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Evaluation of Bacteriological Quality of Indoor Air of Selected Theatres and Wards in a Nigerian Teaching Hospital

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Abstract
The air of confined environments in hospitals may not be a growth medium for microbes but it could be a viable means of transmission of infective nuclei droplets and particulate matter. This research was carried out at the male and female surgical wards of the University of Port Harcourt Teaching Hospital. Sterile blood and MacConkey agar plates exposed at specific points in contact with air at some specified areas of the hospital at a height of one meter above the ground were incubated at 37^{O}C and the isolates were identified following standard bacteriological procedures. The result showed a total of 484 organisms isolated out of which 20% were *Staphyloccocus aureus*, 22% were coagulase negative *Staphyloccocus* species, 15% were *Proteus mirabilis*, 6% *Klebsiella pneumucoccus*, and 5% *Baccillus subtilis*. The presence of these may strongly have impact on the nosocomial and opportunistic infections in the hospital. Therefore, proper hygienic practices and safety equipment should be advocated in confined areas of the hospital that could predispose patients to opportunistic infections. Also, concerted efforts at improving the airborne quality of the hospital environment should be promoted and maintained to enhance public and patient health.
Keywords: Quality, Airborne, Bacteria, Ward, Hospital.

Introduction

Microbes that are found in the air of supposed clean and sterile rooms in the hospital like the theatres and surgical wards were basically contributed by humans including staff, patients and their relatives who makes use of the hospital facility. However, microbes found in hospital confined areas could also originate from various outside sources, as a result of indoor hospital activities as well as environmental conditions such as sunlight, temperature, humidity (Nester *et al*., 1998), number of times the door is opened (Scaltriti *et al*., 2007), occupant density of these rooms (Obbard *et al*., 2003), the location of the hospital or the rooms in the hospital (Obbard *et al*., 2003).

Nevertheless, according to Prescott *et al*., (1999), since bacteria does not grow in the air, those found there may have arisen from human, soil, animal, food, water or plant sources. This makes airborne microbial flora of confined hospital spaces to be transient. Prescott and his colleagues further stated that pathogens can only survive in air when trapped and suspended in air droplets and this can be a source of transmission of these pathogens to humans who inhale them since they can remain viable in air droplets for days.

However, the microbial load of clean rooms in hospitals and clinics such as operating rooms, hospital rooms/wards, intensive care units, maternity wards, etc, could depend on the environmental conditions prevailing at a particular point in time inside and outside the hospital, the nutritional content of the air and the outdoor microbial load (Dong-Uk *et al*., 2013).

This study was targeted at indoor airborne bacteria profile of some selected wards and theatre of the University of Port Harcourt Teaching Hospital, Port Harcourt. This is important as it will help provide data for subsequent researches and planning for arresting the trend of bacteria related nosocomial infections in hospitals.

Methodology

The area of study was the University of Port Harcourt Teaching Hospital, Alakahia, Port Harcourt. This is a tertiary hospital with 500 bed space, designed to accommodate referrals from different health centers across the length and breathe of the metropolitan city of Port Harcourt as well as the south-south geopolitical zone. This research was carried out from theatre 2 and theatre 3 of the main theatre and the female and male surgical wards at the University of Port Harcourt Teaching Hospital, Port Harcourt. The media used in this study are MacConkey agar and blood agar plates. Sterile plates were placed at selected points within the theatres and wards earlier mentioned. The settle plate method was adopted for this study; here, the sterile MacConkey plates and blood agar plates were exposed to selected points in the theatre and the selected wards enumerated above in three consecutive intervals of time and days (ie. morning, afternoon and night as well as three consecutive days in a week; Monday, Tuesday and Wednesday). This was done at heights of 1m above the ground and exposed to the atmospheric conditions of these sites of study for a one hour period. Different plates were exposed for 3 consecutive days in a week in the afternoon and at the various study sites in the hospital. These exposed plates were immediately transferred to the medical microbiology laboratory for incubation at 37^{o}C for 24 hours. Then the different colonies were counted and were sub-cultured for purity. Thereafter, these pure cultures were made

to undergo the following tests; motility, gram stain, Kovac's reagent, catalase, coagulase, Voges Proskauer, oxidase, and methyl-red.

- **Gram Staining Technique**: a smear of the pure culture was made on a clean grease free glass slide and air dried. It was then flamed fixed, allowed to cool and stained according to the procedure outlined by Ochei and Kolhatkar(2000).
- **Motility Test:** each colony was inoculated into a peptone water broth and mixed properly and incubated at 37^0C for 24 hours. A drop of the broth was then placed on a clean cover slip. Plasticine was used to make a ring around the drop of the broth and a grease free glass slide was placed over the plasticine ring and pressed slightly and immediately inverted. It was examined microscopically with X10 and X40 objectives with condenser sufficiently closed.
- **Biochemical tests:** the biochemical tests were carried out on the pure cultures to aid specific organism identification according to their different reactions to the various chemical/enzymatic activities inherent in these biochemical tests following the procedures outlined by Ochei and Kolhatkar, (2000).

Results

The results obtained in this study showed that MacConkey and Blood agar plates sampled at different points in theatre 2 on Monday revealed a mean colony forming units (CFU) per plate of 48, while on Tuesday, 45 CFU were observed and on Wednesday 38 CFU. This produced an average daily colony forming units of 44 per plate. However, theatre 3 showed for all Monday sampling, a mean of 61 CFU/plate, for all the Tuesdays, a mean of 28 CFU/plate were observed, while for all the Wednesdays, a mean of 33 CFU/plate was observed; showing an average daily mean colony forming units of 41 per plate. Nevertheless, the female surgical ward on Monday showed an observable mean of 39 CFU/plate whereas Tuesday yielded a mean of 65 CFU/plate and then, Wednesday produced a mean of 22 CFU/plate. This generally revealed a daily mean colony forming units observable in the female surgical ward to be 42 CFU/plate sampled. Also, the male surgical ward which had a mean of 34 CFU/plate produced a mean of 46 CFU/plate on Mondays, 31 CFU/plate on Tuesdays and 26 CFU/plate on Wednesdays (see Table 1). Obviously, this study have shown the isolation of airborne Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Micrococcus species, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae and coagulase negative Stapylococcus species in varying percentage frequencies. The least being Bacillus subtilis showing 5% prevalence and the highest being coagulase negative Staphylococcus species with 22% prevalence. Generally, the study showed a total of 484 organisms, out of which 20% were Staphylococcus aureus, 10% Escherichia coli, 15% Proteus mirabilis, 12% Pseudomonas aeruginosa, 5% Bacillus subtilis, 22% coagulase negative Staphylococcus species and 6% were Klebsiella pneumoniae (see Table 2).

Table 1: Mean Bacterial Load Isolated At Different Study Sites and Day Intervals

Study Site	Research Days	Mean Bacteria Load (CFU/plate)	Study Site Mean Bacteria Load (CFU/day)
Theatre 2	Monday	48	131
	Tuesday	45	
	Wednesday	38	
Theatre 3	Monday	50	
	Tuesday	51	124
	Wednesday	23	
Female Surgical Ward	Monday	39	
	Tuesday	65	126
	Wednesday	22	
Male Surgical Ward	Monday	46	
	Tuesday	31	103
	Wednesday	26	
			N=484

Table 2: Percentage Bacteria Distribution.

Isolates	Total Percentage Isolation % (Total Isolated)
Staphylococcus aureus	20(97)
Escherichia coli	10(48)
Proteus mirabilis	15(73)
Micrococcus spp	12(58)
Pseudomonas aeruginosa	10(48)
Bacillus subtilis	5(24)
Coagulase negative *Staphylococcus* spp	22(107)
Klebsiella pneumoniae	6(29)
Total	100(484)

Discussion

Air quality in hospital environment has become an important issue in modern society and its assessment appears to be a fundamental tool for public health assessment, disease diagnosis and prevention. The most common airborne microorganisms include bacteria and fungi species, which are responsible for possible multiple infections resulting in allergies and respiratory diseases.

The study showed a high incidence of bacteria isolation in the various sites of sampling at the University of Port Harcourt Teaching Hospital (see Table 3). The available data in table 3 revealed that, the highest prevalence of bacteria was found in theatre 2 with 27.1% bacteria load which was similar to that obtained by Kaoruko *et al*., (2005). This was followed by the female surgical ward with a prevalence of 26%. Whereas theatre 3 and the male surgical ward had a prevalence of 25.6% and 21.3% respectively. Among the different sites sampled within the hospital, the bacteria profile of the organisms were as follows; theatre 2 yielded 17.6% *Staphylococcus aureus,* 12.2% *Escherichia coli*, 12.2% *Proteus mirabilis*, 13.3% *Micrococcus* spp, 13.7% *Pseudomonas aeruginosa*, 19.8% coagulase negative *Staphylococcus* spp and 4.6% *Klebsiella pneumonia*. Similarly, in theatre 3, *Staphylococcus aureus* was seen in 16.9% of the total bacteria isolated. Also, coagulase negative *Staphylococcus* spp was the organism with the highest frequency in theatre 3 and was similar to the study by Kaoruko *et al*., (2005) in Tokyo, Japan.

Table 3: Percentage Distribution of Bacteria Isolates Based on the Study Sites

	Theatre 2 (%)	Theatre 3 (%)	Female Surgical Ward (%)	Male Surgical Ward (%)
Staphylococcus aureus	23(17.6)	21(16.9)	34(27)	19(18.4)
Escherichia coli	16(12.2)	5(4)	21(16.7)	6(5.8)
Proteus mirabilis	16(12.2)	19(15.1)	0(0)	58(12)
Micrococcus spp	18(13.7)	21(16.9)	19(15.1)	0(0)
Pseudomonas aeruginosa	18(13.7)	14(11.3)	0(0)	16(15.5)
Bacillus subtilis	8(6.2)	8(6.5)	8(6.3)	0(0)
Coagulase negative *Staphylococcus* spp	26(19.8)	26(21)	7(5.6)	**Isolate**
Klebsiella pneumoniae	6(4.6)	10(8.1)	13(10.3)	
Total	131(27.1)	124(25.6)	126(26)	103(21.3)

The coagulase negative *Staphylococcus* spp could be important pathogens in cases of bacteria endocarditis and urinary tract infections in sexually active women (Uwaezuoke *et al*., 2003). These results obtained in this study showed that, the theatre is a high risk area as it yielded a bacteria load of 124 and 131 for theatres 3 and 2 respectively. This was in conformity with the submission of Srikanth *et al.,* (2008). However, the male surgical ward had the lowest bacteria load of 103 CFU/day, which was in the range of bacteria load obtained by Kaoruko *et al*., (2005) (50-992 CFU/day) and Fang *et al*., (2007) (70-2210 CFU/day). Although these bacteria loads were less than the 500 CFU/day stipulated by legislation in Portugal. This contamination level lower than the maximum reference level of 500 CFU/m^3 imposed by Portuguese legislation on D.L. 79/2006, 4th April., (Graça *et al*., 2009). This may have been due to the disparity in methods used which was different from the recommended standard slit sampling method. Nevertheless, it is necessary to take note of this result as it could form basis for further studies and hygiene plan. It is important to note that, with modern air conditioning systems that have the capacity to filter the air and remove particulate matter; its advantage can be used to improve the ventilation system of theatres. Proper air handling and ventilation of theatres, will improve the quality of the air enclosed in these theatres.

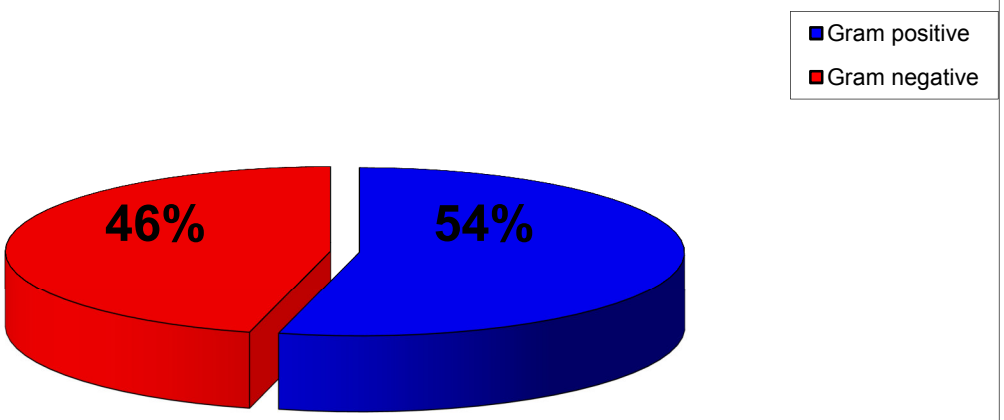

Fig. 1: Distribution of Gram Positive and Negative Organisms in the Study

Gram positive cocci were predominant in the isolates (54%) (Fig. 1) with coagulase negative *Staphylococci* being the highest, followed by *S. aureus* and *Micrococcus* which is similar to the findings of Suchitra *et al.,* (2006) which had *Staphylococci* and *Micrococcus* spp as the most predominant organisms.This study however, recorded more *Proteus mirabilis* 15% (Table 3), next to *Pseudomonas aeruginosa*. This was in contrast to the findings of Suchitra *et al.,* (2008) which had Pseudomonas as the highest occurring organism. The presence of *Bacillus subtilis* is also a potential harmful nosocomial and opportunistic infections (Talaro and Talaro, 2002) and as such could be a potential agent of contamination of open wounds. Similarly, *Pseudomonas* spp, *Klebsiella* spp and *Staphylococcus* were all reported in wounds by Ayodele *et al.,* (2010) from the same site used in this study, the occurrence of these organisms in this study though from air therefore could be significant since some of their subjects were in-patients and need to be taken seriously. It is essential therefore to undertake strict hygiene controls that would ensure minimal bacteria load in the air of hospital confined environments.

Conclusion

This study had shown the range of micro-organisms harbored in the various confined environments that was studied. Also, it represents a contribution on the assessment of the potential biological risks that are inherent to the exposure of patients and hospital workers in these areas of the University of Port Harcourt Teaching hospital. This result had shown that, there is the possibility that some hospital acquired infections could have been contracted through airborne pathogens present in the wards and theatre where patients are kept in hospitals. Obviously, it is basically necessary to apply preventive measures to minimize the risks of exposure to this potential airborne pathogen in the hospital environment.

Recommendations

A careful look at the results has therefore led to the following recommendations:

This study had shown the need for a robust policy to be put in place which will ensure safety and good quality of air in and around the hospitals and especially theatres and the wards. Furthermore, strict adherence to aseptic techniques and infection controls during and after surgeries to limit contamination of wounds by airborne pathogens within the theatres and wards. These policies and or regulations may include; frequent and proper disinfection of hospital wards and high risk areas to ensure good ventilation, avoiding items to cluster the windows/vents. There should be introduction of positive air pressure vents for contaminated areas that requires to be made clean and negative air pressure vents for hospital areas accommodating people with communicable diseases spread through the air. Also, the use of wet mopping to clean hospital wards instead of sweeping should be encouraged this will reduce the generation of aerosols. Similarly, the use of ultraviolet light which has the ability to destroy bacteria in theatres is highly advocated in hospital theatres and other areas that require sterility, particularly when they are not in use. Moreover, there should be the use of highly efficient particle arrester (HEPA) filters in areas with high risk(s) of infection. Hospitals should carry out periodic self assessment of their hospital indoor air quality based on the critical parameters for air quality as given by the World Health Organization, (2006). This includes the following; maintenance/validation of efficiency of filters, pressure gradient across the filter bed and in the operation theatres, air changes per hour, temperature and humidity should be between 20^{o}C - 22^{o}C and 30% - 60% respectively to discourage bacteria multiplication, and routine surveillance for nosocomial infections

Finally, it is imperative for hospital management to create a strategic plan for the continuous

education/awareness of members of staff and the larger hospital community through different media and also institute a strategic monitoring capacity to ensure strict compliance.

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