Hygiene Status of Disinfecting Solutions Used in Floor Cleaning of Five Selected Hospitals in Ogun State, South Western, Nigeria

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Abstract: Poorly disinfected hospital floors are likely reservoir of infectious agents. The in use disinfecting solutions and complete disinfection outfit of five hospitals' floors were quantitatively assessed in this study. Bacterial burden of regularly used mop heads and buckets used for wet mopping were determined on nutrient agar by pour plate method. Following disinfectant addition, in use disinfecting solution were examined quantitatively for bacterial bioburden (reception and wards) at 5 and 15min into cleaning. Isolation of bacteria was done using Centrimide, Eosine methylene blue, Xylose lysine deoxycholate and Mannitol salt agar. Identification was by Standard biochemical tests. The mean and standard deviations for bacterial burden in the wards and the reception mops ranged from $1.09 \times 10^2 \pm 62.7$ to $2.51 \times 10^4 \pm 2.3 \times 10^4$ and 64.7 ± 20.1 to $7.9 \times 10^3 \pm 30.6$ respectively. A reduction in bacterial count ranging from 85% to 96% in wards and 66% to 100% in reception sites of studied hospitals was observed 5minutes into disinfectant addition. Counts at 15min were generally higher in comparison to counts at 5min but remained constant at Hospital A. Decontamination of the cleaning materials caused a substantial decline in counts at all sites, significant at P=0.05. *Staphylocccus aureus* was isolated from four of the sites and other Gram negative bacteria (*Serratia, Citrobacter, Escherichia, Enterobacter, Salmonella, Pseudomonas*) were widely distributed, indicative of hygiene failure.
Key words: Disinfectant, *Staphylococcus aureus,* Bacterial burden, Hospital cleanliness.

INTRODUCTION

Recent decades has witnessed concerted efforts in researches geared towards proper understanding of the factors that drive the spread of hospital acquired infections (HAIs) (Otter *et al*., 2011; Boguz, 2013). The contribution of environmental surfaces is increasingly recognized in this respect.

The hospitals floor is prone to being contaminated with pathogenic microorganisms. This mostly arise from soil-borne pathogen in shoes, wheels, and other objects, spills or vomitus from patients and settling of bacteria present in air and dust (Suleyman *et al.,* 2018). Contaminated surface have been linked to the spread of drug resistant strains of bacteria such as Methycillin resistant *Staphylococcus aureus*, (Suleyman *et al.,* 2018), drug resistant Enterobacteriaceae and various other Gram negative bacteria in hospitals (Kramer *et al*., 2006). Pathogens can survive and persist on floors for days stretching into months especially when protected by organic matter/soil (Rutala *et al*., 2007). In addition, these organisms retain viability and infectivity on dry surfaces for long period of time. This fitness makes them successful pathogens on floors and increases the infection risk for patients in inadequately cleaned hospital environments (Kramer *et al*., 2006; Han *et al*., 2015).

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Chemical disinfectants have found wide application in hospitals for a variety of topical cleaning and have become a critical component of preventive strategy for curbing HAIs. Hospital disinfection is a complex process which requires the balanced combination of physical and chemical materials as well as consistent monitoring strategies to monitor its outcome (Hans, *et al*., 2015; Engelbretch *et al* 2013). A number of factors have been identified as been able to interfere with the efficacy of disinfection; each of which may nullify or limit the efficacy of the process. These include, organic and inorganic load present (Rutala *et al*., 2007); the type and level of microbial contamination, concentration of disinfectants and exposure time to the germicide (Han *et al*., 2015)

An important factor which has so far attracted limited investigation is the influence of the disinfectant carriage tool on cleaning. Previous investigations on microfibers and mops showed them to contribute significantly to the microbial load of disinfecting solution. The recovery of viable bacteria and fungi from mops and laundered microfibers was reported by Sifuentes *et al*., 2013. In that instance, the mops that were meant to clean and deliver disinfectants revert to being fomites. Similarly, the accumulation and protection of trapped pathogens in trapped organic matter within mop fibres and possible neutralization of disinfectants has been reported to affect cleaning (Michael *et al*., 2016).

Moreso, developing countries such as Nigeria, have a bubbly market flooded with a variety of disinfectants and for reasons bordering on outright lack of compliance to standard disinfection practises in hospitals and shortfalls in the economic situation which promotes ''the cheaper the better'' mentality, there is the popular acceptance of the homemade disinfectants in Nigeria hospitals. The consequences of inadvertently seeding hospital surfaces with pathogenic organisms as against expected infectious control may be quite grave to public health. Disinfectants carriage materials as well as active in use disinfecting solution were assessed in this study to know the hygiene status of studied hospitals.

METHODOLOGY

Study hospitals

Five private hospitals were selected from Ijebu Ode for this study. The town is a major city in Ogun State with a bubbly private health-care service business. Selected hospitals have similar architecture in that a large room is used for reception while the rest of the spaces are in rooms used as ward for purpose of admission. The wards consisted of units of two-man to three-man rooms. Cleaning routine consisted of sweeping followed by disinfecting with the use of looped cotton mops.

All five hospitals employed the use of chemical disinfectants. Cleaning protocol involved filling mopping buckets with water and disinfectant were measured in with the caps following manufacturers working use directive. The cleaning personnel performed the exercise in accordance with their usual work routine. From verbal communication with cleaning personnel, average period of disinfecting water change was put at 15min.

Sample collection

Collection of samples of the cleaning outfit which comprised of the cleaning water and mop without the addition of disinfectant was done before the commencement of the cleaning exercise. Briefly the bucket was filled with water. The mop-heads (regularly used for cleaning at respective hospitals) were extracted in a simple protocol that consisted of immersing the mop head into the water, dunking it for five times, followed by squeezing through the wringer. (a) 30ml volumes of samples were taken into labeled sterile sample bottles for laboratory analysis. The addition of disinfectant (which was routinely used for disinfecting at respective hospitals) followed and cleaning commenced immediately.

(b). Samples were also taken 5min into the cleaning exercise and (c). At 15min of cleaning

The samples were collected into labeled sterile containers and transported to the laboratory within the hour for analysis.

Table 1: Disinfectants used in studied hospitals and their active components

Hospitals	Disinfectants	Active ingredients
A	Branded	Orthobenzylchlorophenol+ paracresol
B	Branded	Tar acid phenol+cresyllic creosote
C	Unbranded ''loosely called izal'	Unknown
D	Unbranded loosely called izal	Unknown
E	Unbranded 'Loosely called izal'	Unknown

d. Twice in the course of the study, sampling was carried out differently from the usual protocol. New mops were obtained and decontaminated with hot water (100°C) (Anderson *et al*., 2009). Thereafter, protocol b and c were carried out as earlier described.

Determination of the bacterial burden of cleaning outfit and isolation of bacteria

The bacterial burden of disinfectant carriage materials in this study was determined by first serially diluting the samples in 9ml Dey Engley neutralizing broth (Hi Media) and analyzed by pour plate method in nutrient agar, briefly 1ml aliquot of appropriate dilution of the samples were placed in sterile disposable petridishes, thereafter, sterile cooled nutrient agar was poured into the plates, gently rocked to disperse cells and left to set. Inoculated plates were then incubated at 37°C in an aerobic incubator for 24 hours .

For bacteria isolation, collected samples (abc) were pooled in the laboratory to make one composite sample. Briefly, 1ml of the sample was suspended in the neutralizing broth and serially diluted.

Thereafter, 200µl of appropriate dilutions were plated on the surface; MacConkey, Centrimide, Eosine Methylene blue, Xylose Lysine Deoxycholate agars for the isolation of Gram negative bacteria and Mannitol Salt agar for *Staphylococcus aureus* respectively. Plates were incubated aerobically at 37°C for 24h and subcultured until pure, distinct colonies were obtained. Pure cultures were stored on nutrient agar slants for appropriate biochemical test that aided identification of isolated organisms.

Identification of the organisms was according to the guide of Cowan and Steel manual for identification of Medical Bacteria (Barrow and Feltham (1993). Suspected *Staphylococcus aureus* colonies were identified by observed colony morphology, the slide and tube coagulase test, mannitol fermentation and their heamolytic patterns on blood agar. The Gram negative bacilli were identified based on colony morphology on the selective/differential agar employed in this study, Gram staining characteristic, Beta-galactosidase detection with ONPG discs (Oxoid, UK), catalase, urease production (Urea broth +40% urea), lysine decarboxylation test using Lysine decarboxylase broth tablets (Oxoid, UK), citrate utilization and production of indole and H_2S among others.

RESULTS AND DISCUSSION

The result of the extraction of routinely used mops of the five studied hospitals revealed variable counts though, mostly high. The mean and standard deviations for microbial load in the wards and the reception mops ranged from 1.53 x 10^2 ± 62.7 to 4.8 x 10^4 ± 2.3 x 10^4 and 64.7 ± 20.1 to 7.8 x 10^3 ± 30.6 respectively (Fig 1). Generally the bacterial loads in the wards were higher. Hospital D ward had the highest bacterial counts (4.8 x 10^5± 2.3x10^4cfu/ml) closely followed by hospital C with 7.8 x 10^4 and 7.9 x 10^4 cfu/ml for the ward and reception counts respectively. The least counts were observed from both mops (reception and ward) of hospital A (Fig 1).

The high bacterial burden of test mops is thought to be as a result of long periods between use/contact. Mops in question are used for general cleaning at about 7am in the morning, except for occasional spills, cleaning is not done until another 24 hours. This period may allow for drying out of mop strands which automatically delimit the inhibitory effect of disinfectant. Also, in the situation of excess organic load trapped in mops, the period may support the recovery of damaged cells and cause microorganisms to revert to active. In a study by Sehulster, (2015), the trapping of organic residues in mops was found to impact negatively on outcome of cleaning which often necessitate a combination of thermal efforts and chemical disinfection in order to achieve desired cleanliness. Contaminated cleaning carriage tools have been implicated in the inactivation of chemical disinfectants and consequent cause of hospital outbreak of disease (Cheng *et. al.* 2015). However, Hospital A consistently had a lower bacterial count in this study in spite of similar cleaning regimen. It is thought that the disinfectant in use in this hospital is able to exert a longer residual effect on microbial growth hence the low microbial count. Hospital C and D have the highest counts and these hospitals were, in addition to being cleaned once daily like others, noted for the use of homemade phenol disinfectants (Table 1).

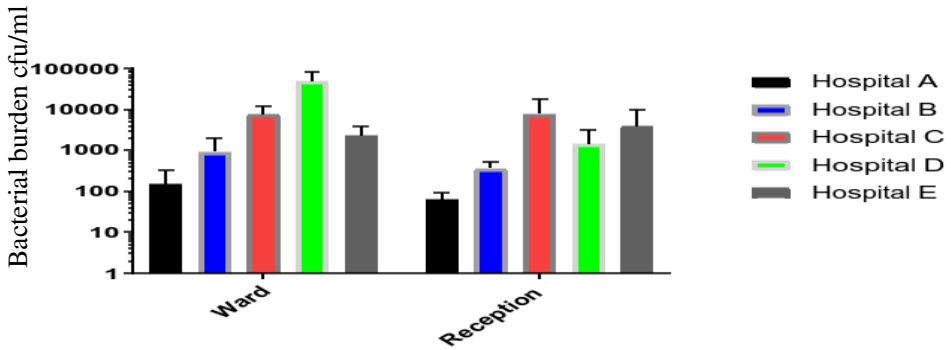

Figure 1: Bacterial burden of routinely used mops at hospital receptions and wards

Result of the sampling carried out at 5min into cleaning revealed evidence of reduction in the bacterial count across all sites following addition of disinfectant into cleaning solution. Bacterial counts ranged from 4.3 ± 7.5 to $3.5x10^3$ ± $1.9x10^3$ cfu/ml and 0 ± 0.0 to $14.3x10^2$± $1.7x10^3$ cfu/ml. for the wards and reception areas respectively (Fig 2 and 3). The reduction was most substantial in hospital A as no viable cell could be recovered from the disinfecting solution at this time. Aerobic count of actively in use disinfectant solution at 15 minutes of cleaning mostly demonstrated increased microbial counts except at Hospital A which consistently had 0cfu/ml at 15min into cleaning. There were no significance differences in counts at 5min and 15 across the ward sites at P=0.05. These high counts may be attributed to build up of organic matter which may have impacted disinfectant's chemistry and reduce its effectiveness. Similar to our findings, other studies have revealed reduction in the levels of active disinfectant over time during cleaning. Boyce *et al.* (2016) in their investigation estimated a 20% decrease in disinfectant activity within the first few minutes of active cleaning. The study reported a startling decline in disinfectant strength by 15minutes comparable to seeding the floor with viable microorganisms. The consistency in the zero viable count in hospital A again, is thought to be attributed to the efficacy of the disinfectant which was able to maintain a 100% (0cfu/ml) disinfecting efficiency throughout the cleaning period (Fig 2). Similar to this trend was hospital B reception where bacterial count was at 11.6±20.2 at 5 min. The count however rose to 3.0 x 10^2±2.71cfu/ml at 15 min (Fig 3).

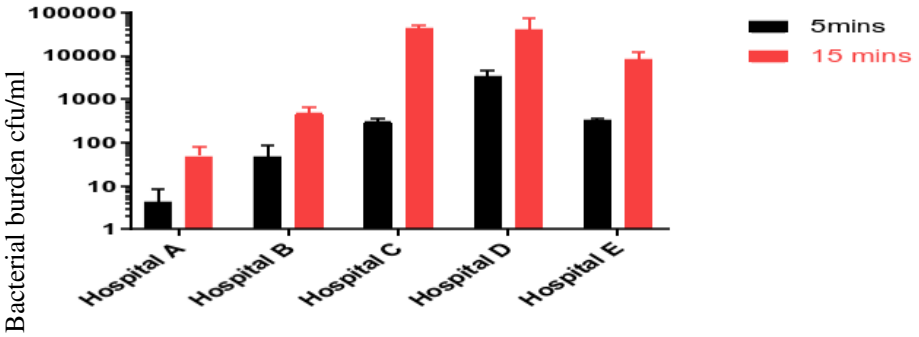

Figure 2: Bacterial burden in ward disinfecting solution at 5 and 15 minutes

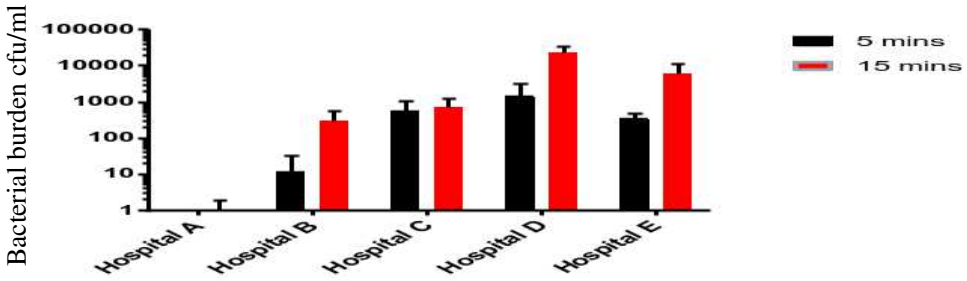

Figure 3: Bacterial burden in reception disinfecting solutions at 5 and 15 minutes

A total of 112 isolates were recovered in this study. This consisted of Gram negative bacteria belonging to the genera *Serratia*, *Citrobacter* sp, *Escherichia*, *Enterobacter*, *Salmonella*, *Pseudomonas; and Staphylocuccus aureus.* The most frequently isolated organisms were the genera *Serratia* spp. and *Pseudomonas* spp., closely followed by *Staph aureus.* These organisms are of public health concern and have assumed epidemiological importance in hospital hygiene. Nosocomial *Serratia marcescens* is increasingly reported in post operative infections especially in pediatric meningitis and wound sepsis (Khanna *et al*., 2013). *Pseudomonas*, due to its nutritional versatility has been reported to be a major cause of contamination of undiluted and in-use disinfectants (Chuanchuen *et al*., 2003). In addition, they have become one of the leading causes of multiple drug resistant nosocomial infections in intensive care units.

Seventeen *Staph aureus* were isolated from four of the five studied hospitals; hospital B (n=2), hospital C (n=7) and E (n=3) and hospital D=(5) respectively While the Gram negative bacteria were distributed across all sampled hospitals. Several yardsticks have been put forward as an encompassing standard to adjudge hospital cleanliness. Though most studies have been majorly quantitative, *Staphylococcus*, has been distinctively investigated and often the focal point of these studies. The isolation of this organism from disinfected hospital surface is regarded as indication of hygiene failure of such sites (Attaway *et al.*, 2012; Dancer *et al*., 2013). This is consistent with our finding in this study as the sites most contaminated by *Staphylococcs aureus* hospital (C, D and E) also had highest bacterial burden, suggestive of hygiene failures in spite of disinfectant use for cleaning in those sites.

Decontamination of mops in this study yielded a startling reduction in the initial bacterial burden of the cleaning materials across all sampled sites which ranged from 0cfu/ml to 5.1cfu/ml. as against (0.64×10^2 to 4.8×10^4) in routinely used contaminated mops. There were significant differences between the counts for decontaminated and routinely used mops across all hospitals at P = 0.05.

Table 2: Bacterial burden of decontaminated mops and resulting counts at 5 and 15 minutes in wards

Hospitals	Decontaminated mop	5MIN	15MIN
A	2.5	(0±0)	(0±0)
B	0	(0±0)	(0±0)
C	1.5	($2.56 \times 10^2 \pm 2.03 \times 10^2$)	($3.02 \times 10^4 \pm$)
D	0	($1.37 \times 10^3 \pm 6.42 \times 10^2$)	($1.54 \times 10^4 \pm 9.0 \times 10^3$
E	0	($2.52 \times 10^2 \pm 0.24 \times 10^2$)	($6.4 \times 10^3 \pm 6.46 \times 10^2$)

Mean values±standard deviation.

Table 3: Bacterial burden of decontaminated mops at 5 and 15 minutes at the reception area

Hospitals	Decontaminated mop	5MIN	15MIN
A	1.5	(0±0)	(0±0)
B	0	(0±0)	(1.6±2.8)
C	3	($0.6 \times 10^2 \pm 0.32 \times 10^2$)	($6.1 \times 10^2 \pm 3.25 \times 10^2$)
D	0	($7.03 \times 10^2 \pm 3.54 \times 10^2$)	($1.94 \times 10^4 \pm 1.17 \times 10^3$)
E	0	($2.76 \times 10^2 \pm 0.46 \times 10^2$)	($3.9 \times 10^4 \pm 4.0 \times 10^2$)

Mean values±standard deviation.

Furthermore, sampling of these areas post addition of disinfectant and 5 minute into cleaning revealed an overall lower bacterial count (0 cfu/ml to 13.4×10^2 cfu/ml) compared to the non- decontaminated mop use (4.3 to 3.5×10^3). This trend was generally maintained at 15min. However statistical difference was not established between the counts in Hospital C, D and E. High microbial loads in these sites in spite of use of sterile mops questions the activity of the disinfectants; three of which were homemade and one branded. This study may not conclusively tie the reduced activity of disinfectants used in C, D, and E to inadequate active ingredients in the homemade disinfectants. It is however certain that the homemade disinfectants lacked any form of quality control necessary to ensure expected quality.

The study showed all three hospitals using homemade disinfectants to have failed the hygiene standard required in a disinfected hospital floor.

CONCLUSION

Four of the five hospitals failed the hygiene test going by the high bacterial burden of the disinfectant solutions and the isolation of *Staphylococcus aureus*. There were also very high bacteria counts of disinfectant carriage materials of the study hospitals. The implication of this is that the hospital floor is being seeded with viable pathogenic microorganisms instead of the expected cleaning. It is notable that the use of new mops revealed reduction in microbial loads across all hospitals. These results generally highlight the need for a major overhaul of disinfection practices in Nigerian hospitals, starting with the disinfectant carriage materials. Work is on going to examine other interplay of factors critical in hospital hygiene.

Recommendations

Going by the findings in this study, the use of disposable mop heads is advised. Mops are advised to be limited to separate rooms (i.e a ward mops shouldn't be used in cleaning the reception area or other wards) in order to contain the spread of infectious agents. Stricter monitoring in form of random cleaning audits is advised to ensure that hospitals comply with standard cleaning procedures as well as ensuring stricter controls on disinfectant production and distribution by appropriate regulatory body.

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