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# **Comparative Evaluation of Nosocomial Infections in Two Major Hospitals in Calabar Metropolis, Cross River State**

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#### **Authors' contributions**

This work was carried out in collaboration between all authors. Authors UEG, CIM, ENM and UOE designed the study. All authors did sample collection, laboratory and data analysis. All authors read and approved the final manuscript.

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# **ABSTRACT**

**Aim:** This study comparatively evaluates nosocomial infections in two major hospitals in Calabar metropolis, Cross River State.

**Place and Duration of Study:** This study was carried out in two major hospitals which were General Hospital (GH) and Infectious Disease Hospital (IDH) located in Calabar, Cross River State, Nigeria. The study lasted for 2 months from samples collection to report writing.

**Methodology:** Bacteria and fungi were isolated using settle plate technique and isolates were subjected to antibiotics sensitivity, minimum and bactericidal concentration tests. Resulting data were analysed using simple descriptive statistics and student t –test.

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Results: A total mean load of 1,002 cfu/m<sup>2</sup>/hr was recorded of which 612.1cfu/m<sup>2</sup>/hr was recorded in General hospital and 389.9 cfu/m<sup>2</sup>/hr was recorded in Infectious Disease Hospital (IDH). Although the wards of both hospitals did not recorded the highest loads, they showed the highest microbial diversities of 23(23.2%) and 19 (21.6%) for GH and IDH, respectively. Salmonella species and Escherichia coli from GH showed resistance to taravid, nalidixic acid, reflacine and ciproflox. E. coli, Salmonella, Klebsiella, Proteus species and P. aeruginosa exhibited a wide range of resistance against tarivid, reflacine, ciproflox, ceporex, nalidixic acid and also moderately to amplicin. Penicillium and Aspergillus species from both hospitals showed higher resistance to ketoconazole than nystatin. Comparism of the mean loads in both hospital showed significance ( $p =$ 0.01). In IDH, S. aureus recorded MICs and MBCs of 1:32 and 1:16, respectively while for Streptococci species it was 1:16-1:64 and 1:8-1:32, respectively. However, in GH, E. coli and Streptococci recorded MICs and MBCs in the range of 1:32-1:512 and 1:16-256, respectively. **Conclusion:** The test isolates when subjected to antimicrobial susceptibility testing exhibited varied patterns of resistance to antibiotics/antifungal agents. This calls for effective monitoring of the air quality in healthcare settings in a bid to reducing nosocomial infections.

Keywords: Nosocomial infections; airborne; microbial load; resistant profile; hospitals; Nigeria.

## **1. INTRODUCTION**

Airborne sources of possible bacterial contamination of the environment of hospitals have long been debated as potential causes of nosocomial infections. This has contributed to the already existing burden of nosocomial infections in the health care settings [1]. Nosocomial Infections (NI) also known as healthcare-associated infections (HCAI) or Hospital-acquired infections (HAI) are infections that arise within few hours of admission into the hospital and were not present at the time of admission. They have been reported globally in hospital environments and they constitute a major hazard confronting patients and personnel within hospital environments [2]. These infections have been reported by Witherspoon [3] to be most often silent while the patient is still in the hospital and account for significant morbidity and mortality.

The units of healthcare environment as revealed by Gupta [4] represent a significant facility in the healthcare settings, providing segregation, healthcare settings, providing segregation,<br>special care, accommodation, succor and special care, accommodation, protection for the sick. Despite advances in human capacity and technology development in the healthcare sector, many hospitals especially in developing countries are still faced with the challenges of nosocomial infections [5]. This may be due largely to the poor infection control practices in these hospitals [6]. The development of nosocomial infections and its severity is linked to several microbial agents. However, the emergence and re-emergence of highly virulent infectious agents further compound the menace, contributing to the increase morbidity and

mortality observed in hospitalized patients; increased burden of discomfort and high socioeconomic cost [7].

Poor disinfection practices, ineffective use of antibiotics, monitoring of the hospital's air and units against overcrowding, poor management and inadequate surveillance teams to manage, sustain and ensure that aseptic hospital ethics has further aggravated the problem [8]. In developing countries including Nigeria, these inadequacies abound in majorities of the hospital settings, creating a safe haven for nosocomial infections [9]. This has been confirmed by several reports including the one recorded by Muhammed et al. [10] who reported high frequency of pathogenic bacteria including Staphylococcus aureus, Proteus species, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Klebsiella species, Salmonella species and Shigella species from hospital sinks, floors, bed covers, toilets and ward walls isolated from Northern Nigeria. Despite the clinical implications associated with nosocomial infections, few studies exist that have evaluated the impact of nosocomial infections within the major hospitals of the state. This study therefore was aimed at evaluating nosocomial infections in two major hospitals in Calabar metropolis, Cross Rivers State.

# **2. MATERIALS AND METHODS**

#### **2.1 Study Site**

This study was carried out in two major hospitals in Cross River State, Nigeria. The hospitals were General Hospital (GH) and Infectious Disease

Hospital (IDH) and are located on latitude  $4^{\circ}59'N$ and longitude 8°15'E, respectively.

#### **2.2 Sources of Samples**

Samples were collected in December 2016 using settle plate technique from various units of these hospitals including pharmacy, theatre, laboratory, blood bank and intensive care units of both hospitals.

## **2.3 Microbiological Analysis**

The air qualities of five units of each hospital was assessed by exposing plates in triplicates containing nutrient agar, sabouraud dextrose agar and blood agar, respectively for 1hour following procedures described by Centre for Disease Centre [11] after which the plates were<br>aseptically packaged and immediately aseptically packaged and immediately transported to microbiology laboratory where they were incubated at 37°C for 24-48 hours. After incubation, the plates were examined for growth and microbial load determined. Purified colonies were identified and characterized following standard microbiological procedures [12].

#### **2.4 Antimicrobial Susceptibility Testing**

This was done following procedures described previously by CLSI, (2004) and CLSI (2014) for fungi and bacteria, respectively [13,14]. Standardized inoculums were inoculated into plates containing freshly prepared Muller Hinton agar and allowed to stand for 15 minutes. Then, antibiotic discs were placed aseptically on the surface of the inoculated plates using sterile forceps and pressed lightly to ensure contact with the agar surface and the plates were incubated at 37°C for 24-48 hours. After incubation, zones of inhibition were measured and compared with appropriate interpretive chats.

# **2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

This was performed following procedures described CLSI [14]. Briefly, 2-3 colonies of the test isolate was inoculated into 5ml of sterile peptone broth/sabouraud dextrose broth and incubated for 30 minutes. Antibiotics of various concentrations were dissolved in sterile test tubes containing 5ml of diluents (distilled water

and dimethyl-sulphur oxide, respectively) to make stock solutions. Doubling dilutions of the antibiotics in the order of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024 were prepared. Standardized inoculums were added to each of the tubes and incubated for 24-48 hrs and the MICs determined. The MBCs were determined by sub-culturing tubes which showed no growth (turbidity) during the MIC tests into plates containing freshly prepared nutrient agar and sabouraud dextrose agar plates, respectively and incubated for 24-48 hrs at  $37^\circ$ .

# **2.6 Data Analysis**

Descriptive analysis such as simple percentages and student t-test was employed in this study. These were all done using Microsoft Excel office 2010.

# **3. RESULTS**

#### **3.1 Microbial Load**

The result of the microbial load and their percentages as observed in various units of general hospital (GH) and infectious disease hospital (IDH) are summarized in Table 1 and Figs. 1 and 2, respectively. In GH, pharmacy had a mean load of 128.90 (21.1%) while ICU had a mean load of 129.9  $(21.2%)$  cfu/m<sup>2</sup>/hr. However, laboratory, blood bank and ward had mean loads and percentages of 124 (20.3%), 136 (22.2%) and 93.7 (15.3%) cfu/m<sup>2</sup>/hr, respectively. Similarly, in IDH, pharmacy had a mean load of 67.40 (17.4%), ICU 59.70 (15.3%), laboratory 115.8(29.7%), blood bank 68.6(17.6%) and ward78.4 (20.1%). Student ttest analysis of the mean loads from the sampled units of both hospitals gave a significant probability value of 0.01 at 95% significance level.

#### **Table 1. Mean bacteria loads (cfu/m<sup>2</sup> /hr) of both hospitals**



significance



**Fig. 1. Percentage microbial load in General Hospital** 

## **3.2 Number and Distribution of Microbial Isolates**

A total of 187 isolates were recovered from both hospitals of which 99 (52.9%) and 88(47.1%) were recovered from General Hospital (Table 2) and Infectious Diseases Hospital (Table 3), respectively. In General hospital, the ward had the highest number of isolate of 23(23.2%) followed by, pharmacy 22 (22.2%), laboratory 21 (21.2%), Intensive care unit 17 (17.2%) and Blood bank 16 (16.1%). Similarly, in Infectious Diseases Hospital (Table 5), Blood bank and ward recorded the highest number of isolates 19 (21.6%), respectively followed by pharmacy 18 (20.5%) while ICU and laboratory recorded 16 (18.2%), respectively.

# **3.3 Antimicrobial Susceptibility Pattern of Isolates Recovered from General Hospital and IDH**

Isolates employed in this study exhibited varying degrees of resistance to commonly used antibiotics as presented in Tables 4 and 5. In General hospital, Salmonella species showed resistance to tarivid and nalidixic acid. Other microbial isolates showed moderate to low resistance to antibiotics and antifungal agents as shown in Table 4. However, in IDH as shown in Table 4, Escherichia coli, Salmonella species, Klebsiella species, Proteus species and P. aeruginosa exhibited a wide range of resistance against tarivid, reflacine, ciproflox, ceporex,

nalidixic acid and moderately to amplicin. These organisms however, showed low resistance to augmentin, gentamycin, septrin and streptomycin as shown in Table 5. In addition, Staphylococcus aureus strains exhibited marked resistance against norfloxacan, ciproflox, streptomycin and levofloxacin while Streptococci showed resistance to norfloxacin, amoxil, ciproflox, chloramphenicol, erythromycin, ampiclox and levofloxacin. Furthermore, Penicillium and Aspergillus species showed resistance to ketoconazole.

# **3.4 Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of Test Isolates**

The MICs and MBCs of microbial isolates are as shown in Tables 6 and 7, respectively. The MICs and MBCs of E. coli, Salmonella species and P. aeruginosa to various antibiotics ranged from 1:16 - 1:64 and 1:8 - 1:32 while that of Klebsiella species ranged from 1:16- 1:128 and 1:8-1:64, respectively. Furthermore, S. aureus recorded MICs and MBCs of 1:32 and 1:16 respectively while Streptococci recorded 1:16-1:64 and 1:8- 1:32 respectively. In addition, Candida and Aspergillus species recorded MICs and MBCs in the range of 1:16-1:32 and 1:8 and 1:16 respectively. However, in GH, E. coli and Streptococci recorded MICs and MBCs in the range of 1:32-1:512 and 1:16-256 respectively while Salmonella species, Klebsiella species and Proteus species exhibited MICs and MBCs in the range of 1:64-1:512 and 1:32-1:256 respectively. Meanwhile, S. aureus and P. aeruginosa had MICs and MBCs in the range of 1:64-1:512, 1:32- 1:128 and 1:32-1:1024 and 1:16-1:64 respectively. Aspergillus species recorded 1:64 and 1:32 as MIC and MBC while Candida albicans recorded 1:32-1:64 and 1:16-1:32 as MICs and MBCs respectively as shown in Table 9.

#### **4. DISCUSSION**

The hospital environment is a complex environment on its own and interactions with different microorganisms lead to infections and re-infections. Several factors may determine which microorganism that will be responsible for a particular infection. Such factors may include the length and nature of which the patient was exposed, virulence and microbial load of microorganism, and also the state of the patients defense [15][16]. Airborne sources of bacterial contamination of the environment of hospital settings have long been debated as an important route of increasing incidence of nosocomial infections [16]. This can contribute to the already existing burden of nosocomial infections in health care set up [17].

The mean load observed in this study is higher than the results obtained by Omoigberale 18] in Ekpoma, Edo State, Nigeria. In addition, a higher mean load (612.1 cfu/m<sup>2</sup>/hr) was observed in General hospital (612.1 cfu/m<sup>2</sup>/hr) compared to

389.9 cfu/m<sup>2</sup>/hr in IDH which are higher the acceptable  $35c$ fu/m<sup>3</sup> per room [16]. In General hospital, blood bank had the highest mean load of 135.6 cfu/m<sup>2</sup>/hr (22.2%) whereas in IDH, the largest was in the laboratory unit which accounted for 115.8  $ctu/m^2/hr$  (29.7%). The higher load in blood bank unit could be due in part to high moisture of the unit, low temperature and possibly poor disinfection of the phlebotomy unit [19]. In addition, the finding of a higher mean load in the laboratory may not be unconnected to the fact that clinical samples containing a vast majority of microorganisms are usually collected and processed there. The temperature, humidity, nutrient media used in the laboratories as well as storage conditions could be contributory factors [20]. Also, this high mean load could also be attributed to poor ventilation in the units. A mean load of 129.9 cfu/m<sup>2</sup>/hr (21.2%) observed in the intensive care unit of GH where patients with critical conditions are kept is worrisome and calls for urgent review of disinfection protocols. Studies have shown that air, temperature, relative humidity, ventilation systems, outdoor penetration and occupant density influence the quantity of airborne pathogens [16,21].

Furthermore, a total of 99 isolates were recovered from General hospital of which 67(67.7%) were bacteria and 32 (32.3%) were of fungal origin while in IDH, a total of 88 isolates were recovered of which 64(72.7%) were bacteria and 24 (27.2%) were fungal isolates. Organism including Escherichia coli, Salmonella species, Klebsiella species, Candida species,



**Fig. 2. Percentage microbial load in Infectious Disease Hospital**



# **Table 2. Distribution of Isolates in General Hospital (GH) units**

**Table 3. Distribution of Isolates in Infectious Disease Hospital (IDH) units** 





## **Table 4. Resistant patterns of microorganisms isolated from IDH**

**Table 5. Resistant patterns of microorganisms isolated from GH** 



Aspergillus sp (9) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_4(44%) \_\_\_\_\_1(11%)<br>Keys: \_\_OFX = TARIVID, \_CEP = CEPOREX, RD = RIFAMPCIN, PEF = REFLACINE, NA = NALIDIXIC ACID, E = ERYTHROMYCIN, CPX = CIPROFLOX, SXT = SEPTRIN,<br>CH = CHLORAMPHENICOL, U =  $S = STREPTOMYCIN, AML = AMOXIL, K = KETOCONAZOLE, NY=NYSTATIN$ 





ND= Not determined.

<b>Organisms</b>	TEST	<b>OFX</b>	<b>PEF</b>	CPX	AU	CΝ		<b>CEP</b>	<b>NA</b>	<b>SX1</b>	<b>PN</b>	<b>NB</b>	<b>AML</b>	<b>RD</b>	Е	CН	<b>APX</b>	LEV	Κ	<b>NY</b>
Escherichia coli	<b>MIC</b>	1:128	:256	:128	:256	1:512	1:128	$\cdot 32$	1:32	1:256	1:128	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	ND	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
	<b>MBC</b>	1:64	1:128	:64	:128	:256	l∶64	1:16	1:16	:128	1:64									
Salmonella species	<b>MIC</b>	1:64	1:64	:512	512: ا	1:64	512: ا	:256	1:64	1:128	:128	ND.	<b>ND</b>	<b>ND</b>	<b>ND</b>	ND	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
	<b>MBC</b>	1:32	1:32	:256	256: ا	1:32	1:256	:128	1:32	1:64	1:64									
Candia species	<b>MIC</b>	<b>ND</b>	ND.	ND	ND.	<b>ND</b>	ND	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	ND	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	1:32	1:64
	<b>MBC</b>																		1:16	1:32
Klebsiella species	MIC	1:512	:512	:256	1:128	1:64	1:64	1:512	1:64	1:128	:256	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
	<b>MBC</b>	1:256	:256	:128	l:64	1:32	1:32	:256	1:32	l:64	1:128									
Proteus mirabilis	<b>MIC</b>	l:64	1:64	:512	1:64	1:256	1:256	:64	1:64	:64	1:256	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	ND	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
	<b>MBC</b>	1:32	1:32	:256	1:32	1:128	1:128	:32	1:32	l:32	1:128									
Pseudomonas aeruginosa	<b>MIC</b>	1:128	1:128	:128	∣:32	1:64	1:32	:64	1:128	:64	1:64	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	ND	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>
	<b>MBC</b>	1:64	1:64	:64	1:16	1:32	1:16	1:32	1:64	1:32	1:32									
Staphylococcus aureus	<b>MIC</b>	<b>ND</b>	<b>ND</b>	1:128	<b>ND</b>	1:128	1:128	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	l:128	1:64	1:64	:256	:64	:128	1:512	ND.	<b>ND</b>
	<b>MBC</b>			1:64		1:64	1:64					1:64	1:32	$\cdot$ :32	∷128	:32	1:64	1:256		
Penicillum species	<b>MIC</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	ND	<b>ND</b>	<b>ND</b>	1:128	1:64
	<b>MBC</b>																		1:64	1:32
Streptococcus species	<b>MIC</b>	<b>ND</b>	<b>ND</b>	1:64	<b>ND</b>	1:32	1:32	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	64: ا	1:32	:64	1:64	1:64	1:64	1:512	<b>ND</b>	<b>ND</b>
	<b>MBC</b>			1:32		1:16	1:16					1:32	1:16	∣:32	1:32	1:32	1:32	1:256		
Aspergillus species	<b>MIC</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	ND	ND	ND	<b>ND</b>	<b>ND</b>	1:64	1:64
	<b>MBC</b>																		1:32	1:32

**Table 7. Summary of MICs and MBCs of test isolates in General hospital** 

ND = Not determined.

Staphylococcus aureus, Aspergillus species, Penicillium species, Proteus mirabilis, Streptococcus species and Pseudomonas aeruginosa were isolated in both hospitals [17]. The most isolated organisms in GH were Candida species and Staphylococcus aureus were the most common in the laboratory and intensive care unit. In IDH, Salmonella sp, Klebsiella species, and Aspergillus species which showed high occurrence in laboratory, pharmacy and blood bank respectively whereas in location two, Generally, fungal isolates accounted for 29.9% of all microbial isolates. However, 32.3% and 27.3% of fungi were isolated from General hospital and IDH, respectively.

In addition, gram negative organisms were more predominant (52.4%) in the environment of these two hospitals than gram positive organisms (17.6%) and fungi (29.9%). This is in line with researches conducted by Musaddiq [22] and Garcia-Cruz [23] where they observed that gram negative organisms were more common in the hospital environment than gram positive ones. The high percentage of gram negative organisms observed in this study is extremely higher than 4.9% reported by Lemmen [24]. The high occurrence of gram negative bacteria in the hospital environment may also be due to their ability to withstand adverse environmental conditions. Also, fungal isolates including Aspergillus, Candida and Penicillum species were the most dominant fungi isolated from these hospitals. This is consistent with findings of other researchers including Garcia-Cruz [23], and Abdollahi and Mahmoudzadeh [25] who confirmed the dominance of Penicillum and Aspergillus species in hospital units.

Furthermore, test isolates were subjected to susceptibility testing using a number of antibiotics. General hospital isolates were observed to be less resistant to commonly used antibiotics than test organisms isolated from IDH. E. coli isolated from IDH showed considerable resistance to antibiotics including tarivid, reflacine, ciprolox, augmentin, gentamycin, streptomycin, ceporex, nalidixic acid, septrin and amplicin compared to Escherichia coli strains isolated in GH where the highest was observed with reflacine. The percentage of P. aeruginosa resistance (30-63%) to ciprofloxacin recorded in this study is in line with 60-70% reported by Kumari et al. [26]. The 33-80% resistance exhibited by gram negative organisms against tarivid in this study is lower than 91% reported

by Gandham and Amatullah [27]. The resistance range of 27-71% of rifampicin against gram positive organism observed in this study is extremely higher than 14% reported by Omoigberale et al. [18]. However, amoxil resistance of 100% against gram positive organism reported previously [18] is consistent with 46-90% observed in this study.

In IDH, Salmonella species showed more resistance (8) to tarivid, reflacine and nalidixic acid while in General hospital, the highest (6) was seen with tarivid and nalidixic acid, respectively. Klebsiella species and Proteus mirabilis isolates showed a peak resistance with tarivid and reflacine respectively in IDH while in General hospital, both isolates were also high. Pseudomonas aeruginosa from IDH showed a higher rate of resistance with amplicin compared to General hospital that showed resistance to both amplicin and gentamycin.

Also, gram positive and fungal isolates from IDH also displayed greater resistance patterns compared to General hospital. This marked resistance of isolates observed in IDH may be due to poor use and misuse of antibiotics in the hospital environments.

The MIC and MBC of the test isolates obtained in this study showed General hospital had the highest MIC and MBC values compared to IDH. Escherichia coli showed a maximum MIC and MBC with ciprofloxacin (MIC 1:128; MBC 1:64) in IDH while in General hospital, gentamycin (MIC 1:512; MBC 256) were the known concentration. Salmonella species has a maximum concentration of MIC and MBC of 1:64, 1:32 with ceporex and amplicin respectively. In IDH, the highest concentration was observed with Salmonella species isolates against ceproflox, augmentin and streptomycin with MIC and MBC of 1:512 and 1:256, respectively. Klebsiella species in General hospital showed MIC and MBC of 1:512 and 1:256 for tarivid, reflacine and ceporex respectively while in IDH, it was just reflacine at a concentration of 1:128 and 1:64. Staphylococcus aureus and Streptococcus species showed the same concentration of MIC and MBC with levofloxacin in General hospital but this varied in IDH. For fungal isolates in IDH, the concentration of MIC and MBC was seen with ketoconazole (MIC 1:64, MBC 1:32) for Penicillium species whereas Penicillium species which is the highest also in General hospital was MIC1:128 and MBC1:64 with the same antibiotics.

# **5. CONCLUSION**

The findings in this study confirm the fact that air quality in hospital environments is an important reservoir of microbes. The mean microbial loads of 389.9 and 612.1 cfu/ $m^2$ /hr reported for both IDH and GH were far above recommended and safe means levels. A total of ninety-nine isolates recovered from both hospitals were distributed amongst bacteria and fungi groups routinely implicated in nosocomial infections. Furthermore, all the isolates showed varying levels of resistance to all the test antimicrobials used in this study. Given these findings, there is need for monitoring of the hospital air especially in the units. Furthermore, hospital management, medical personnel and patients should be encouraged to imbibe high levels of hygiene in order to help reduce nosocomial infections.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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