ANTIBIOGRAM OF INDOOR AIR MICROBES IN A GOVERNMENT HEALTH INSTITUTION

ABSTRACT

Aim: To develop an antibiogram for indoor air bacteria isolated from a government health institution
Place and Duration of Study: The sampled location was the mini mile3 model primary health centre, Port Harcourt with coordinates 4.806°N, 6.992°E for three months (January-March).
Methodology: Indoor air quality was investigated using the kosch’s sedimentation technique which allows Petri plates containing growth media to be exposed to the atmosphere of the study sites. plates were exposed for 15 minutes in each given site. The children, post-natal, outpatient and the injection wards were the studied sites. Nutrient agar and mannitol salt agar were the media used to enumerate the total bacteria and Staphylococci respectively. Commercially prepared antibiotics disc 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.
Results: Four bacterial genera isolated were Bacillus sp (20.41%), Micrococcus sp (28.57%), Serratia sp (10.21%) and coagulase negative and positive Staphylococcus sp (40.82%). The mean count for the total heterotrophic bacteria and Staphylococci counts in log_{10}CFU/m³ for morning sections ranged from (3.41±0.21-3.84±0.09) and (3.15±0.14 - 3.25±0.13) respectively. While counts for the evening section 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. There was a 100% susceptibility rate by the microbes to ciprofloxacin. Serratia species were 100% susceptible to tarivid. Staphylococci and Micrococcus species were 100% resistant to ampicillin which was 100% effective against Bacillus.
Conclusion: The microbial population in this study was very high. Microbes isolated in this study are pathogenic and are known to be associated with nosocomial infections. Ciprofloxacin, gentamycin, pefloxacin and tarivid are best recommended for infections arising from this site.

Key words: microbial contamination, antibiogram, indoor air, Koch’s sedimentation, health centre, nosocomial infections

INTRODUCTION

Microbial contamination of indoor air especially in health institution 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. [2] reported the importance of good air quality within health institution as this institution is known to contain different persons including sick persons. 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]. [11] reported that serious respiratory abnormalities including serious health challenges which include infections, hypersensitivity, pneumonities 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 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]. [12] also reported how the quality of air within the building could be detrimental on the health of persons (about 80-95% who spend their lives indoor) who through inhalation would have inhaled about 10 m³. This biological aerosol is discharged into the atmosphere through various activities such as sneezing, coughing, laughing, and sweeping including other activities. When this aerosol are present in the atmosphere, transmission could either be by inhalation as mentioned earlier or by contact with non-critical surfaces in which this particles must 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 a chart that shows the susceptibility test result of microorganisms against the tested antibiotics. Due to the increasing resistance of pathogenic organisms to the 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 recommended the development of antibiogram annually. Thus, this study is aimed at developing an antibiogram of microorganisms isolated from indoor air of a government health institution.

MATERIALS AND METHOD

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 with coordinates 4.806°N and 6.992°E. There are about eight wards of which only four are constantly used while the other wards are not utilized.

Collection of air samples and Microbial analysis

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 in a day (between 8-10 am; at the peak of working activities and between 4- 6pm; 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, ensuing colonies were counted and enumerated using the kosch’s sedimentation formula as described by [8]. Distinct colonies were isolated by streaking aseptically onto freshly prepared media of 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 Bergy’s manual of determinative microbiology was used to ascertain the identified isolates.

Estimation of the Colony Forming Units
The colonies from each plate were estimated using the Koch's sedimentation formula.

\[ A = \frac{a \times 10^4}{0.2 \times \pi r^2 \times t} \]

- \( A = \text{Cfu/M}^3 \)
- \( a = \text{average number of colonies} \)
- \( r = \text{radius of Petri dish} \)
- \( t = \text{time of exposure of the plate} \)

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 wards.

**Antimicrobial Sensitivity**

The disc diffusion method was employed [1]. The 24 hours old culture of each test organism were seeded 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 24 hours. Zone diameter were measured and recorded after 24 hours of incubation.

### 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 Staphylococcus of the study sites for both the morning and evening sections are presented in figure 1 and 2 respectively. The mean total heterotrophic counts in log_{10}Cfu/m^3 of the morning section ranged from 3.41-3.84 whereas the evening counts ranged from 3.50-3.91 respectively. The highest microbial count was observed in the outpatient ward (3.84 and 3.91) for morning and evening sections respectively. The children ward had the least count with 3.41 whereas the injection ward was least in the evening section with 3.50.

The Staphylococci counts were higher in the evening section. In fig 1, the bacterial counts increased in the evening section except in the injection ward. Despite the higher microbial counts observed in the evening section, there was no significant difference between the morning and evening sections of the total heterotrophic counts in all the wards (table 2) while a significant difference in the Staphylococci count between the morning and evening section exist (table 3). The increase in microbial counts in the evening section (after work activities) has been reported by [7]. It could be that the lack of ventilation in the evening section (after work activities) may be responsible for the high microbial counts observed. The injection ward has a higher microbial count in the morning section (peak of work activities) as compared to the evening section (fig 1). This could be related to the influx of patients including immunization activities which usually take place in the morning sections.

Four bacterial genera belonging to Bacillus (20.41%), Micrococcus (28.57%), Serratia (10.21%) and Staphylococcus (40.82%) species were isolated from the various study sites except Serratia sp which was not isolated in the children ward (table 4). Except for Micrococcus species which is present in this study, all other isolates are similar to most organisms isolated from other studies [7; 2]. Streptococcus sp, including other pathogenic organisms not isolated in this study may not be that these organisms do not occur in this environment.
Fig1. Mean Microbial population in $\log_{10}\text{CFU/m}^3$ of the morning and evening section.

Fig2. *Staphylococci* population in $\log_{10}\text{CFU/m}^3$ of the morning and evening section.

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

In table1, the antimicrobial drugs tested against *Staphylococcus, Bacillus, Micrococcus* and *Serratia* to reveal their susceptibility pattern were pefloxacin, gentamycin, ampiclox, zinnacef, amoxicillin,rocephin, ciprofloxacin, streptomycin, erythromycin, oxacillin, chloramphenicol, augumentin, sparfloxacin and tarivid respectively. All twenty staphylococcus isolates were susceptible to four or more antibiotics with higher susceptibility rate in ciprofloxacin (10%) and pefloxacin (65%). Despite the lower susceptibility rate to other antibiotics, it could be said that vancomycin resistant staphylococcus were not isolated in this study. Literature has revealed that vancomycin resistant staphylococcus is equally resistant to ciprofloxacin (Prescott et al., 2008) which in this study proved to be effective against the *staphylococcus* isolates. For isolates of *Bacillus*, higher susceptibility rate were observed in ciprofloxacin (100%), pefloxacin (100%), zinnacef (100%), amoxicillin (100%), erythromycin (100%), gentamycin (90%) and ampiclox (80%) where as being resistant to rocephin. All isolates of *Micrococcus* were susceptible to pefloxacin, gentamycin, ampiclox and ciprofloxacin. The table also revealed higher susceptibility rate to tarivid, ciprofloxacin, chloramphenicol and septrin by isolates of *Serratia*. The upsurge of drug resistant infectious microorganism is alarming and it is a public health problem. The drug resistant observed in most of the antibiotics by the various microorganisms could be likened to the development of mechanisms that enhanced their resistance. Literature has revealed that microorganisms could develop resistance to drugs either through acquisition of plasmid resistant genes, possessing efflux pumps that aids in expelling drugs or by mutation of drug target sites.

Table1. Susceptibility pattern of isolates of Mini mile 3 Primary health centre

|                | PE | CN | AP | X | A | R | CP | S | S | E | O | AU | OF | CH | SP |
|----------------|----|----|----|---|---|---|----|----|---|---|---|---|----|----|----|----|
| **Staphylococcus** sp | 13(65) | 3(1) | 3(1) | 4(2) | 0 | 2(1) | 20(100) | 0 | 3(1) | 4(100) | NA | NA | NA | NA |
| **Bacillus** sp | 10(100) | 9(90) | 8(80) | 10(100) | 10(100) | 10(100) | 6(80) | 10(100) | N | NA | NA | NA | NA |
| **Micrococcus** sp | 14(100) | 14(100) | 14(100) | 0 | 0 | 0 | 14(100) | 11(7) | 1(1) | N | NA | NA | NA | NA |
Serratia sp 4(8) 2(4) NA NA 2(40) 5(100) 3(0) NA N 0 5(100) 5(100) 5(100)

Antibiogram of various isolates


Table 2: ANOVA test result on mean bacterial concentration difference among different wards of the morning and evening sections

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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</thead>
<tbody>
<tr>
<td>Rows</td>
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<td>3.409814</td>
<td>0.170297</td>
<td>9.276628</td>
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<tr>
<td>Columns</td>
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<td>1</td>
<td>0.015025</td>
<td>1.152783</td>
<td>0.361645</td>
<td>10.12796</td>
</tr>
<tr>
<td>Error</td>
<td>0.039101</td>
<td>3</td>
<td>0.013034</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.187455</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: ANOVA test result on mean TSC population difference among different wards of the morning and evening sections

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rows</td>
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<td>0.074558</td>
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<tr>
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<td>0.025301</td>
<td>13.26763</td>
<td>0.03568</td>
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<tr>
<td>Error</td>
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<td>0.001907</td>
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<tr>
<td>Total</td>
<td>0.070049</td>
<td>7</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: % occurrence of bacterial isolates in various ward

<table>
<thead>
<tr>
<th>Microbial Isolates</th>
<th>% CHW</th>
<th>% OPW</th>
<th>% PNW</th>
<th>% IJW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus species</td>
<td>10</td>
<td>40</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Micrococcus species</td>
<td>7.14</td>
<td>42.85</td>
<td>21.43</td>
<td>28.57</td>
</tr>
<tr>
<td>Serratia species</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>Staphylococci species</td>
<td>20</td>
<td>40</td>
<td>15</td>
<td>60</td>
</tr>
</tbody>
</table>

Conclusion

The microorganisms identified in this study are known to be pathogenic and could be implicated in many hospital acquired infections (nosocomial infections). The high microbial populations could be related to certain physical factors such as humidity, temperature, ventilation as well as hygienic standard of the people including the influx of patients. Also the sensitivity test which revealed some level of resistance against the microorganisms is indicative that microbes in this study site may have acquired resistance against them. Ciprofloxacin, ofloxacin, gentamycin and pefloxacin which were more effective should be considered as the drug of choice for infections of these microbes in this site.
CONSENT

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

REFERENCES


ABBREVIATIONS

Cfu: colony forming unit, M³: cubic metre, THB: total heterotrophic bacteria, ANOVA: analysis of variance, TSC:total Staphylococci count