

Antibacterial Activities of Medicated and Antiseptic Soaps on *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* Isolated from Wound Infection

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Abstract

Soaps and other cleaning chemicals have been widely utilized for various cleaning purposes for a long time. As the skin is the first line of defense, most bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus* reside and are the primary cause of skin infections. The aim of this study was to determine the antibacterial effects of medicated soap (tetmosol) and antiseptic soap (premier cool) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound samples. The antibacterial activity of medicated and antiseptic soaps was investigated against test organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) using agar well and disk diffusion methods. After serial dilution, different concentrations of the various soap samples in the range of 200 mg/ml to 62.5 mg/ml were prepared (using sterile distilled water). The result of this study showed that the antiseptic soap "Premier cool" was found to be most effective against all the bacteria strains tested. The antiseptic soap had the highest zone of inhibition (19.00 ± 1.42 mm) against *Staphylococcus aureus* and 15.00 ± 0.34 mm against *Pseudomonas aeruginosa* at the highest dilution used (200mg/ml). The medicated soap "Tetmosol" exhibited a minimal antibacterial activity against the isolates with a zone of inhibitions of 16.00 ± 0.48 mm 14.00 ± 1.41 mm for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. The result of the minimum inhibitory concentration showed that antiseptic soap (Premier cool) had better MIC and MBC of 12.5 mg/ml and 25 mg/ml, respectively, on *Staphylococcus aureus*. For *Pseudomonas aeruginosa*, the MIC and MBC were 50 mg/ml, respectively. Medicated soap (Tetmosol) had a higher MIC of 25 mg/ml and MBC of 50 mg/ml for *Staphylococcus aureus*. For *Pseudomonas aeruginosa*, the MIC and MBC were 50 mg/ml and 100 mg/ml. The present work has shown that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were susceptible to assayed medicated (Tetmosol) and antiseptic (Premier cool) soaps. This study proved that all the soaps samples had antibacterial activity against all the tested bacterial strains. Still, Premier cool soap is the most effective soap against all the given bacteria and should be the first choice for daily use. It is recommended that further studies should be done on antimicrobial resistance, both phenotypic and genotypic, concerning prolonged use of medicated and antiseptic soaps.

Keywords: *Staphylococcus aureus*; *Pseudomonas aeruginosa*; Antibacterial; Soap; Wound infection.

1. Introduction

Soaps and other cleaning chemicals have been widely utilized for various cleaning purposes for a long time [1]. Handwashing with soap and water has been considered a measure of personal cleanliness for decades. Bacteria may be found in soil, water, air, sewage, and the human body, making them extremely important in terms of health [2]. Soaps are essential for cleansing as well as eradicating microorganisms. Some active substances are added to soap to improve its antibacterial properties [3]. Antibacterial soap can remove about 65-85% of bacterial flora from human skin [4]. Antibacterial activity refers to the capacity to kill or impede the development of germs. This is referred to as cidal or static effects, depending on the situation. This is crucial in avoiding sepsis and skin infections in humans [5]. Soap cleans by attracting molecules to the fatty component of the anions in the soap solution, which are then drawn off the filthy surface and into the water. Additional chemicals are commonly included in antiseptic soaps, which are used to treat skin diseases [6]. Germicidal chemicals such as irgasan, trichlorocarbanlide (TCC), and others are added to antiseptic soaps to boost their antibacterial action [7]. These germicidal ingredients are added in a particular proportion. Their percentages are always listed on the soap case or leaflet, which includes instructions on using the soap for various reasons [8].

As the skin is the first line of defense, most bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus* (*S. aureus*) reside and are the primary cause of skin infections. Handwashing with antibacterial is of more

importance following the health care associates as they may be the leading cause of bacterial contamination either opportunistic or pathogens [9]. Soaps contain active ingredients that have antibacterial activity and also the reducing power against the pyogenic skin infection caused by *Staphylococcus aureus* and other gram-negative species of bacteria [10]. It is studied that antibacterial soap removes bacteria than a plain soap [11]. According to Tong, *et al.* [12] medicated soap contain germicidal ingredients and the usual soap base to boost antibacterial action.

S. aureus is both a commensal bacterium and a human pathogen. Approximately 30% of the human population is colonized with *S. aureus* [13]. According to Ikegbunam, *et al.* [13], *S. aureus* infections range in severity from mild skin infections to severe necrotizing pneumonia. It is simultaneously the leading cause of bacteremia, infective endocarditis (IE), and can also cause osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections. These can vary from superficial skin lesions such as folliculitis to deep-seated abscesses and pyogenic diseases such as endocarditis and osteomyelitis.

A wound is defined as a breach in the skin's integrity or discontinuity due to fracture [14]. Wound healing, or the restoration of skin continuity, is a biological process that involves regeneration, cell proliferation, and collagen production, which can be aided by washing the wound surface and other infected skin lesions, such as atopic dermatitis, with antiseptic soap, which contains phenolic compounds that help keep organisms such as *S. aureus* and *Pseudomonas aeruginosa* away from the sites [15]. According to Becker, *et al.* [16], Antisepsis is the most practical method of avoiding illness by limiting bacterial development. The term "sterilization" refers to the destruction of all living things, and this term is frequently limited to pathogenic organisms that cause devastation.

Several chemical substances are available that can inhibit the development of bacteria and even kill them. These chemicals are in massive quantities, maybe 10,000, with 1000 being regularly utilized in hospitals and households. Solids, liquids, and gases are all forms of chemical compounds. Many chemical groups have been employed to reduce or eliminate microorganisms. Halogens, phenols, soaps, detergents, ammonia compounds, alcohols, heavy metals, acids, and other unusual chemicals are the most significant groupings [17]. Cleaning agents are widely available on the market, and they come in a variety of forms and formulations. Antibacterials such as triclosan, trichlorocarbanilide, and P-chloro-in-xyleneol (PCMX/Chloroxyleneol) are often used in medicated soaps. Unless the substance is identified as antibacterial, antiseptic, or germicidal, they are usually only present at the preservation level [18]. Some people believe that soap's antibacterial component is efficient against germs and may prevent most infectious diseases; however, researchers have discovered that excessive soap use might spread infections rather than control them [19]. Excessive use of medicated soaps may develop a resistant strain, making the user more susceptible to opportunistic skin infections [20]. Therefore it is of paramount importance to evaluate the antibacterial activity of medicated and antiseptic soap on two species of bacteria isolated from wound infection.

2. Materials and Methods

2.1. Sample Collection

The medicated (Tetmosol) and antiseptic (Premier cool) soap samples used for the study were purchased from a known pharmacy (Grace and Mercy pharmacy) in Umuahia, Abia State. The batch numbers, expiry dates and the presence or absence of the manufacturers seal were noted

2.2. Sterilization Methods

All the glass-ware was properly washed and sterilized in a hot air oven at 170°C for 2 hours. Distilled water was sterilized in the autoclave at 121°C for 15mins. Cork-borer and glass rods were sterilized by dipping into 70% alcohol prior to flaming in Bunsen burner. The work bench was swabbed with 75% alcohol before and after each experiment

2.3. Media used/ Preparation.

Manitol salt agar was used to support the growth of *Staphylococcus aureus* from the wound samples, MacConkey agar was used to isolate *Pseudomonas aeruginosa* from the wound samples, Nutrient agar was used for the sub-culturing of the isolates to obtain a pure culture. while Mueller Hinton agar/broth was used for carrying out Agar Disc/well Diffusion method for diameter zone of inhibition, Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) respectively..

All the media used for this research work were prepared according to the manufacturer's instructions and autoclaved for 15minutes at 121°C at 15psi and were aseptically poured into sterile Petri-dishes.

2.4. Source of Microbial Cultures

Cultures are from clinical sources. They were obtained from the wound samples from Linc's laboratory Umuaraga in Ikwuanu LGA, Abia State. They include: one Gram-positive bacterium (*Staphylococcus aureus*) and one Gram-negative bacterium (*Pseudomonas aeruginosa*). The bacterial cultures were maintained in their respective agar slants at 4°C throughout the course of the study and used as stock cultures.

2.5. Confirmation of Test Isolate

The confirmation of the test organisms was done by standard techniques^[21] based on their;

2.6. Morphological Appearance

Parameters such as colour, shape elevation, pigmentation, opacity and nature of edges of the colonies were observed and recorded for each isolate

2.7. Gram Staining

A smear from the sample was made on a clean grease-free slide, air dried and heat fixed. The slide was flooded with crystal violet for 1 minute, and rinsed with water. Lugol's iodine (mordant) was applied for 60 seconds and rinsed. Acetone was used in decolorizing and washed immediately then counter stained with neutral red for 1 minute. It was then rinsed with water, blotted carefully and air dried. Finally the slides were observed under the microscope using oil immersion objectives (x100) [21].

2.8. Motility Test

The test is useful in detecting motile and non-motile organisms. A drop of a 20 hours peptone medium culture of the test organism was placed on a clean grease free slide with the aid of Pasteur pipette. The slide was then covered with a cover slip and viewed under the microscope using x40 objective lens. The movement of small motile bacteria is distinguished from the on-the-spot vibratory movement (Brownian movement) which is shown by all microorganisms and particles when suspended in a fluid. True bacterial motility is the ability of an organism to move itself in different directions or a single direction [21].

2.9. Biochemical Tests

Isolated organisms were identified by standard microbiology identification techniques including Catalase test, Citrate utilization test, methyl-Red test, Voges-Proskauer test, Urease test and Indole test [21].

2.10. Preparation of Soap Samples

A sterile blade was used to scrap one gram (1g) each of the soaps and which quantity was dissolved in 9mls of sterile distilled water to give a stock solution of 10^{-1} . These stock solutions were then stored in a refrigerator in well-sealed containers for future use [15].

2.11. Preparation of Disks with Soap Samples

Disks of diameter 6 mm were bored from Whatman filter paper using disc borer. The discs were then wrapped in foil paper and sterilized in a hot air oven at 100°C for 1 hour and were later soaked in the different soap solutions for a period of one hour to ensure full saturation of the soap preparations. The discs were then aseptically removed from soap solution and allowed to dry in an oven at 25°C. They were then packed into sterile bottles, corked and stored in the refrigerator for future use in susceptibility test [22].

2.12. Antimicrobial Susceptibility Testing

2.12.1. Disk Agar Diffusion Method

The disk agar diffusion method was used with Mueller- Hinton agar plates. Plates were dried with their lids slightly raised at a temperature of 60°C in the oven for 15 minutes. The test organisms from growth on nutrient agar plates incubated at 37°C were suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland Standard. The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates using sterile cotton swab. Different concentrations of the various soap samples in the range of 200 mg/ml to 6.25 mg/ml were prepared (using sterile distilled water) following serial dilution [23]. The plates were left for about 30 minutes; the disks were aseptically placed at equal distance on the sensitivity plates with the aid of a sterile forceps. Within 30 minutes of application, plates were inverted, incubated at 35°C for 24 hrs and then were examined for zone of inhibition around the disk [22].

2.12.2. Determination of Minimum Inhibitory Concentration (MIC)

A previously prepared Mueller-Hinton broth containing various concentrations of the soap sample was inoculated with the standard inoculums of each test organism, followed by incubation of the tube at 37°C for 16-20 hr. Thereafter, the tubes were observed for the presence or absence of growth in each tubes determined by turbidity of the test tubes. The lowest concentration of the extracts resulting in no growth after incubation was taken as the minimum inhibitory concentration (MIC) of the soap sample [24].

2.12.3. Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the soap sample on the bacterial isolates was carried out according to Ajaiyeoba, *et al.* [25]. One (1ml) bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes, which did not show any growth and were sub-cultured onto nutrient agar and incubated at 37°C for 24 hours. After incubation, the concentration at which there was no single colony of bacteria was taken as MBC.

2.12.4. Statistical Analysis

The data obtained from this study were analysed using descriptive statistics in form of means and standard deviation.

3. Results

Table 1 shows the Morphological identification, Biochemical Identification, Gram Reaction and Sugar Utilization Profile of bacterial isolates of choice from the wound swab samples. The bacterial isolates obtained for this study are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 2 shows the diameter of zones of inhibition (mm) produced by medicated soap (Tetmosol) against the test organisms. It was observed that the medicated soap (Tetmosol) was effective in inhibiting *Staphylococcus aureus* with zone of inhibition ranging between 11.0±0.77mm and 16.0±0.48mm as compared to *Pseudomonas aeruginosa* with zone of inhibition ranging between 10.0±1.33mm to 14.0±1.41mm.

Table 3 shows the diameter of zones of inhibition (mm) produced by antiseptic soap (Premier cool) against the test organisms. It was observed that the antiseptic soap (Premier cool) was more effective in inhibiting *Staphylococcus aureus* with zone of inhibition ranging between 12.0±0.84mm to 19.0±1.42mm, as compared to *Pseudomonas aeruginosa* with zone of inhibition ranging between 10.0±1.33mm to 15.0±9.34mm.

Table 4 shows the MIC and MBC values (mg/ml) of the medicated (Tetmosol) and antiseptic (Premier cool) soaps on the test organisms. The result of the minimum inhibitory showed that antiseptic soap (Premier cool) had better MIC and MBC of 12.5 mg/ml and 25 mg/ml respectively on *Staphylococcus aureus*. For *Pseudomonas aeruginosa*, the MIC and MBC were 50 mg/ml and 25 mg/ml. Medicated soap (Tetmosol) had higher MIC of 25 mg/ml and MBC of 50 mg/ml for *Staphylococcus aureus*. For *Pseudomonas aeruginosa*, the MIC and MBC were 50 mg/ml and 100 mg/ml.

Table-1. Morphological identification, Biochemical Identification, Gram Reaction and Sugar Utilization Profile of bacterial isolates from the wound swab samples

S/N	CULTURAL MORPHOLOGY	MICROSCOPI C FEATURES	BIOCHEMICAL TEST										SUGAR FERMENTATION				Gram Reaction	PROBABLE ISOLATES
			Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Methyl Red	Voges-P	Urease	Glucose	Lactose	Mannitol	Sucrose			
2	Golden yellow	Cocci in clusters	+	+	+	-	+	-	+	+	+	AG	AG	AG	AG	+	<i>Staphylococcus aureus</i>	
3	Black spot	Rod shape	+	+	-	-	-	+	-	+	-	NAG	NAG	AG	AG	-	<i>Pseudomonas aeruginosa</i>	

(+) = Positive, (-) = Negative, AG = Acid and Gas Production, A = Acid Production, NAG = No Acid and Gas production

Table-2. Diameter of zones of inhibition (mm) produced by medicated soap (Tetmosol) against the test organisms

Test Organisms	Zones of Inhibition (mm)/Concentration (mg/ml)					
	200	100	50	25	12.5	6.25
<i>Staphylococcus aureus</i>	16.0±0.48	15.0±1.21	13.0±0.93	11.0±0.77	0.0±0.0	0.0±0.0
<i>Pseudomonas aeruginosa</i>	14.0±1.41	12.0±1.21	10.0±0.68	0.0±0.0	0.0±0.0	0.0±0.0

Table-3. Diameter of zones of inhibition (mm) produced by antiseptic soap (Premier cool) against the test organisms

Test Organisms	Zones of Inhibition (mm)/Concentration (mg/ml)					
	200	100	50	25	12.5	6.25
<i>Staphylococcus aureus</i>	19.0±1.42	17.0±1.81	16.0±0.72	14.0±0.97	12.0±0.84	0.0±0.0
<i>Pseudomonas aeruginosa</i>	15.0±0.34	13.0±0.47	11.0±0.32	10.0±1.33	0.0±0.0	0.0±0.0

Table-4. The MIC and MBC values (mg/ml) of the medicated (Tetmosol) and antiseptic (Premier cool) soaps on the test organisms

Test Organisms	Soaps	Soap Concentrations (mg/ml)						MIC	MBC
		200	100	50	25	12.5	6.25		
<i>S. aureus</i>	Tetmosol	-	-	-	-	+	+	25	50
	Premier cool	-	-	-	-	-	+	12.5	25
<i>P. aeruginosa</i>	Tetmosol	-	-	-	+	+	+	50	100
	Premier cool	-	-	-	+	+	+	50	50

Keys:

MIC = Minimum Inhibitory Concentration

MBC = Minimum Bactericidal Concentration

+ = Presence of growth

- = No growth

4. Discussion

Soap is a water-soluble chemical formed by combining caustic soda (sodium hydroxide) or caustic potash (potassium hydroxide) with animal and/or vegetable fats in a process known as saponification (oils). As a cleaning agent, soap can be made in the form of bars, granules, or tablets, as most antiseptic soaps are [8]. Soaps are used to clean and remove microorganisms and dust from a variety of surfaces, including skin, clothes, and utensils [26]. The soaps must be efficient against bacteria while still being gentle on human skin. The antibacterial activity of a medicated soap (Tetmosol) and an antiseptic soap (Premier cold) on *S. aureus* and *Pseudomonas aeruginosa* isolated from wound infection were investigated in this study. The findings of this study demonstrated that the medicinal (Tetmosol) and antiseptic (Premier cold) soaps tested exhibit antibacterial action, but to variable degrees, as evidenced by the suppression of the isolates' development patterns (table 2, 3). The soap extracts are equally broad spectrum in activity as their activities were independent of Gram reaction.

Similarly, Ajaiyeoba, *et al.* [25] reported on the antibacterial activity of some selected medicated soaps such as Dettol, Tura, Sanitol, Safeguard and Tetmosol was determined on *S. aureus* isolated from wound infections. Hence, their results showed that the antibacterial activities of these soaps (Tura, Dettol, Sanitol, Safeguard and Tetmosol) increased as their concentration was increased. Also, Ike [8] carried out a study to determine the antibacterial activity of different types of antiseptic soaps on *S. aureus* isolated from wound infections and eczematous lesions. Nevertheless, these results matched with present study results as these organisms tested showed sensitivity to most of these soaps at higher concentrations.

When the efficacy of the medicated and antiseptic soaps were compared using the disc agar diffusion method, the antiseptic soap "Premier cool" was found to be most effective against all the bacteria strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) tested having the highest zone of inhibition (19.0 ± 1.42 mm) against *Staphylococcus aureus* and 15.0 ± 0.34 mm against *Pseudomonas aeruginosa* at the highest dilution used (200mg/ml) (Table 3). The medicated soap "Tetmosol" exhibited a minimal antibacterial activity against the isolates with zone of inhibitions of 16.0 ± 0.48 mm and 14.0 ± 1.41 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively (Table 2). Imarenezor, *et al.* [27] reported that the Zones of inhibition of the medicated soaps were examined and Tura soap had the highest antibacterial activity 20.00mm (100%), followed by Sanitol 15.00mm (75%), Safeguard 14.00mm (70%), Dettol 12.00mm (60%) and Tetmosol 10.00mm (50%) against *Staphylococcus aureus*. In a similar work, Obi [28] reported that Crusader and Antigal showed antibacterial activity against *S. aureus* and *E. coli* isolates at a higher concentration compared to this work. Similarly, Nwambete and Lyombe [1] also reported that Dettol, Lifebuoy and Tetmosol had inhibitory activities against *E. coli* and *S. aureus* at lower concentrations than that tested in this work. Majority of the assayed medicated and antiseptic soaps have demonstrated satisfactory effect, particularly the antibacterial activity, hence buttressing the information written on the soap labels that they possess antibacterial activity. This observed difference between the soaps might be due to the active ingredients which are incorporated to the various soaps, the geographical distribution of the organisms and may be the sensitivity of the organisms [1].

In the present investigation, the antibacterial activity of medicated and antiseptic soaps was also evaluated against test pathogens (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). It was observed that the antiseptic soap (Premier cool) was more effective in inhibiting *Staphylococcus aureus* with zone of inhibition ranging between 12mm to 19mm, as compared to *Pseudomonas aeruginosa* with zone of inhibition ranging between 11mm to 16mm. In the same sequence, the antibacterial activity of medicated soap (Tetmosol) was evaluated against the test organisms and it effective in inhibiting *Staphylococcus aureus* with zone of inhibition ranging between 11mm and 16mm as compared to *Pseudomonas aeruginosa* with zone of inhibition ranging between 10mm to 14mm. The results obtained in this study are in agreement with the work of Abbas, *et al.* [29] on the antimicrobial activity of three different brands of antibacterial soaps available in the local market. The results showed that three (3) different soap samples tested inhibited the test organisms to variable degrees. The ability of the isolates to withstand the antimicrobial impact of the soaps is shown by the suppression of their growth patterns. However, as the bacterial cell wall is the ultimate target of any antimicrobial drug or disinfectant, these discrepancies might be related to changes in the type and structures of the bacterial cell wall. The antibacterial agents are distinguished by the active component in the soap.

The results of the present study regarding variation of MIC and MBC against different concentrations concurs with a study which reported that the MBC and MIC results obtained against certain strains of bacteria were varied [28]. The lowest MBC (12.5mg/ml) and MIC (6.25mg/10ml) were exhibited by antiseptic soap on *S. aureus*. The result of the minimum inhibitory showed that antiseptic soap (Premier cool) had better MIC and MBC of 6.25 mg/ml and 12.5 mg/ml respectively on *S. aureus*. For *Pseudomonas aeruginosa*, the MIC and MBC were 12.5 mg/ml and 25 mg/ml respectively. Medicated soap (Tetmosol) had higher MIC of 12.5 mg/ml and MBC of 25 mg/ml for *S. aureus*. For *Pseudomonas aeruginosa*, the MIC and MBC were 25 mg/ml and 50 mg/ml than Premier cool. The MIC and MBC values obtained in this study is lower compared to that Obi [28] who reported on the Antibacterial Activities of some medicated soaps on selected human pathogens. This means that this soap is needed in higher concentrations to kill or inhibit the growth of these pathogens.

5. Conclusion

S. aureus and *Pseudomonas aeruginosa* were shown to be vulnerable to tested medicated (Tetmosol) and antiseptic (Premier cold) soaps in this study. This study found that all of the soap samples exhibited antibacterial activity against all of the germs tested, but Premier cool soap was the most effective against all of the bacteria tested.

Tetmosol soap demonstrated the least amount of antibacterial action against any of the microorganisms. As a result, medicated and antiseptic soap with antibacterial activity can be used to prevent skin/wound infections and the spread of skin pathogens. However, long-term usage of these soaps may result in the development of microbial resistance.

Consent and Ethical Approval

The authors declare that all experiments have been examined and approved by the appropriate ethics committee. Informed consents were obtained from all relevant authority.

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Competing Interests

Authors have declared that no competing interests exist.

Authors' Contribution

This work was carried out in collaboration among all authors. Author IUN designed the study and wrote the first draft of the manuscript, EKC wrote the protocol, IVO performed the statistical analysis and UCG and UOG helped with the analyses of the work. All authors read and approved the final manuscript.

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