Antibacterial Activities of Commonly Used Soaps in Nigeria against Hospital Pathogens Isolated from In-patients in a Mission Hospital in Abakaliki, Ebonyi State, Nigeria.

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Abstract

Background: Soaps are used daily for several purposes, especially to control microbial growth as they are capable of killing or inhibiting the growth of microorganisms. This study aimed to determine the antibacterial activities of commonly used soaps against clinical isolates of *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Methods: A total of 10 isolates each from *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were collected from the laboratory unit of Mater Misericordiae Hospital, Afikpo and were re-confirmed following standard microbiological procedures. Soap samples were bought from standard pharmaceutical stores within Abakaliki metropolis. The disc diffusion method was used to assess the antibacterial activities of the soaps at different concentrations of 100, 50, 25, and 12.5 mg/ml. After an overnight incubation, the inhibition zone diameter was measured using a meter rule.

Results: From each of the 10 isolates of Escherichia coli, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* analyzed, a total of 8(80%), 7(70%), and 9(90%) isolates were re-confirmed respectively. The result of the antibacterial activities of medicated soaps, beauty soaps and toilet soaps at different concentrations against pathogenic strains of Escherichia coli, *Klebsiella pneumonia*e and *Pseudomonas aeruginosa* showed that the Medicated soaps were the most active soaps against the clinical isolates.

Conclusion: The soaps showed a geometric decrease in their activities from higher concentration to the lowest concentration.

Keywords: Soaps, Antibacterial activities, Escherichia coli, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

Introduction

Skin is a very important organ in the human body and needs special care and protection against various attacks by microorganisms and other adverse physical or chemical conditions. Skin serves as a protection to other vital organs of the body. The human skin is therefore, the largest organ in the body, forming the outer surface of the entire body and acting to keep the internal tissues free from infections. The protective ability of the skin is achieved by the formation of a physically protective water proof layer which in turn blocks the entry of various microorganisms.[1] Although skin harbors many resident and/transient bacteria, these microorganisms may be displaced from their normal residence to other areas of the skin where they now compete for nutrients, thus leading to unhealthy competition which may lead to various skin infections and probably destruction of this vital organ of the body.

Human beings have several complements of friendly and/or transient bacteria on their skin surface.[2] This is because the skin is a comparatively dry habitat, with available water as the major factor controlling growth.[3] These bacteria include; *Staphylococcusepidermidis*, *S.hominis*, *S. aureus*, *Micrococcusluteus*, *Arcanobacterium haemolyticum*, and *Propioni bacterium acnes*, and other commensals which are part of the *Corynebacterium* group, the *Brevibacterium* species and the *Dermabacter* group.[4] Transient bacteria may also be deposited on the surface of the skin from environmental sources and can lead to skin infections. Examples of such bacteria are *Pseudomonas aeruginosa*, *Bacillus subtilis, E. coli,* and *Staphylococcus aureus.*[5,6] The spread of infection by such bacteria can be prevented by the effective use of antiseptic soaps, as they contain antimicrobial chemicals. However, over use of soaps might result in antimicrobial resistance and even render a person more sensitive to allergies and skin rashes.[7] The increasing trend of microbial resistance especially among Gram-negative bacteria such as *E. coli*, *Klebsiella* spp, *Pseudomonas* spp, *Proteus mirabilis,* and *Acinetobacter baumannii* is a major public health concern, hence the need for microbial growth control.[8]

The antibacterial activity of a particular soap is quite significant with respect to the human body, especially in the prevention and control of skin infections. Soaps are used to remove dirt, including dust, microorganisms, stains and bad smells to maintain health and beauty, and remove bad odor from the body or inanimate objects, including clothes.[9] Despite the enormous use of soaps, there is a paucity of information regarding the antibacterial activities of most of the commonly used soaps among Abakaliki residents and beyond, hence the need for this study.

Material and Methods

Study area

The study area of this research is Mater Misericordiae Hospital, Afikpo, Ebonyi State, Nigeria. The hospital is owned and managed by the Catholic Diocese of Abakaliki and is one of the most popular mission hospitals in the State. Apart from training students of nursing and midwifery, the hospital offers basic medical treatment especially to people of Ebonyi South zone.

Study design

this study used a descriptive cross sectional study design to assess the antibacterial activities of commonly used soaps.

Collection of soaps

Different brands of medicated soaps (Safeguard, MediSoft, Tetmosol), Beauty soaps (Extract, Irish Spring, Idole), and Toilet soaps (Eva, Lux and Ivy) were purchased from standard cosmetics and pharmacy stores at St Magareth Umahi International market, Ebonyi State.

Collection of test organism

A total of ten clinical isolates each of *E. coli*, *K. pneumoniae* and *P. aeruginosa* were randomly collected on nutrient agar slants from the laboratory unit of Mater Misericordiae Hospital, Afikpo and transported immediately to the laboratory unit of the Applied Microbiology Department of Ebonyi State University, Abakaliki for further analysis.

Culture, identification, confirmation and preservation of isolates

The 10 isolates each of *E. coli*, 10 isolates of *K. pneumoniae* and 10 isolates of *P. aeruginosa* were confirmed using standard microbiological methods.[10] Briefly, each of the bacterial isolates was aseptically inoculated into sterile nutrient broth and incubated for 24 hours at 37 ^{0}C. After 24 hours of incubation, the *E. coli* and *K. pneumoniae* isolates in the nutrient broth were plated on Eosin Methylene Blue (EMB) agar while *P. aeruginosa* isolates were plated on cetrimide agar and were further incubated over night at 37 ^{0}C. The isolates were further identified and re-confirmed using standard morphological characteristics, Gram's reaction and biochemical tests such as catalase, oxidase, coagulase test, indole, methyl red and citrate utilization test. The identified bacterial isolates were preserved on nutrient agar slants and stored at 4°C until further use.[10]

Standardization of test inoculum

Overnight cultures of the test organisms were kept ready for antibacterial activity. Each of the isolates was standardized using the colony suspension method.[11] The test organisms from nutrient agar plates, incubated at 37 ^{0}C for 24 hours, were suspended in saline solution (0.85% NaCl). The density of each isolate suspension was matched with 0.5 McFarland standards to give a resultant concentration of 1.5×10^8 cfu/ml.[11]

Preparation of stock solutions and different concentrations of the soap extract

A sterile blade was used to scrape 1g of each of the soaps and each quantity was dissolved in 9 ml of sterile distilled water to give a stock solution of 10^{-1} (dilution factor of the solution which entails combining 1 unit gram of the solute to 9 unit volume of solvent to give 10 units of total volume). These stock solutions (100 mg/ml) were thoroughly mixed and then allowed to stand to settle before other concentrations {50, 25, and 12.5 (mg/ml)}were prepared by pipetting 1 ml of each soap extract to the 2nd test tube containing 9 ml of distilled (50 mg/ml) and then to the 3rd and 4Th test tubes (25 and 12.5 mg/ml) respectively.[12] The importance of testing different soap concentrations against the isolates is to determine if the effectiveness and/or activities of various soaps are concentration bound

Preparation of the disk and antibacterial sensitivity test

The disc diffusion method was used to assess the antibacterial activities of the soaps at different concentrations. A 6mm Whatman paper was cut using a sterile surgical blade and then foiled before autoclaving at 121°C under a saturated steam pressure of 15 psi for 15 minutes to ensure sterility of the disk. Subsequently, the sterilized paper disc was carefully soaked in different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5mg/ml) of the soap extract using sterile forceps. The discs were left to soak in different concentrations of soap extract for up to 1 hour to ensure that the paper discs adequately absorbed the soap extract.[13] The antibacterial sensitivity test of the isolates to different concentrations of the soap extract was carried out by disk diffusion techniques using Mueller Hinton agar as per clinical and laboratory standard institutes (CLSI) criteria.[14]

Results

Cultural, microscopic and biochemical characteristics of isolates

The microbiological analysis of the isolates revealed *P. aeruginosa* as the most frequently isolated pathogen with 9(90 %) isolation compared to *K. pneumonia* with total isolation of 7(70 %) (Table 1).

Antibacterial sensitivity test of different brands of soap against pathogenic strains of *E. coli*

The result of the antibacterial sensitivity studies of medicated soaps showed that Medisoft Soap and Safeguard Soap were more active on *E. coli* than Tetmosol Soaps. Among the beauty soaps analyzed, Extract Soap and Idole Soap were shown to be more active than Irish Spring Soap against *E. coli*. While Lux Soap also had more activity against *E. coli* isolates compared to Premier and Ivy Soaps (Table 2).

Antibacterial sensitivity test of different brands of soap against pathogenic strains of *K. pneumoniae*

In Table 3, the result of antibacterial sensitivity studies of different brands of soaps showed that Medisoft and Safeguard Soaps were more active on *K. pneumoniae* isolates than Tetmosol soap. Extract Soap also showed greater activity than Irish Spring and Idole Soaps while Lux soap showed more activity than Premier and Ivy Soaps among the toilet soaps.

Antibacterial sensitivity test of different brands of soap against pathogenic strains of *P. aeruginosa.*

The analysis of the antibacterial activities of medicated, beauty and toilet soaps against *P. aeruginosa* isolates in Table 4 revealed a higher sensitivity of the medicated soaps than the beauty and the toilet soaps. However among the beauty soaps, Irish Spring showed lower activity compared to Extract soap while Lux and Ivy Soaps were more active than Premier Soap against clinical isolates of *P. aeruginosa.*

Table 1: Cultural, microscopic and biochemical characteristics of isolates

Bacterial isolates (n-10)	Colonial morphology/Microscopy	Gram Stain	Catalase test	Citrate test	Oxidase test	Indole test	Methly red	NPPCBI (n%)	Confirmed isolate
EC 1-10	Raised pinkish colonies on MacConkey agar and metallic green sheen with some dark colours on EMB agar / Tiny/short rods in cluster	-	+	-	-	+	+	8(80)	*E. coli*
KP 1-10	Raised pinkish or purple mucoid colonies with foul smelling odour on EMB agar / Tiny or short rods in cluster	-	+	+	-	-	-	7(70)	*K. pneumoniae*
PA1-10	Greenish colonies on Centrimide agar / short rods in cluster	-	+	+	+	-	-	9(90)	*P. aeruginosa*

Keys:EC1-EC10 *= number of E. coli isolates;* ***K1-K10*** *= number of K. pneumoniae isolates, PA1-10= number of P. aeruginosa isolates* ***NPPCBI****= number and percentage prevalence of confirmed bacterial isolates*

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Table 2. Antibacterial sensitivity test of d ifferent brands of soap against pathogenic s trains of *E. coli*

Group	Soap	Diameter of zone of inhibition (mm) at different concentrations of soap used			
		100 mg/ml (mm)	**50 mg/ml (mm)**	**25 mg/ml (mm)**	**12.5 mg/ml (mm)**
Medicated	Safe guard	20	18	14	12
	MediSoft	22	18	18	15
	Tetmosol	19	15	12	10
Beauty	Extract soap	20	15	12	10
	Irish spring	18	14	10	R
	Idole soap	20	12	10	10
Toilet	Premier Soap	14	12	R	R
	Lux soap	14	13	10	R
	Ivy soap	12	11	R	R

Key: R= Resistance

Table 3. Antibacterial sensitivity t est of different brands of soap against pathogenic strains of *K. pneumoniae*

Group	Soap	Diameter of zone of inhibition (mm) at different concentrations of soap used			
		100 mg/ml (mm)	**50 mg/ml (mm)**	**25 mg/ml (mm)**	**12.5 mg/ml (mm)**
Medicated	Safe guard	20	16	12	10
	MediSoft	21	15	12	10
	Tetmosol	20	15	10	R
Beauty	Extract soap	20	20	15	12
	Irish spring	17	15	10	R
	Idole soap	16	12	10	R
Toilet	Premier Soap	12	12	R	R
	Lux soap	12	10	10	R
	Ivy soap	13	12	R	R

Key: R= Resistance

Table 4. Antibacterial sensitivity test of different brands of soap against pathogenic strains of *P. aeruginosa.*

Group	Soap	Diameter of zone of inhibition (mm) at different concentrations of soap used			
		100 mg/ml (mm)	50 mg/ml (mm)	25 mg/ml (mm)	12.5 mg/ml (mm)
Medicated	Safe guard	18	18	15	11
	MediSoft	18	14	14	10
	Tetmosol	15	12	10	10
Beauty	Extract soap	14	12	10	10
	Irish spring	12	10	R	R
	Idole soap	13	13	10	R
Toilet	Premier Soap	11	R	R	R
	Lux soap	14	10	R	R
	Ivy soap	12	10	R	R

Key: R= Resistant

Discussion

This study evaluated the antibacterial activities of medicated, beauty, and toilet soaps against clinical isolates of *E. coli, K. pneumoniae* and *P. aeruginosa.* These pathogens have been implicated in various nosocomial infections from literature. [6, 8,15,16] *P. aeruginosa* has also been reported as the most frequently cultured source of infection in burn patients, accounting for over half of all severe burn infections and is among the major causes of sepsis after burn trauma.[17] Soaps are generally used by people for different purposes including control of microbial growth. Studies have also shown that soaps can serve as cleaning agents and can be used for the removal of dusts, and microbes present on human skin.[18]

The results of this investigation revealed that the medicated, beauty and toilet soaps assayed have varying degrees of antibacterial activity as shown by their inhibition zone diameter on the isolates tested. Among the medicated soaps assayed, the most active medicated soap against *E. coli* isolates was Medisoft Soap. However, the least active soap against *E. coli* isolates was Tetmosol Soap. The higher activity of Medisoft Soap compared to Tetmosol Soap can be justified by the higher usage of Tetmosol Soap than the Medisoft Soap which may have increased the resistance of microbes to Tetmosol Soap due to their more frequent exposure compared to Medisoft Soap. A study to assess the antibacterial activities of some medicated soaps on selected human pathogens have also shown medicated soaps to be the most effective soap against the bacterial strains tested, having the highest zones of inhibition (IZD) of 25 mm against *S. aureus* and 20 mm against *E. coli*.[15] The susceptibility of *E. coli, K. pneumoniae* and *P. aeruginosa* isolates to medicated soaps (Medisoft, Safeguard and *Tetmosol Soaps)* at all the concentrations in this study is not in agreement with the result of another study which reported non-susceptibility of *P. aeruginosa* isolates to both Sanitol and Premiere Cool soaps.[19] However, the result of this finding is in agreement with the report of another study which showed that medicated

soaps; Tura and Sanitol soaps had an inhibition zone diameter of 20 and 15 mm respectively against *S. aureus* isolates.[20] Also, both soaps were found to be active against *S. aureus* and *E. coli* isolates in another study.[19] Also, another study reported high susceptibility of clinical isolates of *S. aureus, P. aeruginosa* and *Candida albicans* to Dudu Osun in their comparative antimicrobial analysis of indigenous black soap variants.[21] These reports went further to confirm the higher susceptibility of the isolates to medicated soaps tested in this study. In general, studies have shown that medicated soaps contains triclosan, trichlorocarbamide and p-chloro-m-xylenol (PCMX/chloroxylenol) which are common antibacterial ingredients contained in medicated soaps.[22,23] The medicated soaps used in this study were found to contain trichlocarban and triclosan which may be the major ingredients responsible for the higher antibacterial activities of these medicated soaps compared to the assayed beauty and toilet soaps. These chemicals (trichlocarban and triclosan) function by denaturation of the cell wall and interfering with microbial metabolism.[15]Beauty soaps which have also shown varied degree of inhibition to the isolates tested, may also contain active ingredients which act against microbes thereby increasing its role as a skin care agent. This report of antibacterial activities of medicated soaps, beauty soaps and toilet soaps, and several other reports from literature further established the use of soap as a cleaning agent.[24, 25]

A study that evaluated the antibacterial effects of Dettol and Eva Soaps[24] also revealed Eva Soap which is a toilet soap, to have no inhibitory effect on the bacterial isolates tested. The above study is in line with the result of this study where toilet soaps assayed had low activity against *E. coli, K. pneumoniae* and *P. aeruginosa* compared to the beauty an medicated soaps assayed, even at the same concentration. However, in another study, Lifebuoy (toilet soap) exhibited antibacterial activity against isolates of *P. aeruginosa*.[25] In this study, the assayed toilet soaps also proved to be active against the isolates of *E. coli, K. pneumoniae* and *P. aeruginosa* if though only at high concentrations of 100 mg/ml and 50 mg/ml. However, at a reduced concencentration of 12.5 mg/ml, the effects of the toilet soaps where resisted by the pathogens tested. This showed that the effectiveness of these soaps, irrespective of the soap type is concentration bound. However, the medicated soaps have shown from this study to be very effective and efficient against pathogenic strains of *E. coli, K. pneumoniae* and *P. aeruginosa* even at the lowest concentration compared to beauty and toilet soaps which exhibited reduced activity at lower concentrations. The effectiveness of these medicated soaps in this study, justifies their continued usage in healthcare system by healthcare workers who often use them in hand washing. It is also pertinent to state that the effectiveness of these soaps may be microbial cell wall dependent. This means that the type of cell wall possessed by a microbe can contribute to its resistance since the primary target of many disinfectant or any antimicrobial agent is the cell wall. A report has also shown that the nature and structure of bacterial cell wall contributes to the organisms' ability to resist antimicrobial effect of any soap.[15]

Conclusion

The results of this study revealed that the soaps tested possess antibacterial properties and therefore can contribute significantly in the management of various skin infections and microbial growth control. The results further revealed a geometric decrease in the activities of the soaps (medicated, beauty and toilet soaps) from a high concentration of 100 mg/ml to the lowest concentration of 12.5 mg/ml indicating that the more quantity of the soap applied, the more effective it becomes. However, prolonged usage of these soaps could lead to microbial resistance.

Ethical consideration and Sponsorship

We do not intend to use these products as an avenue for any litigation or to devalue any of the products tested but for the advancement of knowledge and deepening scientific study. Also, this study is self-sponsored by the authors and received no funding from any of the producing companies, institutions or organizations.

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