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Microbial contamination of disinfectant solutions in some health care institutions of three towns in Northern Nigeria

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Abstract

Commonly used disinfectants in some health institutions in three major towns of northern Nigeria were examined for presence of bacteria contamination. For each disinfectant, stock, freshly diluted and left-over of used diluted samples were analyzed. All the stock samples were free of bacteria contaminants while 52.17% of hospital freshly diluted and the left over samples had bacteria contents ranging from $3.1x10^1$ - $1.6x10^4$cfu ml^{-1}. Most commonly isolated bacterial contaminants were *E. coli, Ps. aeruginosa*, and *Staph. aureus*. Other organisms also isolated were *Proteus* spp. and *B. subtilis*. Antimicrobial susceptibility studies showed that 90% of the isolates were relatively resistant to chlorhexidine gluconate.

Keywords: Microbial contamination levels; in-use disinfectants

Introduction

Disinfectants which are a diverse group of chemical biocides were introduced with the aim of breaking the chain of infection in homes, food industries and hospitals (Bean, 1967). Hospitals tend to have a high concentration of pathogenic microorganisms brought in mostly by out-patients and in-patients. As a result of this, and coupled with the fact that most inmates are often immuno-compromised, hospitals have come to be recognized as places for easy acquisition of infection (Gaynes, 1998). Hospitals are thus generally associated with higher levels and greater variety of pathogenic organisms (French, 1996). Several reports indicate that a large number of patients acquire infections while on admission through contact with other patients and health professionals, contact with inanimate objects or environment (Sarangi and Rowsell, 1995). Disinfectants and antiseptics have since their introduction, come to be relied upon to prevent or reduce the incidences of hospital-acquired (nosocomial) infections (Rutala and Cole, 1984; Kawana *et al.*, 2002). Due to the high infection potential in hospitals, there is greater use of disinfectants in hospitals to curtail infection transmission.

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 (Burdon and Whitby, 1967; Lilly and Lowbury, 1971; Sogbetan *et al.*,

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1980; Kahan *et al.*, 1984; Gosden and Norman, 1985). In some instances, instead of preventing transmission, hospital use disinfectants have themselves being the vehicle of transmission with fatal consequences (Bassett, 1971).

Some reports have shown that contamination of disinfectants have often arisen from vehicle used during disinfectants dilution, non-adherence to proper techniques in their use, poor personal hygiene, re-use and improper storage (Basset *et al.*, 1970). In Nigeria, Famurewa *et al.* (1996) had also reported on the incidence of microbial contamination of disinfectants in several hospitals located in the south western part of the country. The prevalence of such microbial contamination of disinfectants in health institutions in the northern parts of the country is however not widely reported. This study, therefore, sets out to assess the contamination levels of commonly used disinfectants in health care institutions of three towns in the northern part of Nigeria, with a view of assessing the probable sources of such contamination and recommending preventive measures towards minimizing their occurrence.

Experimental

Culture Media. Various bacteriological media such as Nutrient agar, Nutrient broth, MacConkey agar, Mannitol salt agar, Azide blood agar base, Brain heart infusion broth, Methyl red, Voges-Proskauer and Koser citrate media (all from Oxoid England) and Peptone water (Biotec Laboratories, UK) were used. The dehydrated media were reconstituted with distilled water according to the manufacturer's directive and sterilized by autoclaving at 121°C for 15 minutes. Reagents such as Kovac's reagent, 40% Potassium hydroxide (KOH) solution and 0.04% methyl red solution were used for identification as required.

Collection of samples. Forty (40) milliliters of each stock and the diluted disinfectants (made up of ten brands) were collected from fourteen health institutions located in Kano, Kaduna and Zaria towns of northern Nigeria. The disinfectants sampled were Tiscol Germicide® (TG), Savlon® (S), Tiscol® (T), Z- Germicide® (ZG), Purit® (P), Dettol® (D), Morigad® (M), Hibiscrub® (H), Harpic® (Ha) and Eusol (E). Health institutions in which the disinfectants were sampled comprised 3 University Teaching Hospitals (UTH), 2 University Health Centers (UHC), 2 State Government Hospitals (SGH), 1 Orthopedic Hospital (OH), one Industrial Clinic (IC) and five privately owned clinics (PH).

Microbiological Examinations. The sampled disinfectants were evaluated for their microbial counts and types.

Viable count. The pour plate method was generally used. For phenolic disinfectants, 1ml of the undiluted disinfectant was introduced into 20ml of molten agar at 45°C, gently shaken to ensure distribution and plated out, allowed to solidify, and incubated inverted at 37°C for 24hours. For disinfectants containing chlorhexidine gluconate and cetrimide, 10mls of the disinfectant sample were filtered through a 0.45millipore membrane filter rinsed with two 5ml portions of sterile water and then transferred aseptically to 20ml solidified nutrient agar and incubated at 37°C for 24hours. The mean of the counts in two different plates of the same dilution was taken for each sample and the number of colony forming units (cfu)/ml calculated.

Identification of isolated organisms. The isolates from the diluted biocides were identified based on their morphological characteristics in different diagnostic media and outcomes of their biochemical reactions as described by Hugo & Russell (1987).

Determination of minimum inhibitory concentration. The minimum inhibitory concentration (MIC) of some isolates against chlorhexidine digluconate (Zeneca, UK) was determined by the broth dilution method as described by Sahm and Washington (1990). The MICs were compared with the values obtained for *Ps. aeruginosa* NCTC 6750 and *B. subtilis* NCTC 10342 which were used as reference organisms.

Results

Analysis of the sampled disinfectants showed that most of the brands were used in two or more of the sampled health institutions, Savlon® being the most widely used of all the brands. Dettol®, Harpic®, Hibiscrub® and Tiscol-Germicide® were not widely used. Of a total of 104 samples analyzed 57.69% were contaminated. The breakdown showed that the percentage of contaminated samples in the three towns; Kaduna, Kano and Zaria were 52.5%, 67.5% and 50% respectively. However, all the stock (undiluted) disinfectants solutions were free of microbial contaminants. All the sampled diluted solutions of Dettol® solutions were contaminated (Table 1) with Eusol® and Morigard® hospital diluted solutions having the least percentage of contamination levels. Savlon®, which was the most widely used disinfectant, has 68% of its diluted solution contaminated. Table 2 shows that 52.17% of the diluted solutions were contaminated to varying levels with microbial load ranging from 3.1×10^1 to 1.6×10^4 cfu/ml. Varying levels of contamination was also observed with diluted samples from different collection centers, the highest contamination levels being in samples taken from the Teaching Hospitals. Interestingly, the nine samples taken from one of the private hospital were without contamination. The result presented in Table 3, which showed that the diluted and used disinfectant solutions were generally contaminated across the various hospitals implying that the used dilutions were most probably below the manufacturer's recommended dilution values.

Table 1: Contamination Quotients of disinfectant solutions in some health care institutions in Kaduna, Kano and Zaria.

Source	SAMPLES										
	D	E	H	Ha	M	P	S	T	T-G	Z-G	Total
Kaduna											
IC			4(5)*		1(3)	3(5)	2(3)				10(16)
PH1		1(3)					2(3)				3(6)
PH2		0(3)			0(3)		0(3)				0(9)
GH1	3(3)										3(3)
UTH1						2(3)	3(3)				5(6)
Kano											
OH		3(3)					2(3)			2(2)	7(8)
PH3							3(4)				3(4)
PH4					3(3)					2(4)	5(7)
UHC1					2(3)		3(3)			1(4)	6(9)
UTH2				2(4)	1(3)					3(5)	6(12)
Zaria											
PH5							2(3)			1(3)	3(6)
GH2						2(3)				1(3)	3(6)
UHC2						0(3)		2(3)			2(6)
UTH2									2(3)	2(3)	4(6)
Total	3(3)	4(9)	4(5)	2(4)	7(15)	7(14)	17(25)	2(3)	2(3)	10(24)	60(104)

* value in parenthesis indicates the number of samples analyzed.

Table 2: Contamination Quotient and levels of Disinfectant Solutions Sampled from Health-care Establishments in Kaduna, Kano and Zaria.

Sample Source	Positive Samples	Contamination level (mean)
Kaduna		
IC	10(16)*	$7.9\pm0.6\times10^{1}$
PH1	3(6)	$1.8\pm0.65\times10^{2}$
PH2	0(9)	-
GH1	3(3)	$5.5\pm0.7\times10^{2}$
UTH1	5(6)	$3.7\pm0.14\times10^{3}$
Kano		
OH	7(8)	$4.2\pm0.9\times10^{2}$
PH3	3(4)	$1.9\pm0.2\times10^{2}$
PH4	5(7)	$2.2\pm0.14\times10^{2}$
UHC1	6(9)	$4.8\pm0.9\times10^{2}$
UTH2	6(12)	$4.1\pm0.6\times10^{3}$
Zaria		
PH5	3(6)	$1.3\pm0.9\times10^{2}$
GH2	3(6)	$3.1\pm0.7\times10^{1}$
UHC2	2(6)	$6.3\pm1.2\times10^{2}$
UTH2	4(6)	$1.6\pm0.6\times10^{4}$

- values in parenthesis indicates the number of samples analyzed

Table 3: Contamination level of Disinfectant Samples from Health institutions in Zaria

	Viable count (cfu/ml)							
	UHC		UTH		GH		MH	
	Tiscol	Purit	Tiscol Germicide	Tiscol	Z-Germicide	Purit	Z-Germicide	Savlon
Stock	0	0	0	0	0	0	0	0
Dilute	5.1×10^{2}	0	3.1×10^{2}	3.1×10^{2}	0	2.2×10^{1}	0	4.4×10^{1}
Used	7.4×10^{2}	0	1.83×10^{2}	6.1×10^{4}	3.3×10^{1}	3.9×10^{1}	2.6×10^{2}	6.1×10^{4}
Recommended concn. (v/v)	3.3%	1.8%	0.2%	3.3%	0.08%	1.8%	0.08%	1.8%
Used concn. (v/v)	0.5%	5.0%	0.13%	2.0%	0.2%	0.2%	0.5%	1.0%

Table 4: Distribution of bacterial Contaminants in Sampled Disinfectant solutions

Organism	Number of Isolates			
	Kaduna	Kano	Zaria	Total
E. coli	7	8	4	19 (28.36%)
Ps. aeruginosa	6	5	5	16 (23.88%)
Staph. aureus	7	8	-	15 (22.39%)
Proteus sp.	4	3	4	11 (16.42%)
Bacillus subtilis	-	3	3	6 (8.95%)
Total	24	27	16	67 (100%)

Table 5: MIC of Chlorhexidine gluconate against selected bacteria isolates from the contaminated disinfectant solutions from health care establishments in Kaduna, Kano and Zaria.

Organism (no.)	Minimum Inhibitory Concentration (µg/ml)		
	25	12.5	6.25
E. coli (2)		2	
Ps. aeruginosa. (3)	1	2	
Proteus sp (1)		1	
Staph. aureus.(2)		2	
Bacillus subtilis (2)		1	1
Total (10)	1	8	1

MIC of reference organisms = 6.25µg/ml

This was found to be true in the health institutions sampled in Zaria. Table 4 shows the number and types of the bacterial contaminants isolated from the disinfectant solutions. The gram negative organisms constituted 69% of the microbial contaminants with *E. coli* being the most frequently isolated organism. Analysis of the susceptibility profiles of some of the isolated organisms using the MIC values showed that a high percentage of the isolates (90%) were resistant to chlorhexidine gluconate.

Discussion

The result of this study indicates that a high proportion of dilutions of disinfectants/antiseptics used in health institutions in the three towns are contaminated. This agrees with the findings of a similar work carried out in the south west of the country (Olayemi and Obayan, 1995; Famurewa *et al,* 1996; Ogunsola *et al*, 2002) and in other countries (Cravens *et al,* 1981; Baird, 1981; Keah *et al* 1995; Gajadhar *et al,* 2003).

The fact that all the stock (undiluted) disinfectant samples were free of contamination while the diluted samples were contaminated (irrespective of brand and hospital) implied that the contamination arose most probably during dilution or use. As the analysis of results of samples collected from Zaria showed, contamination most probably arose from over dilution of the stock disinfectant in most of the health institutions. Similar finding was reported by Prince and Ayliffe (1972) who found that 50.5% of the 105 phenolic disinfectant samples analysed were below recommended strength. The use of concentrations of disinfectants lower than the manufacturer's recommended value is fraught with serious consequences, as shown in this study. Users of products must as of necessity, adhere to the manufacturers' recommendation on use of their products, as such recommendations are usually based on carefully considered factors. The use of concentrations below those recommended by manufacturers which could be sub-inhibitory will in the long term, lead to the selection of resistant strains with its dire consequences.

Use of inappropriate diluents such as tap, and other sources of water instead of freshly boiled, distilled or de-ionized water may in itself be a source of microbial contamination of disinfectant solutions. Preparation and storage of diluted disinfectant solutions in large containers may lead to considerable amounts of left-over and the temptation of topping up. Maurer (1969) reported that concentrations of disinfectants solutions which were effective during the first 24hr period were not always effective after being in use for several days. The isolation of *E. coli*, Pseudomonas and Proteus organisms from the disinfectant solution lends support to the assertion that use of impure water as diluents might have been the major sources of contamination of the diluted disinfectant solutions although poor level of hygiene of operators cannot be ruled out

In conclusion, this study shows that a fair number of disinfectant solutions used in health care centers in the towns sampled were contaminated. The reason for contamination observed can be linked to the use of inadequate dilution levels and the use of inappropriate diluents and poor handling of such diluted disinfectant solutions.

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