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PREVALENCE OF AIR BORNE BACTERIA IN PRISON INDOOR ENVIRONMENTS LOCATED IN NSUKKA AND ENUGU METROPOLIS, NIGERIA

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ABSTRACT

Airborne microorganisms are transmitted through the air which can cause respiratory ailments in humans leading to allergies such as asthma and pathogenic infections of the respiratory tract. Most institutions such as prisons and schools accommodate a large number of people and airborne diseases thrive best in overcrowded and unhygienic environment. The study was designed to determine the prevalence of airborne bacterial isolates in Enugu and Nsukka indoor prison environments and possible pathogenic effects on the upper respiratory tract of the inmates. Convenience sampling method was employed. One hundred and forty (140) samples were analyzed consisting indoor air from prison offices (48), inmates cell (28), lavatory (16), furniture (8), nasal swabs (20) and hostels (20). In this study, A6 single impactor with high vaccum pump was used in the collection of indoor air samples; thermometer was used for measuring the temperature of the room and hygrometer for measuring the humidity. Sterile swabs were used for the collection of nasal samples, furniture, walls, toilets and bathroom surfaces. Culture media such as Brain heart infusion agar, Malt extract agar,Sabouraund dextrose agar (0.05µg Chloramphenicol and 1µg of Streptomycin) and Chromagar, Nutrient agar, Blood agar and chocolate agar were used for fungi and bacteria isolation respectively. The Data gathered from this research was analyzed using SPSS statistical software version 23.The results showed that the prevalence of airborne bacteria isolates, were 94.4, 88.9% for bacteria in prison offices in Nsukka and Enugu respectively. In prison cells, the prevalence were 11.1, 5.6% for bacteria in Enugu and Nsukka respectively. There was no significant difference in the distribution of bacterial isolates in Enugu and Nsukka cells at P=0.223, using Chisquare. The indoor temperature and humidity of Enugu and Nsukka were the same at $P > 0.05$ using ANOVA, when compared with the hostels that served as control. It is advocated that proper and adequate measures should be put in place to improve hygienic practices of the prison indoor environments to beef up healthy living and wellbeing of occupants.

KEYWORD: *prevalence, air borne bacteria, prison indoor environments.*

INTRODUCTION

The first indoor environments, built by man over half a million years ago, included caves with leather –draped interiors, fur-carpeted tents and huts covered with animal hides. Microbial predators existed from time immemorial but transmission had always required direct contact because they could not tolerate the sunlight and temperature extremes outdoors (Aneja *et al.*, 2009). Animal husbandry seems to have resulted in a number of pathogens jumping species and then becoming adapted to indoor transmission to the exclusion of outdoor transmission. These include Rhinoviruses, diphtheria, tuberculosis, smallpox, measles and influenza which appears to have come variously from horses, cows, dogs and chickens (Aneja *et al.*, 2009). Most contagious human pathogens have evolved to such dependence on

man's habitats for transmission that they lack any ability to survive outdoors for long. In contrast, the non-contagious pathogens, including the fungi, environmental bacterial and some animal pathogens have maintained the ability to survive in the environment. Even so, direct sunlight is rapidly fatal to almost anything but spores (Mitscherlich and Marth, 1984). Air is a resource that supplies us with oxygen which is essential for our bodies to live. Pure air is a mixture of gases that are invisible, colourless and odourless, consisting 78% nitrogen, 21% oxygen, and other gases as well as varying amounts of water vapour (Murray *et al.*, 1995). This pure air can become contaminated in various ways affecting humans, plants and animals. Microorganisms are transmitted by air, but do not grow and reproduce in air, because the air does not contain the necessary amount of moisture and nutrients needed for growth and metabolism (Aaron and Michael,2013).Air borne pathogens are diseases transmitted through the air and most of the air borne microorganisms originate from natural resources such as soil, lakes, animals and humans. Moreover, agricultural practices, health care units and industrial operations such as sewage treatment, animal rendering, fermentation process and food processing plants also emit viable microorganisms into the air (Aneja *et al.*,2009).Airborne particles are major causes of respiratory ailments of human causing allergies, asthma and pathogenic infections of the respiratory tract. Air pollution plays a role in air borne diseases which is linked to asthma. Both indoor air and outdoor air can become polluted by pesticides. These pesticides contain active and inert substances such as cyclodiene which is associated with symptoms such as dizziness, headaches, weakness, muscle twitching and nausea (Arora *et al.*,2012). Exposure to bio aerosol pollution is now an almost inescapable feature of urban living throughout the world which is associated with a wide range of adverse health effects including contagious infectious diseases *such as* tuberculosis (*Mycobacterium tuberculosis)*,leprosy (*Mycobacterium leprae)*,whooping cough(Bordetella *pertusis and Diphteria, Corynebacterium diphtheria*) along with acute toxic effects, allergies and cancer. Streptococcocus infection, Staphyloccocus infections, Psittacosis and Coccocidiomycosis. This can be caused by exposure to a source; an infected person or animal, mouth, nose cut or needle puncture, therefore it can be spread via respiratory droplets expelled from the mouth and nose. Sinus congestion, coughing and sore throats are examples of inflammation of the upper respiratory air way due to these air borne agents (Laura *et al.*,2008).

Indoor air quality is a term which refers to the air within and around the building and structures especially as it relates to the health and the comfort of its occupants. The quality of air in prisons, in relation to microbial contaminations at a given time period is determined by the quality of air entering the building, number of occupants in the building, physical activities and resultant aerosol generation, human trafficking and the efficiency of ventilations (Adebolu and Vhiterhre,2002).

The concern for quality indoor air is necessary especially in institutionalized settings that accommodate a large number of people such as prison because contaminated air can cause both mild and serious irritating health conditions (Tambeker *et al.*,2007).Environmental monitoring can be done using viable monitoring and non-viable particulate count monitoring. One way of quantitatively ensuring health of indoor air is by the frequency of effective turnover of interior air replacement with outside air, use of air filters can trap some of the air pollutants. Dilution of indoor pollutants with outdoor air is effective to the extent that outdoor air is free of harmful pollutants. Furthermore, linkages between the prison health and national health services would go a long way in addressing the threat of infections to prison population (Khan *et al.*,2003; Simooya *et al.*,2001,).

AIM

This study was aimed at determining the prevalence of air borne bacterial isolates in the two prison indoor environments in Enugu and Nsukka metropolis, Nigeria.

MATERIALS AND METHODS

Study Area

The study was done in Enugu State. Enugu state is densely populated and rated at 460 /km².Enugu prison is in the capital of Enugu state in Nigeria. It is located at 6/24 Kingsway road, Enugu. The city is popularly called coal city known for its coal mining in the past and now is known for cuisine,roburst industries and of course local palm wine. Enugu prison is a maximum security prison which has a carrying capacity of about 638 inmates. Nsukka is a city located in Enugu state, it has an estimated population of inhabitants put at about has 941,000. Nsukka prison is located at Nsukka Local Government Headquarter's Corporate office.It is a medium prison that has a carrying capacity of 363 inmates (Willie and Lizzie, 2008).

Study subjects and enrolment

In this research, the prison inmates and officers were recruited using questionaires. Indoor air samples were collected for bacteria and fungi in prison offices, cells, clinical admission ward and tertiary hostel of Medical Laboratory Science and Technology Daughters of Divine Love Eha-alumona Nsukka and Enugu that served as control. Samples from walls, toilets seat, furniture, and bathrooms surfaces were also collected. In addition, nasal swabs from patients in clinical admission wards of both Nsukka and Enugu prisons were also collected. The research work was carried out in University of Nigeria Teaching Hospital,Ituku Ozalla,Enugu State .The study was done from September 2017 to November 2017.

Inclusion criteria

Subjects who:
i) Are obviously sick (inmates)
ii) Prison inmates who have been previously admitted.

Exclusion criteria
Subjects who
i) Apparently healthy prisoners.

3.3 Ethical approval
Ethical approval was sought for and obtained from the Ethical Committee of University Teaching Hospital Ituku Ozalla in Enugu state.

Informed consent
Oral and Written consent was sought and obtained from all the participants and Controller of prisons.

Study Design
A descriptive cross sectional study was used for this research. The study was carried out to determine the quality of air in prison indoor environment by collecting samples from the air in Prison offices,cells,swab samples from furniture, toilets, bathroom as well as nasal swabs from patients in clinical admission ward with its implication to health status of the prisoners in Enugu and Nsukka. Questionnaire was structured out of extensive literature review on indoor air contamination of fungi and bacteria. The focus of the questionaire was on offices, clinics, cells as well as length of stay in the prison, the number of inmates that occupy a room /apartment that can influence growth and concentration of airborne bacteria and fungi in indoor environment. In Nsukka and Enugu prisons, the size of the rooms were 8.65meters,7.96meters and 8.65meters each. In Nsukka prisons,three apartments contain 60, 61 &63 inmates,two apartments contain 17,16 inmates (7.96m), also another two apartments contain 37, 37inmates(8.65m) respectively. In Enugu, 51 cells were available with population of 1833 males,while Nsukka had (7) seven apartments with population of 363 males. Nasal Swab was collected from patients in Clinical admission ward of both Nsukka and Enugu prisoners that had signs of respiratory problems. Ten rooms each were sampled in a tertiary hostel in School of Medical Laboratory Science and Technology, Daughters of Divine love Eha-alumona Nsukka and Enugu respectively,which served as control.Animal pathogenicity test was done using a total of thirty two (32) adult albino rats. Thirty adult albino rats were used for both fungal and bacterial isolates collected from both the environment and nasal swabs which were treated in three groups, viable, non -viable and immunosuppressed, while two (2) adult albino rats were used as controls.

Sample size
Minimum sample size (N) was obtained by using the formula (Naing *et al.*,2005)**.**

Calculation formula: $N=z^2 \times p\ (1-p)/d^2$

N=minimum sample size

P=prevalence of airborne pathogens (5%)(Ren*et al.*,1999).

D=desired level of significance=0.05 (5%), Z=confidence interval= $1.96(95\%)C.I$, $N=1.96^2\times0.05(1-0.05)/0.05^2=72.9$, approximately 73. A total of 140 samples were used for this study.

Sampling technique: Convenience sampling method was adopted to select 50 sites each from Enugu and Nsukka prisons because of its accessibility and convenience.

Sample collection

METHODOLOGY

Sample collection and preparation
Convenience sampling of indoor air using active monitoring which includes air sampler (Ac single stage impactor with high pressure pump) and sterile swabs stick for collection of samples from furniture(tables, chairs), toilets and bathrooms, Clinical admission wards, Offices and Cells. Samples were collected according to American Conference of Industrial Hygienist(ACGIH) guidelines which accepts sampling of bio aerosols in duplicates or possibly triplicate. An agar plate (20mls) of (Malt extract agar, Nutrient agar, Brain heart infusion blood agar, Sabouraud dextrose agar supplemented with 0.05µg/ml Chloramphenicol and 1µg/ml Streptomycin) was placed inside the AC single stage one at a time which was connected to a pump operating at 28.3L/min.Agar plates were sampled for five minutes each, after which the agar plates were removed and sent to the laboratory for analysis. The temperature and the humidity of indoor environment was measured using wall thermometer and hygrometer. Malt extract agar and Sabouraud dextrose agar,Chromagar(Candida species) were used for culture of fungi while the Nutrient agar and brain heart infusion blood agar for culture of bacteria and fungi.

Laboratory methods and procedure
All reagents were commercially purchased and the manufacturer's instruction strictly followed.

Media preparation.: Malt extract agar, Sabouraud dextrose agar,Brain heart infusion agar, Chromagar (for *Candida* species),Nutrient agar, Blood agar and Chocolate agar were prepared for isolation of fungi and bacteria respectively.Biochemical test was done such as coagulase, catalase, indole urea,methyl red,oxidase,yeast fermentation, germ tube test, identification techniques such as direct microscopy- normal saline, indian ink,potassium hydroxide solution,lactophenol cotton blue and gram stain were employed for identification of the organisms,as well as manual and control charts.

Isolation procedure
The microorganisms were isolated from nasal swab from sick patients, prison offices and prison cells in Enugu and Nsukka prison indoors. Fungi spore for inoculation was prepared by growing each sample in Sabouraund

dextrose agar, Malt extract agar, Chromagar as well as brain heart infusion agar(for both organisms). Blood agar, Chocolate agar and Nutrient agar were used for bacteria isolation respectively. Incubation for fungi growth was left for seven days at 25^{o}C and was checked daily for growth, while incubation for bacteria was left for twenty four hours at 37^{o}C.Both the fungi spores and bacteria were harvested by addition of sterile normal saline, shaking thoroughly in the glass tube. Bacterial isolates inoculated were *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes,* and *Streptococcus pneumoniae.* Fungal spore isolates inoculatedwere *Aspergillius fumigatus, Candida albicans, Penicillum chrysogenum,* and *Cryptococcus neoformans.*

Pathogenicity test procedure: A total of thirty two (32) adult albino wistar rats were used in this study. The rats were purchased and bred in animal house of Nnamdi Azikiwe University, Nnewi Campus. The animals were taken care of, for one week. They were kept under strict hygienic condition during and before introducing the isolates. The cage used for the rats were thoroughly cleaned, three times a week. The adult albino wistar rats were provided with food and clean water and observed daily for any morbidity or mortality. All the animals were adjudged to be healthy. Each albino rat were anaesthesized by placing it in a jar with a wire mesh floor, moistened with chloroform (Abbot laboratories, North Chicago) and observed for decreased mobility and unsteady gait for 20 secs. When fully anaestesized the albino rats were removed from the jar and gently placed on a flat table one at a time. 0.5ml of the suspensions of the different bacteria and fungi spore isolates from the prison indoor air and nasal samples were differently inoculated intra trachea into the rats. One organism each was introduced into three different rats. The suspension was inoculated in triplicates: killed organisms of each isolates, live organisms and one rat each was injected with hydrocortisone to suppress the immunity after which live organism was introduced through the trachea. Two negative controls were used, one each for lung tissue and trachea for both bacteria and fungi respectively, no organism was inoculated in the two adult albino rats. The lungs and trachea of the rats were carefully extracted and checked for any sign of lesion, they were also cultured in brain heart infusion agar for microbiology testing, Some of the lung tissue and trachea were also subjected to histopathology testing. The lung and trachea showed normal histology pictomicrograph. A total of ten organisms were introduced into three groups or triplicates for treatment, as explained above. The treated albino rats were kept for one week before sacrificing. Midline cut through the abdomen was done. Some of the lungs and trachea were carefully extracted and checked for signs of lesions, colourations and pus and were swabbed with sterile swab and cultured in brain heart infusion agar for microbiology testing and incubated for twenty four hours. Some of the lungs tissue and trachea were transferred into a universal container containing 10% buffered formalin for histopathological examination with Haematoxylin and eosin.

Tissue processing; The tissues were dehydrated in different grades of alcohol 80%, 90% and absolute alcohol. The tissues were cleared in xylene and impregnated in paraffin wax to solidify after which they were cut and trimmed into ribbons using the microtome knife The ribbons were floated on the water surface in the water bath with forceps. The slides were smeared with adhesive and inserted into floatation bath where the section was picked and adhered to slides. The tissue section slides were drained for one minute before drying on a hot plate at 56^{o}C for 10mins,regulating the melting point of wax and ready for staining. The sections were hydrated in graded alcohol. The slides were stained using Harris haematoxylin and Eosin staining. Principle: Haematoxylin is a dye which stains acidic structures purplish blue while Eosin a dye that stains basic structure pink red. Commercially prepared Harris haematoxylin (Kelong Scientific and Chemicals.co.Ltd,Nigeria) was used.

Staining procedure: The sections were dewaxed in xylene and hydrated through graded alcohol to water, rinsed in water and stained in Harris haematoxylin for five mins. It was differentiated in 1 % HCL in 70 % alcohol, rinsed in water. The tissues were blued in scott tap water for two minutes and counter stained in 1% eosin stain and washed in running water until eosin is removed after which they were dehydrated in 95% and absolute alcohols. The sections were cleared in xylene and mounted in DPX and examined under the microscope using 100 oil immersion objective.
Results: Nuclei- blue, Cytoplasm-varying grades of pink. Other tissue structures- pink.Red blood cells–red.

Data analysis: The data gathered from this research was analyzed using SPSS statistical software version 23. Pearson correlation coefficient, Spearman's rank correlation, Chi-square and Analysis of variance (ANOVA) were employed. P-value of less than 0.05($p<0.05$) was considered as significant.

RESULTS

Table 1: Frequency of airborne bacteria isolates in Enugu and Nsukka prison offices.

In Enugu prison offices, *Escherichia coli* had the highest percentage of 31.3%*Proteusmirabilis*18.8*%and Pseudomonas pseudomallei*had *lowest percentage* of6.3%distribution.

In Nsukka prison offices,*Staphylococcus aureus had the* highest distribution of 32.4% and *Streptococcus pneumoniae* had the lowest of 2.9%,distribution.

Table 2: Frequency of airborne bacteria isolates in Enugu and Nsukka prison cells.

In Enugu prison cells, *Escherichia coli*had 50%, *Klebsiella pneumoniae* 50%,. In Nsukka prison cells, *Escherichia coli* had 50%,*Staphylococcus aureus* 50%, distribution.

Table 1: Frequency of airborne bacterial isolates in prison offices in Enugu and Nsukka.

Bacteria	Percentage (%) Enugu	Nsukka
Escherichia coli	10 (31.3)	6 (17.6)
Klebisiella pneumoniae	3 (9.4)	5 (14.7)
Proteus mirabalis	6 (18.8)	3 (8.8)
Pseudomonas aeruginosa	0 (0)	2 (5.9)
Pseudomonas pseudomallei	2 (6.3)	2 (5.9)
Staphylococcus aureus	3 (9.4)	11 (32.4)
Staphylococcus saprophyticus	8 (25)	2 (5.9)
Streptococcus pneumonia	0 (0)	1 (2.9)
Streptococcus pyogenes	0(0)	5.9%
Total		32
34		

$\chi^{2=}$15.625,df-8,p=0.048.

χ^2 – Chi square, df – Degree of freedom, p<0.05 is significant.

Table 2: Frequency of airborne bacterial isolates in prison cells in Enugu and Nsukka.

	Percentage (%) Enugu	Nsukka
Escherichia coli	2 (50)	1 (50)
Klebisiella pneumoniae	2 (50)	0
Staphylococcus aureus	0	1 (50)
Total	4	2

χ^2 = 3000, df=8, p=0.223

χ^2 – Chi square, df – Degree of freedom, p<0.05 is significant.

DISCUSSION

In Nsukka prison offices bacterial isolates was higher in percentage than in Enugu offices. This may be due to large turnover of visitors into the prisons, poor sanitation, dust raising activities, temperature and relative humidity of indoor environment, and moisture problem that resulted to proliferation of bacteria in the two prisons. Different airborne bacteria were isolated indoors, some of these organisms were known to be allergenic, pathogenic and toxigenic (Pavan and Manjunth, 2014) such as: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomona aeruginosa, Pseudomonas pseudomallei, Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus pneumoniae, Streptococcus pyogenes*. This is also in line with the findings of Osugwugwu and Onwuka in 2014 that isolated *Staphylococcus epidermis, Proteus mirabils, Pseudomonas aeruginosa,Escherichia coli,Candida species, Streptococcus pneumoniae, Klebsiella pneumoniae, Penicillum,Aspergillus and Bacillus* species which were the most commonly isolated microorganism in indoor environment (Agarwal R, 2011).

In this study, Ac impactor was used in sampling the air from the environment, gram positive and gram negative bacteria were isolated. There was a relationship between the bacteria isolates resident in both prisons of Enugu and Nsukka.

The mean prison indoor temperature and humidity of Enugu and Nsukka offices had moderate temperature and high humidity which favoured the growth of these bacteria and fungi.

According to Thompson *et al*., (2003), Environmental protection agency (EPA), recommends indoor temperature and humidity to be between 30% to 60%.Comfortable room temperature are generally considered to be around 20-22^{o}C.Unbalanced temperature and humidity levels inside home can harm humidity and plays a role in the quality of indoor air. High humidity in a home creates a favorable environment for moulds and dust mites which are powerful allergens and irritants that can lead to respiratory problems like asthma. If the humidity is below 30%, the air is too dry, this can cause irritation of the mucous membranes of the nose and throat and breathing difficulties.

The presence of bacteria in the bathroom and toilet, walls, furniture may be as a result of damp environment and poor sanitation that favours the growth of these microrganisms.

According to Arora *et al*. (2012), the presence of undesirable bio aerosols is often associated with sick building syndrome. These includes furnishings, building materials, fungi contamination with the walls, ceilings and floor cavities by movement of cells, spores and cell fragments through wall openings and gaps at structural joints.

CONCLUSION

Nsukka prison offices had bacteria isolates higher in percentage than in Enugu prison offices overcrowding and inadequate ventilation and poor sanitation in the two prisons may have contributed to poor quality of indoor air.

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