ORIGINAL ARTICLE

Efficacy of Consumer Antibacterial and Non-antibacterial Body Washes on Skin Normal Flora and Pathogen

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ABSTRACT

Introduction: Antibacterial products contain active ingredients that are used to prevent bacterial growth and contamination. Previous studies suggest that antibacterial products are no more effective at removing s kin pathogen compared to plain soap. It is essential to collect the data regarding the effectiveness of antibacterial products with the purpose of continuous surveillance in the detection of emerging resistance pattern. **Method:** *In vitro* antimicrobial activity of six products were established on four species of bacteria namely *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* that represent the bacterial pathogen commonly found on human skin and the surrounding environment. These pathogens are also implicated as the causative organisms for skin infections. **Results:** Product that contains triclosan has the highest bactericidal effect as it is effective against a broad spectrum of bacteria. Body washes without any antibacterial agent also exhibit bactericidal activity but at higher concentrations. Gram-positive bacteria showed more sensitivity compared to gram-negative bacteria. **Conclusion:** Antibacterial and non-antibacterial products have bactericidal effects at different concentration. Different active ingredients showed different antibacterial effects on tested bacteria. Extend usage of antibacterial products pose adverse effects on skin normal flora and can lead to antimicrobial resistance.

Keywords: Antibacterial products, Consumer, Bactericidal, Normal flora, Antimicrobial resistance.

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INTRODUCTION

Antiseptic product is the term used for the products that contain active ingredients which known to inhibit the growth of microbes. It is classified as over-the-counter antimicrobial drug products. Their safety and effectiveness are being authorized by the United States (US) Food and Drug Administration (FDA). Consumer antiseptic drug products are being sold in the marketplaces such as antibacterial soaps, hand washes, antibacterial body washes, sanitizers, surface sprays, and mouthwashes (1). These products contain active ingredients such as chloroxylenol, triclosan and triclocarban, which are listed by the US FDA (2) as over-the-counter antiseptic active ingredients that are still lacking in safety and effectiveness data. Antiseptic or antibacterial body washes are consumer antiseptic products used daily and widely in the community (3). Therefore, it is vital to collect the data regarding the effectiveness of this product, with the purpose of continuous surveillance in the detection of emerging resistance pattern (4).

In previous studies, researchers did the *in vitro* and *in vivo* determination of antimicrobial activity of the active ingredients to collect data for effectiveness (5,6,7,8,9). Although these studies have proven that no significant difference were found in the comparison of bactericidal effects of antibacterial soap with non-antibacterial soap (5,6,9), little is known about their bactericidal activity in our local setting. The choice of bacteria should be that which are classified as the human bacterial pathogen, non-pathogenic and opportunistic pathogens that make up the resident microflora of human skin (microorganisms that are associated with the skin and mucous membranes of every human being from shortly after birth until death i.e *Staphylococcus spp.*, *Streptococcus spp*, *Bacillus spp* and others), food-related bacteria, or non-pathogenic and opportunistic organisms from the environmental area (2). The American Type Culture Collection (ATCC) strains of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa* were used to represent the bacterial pathogen commonly found on human skin (10) and the surrounding environment (11). These pathogens are also implicated as the causative organisms for skin infections (12).

The present study compared the bactericidal activity between antibacterial and non-antibacterial body washes that are commonly found in the local market against the selected organisms. This information will guide the consumer to choose the products wisely since the use of the antibacterial product in daily life is unnecessary (13), while regular soap and water are sufficient to remove naturally acquired bacteria on the skin (14,15). Moreover, the long-term usage of the antibacterial product might result in antimicrobial resistance (15,16).

MATERIALS AND METHODS

Body washes samples

The antibacterial body washes and non-antibacterial body washes used in this study were purchased from the supermarket and drug stores in Selangor, Malaysia. The type of body washes, brands and active ingredient present in the body washes were noted for each sample (Table I).

Table I : Types of body washes, their brands and active ingredients present in their formulation

Type of body washes	Brands	Active ingredient present
Antibacterial	Dettol – Original Antibacterial Body Wash	Chloroxylenol
	Lifebuoy – Total 10 Antibacterial Body Wash	Thymol
	T3 – Acne Body Wash	Triclosan
Non-antibacterial	Shokubutsu – Clean Fresh Shower Foam Sakura Whitening	None
	Lux – White Impress Whitening Shower Cream	None
	Palmolive – Aromatherapy Sensual Shower Gel	None

Test controls

The positive control used in this study was gentamicin 10 µg (CN 10) since it is a broad-spectrum antibiotic, known to inhibit the growth of *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli*. Gentamicin 10 µg gives the zone of inhibition to these bacteria, thus can be a comparison to the discs tested in this experiment. The negative control is sterile filter paper disc because it does not have any antiseptic agent, hence does not produce any inhibition zone. The CN 10 antibiotic discs were supplied by the Microbiology Laboratory of Medical Laboratory Technology Department, UiTM Selangor, Puncak Alam Campus.

Reference bacterial culture

Bacterial cultures used in this study were the American Type Culture Collection (ATCC) strains of *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli*. These organisms were provided by the Microbiology Laboratory, Centre of Medical Laboratory Technology, UiTM Selangor, Puncak Alam Campus. Bacterial cultures were further inoculated separately on the blood agar plate and incubated at 37°C for 24 hours in the incubator, except for *Streptococcus pyogenes*, which was incubated in the carbon dioxide (CO2) incubator. Biochemical tests were performed to confirm the organisms.

Preparation of discs impregnated with body washes samples

The method used for the preparation of discs was modified from the method demonstrated by Abbas et al. (8) and OBI (16). Whatman® Filter Paper Grade 1 was used to make the antibiotic discs impregnated with body washes. Using paper puncher, the filter paper was punched to become 6 mm-diameter discs. The discs were punched into a bijou bottle and were autoclaved. Four different concentrations of each body washes were prepared, which were 1:1, 1:2, 1:4 and 1:8. For 1:1 dilution, the discs were soaked into the initial concentration of the body wash. Serial dilution was made to dilute the initial concentration of body washes into several strengths to study their bactericidal effects after diluting with distilled water. 1:1 to 1:2 dilution were considered as high concentrations while 1:4 to 1:8 were considered as low concentrations.

Disc diffusion antimicrobial sensitivity testing

The disc diffusion method, as described by Bauer et al., (1966), was adopted with some minor modification. The process was done according to the standard approved by the Clinical and Laboratory Standards Institute, CLSI (17). The filter paper discs impregnated with body washes were carefully placed on the inoculated Mueller-Hinton agar plates. On each agar plate, a total of four discs were placed to avoid overlapping of inhibition zone by each body wash concentration. The plates were incubated overnight at 37°C then the zone of inhibition was measured.

Statistical analysis

The data of the inhibition zones were analysed using Statistical Package for Social Sciences, SPSS version 24.0 (IBM corp, Chicago, IL). The data shown represents the means of experiment performed in triplicate. The means were compared using Analysis of Variance (ANOVA) and when it indicated a significant result ($P<0.05$), post-hoc test was done using Scheffe's procedure.

RESULTS

Bactericidal activity of antibacterial body washes

The sensitivity testing of Dettol, Lifebuoy and T3 against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* showed that gram-positive bacteria were sensitive to these antibacterial body washes compared to gram-negative bacteria which almost resistant to them. Dettol was able to inhibit the growth of *S. aureus* and *S. pyogenes* at all concentrations, by drawing the inhibition zone of 16, 15, 12, 11 mm and 13.6, 10.7, 10, 9.7 mm respectively. Lifebuoy, at all concentration, can only inhibit the growth of *S. pyogenes* with inhibition zone measuring 15, 14.6, 11 and 8.3 mm. Meanwhile, T3 was able to inhibit the growth of skin pathogens at all concentration, producing inhibition zones measuring 38.7, 35, 31.3, 28.7 mm, 20, 18.7, 15,13 mm, and 29.3, 28.3, 26.3, 24.7 mm by *S. aureus, S. pyogenes* and *E. coli* respectively. Among all the bacteria tested, *P. aeruginosa* was the most resistant bacteria even at the highest concentration of body washes. S. pyogenes was the most sensitive because the inhibition zone present at all concentration of all body wash tested. Among the antibacterial body washes tested in this study, T3 body wash had the highest bactericidal activity (19.31±13.5 mm). In contrast, Lifebuoy had the lowest bactericidal activity (3.06±5.55 mm) against *S. aureus, S. pyogenes* and *E. coli* (Table II).

Bactericidal activity of non-antibacterial body washes

The sensitivity testing of *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* against Lux, Shokubutsu and Palmolive showed that non-antibacterial body washes possess bactericidal activity even though they do not contain antimicrobial agent in the formulation. However, their bactericidal activities are weaker than antibacterial body washes but are acceptable to inhibit the growth of these pathogens. Lux was able to inhibit the growth of *S. pyogenes* at 1:1 and 1:2 concentrations by producing inhibition zones measuring 10 and 8.3 mm. Shokubutsu can inhibit the growth of *S. pyogenes* at 1:1 and 1:2 concentrations by drawing the inhibition zones of 12 and 9 mm. Palmolive showed bactericidal activity at 1:1 and 1:2 concentrations by inhibiting the growth of *S. aureus* and *S. pyogenes* to produce inhibition zones of 11.6, 9.7 mm and 14, 11 mm respectively. Similar to antibacterial body washes, the zone of inhibition showed that gram-positive bacteria, *S. pyogenes* was the most sensitive to non-antibacterial body washes, while gram-negative bacteria, *E. coli* and *P. aeruginosa* were resistant to all non-antibacterial body washes. Therefore, among the non-antibacterial body washes tested, Palmolive was better at providing bactericidal activity by producing the largest inhibition zone (2.90±5.14 mm), while Lux shows the least bactericidal activity by exhibiting least inhibition zone (1.15±3.09 mm) against these skin pathogens (Table III).

Comparison in bactericidal activities of antibacterial body washes and non-antibacterial body washes

When comparing the bactericidal activity of both types of body washes, the results revealed that antibacterial body washes have the highest bactericidal activity against bacterial skin pathogens. This observation was based on the ANOVA test for the means of inhibition zone produced by these pathogens among the body washes tested in this study (Table IV). The One-Way ANOVA is significant ($P<0.05$) suggest that at least one pair of mean inhibition zones produced among the body wash used were significantly different. The subsequent post hoc analysis (Scheffe's procedure)

Table II : Zone of inhibition (mm) of the skin pathogens against Dettol, Lifebuoy and T3 body wash at the concentration of 1:1, 1:2, 1:4 and 1:8

Bacteria	Dettol				Lifebuoy				T3				Control
	1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8	CN 10
Staphylococcus aureus	16	15	12	11	0	0	0	0	38.7	35	31.3	28.7	25
Streptococcus pyogenes	13.6	10.7	10	9.7	15	14.6	11	8.3	20	18.7	15	13	18
Escherichia coli	0	0	0	0	0	0	0	0	29.3	28.3	26.3	24.7	18
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0	17

Table III : Zone of inhibition (mm) of the skin pathogens against Lux, Shokubutsu and Palmolive body wash at the concentration of 1:1, 1:2, 1:4 and 1:8.

Bacteria	Lux				Shokubutsu				Palmolive				Control
	1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8	CN 10
Staphylococcus aureus	0	0	0	0	0	0	0	0	11.6	9.7	0	0	25
Streptococcus pyogenes	10	8.3	0	0	12	9	0	0	14	11	0	0	18
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0	0	18
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0	17

suggests that the mean inhibition zones produced are significantly different between "Dettol and Lux", "T3 and Dettol", "T3 and Lifebuoy", "T3 and Lux", "T3 and Shokubutsu" and "T3 and Palmolive". We can conclude that T3 was the most bactericidal body wash by having the largest mean inhibition zone, followed by Dettol, Lifebuoy, Palmolive, Shokubutsu, and Lux. Lux was the least bactericidal because it produced the least inhibition zone on the bacterial growth.

The One-Way ANOVA is significant (P<0.05) to suggest that at least one pair among the inhibition zones produced by the skin pathogens were significantly different (Table V). The subsequent post hoc analysis (Scheffe's procedure) suggest that the mean inhibition zones are significantly different between "*S. aureus* and *E. coli*", "*S. aureus* and *P. aeruginosa*", "*S. pyogenes* and *E. coli*", "*S. pyogenes* and *P. aeruginosa*" and "*E. coli* and *P. aeruginosa*". We can conclude that *S. pyogenes* was the most sensitive bacteria by having the largest mean inhibition zones. Meanwhile, *P. aeruginosa* was the most resistant because its growth was not inhibited by any of the antibacterial and non-antibacterial body washes. Therefore, the

Table IV : Comparing mean inhibition zone produced among the body washes used

Body wash	n	Mean(SD)	F-stats[a] (df)	P-value
Dettol	48	6.13(6.42)	45.68(5;282)	P<0.001[b]
Lifebuoy	48	3.06(5.55)		
T3	48	19.31(13.50)		
Lux	48	1.15(3.09)		
Shokubutsu	48	1.31(3.56)		
Palmolive	48	2.90(5.14)		

[a]One-Way ANOVA test

[b]Only "Dettol and Lux", "T3 and Dettol", "T3 and Lifebuoy", "T3 and Lux", "T3 and Shokubutsu" and "T3 and Palmolive" pairs are significantly different by pos-hoc test Scheffe's procedure.

Table V : Comparing mean inhibition zones produced by the bacterial skin pathogen

Skin pathogen	n	Mean(SD)	F-stats[a] (df)	P-value
Staphylococcus aureus	72	8.71(12.71)	17.6(3;284)	<0.001[b]
Streptococcus pyogenes	72	9.33(6.16)		
Escherichia coli	72	4.53(10.33)		
Pseudomonas aeruginosa	72	0.00(0.00)		

[a]One-Way ANOVA test

[b]Only "S. aureus and E. coli", "S. aureus and P. aeruginosa", "S. pyogenes and E. coli", "S. pyogenes and P. aeruginosa" and "E. coli and P. aeruginosa" pairs are significantly different by post hoc test Scheffe's procedure.

ANOVA test showed significant differences (P<0.05) for the inhibition zones produced among the bacterial pathogens and body washes used. However, there is no mean difference in the inhibition zones produced among different concentration tested (P>0.05).

DISCUSSION

This study suggests that among the bacteria tested, gram-positive bacteria produce more sensitivity towards both type of body washes as compared to gram-negative bacteria where most of them do not show any reaction against the body washes except for *E.coli* which sensitive to T3 body wash in all concentrations. According to Nazzaro et al. (18), gram-negative is more resistant than gram-positive bacteria due to the difference in their cell wall structure. Gram-positive bacteria's cell wall has a thick peptidoglycan layer that allows hydrophobic molecules to penetrate its cell wall and acts on both cell wall and within the cytoplasm. Meanwhile, gram-negative bacteria's cell wall consists of a thin peptidoglycan layer covered by an outer membrane that contains lipopolysaccharides. Only the small hydrophilic solute can penetrate the outer membrane, but not hydrophobic antibiotics and drugs. This explained the resistance of *E. coli* and *P. aeruginosa* towards most of the body washes tested in this study.

Dettol can inhibit the growth of gram-positive bacteria, but not gram-negative bacteria. Dettol contains chloroxylenol as its active ingredient. As explained by WHO (19), chloroxylenol has good *in vitro* activity against gram-positive bacteria because its antimicrobial activity attributes to the alteration of bacterial cell walls and inactivation of bacterial enzymes. This is also supported by Berthelot and Zirwas (20), who stated that chloroxylenol has a broad spectrum of antibacterial activity against gram-positive bacteria but moderate activity against gram-negative bacteria. Furthermore, *P. aeruginosa* is resistant to Dettol because chloroxylenol is less effective against the organism (19) but the addition of EDTA can enhance its bactericidal activity (20). A study by Al-Talib et al. (4) revealed that *E. coli* was able to survive against Dettol. Therefore, the resistance of these both gram-negative to Dettol suggest the possible emergence of antibacterial resistance (16).

Furthermore, Lifebuoy can inhibit the growth of *S. pyogenes* only, probably because the active ingredient present in its formulation is thymol, which an essential oil that has antibacterial properties. It is used as a substitution for the active ingredients that have been declared by the US FDA (2) as having insufficient data to be classified as safe and effective, such as chloroxylenol and triclosan (19). A study done by Sfeir, Lefrancois, Baudoux, Debre, and Licznar (21) revealed that thymol showed antibacterial activity against *S. pyogenes*. Therefore, the evidence from a recent study suggests that Lifebuoy is not effective at removing bacterial skin pathogen, but it may show bactericidal activity towards skin normal flora. This finding was supported by the study done by Santhiya and Victoria (22), stating that Lifebuoy was the least effective among the soaps used in their study against daily encountered human skin flora.

T3 shows bactericidal activity on both gram-positive and gram-negative bacteria, except *P. aeruginosa.* This body wash contains a powerful active ingredient, which is triclosan. Triclosan is effective against a broad spectrum of bacteria, by permeating the bacterial cell wall and targets multiple cytoplasmic and membrane sites (23). This includes ribonucleic acid (RNA) synthesis and macromolecules production. In spite of that, the O-methylation of this active ingredient completely abolished its mechanism of action against some pathogenic bacterial species (23). This may contribute to the resistance of *P. aeruginosa* to T3 body wash. The mutation in efflux pump in *P. aeruginosa*, as reported by the previous study regarding its resistance in exposure to triclosan (19), is another possibility for the resistance of the organism towards T3.

The findings of this study suggest that body washes without the antibacterial agent, such as Lux, Shokubutsu, and Palmolive poses bactericidal activity against the bacterial skin pathogens. Nevertheless, their bactericidal activities only shown at higher concentrations where the water used is not exceeded 2 times of the their original volume. This can be expected because bacterial growth that was inhibited at higher concentrations may be due to the presence of preservative in the body washes, that is used to destroy or suppress the growth of microorganisms (15,24). Lux and Shokubutsu inhibit the growth of *S. pyogenes*, while Palmolive inhibits the growth of both gram-positive bacteria, *S. aureus* and *S. pyogenes*. Only gram-positive bacteria were inhibited by these non-antibacterial body washes, which can be explained by the structure of their cell wall that allows the permeability of hydrophilic molecules (18).

Interestingly, *P. aeruginosa* was resistance to all body washes. This pathogen, according to Golemi-Kotra (25), has intrinsic resistance to various agents, including antiseptics and many antibiotics, results from its ability to construct protective biofilms and the nature of gram-negative cell wall structure. Also, a study by D'Arezzo, Lanini, Puro, Ippolito, and Visca (26) reported of a high level of triclosan resistance by *P. aeruginosa*. Hence the usage of the antimicrobial agent in the consumer products should be avoided since this pathogen can become tolerant to increasing concentration of antimicrobial agent (26).

The antibacterial body washes have bacteriostatic and bactericidal effects on some of the skin pathogens only. Nevertheless, the complete resistance was shown by some of the bacterial strains even at higher concentration of the body washes used. Therefore, the information written on these body washes' bottle, claimed that they could kill 99.9% germs could be questioned. This might be due to the overutilization of the antibacterial products in our daily life that might cause antimicrobial resistance (15,16). Besides, the differences in the active ingredient present, type of formulations, and repeated use of the agents might have caused the variability in the bactericidal activity (15).

Although T3 body wash had the highest bactericidal activity among the body washes tested and deem fit to be used for removing bacterial pathogen on the human skin, there should be some limitation for it to be used by the consumer. Since T3 has an intense bactericidal activity to kill most bacteria, it is not suitable for frequent usage because it has a high chance to kill all bacteria present on our skin, including skin normal flora. Disruption to our balanced host-microorganism relationship will lead to a higher opportunity for growth of transient or pathogenic bacteria resulting in skin infections (27), especially in immunocompromised individuals. Therefore, T3 is not suitable to be used by this consumer category. Besides, frequent usage of T3 body wash that contains triclosan as the antimicrobial agent lead to massive exposure of this microbicide to the environment. Accumulation of triclosan is highly toxic to the aquatic environment (28).

Consumers tend to opt for antibacterial products because they believed that antibacterial products could protect themselves from potentially harmful organisms. Meanwhile, there is no scientific evidence that revealed the combination of regular soaps and water have lost its efficacy in preventing the spread of infections (14,15). The consumers failed to realize the potential risks of chemical exposure towards themselves (20, 29) and the environment (28), especially the potential to increase the antibiotic-resistance pathogens (15). Eventually, the use of microbicide in consumer products could exacerbate the problem of clinical antibiotic resistance, and making the treatment of microbial infections even more challenging (15).

Based on the statistical data obtained in this study, the author would like to suggest that the fit body washes to be used by the consumer in their daily bath are Lifebuoy, Palmolive, Lux, and Shokubutsu because they have higher chance to remove bacterial skin pathogen without causing disturbance to our balanced host-microorganism relationship. Moreover, regular body washes are economically more affordable compared to antibacterial body washes. Manufacturers are selling antibacterial products at the higher price, claiming that it can effectively kill 99.9% bacteria while it is actually unnecessary to remove all bacteria on our skin, since the beneficial bacteria present on the skin called normal flora, is able to protect our skin against the pathogen (27).

CONCLUSION

In conclusion, this study was done to determine the bactericidal activity of consumer antibacterial and non-antibacterial body washes against common bacterial skin pathogens. The sensitivity and the resistant of a bacteria against the body washes were very much determined by the active ingredients of the body washes. Based on the result obtained, T3 body wash has the highest bactericidal activity among antibacterial body washes with a mean inhibition zone of 19.31±13.50 mm. Palmolive was the most bactericidal among non-antibacterial body washes drawing mean inhibition zone of 2.90±5.14 mm. The significant difference (P=0.00) were observed for the inhibition zone produced among the pathogens. This current finding proved that antibacterial body washes have higher bactericidal activity than non-antibacterial body washes against bacterial skin pathogen. It is recommended to do further studies to investigate the effects of interaction between skin normal flora with the antimicrobial agent present in the consumer body wash in the long run. This study suggests that the usage of antibacterial products should be limited, unless necessary because their prolonged usage can lead to destruction of skin normal flora thus will cause skin dryness, allergies and antimicrobial resistance. Epidemiological studies are also recommended to collect information regarding the consumer's preference in choosing the bathing products, which is beyond the extent of this study.

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