Efficacy of Some Liquid Antiseptics on *Pseudomonas aeruginosa* Isolated from Wounds

A. M. Deji-Agboola,[1] O. J. Onakalu,[1] A. O. Hassan,[1,2] K. S. Adeboyejo,[1] T. A. Banjo,[1] B. C. Calebs,[1] M. A. Adeleke,[3] A. Oluwadun[2].

[1]Medical Microbiology / Parasitology Department, Olabisi Onabanjo University PMB 2022, Sagamu, Ogun State.
[2]Medical Microbiology / Parasitology Department, LAUTECH Teaching Hospital, PMB 5000 Osogbo Osun state.
[3] Biology Department, Osun State University, Osogbo
mopeagboola@yahoo.co.uk

Abstract: The increasing prevalence of *Pseudomonas aeruginosa* in wound infections have been a major concern, antiseptics are developed to inhibit or reduced the number of bacteria in or on living tissues. Several antiseptics are available in the market with paucity of information on their efficacy. This study therefore determines the efficacies of some liquid antiseptics against *P. aeruginosa* isolated from wounds using both qualitative and quantitative methods. The result of the purity test showed that all the antiseptics were sterile prior to use. The comparative assessment of the zones of inhibition of the diluted antiseptics indicated that Ethanol, TCP and Methylated Spirit were least effective ($F = 799.94$, $p<0.05$). Savlon produced the largest zone of inhibition followed by Purit. The result of the quantitative test using the MBC/MIC ratio showed that 8 (66.7%) were bactericidal with MBC/MIC ratio < 4. The presence of organic matter (plasma) in the undiluted and diluted antiseptics was observed to significantly ($t = 11.48$, $P<0.05$) reduce their zones of inhibition when compared with those without plasma. The antiseptics tested are potent against *P. aeruginosa*, the efficacy are reduced in the presence of organic matters
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1. Introduction

The development of wound infection depends on the integrity and protective function of the skin (Carlvin, 1998). It has been shown that wound infection varies with geographical location which could be prone by resident flora of the skin, clothing at the site of wound, time between wound and examination (Oguntibeju and Nwobu, 2004). The management of infection is a complex and important aspect of wound care.

In recent years, there has been a growing prevalence of Gram negative organisms which have almost replaced *Staphylococcus aureus* in nosocomial infection (Todar, 2004). Of the Gram negative bacilli, *Pseudomonas aeruginosa* has been of particular interest, the incidence of which in wound infection has increased compared to a decade back study (Thanni *et al.*, 2003; De-Quiroz and Day, 2007). The devastating effect of Pseudomonal infection on patient is usually as a result of an excessive immune response and the organism's high resistance to both host defenses and antibacterial agents in general (Loughlin, Jones and Lambert, 2002). The emergence of organisms that are resistant to antibiotic called for the increased use of relevant antiseptic in some cases.

Liquid antiseptics and disinfectants are widely used in hospitals and homes to control the growth or eliminate potentially pathogenic microbes on both living tissues and inanimate objects. They are important aspect of infection control practices and in prevention of hospital acquired infections (Saha *et al.* 2009). Antiseptics are antimicrobial agents that are designed to kill, inhibit or reduce the number of microorganisms (Atiyeh *et al.* 2009). The selection of disinfectants and antiseptics has been a general problem is because response of pathogens to various antiseptics or disinfectants differs (Russell, 1996).

Gibson *et al.* (1998) have suggested the need to test antiseptics, like antibiotics in order to determine their activity against a range of organisms. A lot of dedates have been generated against the use of antiseptics on open wounds, because it has been established that antiseptics are not as effective against bacteria that reside in wounds as they are against bacteria in vitro (Fleming, 1919; Laato, 1988; Rodeheaver, 1989; Dow 1999. Although, several bacteriological studies have shown that antiseptics can decrease bacterial counts within wounds. The presence of exudate, serum, or blood has been reported to decrease their activity (Baker and Breach 1980).

Many brands of antiseptics are available on the counter with paucity of information on their antimicrobial efficacy and factors affecting their efficacy on *P. aeruginosa*. This study was therefore carried out to investigate the antimicrobial effects of

some liquid antiseptics purchased in stores and market on *P. aeruginosa* isolated from wounds.

2. MATERIALS AND METHODS

A market survey of the different types of liquid antiseptics available in the market was carried out, a sample of each antiseptic was purchased and the cost price was noted. A table of the different antiseptics showing class or group, composition and cost price was compiled.

P. aeruginosa was isolated from clinical wound samples received at the Routine Bacteriology Laboratory of Olabisi Onabanjo University Teaching Hospital using standard methods of isolation and identification of bacteria as described by Finegold and Martins (1982). The identified *P. aeruginosa* were subcultured on nutrient agar slant, maintained at 4°C for further use. Standard *P. aeruginosa* ATCC 27853 was used as control organism for the entire test.

The undiluted liquid antiseptics were screened for sterility by aseptically streaking a loopful on Blood agar and MacConkey agar, plates and incubated at 37°C for 24 to 48hrs. The antiseptics solution were diluted according to manufacturer's instruction for use as antiseptic for cleansing and dressing of wounds and their effects on *P. aeruginosa* were determined using qualitative and quantitative methods.

A well dried Muller Hilton Agar was inoculated with overnight old broth culture of *P. aeruginosa,* sterile paper discs soaked with different antiseptics diluted according to manufacturer's instruction were placed on the plate and incubated at 37ºC for 18 – 24 hours. Standard *P. aeruginosa* ATCC 27853 was set up as control; all tests were performed in duplicates. The zones of inhibition produced by the antiseptics were scored as describe by Baker *et al.,* (1975).

The time kill test was used for the quantitative determination of the effect of the antiseptics by evaluating the microbial reduction by an antiseptic as described by (Ogunledun *et al.*, 2008). The Colonies formed by the tests and controls were counted for each contact time using colony counter. The microbial cell reduction rate % was determined using the fomular:

$$\text{Microbial cell reduction rate \%} = \frac{\text{CFU count at t second (control - test)}}{\text{CFU count at t second (control)}} \times \frac{100\ \%}{1}$$

Effect of organic matter on the antibacterial efficacy of antiseptic by adding 1ml of human plasma to 9mls of undiluted antiseptic and antiseptic diluted according to manufacturer's instruction for use. Sterile discs were soaked in the mixture drained to remove excess; these were placed on Muller-Hinton agar plates seeded with 0.5 MacFarland overnight broth culture *of Pseudomonas aeruginosa*. Plates were incubated at 37ºC for 24 and 48hrs standard *Pseudomonas aeruginosa* ATCC 27853 was used as control. The zones of inhibition were measured using a millimeter rule after 24hrs and 48hrs. The chemical composition on the efficacy of the antiseptics was carried out statistically by comparing the chemical composition with the zones of inhibition.

3. RESULTS

The results of the market survey of the various antiseptics with the chemical composition, class or group and cost is presented in Table 1. The antiseptics belong to the phenolic, biguanide, alcoholic, peroxygen and halogen releasing agent groups. The cost price ranges from ₦65 to ₦400 depending on the volume of the antiseptics.

The result of the purity test showed that all the antiseptics were sterile prior to use. Savlon, Purit, Detol and Carex produce the largest zone of inhibition in descending order. The comparative assessment of the zones of inhibition of the diluted antiseptics indicated that Ethanol, TCP (Frichloro-phenyl, methyiodosalicyl) and Methylated Spirit were least effective ($F = 799.94$, $p<0.05$) (Table 2). The result of the quantitative test using the MBC/MIC ratio showed that 8 (66.7) of the antiseptics which includes Tincture of iodine, Eusol and hydrogen peroxide were bactericidal (MBC/MIC ratio ≤ 4) while 4 (33.3) of the antiseptics which includes TCP and Carex were bacteriostatic (MBC/MIC ratio > 4) as presented in Table 3.

The rate of killing of *P. aeruginosa* by the different antiseptics showed that all the undiluted antiseptics exhibited 100% killing of *P. aeruginosa* within 30 seconds exposure time. All the diluted antiseptics exhibited 100% reduction within 30 seconds contact time except Carex and Nixoderm which exhibited 84.6% and 96.6% reduction respectively after 150 seconds (Table 4). The presence of organic matter (plasma) in the undiluted and diluted antiseptics was observed to significantly ($t = 11.48$, $P<0.05$) reduce their zones of inhibition when compared with those without plasma (Table 5). The effect of chemical composition of the antiseptics on their efficacy showed that antiseptics with

chlorinated hydrocarbons were more significantly (t = 8.47, P, < 0.05) effective in terms of antibacterial action against *Pseudomonas aeruginosa* than those without chlorine (Table 6).

Table 1: Market survey of Antiseptics available in Sagamu and Abeokuta

S/N	Trade Name	Active Ingredient	Group/ Class	Volume (mls)	Cost (₦)	Cost/ Litre (₦)
1	Carex	Dichloromethylxylenol terpenol, methylated spirit, castor oli, NaOH, pine oil, caramel deionised water	Phenolic	125		
2	Dettol	Chloroxylenol, Pine oil, denatured spirit	Phenolic	125	220	1,760
3	Ethanol	70%, 50%	Alcohol	Prepared from abs. Ethanol.		
	Eusol	Boric acid chlorinated lime	Halogen releasing agent	100	100	1,000
4	Hydrogen Peroxide	H_2O_2 Contained in 20% w/v	Peroxygen	250	70	280
5	Moko Methylated spirit	Isopropyl alcohol	Alcohol	200	150	750
6	Moko iodine tincture	Iodine 0.025g Potassuim iodine 0.025g Excipient to 1ml	Halogen releasing agent	15	65	4,335
7	Nixoderm	Dichloromethylxylenol, Terpinol	Phenolic	125	220	1,760
8	Purit	Chlorhexdine gluconate cetrimide	Biguanide	250	180	720
9	Savlon	Chlorhexidine gluconate, cetrimide	Biguanide	250	400	1,600
10	TCP (Frichloro-phenyl, methyiodosalicyl)	Phenol 0.175% Halogenated Phenol 0.68%	Phenolic	100	280	2,800

Table 2: Comparative assessment of the zones of inhibition of antiseptics diluted according to manufacturers instruction against *P. aeruginosa*

Antiseptics	N	Mean Zone of Inhibition ± SD	F	P-Value	Least effective antiseptics by LSD
Carex	10	30.40 ± 1.43	799.94	<0.05	
Dettol	10	30.90 ± 1.60			
50% Ethanol	10	11.50 ± 1.35			50% Ethanol
70% Ethanol	10	12.60 ± 1.65			70% Ethanol
Eusol	10	21.10 ± 0.99			
Hydrogen Peroxide	10	19.40 ± 0.84			
Methyleted spirit	10	18.50 ± 3.89			Methylated spirit
Nixoderm	10	24.40 ± 1.35			
Purit	10	35.50 ± 1.35			
Savlon	10	39.80 ± 1.69			
TCP	10	16.10 ± 1.45			TCP
Tincture of Iodine	10	25.20 ± 1.32			

Table 3: MBC/MIC RATIO OF ANTISEPTICS

ANTISEPTIC	MBC	MIC	MBE/MIC RATIO
Carex	¼	1/32	8
Dettol	1/8	1/32	4
50% Ethanol	½	1/16	8
70% Ethanol	½	1/16	8
Eusol	1/16	1/32	2
Hydrogen Peroxide	1/32	1/64	2
Tincture of Iodine	1/32	1/64	2
Methylated spirit	¼	1/16	4
Nixoderm	1/25	1/100	4
Purit	1/8	1/32	4
Savlon	1/17	1/68	4
TCP	¼	1/32	8

Table 4: Rate of killing *P. aeruginosa* by Undiluted and Diluted Antiseptics using Time Kill Test

Antiseptics	Initial conc. X 10^8	% Reductions Time (seconds)						
		Undiluted	Diluted					
		30	**30**	**60**	**90**	**120**	**150**	**180**
TCP	2.92	100	100	100	100	100	100	100
Purit	2.94	100	100	100	100	100	100	100
Savlon	2.90	100	100	100	100	100	100	100
H_2O_2	2.90	100	100	100	100	100	100	100
Detol	2.92	100	100	100	100	100	100	100
Carex	2.94	100	57.5	65.3	70.4	79.3	84.6	89.5
Nixoderm	2.92	100	60.3	79.5	87.3	94.8	96.6	100

Table 5: Effect of organic matter on the efficacy of selected Antiseptics.

Presence of organic Matter	N	Mean zone of Inhibition±LSD	t	P – value
Antiseptics with Plasma	120	10.92 ± 8.60	11.48	<0.05
Antiseptic without Plasma	120	23.78 ± 8.76		

Table 6: Effect of chemical composition of selected Antiseptics on *Pseudomonas aeruginosa*

Chemical Composition of Antiseptic	N	Mean zone of Inhibition ± SD	T	P – value
With chlorine	70	28.31 ± 7.84	8.47	p>0.05
Without chlorine	50	17.44 ± 5.42		

4. DISCUSSION

management is to prevent infection by inhibiting further microbial invasion of the tissue, thus giving the body a chance to repair itself (Brown and Gilbert, 1993). Infections of wound by microorganism may delay healing, cause failure of healing, and even cause wound deterioration (Dow, 1999). Mechanisms that causes delayed wound healing includes persistent production of inflammatory mediators, metabolic wastes, and toxins, and maintenance of the activated state of neutrophils, which produce cytolytic enzymes and free oxygen radicals (Laato, 1988). This prolonged inflammatory response, contributes to host injury and delays healing.

The results of this study showed that the antiseptics available in the markets are affordable depending on the volume. These antiseptics can be used as first aid to reduce bacteria which can compete with host cells for nutrition and oxygen necessary for wound healing (Rodeheaver, 1997; Gehan *et al*., 2009).

The ability of antiseptic to inhibit microorganisms may depend on the mode of action which could be either static or cidal and slow or rapid killer (Tambe *et al*. 2001). The results of the quantitative test using the MBC/MIC ratio showed that 8(66.7%) of the antiseptics tested are bactericidal.

All the antiseptics causes total (100%) cell reduction of *P. aeruginosa* in 30 seconds contact time, except Carex and Nixoderm which causes 100% and 89.5% cell reduction in 180 seconds respectively. Ogunledun *et al* (2008) reported a 100% cell reduction in 60 second for Carex in previous study. The difference in the cell reduction time might be due to storage, Ogunledun *et al*. (2008) obtained the Carex liquid antiseptic directly from the manufacturer and this present study used of the Carex obtained earlier (6months to the expiry date) while other antiseptics were sourced from the stores and markets. They could have suffered poor storage condition and exposure to direct sunlight.

The significant difference observed in antibacterial activities of the antiseptics with chlorine and without chlorine revealed that the antiseptics with chlorine based have higher efficacy than those without chlorine. This is in agreement with earlier observations of De-Quieroz and Day, (2007) which showed that chlorine and alcohol based antiseptics have higher efficacies than those without the two chemicals.

The activities of all the antiseptics (diluted and undiluted) are significantly reduced in the presence of organic matter (plasma). This finding is in agreement with the report of Simon *et al* (2007) who reported that blood reduces the efficacy of iodine by converting it to a non-bactericidal iodide.

In conclusion, eight (66.7%) out of twelve antiseptics were found to be highly potent against *P. aeruginosa*. The chlorine based antiseptics had higher efficacy than those without, the efficacy of the antiseptics is reduced in the presence of organic matter.

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