

**Evaluating the Antimicrobial Activity of Hand Sanitizers
against Bacterial pathogens.
Student Study Project 2021-22**



**To
Department of Microbiology
TARA Government Degree & PG (A) College
Sangareddy**

**EVALUATING THE ANTIMICROBIAL ACTIVITY OF HAND
SANITIZERS AGAINST BACTERIAL PATHOGENS.**

TO

DEPARTMENT OF MICROBIOLOGY



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CERTIFICATE

This is to certify that the project work entitled with the “**Antimicrobial Activity of Hand Sanitizers against Bacterial Pathogens**”, which has been submitted by B.Sc (Microbiology) Students (2021-2022), TARA Govt. Degree College is the original work done by them

The results embodied in this report have not been to any other University or Institution for the award of any degree.

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DECLARATION

We hereby declare that the project report entitled “EVALUATING THE ANTIMICROBIAL ACTIVITY OF HAND SANITIZERS AGAINST BACTERIAL PATHOGENS “ is the work done in the campus at Tara Government College during the academic year 2021-2022 and is submitted in partial fulfilment of the requirements for the degree of Bachelor Of Science by Osmania University , Hyderabad.

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TITLE OF THE PROJECT

**EVALUATING THE ANTIMICROBIAL ACTIVITY OF HAND
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CHAPTER -1

INTRODUCTION

During the year December 2019 several cases of pneumonia of unknown origin spread in China. Later in Jan 2020 it was announced as Corona Disease i.e. severe acute respiratory syndrome CoV-2 (SARS-CoV-2) caused by novel corona virus, which belongs to the Sub family Orthocoronaviridae (order: Nidovirales, family: Coronaviridae). Corona viruses are enveloped viruses with lipid membrane derived from host cells. CoV includes four genera γ , β , α , δ , among the four CoV γ , β infect mammals where as α , δ infect birds. China reported the increasing occurrence of pneumonia in the city of Wuhan, during December 2019. In January 2020, a novel β -CoV was identified as the cause [1] the virus was given the official name of SARS-CoV-2 by the international Committee for Taxonomy of Viruses, while the WHO named the disease caused by the virus, COVID-19

In response to the corona virus disease 2019 pandemic (COVID-19) hand hygiene has taken a prominent role in effects to reduce SARS COV-2 transmission and infection. Hands are the primary mode of transmission of microbes and infection [2]. 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. Hand hygiene helps in preventing the spread of infectious diseases. In situations in which an individual does not have access to soap and water, the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) have recommended the use of alcohol rubs (also known as hand sanitizers) to reduce microbial burden.

To evaluate the utility of sanitizers both the user acceptability and the efficacy need to be evaluated. Very few publications and a little research work available on the efficacy of hand sanitizers against circulating strains of CoV- 2 C. The purpose of this study was to evaluate the effect of antimicrobial activity of hand hygiene agents against Bacterial pathogens.

CHAPTER-2

Hypothesis

Covid Pandemic



Hand Sanitizers



Bacterial Pathogens



Antimicrobial Activity



Disc Diffusion and Zone of Inhibition of Growth



Efficacy of the sanitizer

The emergence of novel pathogens, bacterial or viral, has always posed serious challenges to public health around the globe. One of these dangerous pathogens is “severe acute respiratory syndrome corona virus 2 or SARS-CoV-2, more commonly known for causing corona virus disease 2019 or COVID-19, which has been declared a global pandemic by the World Health Organization in early 2020.

An effective and simple method for reducing transmission of infections in public or healthcare settings is hand hygiene. A range of hand sanitizers are available with various combinations of ingredients and modes of delivery.

Hands are the main pathways of germ transmission during health care. Hand hygiene is therefore the most important measure to avoid the transmission of harmful germs. Hand Sanitizers are a type of disinfectant and antiseptic that is used to destroy microorganism (Pathogens) such as harmful viruses ,Bacteria and Fungi. In 1938, Price⁶³ established that

bacteria recovered from the hands could be divided into two categories, namely resident or transient.

Infectious diseases caused by bacteria, Viruses and fungi are the major cause of morbidity and mortality across the globe. Cholera, tuberculosis, diphtheria, typhoid are some of the infectious diseases caused by bacteria. Influenza, Rabies and HIV are the some of the diseases caused by viruses. Corona viruses are a family of viruses that can cause illnesses such as the common cold, severe acute respiratory syndrome (SARS).

An antimicrobial is an agent that destroys or prevents the growth of microorganisms. Antimicrobial activity can be defined as a collective term for all active principles or agents that inhibit the growth of microorganisms prevent the formation of microbial colonies and may destroy microorganisms

Disc method is most common method used routinely for determination of antibiotic sensitivity of bacteria by measuring zone of inhibition of growth.

Efficacy of a hand sanitizer can be demonstrated as the effect of the application of a hand hygiene formulation when tested in laboratory or in vivo situations. Determination of the Zone of inhibition of growth by Disc method to such formulations against bacteria can be done to estimate the efficacy of hand sanitizer.

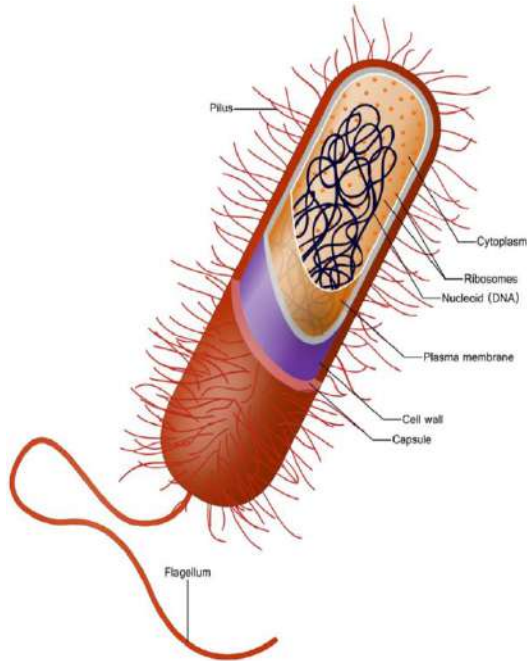


Fig1. Generic structure of a gram-negative bacterium
Image by Ali Zifan, distributed under a CC-BY-SA 4.0 license.

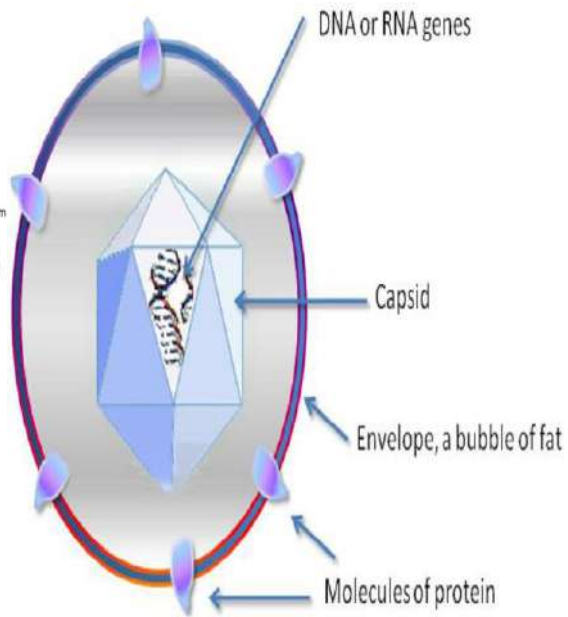


Fig 2. Generic structure of a virus with a lipid envelope.
Image by Graham Beards, distributed under a CC BY-SA 3.0 license.

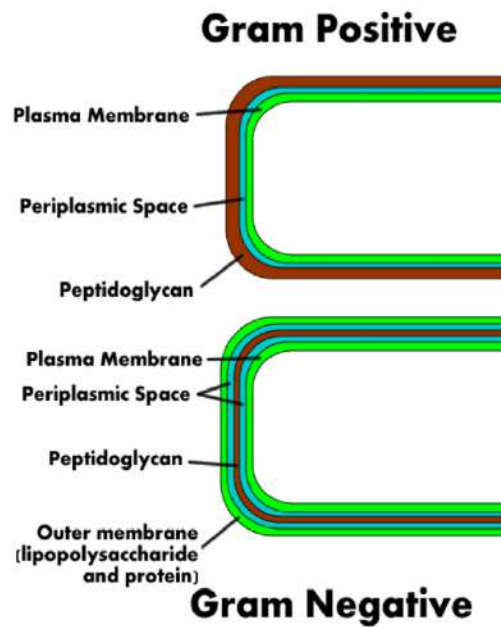


Fig 3. Gram-positive versus gram-negative bacteria. Image by Julian Onions, Wikimedia Commons, Public Domain.

Source: A.P. Golin et al. / American Journal of Infection Control 48 (2020) 1062–1067

CHAPTER-3

AIM & Objectives

To evaluate and compare the antimicrobial activity of three commercially available Hand Sanitizers and one herbal sanitizer against pathogenic bacteria.

- ❖ The emergence of novel Severe Acute Respiratory Syndrome- Corona Virus-2 (SARS-CoV-2) is a serious health issue in public health domain.
- ❖ Non-therapeutic interventions such as practicing good hand hygiene continue to be the mainstay of protection from SARS-CoV-2.
- ❖ Hand sanitizers have been developed as a convenient means to decontaminate an individual's hands of bacterial pathogens in situations in which soap and water are not available
- ❖ The main objective of this project is to evaluate the antimicrobial activity of three commercially available Hand Sanitizers i.e Instant Hand sanitizer, Bactorub, Apollo Hand sanitizer and Herbal sanitizer.
- ❖ Three Bacterial pathogens one Gram positive *Staphylococcus aureus* three Gram negative *Klebsiella pneumonia*, *Proteus vulgaris* and *Escherichia coli*. Used in this study to test the antimicrobial activity.
- ❖ The method applied was Disc method, by using Zone of growth inhibition of bacteria collected from MNR medical college ,Sangareddy.
- ❖ The efficacy of hand sanitizers was tested by the antimicrobial activity Assay with different bacterial with reference to the control standards.



Figure 4. Hand disinfection steps according to EN1500.
doi:10.1371/journal.pone.0111969.g001 Given by WHO
(Source: Rita Babeluk, Hand Hygiene , PLOS ONE,2014)

CHAPTER-4

REVIEW OF LITERATURE

The global pandemic of COVID-19 has renewed public health focus on the efficacy of hand hygiene and respiratory hygiene to limit hand to face (mouth, eyes, nose) and person to person transmission of a highly contagious and novel virus like Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) [Sayandip Mukherjee et al.; 2020].

Skin micro biota, the potential association with health and disease [Rosenthal M, Goldberg D, Aiello A, Larson E, Forman B. 2011]. Effect of hand hygiene on spread of infectious diseases [Kampf G, Kramer A. 2004.] Inactivation of severe acute respiratory syndrome corona virus 2 by WHO-recommended hand rubs formulations and alcohols [Kratzel A, Todt D, V'kovski P, Steiner S, et al., 2020]. Community-based infections and the potential role of common touch surfaces as vectors for the transmission of infectious agents in home and community settings. [Scott E. 2013]. The word hygiene derives from the ancient Greek goddess Hygeia [The goddess of healing Encyclopaedia Britannica Online. <http://www.britannica.com/EBchecked/topic/279225/Hygieia>. Accessed: 2014 Mar 05]. Chemical disinfectants vary in their action mechanism and the majority of disinfectants of a chemical nature target the outer lipid layer of coronaviruses (CoVs) and inactivate the viral particles (Choi et al. 2021). Choi H, Chatterjee P, Lichtfouse E, Martel JA, Hwang M, Jinadatha C, Sharma VK (2021) Classical and alternative disinfection strategies to control the COVID-19 virus in healthcare facilities: a review. *Environ Chem Lett*: 1–7. <https://doi.org/10.1007/s10311-021-01180-4> (In press).

The bacteria reside on hands can be differentiated into resident and transient floras. The genus staphylococcus aureus, staphylococcus epidermidis, and Enterococcus faecalis are the common resident floras. Which have the ability to colonize deep layers of the skin and are resistant to mechanical removal, Transient floras include *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* colonize superficial layers of skin.

Antibiotic susceptibility testing can be done by a standardized single disk method [Bauer, A.W.; Kirby et al 1966]. Antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenza [Fleming, A 1929].

The disc method is the gold standard for confirming the susceptibility of bacteria. Standardised disc diffusion was introduced by Bauer and Kirby's experiments in 1956. After finalizing all aspects of optimization by changing physical conditions [Bauer, A.W.; Kirby, W.M et.al., 2009]. Hand washing with Soap removes the body's own fatty acids from the skin which may result in cracked skin that provides an entry portal for pathogens [Larson El, Hughes CA et al 1998]. In the absence of soap and water, the CDC recommends the use of alcohol-based hand rub containing at least 60% alcohol (w/w) for hand disinfection (<https://www.cdc.gov/coronavirus/2019-ncov/hcp/hand-hygiene.html>). The lipid bilayer present in the enveloped viruses are denatured and destroyed by the natural, synthetic chemical surfactants present in the soaps and liquid sanitizers [Falk N.A. et al 2019].

Community based epidemiological studies have shown that beneficial effect of Hand sanitizer in reducing the transmission of illness. [Reynolds SA, Levy F, Walker ES. 2006]. Evaluation of antibacterial efficacy of some alcohol based hand sanitizers sold in Ilorin [Oke MA, Bello AB, Odebisi MB, El-Imam AM, Kazeem MO. 2013]. Use of alcohol hand sanitizer as an infection control strategy in an acute care facility [Hilburn J, Hammond BS, Fendler EJ, Groziak PA. 2003]

World Health Organization provided clear guidelines on transmission of health care associated pathogens from one patient to another by HCW hands [WHO, 2009]. Comparative evaluations of efficacy of alcoholic and non alcoholic hand sanitizer's studies were done [Madan K et. al., 2012].

In the late December 2019, several cases of pneumonia of unknown origin were reported from China, which in early January 2020 were announced to be caused by a novel coronavirus. The virus was later denominated severe acute respiratory syndrome coronavirus 2 {SARS –COV-2}

and defined as the casual agent of Corona virus Disease 2019 {COVID-19}.Despite intensive , wide-scale attempts to contain the disease in China , the virus had spread around the world in record time , and COVID-19 was thus declared to be pandemic by the World health Oraganization [WHO] in March 2020.

CoV are found globally in humans and many different ferent animal species. They are classified in the Orthocoronaviridae subfamily [order : Nidovirales , subordination : Cornidovirineae , Family : Coronaviridae].CoV can be grouped into 4 genera , including alpha , beta , gamma , delta – CoV and alpha and beta-CoV can infect mammals , while gamma and delta-CoV can primarily infect birds .

CoV are enveloped viruses with a lipid membrane derived from the host cell, in which viral surface proteins are embedded. The proteins protruding from the viral membrane [especially the {S}Spike protein] give theses pathogens their characteristic halo – like appearance under the electron microscope , which has led to the name corona [latin : garland ,crown].

All CoV have in common that their genome is in the form of single –standard ribonucleic acid [RNA] with positive polarity , meaning that the base sequence of the RNA in the 5'-3' orientation and corresponds to the later messenger RNA [m RNA] with the length of 26.4-31.7 kilobases , the genome of CoV is the largest RNA genome of all known RNA viruses .

Besides a number of non structural proteins including the RNA –Dependent RNA Polymerase [RDRP] , the viral RNA encodes four essential structural proteins , namely the nucleocapsid [N] protein surrounding the RNA genome and three membrane proteins : the S-glycoprotein , the matrix [M] , Protein and the envelope [E] protein .The S-Glycoprotein on the surface of CoV can attach to the circular receptor , angiotensin-converting enzyme 2 [ACE-2] on the surface of human cells . ACE-2 is found in the lower respiratory tract individuals , childrens or persons with existing pulmonary diseases , progression to acute respiratory failure can also occur .

The situation completely changed with the appearance of the SARS- COV . The Virus caused serious human respiratory diseases in China in 2002 and 2003 . Approximately 8000 people were affected by this disease at that time , with case fatality rate [Mortality rate] of around 9.5 % . SARS- COV spread could be stopped by the rapid development of a detection method and extensive measures to isolate infected individuals . Subsequent studies in wild animals showed

that SARS – related CoV are found in Bats and civet Cats , Hence it was assumed that the virus spread from the civet cats to humans followed by human to human spread .

While no human infections with original SARS virus have been reported since 2004 ,Another COV dangerous for human emerged in 2012. The MERS-COV was isolated for the first time from a patient who was hospitalized with acute pneumonia in Saudi Arabia . By 2019 around 2500 MERS –COV infection have been reported in humans, About 30% case fatality rate .The main risk area for MERS –COV infections is the Arabian peninsula .Infections were Reported to be both through contact with dromedaries [camels]. These animals appear to represent a reservoir for MERS-COV.

In the end of December 2019, China reported the increasing occurrence of pneumonia in the city of Wuhan , Hubei province. In January 2020 , a novel Beta-CoV found in the bats . SARS-COV -2 is 96.2% identical to a bat CoV RaTG13, whereas it shares 79.5% identity to SARS-COV. SARS –COV -2 is efficiently transmitted from one person to another person and has thus able to spread rapidly across all continents in our globalised world. In the Resulting COVID -19 pandemic , 601,478 people have been infected and 27,961 patients have died so far [as of march 28,2020 , sources : Johns Hopkins University].

As an emerging acute respiratory infectious disease,COVID-19 primarily spreads through the respiratory tract,by droplets ,respiratory secretions,and by direct contact.Inaddition,it has been reported that SARS-CoV-2 was isolated from fical swabs and blood,indicating the possibility of multiple routes of transmission.However,this needs further classification.

The current data suggests an incubation period of 1-14days,in most cases 3- 7days.The virus is highly transmissible in humans and causes severe problems especially in the elderly and people with underlying chronic diseases.COVID-19 patients typically present with specific,similar symptoms,such as fever,malaise,andcough.Most adults and children infected with SARS-CoV-2 have presented with mild flu like symptoms , but few patients are in critical condition and rapidly developed acute respiratory distress syndrome [ARDS], Respiratory failure , multiple organ failure and even death.

According to recent report,the common clinical manifestations of CoV-19 included fever [88.7%], cough[67.8%], Fatigue[38.1%], sputum production[33.4%], shortness of breath

[18.6%], sour throat[13.9%], and Headache[13.6%].A minor number of patients manifested Gastrointestinal symptoms, with Diarrhea [3.8%], and vomiting[5.0%]. Fever and Cough were dominant symptoms, whereas upper respiratory system and Gastrointestinal symptoms were rare. The case fatality rate increases with the severity of illness and can reach up to 49% in critically ill patients. Unfortunately, no specific therapeutic options are currently available. Only supportive measures can be applied at the moment. There is no specific antiviral treatment recommended for COVID-19 and no vaccine is currently available until 2021. But now the vaccines are developed .

The provision of safe water , sanitation and hygiene conditions is essential to protecting human health during all infectious disease outbreaks, including the COVID-19 outbreak. Ensuring good and consistently applied wash and waste management practices in communities, homes, schools, market places and health care facilities with further help to prevent human to human transmission of the COVID-19 virus.

HISTORY OF THE DISK METHOD

Prior to introduction of the disk assay, the most common method for detecting penicillin in body fluid specimens was the cylinder plate method. This method used to small glass or stainless steel cylinder tubes to hold the antibiotic- containing substance on the surface of an agar plate. Zones of inhibition in the agar were measured from the borders of the tubes A modification of this method using filter paper disks was first reported in the literature by Vincent and Vincent in 1944. In addition to being simpler than the cylinder plate method, disc diffusion was found to be more sensitive for detecting penicillin in serum specimens.The authors hypothesised that the improved sensitivity might be due to more consistent contact of Penicillin with the surface of the agar and more even diffusion from the disk. Using *Staphylococcus aureus* as the indicator organism, they reported a lower limit of detection of 1/16[0.0625]U of Penicillin per millimetre to test fluid and noted that the method worked equally well on serum, spinal fluid, and urine specimens.

A 1955 monograph by Grove and Randall described assay methods for all antibiotics commonly in use at the time, including a more sensitive cylinder plate method capable of detecting penicillin concentrations as low as 0.005U/mL with use of *Micrococcus luteus*[formerly

Sarcinalutea] as the indicator organism. Several investigations later modified the methods of Grove and Randall for use with filter paper disks , particularly after the introduction of gentamycin in the 1960s, when the need to determine blood levels for assessment of toxicity became more pressing.

Rhodes J, Hyder JA, Peruski LF, et al. Antibiotic use in Thailand: quantifying impact on blood culture yield and estimates of pneumococcal bacteremia incidence, *Am J Trop Med . Hyg*, 2010, vol.83[pg.301-6]

Shann F . Bacterial pneumonia: commoner than perceived, *Lancet*, 2001, vol.357[pg.2070-2]

Grove DC, Randall WA., *Assay methods of antibiotics*, 1955 New York, NY Interscience Publishers

Sabath LD . *The assay of antimicrobial compounds*, *Hum Pathol*, 1976, vol.7[pg.171-8]

Gratten M, Manning K, Dixon J, et al. Upper airway carriage by *Haemophilus influenzae* and *Streptococcus pneumoniae* in Australian aboriginal children hospitalised with acute lower respiratory infection, *Southeast Asian J Trop Med Public Health*, 1994, vol.25[pg.123-31].

Alderman , D.J. and P.Smith. 2001. Development of draft protocols of standard reference methods for antimicrobial agent susceptibility testing of bacteria associated with fish diseases. *Aquaculture*, 196: 211-243.

NCCLS, 2002. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard- Second Edition*. NCCLS document M31-A2[ISBN 1-56238-461-9]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA.

CHAPTER-5

Research Methodology

Antibacterial Assay:

1. Bacterial cultures

Tes tbacterial cultures were obtained from MNR Medical College, Sangareddy. Clinical isolates of the bacteria ATCC strain - *E.coli* 25922, ATCC strain -*Staphylococcus aureus* 25923*Klebsiella pneumonia* and *Proteus vulgaris* were used in this study. All the cultures were sub cultured and maintained in nutrient agar slants. Each culture was inoculated into nutrient broth and incubated for 24 hrs for each strain.

2. Preparation of Discs

Discs were prepared with Whatmann filter paper No. 1 in size of about 6 cm in diameter with the paper puncturing machine. Blank discs were sterilized by dry heat method (Hot air oven).

3. Testing antimicrobial activity of Hand Sanitizers against bacterial cultures by Agar Disc Method:

Commercially available and most used Hand Sanitizers namely Instant Hand sanitizer ,Bactorub, Apollo Hand sanitizer and Herbal sanitizer prepared by the students used in this study. The commercially available three sanitizers were chosen for this study based on the frequency of their purchase in the local Medical Shops.

Preparation of Herbal Sanitizer:

Composition:

Water	-	1000ml
Neem leaves (<i>Azadirachta indica</i>)	-	20
Turmeric (<i>Curcuma longa</i>)	-	20gm
Camphor	-	2 balls
Aloevera gel-	50gms	

To the 1000ml of water 20 neem leaves were added and boiled for 10 minutes. 20 gm of turmeric, two balls of camphor were added. This solution is kept aside for 5 minutes. Allow the sample to cool then add filtered extract of aloe vera gel.

Each bacterial strain was inoculated into nutrient broth and incubated at 37°C for 18h in order to reach exponential phase. Each culture broth was swab inoculated on to the nutrient agar plates. Agar surface was allowed to dry for few minutes. Four filter paper discs that were soaked in Sanitizers were placed on each nutrient agar and Muller Hinton Antibiotic Assay medium respectively for bacteria and standard antibiotic discs susceptible for that particular organism aseptically. The standard antibiotic discs used were supplied by HiMedia. They are Tetracycline for *Staphylococcus aureus*, Vancomycin for *E.coli*, Pencillin for *Klebsiella pneumoniae* and Tetracyclin for *Proteus vulgaris*. Plates were incubated at 37°C for 24 hours, after incubation zone of inhibition of bacterial growth was observed in terms of the diameter of inhibition zone in mm.

s.no	Name of the sanitizer	Ingredients of the sanitizer
01	Bactorub	Chlorhexidine Gluconate 0.5% W/V + Ethanol 70% W/V
02	Instant Hand Sanitizer	Alcohol Denat, Water, Propylene Glycol, Tetrahydroxy propyl Ethylenediamine, Fragrance, Limonene.
03	Apollo Hand Sanitizer	Ethylalcohol, Aqua, Glycerine, Perfume, Carbomer And Triethanolamine
04	Herbal Sanitizer	Neemleaves, Turmeric, Camphor, Alovera gel.

Table 1. Different Hand Sanitizers used in this study

Figure 5 : Ingredients Of Herbal Sanitizer



Figure 6 :Streaking Method

Schematic Representation Of Agar Disc Test To Determine Susceptibility Of Test Organisms To Hand Sanitizers

Four Different Hand Sanitizers were used and Sterilized. Nutrient , Muller Hinton's Agar plates were inoculated with test organisms.



Sterile cotton swab dipped into tube containing inoculum and excess removed by rotating firmly against inside wall of the tube above the liquid level



Swab was streaked over agar surface three times while rotating the agar plate at an angle of 60° after each application



Sterile 6 mm cork borer used to prepare 4 equally spaced holes in agar plate with one set another set with Disc soaked in Sanitizer placed on another set of the plates and agar plugs discarded using sterile needle



Fifty microliters of the hand sanitizer was then introduced into each of the 4 wells while antibiotic disc was placed to serve as control



This was done for all the test organisms and plates were incubated in incubator for 24 h at 37°C in an upright position



Analysis of zone of inhibition to evaluate Antimicrobial efficacy of different hand sanitizers indicating susceptibility of the respective test organism

Figure 7 :Placing of discs on agar plates



Figure 8 : Zone of inhibition of different Bacterial pathogens



Escherichia coli



Proteus vulgaris



Staphylococcus aureus



Klebsiella pneumoniae

CHAPTER-6

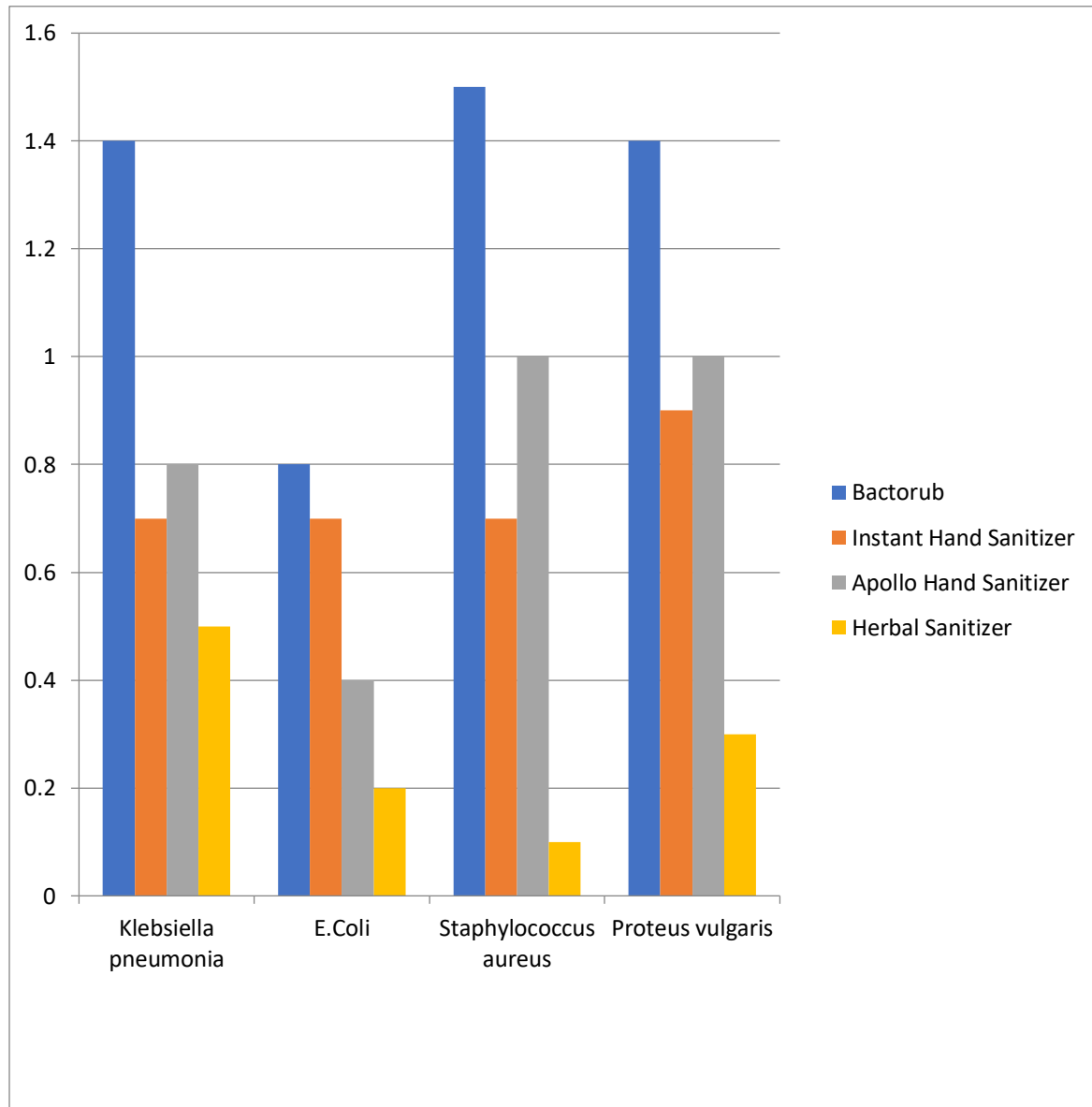
Analysis of Data

The observations made for recorded and represented in the Table 1. Graphs were plotted based on the data.

Table 2. Antimicrobial efficacy of 4 sanitizers against 4 Bacterial Strains

S.NO	Name of the Bacteria	Name of the sanitizer	Zone of Inhibition in cm [Disc Method]
1	Klebsiella pneumonia	Bactorub	1.4
		Instant Hand Sanitizer	0.7
		Apollo Hand Sanitizer	0.8
		Herbal Sanitizer	0.5
		Control[Pencillin]	1.0
2	E.Coli	Bactorub	0.8
		Instant Hand Sanitizer	0.7
		Apollo Hand Sanitizer	0.4
		Herbal Sanitizer	0.2
		Control[Vancomycin]	1.0
3	Staphylococcus aureus	Bactorub	1.5
		Instant Hand Sanitizer	0.7
		Apollo Hand Sanitizer	1.0
		Herbal Sanitizer	0.1
		Control[Tetracycline]	0.6
4	Proteus vulgaris	Bactorub	1.4
		Instant Hand Sanitizer	0.9
		Apollo Hand Sanitizer	1.0
		Herbal Sanitizer	0.3
		Control[Tetracycline]]	1.2

Graph 1 : Effect of four different Hand Sanitizer on four Bacterial strains.



CHAPTER-7

Findings

In the present study Four Hand sanitizers, 3 commercially available one herbal sanitizer were tested for antimicrobial activity against four pathogenic bacteria *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris* respect effective against all the test organisms. The antimicrobial effectiveness was assessed by measuring the zone of inhibition against the particular test organism in one method i.e. Disc method.

Maximum zone of inhibition (in cm) is observed in disc method . Maximum inhibition of growth for *Klebsiella pneumonia* observed was 1.4, 1.0 (in cm) respectively for the Bactorub Sanitizer with reference to the control Pencillin. Minimum growth inhibition was observed with commercially available sanitizers such as Instant Hand Sanitizer , Apollo Hand sanitizer and Herbal sanitizer ,which we prepared in our laboratory .

For the *E.coli* also Bactorub Sanitizer showed maximum growth of inhibition 0.8 , 1.0 (In cm) with reference to the control Vancomycin . Minimum growth inhibition was observed with Instant , Apollo Hand sanitizer and Herbal Sanitizer .

For *Staphylococcus aureus* maximum growth inhibition was observed with 1.5 , 1.0(cm) Bactorub and Apollo Hand Sanitizer compared to the Instant Hand Sanitizer and Herbal Sanitizer respectively (Table.2.)

The observations made were represented in the Table 2 as mean of two values observed on Agar plates Figure. Maximum growth of inhibition was observed with *Klebsiellapneumoniae*, *E.coli*, *Staphylococcus aureus*, *Proteus vulgaris* against Bactorub. The range of inhibition was varied with different commercial hand sanitizers with four bacteria *Klebsiellapneumoniae*, *E.coli*, *Staphylococcus aureus* and *Proteus vulgaris* ..

The inhibition of growth of *Klebsiella* for the effective sanitizer i.e. Herbal Sanitizer against standard antibiotic Tetracycline was slightly low (0.5 , 1.0), same observations are made with other two bacteria *E.Coli* , *Staphylococcus aureus* with standards tetracycline Penicillin respectively .

The effectiveness of hand sanitizers in terms of zone of inhibition of bacteria was shown.

CHAPTER-8

Conclusions

- In the current piece of small work Herbal sanitizer possessed most antibacterial effect in the form of zone of inhibition against Three Gram negative, one Gram positive bacteria strains i.e. *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus* species used in this study.
- Three commercially available Sanitizers i.e Bactorub, Apollohand sanitizer, Instanthandsanitizer. Among these Bactorub has shown more efficacy.
- Even for the herbal sanitizer the efficacy of the sanitizer was less with reference to the standards i.e. Penicillin, Tetracycline respectively which are specific for that particular Bacterium.
- The sanitizers tested in this study for antibacterial activity shown more effect.

Suggestions

- ❖ The present study has its own limitations – as only the antimicrobial efficacy of four different hand sanitizers was assessed. Further studies are required to assess the exact quantity and duration of application of hand sanitizer or disinfectant.
- ❖ There is an urgent need for developing eco-friendly technologies that offer safer and more effective disinfection methods to combat the ongoing pandemic, along with conferring protection to the environment and living beings from the potentially hazardous effects of chemical disinfectants.

CHAPTER-9

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TARA GOVERNMENT COLLEGE(A), SANGAREDDY

PROJECT WORK AWARD LIST

GROUP: B.SC (MZC) SEMESTER VI

SUBJECT:							
S.No	Hall ticket number	Name of the Candidate	Project Report(Max. 70 Marks)	Research design seminar (Max.5 marks)	Work Progression Seminar (Max.5 marks)	Final presentation & Viva voce(Max.20marks)	TOTAL
1	6058-19-457-001	AMRIN					
2	6058-19-457-002	ANGOTH SANGEETHA					
3	6058-19-457-006	DHANNARAM SUDHAKAR					
4	6058-19-457-007	ERIGIPALLY SHIVALEELA					
5	6058-19-457-009	KUNADODDI SHALINI					
6	6058-19-457-011	M PRAGNA					
7	6058-19-457-013	NALLOLLA RANJITHA					
8	6058-19-457-014	NEERADI ANITHA					
9	6058-19-457-016	NELAPATI VIJAYA					
10	6058-19-457-017	PATHRI KIRAN KUMAR					
11	6058-19-457-018	PATNAM PRASHANTH KUMAR					
12	6058-19-457-019	PEDDASAYEB PRAVEEN KUMAR					
13	6058-19-457-020	PERKA SAI CHARAN					
14	6058-19-457-021	PONNA SRIJA					
15	6058-19-457-022	PURAMOLA MADHAVI					
16	6058-19-457-023	RAVULA ABHISHEKASUMALATHA					
17	6058-19-457-024	REGOTI VAMSHI PRIYA					

