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POTENCY OF SEPTOL® AGAINST *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* ASSOCIATED NOSOCOMIAL INFECTIONS IN SPECIALIST HOSPITAL GOMBE

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ABSTRACT
The antimicrobial properties of various dilutions of Septol® against some test and control nosocomial E. coli and Staphylococcus aureus were investigated. Results showed that loss of viability was faster in sterile deionised water (SDW), followed by sterile tap water (STW) and then 10% sheep serum. A higher percentage of the resistant strains (S2 and E2) survived compared to the susceptible strains (S1 and E1). The viability of the cells in the disinfectant was also concentration dependent. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values from the different diluents used were highest for serum, followed by STW, while lowest values were obtained from SDW. This study showed that the organisms exhibited some stable resistance to the disinfectants used; there is therefore a need for review of the dilutions being used. Greater concentration of the disinfectants should be used.
Key wards: Septol®, Disinfection, Susceptibility, bactericidal, viability, Decimal reduction time.

INTRODUCTION

Nosocomial infections affect 5% of all hospitalised patients in some clinical services such as intensive care units. Up to 10% of patients are infected in the developing countries (Madigan *et al*., 2000). Reviewing the incidences and spread of nosocomial infections, Lark (2001) observed that about 5% of patients in the United States developed bacteraemia or fungemia each year, with an associated mortality of 12-50% per episode. Similar incidences have been reported in Europe, Japan, South-east Asia, the Middle East and Australia (Matsuda *et al*., 2003). The most frequent infections are those of surgical sites as well as skin and soft tissue sites, blood, urinary, upper and lower respiratory tract infections. Most of the infections are associated with invasive medical devices or invasive surgical procedure. Factors facilitating the spread of nosocomial infections include impaired immunity, extremities of age, severe illness, treatment with broad spectrum antibiotics, the ever increasing variety of medical procedures and invasive techniques creating potential route of infection and transmission of drug resistant microorganisms among crowded hospital populations (Madigan *et al*., 2000).

The effectiveness of disinfectants in controlling nosocomial infections is often compromised by the fact that many of the disinfectants used in hospitals have been reported to be contaminated with microorganisms (Kahan *et al*., 1984). In some instances, instead of preventing transmission, hospital use disinfectants have themselves been the vehicle of transmission with fatal consequences (Bassett, 1971). Some reports have shown that contamination of disinfectants have often arisen from vehicles used during disinfectant dilution, non-adherence to proper techniques in their uses, poor personal hygiene, re-use and improper storage (Bassett *et al*., 1970). All these have led to the development of resistance to the disinfectants by nosocomial organisms. In this regard the surveillance of nosocomial pathogens and proper use of whatever disinfectant and other antimicrobial agent available cannot be over emphasized. Also, Quite often, studies to determine the efficacy of antiseptics and disinfectants utilize organisms that have little relevance to the hospital environment (ElMahmood, 2006). In the clinical setting, microorganisms are not found in pure cultures, but enveloped in proteinaceous materials such as blood, sputum, faeces or milk. Similarly, distilled water regularly used in the investigations, is not the water that is used in practice to reconstitute the disinfectants (ElMahmood, 2006).

It is worth noting that disinfectants like Septol®, Z-germicide®, and Dettol® etc are being used in cleaning and disinfection at the Specialist Hospital, Gombe, but some nosocomial organisms still persist in the environment (ElMahmood, 2009). There is therefore, a need for studies to be carried out on these disinfectants and microorganisms, in order to determine the true efficacy of these disinfectants on the organisms and also the effectiveness of the various manufacturers' dilutions of the disinfectants, using sterile distilled water, sterile tap water and sheep serum as diluents. This study is aimed at evaluating the efficacy of Septol® disinfectant under use-conditions against nosocomial *Escherichia coli (E. coli)* and *Staphylococcus aureus*.

MATERIALS AND METHODS

The Sampling site and sample collection

The following hospital locations namely; theatre, wards and laboratory surfaces at the Specialist Hospital Gombe served as sample collection sites. Sterile swab sticks dipped in peptone water were used to swab work benches, floors, sits and other surfaces, after which the swab sticks were transported immediately in a closed test tube containing peptone water to the microbiology laboratory of Gombe State University for analysis (Cheesbrough, 2006).

Isolation and identification of the selected organisms

The swabs collected were aseptically inoculated onto MacConkey agar plates, Mannitol salt agar platesand Blood agar plates using the streak plate technique and incubated at 37ºC for 18hrs. After the incubation period, the various growth cultures were then sub cultured on EC medium to select pure cultures of *E.coli*. This was followed by IMvic test to confirm them. Catalase and coagulase tests were carried out for confirmation of *Staphylococcus aureus.* Pure cultures were preserved in nutrient agar slants at 4°C(Cheesbrough, 2006).

Antibiotic susceptibility test

Each of the bacterial isolates was subjected to antibacterial susceptibility tests using the agar diffusion method (Cheesbrough, 2006). One ml of 18hr broth culture suspension of the organisms' equivalent to 0.5 McFarland turbidity standards was poured into different sets of sterile Petri dishes and rocked gently to spread the organisms. Nineteen ml of molten Mueller Hintonagar at 45ºC was then dispensed on to the plates and rocked once again to uniformly mix the contents. The plates were left at room temperature (32-35ºC) for 30mins to solidify and then antibiotic discs (Biotech Lab Ltd. UK) were firmly pressed on to the agar surface at points equidistant to each other. A sterile 6mm filter paper was used as a control; the plates were then incubated at 35-37ºC and observed for 48hrs. The zones of inhibition diameter were measured and interpreted (Baker and Thornberg, 1983). The antibiotics used were: CP=Ciprofloxacin(10µg),NAL= Nalidixic acid(30µg), AMP=Ampicillin(10µg), TET=Tetracycline(30µ), STR=streptomycin (25µg) and GEN=Gentamycin (10µg).

Selection of test and control organisms

Based on the susceptibility results obtained, the organisms were grouped into resistant (*S. aureus* S1 and *E.coli*E1)and susceptible (*S. aureus* S2 andE.coliE2). Resistant organisms were those that show stable resistance to more than 3 antimicrobial agents, while susceptible organisms were those that show stable susceptibility to all drugs tested. S1 and E1 were regarded as the test organisms while S2 and E2 the control organisms (Gupta *et al.*, 2004)

Maintenance of selected test organisms

The selected test and control organisms (10^8cells/ml) were sub-cultured on nutrient agar slants and stored at 4ºC until required. The purity of the organisms was checked at regular intervals of 48 hours by plating and staining (ElMahmood, 2009).

Preparation of cell cultures

The calibration of the organisms was carried out by following the changes in optical densities for a period of 90mins. Cell cultures of the selected organisms in nutrient broth were grown in a shaker water bath maintained at 37ºC and the absorbance values recorded at 15mins intervals using a 722s Spectrophotometer (ElMahmood, 2009).

Determination of the effect of Septol ®on the viability of the selected organisms

Manufacturer's recommended dilutions i.e.1:2000(0.005v/v) and1:500 (0.02v/v) ofSeptol® were used on the selected organisms, *E. coli* (E1 andE2) and*Staphylococcus aureus* (S1and S2).To determine the effect of 1:2000 Septol® on the test organisms, 17.9ml of Sterile tap water (STW) was added to 0.1ml of undiluted Septol® in a 50 ml conical flask. Two ml of prepared cell culture was added to the flask and shaken vigorously and 1ml of the bacterial suspension transferred immediately in to a test tube containing 9ml in-activator solution of 2% 'Tween' 80 plus 1% soy lecithin to inactivate the active component of the disinfectantsand adequately mixed using a test tube shaker and allowed to stand for 1 min for complete inactivation of the disinfectant(ElMahmood, 2009). After the inactivation of the disinfectants, serial dilution of the cell suspensions was carried out in the range of 10^{-1} to 10^{-9}. One ml samples from the 10^{-4} and 10^{-5} dilutions were then cultured in triplicates using the pour plate technique at 5min intervals for 30mins. The culture was then incubated at 37ºC for 24hrs and the colonies counted using a SC6 digital colony counter. The same procedure was repeated using Sterile distilled water (SDW) and 10% sheep serum.

To determine the effect of 1:500 Septol® on the viability of the organisms, 17.6ml of sterile tap water was added to 0.4ml of undiluted disinfectantsin a 50ml conical flask. Two ml of the culture added to the flask and shaken vigorously and the procedure outline above was followed to complete the test.

Determination of MIC of the Septol®

This was carried out using the logarithmic dilution method as described by Croshaw (1983).

Grade volumes of undiluted Septol® (1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8and 3.0ml) and respective calculated volumes of STW (3.8, 3.6, 3.4, 3.2, 3.0, 2.8, 2.6, 2.4, 2.2, 2.0 and 1.8ml) was added to respective 5ml double strength nutrient broth and 0.2ml of test culture(s) to make a total volume of 10ml single strength nutrient broth. This mixture was thoroughly mixed on a whirl mixer. The 12^{th} test tube didnot contain the Septol® and thus served as control. The test tubes were then incubated at 37ºC for 24hrs and observed for growth in form of turbidity. The first test tube that did not show any visible turbidity was regarded as the MIC.

Determination of MBC of the Septol®

One hundred µL of broth culture was removed from the test tubes that did not show any turbidity in theMIC determination after incubation at 37ºC for 24hrs using a micropipette and inoculated on to nutrient agar using the pour plate technique. The plates were then incubated at 37ºC for 24hrs and observed for growth (Waterworth, 1978; Baldry, 1983).The plate that did not show any visible growth is regarded as the MBC.

Calculations

The kinetics of cell deaths has been reviewed by Meynell and Meynell (1970). Microorganisms exposed to a killing agent usually show exponential death with or without an initial shoulder. Figure 1 shows a plot of $\log_{10}$ (N_t/N_o) against time.

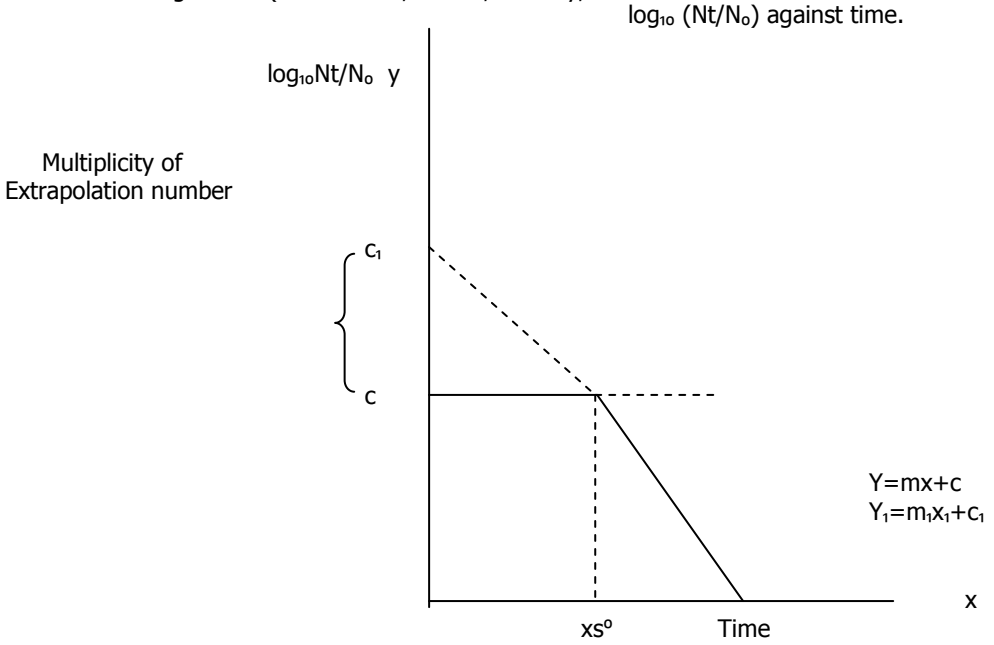

Figure 1: Survival of cells exposed to a lethal agent

Where N_o and Nt are the numbers of viable organisms at time zero and time t respectively. When the kinetics of cell death exhibits a shoulder the graph can be resolved into two straight lines. Such a plot has three important features:

1. The length of the shoulder, Xs is calculated from the intersect of the two straight line portions of the graph.

At the intersect of y=y1 and x=x1.

$Mxs + c = m_1xs + c_1$

$Xs = \frac{c_1 - c}{m - m_1}$

2. The gradient (slope) of the killing curve m_1 is used to calculate the decimal reduction time (DRT) which is the time for a ten- fold reduction in the number of survivors

$DRT = -1/m_1$

The length of the shoulder and the gradient of the killing curve m_1 indicate the resistance of cells to the agent

3. The difference between intercepts c_1 and c is known as the multiplicity of the process or extrapolation number and has been shown to indicate how many molecules of agent interact with one cell to cause death.

The data analysis was done using linear regression and the graphs plotted were used in the calculations (Cove and Holland, 1983).

RESULTS

A total of 39 isolates were obtained from 20 swab samples collected from various locations at the Specialist Hospital Gombe (Table 1). Sixteen of the isolates were identified as *Staphylococcus aureus*, 12 were *E. coli*, *Proteus* sp *3, Pseudomonas* sp *1, Streptococcus* sp *6* and *Klebsiella* sp1.

The antimicrobial susceptibility pattern is shown in Table 2. This showed that 87% of *Staphylococcus aureus* were susceptible to Ciprofloxacin, 75% were susceptible to tetracycline, 50% susceptible to ampicillin, 31% to streptomycin, 25% to gentamycin and 12.5% to Nalidixic acid. While for *E.coli*, it was also observed that 91% were susceptible to ampicillin, 83% were susceptible to tetracycline and the least susceptibility was seen in Nalidixic acid. The MIC and MBC values of the Septol® in STW, SDW and 10% sheep serum are shown in Table 3. The MIC and MBC values of Septol® for S1 in SDW was 1.6 and 2.0ml respectively, while in STW, the values were 1.8 and 2.2ml and in 10% sheep serum, the values were 2.0 and 2.4ml. For the corresponding S2, the MIC and MBC values were also quantitatively similar, showing 2.2 and 2.6ml in STW and 2.0 and 2.6ml in SDW.

Decimal Reduction Time (DRT) in minutes and the slope (M) of the graphs

Values for the Decimal Reduction Time (DRT) and the slope (M) of the graphs are shown in Table 4 for Septol®. The slope for 1:500 (0.02v/v) Septol® for S1 was -0.083 in SDW, -0.081 in STW and -0.078 in serum indicating that the rate of kill was faster in SDW than in STW and 10% sheep serum respectively. For S2, the slope (M) for 1:500 (0.02v/v) was -0.083 in SDW, -0.079 in STW and -0.077 in serum.

The DRT for 1:500 (0.02v/v) Septol® for S1 was 12.00 min in SDW, 12.32 min in STW and 12.78 min in serum. For S2 the DRT was 12.04 min in SDW, 12.50 in STW and 12.94 min in serum. The effect of the use dilutions 1:2000 (0.005v/v) Septol® on the viability of the organisms are shown in Figure 2 for (E1 and E2). For dilutions 1:500 (0.02v/v), the effects are shown in Figure 3 for (S1 and S2).

Table 1: Distribution of isolates based on site of isolation

S/No	Organisms isolated	Laboratory	Theatre	Ward	Total
1.	*Staphylococcus aureus*	5	3	8	16
2.	*E.coli*	4	2	6	12
3	*Proteus* sp	1	0	2	3
4	*Pseudomonas sp*	0	0	1	1
5	*Streptococcus sp*	2	1	3	6
6	*Klebsiellasp*	0	0	1	1
	Total	12	6	21	39

(F^2=1.9, df= 3, >0.01) no significant difference

Table 2: Susceptibility of isolates to antibiotics

S/NO	ISOLATES	N	TET	CP	NAL	AMP	GEN	STR
1.	*Staph. aureus E.coli*	16	12(75%)	14(87.5%)	2(12.5%)	8(50%)	4(25%)	5(31%)
2.		12	10(83.3%)	9(75%)	3(25%)	11(91.7%)	4(33.3%)	4(33.3%)

KEY: CP=Ciprofloxacin(10µg),NAL= Nalidixic acid(30µg), AMP=Ampicillin(10µg), TET=Tetracycline (30µ), STR=Streptomycin(25µg),GEN=Gentamycin(10µg), N: number of isolates

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) values for Septol® (Hydroxydiphenyl methane) against the organisms

	Septol®		
Organisms	**Dilution medium**	**MIC**	**MBC**
S1	SDW	1.6	2.0
	STW	1.8	2.2
	10% sheep serum	2.0	2.4
S2	SDW	2.0	2.4
	STW	2.2	2.6
	10% sheep serum	2.4	2.8
E1	SDW	1.4	1.6
	STW	1.6	1.8
	10% sheep serum	1.8	2.0
E2	SDW	1.8	2.0
	STW	2.0	2.2
	10% sheep serum	2.2	2.4

F=0.12<0.05, no significant difference

KEY: S1: *Staphylococcus aureus* test, S2: *Staphylococcus aureus* control, E1: *E.coli* test, E2: *E.coli* control, STW: sterile Tap water, SDW: sterile deionised water,MIC: Minimum Inhibitory concentration,MBC: Minimum Bacteriocidal concentration

Table 4: Slope (M) of the curves and decimal reduction time (DRT) of the organisms treated with use dilutions of Septol® (Hydroxydiphenyl methane) in STW, SDW and 10% sheep serum

Conc.	DM	S1		S2		E1		E2	
(V/V)		M	DRT	M	DRT	M	DRT	M	DRT
0.005	STW	-0.069	14.49	-0.062	16.25	-0.071	14.08	-0.051	19.78
	SDW	-0.080	12.50	-0.066	15.14	-0.076	13.11	-0.062	16.11
	SERUM	-0.056	17.86	-0.051	19.33	-0.061	16.22	-0.042	23.70
0.02	STW	-0.081	12.32	-0.079	12.5	-0.081	12.33	-0.076	13.22
	SDW	-0.083	12.00	-0.083	12.04	-0.084	11.87	-0.084	11.96
	SERUM	-0.078	12.78	-0.077	12.94	-0.062	16.25	-0.054	18.33

KEY: DM: dilution medium, M: slope, DRT (decimal reduction time in minutes)

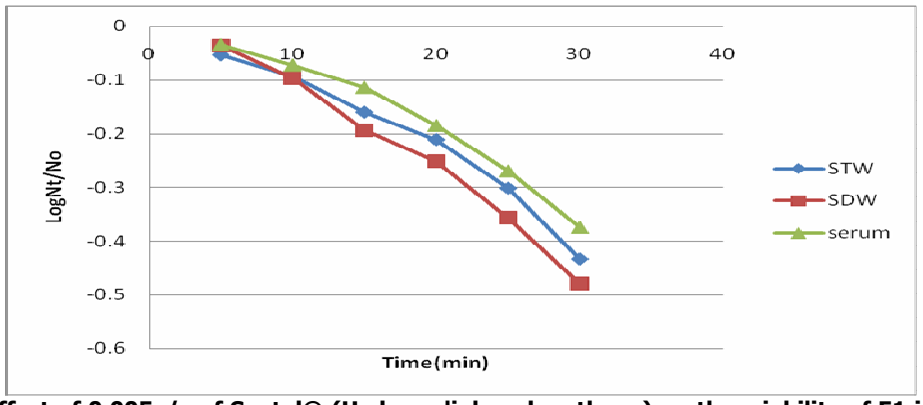

Figure 2a: Effect of 0.005v/v of Septol® (Hydroxydiphenyl methane) on the viability of E1 in STW, SDW and 10% sheep serum at 37°C

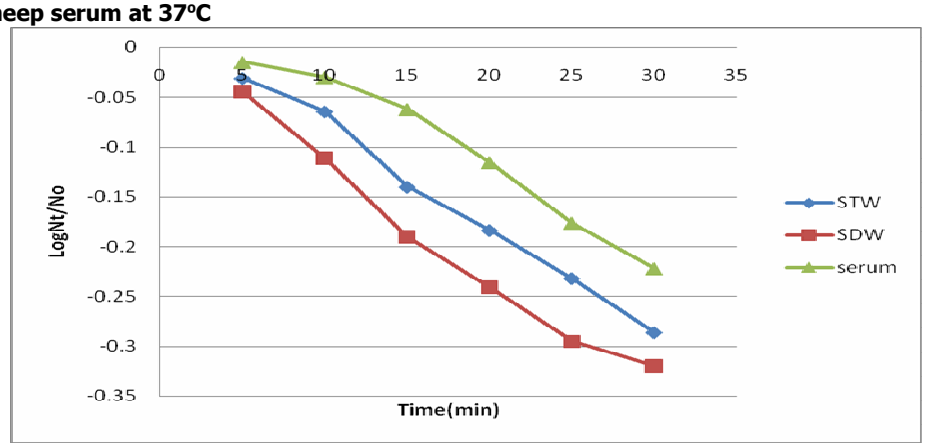

Figure 2b: Effect of 0.005v/v of Septol® (Hydroxydiphenyl methane) on the viability of E2 in STW, SDW and 10% sheep serum at 37°C

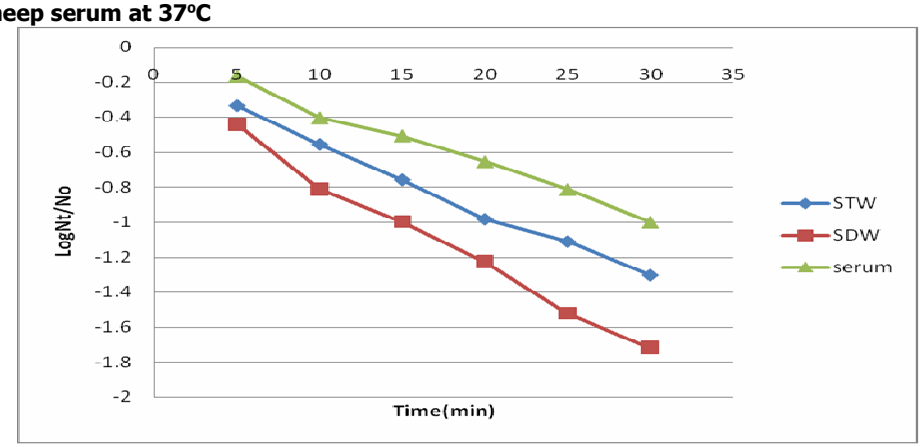

Figure 3a: Effect of 0.02v/v of Septol® (Hydroxydiphenyl methane) on the viability of S1 in STW, SDW and 10% sheep serum at 37°C

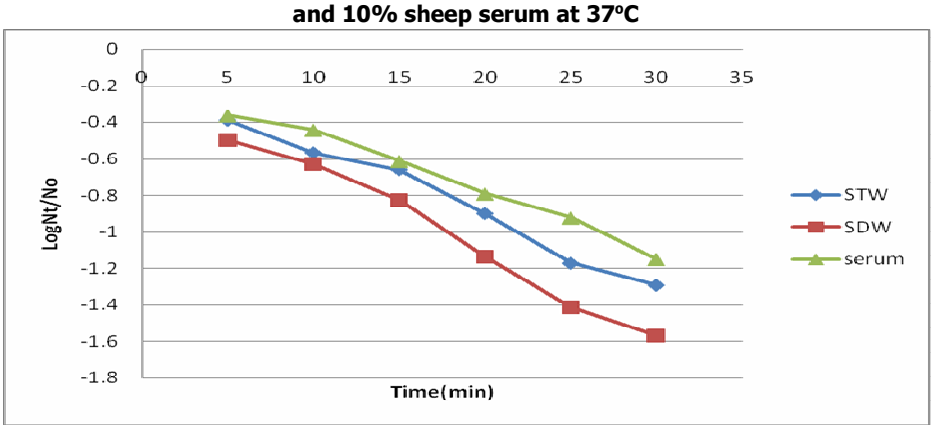

Figure 3b: Effect of 0.02v/v of Septol® (Hydroxydiphenyl methane) on the viability of S2 in STW, SDW and 10% sheep serum at 37°C

DISCUSSION

The result of this study indicates that nosocomial microorganisms are present on environmental surfaces, and these organisms continue to emerge and re-emerge, despite the use of disinfectants and antiseptics in the hospital environment. Microorganisms like *E.coli, Staphylococcus aureus, Proteus sp, Pseudomonas sp* etc implicated in causing nosocomial infections were the same ones isolated from the hospital environment in which this study was carried out. The fact that these organisms were isolated is not unconnected to the fact that inappropriate diluents e.g. tap water was used to dilute the disinfectant and antiseptic in question instead of sterile deionised water (Tyler *et al*., 2006), the use of overnight left over disinfectant which could be contaminated and itself serve as a vehicle for the transfer of these organisms (ElMahmood, 2009) and the fact that the hospital staff that carry out these dilutions may not be well trained or may not adhere strictly to the manufacturer's prescribed dilutions. Some residual amounts of antiseptics and disinfectants found in the hospital environment could contribute to the selection and maintenance of multi-resistant strains of microorganisms.

Several researchers have cautioned that when comparing the frequency of contamination, one should always consider the types and concentration of disinfectants since resistance are known to vary in different organisms (Russell and Chopra, 1996). In this study, the predominantly isolated microorganisms were *Staphylococcus aureus* and *E. coli*. Similar findings on the predominance of these bacteria in disinfectants and hospital environment have been reported by Keah *et al,* (1995).

Colonial morphology and Biochemical analysis carried out confirmed that indeed the isolates were *Staphylococcus aureus, E.coli* and other organisms like *Pseudomonas sp, Proteus sp, Klebsiella sp* and *Streptococcus sp*. The distribution of isolates based on site of isolation, indicated that most of the organisms isolated were from the wards (*S. aureus,* 8 and *E.coli,*6), followed by Laboratory (5 and 4 respectively), while the least number of organisms were isolated from the theatre (3 and 2 respectively). The fact that most of the isolates were isolated from the wards may not be unconnected to the number of people moving in and out of the wards.

The activities of Septol® was assessed by performing viable cell counts at 5 minute intervals on the surviving microbial population for a period of 30 min as shown in the Figures. The log number of cells in both susceptible (S1 and E1) and resistant (S2 and E2) strains were observed to decrease gradually after an initial lag (shoulder), the duration of which is a function of the concentration of the Septol® used and the type of organism. The number of cells decreased faster in SDW than in STW and 10% sheep serum and also in the susceptible than resistant cells with negative slopes. This corresponds with a report by ElMahmood and Doughari (2009). When a microbial population is subjected to the toxic influence of an agent, the number of cells decreases gradually in such a manner that when the logarithm of the number of cells at any time is plotted against time, it gives a descending straight line with a negative slope (Acheampong *et al*., 1988). This is referred to as logarithmic order of death (Essellen and Pflug, 1956). One characteristic of the logarithmic order of death is that there is a linear relationship between logarithm of the number of survivors and time. This means that at any time interval a constant proportion of cells loose viability. All the organisms show or exhibited a uniform response to Septol® as shown by the almost straight line graphs. This is an indication there is no sub-population of cells resistant to the disinfectants in the test and control cultures. Extensive work on the mechanism of death in the presence of several concentration of phenols, diphenyl methane and other halogenated phenols have been documented and the mode of actions of these compounds have been found to be due to their adverse effects on cellular permeability leading to inhibition of enzymes and leakage of intracellular materials out of the cells (Hugo and Bloomfield, 1971). Thus, the cytoplasmic membrane and its components are considered to be the main site of action of Septol®. From the study it was also observed that as the concentrations of the Septol® is increased, the total bacterial counts of the test and control organisms decreases, similar to other studies carried out by (Hani and Adnan, 2009).

Tap water (STW) was observed to increase the MIC and MBC of the Septol®. The presence of serum also tends to increase the MIC and MBC values above that of STW. Tap water used in practice to dilute the disinfectant and serum is a likely organic matter encountered in the hospital environment. In the presence of serum, the MIC and MBC values were considerably higher than without serum. This is not surprising because organic matter have been reported to reduce the activity of most antimicrobial agents (Bean, 1967).Tap water is reported to contain impurities such as ferrous, calcium, magnesium salts and some trace elements (Wilson and Miles, 1964). These impurities might have interacted with the diphenyl methane content of the Septol® to reduce activity. In this study, the test organisms (S1 and E1) were consistently more susceptible to the use dilutions of the disinfectants used than their corresponding index control organisms (S2 and E2). Reports of varying levels of resistance and susceptibility occurring in some species of organisms have earlier been reported and have been attributed to variation in lipid build up (Ducel *et al*., 2002).

CONCLUSION AND RECOMMENDATIONS

This study has shown the improved activity of the disinfectants with increase in use-concentrations. The result also shows the reduction in activity of disinfectants in the presence of potable water and in serum. It was also observed that the decline in the total bacterial count after application of disinfectants is not well pronounced even with higher concentrations (lower dilutions) of disinfectants. This shows that the organisms exhibit some stable form of resistance to the disinfectant in question and there for there is need to review the dilutions used. Greater concentrations of the disinfectants should be used and the water (diluents) used should be first analysed for impurities before use.

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