Isolation, Identification and Antibiotic Susceptibility Pattern of Bacterial Strains from the Surface of Commonly used Soaps of Five Famous Pakistani Brands

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Abstract

This study aimed to isolate and assess the antibiotic susceptibility patterns of bacterial strains from various used soaps. Sixteen sterile soaps from five well-known Pakistani brands (Lifebuoy, Dettol, Safeguard, Lux, and Saba) were utilized in this study and placed in different locations for handwashing purposes. Bacterial strains were isolated and identified using standard microbiological techniques, and their antibiotic susceptibility patterns were determined using the disc diffusion method. Various bacterial strains were isolated from the selected soaps, with a high rate of contamination observed. Staphylococcus aureus was present in 33.3% of the samples, followed by Staphylococcus epidermidis (13.3%), Corynebacterium kutscheri (13.3%), Neisseria mucosa (13.3%), Corynebacterium xerosis (6.6%), Lactobacillus casei (6.6%), Shigella sonnei (6.6%), and Enterobacter aerogenes (6.6%). The antibiotic susceptibility pattern revealed that Staphylococcus aureus exhibited high resistance to penicillin and amoxicillin, followed by Staphylococcus epidermidis, Corynebacterium kutscheri, and Corynebacterium xerosis. Conversely, 4 (26.6%) isolates of Staphylococcus aureus and 2 (13.3%) isolates of Staphylococcus epidermidis were found to be sensitive to levofloxacin. Additionally, 2 (13.3%) multidrug-resistant (MDR) strains of Staphylococcus aureus were observed, showing resistance to gentamicin, amikacin, penicillin, amoxicillin, and erythromycin, isolated

from beauty soaps. Overall, a high rate of contamination was observed in beauty soaps, with the Saba brand showing the highest contamination rate at 37.5%, followed by Lux (25%), Lifebuoy (12.5%), Safeguard (12.5%), and Dettol (12.5%).

Keywords: Beauty soaps, Antibacterial soaps, Staphylococcus aureus, Antibiotic susceptibility

Introduction

From a long time, hand washing is considered as one of the best and easy source of personal hygiene. Among the cleansing agents, soaps are thought to be the dominant agents that have been used around from a long time [1]. They have a key role in removing and killing of microbes as they have some active antimicrobial ingredients that increase their activity [2]. Hand washing is of more importance because it is critically associated to health care of workers and other people and there is a potential risk of cross contamination of bacteria that may be opportunistic or pathogenic [3].

Microorganisms are very much diverse and are present everywhere from soil to water and even in living bodies. These microbes can be beneficial yet harmful and can attack human body. Regarding to health concerns the attacking of bacteria and other microbes are very important. Soap is a general tool used for hand washing and bathing in order to control the spread and attacking of such opportunistic bacteria. Some companies claim their product as antibacterial that can kill bacteria with some antibacterial agents present in soap and a study suggested that antibacterial soaps were more effective than that of plain beauty soaps [4].

Furthermore, it was also suggested that beauty soaps also have some antibacterial ingredients but in a small amount as compare to that of antibacterial soaps. A study conducted on plain soaps advised that washing hands with plain soap can remove many bacteria that may be pathogenic [5].

Materials and Methods

Study Design

This study was conducted from November, 2017 to April, 2018. In the current study n=5 different brands of soaps were tested in which n=3 were antibacterial soaps brands i-e Dettol, Safeguard, and Lifebuoy and n=2 were beauty soaps brands i-e Lux and Saba. A total of n=25 soaps, n=5 from each brand were used in this study. Out of n=25 soaps, n=16 pin packed soaps were selected on the basis of their sterility and were distributed to different locations for washing/cleaning purposes and samples were taken from each on 15th day and on 30th day. The details of locations are given in **table. 1.**

Methodology

Different biochemical tests were done in order to identify different bacterial strains isolated from the surface of used soaps. The biochemical test include gram staining, catalase test, coagulase test, indole test, citrate test, urease test, lactose test, sucrose test, fructose test, glucose test, nitrate reduction test and antibiotic susceptibility pattern was done by disc diffusion method using CLSI guide lines. For gram positive strains, 12 different antibiotic discs were used which include Cefoxitine, Chloramphenicol 30 µg, Doxycycline 30µg, Erythromycin 15 µg, Gentamycin 10 µg, Ciprofloxacin 05 µg, Fosfomycin 50 µg, Amikacin 30 µg, Levofloxacin 05 µg, Ofloxacin 05 µg, Amoxicillin 10 µg, and Penicillin 01 µg. From gram negative 8 different antibiotic discs were used i-e Ciprofloxacin 05 µg, Fosfomycin 50 µg, Amikacin 30 µg, Levofloxacin 05 µg, Ofloxacin 05 µg, Amoxicillin 10 µg, Ceftriaxone 30 µg and Norfloxacin 05 µg.

For the detection of methicillin resistant *Staphylococcus aureus* (MRSA), Muller-Hinton agar (MHA) was used. According to the Clinical and Laboratory Standards Institute (CLSI) the conventional method for the detection of MRSA include cefoxitin disc diffusion method [6].

Data Analysis

Data was analyzed by Microsoft word excel version 7. The data is presented in numbers and percentages n (%).

Results

Effectiveness of Antibacterial and Beauty Soaps

Among the antibacterial soaps, Dettol was found to be the most effective, showing a contamination rate of 6.25%. In contrast, among the beauty soaps, Saba was the least effective, with a contamination rate of 18.75%.

Sampling Results

In the first series of sampling, 4 out of 16 soaps (25%) showed bacterial contamination. The contaminated soaps included two beauty soaps (Saba E22 and Saba E25) and two antibacterial soaps (Safeguard A2 and Lifebuoy C14). In the second sampling, conducted on the 30th day, another 4 out of 16 soaps (25%) showed bacterial contamination, including Dettol D16, Lux B9, Lux B10, and Saba E22. Collectively, 8 out of 16 soaps (50%) were found to be contaminated in this study. **Table 1**

Table 1: *Distribution of soaps at different locations*

Brands	Soaps	Location
Safeguard	A2	Chicken shop
	A3	Boys hostel
	A4	Girls hostel

	A5	Dentist clinic
LUX	B8	Sweets bakery
	B9	Student home
	B10	Student home
Lifebuoy	C11	Microbiology lab.
	C14	Canteen
Dettol	D16	Pharmacy lab.
	D18	Gate keeper's hand washing station
	D20	Pharmacy common room
Saba	E21	Student home
	E22	Student home
	E24	Girls common room (microbio.)
	E25	Student home

Isolated Bacterial Strains

The first series of sampling isolated the following strains: 1 Staphylococcus aureus, 1 Staphylococcus epidermidis, 1 Corynebacterium xerosis, and 1 Corynebacterium kutscheri. The second series of sampling isolated a greater diversity of strains, including 4 Staphylococcus aureus, 1 Staphylococcus epidermidis, 1 Corynebacterium kutscheri, 1 Lactobacillus casei, 1 Shigella sonnei, 1 Enterobacter aerogenes, and 2 Neisseria mucosa. In total, 15 different bacterial strains were isolated during the study. **(Table 2)**

Table 2: *Number of isolated strains of bacteria*

Sample Series	Bacterial Strain	Quantity
1	Staphylococcus aureus	1
	Staphylococcus epidermidis	1
	Corynebacterium xerosis	1
	Corynebacterium kutscheri	1
2	Staphylococcus aureus	4
	Staphylococcus epidermidis	1
	Corynebacterium kutscheri	1
	Lactobacillus casei	1
	Shigella sonnei	1
	Enterobacter aerogenes	1
	Neisseria mucosa	2
Total Number of		15

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Contamination by Soap Brand

Among the 16 soaps tested from various brands (Lifebuoy, Dettol, Safeguard, Lux, and Saba), 8 soaps were found to be contaminated. The beauty soaps showed higher contamination rates, with Saba having a contamination rate of 37.5%, followed by Lux at 25%. The antibacterial soaps showed lower contamination rates, with Lifebuoy, Safeguard, and Dettol each having a contamination rate of 12.5%. **(Table 3)**

Table 3:   *Percentages of brands in total of n=16 soaps*

S.NO.	Soaps	Percentage	Level of contamination (Number of isolated strains)
	Antibacterial brands		
2	Safeguard	6.25%	2
1	Dettol	6.25%	1
3	Lifebuoy	6.25%	3
	Beauty brands		
5	Saba	18.75%	5
4	Lux	18.75%	4

Dominant Bacterial Strains

Among the 15 isolated strains, Staphylococcus aureus was the most dominant, accounting for 33.3% of all isolated strains. Out of the 5 strains of Staphylococcus aureus, 2 were isolated from Safeguard, 2 from Lux, and 1 from Saba soap. Additionally, 2 strains of Staphylococcus epidermidis (13.3%) were isolated from Saba soaps. Corynebacterium kutscheri was isolated from Dettol and Saba soaps, each contributing 13.3% of the total strains. Neisseria mucosa was isolated from Lux and Saba, each also contributing 13.3%. Further isolates from Lifebuoy included Corynebacterium xerosis, Shigella sonnei, and Enterobacter aerogenes, each contributing 6.6%. **(Table 4)**

Methicillin-Resistant and Sensitive Staphylococcus aureus (MRSA and MSSA)

Among the 5 isolated strains of Staphylococcus aureus, 2 (40%) were MRSA, isolated from beauty soaps (Lux and Saba), and 3 (60%) were MSSA, isolated from Safeguard (2) and Lux (1). The MRSA strains were resistant to multiple antibiotics, including gentamicin, amikacin, penicillin, amoxicillin, and erythromycin. **(Table 4)**

Antibiotic Resistance

Among the gram-positive bacteria, all 5 strains of Staphylococcus aureus showed resistance to amoxicillin and penicillin (100%), 4 strains (80%) were resistant to erythromycin and gentamicin, 3 strains (60%) to amikacin, and fewer strains to other antibiotics. Staphylococcus epidermidis showed high resistance to penicillin (100%) and

partial resistance to erythromycin, chloramphenicol, fosfomycin, and amoxicillin. Corynebacterium xerosis was 100% resistant to amoxicillin and penicillin, while Corynebacterium kutscheri showed varying resistance to penicillin, gentamicin, and amikacin. Lactobacillus casei was 100% resistant to gentamicin, amoxicillin, and penicillin. (Table 4)

Antibiotic Sensitivity

The isolated strains of Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium kutscheri, Corynebacterium xerosis, and Lactobacillus casei were highly sensitive to ciprofloxacin, fosfomycin, amikacin, levofloxacin, and ofloxacin. (Table 4)

Table 4: *Antibiotic susceptibility pattern for gram positive isolated bacterial strains.*

S.NO	Antibiotics		Staphylococcus aureus (5)	Staphylococcus epidermidis (2)	Corynebacterium xerosis (1)	Corynebacterium kutschri (2)	Lactobacillus casei (1)
1	Doxycycline (30µg)	S	_	2(100%)	1(100%)	2(100%)	1(100%)
		I	_	_	_	_	_
		R	_	_	_	_	_
2	Erythromycin (15µg)	S	_	1(50%)	1(100%)	2(100%)	_
		I	1(20%)	_	_	_	1(100%)
		R	4(80%)	1(50%)	_	_	_
3	Gentamycin (10µg)	S	1(20%)	2(100%)	1(100%)	1(50%)	_
		I	_	_	_	_	_
		R	4(80%)	_	_	1(50%)	1(100%)
4	Ciprofloxacin (5µg)	S	3(60%)	1(50%)	1(100%)	2(100%)	1(100%)
		I	2(40%)	_	_	_	_
		R	_	1(50%)	_	_	_
5	Fosfomycin (50µg)	S	3(60%)	1(50%)	1(100%)	2(100%)	1(100%)
		I	1(20%)	_	_	_	_
		R	1(20%)	1(50%)	_	_	_
6	Amikacin (30µg)	S	2(40%)	2(100%)	1(100%)	1(50%)	1(100%)
		I	_	_	_	_	_
		R	3(60%)	_	_	1(50%)	_
7	Levofloxacin	S	3(60%)	2(100%)	1(100%)	1(50%)	1(100%)
		I					

#	Antibiotic						
	(05µg)	R	_	_	_	1(50%)	_
			2(40%)	_	_	_	_
8	Ofloxacin (05µg)	S	3(60%)	1(50%)	1(100%)	2(100%)	1(100%)
		I	1(20%)	1(50%)	_	_	_
		R	1(20%)	_	_	_	_
9	Amoxicillin (10µg)	S	_	1(50%)	_	2(100%)	_
		I	_	_	_	_	_
		R	5(100%)	1(50%)	1(100%)	_	1(100%)
10	Penicillin (01µg)	S	_	_	_	_	_
		I	_	_	_	_	_
		R	5(100%)	2(100%)	1(100%)	2(%100)	1(100%)
11	Cefoxitine (30 µg)	S	_	_	_	_	_
		I	_	_	_	_	_
		R	2(40%)	_	_	_	_
12	Chloramphenicol (30µg)	S	3(60%)	_	_	_	_
		I	_	_	_	_	_
		R	2(40%)	_	_	_	_

Gram-Negative Bacteria:

Among the gram-negative bacteria, 2 strains of Neisseria mucosa, 1 strain of Enterobacter aerogenes, and 1 strain of *Shigella sonnei* were isolated. Neisseria mucosa showed resistance to ceftriaxone (100%), levofloxacin, amoxicillin, ofloxacin, and norfloxacin (50% each). Enterobacter aerogenes was 100% resistant to ceftriaxone. Effective antibiotics against Shigella sonnei included ciprofloxacin, amikacin, levofloxacin, amoxicillin, ofloxacin, and norfloxacin, while Enterobacter aerogenes was sensitive to ciprofloxacin, fosfomycin, ofloxacin, and norfloxacin. Neisseria mucosa was sensitive to ciprofloxacin, fosfomycin, and amikacin. **(Table 5)**

Table 5: *Antibiotic susceptibility pattern of gram negative isolated strains.*

Antibiotics		Ciprofloxacin (05)	Fosfomycin (50)	Amikacin (30)	Levofloxacin (05)	Amoxicillin (10)	Ofloxacin (05)	Ceftriaxone (30)	Norflaxacin (05)
Shigella sonnei (1)	S	1(100%)	_	1(100%)	1(100%)	1(100%)	1(100%)	_	1(100%)
	I	_	1(100%	_	_	_	_	1(100%)	_
	R	_	_	_	_	_	_	_	_
Enterobacter aerogene	S	1(100%)	1(100%)	_	_	_	1(100%)	_	1(100%)
	I	_	_	1(100%)	1(100%)	1(100%)	_	_	_
	R	_	_	_	_	_	_	1(100%)	_

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s (1)		_		_			_		
Neisseria	S	2(100%)	2(100%)	1(50%)	_	_	_	_	_
Mucosa	I	_	_	1(50%)	1(50%)	1(50%)	1(50%)	_	1(50%)
(2)	R	_ _	_	_	1(50%)	1(50%)	1(50%)	2(100%)	1(50%)

Discussion

The most shared hand washing agents are soaps. They are mostly stored in a soap container near a wash basin. Many people in daily life share the same soap located next to the washing station. When they are in use these soaps remain moist for a period of time and more often they are rinsed with water making slimy mass which may become a source for bacterial and other microbial growth. Most bar soaps in public areas are used by many people, thus it means that a public soap may come in contact with the different people [7]. So, it is more likely that these soaps become a source for transmission of skin bacteria from one person to another and may anchorage the growth of live pathogenic bacteria and under such environments cross infection can occur [8]. Therefore, to remove microorganisms from skin it is required to practice hand washing properly and to avoid cross infections it is necessary to wash the soap itself before and after use. More, even the use of liquid soaps has a wider satisfactoriness then bar soaps because of the reusable nature of soaps that may grasp bacteria picked up from past use [9]. The present study focused on isolation of contamination from used soaps and from this study it is concluded that beauty soaps are more prone to bacterial germs as compared to antibacterial soaps because beauty soaps were found more contaminated than antibacterial soaps.

A study was conducted in which the antibacterial activity of soaps was checked, and it was concluded that antibacterial soaps were more effective in removal of microorganisms than that of regular beauty soaps [4]. In the current study 80% beauty soaps were found contaminated while antibacterial soaps were found with 20% of contamination.

A study was conducted in which bar soaps i-e (Duru and Turkey) and liquid soaps i-e (Johnson and Turkey) were used. From bar soaps, the isolated strains were (41%) *Pseudomonas aeruginosa* which was found the most dominant followed by (13.6%) *E.coli*, (11.4%) *Acinetobacter baumanii* and from liquid soap the isolated strains were 4(66.6%) *Pseudomonas aeruginosa*, followed by 1(16.6%) *Proteus penneri,* 1(16.6%) *Flavimonas oryzihabitans* [10]. The findings of the previous study are opposite to the findings of the current study regarding to the isolated strains.

Similarly, another study was conducted in which the effect of hand washing with water and soaps were studied on bacterial contamination of hands. The common strains

isolated in the study were *Enterococcus ssp* followed by *Enterbactero ssp* and *E.coli* [11]. In the current study, 2 strains of *Enterbactero ssp* i-e *Enterobacter aerogenes* and *shegilla* were also isolated from the used soaps.

A similar study was done in which the microbial contamination in the soap was checked. *E.coli* was present in almost all samples and some are contaminated with *S.aureus,* followed by *Salmonella* and *Pseudomonas.* In the current study no *E.coli* was isolated and *staphylococcus aureus* was found the dominate bacteria.

Another study was carried out in 2006 in which solid soaps that were in used in 18 different dental clinics were examined for microbial contamination. A total of 32 samples were collected from the soaps which were found 100% positive for microbial growth. A total of eight different genera of microorganisms were isolated and 2 to 5 different genera of microorganisms were isolated from each bar soap. The soaps that were used more were found more contaminated as compared to that which was less used. The dominant strains of the study were *Staphylococcus epidermidis* followed by pathogenic *staphylococcus aureus*, *E.coli* and *klebsiella* [12]. In current study, among the isolated strains of 5(33.3%) *Staphylococcus aureus* 2(40%) pathogenic *Staphylococcus aureus* followed by other pathogenic strains I-e *Neisseria mucosa, shigella, Lactobacillus casei, and Enterobacter aergenes.*

A study was done in which plain soaps and liquid soaps from public toilet were investigated. A total of 84 samples yielded 16 different genera of microbes [13]. Similarly, in present study, 5 different genera from different soaps were isolated which can be compared with the result of the previous study in which 16 genera were isolated. Another study was carried out in which bacteria of different genera were isolated from 14 bar soaps in household setting in which *Staphylococcus* strains and *Enterobacteriaceae* were found the most dominant genera [14]. The results of the previous study can be compared to the findings of the current study in which 2 strain of *Enterobacteriaceae* were isolated i-e *Shegilla* and *Enterobacter aerogenes*. The difference in the genera number may be due to the different locations or settings of soaps of the current study.

In the present study bacterial species isolated from bar soaps shows clear evidence that bar soaps may contribute in transmission of bacteria. Although the strains of bacteria belongs to the normal flora of body but they may become pathogenic when get opportunity such as *Corynbacterium ssp* that were isolated from used soaps in the present study and can lead to skin infections. Also, other pathogenic strains are isolated from the bar soaps including 2(13.3%) *Neisseria mucosa,* 1(6.6%) *Shigella,* 1(6.6%) *Lactobacillus casei and* 1(6.6%) *Enterobacter aerogenes* and out of 5(33.3%)

staphylococcus aureus 2(40%) MRSA were isolated from beauty soaps. Further, bacteria can be transferred from used soaps and little risk was reported in the routine hand washing with the previously used soaps [15]. Moreover, the present study suggested that bar soaps are more colonized as compared to liquid soaps. Therefore, liquid soaps are more suitable for hand washing. Soaps that contain antibacterial agents are also suitable as they reduce bacterial contamination to a significant level [16]. It was also clear from a study in which it was suggested that antibacterial soaps were more effective than that of plain beauty soaps [17].

The difference in isolated strains of other studies and present study is may be due to the difference in normal flora of body of different geographical areas.

Among all the bacterial strains, *Staphylococcus aureus* were found highly resistant to penicillin and amoxicillin which was followed by *staphylococcus epidermidis, Corynbacterium kutchusri* and *Corynbacterium xerosis*. Further *Staphylococcus aureus* were found sensitive toward levofloxacin followed *Staphylococcus epidermidi, Lactobacillus casei* and *Corynbacterium xerosis*.

Conclusion

Out of 25 soaps, 16 soaps were free of bacteria which were distributed at different locations in which 8 soaps were become contaminated after use. Among different isolated bacterial strains (n=15) from used soaps (n=8), *Staphylococcus aureus* was found the dominant strain i-e 5 (33.3%). From this study it is concluded that beauty soaps i-e Saba was found more contaminated with 37.5% contamination followed by Lux 25%, Lifebuoy 12.5%, Safeguard 12.5% and Dettol 12.5%. Hence according to this study it is clear that antibacterial soap i-e Dettol, Lifebuoy and Safeguard were more effective than beauty soaps i-e Lux and Saba because the rate of contamination in antibacterial soaps was less but still it was not fully free from bacteria, also it is cleared that bar soaps become contaminated with use. So, on the basis of this study it is suggested that instead of using bar soaps liquid soaps should be used for hand washing.

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