Miscellany of Hospital Contact Surfaces Microbiome: A Case Study of Selected Hospitals in Owerri South Eastern Nigeria

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Abstract

Hospital contact surfaces are provided for the comfort of the patient and for the convenient of visitors and healthcare workers. They are however reported to be reservoir of an array of microbial species. This study was carried out to understand and elucidate the miscellany of the microbiota of hospital contact surfaces and the potential health challenges they pose to patients, visitors and healthcare workers (HCWs), so as to advice on effective cleaning and disinfectant regime. Using sterile swab sticks moistened in normal saline, 300 samples were collected from contact surfaces of 25 randomly selected hospitals in Owerri South East Nigeria. The samples were cultured in suitable growth media. Using standard laboratory protocols and with reference to standard identification manuals, microbial species were isolated and characterized. *Pseudomonas*, *Enterococci*, *Bacillus*, *Klebsiella*, *Streptococcus*, *Staphylococcus*, *Proteus*, *Micrococcus*, *Corynebacterium*, *E. coli*, *Penicillium*, *Fusarium*, *Trichoderma spp*., *Yeast*, *Mucor*, *Aspergillus*, *Rhizopus* and *Verticillium* were isolated. Mean total aerobic and coliform count in cfu/square swabbed surface of the high and low contact surfaces were $7.4 \times 10^7 \pm 0.03$ and $1.4 \times 10^2 \pm 0.06$, while that of fungal count was $3.4 \times 10^4 \pm 0.03$. Majority of these bacterial and fungal isolates are potentially pathogenic and have been involved in diseases outbreaks, some are opportunistic pathogens and could initiate diseases in immune - suppressed individuals. Continuous cleaning and disinfection of hospital contact surfaces and good hand hygiene, therefore, is recommended to control the spread of hospital acquired infections (HAIs).

Key words: *cleaning, contact, disinfection, hospital, microbiota, surfaces,*

1.Introduction

1.1 Hospital Contact Surfaces

Hospital contact surfaces such as bed, over bed tables, door handle, bed rail, call control bottons, ward screen and low-touch surfaces such as window ledges, window blind and wall [1] are usually provided for the comfort of the patient and or for the convenient of visitors and healthcare workers. Microbes are ubiquitous and are known to inhabit and thrive in every surface, animate or in-animate. Their low generation time enables them, undisturbed, to colonize surfaces, and form biofilms, microbes including Methicilin – Resistant *Staphylococcus aureus* (MRSA), Vancomycin – Resistant *Enterococcus* (VRE,) *Clostridium dificile* etc are reported to be recovered from healthcare facilities after 4 – 5 months [2].

1.2 Humans as Carriers of Some Microbial Species

Humans are known carriers of some microbial species such as the Staphylococcus genus, which inhabit the skin as normal flora. This genus is of great health importance, as they are known to cause an array of debilitating infections, implicated in food poisoning and have been associated with many dis-

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eases out breaks, of note is Methicillin – resistant *Staphylococcus aureus* (MRSA) which has become a major cause of hospital acquired infections [3]. Studies have also shown that those of poor hygiene are carriers of various arrays of microbes depending on the microbial diversity of the environment where they inhabit.

1.3 Microbes in the Environment

The presence of microbes in any environment either built or open is a function of various factors which include: human activities, air circulation [4, 5], dust particles, etc. The microbiome of built environment consist mostly of organisms originating from human, those from the open environment, carried in by air current [4, 6, 5] and those attached to dust particles. Unlike other built environments, microbial communities of hospital environments are reported to survive for a long period of time [7, 8, 9] and are of much interest, due to the nature of the inhabitants of this environment, who are mostly the aged, children and other immune - suppressed individuals.

In the hospital setting, contact surfaces are perfect media for transmission of microbes and pathogens [10, 1], as people constantly inoculate them with variety of microbes and pick up some as they continuously touch them. Although, cleaning and disinfection of these surfaces are known to play a role in the control of microbes, including MRSA [11, 12, 13]. However, despite cleaning and disinfection, microbes and pathogens on these surfaces continue to persist [14], largely due to constant inoculation of these surfaces by people who touch them after cleaning and disinfection [15, 16], thereby making these surfaces a reservoir for microbes and other pathogenic organisms [17, 18, 19, 1], which may play a major role in healthcare acquired infections (HAIs) [20, 21, 22, 23]

However, studies on the microbial ecology of hospital environments including contact surfaces have focused on Meticillin Resistant *Staphylococcus* aureus (MSRA), Vancomycin – Resistant *Enterococci* (VRE) and a few other nosocomial organisms [1,2,5,6], thereby, leaving knowledge gaps on the diversification of microbiome of this environment and the potential health challenges and hazards they may pose to patients with attenuated immune system and other immune - compromised individuals including visitors and health care workers (HCWs). This study, however, is carried out to understand and elucidate the diversity of the microbiota of the high – touch and low – touch contact surfaces of randomly selected hospitals in Owerri, South Eastern Nigeria.

2.0 Materials and Methods

2.1 Sample Collection

25 hospitals within Owerri metropolis were randomly selected, and a total of 300 samples were collected. Using a sterile swab stick moisten in normal saline, a sample was collected in duplicate, each from high-touch contact surfaces: door handle, bed rail and table top in each hospital by swabbing the sticks on the surfaces approximately two square inch area, and replacing the swab stick in its sterile container. Using the same procedure described above, a sample was also collected in duplicate each from low-contact surfaces: under bed, window ledges and wall of each of the 25 hospitals. The samples were properly labeled and transported aseptically to the laboratory for analysis, within an hour of collection.

2.2 Growth Media Preparation

The following media were used for generation, colony count and isolation of organisms: 1 MacConkey Agar (MA) 2 Nutrient Agar (NA) 3 Nutrient Broth (NB) 4 Eosin Methylene Blue (EMB) 5 Potato Dextrose Agar (PDA) 6 Mannitol Salt Agar (MSA) all sourced from MERCK, Germany. EMB Broth (EMBB) was sourced from Sigma-Aldrich, USA and was used for coliform test, Staphylococcus aureus Identification (SAID) Agar was sourced from Oxoid LTD UK, and was used for the selection and Identification of Staphylococcus spp. NA; a general purpose medium was used for the cultivation, isolation and colony count of non fastidious heterotrophic

bacteria, MSA was used for the cultivation, isolation and colony count of Micrococcus, PDA was used for fungal cultivation, isolation and colony count, while the MA and EMB agar were used to cultivate, isolate and colony count of coliforms. Preparation of the media were according to the manufacturer's instructions, they were aseptically poured into Petri dishes, labeled and incubated over night for sterility test.

2.3 Working Stock Preparation

Nutrient Broth was prepared following manufacturer's instructions and 2ml aliquot were dispensed into duplicate bijou bottles for each sample. The swab sticks were sluiced out into each bottle and labeled.

2.4 Analysis of Specimen

The samples were analyzed using spread plate technique by inoculating 0.1ml aliquot of the working stock on to duplicate plates of the different growth media and labeled. The cotton wool of each swab stick were aseptically cut and placed into EMB Broth with inverted Durham tubes for coliform test. PDA plates were cultured at ambient room temperature of 28 ± 2^0C for 3 to 5 days while the other media plates were cultured at the temperature regime of 37^0C for 24 to 48 hours. At the end of incubation, the plates were examined, the morphological characteristics of the organisms observed, and microbial discrete colonies were counted and expressed as colony forming units per ml (cfu/ml) using Gallenkamp England colony counter. These colony forming units per ml (cfu/ml) as calculated are equivalent to colony forming units per square inch swabbed hospital contact surfaces. Repeated sub cultures of some selected discrete colonies were made on freshly prepared Nutrient Agar plates to purify the isolates. The pure cultures obtained were stored on slants, labeled and kept for further analysis.

2.5 Identification of Isolates

Sub cultures were made from the slants onto appropriate medium and incubated for 24 to 48 hours to confirm purity and check for viability. Parameters used for identification of bacteria isolates include colony morphological characteristics, 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 data base was utilized for further identification of the bacteria isolates. Standard keys and atlas data base alongside microscopy and morphological characteristics were employed for fungal isolates identification.

2.7 Statistical Analysis

Obtained data were statistically analyzed and the significance in difference of mean were obtained by Duncan's Multiple Range (DMR) test using SPSS20.0 software for windows SPSS, 2011.

3.0 Results and Discussion

3.1 Results

The result obtained from swabbed high and low contact surfaces of the hospitals investigated, showed an array of microbial diversity they are presented as mean and standard deviation.

Table 1a gives the bacteria isolates from both low and high contact hospital surfaces, which include: *Staphylococcus*, *Bacillus spp*., *Klebsiella spp*., *Streptococcus*, *Micrococcus*, *Pseudomonas*, *Corynebacterium*, *Proteus*, *Enterococcus*, and *E. coli*.

Table 1a: Bacteria Isolates from Swabs of High and Low Hospital Contact Surfaces

Staphylococcus, Klebsiella, Proteus , Pseudomonas
Micrococcus, E. coli, Bacillus, Corynebacterium, and Streptococcus
Micrococcus

Table 1b shows the fungal isolates which include: *Penicillium*, *Aspergillus*, *Yeasts*, *Rhizopus*, *Fusarium*, *Verticillium*, *Mucor*, and *Trichoderma spp.*

Table 2a presents the total aerobic and coliform counts (cfu/ square inch) of swabbed high and low contact hospital surfaces.

Table 3 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	07.0	07.0	06.0	12.0	07.0	07.0	06.0	00.0	10.0
OFL	05.0	19.0	14.0	21.0	16.0	15.0	15.0	18.0	14.0	14.0
STR	10.0	12.0	14.0	14.0	13.0	13.0	14.0	10.0	11.0	12.0
CHL	30.0	15.0	11.0	13.0	12.0	11.0	12.0	11.0	10.0	14.0
CEF	30.0	07.0	00.0	07.0	00.0	08.0	06.0	10.0	00.0	08.0
GEN	10.0	16.0	15.0	18.0	18.0	13.0	18.0	13.0	17.0	14.0
PEF	05.0	10.0	10.0	11.0	01.0	11.0	11.0	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	15.0	18.0	15.0	20.0	17.0	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*;

Table 1b Fungal Isolates from Swabs of High and Low Hospital Contact Surfaces

Yeast, Fusarium, Aspergillus, Rhizopus, Trichoderma,
Penicillin, Mucor,and Verticillium,

Table 2b gives the total Fungi counts (cfu/square inch) of swabbed high and low contact surfaces.

Table 2a Total Aerobic and Coliform Plate Counts (cfu/square surface) of swabbed High and Low Contact surfaces

Contact surfaces	*Total Aerobic Counts*	*Coliform Counts*
High and Low surfaces	$7.4 \times 10^7 \pm 0.03$	$1.4 \times 10^2 \pm 0.06$

Value as mean ± SD of duplicate counts

Table 3 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% 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.

2b Total Fungal Plate Counts (cfu/square surface) of swabbed High and Low Contact surfaces

Contact surfaces	*Total Fungal Counts*
High and Low surfaces	$3.4 \times 10^4 \pm 0.03$

Value as mean ± SD of duplicate counts

3.2 Discussion

3.2.1 Private Healthcare Facilities in Nigeria

The challenging economic climate in the developing world including Nigeria has resulted to very poor medical facilities in this part of the world. Endemic poverty and illiteracy have also been implicated in the dearth of facilities and non availability of high

quality Medicare. To make up for the shortage in this critical sector, private individuals have ventured into the healthcare delivery with private hospitals springing up in and around cities especially in the Nigeria. The locations of most of these healthcare facilities on its own pose a health challenge to patients, healthcare workers and visitor. Most of the hospitals are provided with poor ventilation, poor drainage and some lacking proper refuse disposal system, all these are necessary and sufficient for microorganisms to thrive.

3.2.2 Routine Cleaning in Healthcare Facilities

Although almost all the hospitals sampled engage in routine cleaning of their facility and environs, a few do only on daily basis, despite high number of visitors to these facilities, as the frequency of cleaning is not regulated. However, there is paucity of information on the diversity of microbial communities of the surfaces these people are most likely to come into contact with, and how it might impact their microbiome. The results of this study, however, demonstrate that the high and low contact surfaces in hospitals which are constantly touched by patients; healthcare workers and visitors are highly contaminated with a wide range of bacterial and fungal species. The high microbial load recorded on the contact surfaces could be as a result of the frequency of contact with these surfaces by the colonized visitors, healthcare workers and infected patients who come into contact with them on regular basis [24].

It may also be as a result of poor hygiene on the part of the hospital, as a few only clean and disinfect on daily basis despite the number of visitors, patients and healthcare workers that come into contact with the surfaces. It could also be attributed to the efficacy of the cleaning regime [25, 26], occasioned by the potency of the antimicrobial agent incorporated in the cleaning and disinfecting agents [27], which may not be as effective as expected, or the efficiency of cleaning, as [28] observed that out of 1404 surfaces in 157 patients room that were sampled after routine cleaning, only 47% of the surfaces were actually cleaned. It could, however, be as a result of acquired resistance to the cleaning agent or disinfectant by some of the microbes [29, 30] or a reflection of the microbiota of many of the patients, visitors or healthcare workers that come into contact with the surfaces.

3.2.3 Miscellany of Microbiota of Contact Surfaces

Miscellany of the microbiota of the high – touch and low – touch hospital contact surfaces as observed from this study include *Staphylococcus*, *Bacillus spp*., *Klebsiella spp*., *Streptococcus*, *Micrococcus*, *Pseudomonas*, *proteus*, *Corynebacterium*, *Enterococcus*, and E. coli. The fungal isolates include *Penicillin*, *Aspergillus*, *Yeasts*, *Rhizopus*, *Fusarium*, *Verticillium*, *Mucor*, and *Trichoderma spp*. The *Staphylococcus spp*. and *Corynebacterium* are found in the mucosa of human and animal skin and may have come from the skin of colonized patients, visitors or healthcare workers [31] or from the air, as infected patients shed them as part of epithelial cells [32, 33, 34] the risk of colonization, though, depends on air borne concentrations of the microbes [35]. *Bacillus*, *Micrococcus*, *Pseudomonas* and *Klebsiella* are normally found in water, soil, plants, dust or air, and may have arrived the hospital contact surfaces via air borne dust particle, *Streptococcus* is one of the oral organisms, and may have been deposited as droplets from the mouth by colonized patients, visitors or healthcare workers.

However, *Proteus* which is a saprophyte and are found in decaying organic matter, animal or human feaces, *E coli* which is an enteric organism found also in contaminated food, water, animal and human feaces 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, and may have been carried onto the contact surfaces by air, *yeast* and *Fusarium,* known to inhabit the skin, may have been inoculated by patients, visitors or healthcare personnel. *Aspergillus*, a saprophytic fungus found in decaying organic matter, soil and dust may have been carried onto the sur-

faces by dust particles.

Some of these organisms are pathogenic and of great healthcare importance, while some are opportunistic pathogens and pose great health challenge when introduced into a different anatomical site, *Staphylococcus spp*., for example are known to cause localize Staph infection leading to boil or abscess when they enter through broken skin [36] . They are also implicated in food poison [37], due to their enterotoxin [38]. Methicillin - resistant *Staphylococcus aureus* (MRSA) is well known due to its resistance to antibiotics [39] and contact route has been implicated in its transmission [40]. *Bacillus* are known to cause septicemia [41, 42] and are implicated in catheter – related bacteremia and in muscoloskeletal infections [43, 44]. Klebsiella, expercially *K. pneumonia* and *proteus mirabilis* are opportunistic pathogens and cause urinary tract infection [45, 46], especially in the elderly and the immune – suppressed individuals.

Streptococcus is implicated in throat infection, pneumonia, skin, wound infection and pharyngitis and toxic shock syndrome ([47, 48]. Micrococcus an opportunistic pathogen is involved in pulmonary infection in severe immune – suppressed individuals, [49], *Pseudomonas* also are known to colonize catheters and other medical implants, causing cross infections in the elderly and the immune – suppressed individuals [50]. *Corynebacterium striatum* have been isolated from patients with chronic obstructive pulmonary disease (COPD) [51], Enterococcus eg *E. Faecalis* and *E. faecium* are implicated in a wide range of infections including wound infection, prostatitis, endocarditis, [52] urinary tract infection etc, while *E. coli* have been established to cause urinary tract infections and diarrhea [53, 54, 55] with occasional outbreaks.

Most of the fungal species identified are known to cause disease in humans especially people with suppressed immune system. Example, Yeast especially *Candida spp* is associated with vaginal yeast infection [56], *Fusaruim* is implicated in Keratitis, Sinusitis and Mycotoxicosis [57, 58], endophthalmitis, musculoskeletal infections etc. *Rhizopus* is associated with mucromycosis [59, 60] while *Aspergillus* is known to cause pulmonary disease [61] and aspergillosis [62].

4.0 Conclusion

With the etiology of bacterial and fungal diseases known, and the miscellany of the microbiome of hospital high – touch contact surfaces and low – touch contact surfaces now elucidated, it is therefore, of great public health importance to ensure that hospital contact surfaces are continuously disinfected, to reduce microbial load and cultural yield, although, it is almost entirely impossible to achieve this due to time pressure on healthcare workers [22], shortage of staff and people flow in and out of the hospital, which presents an opportunity to re – inoculate these surfaces with microbes after disinfection [15, 16], the culture of hand hygiene using alcohol gel should be established in every hospital for both healthcare workers and visitors [63]. Future studies on the possibility of incorporating nano materials loaded with anti microbial agents in these surface coatings is now necessary [64, 65]. These bactericidal nano material coatings may gradually release in cascades, disinfecting agents, thereby making the surfaces self disinfecting, thus eliminating entirely manual disinfection, with its associated problem of time pressure on healthcare workers, and personnel cost.

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Conflict of interest

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