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An Approach to Effective Disinfection of Salon Items [clippers, combs and scissors]

O.S. OLONITOLA^{1*}, P.F. OLURINOLA² AND J.O. EHINMIDU²

¹Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria.
²Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Antibacterial susceptibility patterns of some bacteria isolated from selected barbing salons in parts of Northern Nigeria was studied for the purpose of developing a disinfectant system suitable for use in salons. The K-values for selected cells ranged from 0.0072 to 0.0399 (benzalkonium chloride), and from 0.0062 to 0.1338 (propylene glycol) as against a range of 0.0147 to 0.1343 when the two chemicals were combined. The observed D-value range of 64.5 to 99 (benzalkonium chloride), 31.5 to 105 (Propylene glycol) and 21 to 24 (admixture of the two chemicals) were exploited in the development of "SALONSAFE" – a salon disinfectant that was able to eliminate 10^5 cfu/ ml (of the most resistant bacterial isolated in these studies) completely in 60 seconds.

Key words: Salonsafe, antibacterial, disinfectant, salon, K-value, D-value.

INTRODUCTION

In the recent past, several reports have identified resistances among pathogenic bacteria and fungi [1-6]. This may be due to the fact that antimicrobial resistant and stress-modified strains of microorganisms frequently emerge in nature [7].

Bacteria display an amazing capacity to survive in extremely hostile environment. Entire group of microorganisms have adapted their lifestyles to prefer these extreme environments (examples are thermophiles, halophytes, acidophiles). For most, the tolerance can be pushed to maximum limits if the cell is provided sufficient opportunity to sense and adapt to a deteriorating condition [8]. Inducible tolerances to temperature, salt, DNA-damaging agents and oxidative stress are widely known and well studied.

The desire to release some genetically modified microorganisms (useful as biological control agents or for degradation of pollutants in land reclamation) into the environment has caused concerns over potential environmental damages [9, 10]. This calls for measures to ensure minimum damages from such releases [11].

Very few known pathogenic microorganisms have become extinct despite worldwide efforts to eradicate them [12]. This is due mainly to their ability to perpetuate themselves and

survive in nature depending on such factors as their readiness to escape from the infected animal in life or at death, ability to survive in the environment outside the animal, their transmission to susceptible hosts and their ability to colonize and cause infection in the new hosts.

Barbing salons have been reported to harbour pathogenic microorganisms [13, 14] many of which were resistant to both antibiotics and chemical (none-antibiotic) anti microbial agents. Such observations have led to word-wide concern about the emergence of antimicrobial resistances in common pathogens of community [15].

Available evidence show that significant proportion of the populace patronize barbing salons where the barbers do not employ hygienic practices. The soaps, mixtures or even the barbers' water may be reservoirs of pathogenic microorganisms from other clients thus subjecting susceptible clients to great risks. It is therefore very essential to develop potent antimicrobial products with increased value over those currently available. Such products may be better achieved by exploiting the phenomenon of synergism between two or more antimicrobial agents [16].

In this paper, we present the report of studies leading to the formulation and compounding of a new disinfectant product based on the

^{*} Author to whom the correspondence may be addressed

observed synergistic effects between Benzalkonium chloride and Propylene glycol.

EXPERIMENTAL

Organisms

Field isolates of bacteria obtained from barbing salons located in Zaria, Kano and Kaduna towns of Nigeria were utilized. The bacteria were maintained on nutrient agar slants. When needed, inocula were grown overnight in nutrient broth and prepared by washing the cells three times in normal saline with centrifugation at 5,000 rpm for five minutes and spectrophotometrically standardized to 108 cfu/ml.

Rate of cell kill

The rate of kills of test bacterial isolates by the chemicals being investigated was assessed alone and in combination. An aliquot (20 mls) of the desired concentration of the test chemicals was prepared aseptically. These were inoculated with 108 cfu/ml of the bacteria under test. At different time intervals, 1 ml was withdrawn from the reaction mixtures and ten fold dilutions made in inactivating diluents (sterile normal saline with 3 % Tween 80). One milliliter of each dilution was mixed with 0.5 % yeastextract enriched melted nutrient agar (45 °C) and plated out in triplicates. The rate of cell kill was characterized by means of the rate of cell death (K-values) and one log cycle reduction in cell population (D-values).

RESULTS AND DISCUSSION

This report and previous others have indicated the presence of pathogenic bacteria in barbing salons. The commonly isolated genera are Staphylococcus, Escherichia, Citrobacter, Serratia, Klebsiella, Bacillus and Actinomyces (Table 1).

Table 1: Distribution patterns of the bacterial isolates in barbing salons

Organisms	Isolated from					
Organisms	Soaps	Water	Clippers			
Bacillus spp	+	+	+			
Klebsiella spp	-	+	-			
Serratia spp	-	+	-			
Citrobacter spp	-	+	-			
Escherichia spp	-	+	-			
Staphylococcus spp	+	+	+			
Actinomyces spp	-	-	+			

-: organisms not isolated, +: organisms isolated

The antibacterial activities of the test chemicals against the bacterial isolates are presented in Table 2. Analysis of the K-values and the D-values also confirms that the agents were more potent when used in combination than when used alone. Generally, a higher proportion of the cell population was killed [after 60 minutes contact] by a combination of Benzalkonium chloride and propylene glycol than when the drugs were used alone. Similar patterns (Staphylococcus aureus) were obtained for in Figures 1, 2 and 3 as shown in isolates-except for Escherichia coli (Figure 4), which was the most resistant isolate recovered in this study.

Table 2: Rate of kill of 10⁸ cfu/ml of test bacteria (K-values) and one log cycle reduction time (D values) of test antibacterial agents.

	Antibacterial activities against							
Antibacterial Agent	Kleb. Aerogenes		Serr.liquifaciens		Esch. coli		Staph.aureus	
	K-value	D-value	K-value	D-value	K-value	D-value	K-value	D-value
Benzalkonium chloride (0.003 % w/v)	0.0399	69	0.0228	64.5	0.0072	99	0.0275	81
Propylene glycol (20 % v/v)	0.1338	31.5	0.0233	63	0.0062	105	0.0259	70.5
Benzalkonium chloride (0.0003 % w/v) Propylene glycol (20 % v/v)	0.1343	21	0.038	24	0.0147	91.5	0.0321	22.5

Increasing the concentration of Benzalkonium chloride, gradually, from 1X 10⁻⁴ ug/ml to 20 X 10⁻⁴ ug/ml did not lead to significant increase in cell deaths (Figure 1), but when a sub-lethal concentration (0.0003 % w/v) Benzalkonium chloride was combined with increasing concentrations (Figure 2) or with a constant low concentration (20 % v/v) of propylene glycol (Figure 3), a significant increase in the rates of cell deaths were recorded.

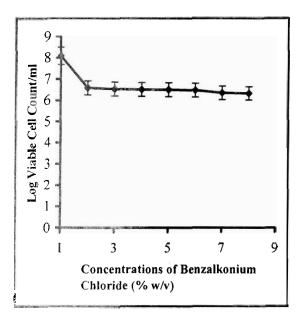


Figure 1: Effect of increasing the concentrations of bc on cell survival [Staphylococcus aureus]

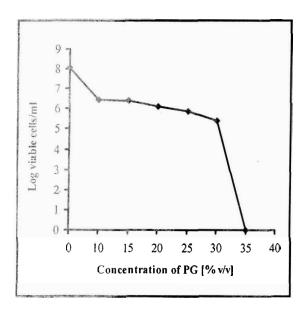


Figure 2: Effect of 0.0003 % [w/v] bc + varied Concentrations of pg on cell survival (Staphyceons cureus)

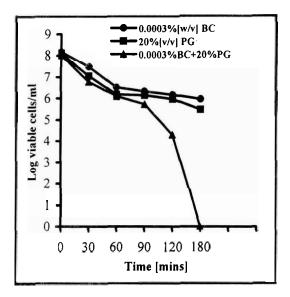


Figure 3: Effects of 0.0003 % [w/v] bc and 20 % [v/v] pg (alone and in combination) on cells survival with time (Staphylococcus aureus)

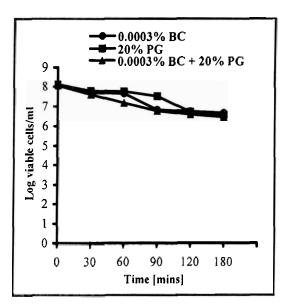


Figure 4: Effects of 0.0003 % [w/v] bc and 20 % pg (alone and in combination) on cells survival with time (*Escherichia coli*)

For example, there was a one-log cycle reduction in the population of *Staphylococcus aureus* from 81 when Benzalkonium chloride was used alone to 22.5 when combined with propylene glycol (Table 2).

The observed disparity in the responses of *E. coli* (the most resistant isolate in this investigation) may be due to the fact that the isolate could have acquired some peculiar

resistant characteristics; depending on the environment from which it originated [17] or possibly because of constant contact with sublethal doses of the antimicrobial agents in nature [18] as it is a common knowledge that these chemicals are widely used as preservatives in cosmetics and cells are liable to making contacts with them in sub-lethal doses.

Benzalkonium chloride has found extensive use as an anti-microbial agent because of its rapid bactericidal effects [19] but the ease with which microorganisms developed resistance to otherwise potent antimicrobial agents has led to a suggestion that formulations be designed to contain two or more antimicrobial agents [16].

Indeed, resistance to disinfectants based on quaternary ammonium compounds has been reported to be widespread among clinical strains of staphylococci in studies from many countries [20]. This observation justifies the need to use two or more antimicrobial agents in new formulations because of stated advantages of such practice- particularly with use of agents that are synergistic in action.

The phenomenon of synergism often enables a reduction of effective doses of chemotherapeutic agents [5], and this is believed to be brought about via three mechanisms namely increase by one agent of the permeability of cell wall and membranes to the second, inhibition of enzymes able to degrade the second agent and double blocking by the two, of components of successive steps in the metabolic sequences [21, 22].

The recovery of various bacterial species in these studies (Table 1) confirms an earlier report on the incidence of various bacterial species [13] in barbing salons. Many of these salon isolates have been found to be resistant to most chemical preservatives used in cosmetics.

These observations were built into the formulation of a disinfectant with reference name "Salonsafe" which contained benzalkonium chloride and propylene glycol as active anti-microbial constituent, citronella oil (fragrance) and iodine (colorant) in methanol vehicle-which also acted as drier. This product was challenged with 10⁵cfu/ml of the most resistant cells, which were completely killed at a short time of 60 seconds (Figure 5). This time is

by far less than the time that would be needed to prepare another client for barbing, thus limiting the risks of transferring infections among clients.

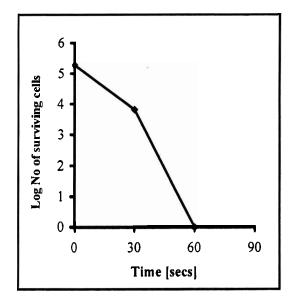


Figure 5: Log survival of Escherichia coli in "salonsafe"- a laboratory formulated disinfectant

It must be noted that currently, barbers "sterilize" their clippers, combs and scissors by cleaning them with methylated spirit. Studies in our laboratories have shown that this approach is ineffective. It is therefore essential to develop effective products for use as salon disinfectants.

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