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Antibacterial activities of medicated soaps on selected clinical bacterial isolates

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Abstract

Soaps have been established generally as cleansing agents because of their antimicrobial activities. The antibacterial activities of three different soaps namely; Sanitol, Premier Cool and No.1 antiseptic were investigated on four test clinical bacterial isolates that were tentatively identified as: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus* spp. Antimicrobial susceptibility test was carried out using the agar disc diffusion technique at concentrations of 400, 200, 100, 50 and 20 mg/mL. *Pseudomonas aeruginosa* was resistant to all the soaps at all concentrations while the other bacteria were inhibited at various concentrations. The minimum inhibitory concentrations (MIC) ranged from 12.5-100 mg/mL of distilled water for the different isolates while 25 mg/mL of Sanitol and Premier Cool was established as the minimum bactericidal concentration (MBC) against *Bacillus* spp. Sanitol, having displayed the highest activity at low concentrations, can be considered a good antibacterial agent for cleansing purposes.

Keywords: Antimicrobial activity, bacteria, soap, active ingredient, MIC, MBC

1 Introduction

Soaps are the mixtures of oils and fats (of vegetable or animal origin) and salts. Soaps are generally salts formed during saponification between alkaline substances and free fatty acids in fats and oils. The different types of soaps are produced when other substances are added to these salts of free fatty acids or soap base [1]. Soaps play an important role in removing and killing bacteria. Even though the major ingredients of soaps are fats and oils, some detergents are added to enhance the antibacterial activities of soaps [2]. It has been a common practice to wash hands with soap and water and this practice is taken as a measure of personal hygiene [3]. Bacteria live in diverse environments such as soil, water, sewage, standing water and human body. Bacteria that attack human body is of great importance with reference to public health [4]. Although bacteria that attack human body are of great importance with reference to health, Fuls et al. [5] reported the inhibitory potential of antimicrobial and non-antimicrobial soaps in clinical cases. When used properly, washing with soap could reduce *Propionibacterium acnes* and prevent secondary infections in acne skin and healthcare-associated transmission of contagious diseases more effectively. Cleansing agents have been used around us for a long time and among them soap, liquid hand-wash, detergent, etc., are noteworthy. As skin is the first line of defense, most of the bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus* reside on skin and is the major cause of skin infections [3]. Scrubbing hands or body, especially with soaps, is the first line of defense against bacteria and other pathogens that can cause flu, colds, skin infections and even deadly communicable diseases [6]. Soaps contain active ingredients (Table 1) that have an antibacterial activity and also the reducing power against the pyogenic skin infection caused by *Staphylococcus aureus* and other Gram-negative species of bacteria [7]. At present, little or no information exists concerning the antibacterial activities of Sanitol, Premier cool and No.1 antiseptic in Nigeria. Therefore, the aim of this study was to evaluate the antimicrobial activities of the above medicated soaps against clinical bacterial isolates namely: *Staphylococcus aureus*, *Escherichia coli.*, *Bacillus* spp and *Pseudomonas aeruginosa*.

Table 1. Assayed soaps and their active ingredients as per label disclosure

Name of soap sample	Active ingredient	Categories of soap
Sanitol	Trichlorocarbanilide (0.5%)	Medicated soap
Premier Cool	Phenoxyethanol (0.1%)	Medicated soap
No .1	Monosulfiram (0.1%)	Medicated soap

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2 Materials and methods

2.1 Sample collection

The medicated soap samples used for the study (Sanitol, Premier Cool and No.1 medicated soap) were purchased from standard cosmetics and pharmacy stores in Ogige market, Nsukka, Enugu State. The batch numbers, expiry dates and the presence or absence of the manufacturers seal were noted.

2.2 Collection of test organisms

The test organisms were aseptically obtained from the General Medical Microbiology Laboratory, University of Nigeria, Nsukka in sterile agar slants. The organisms were revived and the identities were confirmed using standard bacteriological methods [8]. The test organisms collected were tentatively identified as *Staphylococcus aureus, Bacillus* spp, *Escherichia coli* and *Pseudomonas aeruginosa*.

2.3 Preparation of soap-impregnated disks

Whatman number 4 filter paper was cut into discs of uniform size (6 mm, diameter) using a perforator and were sterilized in the autoclave at 121 °C for 15 min. The sterile discs were soaked in each of the soap solutions of different concentrations 400, 200,100, 50 and 25 mg/mL for 30 min and the excess solution was drained off.

2.4 Antimicrobial sensitivity test

Antimicrobial activities of the selected soaps on the clinical test isolates were carried out using the agar-disk diffusion method [9]. Briefly, standardized bacterial isolates (0.1 mL $=10^8$ cells) were aseptically inoculated on the surface of freshly prepared Mueller-Hinton agar. After about five minutes, soap-impregnated disks of different concentrations were aseptically placed on the agar surface and the culture was incubated at 37 °C for 18-24 hr. After incubation, the plates were observed for the presence or absence of inhibition zones. Diameters of inhibition were measured to the nearest millimetres using a transparent metre rule. Distilled water served as the control.

2.5 Minimum inhibitory concentration determination (MIC)

MIC was determined only for samples that showed inhibitory activity on the test isolates by the presence of inhibition zones. The MICs of the selected soaps were determined by the standard macrobroth dilution method in Mueller–Hinton broth [10]. The soaps were dissolved in 10% DMSO. Subsequently, a stock solution containing 200 mg/mL in distilled water was made. A two-fold dilution was performed by taking 0.5 mL from the stock solution (200 mg/mL) and successively pipetting the same volume into sterile test tubes containing 0.5 mL of Muller Hinton broth to obtain concentrations: 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL. A ten-fold dilution of the standardized inoculum was done by pipetting 0.1 mL of the standardized inoculum into 9.9 mL of Muller Hinton broth and the tubes inoculated with 100 µL of each of the test bacterial isolates. A tube containing gentamicin and the test isolates served as the positive control while a tube containing the broth and test isolates served as the negative control. All cultures were incubated at 37 °C for 24 hr. The MIC was taken as the lowest soap concentration that allowed no visible growth in the Mueller–Hinton broth. All data were reported as mean ± standard deviation of duplicate experiments.

2.6 Minimum bactericidal concentration determination

The minimum bactericidal concentrations (MBC) assay was determined by inoculating fresh nutrient agar plates with one loopful of culture taken from each of the broth cultures that showed no growth in the MIC tubes. The plates were incubated at 37 °C for 24 hr. After the incubation period, the lowest concentration of the soap that killed the bacteria on the solid medium was established as MBC values for each soap sample.

3 Results

3.1 Antimicrobial susceptibility patterns of the test isolates

The antimicrobial activity patterns of the soaps against clinical bacterial test isolates are presented in Table 2. Sanitol was active against *Bacillus* spp*, E. coli, Staphylococcus aureus* at all concentrations. *Pseudomonas aeruginosa* was resistant to all the soaps used. Premier Cool and No.1 antiseptic were active against *E. coli* and *Bacillus spp*. The zones of inhibition ranged from 8 mm (Premier Cool) to 15 mm (Sanitol) on *Bacillus spp*; 7 mm (No.1) to 18 mm (Sanitol) for *Escherichia coli*; 18 mm (Sanitol) to 14 mm (Sanitol) for *Staphylococcus acureus*. *Staphylococcus aureus* was also resistant to No.1 and Premier Cool as no zone of inhibition was recorded for it.

3.2 Minimum inhibitory concentrations of soap against bacteria

The MIC values of the different soaps against the test bacterial isolates are presented in Table 3. Sanitol inhibited the growth of *Bacillus* spp and *E. coli* at 100 mg/mL, 50 mg/mL and 25 mg/mL making 25 mg/mL as MIC value. Sanitol displayed an MIC value 25 mg/mL for *Staphylococcus aureus*. Premier antiseptic soap displayed the MIC at 25 mg/mL for *Bacillus*. *E. coli* was inhibited at 50 mg/mL for Premier Cool. No.1 Antiseptic inhibited *Bacillus* and *E. coli* at 100 mg/ml and 50 mg/ml making 50 mg/ml the MIC.

Table 2. Zones of inhibition (mm) of three medicated soaps on the three clinical isolates

		Mean ± S.D.			
Test isolates	**Conc. (mg/mL)**	**Sanitol**	**Premier Cool**	**No.1 Antiseptic**	**Water (control)**
***Bacillus* spp**	400	15.00 ± 0.56	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	200	10.00 ± 0.02	8.00 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
	100	11.00 ± 0.00	11.00 ± 0.05	10.00 ± 0.00	0.00 ± 0.00
	50	11.00 ± 0.04	6.00 ± 0.12	11.00 ± 0.00	0.00 ± 0.00
	20	11.00 ± 0.03	10.00 ± 0.07	12.00 ± 0.09	0.00 ± 0.00
Staphylococcus aureus	400	18.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	200	15.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	100	14.00 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	50	12.00 ± 0.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	20	11.00 ± 0.45	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Escherichia coli	400	21.00 ± 0.00	15.00 ± 0.01	10.00 ± 0.00	0.00 ± 0.00
	200	17.00 ± 0.00	13.00 ±0 .04	8.00 ± 0.01	0.00 ± 0.00
	100	17.00 ± 0.12	11.00 ± 0.03	9.00 ± 0.05	0.00 ± 0.00
	50	10.00 ± 0.03	11.00 ± 0.05	7.00 ± 0.05	0.00 ± 0.00
	20	6.00 ± 0.05	9.00 ± 0.05	7.00 ± 0.01	0.00 ± 0.00

Table 3. Minimum inhibitory concentration (MIC) of soap samples

Soaps	**Test organisms**	**MIC (mg/ml)**
Sanitol	*Bacillus* spp.	25
	Escherichia coli	25
	Staphlococcus aureus	12.5
Premier Cool	*Bacillus* spp	25
	Escherichia coli	50
No.1 Antiseptic	*Bacillus* spp	50
	Escherichia coli	50

3.3 Minimum bacteriocidal concentration (MBC) of soap samples

Table 4 shows the minimum bactericidal concentration of the soaps against the test bacterial isolates. Twenty-five (25) mg/mL was recorded as MBC for Sanitol and Premier Cool against *Bacillus* spp

Table 4. Minimum bacteriocidal concentration (MBC) of soap samples

Soaps	**Test organisms**	**MBC (mg/ml)**
Sanitol	*Bacillus* spp	25
Premier Cool	*Bacillus* spp	25

4 Discussion

Several studies on antimicrobial potentials of medicated soaps have proven that medicated soaps have antimicrobial properties [3, 11, 12]. It is however note-worthy that antimicrobial activities vary with soap and the bacteria involved. In the present study, the antibacterial activities of different soaps namely; Sanitol. Premier Cool and No.1 medicated soaps were investigated. It is clear that the soaps have different active ingredients at different concentrations (Table 1) and presumably, these differences would have contributed to the varying activities of the soaps on the test isolates. (Table 2). It was observed in the present study that the inhibition zone diameters were in the order: Sanitol > Premier Cool > No.1 antiseptic soap. Furthermore, the observed variation in antimicrobial activity may be due to difference of chemical contents, type of formulations, and probably repeated uses of the agents, which might have made the microorganisms resistant. Sanitol contains the highest concentration of active principle (Table 1). This could account for the highest inhibition zone diameters that was consistently observed among all isolates (Table 2). The observed trend is consistent with earlier works who reported that activities of antimicrobial agents are concentration-dependent [13, 14]. It was also noted for No 1 antiseptic soap that higher concentrations produced no zone of inhibition. No inhibition was recorded at 200 and 400 mg/mL (Table 2). The activities of some antimicrobials have been found to decrease with concentration. This can be attributed specifically to the active principle used in formulating No 1 antiseptic soap.

In the present study, Sanitol was active against *Staphylococcus aureus* even at the lowest concentration (12.5 mg/mL) (Table 3). As a skin-inhabiting bacterium, *Staphylococcus aureus* has been implicated in many wound infections. Also, Sanitol was active against 75% of the test bacteria while Premier Cool and No 1 antiseptic soaps were active on 50% of the test bacteria. It stands to reason therefore, that Sanitol proved satisfactorily effective as an antibacterial soap and can be recommended for treatment of wounds associated with staphylococcal infection. It was observed that *Pseudomonas aeruginosa* was resistant to all the soaps at all concentrations tested. Similar observation has been documented [14]. However, it is possible that Sanitol might have some activity against *Pseudomonas aeruginosa* if the concentration was increased. Riaz et al. [13] recorded some cidal activities against *Pseudomonas aeruginosa* at 500 mg/mL using 'safeguard' soap. Premier Cool and No 1 antiseptic soap, having shown some activities on the test isolates at higher concentrations suggest that some soaps are effective only at higher concentrations. *Bacillus* spp was the only organism that was killed by Sanitol and Premier Cool even at 25 mg/mL (Table 4). It was noted that the MIC and MBC values were equal both for Sanitol and Premier Cool (25 mg/mL) against *Bacillus* spp (Tables 3&4). This suggests therefore that the two soaps

are highly bacteriocidal. Bioactivity increases as MBC draws closer to MIC and vice-versa.

From a clinical standpoint, all test isolates except *Bacillus* spp belong to the ESKAPE group of organisms which are the leading causes of nosocomial infections and as such might have acquired some level of resistance against antimicrobial agents. This might explain the reason why *Bacillus* spp was killed even at the lowest concentration (Table 4). Even though No 1 antiseptic soap was active against *Bacillus* spp and *Escherichia coli*, its activity was only found to be bacteriostatic as no MBC was recorded for it (Table 4). Therefore, while some soaps serve the dual function of removing and killing bacteria [13], some serve only for removing bacteria. The latter is the case with bacteriostatic soaps.

5 Conclusion

In this work, the antibacterial activities of some selected soaps namely: Sanitol, Premier Cool and No 1 antiseptic soap, against clinical bacterial isolates belonging to the genera *Bacillus, Escherichia, Pseudomonas* and *Staphylococcus* were investigated. The activities were generally found to be concentration-dependent and also varied with type and formulations of soap. *Pseudomonas aeruginosa* was resistant to all the soaps at all concentrations tested while *Bacillus* spp was killed by Sanitol and Premier Cool. No 1 antiseptic exhibited only inhibitory effect on *Bacillus* spp and *Escherichia coli*. The activities of the soaps were in the order: Sanitol > Premier Cool > No 1 antiseptic soap.

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