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Investigating the Bioburden of "Neglected" Hospital Low Contact Surfaces

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Abstract

Microbes inhabit every surface, reproduce, and if undisturbed, could form biofilm. Hospital contact surfaces have been reported to play a major role in the spread of healthcare-acquired infections (HAIs). Most studies on these surfaces as a route for the spread of nosocomial infections have focused on the high-contact surfaces. There is a paucity of information on the bioburden of "neglected" low-contact surfaces such as bedside bible, ward television, and ward clock, etc. This study was carried out to investigate the bioburden of "neglected" low-contact hospital surfaces and compare it with that of the high-contact surfaces. Using a sterile swab stick moistened in normal saline, we collected 400 samples from contact surfaces of 20 randomly selected hospitals in Owerri, southeast in Nigeria, and by standard microbiological methods and with reference to standard identification manuals, microbial species were isolated and characterized. The results show that the mean of the bioburden in cfu/square swabbed surface of these "neglected" low-contact surfaces is significantly higher ($p = 0.005$) than that of the high-contact surfaces which may be a result of target hygienic cleaning, with attention on the high-contact surfaces and the low-contact surfaces are often "neglected". This result gives an insight into the continued prevalence of hospital-acquired infections as these "neglected" low-contact surfaces continue to serve as a reservoir for pathogenic microbes and a source of continued microbial contamination of hospital surfaces. It therefore calls for a revamp of existing hospital cleaning protocols and redesigning of cleaning regimes.

Keywords

Cleaning, Contact, Infections, Microbiota and Surfaces

1. Introduction

In every health care institution, contact surfaces have continued to pose a health challenge. Some are constantly touched by patients, visitors, and other health-care workers as they carry out their daily routine, or are on a visit to the health-care facility and are known as high-touch surfaces, such as bed lining, door handle, over bed table, sink, tabletop, etc. while some are in rare contact with visitors and personnel and are referred to as low-touch surfaces, such as window ledges, window blind, ward screen, bedside bible, ward television, clock, etc. [1] (Huslage *et al.*, 2010). Some of these low contact surfaces are often forgotten or neglected during routine cleaning and disinfectant schedule and may serve as a reservoir for pathogenic and non-pathogenic microbes.

Microbes are known to thrive and inhabit surfaces. They are ubiquitous and can easily colonize any fomite they are in contact with, and in cases exhibiting resistance to some known disinfectants and antibiotics. Humans are known to carry some microorganisms on their skin and mucosa and shed them onto surfaces they come in contact with. Human activities, air circulation, and other factors have been reported to aid the spread of microbes in any environment [2] (Creamer *et al.*, 2014). Microbiota of hospital environments including contact surfaces are reported to survive for a long period of time [3] [4] (Otter and French 2009, Kramer *et al.*, 2006) and have become an issue of concern and of great interest to scholars, due to increase in the cases of nosocomial infections and its associated economic challenges including loss of man-hour and pressure on hospital bed spaces.

Studies have implicated hospital contact surfaces in the transmission of microbes and pathogens [5] (Dancer 2009), they are inoculated regularly by healthcare workers [6] [7] [8] (Bhalla *et al.*, 2004, Dancer *et al.*, 2009, Hayden *et al.*, 2006) and other users of the healthcare facility. Routine cleaning and disinfection of these surfaces, though, deplete its bioburden and aid the management of nosocomial infections [9] (Kundrapu *et al.*, 2012). However, they have failed to make the surfaces sterile, as microbial contaminants continue to persist [5] (Dancer 2009), occasioned by continuous re-inoculation of microbes onto these surfaces by visitors, patients, and health care workers. [10] (Bogusz *et al.*, 2013), with the surfaces becoming a reservoir for microbes and other disease-causing organisms [6] (Bhalla *et al.*, 2004).

Studies on the aetiology of nosocomial infections and the role of hospital contact surfaces have focused on the high contact surfaces probably due to their high frequency of contact by patients, visitors, and healthcare workers [11] (Riggs *et al.*, 2007). There is, therefore, a paucity of information on the bioburden of some "neglected" low contact surfaces such as bedside bible, ward television, ward clock, window blind, and ward screen which are out of rich to visitors, and not regularly in contact with healthcare workers and thus are seldom cleaned and disinfected by cleaning staff and their potential health risks to patients, immune-compromised individuals, visitors and healthcare workers [HCWs] including their role in the spread of nosocomial infections. No study,

though, has compared the bioburden of these contact surfaces, so as to enable informed decisions on cleaning and disinfecting schedules for these surfaces, in order to minimize the risks of hospital-acquired infections.

This study, however, is carried out to investigate and compare the bioburden of the high-touch and some "neglected" low-touch contact surfaces of randomly selected hospitals in Owerri, South Eastern Nigeria, with a view to establishing the extent of the risks these contact surfaces pose to critical care patients, visitors and other healthcare workers and also advise on the appropriate cleaning regime and disinfection necessary to prevent the spread of healthcare-acquired infections via these surfaces.

2. Materials and Methods

2.1. Sample Collection

400 samples were collected from 20 randomly selected hospitals within the Owerri metropolis. Using normal saline moisten sterile swab stick, a sample was collected in duplicate, each from 5 high-touch contact surfaces: door handle, bed rail, sink, bed lining and tabletop in each hospital by swabbing the sticks on the surfaces, approximately two square inch area of the surfaces mentioned above and replacing the swab stick in its sterile container. Using the same procedure described above, a sample was also collected in duplicate each from 5 low-contact surfaces: bedside bible, window blind, ward clock, ward television and ward fan from each of the 20 hospitals. The samples were labeled and transported to the laboratory for analysis.

2.2. Media Preparation

MacConkey Agar [MA], Nutrient Agar [NA], Nutrient Broth [NB], Eosin Methylene Blue [EMB], Potato Dextrose Agar [PDA], and Mannitol Salt Agar [MSA] were used for this study, and were all sourced from MERCK, German; EMB Broth [EMBB] used for the coliform test was sourced from Sigma-Aldrich USA, the media used for Staphylococcus aureus Identification, [Staphylococcus aureus Identification [SAID] Agar] was sourced from Oxoid LTD UK. Nutrient Agar [NA] was used for the cultivation, isolation and colony count of non-fastidious heterotrophic bacteria, Cultivation, isolation and colony count of Micrococcus was carried out using MSA, Potato Dextrose Agar [PDA] was used for fungal cultivation, and that of coliform was carried out using MA and EMB Agar. Manufacturer's instructions were adhered to during the preparation of the media. They were aseptically poured into Petri dishes, labeled and incubated overnight for sterility test.

2.3. Working Stock Preparation

Manufacturer's instruction was adhered to in the preparation of nutrients both used as working stock. 2 ml aliquot of the broth was aseptically dispensed into duplicate bijou bottles for each sample; the swab sticks were sluiced out into each bottle and labeled.

2.4. Analysis of Specimen

Spread plate technique was used to analyze the samples by inoculating 0.1 ml aliquot of the working stock onto labeled duplicate plates of the different growth media (Figure 1). Coliforms were tested by aseptically removing the cotton wool of each swab stick and placing it into EMB Broth with inverted Durham tubes. The Potato Dextrose Agar [PDA] plates were cultured at ambient room temperature of 28˚C ± 2˚C for 3 to 5 days. The rest of the media plates were incubated at 37˚C for 24 to 48 hour, after incubation, an examination of the plates was done, and morphological characteristics of the organisms were observed and recorded. Enumerations of discrete microbial colonies of generated species were carried out using the Gallenkamp England colony counter, and total counts were expressed as colony forming units per ml [cfu/ml]. These colony forming units per ml [cfu/ml] as calculated are equivalent to colony forming units per square inch swabbed hospital contact surfaces. Isolates were purified by repeated subcultures of some selected discrete colonies from the various plates on Nutrient Agar. The pure cultures obtained were stored on labeled slants and preserved for further analysis.

2.5. Identification of Isolates

From the slants, subcultures of the various organisms were made onto an appropriate medium and incubated for 24 to 48 hours at 37˚C to confirm the purity of the organisms, and the same procedure was used to check for their viability. Identification of bacteria isolates was based on colony morphological characteristics, gram stain reaction, and microscopy and biochemical tests: indole test, catalase test, methyl red production, citrate utilization, Vogues-Proskauer test, urease production, coagulase test, oxidase test, gelatin liquefaction, sugar fermentation, starch hydrolysis, temperature, salt tolerance and motility test. The Analytical Profile Index [API] system [Biomerieus sa] with reference to standard identification database formed the basis for further identification of the bacteria isolated. Fungal isolates were identified using standard keys and atlas database including microscopy and morphological characteristics.

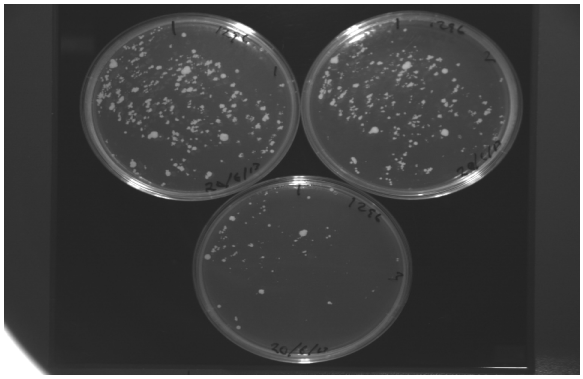

Figure 1. Plates of aerobic microbial count. Plates 1296 (1) and (2) are plates of swab from low contact surfaces while plate 1296 (3) is plate of swab from a high contact surface.

2.6. Statistical Analysis

Statistical analysis of the data was carried out and the significance in difference of mean was obtained by Duncan's Multiple Range [DMR] test using SPSS 20.0 software for windows SPSS, 2011.

3. Results

The results from this study reveal an array of microbial diversity obtained from swabbed high and low contact surfaces of the hospitals investigated. The bioburden of the contact surfaces under investigation is presented as mean and standard deviation in cfu/square inch of the swabbed surface as shown below.

Table 1 gives the mean of the colony count of Aerobic and coliform isolates in [cfu/square inch] of swabbed high contact surfaces. The bacteria isolates include Staphylococcus, *Bacillus sp.*, *Klebsiella sp.*, Streptococcus, Micrococcus, Pseudomonas, Corynebacterium, Proteus, *Enterococcus*, and *E. coli.*

Table 2 shows the mean of the colony count of fungal isolates in [cfu/square inch] of swabbed high contact surfaces, which includes *Penicillium*, Aspergillus, Yeasts, *Rhizopus*, Fusarium, Verticillium, Mucor, and *Trichoderma sp*.

Table 3 presents the mean of the colony count of aerobic and coliform isolates in [cfu/square inch] of the swabbed low contact surfaces. The bacteria isolates include Staphylococcus, *Bacillus sp.*, *Klebsiella sp.*, Streptococcus, Micrococcus, Pseudomonas, Corynebacterium, Proteus, *Enterococcus*, and *E. coli.*

Table 4 shows the mean of the colony count of fungal isolates in [cfu/square inch] of swabbed low contact surfaces. The isolates include *Penicillium*, Aspergillus, Yeasts, *Rhizopus*, Fusarium, Verticillium, Mucor, and *Trichoderma sp*.

Table 5 presents the result of the susceptibility test of the bacteria isolates on routinely used antibiotics. All the isolates were resistant to a number of antibiotics tested. 85.1% of isolates were resistant to four antibiotics, 58.5% were resistant to five of the antibiotics tested while 24% were resistant to 7 out of the ten antibiotics tested. It also shows that over 90% of the isolate were susceptible to Gentamycin, Ciprofloxacin and Ofloxacillin.

Table 1. Mean and standard deviation of total aerobic and coliform plate counts (cfu/square surfaces) of swabbed high contact surfaces.

Contact Surfaces	Total Aerobic Microbial Counts	Coliform Counts
Door Handle	$5.6 \times 10^3 \pm 0.02$	$2.2 \times 10^4 \pm 0.04$
Bed Rail	$4.3 \times 10^3 \pm 0.04$	$1.7 \times 10^3 \pm 0.03$
Table Top	$5.4 \times 10^4 \pm 0.05$	$2.0 \times 10^3 \pm 0.03$
Sink	$4.6 \times 10^4 \pm 0.05$	$1.8 \times 10^3 \pm 0.05$
Bed Lining	$4.2 \times 10^3 \pm 0.04$	$1.6 \times 10^4 \pm 0.04$

Value as mean ± SD of duplicate counts.

Table 2. Total fungal plate counts (cfu/square surfaces) of swabbed high contact surfaces.

Contact Surfaces	Total Fungal Counts
Door Handle	$4.6 \times 10^5 \pm 0.04$
Bed Rail	$3.0 \times 10^6 \pm 0.05$
Tabletop	$2.1 \times 10^5 \pm 0.03$
Sink	$3.8 \times 10^6 \pm 0.04$
Bed Lining	$3.5 \times 10^6 \pm 0.06$

Value as mean ± SD of duplicate counts.

Table 3. Total aerobic and coliform plate counts (cfu/square surfaces) of swabbed low contact surfaces.

Contact Surfaces	Total Aerobic Microbial Counts	Coliform Counts
Bedside Bible	$7.6 \times 10^8 \pm 0.03$	$3.2 \times 10^6 \pm 0.04$
Ward Television	$5.3 \times 10^7 \pm 0.04$	$3.7 \times 10^5 \pm 0.03$
Ward Fan	$5.4 \times 10^8 \pm 0.02$	$4.0 \times 10^5 \pm 0.03$
Ward Clock	$4.6 \times 10^7 \pm 0.02$	$4.4 \times 10^5 \pm 0.05$
Window Blind	$1.2 \times 10^7 \pm 0.05$	$2.6 \times 10^6 \pm 0.04$

Value as mean ± SD of duplicate counts.

Table 4. Total fungal plate counts (cfu/square surfaces) of swabbed low contact surfaces.

Contact Surfaces	Total Aerobic Counts
Bedside Bible	$6.6 \times 10^7 \pm 0.02$
Ward Television	$4.7 \times 10^8 \pm 0.04$
Ward Fan	$4.4 \times 10^8 \pm 0.05$
Ward Clock	$5.8 \times 10^7 \pm 0.05$
Window Blind	$3.1 \times 10^7 \pm 0.04$

Value as mean ± SD of duplicate counts.

Table 5. Susceptibility of bacterial isolates from swabs of high and low hospital contact surfaces.

Antibiotic	Conc. (g)	*E. coli*	*K. pneu*	*S. epider*	Micro	*S. aureus*	*Proteus spp.*	*B. cereus*	*P. aerug*	Control *S. aureus* (virgin)
AMX	25.0	080	07.0	06.0	11.0	07.0	07.0	07.0	00.0	10.0
OFL	05.0	18.0	15.0	19.0	15.0	17.0	14.0	17.0	15.0	14.0
STR	10.0	11.0	13.0	15.0	12.0	13.0	12.0	11.0	13.0	12.0
CHL	30.0	15.0	14.0	12.0	14.0	12.0	13.0	10.0	11.0	14.0
CEF	30.0	07.0	00.0	08.0	00.0	07.0	08.0	12.0	00.0	08.0
GEN	10.0	15.0	16.0	17.0	18.0	14.0	17.0	14.0	18.0	14.0
PEF	05.0	10.0	11.0	10.0	02.0	12.0	13.0	11.0	08.0	11.0
COT	25.0	07.0	08.0	08.0	05.0	06.0	05.0	10.0	00.0	10.0
CPX	10.0	16.0	78.0	16.0	21.0	18.0	20.0	12.0	14.0	14.0
ERX	05.0	11.0	12.0	11.0	13.0	08.0	08.0	00.0	00.0	11.0

Key: AMX = Amoxicillin; OFL = Ofloxacillin; STR = Streptomycin; PEF = Pefloxacin; CHL = Chloramphenicol; CEF = Ceftriazone; GEN = Gentamicin; COT = Cotrimazole; CPX = Ciprofloxacin; ERX = Erythromycin; *K. pneu* = *Klebsiella pneumonia*; *S. epider* = *Staphylococcus epidermidis*; Micro = Micrococcus; *P. aerug.* = *Pseudomonas aeruginosa*.

4. Discussion

Almost every hospital sampled engages in routine cleaning and has elaborate cleaning and disinfectant schedule for their facility and environs, but some low contact surfaces are neglected, resulting in heavy deposits of dust particles seen on these surfaces. The contact surfaces of these hospitals, however, continue to harbour microorganisms that exhibit a wide array of diversity and may reflect the microbiome of those that come into contact with them. The result of this study, however, indicates that the bioburden of these "neglected" low contact surfaces is significantly higher [$p = 0.005$] than those of high contact surfaces.

Considering the frequency of touch, the microbial yield from these low contact surfaces is unexpectedly high when compared with those of high contact surfaces. However, due to negligence on the part of the hospitals, as they do not routinely clean and disinfect these surfaces, microbes have continued to persist on them. The window blind, though, which is occasionally cleaned and disinfected when they are dirty, have a microbial yield of $1.2 \times 10^7 \pm 0.05$ for the aerobic count, $2.6 \times 10^6 \pm 0.04$ for coliform count and $3.1 \times 10^7 \pm 0.04$ for fungal count. This is lower than the microbial yield from other "neglected" low contact surfaces which peak for aerobic count at $7.6 \times 10^8 \pm 0.03$, for coliform count at $4.4 \times 10^5 \pm 0.05$ and fungal count at $6.6 \times 10^7 \pm 0.02$ and is higher than those of the high contact surfaces under study, thus underscoring the importance and efficacy of routine cleaning regime in depleting bioburden of surfaces.

This study revealed the diversity of the microbiome of the contact surfaces under study to include bacteria such as Staphylococcus, *Bacillus sp.*, *Klebsiella sp.*, Streptococcus, Micrococcus, Pseudomonas, Proteus and *E. coli* and fungi such as Penicillin, Aspergillus, Yeasts, Rhizopus, Fusarium, Verticillium, Mucor, and *Trichoderma sp.* The Staphylococcus spp. are resident in the mucosa of human and animal skin as normal flora and may have been shed by colonized patients, visitors or healthcare workers [12] (Wilson *et al.*, 2011) or from the air, as carriers may shed them as part of epithelial cells which have been atomized [13] (Gehanno *et al.*, 2009) the risk of colonization, though, depends on airborne concentrations of the microbes. Bacillus, Micrococcus, Pseudomonas and Klebsiella are normally found in water, soil, plants, dust or air, and may have contaminated the hospital contact surfaces via airborne dust particles.

Proteus which is a saprophyte is known to inhabit decaying organic matter, animal or human feaces, *E. coli*, an enteric organism found also in contaminated food, water, animal and human feces and *Enterococcus* found in fecal material may have been inoculated onto the surfaces by individuals with poor toilet hygiene or who have touched contaminated uncooked food. The fungal species including Rhizopus [bread mould], produce spores may have been carried onto the contact surfaces by air currents or with dust particles, yeast and Fusarium, inhabit the skin, may have come from infected patients, visitors or healthcare personnel. Aspergillus, a saprophytic fungus found in decaying organic matter, soil and dust may have been carried onto the surfaces by dust particles.

Most of these organisms are pathogenic and can initiate diseases, hence are of great healthcare importance, some are however, opportunistic pathogens and could initiate a disease condition when introduced into a different anatomical site. Studies have revealed that *Staphylococcus spp.* cause localized Staph infection leading to boil or abscess when they penetrate through broken skin [14] (Liu *et al.*, 2011). Due to their enterotoxin [15] (Bergevin *et al.*, 2017), they cause food poisoning and have been implicated in several outbreaks [16] (Okuyama and Yoshida 2012).

Meticillin-resistant Staphylococcus aureus [MRSA] is an established antibiotics resistance strain [17] (Jensen and Lyon 2009) and the contact route has been implicated in its transmission [18] (Siegel *et al.*, 2007). Bacillus is known to cause septicemia [19] [20] (Mazza *et al.*, 2009, Kiss *et al.*, 2021) and has been isolated in catheter-related bacteraemia and in musculoskeletal infections. Klebsiella, especially the strain *K. pneumonia* and *Proteus mirabilis* are established opportunistic pathogens that cause urinary tract infection [21] (Liverelli *et al.*, 2006), mostly in the elderly and those with suppressed immune systems.

Streptococcus is a known cause of throat infection, pneumonia, skin, wound infection, pharyngitis and toxic shock syndrome [22] [23] (Marylin *et al.*, 2018, Baxter and McChesney 2000). Micrococcus an opportunistic pathogen is involved in pulmonary infection in severe immune-suppressed individual patients, [24] (Smith *et al.*, 2020), Pseudomonas, however, colonizes catheters and other medical implants leading to cross infections, especially in the elderly and the immune-suppressed individuals [25] (Wagner *et al.*, 2006). E. coli have been established to cause urinary tract infections and diarrhea [26] [27] (Hien *et al.*, 2008, Kang *et al.*, 2018) with occasional outbreaks in places such as schools, prisons, etc.

The fungal species isolated in the contact surfaces are known to cause disease in humans, especially in the immune-suppressed individuals and the elderly. The Yeast species, especially Candida spp, is known to cause vaginal yeast infection [28] (Sobel 2007), Fusaruim causes Keratitis, Sinusitis and Mycotoxicosis [29] (Okafor *et al.*, 2021), endophthalmitis, musculoskeletal infections etc, *Rhizopus* is associated with mucromycosis [30] (Lee *et al.*, 2009) while Aspergillus is implicated in pulmonary disease [31] (Soubani, and Chandrasekar 2002) and aspergillosis [29] [32] (Rankin 1953, Okafor *et al.*, 2021).

5. Conclusion

Having known the medical importance of the microbiota of these near-forgotten low contact surfaces including ward clock, ward fan, ward television, bedside bible, and window blinds with a very high bioburden ranging between $1.2 \times 10^7 \pm 0.05$ to $7.6 \times 10^8 \pm 0.03$ for the total aerobic count, $2.6 \times 10^6 \pm 0.04$ to $3.2 \times 10^6 \pm 0.04$ for the total coliform count and $3.1 \times 10^7 \pm 0.04$ to $6.6 \times 10^7 \pm 0.02$ for total fungi count. Comparing it with the low bioburden of the high contact surfaces, it is therefore necessary for governments and non-governmental agencies to carry

out a public health awareness campaign in order to educate and inform the general public and health care facility managers in particular to implement cleaning and disinfectant regime that will target these near-forgotten hospital surfaces. This study has shown that "neglected" contact surfaces have very high bioburden, when compared with high contact surfaces and therefore, are reservoirs to potential pathogenic microbes and may have been playing a significant role in the persistence of hospital-acquired infections.

Recommendations

♦ Hospital low contact surfaces should be atarget during routine cleaning and disinfection

♦ High importance should be placed on hand hygiene for both healthcare workers and visitors

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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