove fat and food debris from soiled hands (FDA, 2009).

Dentists are exposed to different types of infectious microorganisms daily. A large number of pathogens are localized in the oral cavity, which can be transmitted in different ways during dental procedures (Decraene, 2008) usually by means of air/water syringe and high-speed instruments (Sotiriou, 2008). Two basic ways for spreading pathogenic microorganisms in a dental practice are blood and saliva through droplet aerosol of infected patients (Nejatidanesh, 2013). It has been shown that the most intensive aerosol

and splash production occurs during the work of an ultrasonic scaling unit and high-speed handpiece (Leggat, 2001).

Simply rubbing or friction and using water thoroughly can reduce the load of bacteria as well as dirt. But nowadays different antimicrobial agents (triclosan, para-chloro-meta-xylinol, chlorhexidine gluconate) are used in soaps to improve the effectiveness of soap during hand washing which adds an extra advantage of killing or inhibiting bacteria but with lower effectivity for gram negative bacteria (Larson, 1995; Jones, 2000).

2.5 Agar well diffusion test for determining antimicrobial efficacy.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Magaldi, 2004; Valgas, 2007) The well variant of agar disk diffusion test using Mueller-Hinton agar was used for evaluating the antimicrobial efficacy of hand sanitizers. McFarland 0.5 turbidity standard was taken as reference to adjust the turbidity of bacterial suspensions. Fifty microliters of the hand sanitizer was introduced into each of the 4 wells while the 5^{th} well incorporated with sterile water served as a control. This was done for all the test organisms and plates were incubated in an incubator for 24 hr at 37℃. After incubation, antimicrobial effectiveness was determined using digital caliper (mm) by measuring the zone of inhibition (Jain, 2016).

2.6 Hand sanitizers efficacy against bacteria and fungi

Traditionally, bacteria on hands can be categorized as resident and transient floras. Common resident floras include *Staphylococcus aureus, Staphylococcus epidermidis*, and *Enterococcus faecalis*, which colonize deep layers of the skin and are resistant to mechanical removal (Jain, 2016). On the other hand, transient floras such as *S. aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, colonize the superficial layers of skin. (Jain, 2016). There are also numerous bacterial strains that can be transmitted to the host from other sources that can potentially develop into a variety of bacterial infections. ABHS are very effective for quickly destroying many pathogens by the action of the aqueous alcohol solution without the need for water or drying with towels. According to the Centers for Disease Control and Prevention (CDC), ABHS have excellent in vitro antimicrobial activity, including multidrug-resistant pathogens, such as methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus* (CDC, 2002). Specific in vitro studies show that hand sanitizers containing 60%-80% ethanol produced 4 to 6 log reduction in 15-30 seconds against a range of bacterial and fungal species (Fendler,

2002). Numerous studies have also documented in vivo antimicrobial activity from contaminated hands (Di Muzio, 2015; Ramasethu, 2017) While different alcohol-based hand sanitizers all demonstrated antimicrobial effects against various gram-positive and gram-negative bacteria using the Kirby-Bauer method, which uses antibiotic-impregnated disks to test the susceptibility of strains, propanol-based sanitizers were more effective compared to ethanol with the greatest zone of inhibition (Gold, 2018; Jain, 2016).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Materials

All the materials including chemical, microbiological media are given in Appendix IV.

3.1.1 Sample collection and size

Thirty one hand sanitizers used in the study were purchased and collected in their sealed form available in the local markets of Kathmandu. Hand sanitizers that are being sold in markets were included or without sealed or expired and unlabeled hand sanitizers were excluded from the study. All the hand sanitizers were represented by their symbols viz: ; MA, YS, AU, IT, BA, SR, CS, AW, PC, VS, SS, HA, SH, SA, RB, IC, GR, KL, PSK, AK, WF, FL, MK, KS, ST, PJ, CR, TP, IS, SP and NY. Their composition are given in Appendix VII.

3.1.2 Description of the research site

Thirty one hand sanitizers were purchased and collected from the local markets of Kathmandu. Their efficacy were tested against the standard American Type Culture Collection (ATCC) in the laboratory of Department of Microbiology, Amrit Campus, Kathmandu.

3.2 Methods

3.2.1 Research design

This study has descriptive and quantitative type of research design. It mainly focuses on obtaining information about the efficacy of hand sanitizers against the standard ATCC cultures.

3.2.2 Collection of standard ATCC cultures and preparation of stock culture

A total of four standard ATCC cultures i.e; *E. coli* 35218, *E. coli* 25922, *K.* pneumoniae 700603 and *S. aureus* 43300 were collected from Central Department of Microbiology, Tribhuvan University and isolated using selective media**.** All the standard ATCC cultures were isolated to culture technique for standard stock culture. Nutrient broth and nutrient agar medium were used for bacterial isolate preservation. The turbidity of the inoculum was maintained with 0.5 Mc Farland turbidity standard by adding more bacteria or nutrient broth (Vandepitte, 2003).

3.2.3 Agar Well Diffusion Test

The antibacterial effect of the standard ATCC cultures to the hand sanitizers was performed using the well variant of the agar diffusion method described by (Valgas *et al*, 2007). Sterile Mueller-Hinton agar was inoculated in the petri plates. A sterile cotton swab was dipped into the test tube containing inoculum. Excess inoculum was removed by firmly pressing the cotton swab against the wall of the test tube. The cotton swab was streaked all over the agar surface by rotating the plate at an angle of 60°. Then, it was left to dry at room temperature with the lid closed. With the help of cork borer, 5 equally spaced holes were bored in the agar plates. The agar plugs were discarded with the help of sterile needle. Fifty microliters of hand sanitizers was inoculated in the four wells with sterile water of equal volume in the central well. The plates were incubated at 37°C for 24 hrs in an upright position. After 24 hrs the zone of inhibition was observed which shows the degree of susceptibility and resistance of the standard ATCC culture. Similarly, the test was also carried out in hundred microliters and one hundred fifty microliters respectively. The zone of inhibition was measured in mm with the help of ruler.

3.2.4 Data collection and analysis

The data collected from the study were analysed using Genstat 12th edition.

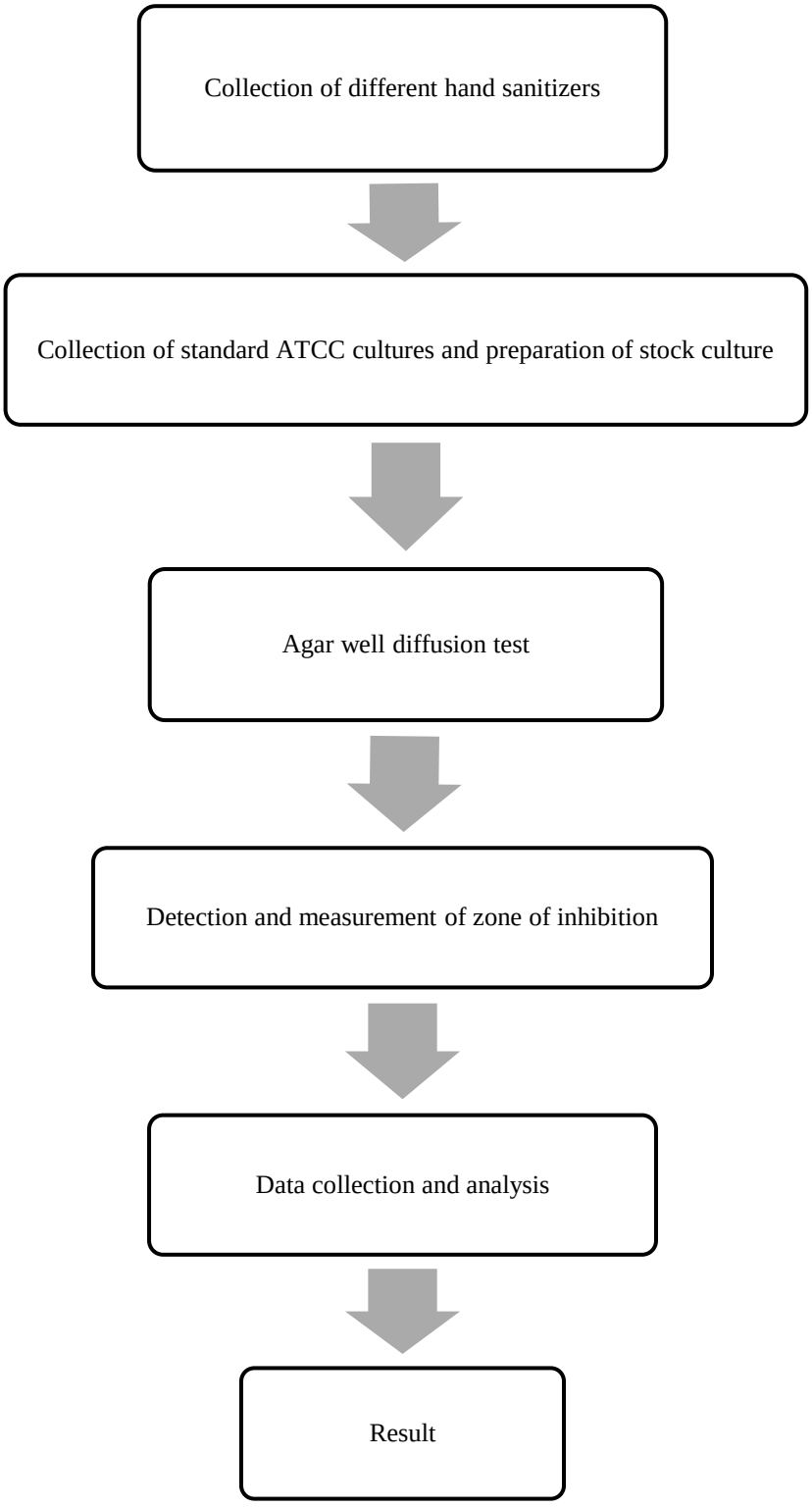


Figure 1: Flow chart diagram of research methodology (Jain, 2016)

CHAPTER 4

4. RESULTS AND DISCUSSION

In the study, isopropyl alcohol having 70% v/v is seemed to be more effective against standard ATCC cultures. Comparatively liquid based hand sanitizers showed more efficacy than gel based hand sanitizers. Also, the hand sanitizers containing alcohol and additional ayurvedic ingredients showed high antibacterial effect than hand sanitizers containing alcohol. The given hand sanitizers were effective against both gram positive and gram negative bacteria. Most of the hand sanitizers inhibited *E. coli* 25922 and *S. aureus* 43300.

4.1 Zone of inhibition (in mm) at 50 µl hand sanitizer sample

Table 1: Zone of inhibition of liquid based hand sanitizers of 50 µl volume against standard ATCC cultures.

Type of hand sanitizer	**Sample code**	**Zone of inhibition (in mm) at 50 µl sample**			
		***E. coli* 35218**	***E. coli* 25922**	***S. aureus* 43300**	***K. pneumoniae* 700603**
B	MA	14	17	17	15
B	YS	-	12	15	-
A	AU	11	12	-	-
A	IT	14	16	12	12
A	BA	12	16	-	11
A	SR	13	17	18	15
A	CS	-	9	-	-
A	AW	23	22	27	19
A	PC	11	13	-	-
B	VS	9	12	12	-
A	SS	-	10	-	-
B	HA	-	12	11	10
B	SH	-	11	-	10
B	SA	12	18	13	11
A	RB	11	16	16	13

Note: A and B represents liquid based and liquid with ayurvedic ingredients based hand sanitizers respectively.

In the volume of 50 µl liquid hand sanitizers, (46.67%, 60%, 26.67% and 33.33%) liquid based and (20%, 40%, 33.33% and 26.67%) liquid with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. Highest and lowest zone of inhibition was shown by AW (against *S. aureus* 43300) and VS (against *E. coli* 35218) respectively. ANOVA revealed that there is significant difference at 5% level of significance (<0.01; P<0.05) between the liquid based hand sanitizer's components in antibacterial activity using 50 µl sample. The ANOVA table is given in Appendix II.

Table 2: Zone of inhibition of gel based hand sanitizers of 50 µl volume against standard ATCC cultures.

Type of hand sanitizer	**Sample code**	**Zone of inhibition (in mm) at 50 µl sample**			
		***E. coli* 35218**	***E. coli* 25922**	***S. aureus* 43300**	***K. pneumoniae* 700603**
B	IC	-	-	-	-
A	GR	-	8	8	-
A	KL	-	-	-	-
A	PSK	-	-	-	-
A	AK	-	-	-	-
A	WF	18	-	-	-
B	FL	-	-	-	-
A	MK	-	-	-	-
A	KS	-	-	-	-
B	ST	-	-	-	-
B	PJ	29	28	38	20
B	CR	-	-	-	-
A	TP	12	18	10	9
A	IS	-	9	9	8
A	SP	-	-	-	-
B	NY	10	9	12	-

Note: A and B represents gel based and gel with ayurvedic ingredients based hand sanitizers respectively.

In the volume of 50 µl gel hand sanitizers, (12.5%, 18.75%, 18.75% and 12.5%) gel based and (12.5%, 12.5%, 12.5% and 6.25%) gel with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. Highest and lowest zone of inhibition was shown by PJ (against *S. aureus* 43300) and GR (against *E. coli* 25922, *S. aureus* 43300 and *Klebsiella* 700603) respectively. ANOVA revealed that there is significant difference at 5% level of significance (<0.01; P<0.05) between the gel based hand sanitizer's components in antibacterial activity using 50 µl sample. The ANOVA table is given in Appendix II.

4.2 Zone of inhibition (in mm) at 100 µl hand sanitizer sample

Table 3: Zone of inhibition of liquid based hand sanitizers of 100 µl volume against standard ATCC cultures.

Types of hand sanitizer	**Sample code**	**Zone of inhibition (in mm) at 100 µl sample**			
		***E. coli* 35218**	***E. coli* 25922**	***S. aureus* 43300**	***K. pneumoniae* 700603**
B	MA	19	20	35	20
B	YS	13	12	20	11
A	AU	12	13	10	-
A	IT	15	18	27	13
A	BA	14	19	-	13
A	SR	17	19	35	18
A	CS	-	10	-	-
A	AW	31	31	33	28
A	PC	13	15	12	10
B	VS	12	15	16	12
A	SS	8	12	11	9
B	HA	-	13	18	11
B	SH	-	10	12	11
B	SA	14	20	28	15

A	RB	13	18	33	15

Note: A and B represents liquid based and liquid with ayurvedic ingredients based hand sanitizers respectively.

In the volume of 100 µl liquid hand sanitizers, (53.33%, 60%, 46.67% and 46.67%) liquid based and (40%, 40%, 40% and 40%) liquid with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. Highest and lowest zone of inhibition was shown by MA (against *S. aureus* 43300) and GR (against *E. coli* 35218) respectively. ANOVA revealed that there is significant difference at 5% level of significance (<0.01; P<0.05) between the liquid based hand sanitizer's components in antibacterial activity using 100 µl sample. The ANOVA table is given in Appendix II.

Table 4: Zone of inhibition of gel based hand sanitizers of 100µl volume against standard ATCC cultures.

Types of hand sanitizer	**Sample code**	**Zone of inhibition (in mm) at 100 µl sample**			
		***E. coli* 35218**	***E. coli* 25922**	***S. aureus* 43300**	***K. pneumoniae* 700603**
B	IC	-	-	-	-
A	GR	10	9	9	9
A	KL	9	9	8	8
A	PSK	-	8	8	8
A	AK	9	9	9	8
A	WF	21	14	8	10
B	FL	11	11	12	11
A	MK	8	10	10	9
A	KS	10	11	11	9
B	ST	9	9	9	8
B	PJ	33	34	40	30
B	CR	-	9	-	-
A	TP	13	19	12	9
A	IS	-	10	10	10

A	SP	11	12	12	11
B	NY	12	13	15	12

Note: A and B represents gel based and gel with ayurvedic ingredients based hand sanitizers respectively.

In the volume of 100 µl gel hand sanitizers, (50%, 62.5%, 62.5% and 62.5%) gel based and (25%, 31.25%, 25% and 25%) gel with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. Highest and lowest zone of inhibition was shown by PJ (against *S. aureus* 43300), KL (*S. aureus* 43300 and *K.* pneumoniae 700603), PSK (*E. coli* 25922, *S. aureus* 43300 and *K.* pneumoniae 700603) and AK (*K.* pneumoniae 700603) respectively. ANOVA revealed that there is significant difference at 5% level of significance (<0.01; P<0.05) between the gel based hand sanitizer's components in antibacterial activity using 100 µl sample. The ANOVA table is given in Appendix II.

4.3 Zone of inhibition (in mm) at 150 µl hand sanitizer sample

Table 5: Zone of inhibition of liquid based hand sanitizers of 150 µl volume against standard ATCC cultures.

Types of hand sanitizer	**Sample code**	**Zone of inhibition (in mm) at 150 µl sample**			
		***E. coli* 35218**	***E. coli* 25922**	***S. aureus* 43300**	***K. pneumoniae* 700603**
B	MA	24	25	35	23
B	YS	16	16	22	13
A	AU	13	14	12	12
A	IT	20	20	28	20
A	BA	16	21	15	16
A	SR	22	22	35	25
A	CS	9	12	11	11
A	AW	32	32	35	29
A	PC	17	15	14	12
B	VS	16	16	17	17
A	SS	14	14	13	11

B	HA	12	15	20	13
B	SH	12	16	13	13
B	SA	20	23	32	17
A	RB	20	23	35	25

Note: A and B represents liquid based and liquid with ayurvedic ingredients based hand sanitizers respectively.

In the volume of 150 µl liquid hand sanitizers, (60%, 60%, 60% and 60%) liquid based and (40%, 40%, 40% and 40%) liquid with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. Highest and lowest zone of inhibition was shown by MA, SR, AW, RB (against *S. aureus* 43300) and CS (against *E. coli* 35218) respectively. ANOVA revealed that there is significant difference at 5% level of significance (<0.01; P<0.05) between the liquid based hand sanitizer's components in antibacterial activity using 150 µl sample. The ANOVA table is given in Appendix II.

Table 6: Zone of inhibition of gel based hand sanitizers of 150 µl volume against standard ATCC cultures.

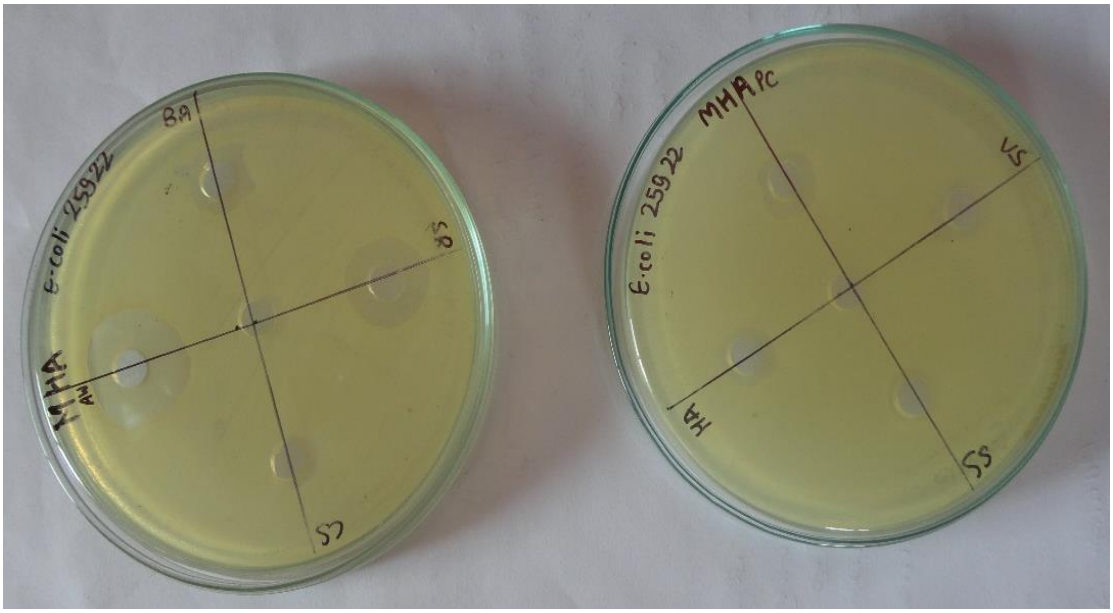
Types of hand sanitizer	**Sample code**	**Zone of inhibition (in mm) at 150 µl sample**			
		***E. coli* 35218**	***E. coli* 25922**	***S. aureus* 43300**	***K. pneumoniae* 700603**
B	IC	-	-	-	-
A	GR	12	12	12	11
A	KL	12	13	13	12
A	PSK	12	13	13	13
A	AK	13	14	15	13
A	WF	23	17	16	14
B	FL	15	14	14	12
A	MK	12	12	13	12
A	KS	12	13	12	11
B	ST	12	13	11	12
B	PJ	35	36	43	35

B	CR	11	13	11	11
A	TP	14	22	15	13
A	IS	15	15	15	13
A	SP	15	14	17	14
B	NY	18	17	19	14

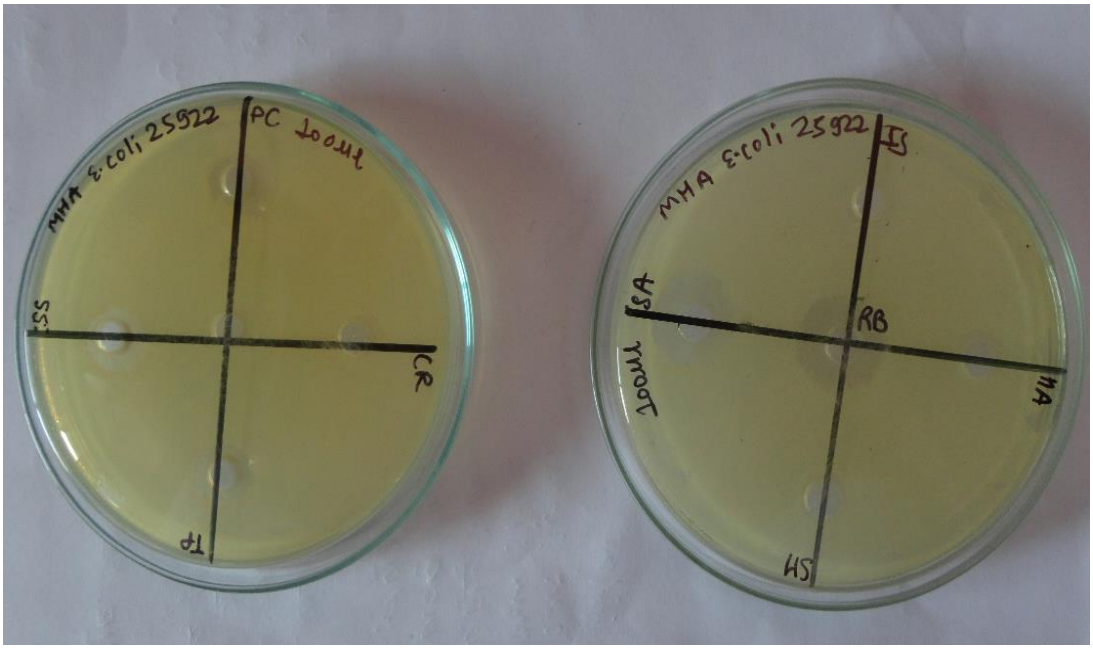
Note: A and B represents gel based and gel with ayurvedic ingredients based hand sanitizers respectively.

In the volume of 50 µl gel hand sanitizers, (62.5%, 62.5%, 62.5% and 62.5%) gel based and (31.25%, 31.25%, 31.25% and 31.25%) gel with ayurvedic ingredients based hand sanitizers showed the antibacterial effect against *E. coli* 35218, *E. coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603 respectively. Highest and lowest zone of inhibition was shown by PJ (against *S. aureus* 43300) and GR (against *K.* pneumoniae 700603), KS (against *K.* pneumoniae 700603), ST (against *S. aureus* 43300) and CR (against *E. coli* 35218 and *S. aureus* 43300) respectively. ANOVA revealed that there is significant difference at 5% level of significance (<0.01; P<0.05) between the gel based hand sanitizer's components in antibacterial activity using 150 µl sample. The ANOVA table is given in Appendix II.

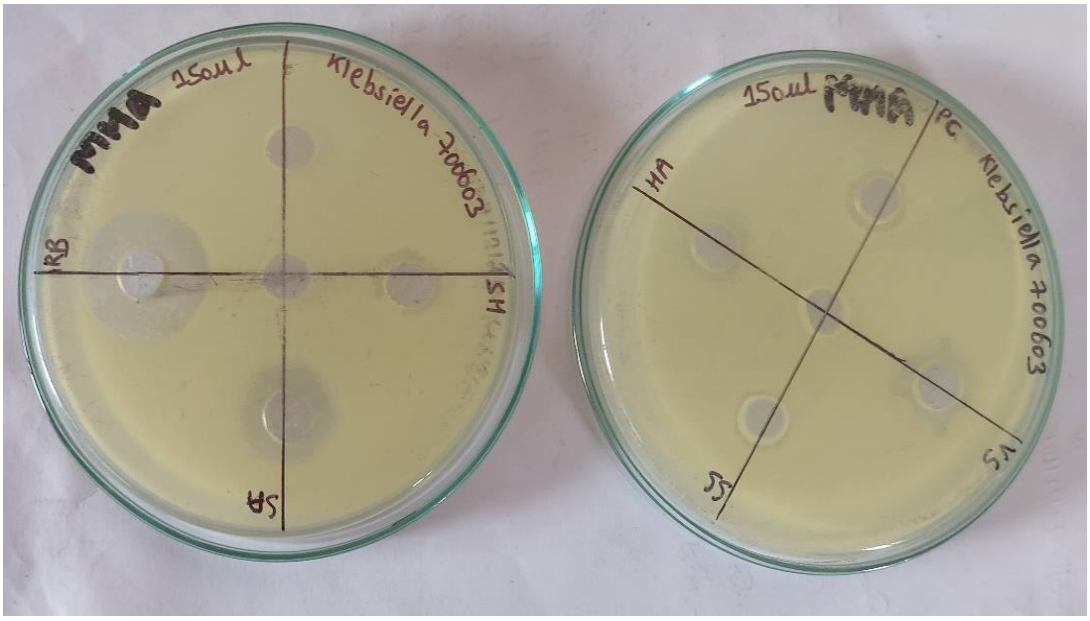
PHOTOGRAPHS



Photograph 1: Zone of inhibitions shown by hand sanitizers at 50 µl against ATCC culture *E. coli* 25922.



Photograph 2: Zone of inhibitions shown by hand sanitizers at 100 µl against ATCC culture *E. coli* 25922.



Photograph 3: Zone of inhibitions shown by hand sanitizers at 150 µl against ATCC culture *K.* pneumoniae 700603

Photograph 4: Inoculation of hand sanitizers using agar well diffusion method

4.4 Discussion

There are various microorganisms which are present in our body as well as the environment. Some may be harmful and some may be harmless as well. Our body always harbors some microorganisms in our body which plays an important role in maintaining our human health. Some of the microorganisms which are present in our hands may lead to various infections and diseases. So, to prevent such infections and diseases various measures are used like hand washing and use of hand sanitizers. The use of hand sanitizers has recently gained popularity due to the outbreak of corona virus. Due to the corona virus, people have been aware of the spread of diseases and its consequences. So, people have been using hand sanitizers as an easy and convenient measure for preventing various diseases. But the efficacy of the available hand sanitizers is still unknown.

In the alcohol based hand sanitizers, the main active ingredient is alcohol which exerts antibacterial activity by causing protein denaturation, disruption of tissue membranes, and dissolution of several lipids (Kar, 2008). The alcohol concentration ranges from 66% to 95% in the given hand sanitizers. Different types of alcohol are used like ethyl alcohol and isopropyl alcohol are used. In this study, isopropyl alcohol having 70% v/v is seemed to be more effective against ATCC cultures. It was similar to the findings of (Kar, 2008). Similarly, ethyl alcohol having 75 % v/v volume also seemed to be effective against different ATCC cultures. These results are similar to (Chojnacki, 2021) and (Edmond, 2011).

Traditionally, agar diffusion method and agar dilution method are commonly employed for assessment of the antimicrobial activity of any material. The advantages of agar disk diffusion method are chemical properties of the sanitizer remains unchanged, an easy and less technique sensitive method (Aravind, 2006; Pumarola, 1992)

Similarly, some hand sanitizers may contain hydrogen peroxide which is used to kill the bacteria or other microorganisms. In the present study, sample BA showed antibacterial effect against *E. coli* 35218, *E. coli* 25922 and *K. pneumoniae* 700603; CS showed antibacterial effect against *E. coli* 25922 only whereas SR showed antibacterial effect against all ATCC cultures. This indicates that the absence of active ingredient in product (hydrogen peroxide) may limit the cidal effect of the alcohol from attainment the bacterial cells (Manaye, 2021). Effects of the non-active ingredients such as fragrance,

emollients, humectants, gelling and thickening agents which could prevent the cidal effect of the products from reaching the microorganisms (Oke *et al*., 2013).

The present study was carried out on the basis of volume of hand sanitizers against the ATCC cultures, in contrary to it (Chojnacki *et.al.,* 2021) had studied based on exposure time against *S. aureus* ATCC 29213 and USA 300 (LAC) and *E. coli* ATCC 25922 and ATCC 51435. However, it didn't covered *K. pneumoniae* 700603. In this study (50 µl), the liquid based hand sanitizers were more effective against the test ATCC bacterial cultures than the gel based hand sanitizers. Similarly, liquid based hand sanitizer with ayurvedic ingredients showed more effective against the test ATCC bacterial cultures than gel based hand sanitizer with ayurvedic ingredients; similar to the study by (David *et al*., 2017). However, in case of 100 µl and 150 µl sample, the gel based hand sanitizers were more effective against the test ATCC bacterial cultures than the liquid based hand sanitizers. But, liquid based hand sanitizer with ayurvedic ingredients showed more effective against the test ATCC bacterial cultures than gel based hand sanitizer with ayurvedic ingredients similar to the study by (David *et al*., 2017). Overall, liquid based hand sanitizers were more effective than gel based hand sanitizers and hand sanitizers with ayurvedic ingredients were also more effective against the test ATCC bacterial cultures. This is supported by the ANOVA analysis (Appendix II). Beside this, liquid based hand sanitizers spread easily on hands, gives high coverage and act effectively leaving no residual substance on hands but gel based hand sanitizers require time to spread on the surface of hands, some of the areas could get missed and it also requires some time to show it's efficacy. This could also be the reason for less efficacy of gel based hand sanitizers than liquid based hand sanitizers. This result is similar to the study by (Kramer *et al*., 2002; Dharan *et al*., 2003). Hand sanitizers containing alcohol with ayurvedic ingredients seemed to have more efficacy than alcohol based hand sanitizers. The ayurvedic ingredients have antibacterial and antifungal properties which helps to kill bacteria and fungi. Due to this property, the alcohol based with ayurvedic ingredients showed more efficacy than alcohol based hand sanitizers. In this study, sample PJ with alcohol and ayurvedic ingredients has effective antibacterial effect against all ATCC cultures; which is similar to Mondal, (2004) where herbal hand sanitizer was found to be effective, safe, and less likely to cause adverse skin reactions and saves time and human resources. According to Mondal (2004), the formulated herbal hand sanitizer completely inhibited the growth of microorganisms on agar medium and also exhibited a high

antimicrobial efficacy in inhibiting the growth of all the test organisms. In this study, maximum zone of inhibition was shown by PJ in lowest volume. The isopropyl alcohol present in PJ acts as a main active ingredient to kill the microorganisms. Propanediol, acrylates/ C10-30 alkyl acrylate crosspolymer, Ethyl hexyl glycerin acts as a moisturizer which adds moisture to the skin. Phenoxyethanol is used as a stabilizer. Undeclycenic acid, CL 19140, CL42090 is used to treat the fungal infection. Sugandhitdravya is used to provide fragnance.

Despite of the ingredients information given in sample IC, it doesn't show any zone of inhibition (ZOI) in different volume from 50µl to 150µl. According to it's label, it contains Isopropyl alcohol, Aloe vera gel, Glycerine, Water, Lemon essenitial oil. This indicates that the sample IC might not contain the specified ingredients. Besides this, the concentration of the Isopropyl alcohol and other ingredients in this hand sanitizer was not mentioned. The addition of water causes the dilution of the alcohol which could be the reason for its no efficacy. This was in accordance with Harmanci (2016), i.e: the efficacy of alcohol-based hand sanitizers gets affected by different factors like the type of alcohol used, concentration of alcohol or amount of alcohol used, the possible contact time.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusions

In this study, antibacterial activity of thirty one different hand sanitizers found in the local markets of Kathmandu was assessed against *E.coli* 35218, *E.coli* 25922, *S. aureus* 43300 and *K. pneumoniae* 700603. All the hand sanitizers showed zone of inhibitions except one gel based hand sanitizer. Liquid based hand sanitizers showed more antibacterial activity than gel based hand sanitizers in 50 µl, 100 µl and 150 µl volumes. The highest ZOI was observed in alcohol with ayurvedic based hand sanitizers in different volumes. Similarly, the lowest zone of inhibition was shown by alcohol and gel based hand sanitizers. Also, most of the liquid based hand sanitizers showed zone of inhibition against different ATCC cultures than gel based hand sanitizers. It showed that the liquid based hand sanitizers were more effective than gel based hand sanitizers. Similarly, the alcohol with ayurvedic based hand sanitizers were more effective than alcohol based hand sanitizers.

5.2 Novelty and National Prosperity aspect of Project Work

Various studies and literatures have been made on this topic around the world. Those literatures have its own result, significance and limitation. But it could be useful topic for project work in Nepal because only few studies have been made on this topic relating to the current situation of corona virus around the world it could be useful information for the people. The literatures and studies have been helpful in determining the pathway for this project.

5.3 Limitations of the Work

The present study has its own limitations like the antimicrobial efficacy of the hand sanitizers was known. Only four bacterial microorganisms were used for testing the efficacy of the hand sanitizers which means the efficacy against other microorganisms is not known. Also only the hand sanitizers which are available in the market have been used for determining the efficacy of hand sanitizers. Due to the lack of time, sample size were least and unable to cover all the test samples including other bacterial strains and microbial samples i.e; fungi.

5.4 Recommendations for further work

The hand sanitizers which are prepared in the manufacturing company should be tested after preparation to maintain the proper efficacy of the hand sanitizers which are claimed by the company. Only the hand sanitizers which show proper efficacy of hand sanitizers should be sold in the market to protect the consumers from buying poor quality hand sanitizers. The government should also enforce the strict laws for maintaining the quality of hand sanitizer.

This study was only confined to antimicrobial activity. However, there are many reasons that further works can be carried out. They are as below;

1. The effectiveness of hand sanitizers are also governed by its composition. So, further proximate chemical analysis of hand sanitizers can be done.
2. The study was only confined in the trial against only four ATCC bacterial cultures which may be increased and can cover many strains. This leads to reveal the broad spectrum of hand sanitizers.
3. Only bacterial cultures were tested. So, fungi and even virus can be tested for further work.
4. The adverse effects of hand sanitizers on skin can be studied further.
5. Only 31 hand sanitizers were used. So, it would be better to cover a large number of hand sanitizers for detail study in future for further research work.

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APPENDIX – I

CHARTS OF ZONE OF INHIBITION SHOWN BY HAND SANITIZER AGAINST ATCC CULTURES

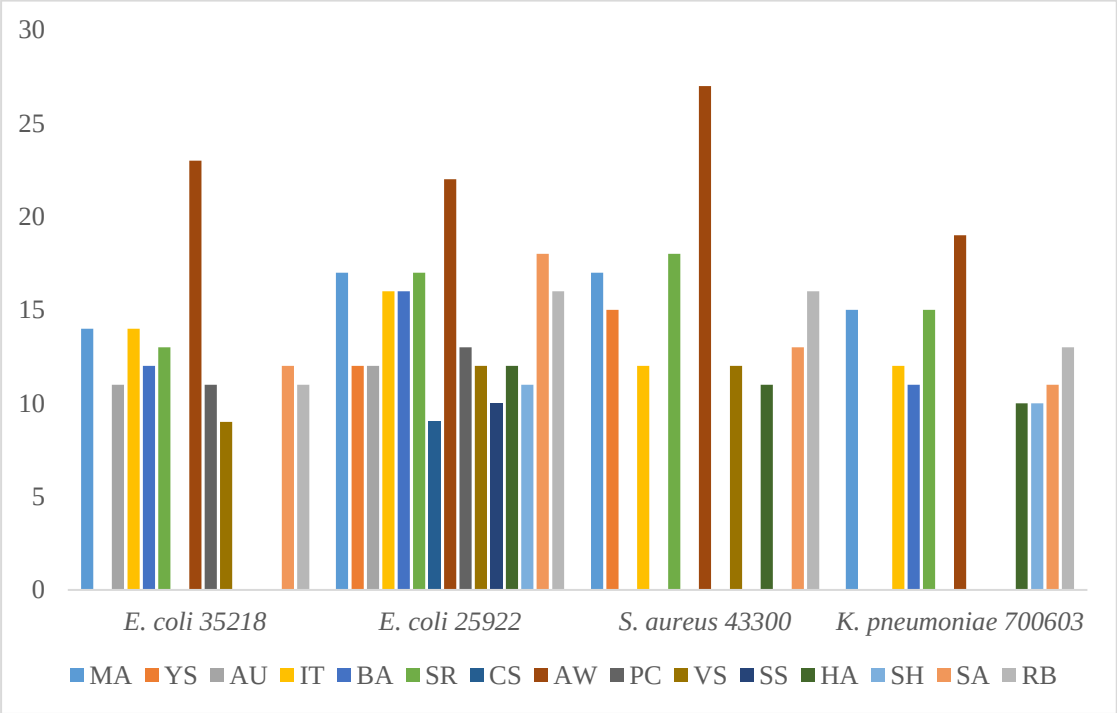

Figure 2: Chart of zone of inhibition at 50 µl volume of liquid based hand sanitizers against standard ATCC cultures.

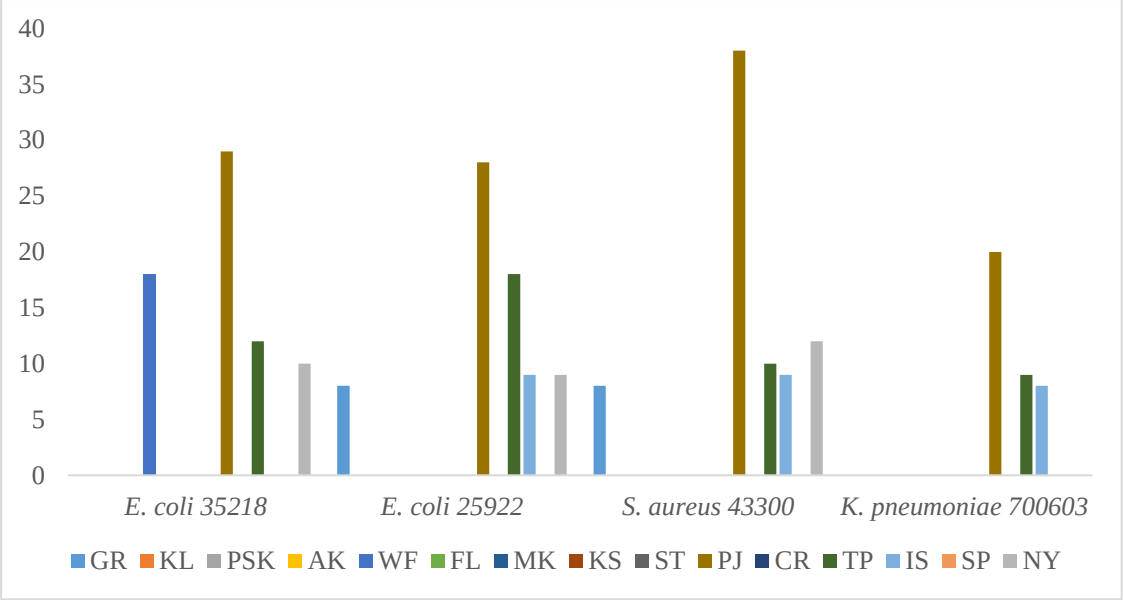

Figure 3: Chart of zone of inhibition at 50 µl volume of gel based hand sanitizers against standard ATCC cultures.

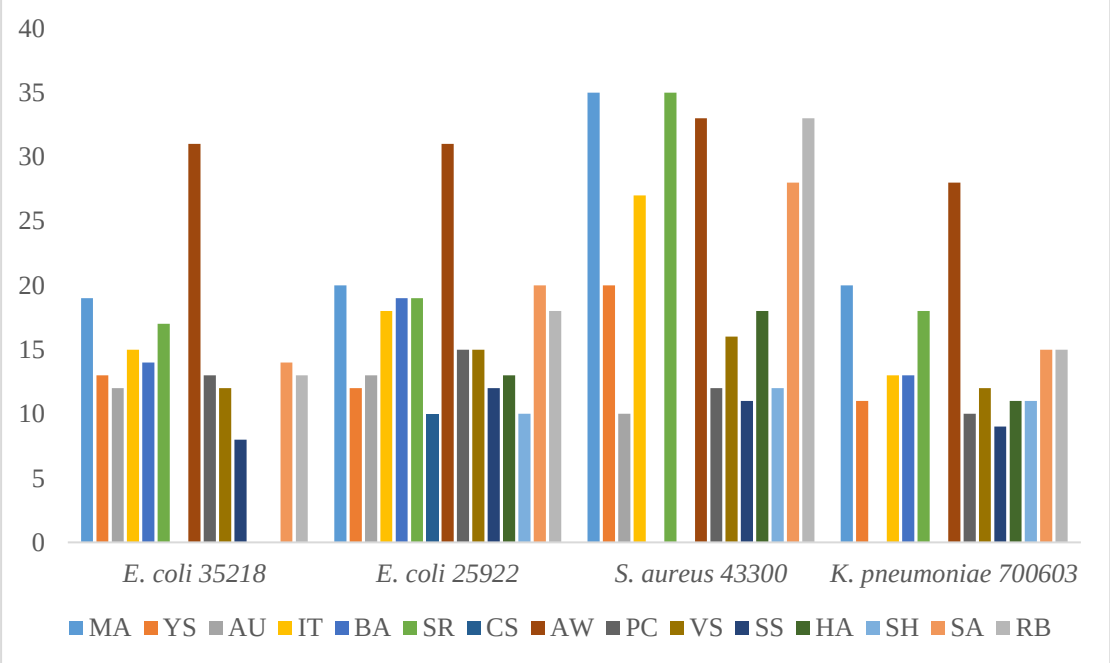

Figure 4: Chart of zone of inhibition at 100 µl volume of liquid based hand sanitizers against standard ATCC cultures.

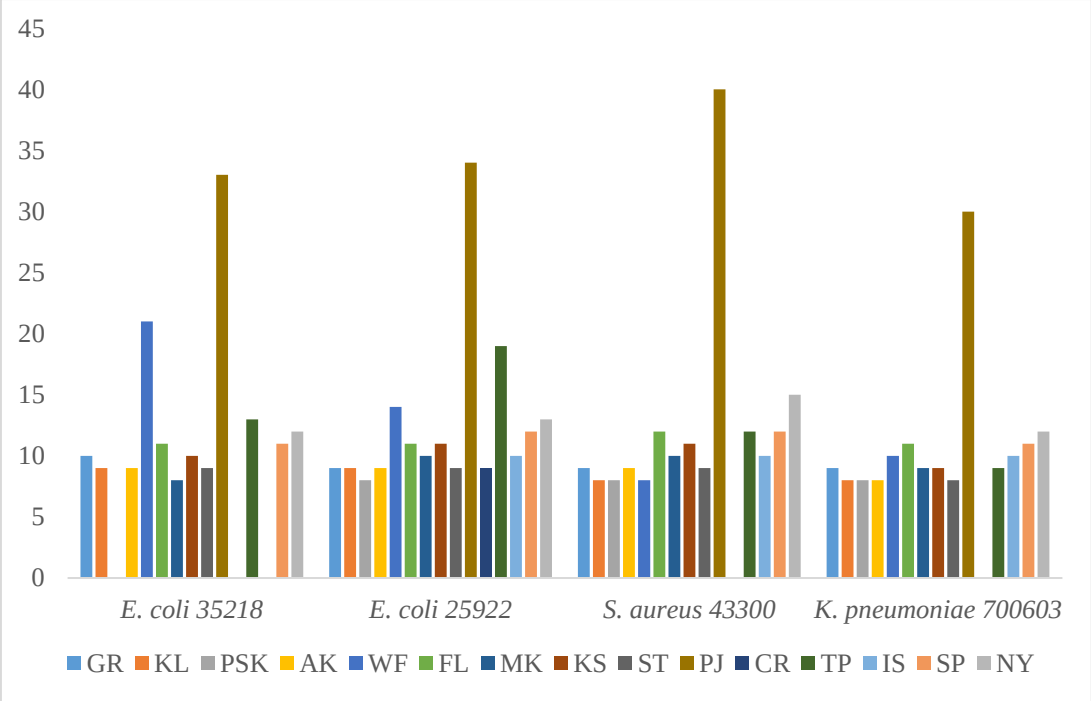

Figure 5: Chart of zone of inhibition at 100 µl volume of gel based hand sanitizers against standard ATCC cultures.

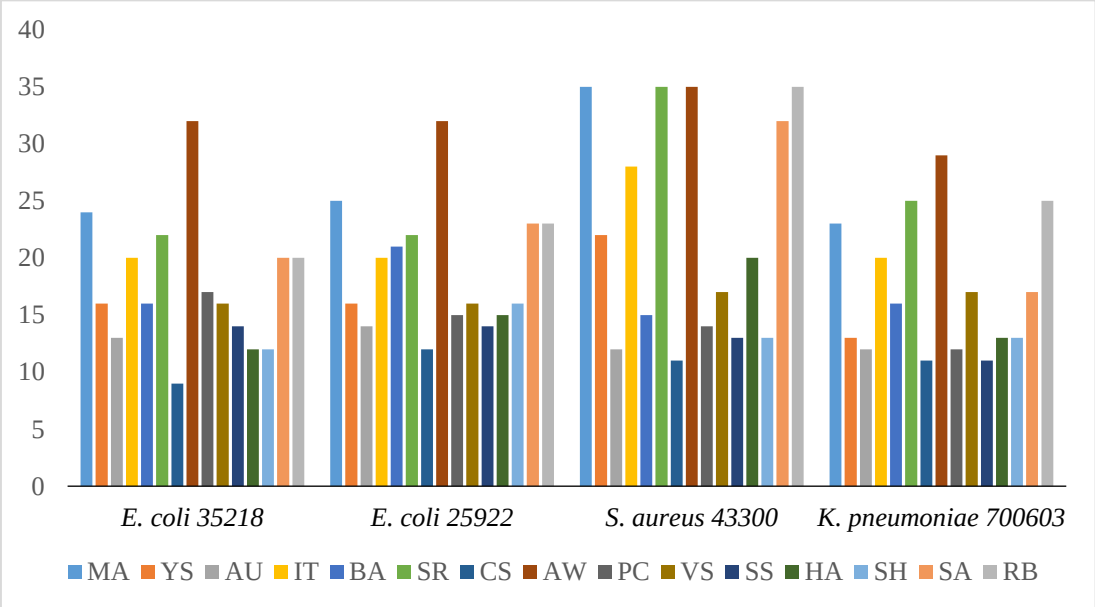

Figure 6: Chart of zone of inhibition at 150 µl volume of liquid based hand sanitizers against standard ATCC cultures.

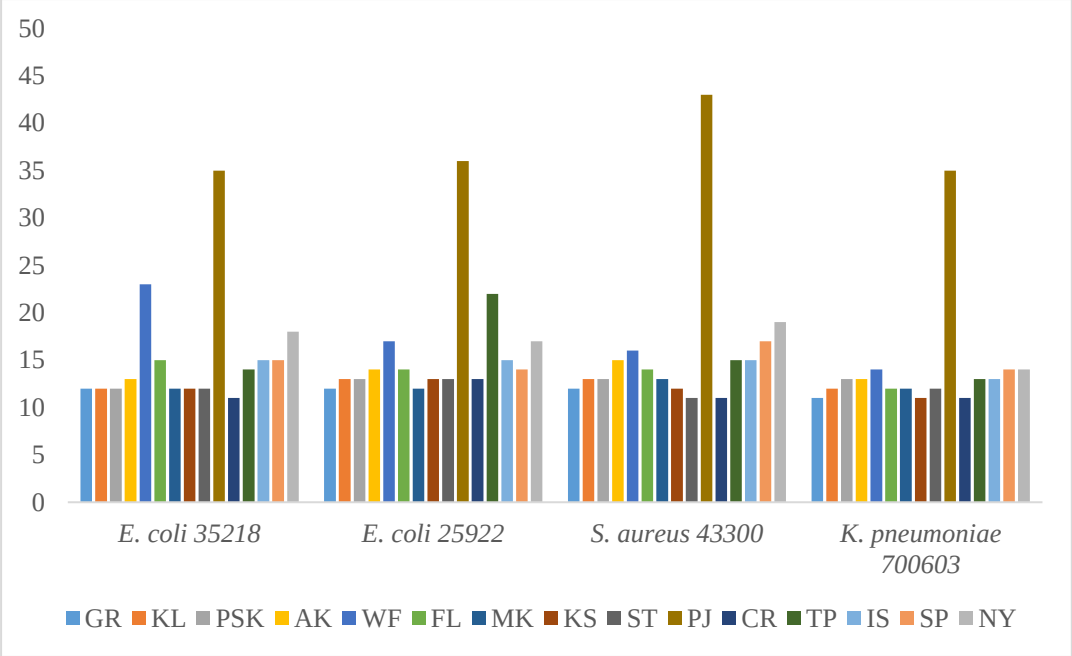

Figure 7: Chart of zone of inhibition at 150 µl volume of gel based hand sanitizers against standard ATCC cultures.

APPENDIX – II

ANOVA RESULT

1. Liquid based hand sanitizers

Analysis of variance

Variate: ZOI mm at 50µl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	14	1835.50	131.11	5.11	<.001
Residual	45	1154.50	25.66		
Total	59	2990.00			

Variate: ZOI mm at 100 µl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	14	2706.40	193.31	6.33	<.001
Residual	45	1375.25	30.56		
Total	59	4081.65			

Variate: ZOI mm at 150 µl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	14	2314.10	165.29	11.54	<.001
Residual	45	644.75	14.33		
Total	59	2958.85			

2. Gel based hand sanitizers

Analysis of variance

Variate: ZOI mm at 50µl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	15	3444.73	229.65	16.70	<.001
Residual	48	660.25	13.76		
Total	63	4104.98			

Variate: ZOI mm at 100µl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	15	3397.609	226.507	31.63	<.001
Residual	48	343.750	7.161		
Total	63	3741.359			

Variate: ZOI mm at 150µl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	15	3104.359	206.957	58.52	<.001
Residual	48	169.750	3.536		
Total	63	3274.109			

APPENDIX - III

Composition of Hand sanitizers According to Nepal Bureau of Standards and Metrology -2020 and WHO guideline

Formulation I

a. Ethanol (with a purity 96% v/v),

b. Hydrogen Peroxide 3% v/v,

c. Glycerol 98% v/v

Formulation II

a. Isopropyl alcohol (with a purity of 99.8% v/v),

b. Hydrogen Peroxide 3% v/v,

c. Glycerol 98% v/v

APPENDIX – IV

MATERIALS USED

Equipments

1. Autoclave (Life, India)
2. Incubator (Memmert, Germany)
3. Hot Air Oven (Ambassador)
4. Refrigerator (LG, India)
5. Electronic weighing balance (Phoenix instrument, Germany)
6. Bunsen burner
7. Cork borer
8. Micropipette

Glass-wares/ Plastic-wares

1. Beakers
2. Conical flasks
3. Petriplates
4. Pipettes
5. Measuring cylinders
6. Test tubes
7. Microtips

Microbiological media (Hi- Media Laboratories Pvt. Ltd.)

1. Mueller Hinton Agar (MHA)
2. Nutrient broth
3. Nutrient agar

Miscellaneous

1. 0.5 Mc Farland Turbidity Standard
2. Inoculating loop

3. Forceps
4. Labelling tags
5. Cotton
6. Aluminium foil
7. Tray
8. Test tube rack
9. Permanent marker
10. Lighter
11. Record book
12. Pens/Pencils
13. Distilled water

APPENDIX - V

COMPOSITION AND PREPARATION OF DIFFERENT CULTURE MEDIA

1) Nutrient agar Composition

Ingredients	**gms/liter**
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Agar	15.00

Final pH (at 25°C) 7.4±0.2

Preparation

28 grams of Nutrient agar was suspended in 1000 ml distilled water and was boiled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 mins and cooled at 45-50°C. Then, it was mixed and poured into petriplates.

2) Nutrient broth Composition

Ingredients	**gms/liter**
Peptic digest of animal tissue	5.00
Yeast extract	1.50
Beef extract	1.50
Sodium chloride	5.00

Final pH (at 25°C) 7.4±0.2

Preparation

13 grams was suspended in 1000 ml distilled water and heated to dissolve the medium completely. Then, the medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

3) Mueller Hinton Agar Composition

Ingredients	**gms/liter**
Beef infusion form	300.00
Casein acid hydolysate	17.50
Starch	1.50
Agar	17.00

Final pH (at 25°C) 7.4±0.2

Preparation

38 grams was suspended in 1000ml distilled water and heated to dissolve the medium completely. Then, the medium was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

APPENDIX – VI

Preparation of 0.5 Mc Farland Turbidity Standard

0.5 McFarland turbidity standard was prepared in a test tube from the mixture of barium chloride dehydrate ($BaCl_2.2H_20$) solution and sulfuric acid (H_2SO_4). The accuracy of density was verified using spectrophotometer. The absorbance of the 0.5 Mc Farland Turbidity Standard at a wavelength of 625 nm was 0.08-0.10. The prepared tube was stored in a sealed container at room temperature in a dark place (Cheesbrough, 2006).

APPENDIX-VII

COMPOSITION OF DIFFERENT HAND SANITIZERS

1. MA

Ingredients	**Percentage (%) v/v**
Ethyl alcohol	75
Aroma (lemongrass essential oil)	0.1

2. YS

Ingredients	**Percentage (%) v/v**
Iso propyl alcohol	N/A
Perfumed aqua	N/A
Aloe vera gel	N/A
Alcohol	70

3. AU

Ingredients	**Percentage (%) v/v**
Ethyl alcohol	75
Carbopol	N/A
Propylene glycol	N/A
Perfume	N/A
Tea	N/A
Aqua	N/A

4. IT

Ingredients	**Percentage (%) v/v**
Ethyl alcohol	72
Glycerol	2
Water	N/A
MPS	N/A
PPS	N/A

5. **BA**

Ingredients	**Percentage (%) v/v**
Isopropyl alcohol	75
Hydrogen peroxide	0.125
Glycerol	1.45
Purified water (q.s. colour)	N/A

6. **SR**

Ingredients	**Percentage (%) v/v**
Ethyl alcohol	75
Hydrogen peroxide	0.125

7. **CS**

Ingredients	**Percentage (%) v/v**
Ethanol BP	80
Hydrogen peroxide	0.125
Glycerine	1.45

8. **AW**

Ingredients	**Percentage (%) v/v**
Isopropyl alcohol	70
Chlorhexidine gluconate solution	2.5
Chlorhexidine gluconate	0.5 w/v

9. **PC**

Ingredients	**Percentage (%) v/v**
Ethyl alcohol	73
Aqua	N/A
Carbomer	N/A
Triethanolamine	N/A
Glycerine	N/A
Perfume	N/A
Disodium EDTA	N/A

10. VS

Ingredients	Percentage (%) v/v
Isopropyl Alcohol	70
Vitamin E	N/A
Carbopol 940	N/A
Triethanolamine	N/A
Neem extract	N/A
Aloe Vera Extract	N/A
Perfume	N/A
Colour	N/A
Distilled water	N/A

11. SS

Ingredients	Percentage (%) v/v
Ethyl alcohol	95
Denatured alcohol	70
Aqua triethylamine	N/A
Carbopol	N/A
Glycerine	N/A
Fragnance	N/A

12. HA

Ingredients	Percentage (%) v/v
Ethanol	75
Distilled water	21.5
Glycerol	1.45
Aloe vera	0.5
Azadirachtaindica	0.5
Curcuma longa	0.5
Hydrogen peroxide	0.125
Cinnamomumcamphora	0.425

13. SH

Ingredients	Percentage (%) v/v
Vitamin E	N/A
Aloe vera extract	N/A
Glycerine	N/A
Ethyl Alcohol	N/A
Triethanolamine	N/A
Aqua	N/A
Hydrogen peroxide	N/A
Colour	N/A
Perfume	N/A

14. SA

Ingredients	Percentage (%) v/v
Isopropyl alcohol	70
Hydrogen peroxide	0.125
Glycerine	0.5
Water	N/A
Aloe vera	N/A
Perfume	N/A

15. RB

Ingredients	Percentage (%) v/v
Ethyl alcohol	83.33
Hydrogen peroxide	4.17
Glycerol	1.45
Aqueous base QS	N/A
Emollients	N/A
Moisturizer	N/A

16. IC

Ingredients	Percentage (%) v/v
Isopropyl alcohol	N/A
Aloe vera gel	N/A

Glycerine	N/A
Water	N/A
Lemon essenitial oil	N/A

17. GR

Ingredients	**Percentage (%) v/v**
Ethyl Alcohol	95
Isopropyl Alcohol/absolute alcohol	76/72.34
Perfumed gel base QS,	N/A

18. KL

Ingredients	**Percentage (%) v/v**
Ethyl Alcohol (Denaturated)/ Absolute alcohol,	95
Perfumed gel base	N/A

19. PSK

Ingredients	**Percentage (%) v/v**
Ethyl alcohol	95
Isopropyl Alcohol	62 (w/w)
Perfumed gel base QS	100 (w/w)

20. AK

Ingredients	**Percentage (%) v/v**
Alcohol	66
Water	N/A
Glycerine	N/A
Acrylates/C10-30 Alkyl Acrylate Cross Polymer	N/A
Triethanolamine	N/A
Fragnance	N/A

21. WF

Ingredients	**Percentage (%) v/v**
Ethyl alcohol /Denaturated	75
Di-sodium EDTA	N/A

Triethhanolamine	N/A
Water	N/A
Glycerine	N/A
Carbomer	N/A

22. FL

Ingredients	**Percentage (%) v/v**
Ethyl Alcohol (Denaturated)/ Absolute alcohol	75
Perfume	N/A
Glycerine	N/A
Water	N/A
Propylene Glycol	N/A
Carbopol	N/A
Triethanolamine	N/A
Tocopheryl Acetate	N/A
Aloe vera juice extracts	N/A
CL No: 42090	N/A
CL No: 19140	N/A

23. MK

Ingredients	**Percentage (%) v/v**
Ethanol	74 (w/w)
Aqua	N/A
Glycerine	N/A
Carbomer	N/A
Aminomethyl propanol	N/A
Perfume	N/A

24. KS

Ingredients	**Percentage (%) v/v**
Ethyl Alcohol (Denaturated) /Absolute alcohol	72.34
Perfumed gel base	N/A

25. ST

Ingredients	Percentage (%) v/v
Alcohol	75
Water	N/A
Aloe vera	N/A
Ethanol	N/A
Carbomer	N/A
Triethanoamine	N/A

26. PJ

Ingredients	Percentage (%) v/v
Isopropyl alcohol	70
Propanediol, Acrylates/ C-10-30 Alkyl Acrylate Crosspolymer	N/A
Ethylhexylglycerin	N/A
Phenoxyethanol	N/A
Undeclycenic acid	N/A
CL 19140	N/A
CL42090	N/A
Sugandhitdravya Q.S.	N/A

27. CR

Ingredients	Percentage (%) v/v
Alcohol	75
Aqua	N/A
Aloe barbadensis leaf extract	N/A
Carbomer	N/A
Curcuma longa extract	N/A
Fragnance	N/A
Glycerine	N/A
Polysorbate 20	N/A
Triethanolamine	N/A
Tocopherol	N/A

28. TP

Ingredients	Percentage (%) v/v
Ethyl Alcohol (Denaturated)	75
Absolute Alcohol	N/A
Water	N/A
Glycerine	N/A
Propylene	N/A
Glycol	N/A
Carbomer	N/A
Triethanolamine	N/A
Perfume	N/A
Tocopheryl acetate	N/A

29. IS

Ingredients	Percentage (%) v/v
Ethyl Alcohol (Denaturated)/ Absolute alcohol	75
Water	N/A
Glycerine	N/A
Propylene glycol	N/A
Carbomer	N/A
Triethanolamine	N/A
Perfume	N/A
Tocopheryl acetate	N/A

30. SP

Ingredients	Percentage (%) v/v
Isopropyl alcohol	70
Propylene glycol	N/A
Carbomer	N/A
Glycerine	N/A
Perfume	N/A

31. NY

Ingredients	Weight (mg)
Aloe vera leaves ext	20
Neem leaves distillate	20
Lime fruit peel distillate	1
Ushira root distilla	2
Hrivera root distillate	1
Carbomer, Polyquanternium 7	N/A
Polysorbate 20	N/A
Diethanol amine	N/A
Perfume	N/A
Glycerine	N/A
Propylene glycol	N/A
Iso propyl alcohol	N/A
Purified water	N/A