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(RESEARCH ARTICLE)



## Microbiological assessment of environmental surfaces in a healthcare facility

Amaka M Awanye \* and Believe Amrasawore

Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria.

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### Abstract

Hospital-acquired infections (HAIs) are infections occurring within 48 hours of hospital admission, 3 days of hospital discharge or 30 days after a surgical procedure. HAIs are a major cause of death and disability within the hospital. Inanimate surfaces can serve as sources of HAIs and can significantly contribute to the spread of pathogens within hospitals. Swab samples were obtained from 53 inanimate surfaces such as air, bed pan, ward doorknob, nurses' glove, sink, sink tap, cistern lever, toilet seat and wheel chairs located in the various wards of the Accident and Emergency Unit as well as Plastic and Burn unit of, University of Port Harcourt Teaching Hospital (UPTH). The samples obtained were screened for the presence of pathogenic microorganisms and their susceptibility to frequently prescribed antibiotics using disc diffusion method. Our findings showed highest level of contamination was the cistern lever (15 %), and the least was from nurses' gloves (6 %). Microbial identification revealed the presence of 161 microorganisms comprising *Pseudomonas aeruginosa* (27 %), *Staphylococcus spp.* (22 %), *Salmonella spp.* (16 %), *Klebsiella spp.* (13 %), and *Candida spp.* (21 %). Interestingly, 83 % of isolated staphylococci were resistant to cefoxitin, 57 % of *Pseudomonas spp.* was resistant to levofloxacin, and both *Klebsiella spp.* and *Salmonella spp.* were most resistant to ceftriaxone (81 % and 77 % respectively). The observed high prevalence of pathogenic and antibiotic resistant microorganisms calls for implementation of regular surveillance and effective infection control measures within the hospital.

**Keywords:** Nosocomial Infections; Hospital-Acquired Infections; Inanimate Surfaces; Fomites; Antimicrobial Resistance

### 1. Introduction

Hospital-acquired infections (HAIs) also known as nosocomial infections or healthcare-acquired infections are those infections that were acquired while receiving care in a hospital or healthcare facility. They include infections that occur after 48 hours of hospital admission, within 30 days of a surgical operation, 3 days after discharge from a hospital, and infections that arise other than the primary cause of hospitalization (1). HAIs are major causes of mortality and morbidity in hospitals. It has been reported that HAIs caused by resistant microorganisms increase the cost of treatment, duration of treatment, and mortality rate (2). The hospital environment especially the accident and emergency unit is a major source of infection. This is because people with various medical-related emergencies and health conditions first present to this unit before they are transferred to the appropriate department for treatment. Patients with burn injuries are at higher risk of opportunistic infections due to the loss of skin, a protective barrier to infection. Bacterial infections in patients with burn injuries increases the risk of mortality (3). The hospital environment is predisposed to harbour microorganisms considering the traffic of patients, patient-care activities performed by healthcare providers and the medical equipment used that will require frequent cleaning. Despite routine cleaning, many studies have revealed the presence of opportunistic pathogens including resistant strains in many inanimate surfaces in the hospital (4-10). Many pathogens that are involved in HAIs are harboured on inanimate surfaces. They are able to form biofilms that protect them from clearance by disinfectants.

\* Corresponding author: Awanye Amaka M

Antimicrobial resistance (AMR) has become a huge global challenge as microorganisms have developed resistance to virtually all antibiotics available. AMR increases duration and cost of treatment, reduces therapeutic outcome, reduces quality of life and increases mortality (11). Many multi-drug resistant pathogens have been identified and are implicated in many hospital-acquired infections (12). The Plastic and Burn Unit as well as Accident and Emergency unit frequently have patients in critical conditions that are more at risk of contracting infections caused by antimicrobial resistant pathogens. Hence, it is imperative that while receiving care in a hospital, that patients do not encounter pathogens that may cause opportunistic infections and complicate their already existing medical condition. Identifying inanimate sites that harbour anti-microbial resistant pathogens and the common microbial contaminants may be pivotal to the promotion and implementation of effective infection control practices within the University of Port Harcourt Teaching Hospital (UPTH).

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## 2. Material and methods

### 2.1. Study site

The study was carried out in the various wards of the accident and emergency and plastic and burn units of University of Port Harcourt Teaching Hospital (UPTH) Port Harcourt, Nigeria.

### 2.2. Sample collection

Samples were collected from inanimate surfaces or fomites such as air, clean patient's bed pan, ward doorknob, nurses' gloves, sink, sink tap, cistern lever, toilet seat and wheel chairs located in the selected wards. A description is shown in Table 1. The samples were obtained by rubbing sterile swabs sticks pre-soaked in 0.9% physiological saline on the inanimate surfaces (5 cm x 5 cm). The swabs sticks were replaced into the collection tubes and labeled appropriately. All samples were then transported to the research laboratory and inoculated in 5 ml nutrient broth (for bacterial isolation) and Sabouraud dextrose broth (for fungal isolation) within 6 hours of sample collection. Bacterial cultures were grown at 37 °C for 24 hours and 25 °C for 48 hours for fungal cultures. Exposing sterile nutrient agar and Sabouraud dextrose agar plates for 10-15 minutes collected air samples.

### 2.3. Microbial isolation and identification

All samples were cultured on selective and differential media such as cetrimide agar, mannitol salt agar, MacConkey agar and, Salmonella-Shigella agar (Titan, India) and incubated at 37°C for 24 hours for bacterial growth. Fungal culture plates were grown on Sabouraud dextrose agar (SDA) for 1 – 3 days at 25°C. The microbes were identified according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (13, 14). Species identification was based on colony morphology, Gram staining, microscopy and biochemical testing.

### 2.4. Antibacterial susceptibility testing

Determination of susceptibility of bacterial isolates to commonly prescribed antibiotics was evaluated using the Kirby-Bauer agar diffusion assay (15). In brief, bacterial inoculum is standardized with peptone water to match 0.5 McFarland solution. A 0.1 ml volume of standardized bacterial culture was spread on sterile Mueller Hinton Agar plate. Using sterile forceps, single antibiotic discs (Oxoid, UK) namely gentamicin (10 µg), amoxicillin/clavulanate (30 µg), doxycycline (30 µg), levofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), ceftiofur (30 µg) and ceftriaxone (30 µg) were placed on the surface of the agar and incubated at 37 °C for 24 hours. After incubation, the clear zones of inhibition around the discs were measured and the degree of susceptibility was determined based on the guidelines recommended by CLSI (13, 14).

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## 3. Results

### 3.1. Microbial distribution profile on fomites

A total of 53 swab samples were collected from 9 different inanimate collection sites in the 7 wards of the burn unit and accident and emergency wards of UPTH (Table 1). As shown in Table 2, microbial culture and identification revealed a total of 161 microbial isolates (127 bacterial isolates and 34 fungal isolates) from all 53 fomites. The microbes isolated from all wards include *Pseudomonas spp.* (44), *Klebsiella spp.* (21), *Staphylococcus spp.* (36), *Salmonella spp.* (26) and *Candida spp.* (34). Most wards had high levels of contamination and most sites showed evidence of poly-microbial contamination. More microbes were isolated from the general wards of the accident and emergency unit: 31 (19 %) each from the male and female wards, 23 (14 %) from the resuscitation ward and the least number 7 (4 %) were from

the theatre. In the burn unit, 33 (20 %) microbes were isolated from the female ward, 25 (15 %) from the male ward and 15 (9 %) from the children's ward.

**Table 1** Sample collection sites from various wards in the accident and emergency and plastic and burn units of UPTH

Collection site	Accident and emergency unit				Plastic and burn unit			Total Samples
	Male ward	Female ward	Resuscitation ward	Theatre	Male ward	Female ward	Children's ward	
Air	+	+	+	+	+	+	+	7
Bed pan	+	+	+	-	+	+	-	5
Cistern lever	+	+	+	+	+	+	+	7
Doorknob	+	+	+	+	+	+	+	7
Nurses' gloves	+	+	+	-	-	-	-	3
Sink	+	+	+	+	+	+	+	7
Sink tap	+	+	+	+	+	+	+	7
Toilet seat	+	+	+	+	+	+	+	7
Wheel chair	+	+	+	-	-	-	-	3
Total	9	9	9	6	7	7	6	53

(+): Sample collected; (-): No sample collected

**Table 2:** Distribution profile of microbes isolated from the various fomites

	Accident and emergency unit				Plastic and burn unit			Total isolates
	Male	Female	Resuscitation	Theatre	Male	Female	Children's	
<b><i>Staphylococcus spp.</i></b>								
Air	+	+	+	-	-	+	-	4
Bed pan	+	+	-	-	-	+	-	3
Cistern lever	+	+	+	-	-	+	-	5
Doorknob	+	+	+	-	+	+	+	6
Nurses' gloves	+	+	+	-	-	-	-	3
Sink	+	+	-	-	+	+	+	4
Sink tap	+	+	-	-	-	+	-	4
Toilet seat	+	+	+	-	+	+	-	4
Wheel chair	+	+	+	-	-	-	-	3
Total isolates	9	9	6	0	3	7	2	36
<b><i>Salmonella spp.</i></b>								
Air	-	-	-	-	-	+	-	1
Bed pan	+	+	-	-	+	+	-	4
Cistern lever	+	-	-	-	-	+	+	3
Doorknob	+	-	-	+	+	+	-	4
Nurses' gloves	+	+	-	-	-	-	-	2
Sink	+	-	-	-	+	+	+	4

Sink tap	+	-	-	-	-	+	+	3
Toilet seat	+	-	-	-	+	+	+	4
Wheel chair	+	-	-	-	-	-	-	1
Total isolates	8	2	0	1	4	7	4	26
<b><i>Pseudomonas spp.</i></b>								
Air	-	-	+	+	+	+	+	5
Bed pan	+	+	+	-	+	+	-	5
Cistern lever	+	+	-	+	+	+	+	6
Doorknob	-	+	-	+	+	+	-	4
Nurses' gloves	+	+	-	-	-	-	-	2
Sink	+	+	+	+	+	+	+	7
Sink tap	+	+	+	+	+	+	+	7
Toilet seat	+	+	-	+	+	+	-	5
Wheel chair	+	+	+	-	-	-	-	3
Total isolates	7	8	5	6	7	7	4	44
<b><i>Klebsiella spp.</i></b>								
Air	-	-	+	-	-	+	-	2
Bed pan	-	-	-	-	+	+	-	2
Cistern lever	-	+	+	-	-	+	+	4
Doorknob	-	+	+	-	-	+	-	3
Nurses' gloves	-	-	+	-	-	-	-	1
Sink	-	+	+	-	-	+	-	3
Sink tap	-	-	-	-	-	+	-	1
Toilet seat	+	-	+	-	+	-	-	3
Wheel chair	+	+	-	-	-	-	-	2
Total isolates	2	4	6	0	2	6	1	21
<b><i>Candida spp.</i></b>								
Air	-	+	+	-	-	-	+	3
Bed pan	-	-	-	-	+	+	-	2
Cistern lever	+	+	+	-	+	+	+	6
Doorknob	+	+	-	-	-	+	-	3
Nurses' gloves	-	+	+	-	-	-	-	2
Sink	+	+	+	-	+	+	-	5
Sink tap	-	+	-	-	+	+	+	4
Toilet seat	+	+	+	-	+	+	+	6
Wheel chair	+	+	+	-	-	-	-	3
Total isolates	5	8	6	0	5	6	4	34
<b>Frequency</b>	<b>31</b>	<b>31</b>	<b>23</b>	<b>7</b>	<b>25</b>	<b>33</b>	<b>15</b>	<b>161</b>
<b>Relative frequency (%)</b>	<b>19</b>	<b>19</b>	<b>14</b>	<b>4</b>	<b>15</b>	<b>20</b>	<b>9</b>	<b>100</b>

Following the results obtained as presented in Table 3, it shows that *Pseudomonas spp.* was the most prevalent microbe isolated (27 %), followed by *Staphylococcus spp.* (22 %), *Candida spp.* (21 %), *Salmonella spp.* (16 %) and the least was *Klebsiella spp.* (13 %). *Pseudomonas spp.* unlike other microbes isolated was obtained from every ward including the theatre in the accident and emergency unit (Table 2). Our findings also show that the toilet area poses the highest risk of nosocomial infection and that the highest number of microbial pathogens were isolated from the cistern lever (15 %) followed by toilet seat (14 %), sink (14 %), sink tap (12 %) and door knob (12 %). The least number of microbes were isolated from nurses' gloves (6 %).

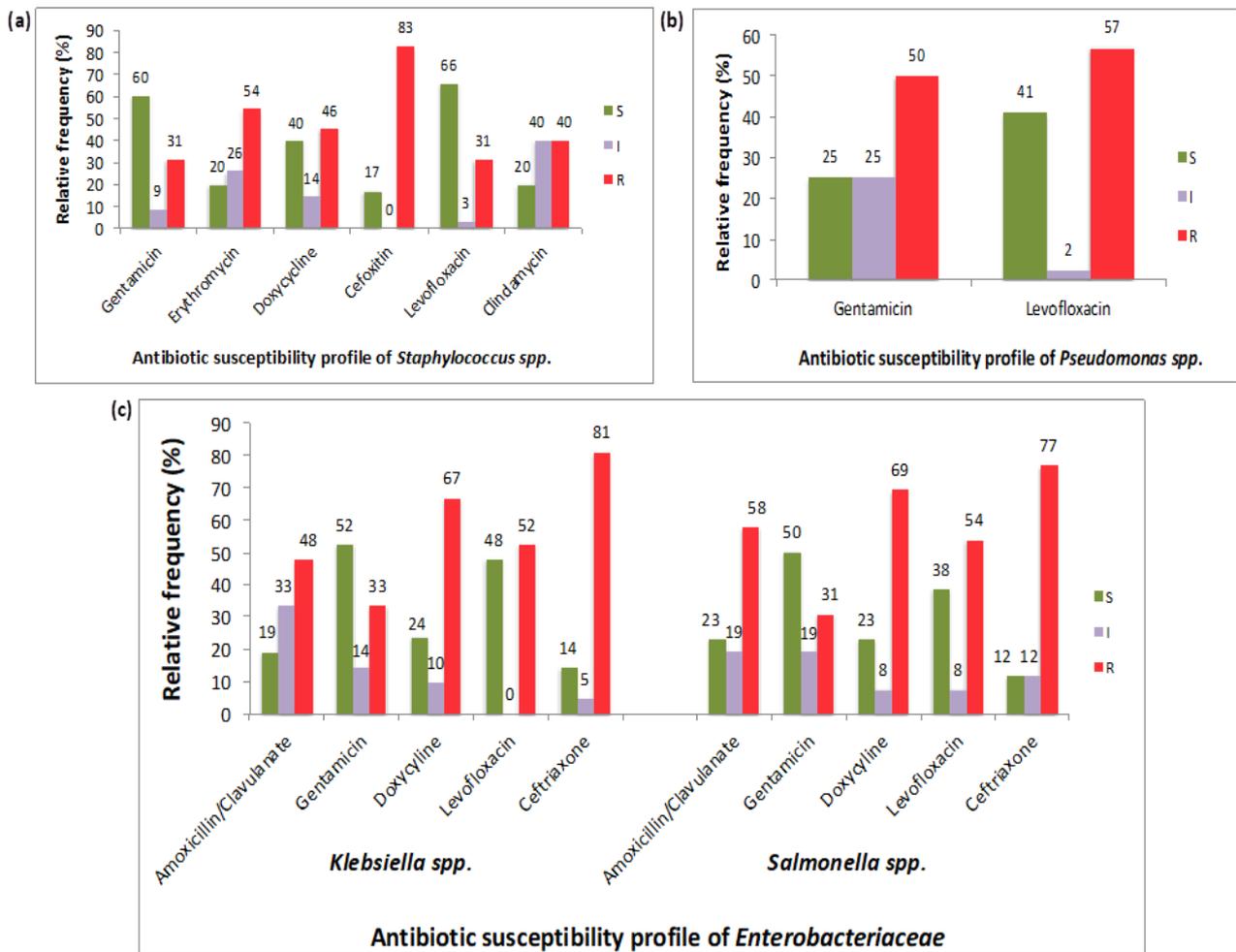
**Table 3:** Relative frequency of occurrence of isolates

	<i>Staphylococcus spp.</i>	<i>Salmonella spp.</i>	<i>Pseudomonas spp.</i>	<i>Klebsiella spp.</i>	<i>Candida spp.</i>	Total frequency (N)	Relative frequency (%)
Air	4	1	5	2	3	15	9
Bedpan	3	4	5	2	2	16	10
Cistern lever	5	3	6	4	6	24	15
Doorknob	6	4	4	3	3	20	12
Nurses' gloves	3	2	2	1	2	10	6
Sink	4	4	7	3	5	23	14
Sink tap	4	3	7	1	4	19	12
Toilet seat	4	4	5	3	6	22	14
Wheel chair	3	1	3	2	3	12	7
Total microbes isolated	36	26	44	21	34	161	100
Relative frequency of microbial isolation (%)	22	16	27	13	21	100	

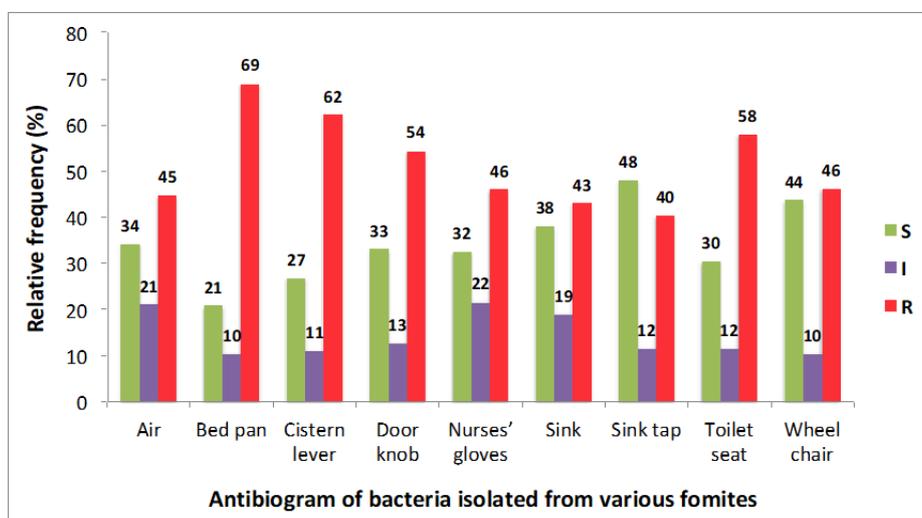
### 3.2. Antibigram profile of isolated bacteria

Antibacterial susceptibility testing carried out on all bacteria isolated from various fomites showed varying degrees of susceptibility to commonly prescribed antibiotics (Fig. 1). Isolated staphylococci showed highest level of sensitivity to levofloxacin (66 %) followed by gentamicin (60 %), doxycycline (40 %), erythromycin (20 %), and clindamycin (20 %). Interestingly, 83 % of isolated *Staphylococcus spp.* was resistant to ceftiofur, indicating the presence of methicillin resistant *Staphylococcus aureus*. The susceptibility of isolated *Pseudomonas spp.* to the 2 anti-pseudomonal drugs tested showed a higher level of susceptibility to levofloxacin (41 %) than gentamicin (25 %). Both *Klebsiella spp.* and *Salmonella spp.* showed high levels of resistance against amoxicillin/clavulanate, doxycycline, levofloxacin and ceftiofur. The highest level of resistance in *Klebsiella spp.* and *Salmonella spp.* was recorded for ceftiofur, 81 % and 77 % respectively. And both *Enterobacteriaceae* were most sensitive to gentamicin 52 % and 50 % respectively.

The antibiotic susceptibility pattern for every bacterium isolated from the sampled fomites showed a diverse distribution pattern (Fig. 2). The highest amount of resistant bacteria were isolated from bed pans (69 %), followed by cistern lever (62 %), toilet seats (58 %), door knobs (54 %), nurses' gloves (46 %), wheel chair (46 %), air (45 %), sink (43 %) and least from sink tap (40 %). Conversely, the sink tap (48 %) harboured more susceptible bacterial than the bedpan (21 %).



**Figure 1:** Antibiotic susceptibility pattern of isolated bacteria. Test was carried out using the disc diffusion method and susceptibility cut-offs used were in accordance with the recommendation by CLSI. All tests were performed in duplicates. S: sensitive; I: intermediate susceptibility; R: resistance.



**Figure 2:** Antibigram of bacteria isolated from fomites in various wards of accident and emergency ward plastic and burn units of UPTH. S: sensitive; I: intermediate susceptibility; R: resistance.

#### 4. Discussion

Despite good infection control practices employed in the hospital, our findings revealed the presence and prevalence of pathogenic microorganisms in various fomites at the Accident and Emergency Unit and Plastic and Burn Unit of University of Port Harcourt Teaching Hospital. Different pathogenic bacteria and fungi from various fomites were obtained from all wards sampled. The study showed more contaminated surfaces in the Accident and Emergency Unit than the Plastic and Burn Unit. The higher incidence of contaminated fomites in the Accident and Emergency Unit may be attributed to high patient traffic, rapid patient turnover and even overcrowding compared to the Plastic and Burn Unit. Pathogenic microorganisms isolated from the 53-screened fomites include: *Staphylococcus spp.*, *Pseudomonas spp.*, *Salmonella spp.*, *Klebsiella spp.* and *Candida spp.* This suggests that these contaminated fomites are capable of giving rise to nosocomial infections. The distribution showed that *Pseudomonas spp.* had the highest frequency of occurrence (27 %), followed by *Staphylococcus spp.* (22 %), then *Candida spp.* (21 %), *Salmonella spp.* (16 %) and then the least occurring microbe was *Klebsiella spp.* (13 %). Amongst all wards screened, fomites sampled in the theatre showed the lowest level of contamination (4 %). This is probably due to a stricter infection control practice within the theatre as well as restriction to unauthorized personnel as it is only patients undergoing surgery and the healthcare providers that have access into the theatre. It is worthy to note that 6 out of 7 microbes (86 %) isolated from the theatre were *Pseudomonas spp.* This implies that the infection control strategy such as the disinfectant used in the theatre is not as effective in combating *Pseudomonas spp.* as it is with other microbes.

Furthermore, the toilet area poses a higher risk of exposure to nosocomial pathogens. The frequency of isolating pathogens were highest from the toilet area namely cistern lever (15 %), toilet seat (14 %), sink (14 %), and sink tap (12 %). A study has shown that toilet flushing can aerosolize faecal matter thereby contaminating the air and surfaces (16). Besides the surfaces in the toilet area, other surfaces screened for microbial contamination include the doorknob leading into the wards, bedpans, air, and wheelchair handles. All surfaces screened are commonly in contact with patients and healthcare providers at the accident and emergency unit and plastic and burn unit of UPTH. The presence of microbial contamination in these surfaces creates a possibility of transfer of infection.

Several studies have also shown the presence of pathogenic bacteria in various inanimate surfaces within hospitals premises such as medical charts (4), stethoscopes (5), thermometers (6), work surfaces (7), medical equipment (8), medical devices (9), white coats (10), mobile phones (17). Many microbes survive for long periods of time on inanimate surfaces and contribute to challenges associated with the control of hospital-acquired infections. In a systematic review, Kramer and colleagues concluded in their study that nosocomial pathogens may persist on inanimate surfaces for months and can thereby serve as a continuous source of transmission if regular disinfection is not performed (18). The quality and quantity of microbes on surfaces depends on many factors such as type of contaminating microorganism, their ability to form biofilms, and their resistance to disinfectant used. In addition, the nature of the surface and the presence of cracks on surfaces can favour microbial colonization. Furthermore, the quality of microbes on any surface depends on the quality of air. This is because airborne pathogens will inevitably settle on surfaces. Hence, despite adequate cleaning of surfaces, poor air treatment will impact negatively on the cleaning efficiency.

Air quality and inanimate surfaces contribute significantly to many infections acquired in the community and hospital environments. The recent outbreak of the novel 2019 coronavirus disease (COVID-19) has shown high incidence of hospital transmission of the infecting virus (SARS-CoV-2) among healthcare workers (19). Transmission is thought to be mostly through contact with contaminated surfaces and inhalation of airborne droplets. This necessitated the public health recommendation for preventing its spread by maintaining social distancing of at least 6 feet, frequent hand washing and use of facemasks for infected patients and healthcare providers (20). The role of air and surfaces in the transmission of nosocomial infections was highlighted in a study carried out by Wang and co-workers. Healthcare workers directly involved in treatment of COVID-19 patients that wore facemasks and frequently washed their hands were protected more against SARS-CoV-2 infection despite having a significantly higher risk of exposure. As compared with a higher incidence of COVID-19 in other healthcare workers within the same hospital that did not wear facemask, washed their hands occasionally and were not involved in the treatment of COVID-19 patients (21).

Furthermore, the findings of this study revealed a high prevalence of multidrug resistance involving both Gram-negative and Gram-positive bacteria. A very striking observation was the high level of resistance (83 %) to cefoxitin, an indication of the presence of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital. *S. aureus* is a commonly isolated hospital pathogen and the cause of many nosocomial infections (22). *Pseudomonas spp.* was the most prevalent microbe isolated. It was isolated from every ward sampled including the theatre and poses a huge risk of contracting post-operative surgical infections. *Pseudomonas aeruginosa* is an opportunistic organism in immuno-compromised individuals and a common nosocomial pathogen implicated in many chronic infections such as pneumonia (23). It exhibits intrinsic resistance to a wide range of antibiotics. The high level of resistant to both anti-pseudomonal

antibiotics tested calls for public health concern. Both *Klebsiella spp.* and *Salmonella spp.* isolated were highly resistant to ceftriaxone, a commonly administered antibiotic in UPTH. *Klebsiella spp.* is a common hospital pathogen implicated in many respiratory tract and urinary tract infections. Infections caused by *Salmonella spp.* are often spread through oral-faecal route. Presence of *Salmonella spp.* is indicative of poor hygiene practices within the hospital environment.

The high incidence of antibiotic resistance in isolated bacteria to commonly prescribed antibiotics is of great public health concern. Frequent antibiotic use in hospitals exerts selective pressure on the development of antibiotic resistant bacteria. It has been shown that increase in antibiotic use increases the development and spread of antibiotic resistance (24, 25). As a result, antimicrobial resistance increases number of drugs used in treatment, cost of treatment, duration of treatment, and in severe cases, may be life-threatening if infection is uncontrolled (2, 26, 27). Hence, effective infection control strategies should be adopted in the hospital such as frequent and deep cleaning (28); use of motion sensors to control cistern levers, sink taps and doorknobs; use of automatic UV room disinfectant systems (29) and the use of antimicrobial coated surfaces such as copper, silver (30, 31).

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## 5. Conclusion

The study reveals high levels of microbial contamination in inanimate surfaces within the hospital including pathogenic organisms that have the potential to cause hospital-acquired infections. It is recommended that adequate surveillance programs should be set up to monitor and prevent development of hospital-acquired infections.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there are no conflicts of interest.

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