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Microbiological Air Quality of Female Medical and Female Surgical Wards of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria

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ABSTRACT

The quality of the hospital environment, if not controlled, can potentially place patients, hospital workers and patients' relatives at risk. This research was aimed at investigating the air quality of the female medical and female surgical wards of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. Air samples were collected twice daily, in the morning (7am - 8am) and in the evening (4pm - 5pm) from July, 2014 to September, 2014, using the settled plate methods. The airborne bacterial counts in the female medical and female surgical wards ranged from 3.2cfu/min to 13.2cfu/min and 3.0cfu/min to 8.2cfu/min respectively. The airborne fungal counts in the female medical and female surgical wards ranged from 3.0cfu/min to 6.7cfu/min and 3.0cfu/min to 5.7cfu/min respectively. However, there was no statistically significant difference ($p>0.05$) between the microbial counts obtained in both wards. Four (4) airborne fungal isolates (Penicillium notatum, Aspergillus flavus, Aspergillus niger, Mucor spp.) and four (4) airborne bacterial isolates (Staphylococcus aureus, Bacillus spp., Micrococcus spp. and Pseudomonas spp.) were characterized and identified. Staphyloccocus aureus was the only airborne bacterial isolate that was sensitive to cefdinir and ciprofloxacin while Aspergillus flavus and Aspergillus niger were the only airborne fungal isolates that were sensitive to fluconazole. Some isolates had similar plasmids and DNA bands with the respective primers. The presence of these airborne microorganisms could pose serious health hazards. Therefore, it is recommended that the practice of good hygiene should be encouraged in public places, especially hospitals.

1. INTRODUCTION

A hospital is an institution where the sick, injured are given medical or surgical attention. The sanitary state of the hospital especially the wards where the patients are kept, usually influences the kind and type of

infections that would occur. The airborne microorganisms that raise public health concerns are the causative agents of infectious diseases and allergies that may include viruses, bacteria and fungi (Ekhaise *et al.*, 2008). Their estimation is important for use as an index of cleanliness for any particular environment and to determine the relationship they bear on human health. It is however documented that the hospital environment is a source of acquired infections (Ekhaise *et al.*, 2008). Airborne microorganisms that are present in the hospital environment are responsible for a number of nosocomial infections which are infections acquired in a hospital environment that were not present at the time of admission (Claudete *et al.*, 2006). Reports have shown that hospital attendants and personnel help spread these nosocomial infections within the hospital environment, since they move from ward to ward, and from patient to patient (Pollack, 2010). Infections of the urinary tract, pneumonia and that of surgical sites are the most commonly known nosocomial infections.

This research was aimed at investigating the microbiological air quality of the female medical and female surgical wards of University of Benin Teaching Hospital (UBTH), Benin City.

2. MATERIALS AND METHODS

2.1. Study Area

This work was carried out in the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The two study sites were the Female Medical ward (FM) and Female Surgical ward (FS). The female wards were chosen for this study due to the peculiarity of the anatomic structure of the female gender that is prone to both fungal and bacterial infections and also the frequent movement of persons in and out of the wards.

2.2. Sampling Method

Air samples were collected twice daily (morning and evening) within the hours of 7am – 8:00 am and 4 pm – 5:00 pm for three months (July, August and September, 2014), using the settled plate method (Ekhaise *et al.*, 2008). The plate containing Nutrient Agar (NA) incorporated with griseofulvin, to inhibit the growth of fungi, and Sabaroud Dextrose Agar (SDA) containing chloramphenicol to inhibit the growth of bacteria were used for the isolation of the airborne bacterial and fungal isolates respectively. Each plate was exposed at a height of 1m to 1.5m from the ground for a period of 15minutes. The nutrient agar plates were incubated at 37^{o}C for 24 – 48hr while the Sabaroud Dextrose Agar plates were incubated at room temperature of 28^{o}C for 3 – 4 days after which the airborne microbial isolates where enumerated and identified.

2.3. Identification of the Airborne Microbial Isolates

After incubation, the total number of colony forming unit per/minutes (cfu/mins) for the airborne bacterial and fungal isolates were enumerated and characterized by using the cultural, morphological and microscopic examinations. Further characterization of the isolates was done using biochemical and physiological examinations (Ekhaise *et al.*, 2008).

2.4. Sensitivity Test for the Isolated Microorganisms

2.4.1. Bacterial isolates

The bacterial isolates were inoculated in nutrient agar plates in additional to the conventional multi sensitivity discs in appropriate aseptic conditions and incubated for 48 hours. It was then observed for resistivity and sensitivity.

2.4.2. Fungal isolates

The antifungal drug (Fluconazole) was evaluated at one and two capsules on SDA using poisoned food technique (Ali-Shtayeh and Abu Ghdeib, 1999). SDA medium without the drug served as control. Each plate was inoculated with mycelial disc of pathogen (5 mm) taken from the periphery of 7 day old cultures grown on SDA. The inoculated plates were incubated at 28 ± 1°C till the fungus growth covered the plate in case of control. The growths were visually determined for each isolate at day 1, day 2 and day 3 and results recorded.

2.5. Plasmid Isolation

A volume of 1.5ml of overnight culture for 1 minute was spun in a micro-centrifuge to pellet cells which was suspended in 200µl of solution A (100 mM glucose, 50 mM Tris hydrochloride (pH 8), 10 mM EDTA) containing 10 mg of lysozyme per ml and incubate for 30 min at 37°C. A volume of 400µl of freshly prepared 1% sodium dodecyl sulfate in 0.2N NaOH was added and mixed. Subsequently, 300µl of a 30% potassium acetate solution (pH 4.8) was added and mixed by vortexing. After incubating on ice for 5 minutes, the debris was removed by a 5-minutes centrifugation. The supernatant was transferred and extracted once with a phenol-chloroform mixture (1:1). The plasmid DNA was precipitated with an equal volume of isopropanol and allowed to dry and dissolved with Tris-EDTA buffer.

2.6. Chromosomal DNA Extraction

DNA extraction was carried out directly from the samples by boiling as follows. A volume of 1.5ml of the organisms in broth was centrifuged at 10,000rpm for 5 minutes. The supernatant was discarded and the pellets were washed twice with sterile water. After this, 200µl of sterile water was added to the pellets, the pellets were vortexed to homogenize and boiled in a dry bath at 100°C for 10 minutes. This was followed by vortexing and centrifugation at 12,000rpm for 5 minutes. The supernatant containing the DNA were transferred to another tube and stored in a refridgerator at -20°C. The concentration and purity of the extracted DNA was estimated using a Nanodrop spectrophotometer (Analytik Jena, Jena, Germany).

2.7. PCR Amplification of the S Gene

PCR amplification was carried out using the RAPD operon primers OPR-02 (GATGACCGCC), OPC-10 (TGTCTGGGTG) and OPI 05 (GGTTCCACGC). The reaction was carried out in a 20µl reaction mixture containing x1 PCR buffer (Solis Biodyne, Estonia), 2.5mM Magnesium Chloride (Solis Biodyne), 200µM of each dNTP (Solis Biodyne), 50pmol of each primer, and 2U Taq DNA polymerase (Solis Biodyne). Amplification was carried out in an Eppendorf Thermal Cycler (Nexus Vapo protect series) using the following cycling parameters: An initial denaturation at 95ºC for 5 minutes and 40 cycles of 95ºC for 1 minute, 30ºC for 1 minute and 72ºC for 2 minutes. This was followed by a final extension of 72ºC for 10 minutes. The PCR products were separated on a 1.0 agarose gel and 1Kb DNA ladder was used as DNA molecular weight standard.

2.8. Agarose Gel Electrophoresis

DNA gel electrophoresis is a technique used for the detection and separation of DNA by applying an electrical field to move the charged molecules through an agarose matrix and the DNA is separated by size in the gel matrix. Agarose powder (0.8g) was weighed out, mixed with 100 ml of TBE buffer and dissolved by boiling using a magnetic stirrer. It was allowed to cool to about 60^0C, then 10 µL of ethidium bromide was added and mixed gently. The mixture was poured into the electrophoresis tank with the comb in place to obtain a gel thickness and to avoid bubbles, allowed to solidify for 20 minutes and the comb was removed. The tray was placed in the electrophoresis tank containing TBE buffer to ensure that the buffer covers the

surface of the gel. About 15 µL of the sample was mixed with 2 µL of the loading dye and carefully loaded into the wells created by the comb. The electrodes were connected to the power pack in such a way that the negative terminal is at the end where the samples were loaded. Electrophoresis was run at 60V – 100V until the loading dye migrated about three quarters of the gel. The electrophoresis tank was turned off and the electrodes disconnected and observed on a UV- transilluminator (QIAGEN, 2013).

2.9. Statistical Analysis

The mean airborne microbial counts were subjected to one-way analysis of variance (ANOVA) using SPSS version 25.0 software. This was done to determine if there were significant differences ($\alpha=0.05$) between the microbial counts in the morning and afternoon sessions in both wards.

3. RESULTS AND DISCUSSION

Table 1 shows the total airborne bacterial isolates in the female medical and female surgical wards which ranged between 3.0 x 10^3 to 13.2 x 10^3 cfu/min. It was observed that in the month of July 2014, the female medical ward recorded the highest concentration of airborne bacterial isolates in the afternoon session, while the female surgical ward recorded the lowest concentration of airborne bacterial isolates in the month of August and September both in the afternoon and morning sessions respectively. The high concentration of bacteria in the female medical ward was attributed to high human population (patients and visitors), increased human activity, inflow of bacteria from outdoor air via ventilation and improper sanitation procedures. This result is similar to that of Ekhaise and Ogboghodo (2011) which stated that human population and activity may contribute to increased bacterial concentration.

Table 1: Total airborne bacterial counts in the female medical and female surgical wards

Weeks	Period of day	Ward	July CAI (cfu/min)	August CAI (cfu/min)	September CAI (cfu/min)
2	Morning	F m	3.8 x 10^3	3.2x 10^3	3.2x 10^3
		F s	3.3x 10^3	3.3x 10^3	3.0x 10^3
	Afternoon	F m	13.2x 10^3	3.5 x 10^3	3.2x 10^3
		F s	8.2x 10^3	3.0 x 10^3	3.5x 10^3
4	Morning	F m	3.5 x 10^3	3.5x 10^3	3.2x 10^3
		F s	3.2x 10^3	3.0x 10^3	3.2x 10^3
	Afternoon	F m	3.5x 10^3	3.2x 10^3	3.5x 10^3
		F s	4.5x 10^3	3.3x 10^3	3.3x 10^3

Fm: Female medical, Fs: Female surgical, CAI: Concentration of airborne isolates, Cfu/min: Colony forming units per minutes

Table 2 shows the percentage occurrence of the airborne bacterial isolates. It was observed that in the month of July 2014, *Staphyloccocus aureus* recorded the highest percentage occurrence in the female surgical ward while *Micrococcus* spp. recorded the lowest percentage occurrence in the female medical ward throughout the sampling periods. The results obtained are similar to those obtained by Ekhaise *et al.* (2010). They stated that among the bacterial isolates observed, *Staphylococcus aureus* was most prevalent in hospitals. *Staphylococcus aureus* is a causative agent of soft tissue and respiratory infections. It is an accepted cause of nosocomial infections.

Table 2: Percentage occurrence of the airborne bacterial isolates

Isolates	Ward	July		August		September	
		Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
Bacillus sp	Fm	62.5	25	50	50	50	50
	Fs	37.5	0	12.5	37.5	50	50
Pseudomonas sp	Fm	0	0	37.5	0	37.5	12.5
	Fs	25	25	0	12.5	12.5	25
Staphylococcus aureus	Fm	37.5	37.5	37.5	25	37.5	25
	Fs	87.5	12.5	25	37.5	50	75
Micrococcus sp	Fm	0	0	12.5	0	0	25
	Fs	37.5	12.5	50	0	12.5	12.5

Fm: Female medical, Fs: Female surgical

Table 3 shows the total airborne fungal counts in the female and medical wards. It was observed that in the month of July 2014, the female medical ward recorded the highest concentration of airborne fungal isolates in the afternoon session, while the female surgical ward and the female medical ward both recorded the lowest concentration of airborne bacterial isolates in the month of August and September both in the afternoon and morning sessions respectively. The high concentration of fungi was attributed the influence of the physical factors such as temperature and relative humidity during the sampling months which play significant role in the release or dispersal of fungal spores, especially in the indoor environments like the hospitals.

Table 3: Total airborne fungal counts of the female medical and female surgical wards

Weeks	Period of day	Ward	July CAI (cfu/min)	August CAI (cfu/min)	September CAI (cfu/min)
2	Morning	F m	3.3×10^3	3.0×10^3	3.2×10^3
		F s	3.3×10^3	3.2×10^3	3.5×10^3
	Afternoon	F m	4.2×10^3	3.0×10^3	3.5×10^3
		F s	3.7×10^3	3.2×10^3	3.0×10^3
4	Morning	F m	4.7×10^3	3.0×10^3	3.2×10^3
		F s	3.3×10^3	3.0×10^3	3.5×10^3
	Afternoon	F m	6.7×10^3	3.3×10^3	3.2×10^3
		F s	5.7×10^3	3.0×10^3	3.0×10^3

Fm: Female medical, Fs: Female surgical, CAI: Concentration of airborne isolates, Cfu/min: Colony forming units per minutes

It was observed that in the month of July 2014, the fungal isolate *Aspergillus niger* in the female medical ward recorded the highest occurrence while *Penicillium notatum* recorded the lowest percentage occurrence in the female medical in the month of August and September. The results obtained are similar to those obtained by Ekhaise *et al.* (2010). They stated that *Aspergillus niger* and *Aspergillus flavus* were the most prevalent fungal isolates. *Aspergillus niger* and *Aspergillus flavus* were also among the isolated fungal organisms obtained from the study by Jaffal *et al.* (1997) in a hospital environment. Although *Aspergillus* spp. may be tolerable for healthy individuals, it may be dangerous in immune-compromised patients thereby worsening their health status.

Table 4: Percentage occurrence of Fungal isolates of the female medical and female surgical wards

Isolates	Ward	July 2014		August 2014		September 2014	
		Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
Mucor spp.	Fm	25	25	12.5	37.5	12.5	50
	Fs	37.5	75	25	12.5	12.5	25
Aspergillus niger	Fm	0	100	25	50	25	25
	Fs	0	0	62.5	25	50	50
Aspergillus flavus	Fm	12.5	50	37.5	25	50	37.5
	Fs	0	25	0	25	50	25
Penicillium notatum	Fm	50	0	0	0	0	25
	Fs	50	50	25	12.5	25	25

Fm: Female medical, Fs: Female surgical

Table 5 shows the sensitivity and resistance of the airborne bacterial and fungal. The bacterial isolate *Staphylococcus aureus* was the only organism that was sensitive to Ciprotab (Ciprofloxacin) and Ranicef (Cefdinir) antibiotics and resistant to the other drugs while *Aspergillus flavus and Aspergillus niger* were the only fungal organisms that were sensitive to the fungal drug Fluconazole. This may be as a result of the fact that these isolates do not have plasmids which would have conferred some resistance to the drugs. Genes for antibiotic resistance in bacteria is not only plasmid mediated but also occur on the chromosome. Mobile genetic elements can help in the distribution of these resistant genes in the environment. Also, resistance of microorganisms has been shown to be attributed to the misuse of antibiotics and also the presence of resistant genes.

Table 5: Sensitivity and Resistance of isolated bacterial and fungal organisms using some antibiotics drugs for the bacterial isolates and antifungal drug for the fungal isolates

Organisms	GEN	ERY	AUG	OFL	CRX	CXC	CAZ	CPT	CTR	RNF	FLU
Bacillus spp	R	R	R	R	R	R	R	R	R	R	
Pseudomonas spp	R	R	R	R	R	R	R	R	R	R	
Staphyloccocusaureus	R	R	R	R	R	R	R	S	R	S	
Micrococcus spp	R	R	R	R	R	R	R	R	R	R	
Mucor spp.											R
Asppergillus niger											S
Asppergillus flavus											S
Penicillium notatum											R

R = RESISTANCE, S = SENSITIVITY, GEN – GENTAMICIN, ERY- ERYTHROMYCIN, AUG- AUGMENTIN, OFL – OFLOXACIN, CRX – CEFUROXIME, CXC – CLOXACILLIN, CAZ – CEFTAZIDINE, CPT – CIPROFLOXACIN, CTR – CEFTRIAZONE, RNF – RANICEF, FLU – FLUCONAZOLE

Plate 1, shows the plasmid bands of the bacterial isolates' numbers 1- 4 and fungal isolates labelled A – D. Organisms number 1 and 2 had similar plasmid bands of 23,130bp. The bacterial isolates 1 and 4 had similar bands when compared with the plasmid band of the marker 21,130bp, which means that the organism possesses the base sequence present in the marker. Plasmids are small circular pieces of DNA found in organisms. Some plasmids confer resistance to antimicrobial drugs. This characterization enables one to compare the bands in a given DNA or plasmid samples with the bands in the reference ladder/marker. The

plasmid results show that bacterial isolates 1 and 4 had similar bands when compared with the plasmid band of the marker 21,130bp, which means that the organism possess the base sequence present in the marker. These similarities in DNA and plasmids bands of these bacterial and fungal isolates indicate that they may possess similar base sequence in their genome.

Plate 2, clearly shows the DNA bands of isolated bacterial organisms numbered 1 - 4 and isolated fungal organisms labeled A – D using three different primers/ markers (OPR-02, OPC-10, and OPI - 05).

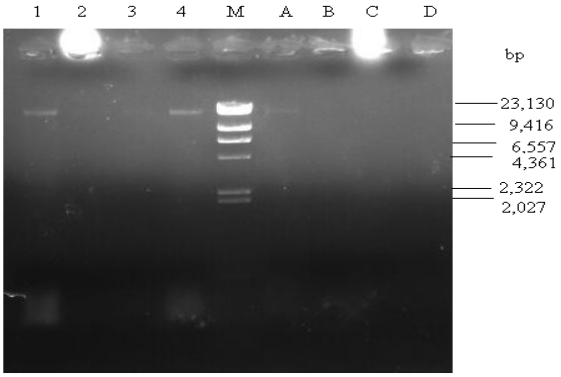

Plate 1: The Plasmids analysis of the airborne bacterial and fungal isolates

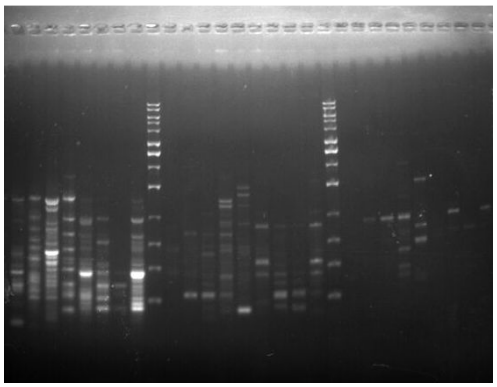

Plate 2: The DNA analysis of the bacterial and fungal isolates

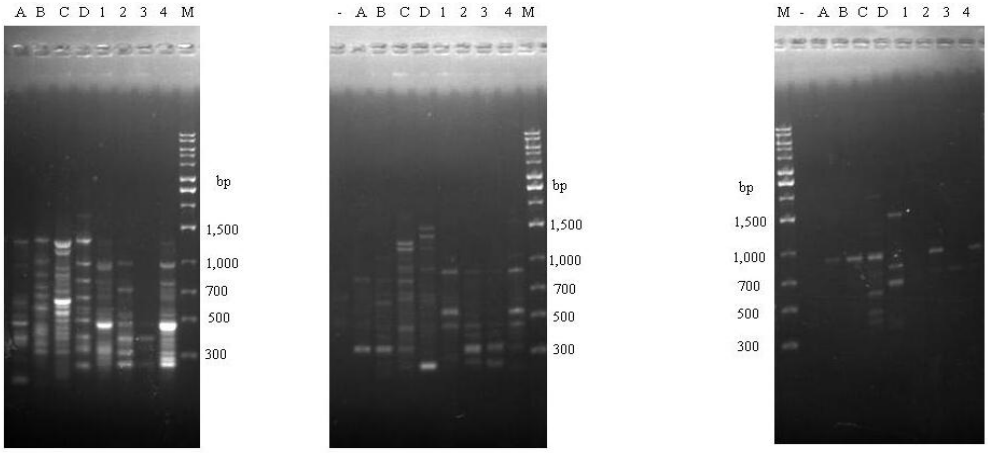

Plate 3: The DNA bands of isolated organisms (bacteria and fungi) in the study. (a) shows complete DNA bands with the three markers. (b) a section of the DNA bands using OPR-02 marker with a base sequence – GATGACCGCC, (c) a section of DNA bands using OPC-10 marker with base sequence – TGTCTGGGTG, (d) a section of DNA bands using OPI-05 marker with base sequence – GGTTCCACGC.

The DNA results show that with Marker/Primer OPR-02, fungal isolate D (*Asppergillus niger*) and bacterial isolate 2 (*Bacillus* spp*)* had similar DNA bands with the marker of 1000bp; fungal isolate C (*Mucor* spp*),* bacterial isolates 2 (*Bacillus* spp*)* and 4 (*Micrococcus* spp.) had similar bands with marker of 700bp; bacterial isolates 1 (*Pseudomonas* spp*),* 2 (*Bacillus* spp*),* and 4 (*Micrococcus* spp) had similar DNA bands with the marker of 300bp. With Marker/Primer OPC-10, the fungal isolates D (*Aspergillus niger*) has similar band with the marker of 1500bp; bacterial isolates 1 (*Pseudomonas* spp*)* and 4 (*Micrococcus* spp.) had similar DNA bands with the marker of 900bp; fungal isolates A (*Aspergillus flavus*) and B (*Penicillium notatum),* and bacterial isolates 2 (*Bacillus* spp*)* and 3 (*Staphylococcus aureus)* had similar bands with the marker of

300bp. With Marker/Primer OPI-05, fungal isolates C (*Mucor* spp) and D (*Aspergillus niger)* have similar bands with the marker of 1000bp while bacterial isolate 1(*Pseudomonas* spp*),* had similar band with the marker of 700bp.

4. CONCLUSION

This study has shown that air contains both bacterial and fungal microorganisms especially in the wards of a hospital environment which could lead to increased infection of the patients. As a result, maintaining high hygienic standards of hospital wards and surroundings should be of utmost importance for medical personnel and patients within and outside the hospital environment. Thorough washing of hands with soap and running water as well as the use of hand sanitizers or alcohol disinfectants are highly recommended to limit the spread of these infectious organisms both within and outside the hospital environment. This study also shows that organisms may have similar plasmids and DNA segments. This implies that some of the diseases caused by these nosocomial organisms can be treated with combined therapy of antibiotics or antifungal drugs. General individual hygiene and healthy living is also important, because it would help build the body immunity strong enough to withstand certain bacterial and fungal infections.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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