EVALUATION OF THE EFFICACY OF A COMMONLY USED ANTISEPTIC SOAP IN REDUCING THE BACTERIAL LOAD ON THE PALMS OF SOME UNDERGRADUATE STUDENTS OF ALEX EKWUEME FEDERAL UNIVERSITY NDUFU ALIKE IKWO, EBONYI STATE.

ABSTRACT

INTRODUCTION: The human hand serves as a carrier of microorganisms, comprising both transient and normal flora. The present study aims at evaluating the efficacy of a commonly used antiseptic soap in reducing the bacterial load on the palms of some undergraduate students of Alex Ekwueme Federal University Ndufu Alike Ikwo, Ebonyi State.

METHODS: Handwashing with the antiseptic soap was carried out according to the World Health Organisation protocol on handwashing. Bacterial identification was done using morphological and biochemical characteristics, also antimicrobial susceptibility test was carried out on the identified bacterial species using agar diffusion according to the method of Clinical Laboratory Standard Institute.

RESULTS: The result of the bacterial load indicated bacterial load of 2.0×10^2 before handwashing and 0.96×10^2 after handwashing, on plate count agar and bacterial load of 7.7×10^1 before handwashing and 3.2×10^1 on MacConkey agar, the results were statistically analyzed using Chi-square test. The following bacteria species were identified from the palms of the students after handwashing; *Strep. pneumoniae, Staph. aureus, Staph. spp, Strep. spp, A. baumannii, E. coli*.

CONCLUSION: The result of the statistical analysis showed that sanitol antiseptic soap significantly reduced the bacterial load on the palms of the participants.

Keywords: Antiseptic, Hand hygiene, Efficacy.

INTRODUCTION

Hand hygiene refers to the procedure for cleaning and decontaminating the hands using alcohol-based hand-rub or plain soap and water. It is an essential clinical action that fits into everyday practice and is considered the most effective measure in preventing healthcare-associated infections and, indeed, in saving lives [10,5].Cross-transmission of microorganisms that can be pathogenic involves five sequential actions, but there are many opportunities to break this chain [4]. If the actions are not taken, the hands become the main route of microbial transmission [8].

The importance of hand hygiene as a fundamental preventive measure cannot be overstated. Hands serve as common vectors for the transmission of various pathogens, including bacteria, viruses, and fungi [7]. Consequently, effective hand hygiene practices have emerged as an indispensable strategy for breaking the chain of infection transmission.

Hand antiseptics, particularly those based on alcohol formulations recommended by the World Health Organization (WHO), play a pivotal role in breaking the chain of pathogen transmission from humans to humans and from the environment to humans. The need for high-quality hand antiseptics is imperative as they serve as a front-line defence against infectious diseases in various settings, including healthcare facilities, public spaces, and everyday life. In this context, the research conducted by [1], contributes significantly to our understanding of the performance of these essential hygiene products.

Within the academic context, undergraduate students represent a dynamic and diverse population, regularly engaging in a multitude of activities. Their hands are exposed to a plethora of environmental contaminants, pathogens, and communal surfaces as they participate in classroom learning, laboratory work, sports, and social interactions. These activities can lead to the accumulation of microorganisms on their hands, making them potential carriers of infectious agents within the university community [9].

Sanitol Antiseptic Soap, like many other commercial antiseptic soaps, claims to possess potent microbicidal properties. These products are marketed as effective tools for reducing microbial contamination on the skin, particularly on the hands, where pathogens often gain entry into the body [4].

MATERIALS AND METHOD

Three (3) students participated in the study, the palms of the participants were swabbed with sterile swab sticks before and after handwashing with the antiseptic soap.

MEDIA PREPARATION

All media used was prepared according to manufacturer's guide, it was weighed, dissolved in a conical flask containing distilled water and stirred properly to allow complete dissolution of the dehydrated powder. The flask containing the media was autoclaved at 121^{o}C for 15 minutes for sterility. It was allowed to cool at 45^{o}C and dispensed 15mls into 20mls petri dishes and allowed to solidify.

ISOLATION, COLONY COUNT AND IDENTIFICATION OF BACTERIA SPECIES FROM THE PARTICIPANTS PALMS.

Innoculation of broth containing microorganisms from participants palms onto petri dishes was carried out, colony count, gram staining and the following biochemical test were carried out (catalase test, indole test, methyl-red test, citrate utilization test, gel liuefaction test, oxidase test, coagulase test).[11,12]

ANTIMICROBIAL SUSCEPTIBILITY

Antimicrobial susceptibility test was carried out on the identified bacterial species using agar diffusion according to the method of Clinical Laboratory Standard Institute.

DATA ANALYSIS

Chi-square test was used to statistically analyse the data gotten from the palm of students before and after handwashing, so as to determine if there was a significant difference in microbial load between the critical value representing the control group and the calculated value representing the experimental group.

RESULT

Colony Count on PCA before handwashing

Table 1 below showsthe colony counts of organisms isolated from the palms of the participants which were cultured on Plate Count Agar before handwashing with Sanitol antiseptic soap. The organisms were counted and expressed in colony forming unit (CFU).

Table 1: Bacterial Load On Plate Count Agar (PCA) From The Palms Of The Participants Before Handwashing With Sanitol Soap.

Participants	No_ Of Colonies	Colony Forming Units/ml
Participants 1	200	2.0×10^2
Participants 2	150	1.5×10^2
Participants 3	250	2.5×10^2

Key Interpretations

No: Number

ml: Mililitres

Colony Count on MAC before handwashing

Table 2 below showsthe colony counts of organisms isolated from the palms of the participants which were cultured on MacConkey Agar before handwashing with Sanitol antiseptic soap. The organisms were counted and expressed in colony forming unit (CFU).

Table 2. Bacterial Load On MacConkey Agar (MAC) From The Palms Of The Participants Before Handwashing With Sanitol Soap.

Participants	No_ Of Colonies	Colony Forming Units/ml
Participants 1	72	7.2×10^1
Participants 2	64	6.4×10^1
Participants 3	79	7.9×10^1

Key Interpretations

No: Number

ml: Mililitres

Colony Count on PCA after handwashing

Table 3 below showsthe colony counts of organisms isolated from the palms of the participants which were cultured on Plate Count Agar after handwashing with Sanitol antiseptic soap. The organisms were counted and expressed in colony forming unit (CFU).

Table 3. Bacterial Load On Plate Count Agar (PCA) From The Palms Of The Participants After Handwashing With Sanitol Soap.

Participants	No_ Of Colonies	Colony Forming Units/ml
Participants 1	98	9.8×10^1
Participants 2	84	8.4×10^1
Participants 3	106	1.1×10^1

Key Interpretations

No: Number

ml: Mililitres

Colony Count on MAC after handwashing

Table 4 below showsthe colony counts of organisms isolated from the palms of the participants which were cultured on MacConkey Agar after handwashing with Sanitol antiseptic soap. The organisms were counted and expressed in colony forming unit (CFU).

Table 4. Bacterial Load On MacConkey Agar (MAC) From The Palms Of The Participants After Handwashing With Sanitol Soap.

Participants	No_ Of Colonies	Colony Forming Units/ml
Participants 1	32	3.2×10^1
Participants 2	37	3.7×10^1
Participants 3	26	2.6×10^1

Key

Interpretations

No: Number

ml: Mililitres

Statistical analysis for isolates on PCA before and after handwashing

Table 5 below shows the result of the statistical analysis performed using the colony count from the isolates on Plate Count Agar before and after handwashing with Sanitol antiseptic soap. The Chi-square result was statistically insignificant (p = 0.6).

Table 5: Chi-square Test For The Bacterial Load On Plate Count Agar (PCA), Before And After Hand Washing With Sanitol Soap.

	Participant 1	Participant 2	Participant 3	Chi-square	Decision
Before	2.0×10^{2}	1.5×10^{2}	2.5×10^{2}	0.60	
After	9.8×10^{1}	8.4×10^{1}	1.1×10^{2} 1.2	1.8÷2 = 0.9	did not Significantly reduce bacteria load.

Statistical analysis for isolates on MAC before and after handwashing

Table 6 below shows the result of the statistical analysis performed using the colony count from the isolates on MacConkey Agar before and after handwashing with Sanitol antiseptic soap. The Chi-square result was statistically insignificant (p = 0.6).

Table 6: Chi-square Test For The Bacteria l Load On MacConkey Agar (MAC), Before And After Hand Washing With Sanitol Soap.

	Participant 1	Participant 2	Participant 3	Chi-square	Decision
Before	7.2×10^{1}	6.4×10^{1} 7.9×10^{1}	1.05		
After	0.32×10^{1}	0.37×10^{2}	0.26×10^{2} 2.4	3.45÷2 = 1.725	Significantly reduced bacteria load.

ISOLATED MICROORGANISMS, THEIR GRAM REACTIONS AND BIOCHEMICAL CHARACTERISTICS.

Table 7 shows the microorganisms isolated from palms of the participants on Plate Count Agar, their gram reactions and biochemical characteristics. Different organisms such as *Staphaureus*, *P. mirabilis*, *A. baumannii*, *Strep. spp* were obtained and they were identified using several biochemical test such as catalase, oxidase, gel liquefaction, citrate, methyl-red and indole tests. Coagulase test was only carried out for one organism (*Staph. aureus*).

Table 7. Morphological And Biochemical Characteristics Of Bacteria Species On Plate Count Agar (PCA) Isolated From The Palms Of The Participants After Handwashing With Sanitol Antiseptic Soap .

Isolates	Cell Shape	Cell Arrang	Gram Reac -ment-tion	Cata -lase Test	Oxid -ase Test	Coagu -lase Test-faction	Citrate Test	Gel Lique	Methyl -red	Indole Test	Most Probable Organisms
$P1_1$	Cocci	Pairs	+ve	+ve	-ve		+ve	-ve	+ve		Strep. Pneumoniae
$P1_2$	Cocci	Clusters	+ve	+ve	-ve	+ve	+ve	-ve			*Staph. aureus*
$P1_4$	Cocci	Single	+ve	+ve	-ve		-ve	-ve			Staph. *aureus*
$P2_2$	Cocci	Pairs	+ve	+ve	-ve						*Staph. spp*
$P2_3$	Rod	Single	-ve	-ve	+ve		-ve	-ve	+ve	-ve	*P. mirabilis*
$P3_1$	Cocci	Chains	+ve	-ve	+ve		-ve	-ve			*Strep. spp*
$P3_3$	Cocci	Chains	-ve	+ve	+ve		+ve	-ve			*A. baumannii*

Key Interpretations

+ve: Positive

-ve: Negative

Staph. aureus: Staphylococcusaureus

Staph. spp: Staphylococcus specie

Strep. spp: Streptococcus specie

A. baumannii: Acinetobacter

P. Mirabilis: Proteus mirabilis

ISOLATED MICROORGANISMS, THEIR GRAM REACTIONS AND BIOCHEMICAL CHARACTERISTICS.

Table 8 below shows the microorganisms isolated from palms of the participants on Plate Count Agar, their gram reactions and biochemical characteristics. Different organisms such as *E. coli, M. morganii* and *Strep. spp* were obtained and they were identified using several biochemical test such as catalase, oxidase, citrate, gel liquefaction, methyl-red and indole tests.

Table 8. Morphological And Biochemical Characteristics Of Bacteria Species On MacConkey Agar (MAC) Isolated From The Palms Of The Participants After Handwashing With Sanitol Antiseptic Soap .

Isolates	Cell Shape	Cell Arrang-ment	Gram Reac-tion	Cata-lase Test	Oxid-ase Test	Coagu-lase Test	Citrate Test	Gel Lique-faction	Methyl-red Test	Indole Test	Most Probable Organisms
$P2_6$	Rod	Chains	-ve	-ve	+ve		-ve	-ve	+ve	+ve	*E. coli*
$P2_7$	Rod	Single	-ve	-ve	+ve		-ve	-ve	+ve	+ve	*E.coli*
$P3_2$	Cocci	Pairs	-ve	-ve	-ve		-ve	-ve			*Strep. spp*

Key Interpretations

+ve: Positive

-ve: Negative

E. *Coli: Escherichia coli*

ANTIBIOTIC SUSCEPTIBILITY TESTS

Table 9 below shows the inhibition zone diameters in millimetre of the isolates against the different antibiotics used.

Table 9: Inhibition Zone Diameters (IZD) Of The Isolated Bacterial Species Against The Different Antibiotics Used.

Antibiotics Used And IZD In (mm)						
Isolates	**IMI**	**CIP**	**TE**	**CN**	**E**	**AMP**
Strep. Pneumoniae	19	14	12	28	7	14
Staph. aureus	12	27	-	23	10	8
Staph.aureus	23	26	12	-	-	17
Staph. spp	32	28	20	28	28	23
P. mirabilis	21	25	10	29	18	7
E. coli-	23	11	27	17	7	
M. morganii	27	10	15	31	-	27
*Strep. spp*30	-	16	24	10	17	
Strep. spp	26	31	16	30	22	34
A. baumannii	27	10	18	33	-	29

Key Interpretations

Staph.aureus: Staphylococcus aureus

Staph. spp: Staphylococcus specie

Strep. spp: Streptococcus specie

A. baumannii: Acinetobacter baumannii

P. Mirabilis: Proteus mirabilis

Strep. spp: Streptococcus specie

E. coli: Escherichia coli

M. morganii: Morgenella morganii

IMI: Imipenem

CIP: Ciprofloxacin

CN: Gentamicin

AMP: Ampicillin

E: Erythromycin

TE: Tetracycline

ANTIBIOTIC SUSCEPTIBILITY PROFILE

Table 10 shows the susceptibility or resistivity of the different isolates to the different antibiotics used. The antibiotics used were Imipenem, Ciprofloxacin, Erythromycin, Ampicillin, Gentamycin, Tetracycline.

Table 10:Antimicrobial Susceptibility Profile Of The Isolated Bacteria Species To The Different Antibiotics Tested.

Isolates	IMI	CIP	TE	CN	E	AMP
Strep. Pneumoniae	R	R	R	S	R	I
Staph. aureus	R	S	R	S	R	R
Staph. aureus	S	S	R	R	R	S
Staph. spp	S	S	S	S	S	S
P. mirabilis	I	S	R	S	I	R
*E. coli*R	S	R	S	I	R	
M. morganii	S	R	I	S	R	S
*Strep. spp*S	R	I	S	R	S	
Strep. spp	S	S	I	S	I	S
A. baumannii	S	R	I	S	R	S

Key Interpretations

Staph. aureus: Staphylococcus aureus

Staph. spp: Staphylococcus specie

Strep. spp: Streptococcus specie

A. baumannii: Acinetobacter baumannii

P. Mirabilis: Proteus mirabilis

Strep. spp: Streptococcus specie

E. coli: Escherichia coli

M. morganii: Morgenella morganii

R: Resistant

S: Susceptible

I: Intermediate

IMI: Imipenem

CIP: Ciprofloxacin

CN: Gentamicin

AMP: Ampicillin

E: Erythromycin

TE: Tetracycline

DISCUSSION

In this study, handwashing was done according to WHO, CDC and UNICEF standards using 200mls of water for 50 seconds after which samples were collected from the palms using a sterile swab stick. Subsequent assessment of bacterial colonies on the participant palms revealed that the bacterial colony counts was 2.0×10^2 and 0.96×10^2 colony forming unit (CFU) on plate count agar and 7.7×10^1 and 3.2×10^1 before and after handwashing respectively with sanitol antiseptic soap. The results on the bacterial colonies can be related to a study conducted by [3]. whose study based on investigating the variance of bacterial colony counts on students palms resulting from using plain soap and antiseptic soap for handwashing, the results showed that bacterial colony counts for plain soap was 45.5 and that of antiseptic soap to be 38.8 colony forming unit respectively. [3]

Bacterial colonies were identified and gram stain as well as other biochemical tests were carried out for secondary identification of the isolates. The results of the bacterial isolates from the palm swab samples indicated the presence of coagulase positive *Staphylococcus aeurus, Proteus mirabilis, Escherichia coli, Morgenella morganii, Streptococcus pneumoniae, Acinetobacter baumanni, staphylococcus species and streptococcus species*. This isolates gotten from this study are consistent with the isolates gotten from a study conducted by [2] among female students in Madonna University Elele, Rivers State which was aimed at assessing the antibacterial effects of various medicated soap on bacterial isolates from the skin.

Some of these bacterial isolates obtained from the present study are not normal flora and they includes; *Escherichia coli* which is not part of the normal flora of the skin. It is primarily found in the intestines but can be transiently found on the skin due to contact with fecal matter. It is not a typical cause of skin infections. *Proteus mirabilis* also is not a resident of the skin. It is more commonly associated with urinary tract infections and wound infections than skin flora [6].

Futhermore, the bacterial specie *Acinetobacter baumannii* is not a part of the normal skin flora and is more often associated with healthcare-associated infections, especially in hospitalized patients. *Streptococcus pneumoniae* is not typically part of the normal skin flora and is primarily found in the respiratory tract. It can cause respiratory infections but is not a common cause of skin infections.

To discern if a significant reduction existed in bacterial colony counts from the 'before' and 'after' handwashing, a Chi-Square statistical test was employed. The resultant probability value was 0.9 and 1.725, surpassing the predefined significance level of 0.50 (1.386). Consequently, this indicated that there was a significant reduction of the bacterial load on the palms.

Antimicrobial susceptibility tests using agar diffusion followed the Clinical Laboratory Institute method. After 24 hours, results showed susceptibility of *Streptococcus pneumoniae*, *Proteus mirabilis, Escherichia coli*, and *Morgenella morganii* to gentamycin. They exhibited resistance to tetracycline, ampicillin, imipenem, and erythromycin respectively. *Staphylococcus* and *Streptococcusspp* were

susceptible to imipenem and resistant to ciprofloxacin, respectively. Both *Staphylococcusaureus* isolates were susceptible to ciprofloxacin but resistant to ampicillin. *Acinetobacter baumannii* and *Streptococcus aureus* were susceptible to ampicillin, while the latter showed resistance to erythromycin.

The isolation of bacteria from the palms after handwashing with antimicrobial soaps in this research raises significant implications for both individual and public health. While antimicrobial soaps are designed to reduce microbial contamination, the persistence of bacteria on the hands suggests potential shortcomings in the efficacy of the handwashing process. Effective handwashing is expected to significantly reduce the number of microorganisms on the hands but this vary widely depending on several factors, including the initial microbial load on the hands, the effectiveness of the handwashing process, the type of soap or antimicrobial agent used, and individual hygiene practices. However, it is uncommon to achieve complete sterility, and a residual population of microorganisms may persist. The goal of handwashing is to reduce the microbial load to a level that is considered safe and unlikely to contribute to the transmission of infections.

CONCLUSION

In conclusion, isolates from the participants palms indicated the presence of beneficial (normal flora) microorganisms and non beneficial microorganisms as they exhibited various characteristics in reaction with the different biochemical test carried out which is in line with a study conducted by [2]. The bacteria colonies gotten before and after handwashing with Sanitiol antiseptic soap indicated that there was a significant reduction after handwashing which aligns with a study by [3]. After adequate

statistical evaluation using Chi-square analysis was done, it was concluded that Sanitol antiseptic soap significantly reduced the bacterial load on the palm.

It is advised that proper handwashing be done with antiseptic soaps so as to effectively reduce bacterial load on the palms and generally prevent hand associated infections.

Disclaimer (Artificial intelligence)

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Details of the AI usage are given below:

1. ChatGPT 3; provide an outlined structure of a research work
2. ChatGPT 3; write briefly on Normal flora of the hand.
2.
3.

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