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Full Length Research Paper

Bacteriological examination of some diluted disinfectants routinely used in the Specialist Hospital Yola, Nigeria

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Five frequently used disinfectants in our hospital (Specialist Hospital, Yola, Nigeria) were analyzed for bacterial contamination and sensitivity to antibiotics. For each disinfectant, 20 different samples of stock and left-over diluted solutions were used for the analysis. All the stock undiluted samples were free from any bacterial growth. However, all the left-over disinfectant samples were found to have significant bacterial contamination, predominantly gram negative bacteria and the contamination level varied from 2.6 x 10^5 to 3.5 x 10^8 cfu/ml. Amongst the 5 different disinfectants analyzed, Purit was found to be highly contaminated (30%), followed by Dettol (25%), Parazone (20%), Z-germicide (15%), Septol (10%). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the isolates were significantly higher than that of the control sensitive strains, but were lower than the values quoted by the manufacturers of these disinfectants. All the isolates showed variable sensitivity to antibiotics with each disinfectant showing sensitivity to at least four antibiotics tested in this study.

Key words: Disinfectant, bacterial contamination, left-over diluted samples, antibiotic susceptibility.

INTRODUCTION

Despite best efforts to identify and eliminate infectious microorganisms, they continue to emerge and re-emerge. These pathogenic bacteria significantly contribute to human illness and death especially as a result of hospital acquired infection/s (French, 1996). The successful eradication of these pathogens with antibiotics has been complicated by the development of highly resistant strains as well as the appearance of new virulent pathogens. Some non antibiotic antimicrobial agents of various preparations have been developed and introduced with the aim of breaking the chain of infections in homes, industries and hospitals (Bean, 1967).

Many chemical agents are now available commercially as disinfectants and antiseptics. These preparations include halogen compounds, phenols and halogenated phenolic and substituted phenolic compounds. Additional preparations are Tar acid phenol, biguanides, alcohols, aldehydes, peroxygens and quaternary ammonium compounds chlorohexidine gluconate. Considerable progress has been made in the understanding of the mechanisms of antimicrobial actions of antiseptics and disinfectants (Russell and Chopra, 1996; Russell et al., 1997)

As a result of extensive use, a significant proportion of the pathogens have not only developed resistance, but they also grow in the solutions of these biocides. There is enough scientific evidence in the literature describing not only the growth, but also the concentration of the colony-forming units of bacteria at sites of application of disinfectants and antiseptics (Oieand and Samiya, 1996; Gajadhar et al., 2003). Bacteria have a survival strategy by colonizing at the surfaces and grow as biofilm communities embedded in a gel-like polysaccharide matrix. Several reports attribute the emergence and spread of nosocomial infections to contaminated disinfectants, antiseptics and their use on the skin of patients and health care providers. The use of contaminated instruments and appliances on the patients after treatment with these disinfectants and antiseptics is another for hospital acquired infections in the hospitalized patients (Keah et al., 1995). Ayliffe (1987) had reported that bacteria isolated from contaminated disinfectant solutions and antiseptics

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exhibit increased resistance to commonly used antibiotics. This constitutes a serious public health problem, given the fact that bacteria have the ability to share resistance markers, and once a resistance develops for one agent, cross-resistance to other agents can occur. Several studies have also shown that pathogenic strains may be derived from commensal strains by acquiring chromosomal and extra-chromosomal virulence operons. In some instances, instead of preventing transmission, hospital used disinfectants have themselves being the vehicle of transmission with fatal consequences (Bassett, 1971). The activity of biocides against microorganisms is not always consistent due to several basic methodological problems as well as high intrinsic resistance due to differences in membrane structure. Some reports have shown that contamination of disinfectants have arisen from vehicle used during disinfectants dilution, non-adherence to proper techniques in their use, re-use and improper storage (Keah et al., 1995).

Our hospital had been using various disinfectants extensively, but there is no report on the microbial contamination of these biocides from any referral hospital from this part of the country. This study was aimed to determine the contamination level in the commonly used disinfectants, isolation of the bacteria and evaluation of sensitivity of the isolated micro-organisms to few of the antibiotics in routine patients' use at this hospital.

MATERIALS AND METHODS

Selection of disinfectants

The disinfectants used in this study included Dettol (Batch 3096, Beckith benckiser Pharmaceuticals ltd, South Africa), Purit (BN 231, Chemical and Allied Products plc, Lagos Nigeria), Parazone, Zgermicide and Septol (all of Gongoni Co. ltd, Kano, Nigeria) and were selected based on wide acceptability and frequency of use in the hospital.

Collection of diluted disinfectants samples

Fifty milliliters (50 ml), each of the five brands of diluted disinfectants was collected inside a sterile container and transferred immediately in ice-cooled packs to the Microbiology Laboratory (Federal University of Technology, Yola, Nigeria) for microbiological analysis.

Microbiological examinations

The collected samples were evaluated for their bacterial counts, types and susceptibility to commonly used antibiotics.

Enumeration of bacterial colonies

The pour plate method was used and for each diluted disinfectant collected from the hospital, 1.0 ml of the sample was added to a test tube containing 9 ml sterile tryptone soy broth and the contents mixed thoroughly on a votex mixer (El-Mahmood and Doughari, 2007). Subsequent dilutions were made in 9.0 ml sterile tryptone soy broth to obtain countable colonies and 1.0 ml of the final dilution plated on nutrient agar plates. The plates were incubated at 37ºC for 48 h and colonies counted on a Gallenkamp Colony counter. Mean of triplicate results taken as the average number of colonies and were multiplied by the dilution factor to obtain the total number of organisms per milliliter of the stock. Serial dilutions of 1 ml of each of the disinfectants (undiluted) were used as positive control and 1.0 ml of sterile distilled water were used as negative control.

Isolation and identification of the bacteria

A sterile wire loop was used to pick a colony of bacteria on the cultured plates and then sub-cultured on a sterile nutrient agar by streaking. The inoculated plate was incubated at 37^{o}C for 24 h. The bacteria were identified using standard procedures as described by Baker and Thornberg, 1983).

Inoculum preparation

The method of Baker and Thornberg (1983) was used and two colonies from the 18 h old culture were aseptically transferred into 9.0 ml single strength nutrient broth (NB, Oxoid) contained in sterile test tubes and incubated at 37ºC for 24 h. The overnight growth culture was gradually added into sterile normal saline contained in 20.0 ml universal bottle till turbidity similar to McFarland 0.5 which is equivalent to 1 x 10^8 cells was obtained.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the disinfectants against the isolates

The minimum inhibitory concentration of Dettol against *Escherichia* coli was determined using the arithmetic dilution method of Croshaw, (1983). To sets of test tubes containing 5.0 ml double strength nutrient broth, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2 and 3.1 ml of sterile distilled water and 1.0 ml of cell culture containing 1 x 10^8 cfu/l ml were added. To each of the tubes 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.2 ml of Dettol were then added and the content thoroughly mixed on a Gallen kamp whirl mixer. To the 8th test tube (control) no Dettol was added. Both the control and treated cultures were incubated at 37ºC for 24 h and observed for growth by visual inspection. The test tube that shows no visible growth or turbidity after incubation was considered the MIC. The same procedure was repeated for each of the other bacterial isolates and disinfectants.

The minimum bactericidal concentration of Dettol against *Escherichia coli*

Two loopfuls from the test tubes that showed no visible growth were inoculated into solidified nutrient agar (NA, Oxoid) plates and incubated at 37ºC for 24 h. The concentration at which no growth was observed visibly from the plates was recorded as the MBC of the dettol. The same procedure was repeated for each of the other bacteria and other disinfectants.

Susceptibility test

Each of the bacterial isolates was subjected to Antimicrobial susceptibility tests using the agar diffusion method. 1.0 ml of 18 h broth culture suspension of the test organisms (0.5 McFarland tur-bidity standards) was poured into different sets of sterile Petri dishes and rocked slightly to spread the organisms. 19.0 ml of molten NA at 45ºC was then dispensed onto the plates and rocked once again for uniform mixing of the contents. The plates were left at room

Table 1. Contamination levels of the in-use diluted disinfectants.

Disinfectant	Total number	Number of		Bacteria Isolated	Total plate count cfu/ml
		Isolates positive	Percent positive		
Dettol	20	5	25	*P. aeruginosa* *E. coli*	$5.4x10^8$
Purit	20	6	30	*S. aureus* *P. mirabilis* *E. coli*	$6.3x10^7$
Parazone	20	4	20	*S. aureus* *P. aeruginosa* *P. mirabilis*	$7.1x10^7$
Z-germicide	20	3	15	*S. aureus* *E. coli*	$4.9x10^6$
Septol	20	2	10	*S. aureus* *S. aureus* *P. aeruginosa*	$7.5x10^5$

Table 2. The MIC and MBC of the disinfectants against their isolates.

Disinfectant	Organism	MIC		MBC	
		Isolate	Control	Isolate	Control
Dettol	*P. aeruginosa*	0.16	0.08	0.18	0.10
	E. coli	0.12	0.08	0.14	0.08
	S. aureus	0.10	0.06	0.12	0.06
Purit	*P. mirabili*	0.10	0.06	0.12	0.08
	S. aureus	0.08	0.06	0.10	0.06
	E. coli	0.2	0.08	0.14	0.08
Parazone	*P. mirabilis*	0.12	0.06	0.14	0.08
	S. aureus	0.10	0.06	0.10	0.08
	P. aeruginosa	0.14	0.08	0.18	0.10
Z-germicide	*E. coli*	0.14	0.08	0.16	0.08
	S. aureus	0.12	0.08	0.14	0.06
Septol	*P. aeruginosa*	0.12	0.06	0.16	0.08
	S. aureus	0.10	0.06	0.14	0.08

temperature (32-35ºC) for 30 min to solidify and then antibiotic (ampicillin 25 mg, cotrimoxazole 20 mg, gentamycin 10 mg, nalidixic acid 30 mg, nitrofurantoin 20 mg, colistin 25 mg, streptomycin 25 mg, tetracycline 25 mg, chloramphenicol 25 mg, ciprofloxacin 10 mg) discs (procured from Biotech laboratories Ltd., UK) were firmly pressed on to the agar surface at points equidistant to each other. A sterile 4 mm filter paper was used as control; the plates were then incubated at 35-37ºC and observed till 48 h of incubation period. The zone of inhibition diameter were measured and interpreted as described by (Baker and Thornberg, 1983).

RESULTS AND DISCUSSION

Twenty samples, from each of the 5 disinfectants used in the hospital (Table 1) were collected from various service units, including the operation theatres, patients' wards, accident/emergency units. A total of 100 reconstituted samples (disinfectants) were collected. The culture analysis revealed that, 20% of the samples were found to be contaminated and the isolated bacterial type/s has been presented in Table 1. Tytler et al. (2006) have reported higher percentage of disinfectants were contaminated when sampled for similar analysis from three different Northern Nigerian hospitals that is Kaduna (52.5%), Kano (67.5%) and Zaria (50%).

The present study indicated that all the diluted disinfectants were contaminated (Table 2) implying that the manufacturers recommended dilution values were not adhered to strictly. Purit was found to be highly contaminated (30%), followed by Dettol (25%), Parazone (20%), Z-germicide (15%), Septol (10%).The contamination levels observed in the present study were higher when compared with that of the hospitals in other countries such as 6.1% Trinidad, 3% in Danish hospitals (Christensen et al., 1982) and 7.9% in Malaysian hospitals (Keah et al.,1995). In the previous studies, a rate of 34.4% has been recorded in some health centers in Ilorin, Nigeria

(Olayemi et al., 1994) and 43% in Japan (Oie et al., 1996). The fact that all the stock solutions of the disinfectants were not contaminated implied that the contamination arose probably during dilution or use. Various reports in the scientific literature have linked the contamination of disinfectants in the hospital environment to sub-optimal sanitary practices during preparation and distribution of these biocides (Ojajarvi, 1980). Some residual amounts of antiseptics and disinfectants found in the hospital environment could contribute to the selection and maintenance of multiresistant strains. Several researches have cautioned that when comparing the frequency of conta-mination, one should always consider the types and concentration of disinfectants since resistance are known to vary in different microorganisms (Russel and Chopra, 1996).

The total bacterial count (TBC) ranged from 7.5×10^5 to 6.3×10^8. Other scholars had reported a range of 10^2-10^8 cfu/ml (Oie and Samiya, 1996 and Zembizuska-Sadkowska, 1995). The implication of these high bacterial colony counts in the samples is that there is a likelihood of attaining an infective dose at the site of application of an antiseptic and or establishing an infection. Such risks would be more evident when the disinfectants are used in the wards where compromised patients live, because the resulting nosocomial infections would be disastrous (Wishart and Riley, 1976). Hand washing with a skin disinfectant that is contaminated is also dangerous for the staff themselves.

Among the disinfectants purit, whose main constituents is chlorohexidene gluconate was the most contaminated, with 30% of the samples yielding bacterial growth. Since preparation of the in-use dilutions of the disinfectants were done in a similar way, it would be thought that the others, especially septol with only 10% and z-germicide with 15% would be contaminated at the same level. The observed differences may be due to the low concentration of chlorohexidine in the formulation of purit. Some outbreaks of *Pseudomonas maltophilia* infections associated with contaminated solutions of chlorohexidine gluconate and cetrimonium bromides have been reported in some Australian hospitals (Wishart and Riley, 1976).

The manufacturers of dettol, purit and parozone recommended the use of these biocides in skin disinfection. The high level of contamination poses serious problems in patients with open wounds. All the concentrated solutions of the disinfectants did not allow the growth of any bacteria. This may be partly due to the high concentration of the active ingredients in the undiluted portions and partly because they are not exposed to potential environmental contaminants. On periodic inspections, it was observed that instead of preparing the diluted portions in the pharmacy, concentrated solutions were sent to the wards and dilution done by unqualified ward Attendants with little or no supervision by the staff nurses. The use of inappropriate types of water and over dilution of portions of the disinfectants were the main contributory factors for the contamination. The water of variable sources that were used to dilute the disinfectants often contains dissolved impurities and other organic substances that might interfere with the efficacy of the disinfectants. Additionally, the storage of diluted disinfectant solution in large containers for longer periods may lead to the observed levels of bacterial contamination. (Maurer, 1969) reported that concentration of disinfectants which were effective during the first 24 h period were not always effective when thereafter.

In this study, the predominantly isolated microorganisms were *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa.* Similar findings of the predominance of these bacteria in disinfectant and antiseptic solutions and the different strains exhibiting variable resistance to disinfectants and antiseptics have been reported earlier (Keah et al., 1995). Other strains like *Pseudomonas mirabilis* and *E. coli* have also been isolated and linked to nosocomial outbreaks (Keah et al., 1995). Amongst the major contaminants of their disinfectants, Tytler et al. (2006) reported that Gram-negative bacteria constituted 69% of the microbial contaminants and *E. coli* remains the most predominant one.

The antimicrobial properties of dettol (chloroxylenol) purit (chlorohexidine gluconate) parozone (sodium hypochloride) z-germicide (tar acid phenol) and Septol (5-chloro2-hydroy diphenyl methane) have been described by several authors including Baldry (1983). The mechanism of action of disinfectant or antiseptic on the micro-organism remains the same irrespective of the type and is exerted through the penetration into the cell and action at the target site(s). The latter can produce a significant effect on the viability as most of the biocides appear to act through intra-cellular mechanism (Russell and Chopra, 1996). The sensitivity or resistance at the level of the bacterial cell membrane, therefore, can be very important factor in determining the final outcome of the treatment with the proposed disinfectant in the hospital practice. Some of these disinfectants also work by production of destructive chemicals against various pathogenic bacterial to attack membrane lipids, DNA and other essential cell components (Rutala, 1996).

The effectiveness of disinfectants in controlling nosocomial infection is often compromised by the fact that many of the disinfectants used in hospitals have been reported to be contaminated with organisms during the preparation processes (Burden and Whitby, 1967). The MIC and MBC values of the disinfectants against the organisms are shown in Table 2. Most antimicrobial agents show both inhibitory and lethal effects depending on the concentration used and other factors such as degree of contamination and duration of treatment. The MIC is a helpful parameter used to assess the bacteriostatic activity of a given disinfectant, while the MBC is used to detect bactericidal activity under similar conditions. The MIC and MBC values of dettol, purit, parazone z-germicide and septol obtained in this study showed that concentra-

Table 3. Antibacterial susceptibility of the isolates.

S/No.	Disinfectant	Bacteria	AMP	COT	GEN	NAL	COL	NIT	STR	TET	ERT	CHL	CPX
1	Dettol	*E. coli*	5	6	11	2	5	3	12	5	7	11	13
		S. aureus	6	4	3	6	7	8	10	4	4	5	12
		P. aeruginosa	4	5	5	6	4	5	7	5	6	6	7
2	Purit	*P. mirabilis*	7	10	7	8	7	6	8	7	6	8	9
		E. coli	5	8	6	9	4	5	7	8	6	9	8
		S. aureus	8	10	15	4	7	9	13	12	10	11	9
3	Parozone	*P. aeruginosa*	5	4	6	8	4	5	4	6	7	4	6
		P. mirabilis	9	6	7	9	12	7	9	8	11	9	10
		S. aureus	9	5	6	2	8	4	5	5	7	9	13
4	Zgermicide	*E. coli*	8	9	8	7	6	3	4	4	4	5	7
		S. aureus	8	7	8	5	7	5	11	13	5	8	11
5	Septol	*P. aeruginosa*	4	6	7	4	5	4	3	6	4	6	6
		S. aureus	5	8	14	3	6	5	15	5	13	11	8

AMP = Ampicilin 25 mg; COT = Cotrimoxazole 25 mg; GEN = Gentamcin 10 mg; NAL = Nalidixic acid 30 mg; COL = Colistin 25 mg; NIT = Nitrofurantoin 20 mg; STR = Streptomycin 25 mg; TET = Tetracyclin 25 mg; CHL = Cloramphenicol 25 mg; CPX = Ciprofloxaxin = 10 mg

tion of the active ingredients in the recommended dilutions of the disinfectants is lethal to the organisms. The MIC and MBC values are more than manufacture's recommended values. The relationship between the MIC and the content of the disinfectant is considered to be a useful property of the agents. The MIC of the disinfectants against the organisms was lesser as compared with the MBC values and increased with increase in the concentration of the agent used. Ashley (1983), who studied the effect of two mouths washes that is chlorohexidine and hexidine against some buccal organisms, reported similar findings that MIC was lower than the MBC for these two preparations. The MIC of dettol against *E. coli* was (0.10 ml) and MBC (0.14 ml), the MIC of purit against *S. aureus* was (0.08 ml) and MBC (0.14 ml), the MIC of parazone against *P. aeruginosa* was (0.08 ml) and MBC (0.12 ml), the MIC of z-germicide against *S. aureus* was (0.12 ml) and MBC (0.14 ml), the MIC of septol against *S. aureus* was (0.10 ml) and MBC (0.14 ml). Unlike the antibiotics, increase in the MIC of biocides does not necessarily correlate with therapeutic failure. Issues such as the pleiotropic action of most biocides, bactericidal activity, concentrations used in the products, direct product application and formulation must be considered in evaluating the clinical implications of such observations. Increased resistance to antiseptics and disinfectants have been associated to mutation and or presence of plasmids (Candal and Eagon, 1984, Kaulfers et al., 1987) and both have been observed in some strains of *S. aureus* (Sasatsu et al., 1995,) *P. aeruginosa* (Sulton and Jacoby, 1978), *Proteus* spp. (Stickler et al., 1983) and *E. coli* (Roussou and Rowbury, 1984). Susatsu et al. (1994) have described high-level of resistant strain of *S. aureus* for which the MICs of chlorohexidine, CTAB and butylparaben were the same. Irizarryi et al. (1996) compared the susceptibility of methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* MSSA. On the basis of MIC, it was reported that MRSA strains were four times more resistant to chlorohexidine and five times more resistant to QACs than MSSA strains.

The results of susceptibility tests of the isolates to the commonly prescribed antibiotics are presented in Table 3. Out of the eleven antibiotics tested, *E. coli* was susceptible to four (chloramphenicol, gentamycin, streptomycin and tetracycline) antibiotics and resistant to six. *S. aureus* was susceptible to five (nitrofurantoin, tetracycline, gentamycin, ciprofloxacin, and streptomycin) antibiotics and resistant to six. *P. aeruginosa* was susceptible to six (gentamycin, tetracycline, erythromycin, ampicilin, cotrimoxazole, chloramphenicol) antibiotics. Some commonly prescribed antibiotics in the hospital such as tetracycline inhibited the growth of all the isolates but colistin has no inhibitory effects on any of the isolates investigated in this study.

In conclusion, this study showed that some of the disinfectants' dilutions used in this referral hospital had sub-optimal concentrations and were contaminated. The use of concentration of disinfectant lower than that quoted by the manufactures might have serious consequences in the post-traumatic or post-surgical managements of the patients in the tertiary care referral hospitals. Further, the use of sub-optimal concentrations might lead to the development of resistant and virulent strains. As a part of the 'good hospital practice', an utmost care should be taken to use the optimal concentrations of the disinfectants to reduce the incidence of the 'hospital acquired infections' for the better patients' management and survival.

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