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Evaluation of Indoor Air for Bacteria Organisms and their Antimicrobial Susceptibility Profiles in a Government Health Institution

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAW designed and supervised the study. Author VKR performed the work, statistical analysis, wrote the protocol, managed the analyses and literature searches of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate environmental bacteria isolates from a government health institution and their antimicrobial profile.
Place and Duration of Study: The sampled location was the mini Mile 3 Model Primary Health Centre, Port Harcourt, Nigeria for three months (January-March).
Methodology: Indoor air quality was investigated using the sedimentation technique in which Petri plates containing growth media were exposed to the atmosphere of the sites under study. Plates were exposed for 15 minutes at each given site. The children, post-natal, outpatient and the injection wards were the sites under study. Nutrient agar and Mannitol Salt agar were the media used to enumerate the total bacteria and *Staphylococci* respectively. Commercially prepared antibiotic discs with known concentrations were used to test for susceptibility of these microbes using the disc diffusion technique and test isolates were standardized using the McFarland standard.

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Results: Four bacterial groups isolated were *Bacillus* sp (20.41%), *Micrococcus* sp (28.57%), *Serratia* sp (10.21%) and *Staphylococcus* sp (40.82%). The mean count for the total heterotrophic bacteria and *Staphylococci* counts in $log_{10}Cfu/m^3$ for morning sections ranged from (3.41±021-3.84±0.09) and (3.15±0.14 - 3.25±0.13) respectively; while counts for the evening sections ranged from (3.50±0.11- 3.91±0.05) (3.24±0.42 – 3.48±0.04) respectively. There was a significant difference between the morning and evening hours of the total *Staphylococci* at *P*=0.05. All the bacterial isolates were 100% susceptible to ciprofloxacin. *Serratia* species were 100% susceptible to ofloxacin. *Staphylococci* and *Micrococcus* species were 100% resistant to ampicillin which was 100% effective against *Bacillus* sp.
Conclusion: The microbial loads in this study were very high. Microbes isolated in this study are pathogenic and are known to be associated with nosocomial infections. Ciprofloxacin, gentamycin, pefloxacin and ofloxacin are best recommended for infections arising from this site.

Keywords: Microbial contamination; antimicrobial profile; indoor air; sedimentation; health centre; nosocomial infections.

ABBREVIATIONS

Cfu : colony forming unit,
M^3 : cubic metre,
THB : total heterotrophic bacteria,
ANOVA : analysis of variance,
TSC : total Staphylococcal counts

1. INTRODUCTION

Microbial contamination of indoor air especially in health institutions has become a global or public health concern. Studies have revealed the presence of bioaerosols in indoor air which are pathogenic and are able to cause serious health challenges. Agbagwa and Onyemaechi reported the importance of good air quality within health institutions as these institutions are known to contain different persons including sick persons [2]. As reported by Mahmoud et al., in other to ensure good air quality within the health care facility, there should be strict adherence to the type and quality of air within this environment so as to protect workers and patience from hospital acquired infections [9]. Yassin and Almouqatea reported that serious respiratory abnormalities and health challenges which include infections, hypersensitivity, pneumonia and toxic reactions could occur when these biological materials (bacteria, fungi, viruses, etc) or their by-products come in contact with man. Also in respect to exposure of biological particles into the atmosphere, air pollution and population health is regarded as a public health problem [5]. Studies on evaluation of air quality have revealed serious health problems that are caused by the contamination of air with pollutants especially biological particles. This poses a threat both to the environment, quality of life as well as the health of the growing population [10]. Zemichael et al also reported how the quality of air within the building could be detrimental to the health of persons (about 80-95% who spend their lives indoor) who through inhalation would have inhaled about 10 m^3. This biological aerosol is discharged into the atmosphere through various activities such as sneezing, coughing, laughing, and sweeping including other activities. When these aerosols are present in the atmosphere, transmission could either be by inhalation as mentioned earlier or by contact with non-critical surfaces which these particles may have settled on. The air though not a natural medium for microbes serves as a carrier of particles including dust and droplets which heavily contaminates it. Factors such as sunlight, temperature, humidity, size of microbes determine the availability including the population and type of microorganisms within that building [4].

An antibiogram is however a chart that shows the susceptibility test result of microorganisms against the tested antibiotics. Due to the increasing rate of resistance of pathogenic organisms to commonly used antibiotics, the need to test microorganisms against these commonly used antibiotics would be of immense help to the physicians in prescribing specific drugs and thereby reduce the use of broad spectrum drugs. The clinical and laboratory standard institute [6] provided guidelines for antimicrobial susceptibility testing and have recommended the development of antibiogram annually. Thus, this study is aimed at determining the bacteria organisms from indoor air and their antibiotics susceptibility profile in a government health institution.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Mini Mile 3 Model Primary Health Centre which is located along the building material axis of the mile 3 market in Port Harcourt Local Government Area of Nigeria. There are about eight wards of which only four are constantly used while the other wards are not utilized. The four most used wards are the children, outpatient, post-natal and injection wards which are the studied sites.

2.2 Microbiological Analysis of Air Samples

The plate exposure technique also referred to as Kosch's sedimentation method was employed as sample collection method [8]. In this method, Petri dishes containing freshly prepared growth media (Nutrient agar and Mannitol salt agar) in duplicates were exposed above one metre from the ground [4] to the ambient air of the various study sites for fifteen (15) minutes. Sampled plates were closed at the end of sampling and transferred to the Microbiology Laboratory of the Department of Microbiology, Rivers State University, where they were incubated at 37°C for 24 hours. Sampling was carried out twice a day (between 8- 10 am; at the peak of work activities and between 4- 6 pm; after the end of work activities) in each of the study sites. The study was for a period of three months (January-March) from dry season to the beginning of the rainy season. The studied sites were the children ward, outpatient ward, post natal ward and injection/immunization ward. At the end of incubation, positive bacteria colonies were counted and enumerated using the Kosch's sedimentation formula as described by Latika and Ritu [8]. Distinct colonies were isolated by streaking aseptically onto freshly prepared nutrient agar and mannitol salt agar. Cultural, morphological and biochemical characteristics were employed for identification of isolated colonies as described by other researchers [3,4]. At the end of identification, the Bergey's manual of determinative microbiology was used to ascertain the identified isolates.

2.3 Estimation of the Colony Forming Units

The colonies from each plate were estimated using the koch's sedimentation formula.

$$A = a \times 10^4 / 0.2 \times \pi r^2 \times t$$

A= Cfu/M^3
a= average number of colonies
r = radius of Petri dish
t = time of exposure of the plate

The collected data were statistically analyzed using IBM SPSS version 22 statistical tool. ANOVA without replication was used to check for significant differences between mean of both morning and evening sections for the studied sites.

2.4 Antimicrobial Susceptibility Tests (AST)

The disc diffusion method as described by Aayesha et al was employed [1]. The 24 hours old cultures of each test organism were spread evenly onto the surface of Mueller-Hinton agar and allowed to dry. Wafers containing the antibiotics were aseptically placed on the surface of the dried agar plates and incubated for 18-24 hours, following which their zone of inhibition diameter were measured and recorded. Susceptibility to conventional antibiotics was determined using the standard described by CLSI [6].

3. RESULTS AND DISCUSSION

In the present study, a total of 192 air samples were collected from the indoor air of the various study sites. The result revealed the presence of microorganisms in the various study sites. The mean bacterial counts in $log_{10}Cfu/m^3$ for the total heterotrophic bacterial and *Staphylococcal* load of the study sites for both the morning and evening sampling (hours) are presented in Figs. 1 and 2 respectively. The mean total heterotrophic counts in $log_{10}Cfu/m^3$ of the morning sampling ranged from 3.41±0.21 to 3.84±0.09 while the evening microbial load ranged from 3.50±0.11 to 3.91±0.05 respectively. The highest microbial counts were observed in the outpatient ward (3.84±0.09 and 3.91±0.05) for morning and evening sampling respectively. This increased microbial load in this area could be related to the dual purpose of the ward as it is also used as the reception containing vast number of different persons who shed their normal flora into the ambient air. The children ward had the least total heterotrophic counts of 3.41±0.21 whereas the injection ward was least in the evening sampling with 3.50±0.11. Literatures haven't revealed any unanimous globally acceptable standard on the levels of

bacterial loads in indoor air. In an assessment of health risks of biological agent in indoor environment conducted by some WHO experts, it was suggested that the total microbial load should not exceed 1000 Cfu/M^3 [12]. Thus, microbial load above this standard could be hazardous to the health of people within the building. The *Staphylococcal* counts were higher in the evening sampling (Fig. 2). In Fig. 1, the total heterotrophic bacterial loads increased during the evening sampling in all the study sites except in the injection ward which was higher during the morning hours. The observed increase in the morning hours in the injection ward could be attributed to immunization and injection activities which are mostly done in the morning hours. Varied number of persons coming in for immunization/injection may have shed part of their normal flora via sneezing, talking, coughing into the indoor air. Despite the higher microbial counts observed in the evening sampling, there was no significant difference between the morning and evening sampling of the total heterotrophic counts in all the wards at *P=0.36* while a statistical significant difference in the *Staphylococci* count between the morning and evening sampling existed at *P = 0.004.* The increase in microbial counts in the evening hour (after work activities) has been reported by [72,]. The children ward had the least microbial load and this could be attributed to the low hospitalization of children observed in the health centre. The microbial load in this study is higher than those reported by Agbagwa and Onyemachi [2]. The study sites were ventilated both mechanically and by natural air (via windows and open doors) during the morning hours (peak of work activities) except for the evening hours that ventilation is only dependent on natural air or none at all. This factor could be responsible for the high microbial populations observed in the evening hours compared to those of the morning hours. Insufficient ventilation has been reported as a contributing factor to the increase in microbial load [15,16]. Though the temperature and humidity of the study sites was not determined, scholars have revealed that favourable temperature and humidity could influence microbial populations, thus, leading to the survival and eventual growth of microorganisms [12], this may have influenced the high levels of microbial load in this study (Figs. 1& 2).

Four bacterial groups belonging to *Bacillus* species (20.41%), *Micrococcus* species (28.57%), *Serratia* species (10.21%) and *Staphylococcus* species (40.82%) were isolated from the various study sites. *Serratia* species was not isolated in the children ward (Table 2), *Staphylococcus* and *Bacillus* species were more predominant in the children wards, while *Micrococcus* species were more predominant in the outpatient and post-natal wards. *Serratia* species were more predominant in the injection ward (Table 2). *Bacillus* species are known to possess endospores which increase their chance of survival; thus this may have led to their abundance and presence in all the wards while *Staphylococcus* is a known cause of skin diseases, food borne infections and urinary tract infections. It is readily transmitted either by fomites to persons, person to person contact or via inhalation. This could be the reasons for its high occurrence and predominance [2]. Except for *Serratia* species which is present in this study, all other isolates are similar to most organisms isolated from other studies [7,2].

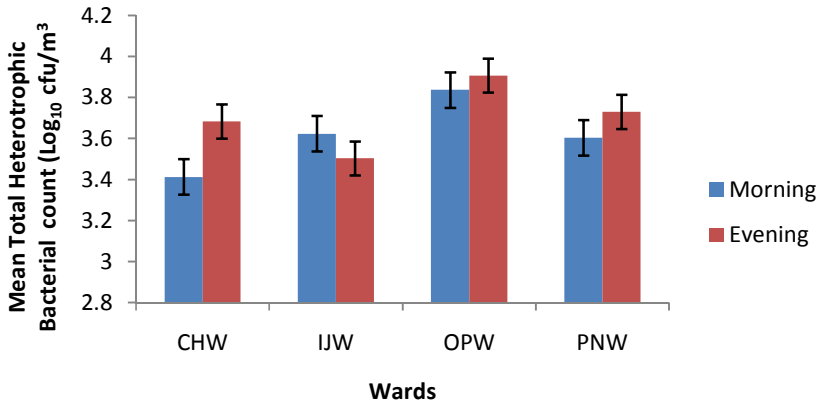

Fig. 1. Mean Microbial population in log_{10}Cfu/m^3 of the morning and evening section

Table 1. Susceptibility pattern of isolates of Mini mile 3 Primary health centre

Isolates (N)	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	OX	AU	OFX	CH	SP
	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)
Staphylococcus sp (20)	65	15	15	20	0	10	100	0	15	10	20	NA	NA	NA	NA
Bacillus sp (10)	100	90	80	100	100	0	100	60	80	100	NA	NA	NA	NA	NA
Micrococcus sp (14)	100	100	100	0	0	0	100	78.6	78.6	7.1	NA	NA	NA	NA	NA
Serratia sp (5)	80)	40	NA	NA	40	NA	100	60	0	NA	NA	0	100	100	100

**Antibiogram of various isolates*

Keys: Pef: Pefloxacin, CN: Gentamycin, APX: Ampiclox, Z: Cefuroxime, AM: Amoxicillin, R: Ceftriaxone, CPX: Ciprofloxacin, S: Streptomycin, SXT: Trimethroprime-Sulphamethaxole, E: Erythromycin, OX: Oxacillin, CH: Chloramphenicol, AU:Amoxicillin-clavulanic acid, OFX:ofloxacin, SP:sparfloxacin. (N): number of bacterial isolates, S: susceptibility.

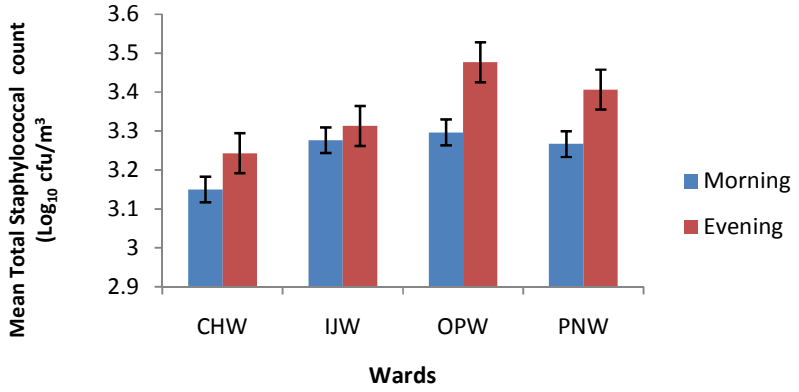

Fig. 2. *Staphylococcal* population in $log_{10}Cfu/m^3$ of the morning and evening sections

Keys: CHW= children ward, IJW= injection wards, OPW=outpatient ward, PNW= post-natal ward

Table 2. % occurrence of bacterial isolates in various ward

Microbial Isolates	% CHW	% OPW	% PNW	% IJW
Bacillus species	20	40	15	25
Micrococcus species	7.14	42.85	21.43	28.57
Serratia species	0	20	20	60
Staphylococci species	20	40	15	25

Results of susceptibility tests of antimicrobial drugs against *Staphylococcus, Bacillus, Micrococcus and Serratia* species revealed their susceptibility pattern to pefloxacin, gentamycin, ampiclox, cefuroxime, amoxicillin,Ceftriaxone , ciprofloxacin, streptomycin, erythromycin, oxacillin, chloramphenicol, amoxicillin-clavulanic acid and tarivid respectively (Table1). All twenty *Staphylococcal* species isolates were susceptible to nine antibiotics with higher susceptibility rate in ciprofloxacin (100%) and pefloxacin (65%). Erythromycin has the least susceptibility rate at 10%, while high resistance rate was observed in Amoxicillin and Streptomycin respectively (Table1). The drug resistance could be attributed to factors such as multidrug efflux pump system, alterations of drug target sites or mutations in the nucleic acid sequence. Ten *Bacillus* species isolates tested were susceptible to pefloxacin, gentamycin, ampiclox, zinnacef, amoxicillin, ciprofloxacin, streptomycin, septrin and erythromycin in the following order- 100%, 90%, 80%, 100%, 100%, 100%, 60%, 80%, and 100% respectively. The ten Bacillus species isolates were not sensitive to Rocephin (Table1). Ampicillin, gentamycin and erythromycin have been reported to possess some remarkable level of antimicrobial activitiy on *Bacillus* and *Micrococcus* species [16]. *Micrococcus* species were highly susceptible to pefloxacin, ampiclox, gentamycin, and ciprofloxacin and their actions were 100%. Second most effective drugs were observed to be streptomycin and septrin with activity of 78.6% while erythromycin had the least antimicrobial action of 7.1%. Zinnacef, ampicillin and rocephin were not very effective drugs as complete resistance was observed. *Serratia* species were completely sensitive to ciprofloxacin, tarivid, chloramphenicol and sparfloxacin respectively while complete resistance was observed in septrin and augmentin (Table1). This study revealed that some commonly used antibiotics are becoming less effective to pathogens in these study sites. Indiscriminate use of drugs as well as the co-habitation (where resistance genes are transferred to other organisms in the same niche) could be responsible for the observed resistance as well as susceptibility. This statement is in agreement with the work reported by (14).

4. CONCLUSION

The microorganisms identified in this study are known to be pathogenic and could be implicated in many hospital acquired infections. The microbial population in this study is very high as compared to suggested standards and could be caused by poor ventilation systems as well as hygienic standard of the people including the influx of patients and staffs. The susceptibility pattern of the isolates revealed that ciprofloxacin, ofloxacin, gentamycin and pefloxacin were more effective drugs; thus, should be considered the drug of choice for infections caused by these microbes in these sites.

CONSENT

All authors declare that 'written informed consent to undertake the research was obtained from the Head medical officer Port Harcourt city local government Area, Rivers State, Nigeria.

ETHICAL APPROVAL

The ethical consideration to undertake the research was sought and obtained from the ethics committee of the Rivers State Primary Health Care Management Board, Port Harcourt and permission to perform the study at these sites was given by the Head of Medical Officer, Port Harcourt City Local Government Area.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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