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# BIOASSESSMENT OF INDOOR AIR IN THREE HOSPITALS (SECONDARY HEALTH CARE FACILITIES) IN DELTA CENTRAL SENATORIAL DISTRICT, DELTA STATE, NIGERIA

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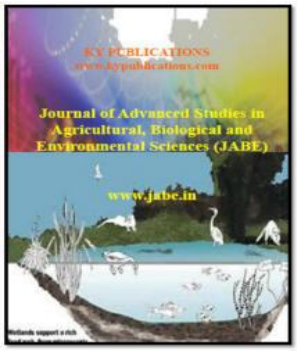


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**ABSTRACT**
The airborne microflora of indoor air in three hospitals in Delta Central Senatorial District of Delta State, Nigeria, was assessed by sampling the indoor air of female, male and children wards as well as theater of each hospital for a period of sixty days using the exposed plate technique. Thew three hospitals were designated Hospital A, B and C. Nine hundred and thirteen bacterial isolates and one hundred and ten fungi isolates were obtained. The bacterial isolates belonged to seven genera which were Bacillus, Escherichia, Pseudomonas, Proteus, Staphylococcus, Klebsiella and Streptococcus. The fungi isolates include *Aspergillus* spp, *Mucor* spp and Penicil*l*ium spp. Results obtained revealed that although, the microbial flora (bacteria and fungi) in the indoor air of various units varied from hospital to hospital, they were highest in Hospital C and least in Hospital A . However, in all hospitals the microbial load decreased in the order: female ward ≥ children ward > male ward > theater. Also, in all the units studied the concentrations of bacteria population were higher than fungi population. The prevalence of the organisms obtained in the three hospitals decreased as follows: *Staphylococcus aureus* > *Pseudomonas* spp > *Proteus* spp > *Streptococcus* spp > *Bacillus* spp > *Aspergillus* spp ≥ *Escherichia coli* ≥ *Klebsiella* spp ≥ *Mucor* spp > *Penicillium* spp. The result of the antibiotic susceptibility test demonstrated that isolates belonging to each bacterial genus possessed varied degree of multiple antibiotics resistance. Also, survival of both fungi and bacteria decreased with increase in concentration of dettol and izal.

Key words: Hospital Indoor air microflora bioassessment

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## INTRODUCTION

Atmospheric pollution is one of the most drastic problems of our age [1]. Air pollution by microbiota entities is a growing menace to human health throughout the world. Various allergic and infectious ailments of man are caused by air borne microbes [2]. In recent years, a number of factors have stimulated an increased awareness of the presence of potentially pathogenic bioaerosols in indoor and outdoor environments and the detrimental health effects associated with them. Although, indoor environments are considered to be protected, they can become contaminated with particles that present different and sometimes more serious risks when their concentrations exceed recommended maximum limits than those related to outdoor exposures [3]. The

recommended maximum limits are 1000 $cfum^{-3}$ for the total number of bioaerosol particles as set by the National Institute of Occupational Safety and Health (NIOSH) while the American conference of governmental Industrial Hygienists (ACGIH) stipulated that the culturable count for total bacteria should not exceed 500 $cfum^{-3}$ [4].

The microbiological quality of indoor air in hospitals is an issue with increased emphasis than in other type of building because of the potential severity of the consequences of nosocomial infections [2]. The problem of nosocomial infections is generally largest in older hospital which have large wards and poor or no mechanical ventilation and the situation is even more difficult in developing countries [5,6]. The incidence of hospital acquired infection is a serious and widespread problem with an estimated 1 in 10 patients acquiring an infection during a hospital stay.

The level of airborne microbial load of hospital indoor air in Delta Central Senatorial District (DCSD) of Delta State, Nigeria, is unknown. Microbial monitoring of such environments is important for the quality of life of humans within the hospital community (such as patients, patient attendant and medical or health workers). Therefore, this study was aimed at determining the quality and quantity of airborne microflora in three hospitals in Delta Central Senatorial District and assessing the responses of these organisms to commonly used antibiotics and disinfectants.

**MATERIALS AND METHODS**

**Study site**

Three hospitals (one private and two government owned) in Delta Central Senatorial district were selected for this study. One each was located in Eku, Abraka and Ughelli and were designated as Hospital A, Hospital B and Hospital C respectively. The description of the various wards in each of the hospital is as follows:

**Hospital A:** Although established over 50 years ago, the wards had just been renovated with floors properly tiled at the time of the experiment. The wards were large, beds were about 2m apart and a window by each bed. Also the floors of various wards were cleaned routinely (daily) with disinfectant.

**Hospital B:** Established over 20 years ago, no renovation, floors not tiled but were cleaned with disinfectant on daily basis. Wards were quite small.

**Hospital C:** Established over 30 years, no renovation since then and floors not tiled. Athough wards were large, beds were located less than a meter apart.

**Media used:** Media used in this study include: nutrient agar (for bacteria isolation), sabauraud dextrose agar (fungal isolation) and Muller Hinton agar (used to assay the potency of some commonly used antibiotics against isolates obtained).

**Air sampling:** The exposed plate technique described by [7] was adopted. Sampling was done once in a day (usually between 1:00 and 3:00 pm) for 60 days at intervals of Day 1, 2, 3, 7, 14, 21, 28 and 60. This was done by exposing plate containing the appropriate media in various wards. Exposure was done for 5 minutes after which, plates were transported in pre-sterilized container to the laboratory for further analytical procedures. All plates were incubated at room temperature for 24 to 96 hours. At the end of incubation plates were counted and discrete colonies were sub-cultured for characterization. Fungal colonies were identified based on the criteria in [8] while [9]as well as [10] were used in bacterial identification.

The total microbial load was estimated thus:

Number of organisms (CFU/ml) = a.1000/ p.t.0.2.

Where a = the number of colonies in the plate

P = the surface area of the petri-dish

t = the duration of exposure of the Petri-dish

**Evaluating the response of test isolates to disinfectant**

The test tube dilution method was adapted. One milliliter of standardized inocula of test isolate were respectively introduced into 9ml of various concentration of dettol and izal ( 1%v/v, 5%v/v, 10%v/v, and

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20%v/v). All the test tubes were allowed to stand at room temperature for 24 hours. Survival of bacteria and fungi was determined by sub-culturing 1ml from various test tube and inoculating into nutrient agar and Sabauraud dextrose agar plates respectively using the pour plate method. Plates were incubated at room temperature for 24 to 48 h after which they were observed for growth

**Preparation of standard inoculum for the determination of the sensitivity of various test isolate to disinfectant**

A loopful of each bacterial and fungal isolate was introduced into 9ml deionized water and serial dilution was performed after which 0.1ml from each dilution was introduced into nutrient agar or Sabauraud Dextrose agar plates (as the case may be) using spread plate technique. Incubation followed immediately at room temperature for 24 hours and dilution that produced 70-100 colonies were chosen as standard inoculum.

**Determination of the sensitivity of bacterial isolates to antibiotics.**

Three colonies each of the bacterial test isolate was inoculated into 3ml freshly prepared peptone water. This was incubated at 37$^{o}$C until turbidity marched that of 0.1% $BaSO_4$ solution then a swab stick was used to inoculate onto the surface of freshly prepared nutrient agar plates. A multiple antibiotics disc was placed on the already seeded agar plate (The disc contained the following antibiotics; ciprofloxacin (10µg), streptomycin (30 µg) septrin (30 µg), ampliclox(30 µg), zinnacef(20 µg), amoxicillin(30 µg), tarivid(10 µg), gentamycin(10 µg),rocephin(25 µg), augumentin(30 µg), erythromycin(10 µg), perfloxacin(10 µg) and sarfloxacin(10 µg) . Then, plates were incubated at 37$^{o}$C for 24h, at the end zones of inhibition were measured. Zones less than 14mm were taken as resistant.

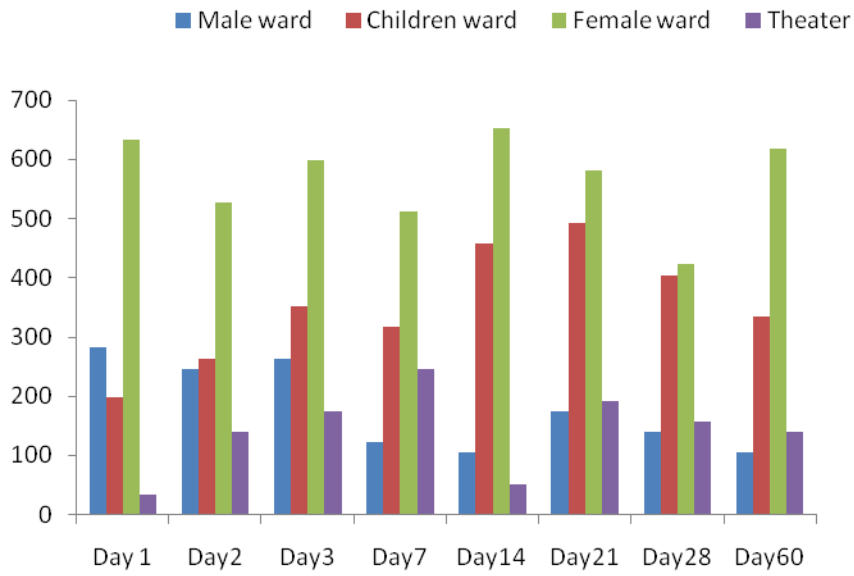


**Fig. 1: Bacteria load in hospital A**

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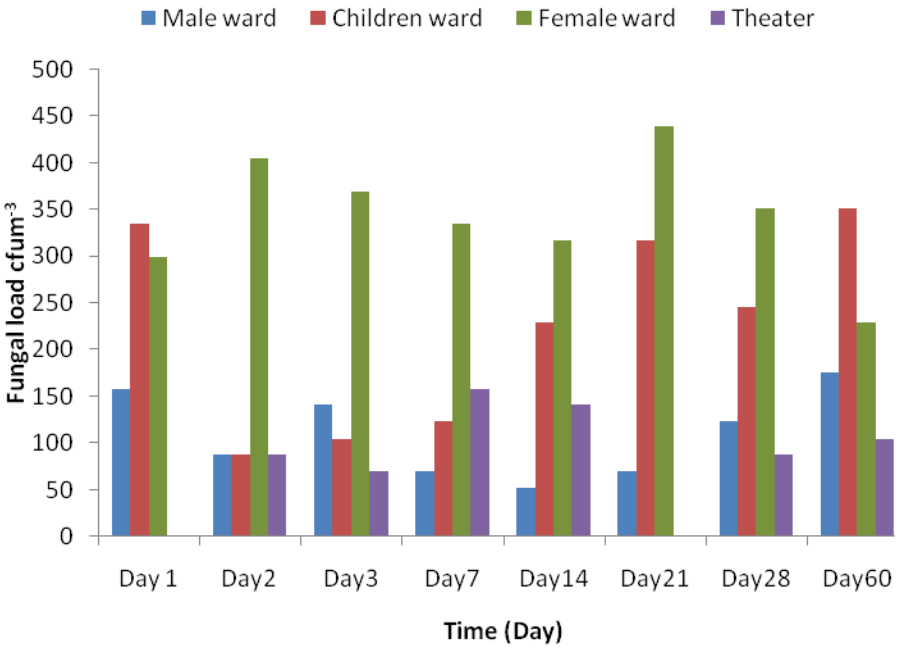


**Fig. 2: Fungal load in hospital A**

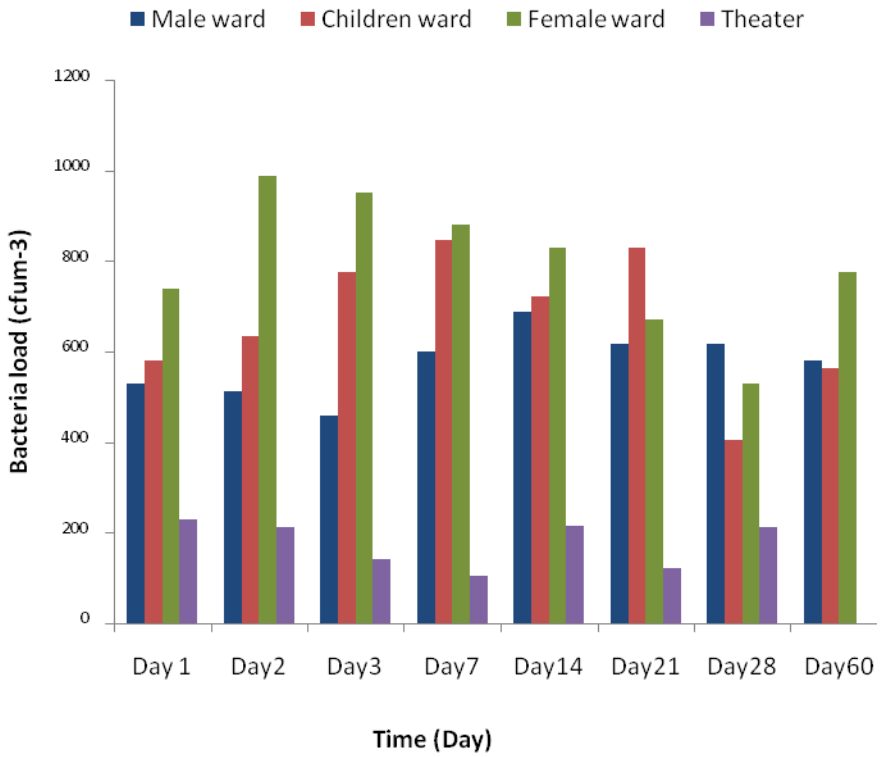


**Fig. 3:Bacteria load in hospital B**

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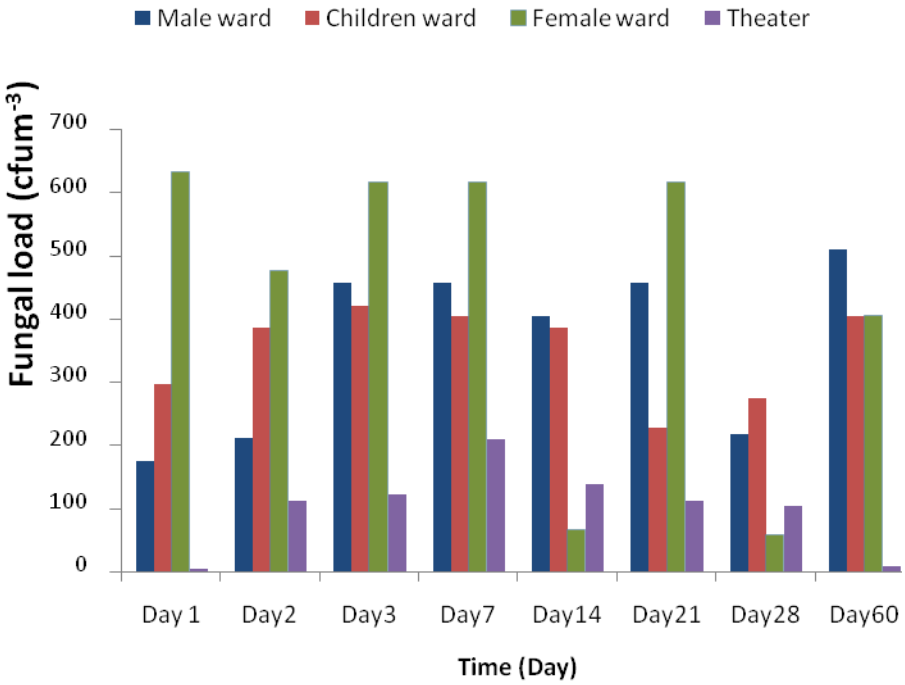


**Fig.4: Fungi load in hospital B**

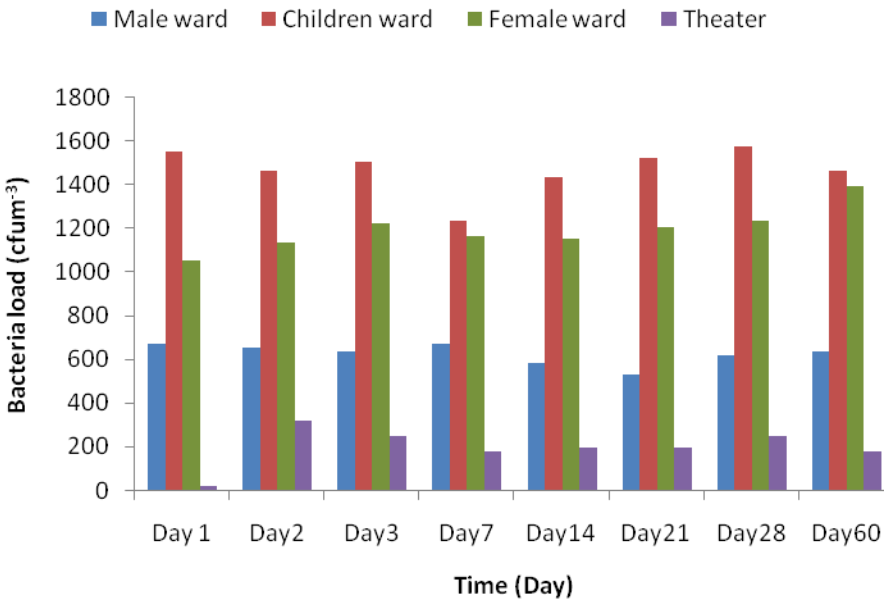


**Fig. 5: Bacteria load in Hospital C**

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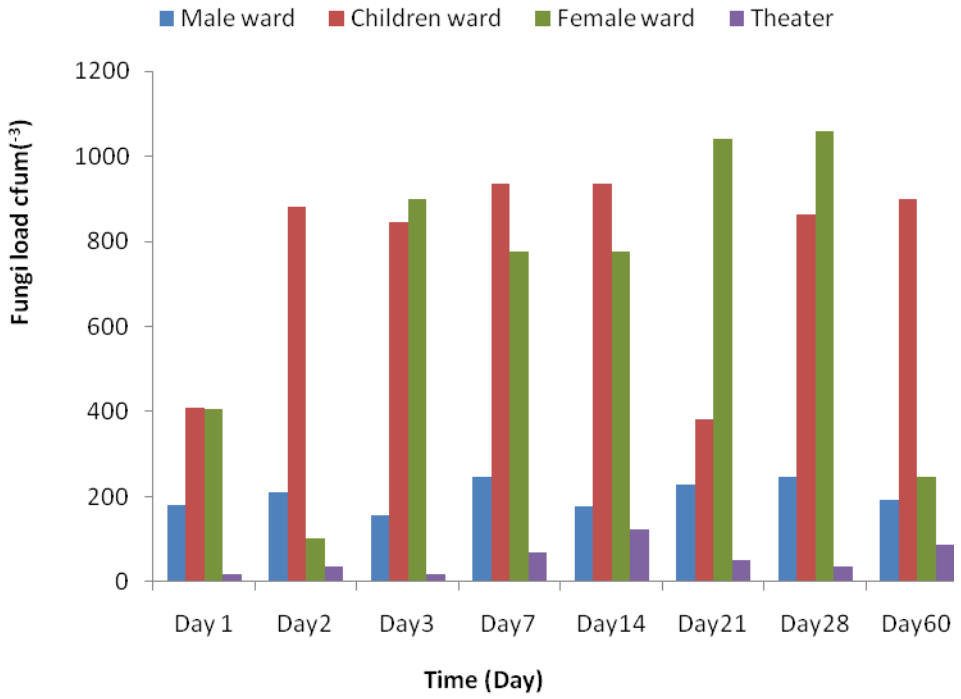


**Fig. 6: Fungal load in hospital C**

Table 1: Prevalence of bacterial isolates in various units studied in each hospital

| Organism | Hospital A | | | | Hospital B | | | | Hospital C | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MW | FW | CW | T | MW | FW | CW | T | MW | FW | CW | T | |
| Bacillus | 4 (0.44%) | 9 (0.99%) | 3 (0.33%) | 0 (0%) | 8(0.88%) | 12 (1.31%) | 14 (1.55%) | 4(0.44%) | 10(1.1%) | 16 (!.75%) | 12 (1.31%) | 6 (0.66%) | 98 (10.73%) |
| Esherichia | 0 (0%) | 0 (0%) | 6 (0.66%) | 0 (0%) | 0 (0%) | 3 (0.33% | 6 (0.66%) | 0 (0%) | 7(0.77%) | 8(0.88%) | 10(1.1%) | 0 (0%) | 40(4.38%) |
| Pseudomonas | 5(0.55%) | 16 (!.75%) | 10((1.1%) | 2(0.22%) | 22(2.41%) | 18(1.94%) | 30(3.29%) | 5 (0.54%) | 11(2.30%) | 35(3.83%) | 26(2.85%) | 8(0.88%) | 188(20.59%) |
| Proteus | 1(0.11%) | 14(1.53%) | 9().99%) | 1(0.11%) | 10(1.1%) | 19(2.08%) | 13(1.42%) | 2(0.22%) | 15(1.64%) | 20(2.19%) | 19(2.08%) | 7(0.77%) | 130(14.24%) |
| Staph | 19(2.08%) | 27(2.95%) | 17(1.86%) | 8(0.88%) | 35(3.83%) | 30(3.21%) | 25(2.74%) | 16(1.75%) | 39(4.27%0 | 47(5.15%) | 31(3.4%) | 11(1.2%) | 305(23.45%) |
| Strept | 8(0.88%) | 7(0.77%) | 7(0.77%) | 0 (0%) | 5(0.55%) | 10((1.1%) | 7(0.77%) | 6(0.66%) | 15(1.64%) | 12(1.31%) | 17(1.86) | 10(1.1%) | 114(12.49%) |
| KLebsiella | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 4(0.44%) | 14(1.53%) | 10(1.1%) | 1(0.11%) | 2(0.22%) | 5(0.55%) | 1(0.11%) | 1(0.11%) | 38(4.16%) |
| Total | 37(0.04%) | 73(8%) | 52(5.7%) | 11(1.2%) | 84(9.2%) | 106(11.61%) | 105(11.5%) | 34(3.72%) | 99(10.84%) | 143(15.66%) | 116(12.71%) | 43(4.71%) | 913(100%) |

Key: MW = male4 ward FW= Female ward, CW = children's ward = theater, Staph b= *Staphylococcus* spp, Strept = *Streptococcus* spp

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Table 2: Prevalence of fungal isolates in various units studied in each hospital

| Organism | Hospital A | | | | Hospital B | | | | Hospital C | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MW | FW | CW | T | MW | FW | CW | T | MW | FW | CW | T | |
| A | 3(2.73%) | 2(1.82%) | 3(2.73%) | 0(0%) | 4(3.64%) | 7(6.36%) | 5(4.55%) | 0(0%) | 5(4.55%) | 8(7.27%) | 10(9.09%) | 1(0.91%) | 48(43.64%) |
| B | 0(0%) | 4(3.64%) | 0(0%) | 0(0%) | 2(1.82%) | 5(4.55%) | 3(2.73%) | 2(1.82%) | 3(2.73%) | 5(4.55%) | 8(7.27%) | 0(0%) | 32(29.09%) |
| C | 0(0%) | 2(1.82%) | 2(1.82%) | 0(0%) | 3(2.73%) | 8(7.27%) | 6(5.45%) | 2(1.82%) | 0(0%) | 3(2.73%) | 3(2.73%) | 1(0.91%) | 30(27.27%) |
| Total | 3(2.73%) | 8(7.27%) | 5(4.55%) | 0(0%) | 9(8.18%) | 20(18.18%) | 14(12.73%) | 4(3.64%) | 8(7.27%) | 16(23.64%) | 21(19.09%) | 2(1.82%) | 110(100%) |

Key: MW = male ward FW= Female ward, CW = children's ward, T = theater, a = *Aspergillus* spp, b =*Mucor* spp, c = *Penicillium* spp

Table 3: Percentage of microbial isolate that showed growth in various concentrations of dettol

| Organism | Total Number of isolates | Percent that showed growth in various disinfectant concentrations | | | | |
|---|---|---|---|---|---|---|
| | | 1 %v/v | 5 %v/v | 10 %v/v | 15 %v/v | 20 %v/v |
| *Bacillus* spp | 98 | 90(91.84%) | 71(72.45%) | 31(31.63%) | 13(13.27%) | 17(17.35%) |
| *Escherichia coli* | 40 | 30(75%) | 27(67.5%) | 18(45%) | 11(27.5%) | 5(12.5%) |
| *Pseudomonas* spp | 188 | 120(63.83%) | 100(53.19%) | 92(93.88%) | 70(37.23%) | 53(28.19%) |
| *Proteus* spp | 130 | 100(76.92%) | 65(50%) | 38(29.23%) | 31(23.85%) | 19(14.12%) |
| *Staphylococcus aureus* | 305 | 250(81.97%) | 210(68.85%) | 109(35.74%) | 84(27.54%) | 6717.54%) |
| *Streptococcus* spp | 118 | 89(78.07%) | 80(70.18%) | 58(50.88%) | 14(15.73%) | 20(42.11%) |
| *Klebsiella* spp | 38 | 37(97.37%) | 30(78.95%) | 27(71.05%) | 28(75.68%) | 16(33.33%) |
| *Aspergillus* spp | 30 | 30(100%) | 21(70%) | 26(86.67%) | 15(50%) | 10(27.08%) |
| *Mucor* spp | 48 | 40(83.33%) | 36(75%) | 26(75%) | 20(54.17%) | 13(27.08%) |
| *Penicillium* spp | 32 | 20(62.5%) | 18(56.25 | 10(31.25%) | 12(37.5%) | 10(31.25%) |

Table 4: Percentage of microbial isolates that showed growth in various concentrations of izal

| Organism | Total Number of isolates | Percent that showed growth in various disinfectant concentrations | | | | |
|---|---|---|---|---|---|---|
| | | 1 %v/v | 5 %v/v | 10 %v/v | 15 %v/v | 20 %v/v |
| *Bacillus* spp | 98 | 98(96.94%) | 64(65.31%) | 10(10.20%) | 5(5.10%) | 3(3.06%) |
| *Escherichia coli* | 40 | 32(80%) | 15(37.5%) | 4(10%) | 2(5%) | 6(15%) |
| *Pseudomonas* spp | 188 | 150(53.19%) | 74(39.36%) | 50(26.6%) | 24(12.76%) | 20(10.64%) |
| *Proteus* spp | 130 | 102(78.46%) | 100(76.92%) | 26(20.0%) | 10(7.69%) | 14(10.77%) |
| *Staphylococcus aureus* | 305 | 208(68.2%) | 164(53.97%) | 61(20.0%) | 49(16.07%) | 30(9.84%) |
| *Streptococcus* spp | 118 | 60(52.63%) | 37(32.46%) | 41(35.96%) | 21(18.42%) | 5(4.39%) |
| *Klebsiella* spp | 38 | 31(81.58%) | 23(60.53%) | 15(39.47%)) | 16(42.11%) | 10(26.32%) |
| *Asppergillus* spp | 30 | 22(73.33%) | 16(53.33%) | 10(33.33%) | 11(36.67%) | 6(20%) |
| *Mucor* spp | 48 | 40(83.33%) | 29(60.42%) | 14(29.11%) | 8(16.66%) | 7(14.58%) |
| *Penicillium* spp | 32 | 14(43.75%) | 10(56.25 | 5(15.63%) | 2(6.25%) | 2(6.25%) |

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Table 5: Percentage of Gram positive bacterial isolates that showed resistance to various antibiotics

| Organism | Total number of isolate | Percent that showed resistance | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CIP | STR | SEP | AMP | ZIN | GEN | ROC | ERY | PER |
| Bacillus spp | 98 | 20.41% | 10.20% | 6.12% | 40.82% | 4.08% | 11.23% | 8.16% | 13.27% | 2.04% |
| *Staphylococcus aureus* | 305 | 22.95% | 16.72% | 22.95% | 65.57% | 15.41% | 32.79% | 21.97% | 7.87% | 4.92% |
| *Streptococcus* spp | 114 | 14.04% | 43.86% | 40.35% | 32.46% | 17.54% | 17.54% | 16.67% | 11.40% | 8.77% |

Key: CIP = ciprofloxacin, STR = streptomycin, SEP = septrin, AMP = ampliclox, ZIN = zinnacef, GEN = gentamycin, ROC = rocephin, ERY – erythromycin and PER = perfloxacin.

Table 6: Percentage of Gram negative bacteria isolates that showed resistance to various antibiotics

| Organism | Total number of isolates | Percent that showed resistance | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CIP | STR | SEP | AMO | TAR | GEN | AUG | SAR |
| *Escherichia coli* | 40 | 72.50% | 37.50% | 25.00% | 75.00% | 32.50% | 42.50% | 63.50% | 2.50% |
| *Pseudomonas* spp | 188 | 45.74% | 31.91% | 31.91% | 38.83% | 26.00% | 21.81% | 18.62% | 15.43% |
| *Proteus* spp | 130 | 24.62% | 7.69% | 3.85% | 9.23% | 4.62% | 6.92% | 13.07% | 11.54% |
| *Klebsiella* spp | 38 | 78.95% | 73.68% | 65.79% | 65.79% | 71.05% | 89.58% | 52.63% | 47.37% |

Key: CIP = ciprofloxacin, STR = streptomycin, SEP = septrin, AMO = amoxicillin, TAR = tarivid, GEN = gentamycin, AUG = augumentin, perfloxacin and SAR = sarfloxacin.

**RESULT**

The fungal and bacteria load obtained in the three hospitals studied are presented in Figs. 1 to 6. The total bacteria load was higher in each ward than the total fungi load. Also the bacterial and fungal load obtained from the various wards varied from day to day and also from hospital to hospital. In all the hospitals bacterial and fungal load decreased in the order: Female ward ≥ children ward > male ward > theater. The frequencies of occurrence (prevalence) of the isolates in each ward in the three hospitals are shown in Tables 1 and 2. A total of nine hundred and thirteen bacteria (Table 1) and one hundred and ten fungi (Table 2) isolates were obtained. The bacteria isolates belonged to the following genera: Bacillus, Escherichia, Pseudomonas, Proteus, Klebsiella, Staphylococcus and Streptococcus while the fungi isolates included Aspergillus, Penicillium and Mucor. There was no significant difference among the counts of both bacterial and fungi isolates obtained in the different days of the study in each hospital. However microbial count obtained in Hospital C were higher than in hospital B and least in hospital A. The prevalence of the organisms obtained in the three hospitals decreased as follows: *Staphylococcus aureus* > *Pseudomonas* spp > *Proteus* spp > *Streptococcus* spp > *Bacillus* spp > *Aspergillus* spp ≥ *Escherichia coli* ≥ *Klebsiella* spp ≥ *Mucor* spp > *Penicillium* spp.

The result of the effect of disinfectant on the survival of each microbial isolate is shown in Tables 3 and 4. The percentage of isolates of each organism that showed growth decreased as the concentration of each disinfectant increased. The results obtained indicated that a high percentage of the isolates of each organism were susceptible to the disinfectants used. However, the isolates were more susceptible to izal than dettol. There was a significant difference at $p < 0.05$ among the percent growth of isolates exposed to various concentrations of each disinfectant and also between growth of organisms exposed to dettol and izal.

The results of the susceptibility of the gram positive and gram negative bacteria isolates to the tested antibiotics are presented in Tables 5 and 6 respectively. All the bacteria isolates showed varying degree of resistance to the antibiotics tested. Percentage of Bacillus, Staphylococcus and Streptococcus isolates that exhibited resistance to each antibiotics were less than 50% with the exception of ampicillin to which 65.57% of Staphylococcus aureus isolates showed resistance. Also, of the Gram negative bacteria isolates, the percentage of Klebsiella isolates that showed resistance to the various antibiotics was highest. This was followed by *Escherichia coli* and percentage of *Proteus* isolates that showed resistance to various antibiotics was least.

**DISCUSSION**

Although, the microbiological quality of the indoor air of the three hospitals investigated varied, they all exceeded the set standards as established by NIOSH and ACGIH. The quality of air in these hospitals might have been influenced by factors such as age of the building, degree of cleanliness and disinfection, type of disinfectant used, nature of floors (whether tiled or not) and control of influx as well as efflux of people into the various units. Hence, the airborne bio-contaminants in hospital A that was newly renovated with adequate ventilation system, was least. The high rates of microbial load observed in hospitals B and C may also be attributed to the smaller size of the wards (higher number of beds per $m^3$ of ward) [2] and occurrence of crevices in the floors, ceiling and walls of these hospitals. Also, specific activities like talking, sneezing, walking and washing can generate airborne biological particulate matter [4].

The high number of bacteria and fungi population observed in female and children wards in all the hospitals may be attributed to the high number of occupants (patients, patient attendant, personnel and visitors) at all times while the low microbial population recorded in the theater of the three hospital may be as a result of the location of theater in these hospitals, controlled entry and the high degree of disinfection that usually occur in this unit in hospitals. Similarly, [2,11,12] had reported that patient room had the highest microbial count in hospitals.

The high incidence of Staphylococcus observed in this study could be as a result of the fact that Staphylococcus is a normal flora of the skin and nasopharynx and thus can easily be shed off by hospital occupants and hence contribute greatly to the hospital indoor air flora. The prevalence of E coli was quite low but more often was isolated from the children's ward. This is likely due to passage of fecal matter into surrounding as well as the use of focally contaminated water in cleaning floors or other activities in the wards or could even be generated by toilet flushing. [12]had also reported similar results. The occurrence of pathogens such as *Pseudomonas* spp, *Proteus* spp, *Klebsiella* spp, *Streptococcus* spp and *Aspergillus* spp is suggestive that the patients in the wards might be responsible for shedding these organisms into the hospital indoor air

In all the units studied, the bacterial load was consistently, higher than the fungi load. This might have been favored by the high environmental temperature at the period of the study. It could also be attributed to the larger size of fungi which might have affected their floating capacity.

The result of the antibiotics susceptibility test suggest that many of the bacteria contaminating the indoor air of the three hospitals are of human origin and that the indiscriminate use of antibiotics may explain the emergence of antibiotics resistance in these organisms. The resistance to various antibiotics observed in a great percentage of *Klesiella* spp may be as a result of the occurrence of capsule in this bacterium. Additionally, the difference noticed in the susceptibility of organisms to dettol and izal, could possibly be as a result of the differences in the chemical composition of these disinfectants. However, on a general note, both

disinfectants reduced the concentration of the population of each organism considerably especially at high concentrations. The results demonstrated the effectiveness of these disinfectants against fungi and bacteria.

Conclusively, indoor air monitoring that is focused on the presence of bacteria and fungi cannot be over emphasized. Therefore, efforts should be made to improve the hygiene of hospital environments. It is also necessary to raise the awareness of medical personnel to reduce the hazard of transmission of potentially pathogenic airborne microorganisms. Perhaps, this could be by developing a good disinfection strategy which will be beneficial to reducing the airborne microbial load. Furthermore, it is advisory to obtain an antibiogram in an event where by any of these organisms are implicated in nosocomial infection outbreak for effective combat.

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