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Evaluation of hospital disinfection as a means of controlling endemic nosocomial pathogens in a University Teaching Hospital in Nigeria

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Abstract
Evaluation of hospital environment disinfection as a means of controlling endemic nosocomial pathogens in a University Teaching Hospital in Nigeria was evaluated. Disinfectant used in the Hospital was collected from the Infection Control unit and prepared in different concentrations. The isolated bacterial species from the hospital environment were exposed to graded concentrations of the disinfectants and the most effective concentration on each isolate was noted. This procedure was carried out in two successive years (2006 and 2007). Killing rate of the isolates that were resistant to the disinfectants was also carried out and likely effective exposure time was determined. The following bacterial species were isolated: *Staphylococcus epidermidis, Klebsiella Pneumoniae, Klebsiella spp., Bacillus subtilis, Enterobacter spp., Serratia marcescens, Pseudomonas aeruginosa, Pseudomonas spp., Escherichia coli, Serratia spp., Bacillus cereus, Citrobacter freundii, Proteus mirabilis, Staphylococcus aureus, Bacillus megaterium, Streptococcus pyogenes,* and *Streptococcus spp*. Minimum Effective Dilution (MED) of the disinfectant on all isolates ranged from 1:300 to1:1000. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most resistant isolate with MED of 1:400 and 1:300 respectively. Result of killing rate on the two most resistant isolates showed that *Staphylococcus aureus* and *Pseudomonas aeruginosa* required 80 and 120 minutes of exposure respectively to the disinfectant to bring about almost total killing of these resistant isolates. The results show that improper disinfections, degradation of disinfectant and lack of routine standardization of disinfectants are responsible for failure of chemical disinfection as a means of controlling nosocomial infections in the hospital.

Keywords: Nosocomial pathogens; Hospital disinfections; Disinfectants and pathogens resistance.

Introduction

The role of the inanimate hospital environment (e.g., surfaces and equipment) in the spread of nosocomial infection has remained controversial. Although contamination of the inanimate environment by microorganisms has long been recognized, its significance in nosocomial infections still remains unclear. For example, it was noted in one medical center that, the decrease in environmental contamination that occurred after a move to a new hospital was not associated with any change in nosocomial infection rates (Panutti, 1997). According to

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the report of Coza *et al*., 2003, molecular techniques provide the most concrete evidence of transmission of *C. difficile* from environmental surfaces to patients. Based on The molecular analysis of endemic *C. difficile* Sidwell *et al.,* (1967) observed as follows: (1) *C. difficile* was present on the hands of health care workers, (2) there was a correlation between the degree of colonization of health care workers' hands and environmental contamination with *C. difficile,* and (3) there was differential contamination of the environment by individual strain types. Among colonized patients, a single, predominant isolate was found and was more likely to contaminate the environment than were isolates that sporadically colonized patients. This finding was reproduced in a study in which (1) despite endemicity of *C. difficile,* a single genotype predominated in the inanimate environment, and (2) the incidence of *C. difficile* infection correlated well with environmental contamination (Liao and Sapers, 2000). These data suggest that environmental surfaces serve as a reservoir that permits the cross-colonization of patients after they have had contact with a health care worker and that, in environments in which *C. difficile* is endemic, specific isolates likely predominate (Sidwell *et al*., 1967, Liao and Sapers, 2000).

According to current scientific knowledge, microbial contamination of the patient's inanimate environment seems to be only a minor causative factor within the complex nature of nosocomial infection. (Ayliffe and Babb, 1996), Maki and coworkers (Maki *et al.,* 1982) published findings that suggest that microorganisms in the inanimate hospital environment make a negligible contribution to endemic hospital-acquired infection rates, and numerous other studies (Daschner *et al.,* 1980; Danforth *et al.,* 1987 and Dharan *et al.,* 1999) have established that the use of disinfectants does not impact on the incidence of hospital-acquired infections. While some studies (Pittet *et al*., 2000, Boyce, 2002) have shown hygiene (especially hand hygiene) and targeted disinfection regimens to be useful in the eradication of antibiotic-resistant organisms in the hospital, the use of disinfectants for routine surface disinfection is not recommended by any national centers for nosocomial infection control in Europe, and, to our knowledge, not a single study has ever shown that routine use of disinfectants has prevented infections acquired in households (Allerberger *et al.,* 2002).

This work therefore examined role of effective disinfection of the hospital environment as a means of controlling endemic hospital nosocomial pathogens.

Experimental

Determination of susceptibilities of isolates to the disinfectant. The disinfectant commonly used in the University Teaching Hospital studied is Izal. Effect of different concentrations of the disinfectant was measured on isolated bacteria by preparing different concentrations of the disinfectant. By following the manufacturer's instructions, the following concentrations were prepared, 1:300, 1:400, 1:500, 1:600, 1:700, 1:800, 1:900, and 1:1000. The test procedures described by Lamikanra (1989) were followed. One milliliter of organism under test which had been standardized to 10^6cfu/ml was mixed thoroughly with 20ml of molten Mueller-Hintin agar in a sterile Petri dish and allowed to set. A sterile cork borer (8mm in diameter) was then pushed into the agar and agar plugs were removed with a sterile loop. The cup formed were then filled with different concentrations of Izal solution, using a sterile dropping pipette and plates used for all isolate were incubated at 37oC for 24 to 48hours. The plates were observed for presence and absence of zones of inhibition. The inhibition zone diameters were measured and recorded.

Determination of rate of killing by disinfectants on most antibiotics resistant isolates. Rate of killing was carried out on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (been the most resistant isolates found in this work) by using Kelsey and Maurer (1974) method. A 0.5ml of different concentrations of 10^6 cfu/ml of test isolates was mixed with 4.5ml of different concentrations izal solution. A 0.1ml from the mixture was taken with sterile 1ml syringe and needle and plated on dried sterile nutrient agar at time interval of 0, 5, 10, 15, 20, 25 and 30 minutes. The plates were incubated at 37^oC for 24hours, surviving bacteria were counted and logarithms of the survivors was taken and plotted against time.

Determination of effectiveness of disinfections carried out by both the Hospital cleaners and the researcher. Effectiveness of disinfections carried out by the hospital cleaners and the investigator was carried out. This was done by swabbing a squared meter of the hospital floor before disinfection and 1hr after disinfection, by using sterile moistened cotton swab stick. The swab was then inoculated in 9ml peptone water broth, which was then serially diluted up to 10^{-5}. Each dilution was plated on nutrient agar and incubated at 35^oC for 24hrs. The numbers of colonies developed were counted and recorded.

Results

Comparison of dilution ability of the disinfectant is shown on Table 1.0. Decrease in ability of the disinfectant was observed from 2006 to 2007 data but *Strept. pyogenes* still required the same concentration of the disinfectant.

Table 2 showed effectiveness of disinfections made by the hospital cleaners. T- test statistical analysis showed no significant difference before and after disinfection ($p<0.05$). In W1 and W5, bacterial counts before disinfection were $3.14x10^3$ and $2.14x10^2$; these were increased to $3.36x10^3$ and $2.93x10^2$ after disinfections respectively. In other words, there were no significant observable changes in bacterial counts before and after disinfections.

There were significant observable differences ($p>0.05$) in bacterial counts before and after disinfections (Table 3.0). Bacterial loads were drastically reduced after disinfection.

The Fig. 1 showed the killing rate of *S. aureus* as one of the most resistant isolates to the disinfectants. The curve showed that the total killing of the organism can only be affected eighty (80) minutes after exposure to the minimum concentration of the disinfectant. As the number of exposed cells decreased from 10^6 to zero within 80 minutes of exposure.

Effect of Izal on *Ps. aeruginosa* is illustrated on fig 2 From this illustration, it can be observed that the number of surviving cells decreased from 10^6 cfu/ml to zero within 120-140 minutes of exposure to Minimum Effective Concentration of the disinfectant.

Discussion

Hospital environments are abode of pathogenic and antibiotic resistant bacteria. The endemicity of these pathogens in hospital environment has made effective control of hospital acquired infections difficult, therefore chemical disinfection of hospital environment has been one of the major ways of reducing these endemic drug resistant bacterial pathogens in the hospital and hence control of nosocomial infections. However there have been arguments for and against the use of chemical disinfectant as means of controlling nosocomial infection. In the study reported by Daschner (1984), he found that rate of nosocomial infection was 15.6% and 15.5% when detergent solution and chemical disinfectant were respectively used in floor disinfection of the studied hospital.

Table 1 Maximum Dilutions of Izal that Inhibited Growth of the isolates from the Floor of Wards and Theatre of the University Teaching Hospital in 2006 and 2007

Organism	Minimum Effective Dilution of Izal	
	2006	2007
S .epidermidis	1:800	1:500
Kl. pneumoniae	1:800	1:500
Klebsiella spp.	1:800	1:600
B. subtilis	not tested	1:400
Enterobacter spp.	not tested	1:800
Ser. marcescens	not tested	1:900
Ps. aeruginosa	1:500	1:300
Pseudomonas spp.	1:500	1:400
E. coli	1:600	1:700
Serratia spp.	Not tested	1:800
B. cereus	1:700	1:600
Cit. freundii	Not tested	1:700
Pr. mirabilis	1:1000	1:400
S. aureus	1:600	1:400
B. megaterium	Not tested	1:400
Str. pyogenes	1:600	1:600
Strept spp.	1:1000	1:600

Table 2. Average bacterial counts of the Wards and Theatre floors before and after disinfection with Izal by the hospital cleaners.

Wards	Bacterial counts cfu/ml	
	Before	After
W1	$3.14x10^3$	$3.36x10^3$
W2	$3.45x10^2$	$3.19x10^2$
W3	$4.23x10^3$	$4.91x10^2$
W4	$3.84x10^2$	$3.10x10^2$
W5	$2.14x10^2$	$2.93x10^2$
W6	$3.92x10^2$	2.27x102
W7	$4.82x10^2$	$3.98x10^2$
W8	$3.78x10^2$	$2.19x10^2$
Theatre	$2.67x10^2$	$1.34x10^2$

Table 3. Average bacterial counts of the Wards and Theatre floors before and after disinfection with Izal by the Investigators..

Wards	Bacterial counts cfu/ml	
	Before	After
W1	$3.98x10^3$	$2.136x10^2$
W2	$2.995x10^2$	$1.86x10^1$
W3	$3.68x10^3$	$3.90x10^2$
W4	$4.10x10^2$	$3.98x10^1$
W5	$3.18x10^2$	$2.83x10^1$
W6	$3.00x10^2$	$1.48x10^2$
W7	$3.23x10^2$	$3.11x10^1$
W8	$2.88x10^2$	$2.91x10^1$
Theatre	$2.10x10^2$	$1.5x10^1$

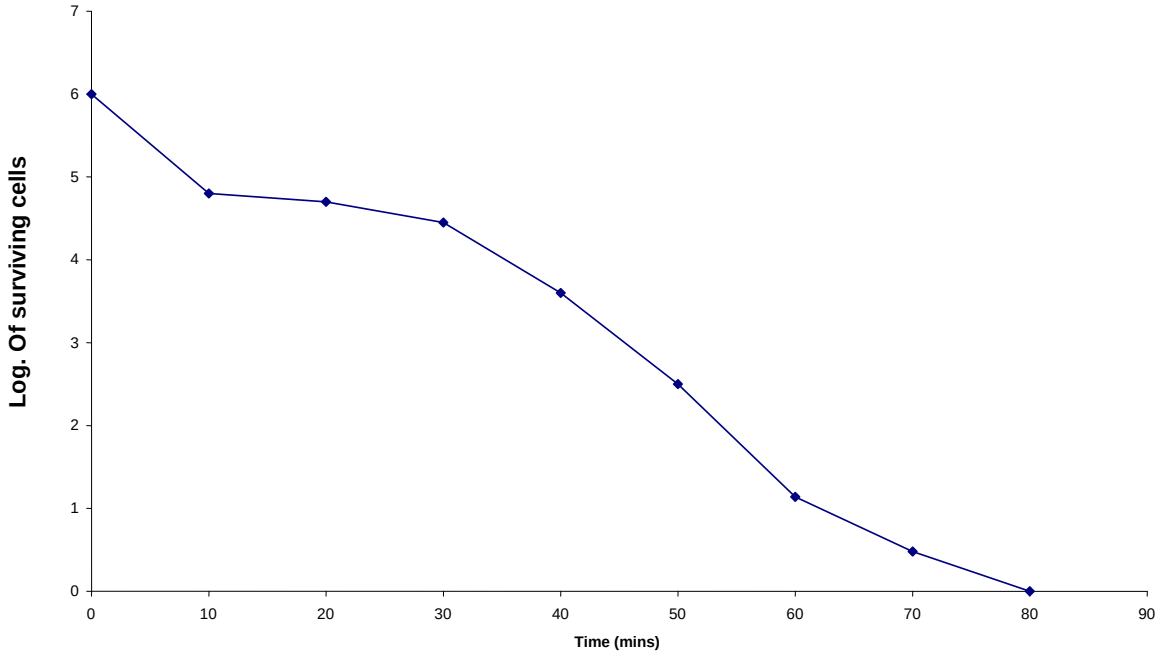

Fig 1.0 Effect of Izal on *S.aureus* (One of the antibiotics and disinfectants most resistant isolates)

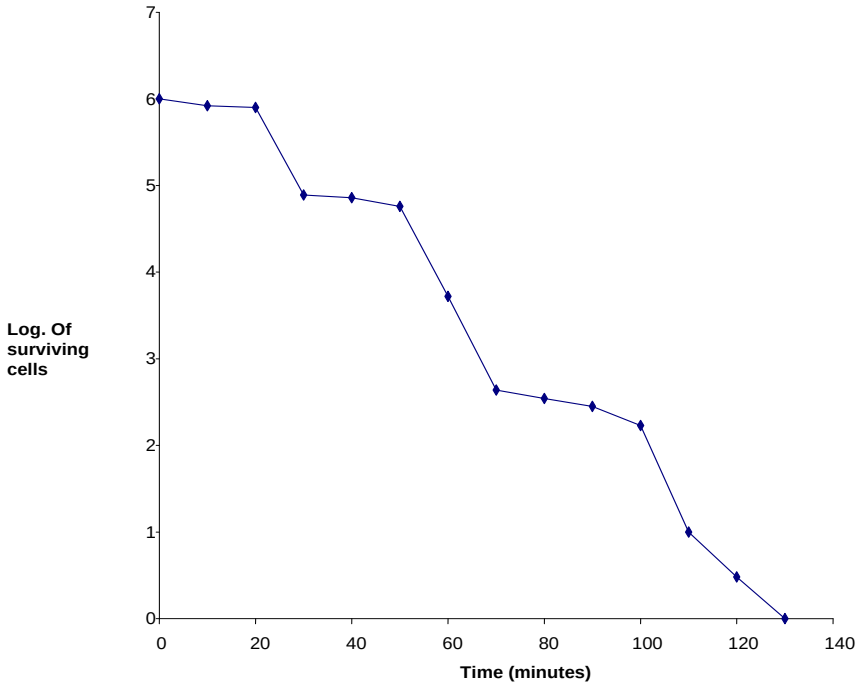

Fig 2.0 Effect of Izal on *Ps.aeruginosa*

He concluded that use of chemical disinfectant did not impact any significant effect in control of nosocomial infections in the hospital.

Franz and Armin, (2004) and other researchers also reported as follow; "disinfection of certain environmental surfaces is in certain instances an established component of hospital infection control, we disagree with the fact that disinfectants provide an incremental public health benefit, as disinfectants may lead to the development of resistance and allergies and because they constitute an environmental load, indiscriminate or excessive use of antibiotics has been widely blamed for the appearance of so-called superbugs. Disinfectants that contain biocidal agents such as quaternary ammonium compounds (quats), as well as triclosan, a widely used potent antibacterial and antifungal agent, which has so far been considered to be harmless. While it was formerly thought that triclosan killed cells only by nonspecific mechanisms, now it is known that triclosan, like antibiotics, can interact with well-defined molecular targets, thus leading to the development of resistance. Researchers warn that bacterial resistance to triclosan is a distinct possibility and that its widespread use may be unwise. Overuse of biocides in an effort to produce a germ-free environment may result in lowered natural immunity to common pathogens and in increased resistance by pathogens to frequently used biocides (Kennedy, 2000; Alielo and Larson (2003); McMurray et al., 1998; Levy, 2000)".

In contrast to the above, Cozad et al., (2003), reviewed reports many researchers and stated that, Quaternary ammonium (quat), iodine, alcohol, aldehyde, organic acid, peroxide, and halogenated compounds have proven effective against a broad spectrum of microorganisms. The disinfection of water, medical devices, food products, fabric/laundry, and hard surfaces in domestic as well as institutional settings has been detailed (Gilbert and Maurer, 1968; Nicholes, 1970; MacCullouch, 1972; Cogan et al., 1999; Neely and Maley, 2000; Nomura *et al.*, 2000) Likewise, the improper use of disinfectants and the limited spectrum of certain germicides have been documented in various studies. The nature of the surface to be disinfected may influence the degree of disinfection that can be achieved. The germicide-surfactant system, germicide concentration, and contact time also can significantly affect antimicrobial activity. Vesley and Michaelsen (1964) showed that phenolic and quat disinfectants killed bacteria on the hospital floor but were not significantly better than detergent and hot water. In contrast, a 6-month hospital study by Kundsin and Walter (1961) showed that use of a phenolic germicide on floors decreased the number of microorganisms in the environment and maintained low levels of organisms in patient and operating rooms. Studies (Krog and Marshall, 1940, Krysinski et al., 1992) in commercial settings showed the reduction of fecal bacteria on drinking glasses by quat disinfection and removal of *Listeria monocytogenes* biofilm from food processing surfaces (e.g. stainless steel and polyester/ polyurethane) by detergent/germicide combinations such as enzyme cleaner plus iodophor, quat, acid anionic or chlorine sanitizer and alkaline cleaner plus quat sanitizer. Similarly, domestic site studies (Josephson and Rubino, 1997) showed reductions in the number of microbially contaminated sites through quat or hypochlorite disinfection.

In this work effectiveness of disinfection of hospital environment in a University teaching hospital in Nigeria was evaluated. The results revealed a number of reasons why disinfections carried out by hospital cleaners most time are not effective.

We found that no written disinfection procedure, a situation that gives room for

improper mixing of the disinfectant and hence non-effective disinfections.

The disinfectant was not standardized. In this study we found that the disinfectant lost potency over the time of study as demonstrated in observed changes in their minimum effective concentration on the test isolates between 2006 and 2007. For this reason, it become necessary that the amount of disinfectants to be used must be standardized against most resistant isolate found within the same hospital environment. This goes to say that assumption that the same concentration of a disinfectant can be used all years round is wrong, and probably explain one of the reasons why disinfection in some of our hospital have not significantly different from using ordinary detergent in the disinfecting the hospital.

Cleaners did not know that mobbing stick must be properly washed and kept dried after use, before keeping for next day work. These facts were observed when disinfection carried out by the investigators and one carried out by the hospital cleaners were compared, where it was found that microbial loads were significantly reduced in disinfection carried out by the investigators when compared with the hospital cleaners. The reason for the difference was that after each day disinfection the investigators properly clean and dried the mobs before keeping in it in its corner. The hospital cleaners did not know that such mops provide good menstrum for microbial proliferation, increasing the microbial bioburden of disinfectants during use and spreading such organisms all over the ward surfaces.

We conclude that chemical disinfection remain one of the most effective ways of reducing nosocomial pathogens in hospital environment as demonstrated in the results of this work. However the use of chemical disinfectants must be done with caution, otherwise using chemical disinfectants may create more problems than solving. The assumption that disinfection of hospital environment is a job for illiterates must change; it should rather be job for properly educated or trainable personnel. There must be written standard procedure for mixing the disinfectants and as well as disinfection proper, this is to ensure that right materials are used in right quantities. From time to time potency of the disinfectant in use must be evaluated in order to keep pace with degradation of the disinfectant with normally occur with time. Adherence to good disinfection practices as enumerated above would reduce environmental microbial levels of the wards, and substantially lower incidences of nosocomial infections.

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