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Contamination levels of in-use disinfectants in a teaching hospital in Lagos, Nigeria

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Summary
In-use testing of the disinfectants; Hibitane (5% w/v Chlorhexidine gluconate), Hibiscrub (4% w/v Chlorhexidine gluconate), Savlon (3% w/v Chlorhexidine /Cetrimide), hydrogen peroxide (6% w/v hydrogen peroxide with stabilizer) and a common household bleach Jik (3.5% w/v sodium hypochlorite), was carried out over a two-month period at a university teaching hospital in Nigeria. Contamination levels were high with 82 (63.1%) of the 130 in-use disinfectants contaminated. However, a few of the stock solutions remained sterile. One hundred and thirty-four isolates were obtained of which 120 (91%) were gram–negative with *Pseudomonas* species being the commonest, constituting 67.2% of all the isolates. Gram-positive organisms made up the remaining 12 (9.0%) isolates. All the *Pseudomonas* spp. were resistant to gentamicin, ceftazidime, nalidixic acid and perfloxacin. Contributory factors for the high contamination levels were dilution of disinfectants with tap water, inadequate care of stock solution bottles and long storage of the diluted disinfectants in the wards.

Keywords: *Disinfectants, in-use, contamination*

Résumé
L'essai pratique des désinfectants Habitera (5% w/v Chlorhexidire gluconate), Hibiscrub (4% w/v Chlorhexidire / centrimide), hydrogène peroxyde plus stabilisateur) et l'eau de Javel de marque Jik (3,5% w/v sodium hydrochlorite), était mené pendant une période de deux mois dans un centre hospitalier universitaire au Nigeria. Les niveaux de contamination étaient très élevés par 82 (63,1%) parmi les 130 désinfectants contaminé utilisé. Pourtant, quelque solutions reste stérile cent trente-quatre isolés sont obtenus parmi lesquels 120 (91%) sont gramme négatif avec les espèces pseudomonas faisant partie de plus commun, constituant & 67,2% de tous les isolés. Tous les spp pseudomonas résistent au gentamicin, ceftazidine, acide nalidixic of perfloxacin. Les facteurs contributives au niveau élevé de contamination étaient la dilution des désinfectant avec l'eau de robinet, la maintenance inadéquate des bouteilles de solution stockée et l'entreposage des désinfectants dilués dans des salles.

Introduction

Despite improvements in surgical and medical techniques, hospital-acquired infections still remain one of the main causes of morbidity and mortality [1]. It has led directly or indirectly to an enormous increase in the cost of hospital care and the emergence of new pathogens that can be transmitted by a variety of routes [1,2,3,4]. Chemicals have been used to reduce the microbial load on various medical and surgical devices that cannot withstand the heat of sterilization [5].

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To use disinfectants, a thorough understanding of the factors that enhance and limit their effectiveness is required. Adequate disinfection is directly related to the contact time, temperature, concentration and type of the disinfectant [1]. It has been previously shown that storage of dilute disinfectants and incorrect dilution, have resulted in inadequate disinfection [6]. They have been implicated as occasional vehicles of hospital infections and pseudo-epidemics resulting primarily from disinfection failures [7,8,9,10,11]

This study was therefore carried out to assess the level of in-use contamination of disinfectants used in the hospital and identify the possible factors contributing to their contamination.

Materials and methods

Sixty-five stock solutions (used as controls) and 130 samples of in-use disinfectant solutions were collected over a two-month period (June 1st to July 31st 1999) from the shelves, galley pots or discard jars in different departments, wards, and clinics at the hospital.

These departments included the Pharmacy, Paediatric, Surgical, Medical, Obstetric and Gynecological wards (Obgyn). Others were the Guinness Eye Center (G.E.C), Modular theaters, Ear, Nose and Throat (E.N.T) and the family planning clinics (F.P.C). Two stock solutions each of 5% w/v Chlorhexidine gluconate (Hibitane), 4% w/v Chlorhexidine gluconate (Hibiscrub), 3% w/v Chlorhexidine/cetrimide (Savlon), 6% w/v hydrogen peroxide with stabilizer (hydrogen peroxide) and 3.5% w/v sodium hypochlorite (JIK, a proprietary household bleach) as well as two samples each of boiled, cooled water and plain tap water (normally used as diluent for the disinfectants), were also used as controls.

The names of the disinfectants, dilution factors (if applicable), date of collection, length of storage and site of sample collection, were recorded and labeled neatly on each sample bottle.

Transportation of disinfectant

About 1ml of each of disinfectant solution was transferred with a sterile pipette from a discard jar, galley pot, or stock solution bottle, into a sterile universal bottle, containing 9ml of sterile tween 80-broth (diluent). The inner surface of each stock solution bottle was swabbed with sterile swab-sticks. All samples were immediately transported to the laboratory where they were processed within 1hr of collection. All the disinfectants were processed using a modification of the Kelsey and Maurer method [12] while the swabs were cultured on nutrient agar (Oxoid).

Bacteriological testing of water

About 100ml each of the water (boiled and unboiled) used as diluent were collected in sterile bottles and transported immediately to the laboratory and processed using the membrane filter method described by Milipore [13].

Table 1: Contamination levels of disinfectants

Disinfectant	Dilution factor	Stock	Contamination (%)	In-use	Contamination (%)	Organisms
Hibitane	NA	2	0			
	1:200	5	3(60)	11	7(63.3)	Ps, Pr. Eco, Staph
	1:250	5	3(60)	10	8 (80)	Ps, B, Eco, Staph
	1:500	5	3(60)	11	9(81.8)	Ps, Pr. Eco K, B.
	1:1000	5	3(60)	11	9(81.8)	Ps, Pr, Eco Staph, En,K
	1:2000	5	4(80)	15	13(86.7)	Ps, Pr. Eco, Staph, En,K,B
Hibiscrub	NA	12	5(41.7)	20	9 (45)	Ps, B, Pr, Staph
Hydrogen peroxide	NA	11	7(63.3)	22	14(63.6)	Ps, Pr, Staph, En, K,B
Salvon	NA	13	5(38.5)	25	10 (40)	Ps, Pr, K, B.
JIK	NA	2	0	-	-	
	1:10			5	3 (60)	Ps, Pr, Eco, Staph, B
Total		65	33(50.8)	130	82(63.1)	

Key: Ps, Ps aeruginosa; Pr, Proteus mirabilis; Eco, Ecoli; Staph, Staphylococci :spp.: B,Bacillus spp.: K. kk, aerognes; Enb, Enterobacter spp.

Culture of disinectants

A small quantity of the disinfectant/tween 80 broth mixture was withdrawn from the sterile universal bottle with separate sterile 50 dropper pipettes and 5 drops of the broth mixture placed on the surface of two well dried sterile nutrient agar plates. One of the plates was incubated at 37°C and the other at room temperature, overnight. Plates with no growth were further incubated for 72 hours before being discarded. The number of live organisms / ml was determined from the nature of growth and number of colonies counted. The formula shown below was used to quantify the bacterial load of each sample solution.

Organisms/ml of solution = no. of colonies counted/ diluted factors.

Interpretation of results

a) Counts of 4 colonies or less from either or both plates indicated that the disinfectant was effective.
b) Counts of 5 colonies on either or both plates suggested the presence of about 500 cfu/ml of the solution, and implied inadequate disinfection.
c) Counts of more than 5 colonies or growth of non-discrete colonies on either or both plates indicated heavy contamination and a general failure of disinfectant activity

Identification

Organisms isolated from the primary culture plates were subcultured into blood agar (Blood agar base 2, (Oxoid) + 5% human blood) and MacConkey agar (Oxoid) and incubated at 37°C in air, overnight. All isolates were identified by standard microbiological methods (ASM). *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-29213) and *Psuedomonas aeruginosa* (ATCC-27853) were used as controls for the experiments.

Antibiotic susceptibility testing

Antibiotic susceptibility tests were carried out on all the *Pseudomonas spp.* because they were the most frequently isolated organisms and had the most significant growth in this study. The Kirby Bauer disk diffusion method [14] was employed, using ceftazidime, gentamicin, nalidixic acid and perfloxacin antibiotic disks, respectively and the interpretation carried out according to NCCLS (1993) [15].

Statistical analysis

The chi-squared test and correlation coefficient was used to determine the significance of the results.

Results

There was a statistically significant difference in contamination levels ($P>0.01$) among the various in-use disinfectant solutions (Table I). Hibitane (diluted) was the most widely used and most often contaminated disinfectant, while Savlon and Hibiscrub were the least contaminated. There was no statistically significant difference observed in contamination levels of the different dilutions of in-use Hibitane, as all were heavily contaminated.

Table 2: Effect of storage time on levels of contamination

Length of storage (hrs)	No of samples examined	Samples contaminated (%)
1 - 24	38	14 (36.8%)
24 - 48	41	27 (65.9%)
> 48	51	41 (80.4%)
Total	130	82 (63.1%)

There was a direct correlation ($r = +0.9$) between the duration of storage of disinfectant solutions on ward shelves and their levels of contamination (table 2) and most of the in-use disinfectants were heavily contaminated after 48 hours. On the other hand, there was no significant difference ($P > 0.01$) between contamination levels of the different types of disinfectants sampled from different units. Stock solutions in the wards (pre-diluted in the pharmacy) were also mostly contaminated though a few remained consistently sterile (Table 3).

About 91.0% of isolates were gram-negative bacilli, with *Pseudomonas* species constituting 67.2% of all isolates. In 15.2% of disinfectants, contamination was polymicrobial while gram-positive isolates were recovered from only 9.0% of contaminated disinfectants (Table 4).

Table 3: Contamination levels in various departments

Departments	Stock samples		In-use samples		Organisms
	Total number	Number contaminated	Total Number	Number contaminated	
Pharmacy	20	7(58.3)	NA	NA	Ps., Prot., E.coli, Staph, Enterob, Ba
Paediatrics	5	3(60)	15	10 (66.7)	Ps., Prot., E.coli, Staph, Ba
Obs and Gynae	5	3(60)	27	17 (63)	Ps. Prot., E.coli, Staph, Ba *Klebsiella aerogenes*
Medicine	8	5(62.5)	17	11 (64.7)	Ps. Prot., Staph, Ba
Guinness Eye Center	7	5(71.4)	18	13 (72.2)	Ps., Prot., E.coli, Staph, Ba, *Klebsiella aerogenes*
Surgery	8	4(50)	25	14 (56.0)	Ps., Prot., Enb, Ba
Accident and emergency	5	3(60)	13	9 (69.2)	Ps., Prot., E.coli, Staph, Enb,
ENT clinic	5	3(60)	10	5 (50)	Ps., Prot., E.coli, Staph, E. coli,
Family Planning Clinic	2	0(0)	5	3 (60)	Ps., Enterobacter, E. coli, Staph, Ba, Klebsiella
Total	65	33 (50.8)	130	82 (63.1)	*aerogenes.*

Key: Ps, *Pseudomonas aeruginosa*; Prot. *Proteus*; Staph, *Staphylococci*, Enterob. *Enterobacter* spp. Ba. *Bacillus* spp.

Table 4: Frequency of bacterial isolates

Total number of isolates (n= 134)			
Gram-negative Isolates	Number (%)	Gram-positive Isolates	Number (%)
Pseudomonas aeruginosa	82(67.2)	*Staphylococcus spp.*	7(58.3)
Proteus mirabilis	20(16.4)	*Bacillus spp.*	5(41.7)
Enterobacter spp.	8 (6.6)		
E. coli	6 (4.9)		
Klebsiella spp.	6 (4.9)		
Total	122 (91)		12(9.0)

Discussion

The disinfectants used in the hospital are prediluted in the pharmacy and it was found that either unboiled or cooled, boiled tap water was used for diluting the disinfectants. The water grew mainly *Pseudomonas spp.* and coliforms and the majority of the diluted disinfectants were contaminated with similar organisms suggesting that the contamination was introduced at the time of dilution. This fact was borne out by the fact that there was no significant difference in contamination levels between wards and that unopened but pre-diluted disinfectants were also contaminated. Most of the diluted disinfectants showed heavy growths after 48hours (two days) of storage, which probably relates to the deterioration and reduction of effectiveness of the diluted disinfectants on prolonged storage. This finding correlates with previous studies [11,16,17]. The lack of a statistically significant difference in contamination levels among the various dilution strengths of hibitane used, also supports the conclusion that the contamination was from the water used as diluent though the effect of prolonged storage as a predisposing factor cannot be ruled out [17]. A third factor that could be associated with the high contamination rates is the observation that stock bottles were not always washed before they were refilled and swabs from inside bottles grew various organisms confirming its role as a possible contaminant. These three factors: prolonged storage of diluted disinfectant, tapwater as diluent and unwashed stock bottles are probably responsible for the high contamination rates.

The most commonly isolated organisms were *Pseudomonas spp. Proteus spp., Klebsiella spp. and Escherichia coli* and coincidentally, these are the organisms most often associated with with nosocomial infections in this hospital [11,18,19,20] The most prevalent isolate was *Pseudomonas aeruginosa,* an organism commonly found growing in diluted disinfectants, which can be attributed to their hardy nature and nutritional versatility [21]. The repeated isolation of *Pseudomonas* species from the different solutions in the various departments poses serious infection risks and disinfectant-associated pseudomonas post-operative wound infections have been previously reported in this hospital [11,19].

It is therefore important to design an effective disinfection policy that will ensure proper management of disinfectants while ensuring regular in-use testing of disinfectants.

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