Assessment of Sedimentation Rate of Aero-Pathogens in Health Centres in Port Harcourt

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Abstract
Air sampling was done in Rivers State health centres using the direct and indirect sedimentation method. In the indirect method 20ml of normal saline was measured in a sterile beaker and exposed to air for 24 hours at three different locations in the hospitals where patients are frequently found, while the direct sedimentation method involve the direct exposure of growth media to air for 10minutes. The media used were Nutrient agar, MacConkey agar and Sabouraud Dextrose agar for the cultivation of heterotrophic bacteria, enteric bacteria and fungi. The sedimentation rates per colony (Øcfu/min) of the isolates were; bacteria: Proteus penneri 3.422 > Escherichia coli = Shimwellia blattae 3.360 > Photorhabdus luminescens 2.472 > Providencia rustigianii 2.250 > Micrococcus luteus 2.138 > Pragia fontium 2.112 > Providencia stuartii 1.862 > Tatumella punctata 1.696; while fungal species were Cladosporium spp = Mortierella spp 1.833 > Mucor spp 1.750 > Penicillium spp. 1.667 > Aspergillus spp. 1.500 > Chrysosporium spp. 1.333. The differences in their sedimentation rate on the media plates could be due to size, shape, buoyancy structures, air flow direction/source, prevalent types of patients' diseases and exposure. The percentage (%) frequency of first three most occurring bacterial isolates were Proteus penneri (15.28), Escherichia coli (14.79), Shimweillia blattae (14.79) while fungi Cladosporium spp = Mortierella spp (18.49), Mucor spp (17.65). The least occurring bacterium Tatumella punctata (7.46) while fungus Chrysosporium spp.(13.44). The occurrence of pathogenic microorganisms like Aspergillus fumigatus, Proteus penneri and Escherichia coli in the air of a hospital possess a great health threat and should be given strict attention.

Keywords: *Sedimentation rate, aero-pathogens, health centre, Direct and indirect sedimentation method, Escherichia coli*

1.0 Introduction

Aerosols are a suspension of solid or liquid particles in a gas, with particle size from 0.001 to over 100 mm (Hinds, 1982). Infectious aerosols contain pathogens. Air is not an appropriate medium for the survival of disease causing bacteria, so any pathogenic bacteria found in the air must have a source of where it originated from (Ishida *et al.,* 2006). Many pathogenic bacteria can cause nosocomial infections (hospital acquired infections) but those that thrive in the hospital environment for a long period of time and can resist disinfections are those of primary concern. More people die every year due to hospital infections (Johnson, 2002). When the temperature, moisture content are favourable and even dust on surfaces of various object is enough to provide nutrient that will enhance the growth of these microorganisms (Korpi *et al.,1997*, Pasanen *et al.,* 1997a, Nrior and Adiele, 2015), and this growth starts within hours ((Pasanen *et al.,* 1997b, Nrior and Chioma, 2017). Microorganisms produce of volatile metabolite of which the composition depends on the kind of nutritional and environmental conditions (Korpi *et al., 1998).* Some fungi are capable of producing

mycotoxin which is present in hyphal fragments and spores. Though, the direct health outcomes that is caused by these metabolites at concentrations in closed room are difficult to state (Gottschalk *et al.,* 2008, Korpi *et al.,* 2009,). Moulds have the ability to cause great health effects such as allergic reactions, irritation of the skin, difficulties in breathing, severe headache and aggravation of asthma symptoms (US Environmental Protection Agency, 2001).

People living in a populated and closed place are subject to microbial contact. It has been shown that the average concentrations of bacteria in the air in closed room were higher in the presence of people and furnishing compared to that of empty rooms (Sessa *et al.,* 2002).
Nosocomial infection (Greek *noscos,* disease and *Komeion,* to take care of) also known as hospital-acquired infection (HAI), result from pathogens acquired by patients while in a hospital. Most health-care-associated infections become clinically apparent while patients are still hospitalized (Prescott *et al.,* 2013). To emphasize both hospital and nonhospital settings, it is sometimes instead called a health care–associated infection (HAI or HCAI). Such an infection can be acquired in hospital, nursing home, rehabilitation facility, outpatient clinic, or other clinical settings. Infection is spread to the susceptible patient in the clinical setting by various means. Health care staff can spread infection, in addition to contaminated equipment, bed linens, or air droplets. The infection can originate from the outside environment, another infected patient, staff that may be infected, or in some cases, the source of the infection cannot be determined. In some cases the microorganism originates from the patient's own skin microbiota, becoming opportunistic after surgery or other procedures that compromise the protective skin barrier. Though the patient may have contracted the infection from their own skin, the infection is still considered nosocomial since it develops in the health care setting. In the United States, the Centers for Disease Control and Prevention estimated roughly 1.7 million hospital-associated infections, from all types of microorganisms, including bacteria and fungi combined, cause or contribute to 99,000 deaths each year (*Klevens. et al.,2007).* Types of nosocomial infections include: Hospital-acquired pneumonia (Ventilator-associated pneumonia), Urinary tract infection, Gastroenteritis, Puerperal fever; Tuberculosis, Legionnaires' disease caused by *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Clostridium difficile*, *Escherichia coli,* Vancomycin-resistant *Enterococcus*. The spread of microorganisms is of great concern in the hospital environment. There, nosocomial infections call for a very stringent control because of immune-incompetent persons. Fungal spores and metabolites possess great detrimental effect not only to the immunocompromised persons (Ortiz, 2009). This research is aimed at assessing of sedimentation rate of aero-pathogens in health centres (hospitals) in Port Harcourt, Nigeria.

2.0 Material and Methods

Study Area

The study Area was three different Health Centres in Port Harcourt, Rivers State, Nigeria

Sample Collection

The air sample was collected from three different locations from health centres using direct and indirect sedimentation method. The direct method involves direct ascetical exposure of the growth media to air for 10 minutes while in the indirect method, 20ml of sterilized normal saline was measured into a sterilized beaker and exposed at three different locations in the health centres for 24 hours; after which, it was used for serial dilution according to methods used by Nrior and Adiele (2015)

Sample Storage

The normal saline, which was already exposed to air was stored in a cool air pack and transported from the site of collection to the laboratory for analysis within two hours after collection. Also the sampled agar plates were kept in separate cool air pack and transported to the laboratory for incubation.

Preparation of analytical media

Nutrient agar, MacConkey agar and Sabouraud dextrose agar was used for this research work.

[i] Nutrient Agar (NA)

This general purpose medium was used for the culture of less nutritionally fastidious bacterial isolates. It was prepared by adding 28g of nutrient Agar to 1000ml of distilled water. This was autoclaved at 121°C, 15psi for 15mins and allowed to cool. The plates were poured into sterile Petri dishes, allowed to cool and solidify and then surface dried in a hot air oven.

[ii] MacConkey agar (MCA)

This is a selective medium used for the cultivation of enteric bacterial isolates. It was prepared by adding 48g of MacConkey agar to 1000ml of distilled water, then autoclaved at 121°C, 15psi for 15minutes and poured into sterile Petri dishes

[iii] Sabouraud Dextrose Agar

This is a selective medium used for the cultivation of fungal isolates. It was prepared by adding 68g of the agar to 1000ml of distilled water, then autoclaved at 121 °C, 15psi for 15 minutes and poured into sterile Petri dishes.

2.4.4 Normal Saline (diluent) for indirect sedimentation method and serial dilution

Sodium chloride (8.5g) was dispensed into 1000ml (1 litre) of distilled water. The diluents were dispensed into Beakers and test tubes and sterilized in an autoclave at 121°C, 15psi for 15minutes. (20ml dispense into beakers for indirect sedimentation sampling while 9ml per test tube were used for serial dilution).

Sterilization

All materials that were used during the research work were adequately sterilized before and after use. Glass wares such as test tubes, pipettes, conical flasks, etc. were thoroughly washed with detergent, properly rinsed and drained. They were then wrapped in aluminum foil and sterilized in hot air oven at a temperature of 170°C for 2 hours. Inoculating needles and wire loops were sterilized by heating them to red hot with direct flame before and after use. The bench on which all analysis was carried out was sterilized by swabbing with cotton wool soaked in ethanol. All the media used were sterilized in an autoclave at 121°C for 15 minutes. Culture plates and media were destroyed by autoclaving at 121°C for 20 minutes. All inoculation and isolation were done near open flame in the inoculating chamber to avoid or limit contamination of culture plates.

Determination of Bacterial and Fungal Count

Direct Sedimentation agar plated colonies were counted after incubation at appropriate temperature and period [NA was incubated at 37±0.2C for 24hours for Heterotrophic bacteria, MCA incubated at 44±0.5 for enteric bacteria while SDA was incubated at room temperature (27±2^{0}C) for 3-5 days for Fungi].

In the indirect sedimentation, Spread plate method was adopted for enumeration of the

microbes. Nutrient Agar (NA) was used for Heterotrophic bacteria, Mac Conkey Agar (MCA) for Enteric bacteria while Sabouraud Dextrose Agar (SDA) was used for fungi. An aliquot (0.1ml) of the diluted sample was plated out on the different agar plates in triplicate. NA was incubated at 37±0.2C for 24hours for Heterotrophic bacteria, MCA incubated at 44±0.5 for Enteric bacteria while SDA was incubated at room temperature (27±2^{0}C) for 3-5 days for Fungi. Colonies were counted and recorded after incubation (Nrior and Adiele, 2015).

Isolation and preservation of Pure Culture

From the primary plates, discrete colonies of the organisms isolated (for bacteria) were selected and sub-cultured from the plates to respective nutrient agar plates and incubated at 37°C for 24 hours. For the filamentous fungi, appropriate spore dilutions of the fungal isolates were surface-spread in triplicate on SDA plates and incubated at room temperature for 3-5 days. The pure cultures of isolates were maintained on sterile nutrient agar and Sabouraud dextrose agar slant; and 10% glycerol stored in the refrigerator at 4°C.

Identification of Isolates

Bacterial colonies were identified based on their morphological and biochemical characteristics. Discrete colonies were studied on the various microbiological plates after 24hours, 48hours and 72 hours of incubation and characterized based on Bergey's Manual of Systematic Bacteriology, 1994.

Fungi identification were carried out based on macroscopic and microscopic characteristics; A few drops of lacto phenol -cotton blue was place on a clean microscope slide, and a sterile forceps was used to pick part of the fungal colony and placed on the lacto phenol cotton blue. A clean cover slip was used to cover the slide and the observed under the microscope (Cheesbrough, 2004)

Analytical formula:

Note: The standard time for bio-aerosol sampling is ten minutes (10min)

For Direct sedimentation method:

$$\text{Cfu/10min/m}^2 = \frac{\text{No. of colonies x 10 x } 3.142r^2}{\text{Time of exposure (min)}}$$

where:

r = radius of media plate used (in meters).

For Indirect sedimentation method:

$$\text{Cfu/10min/m}^2 = \frac{\text{No. of colonies x 10 x } 3.142r^2 \text{ x } V_D}{\text{Time of exposure (min) x dilution x } V_P}$$

where:

r = radius of beaker used (in meters)

V_D = Volume of diluent (*usually normal saline 100ml*) in beaker

V_P = Volume plated (*usually 0.1ml aliquot/inoculum*)

3. 0 Results and Discussion

Total Heterotrophic Bacteria (THB) and Total Hereotrophic fungi (THF) results from air sampling carried out in Rivers State health centres using the direct and indirect sedimentation method were shown in Fig. 1. The direct sedimentation method involve the direct exposure of growth media to air for 10minutes while in the indirect method, 20ml of normal saline was

measured in a sterile beaker and exposed to air for 24 hours at three different locations in the hospitals where patients are frequent – Reception, Toilet and Laboratory. The media used were Nutrient agar, MacConkey agar and Sabouraud Dextrose agar for the cultivation of heterotrophic bacteria, enteric bacteria and fungi respectively.

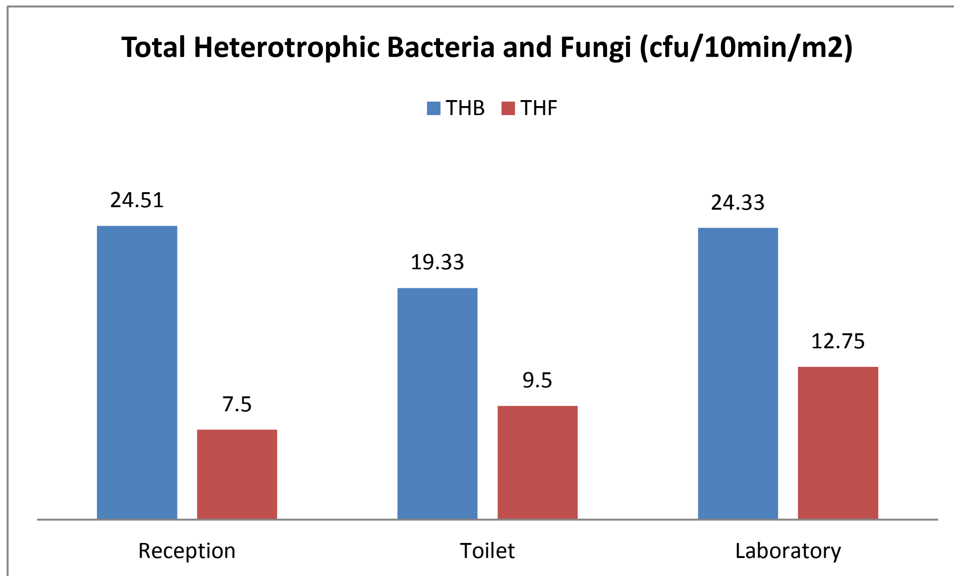

Fig 1: Total Heterotrophic Bacteria (THB) and Total Heterotrophic Fungi (THF) (cfu/10min/m^2) from hospital aerosol.

It was observed that the hospital reception hall has the highest Total Heterotrophic bacteria (THB) (24.51cfu/10min/m^2) followed by laboratory (24.33cfu/10min/m^2) and least in Toilet (19.33cfu/10min/m^2) (Fig. 1). The reduced THB load in toilet may be due to regular washing and disinfection. This is an eye opener that the seemingly overlook reception as regards contamination could harbor such high bioaerosol; this could be due to negligence of regular disinfection thinking that reception is just to receive patients and visitors and more attention is given to toilets. Total Heterotrophic Fungi (THF) showed alternate report having highest THF in Laboratory (12.75cfu/10min/m^2) followed by Toilet (9.5cfu/10min/m^2) and least in reception (7.5cfu/10min/m^2) (Fig.1). The high THF count in the laboratory could be due to its materials/cultures that could encourage fungal growth coupled with relative low temperature and high moisture content (humid environment)
The result of bacterial and fungal species using the direct and indirect sedimentation method were shown in Table 1-4. Evaluating the direct method in relation to bacterial isolates,

Table 1: Bacterial isolates frequency (cfu/10mins) from direct Sedimentation

Direct Sedimentation method	**Reception**		**Toilet**		**Laboratory**	
Bacterial Isolates	**Hospital 1**	**Hospital 2**	**Hospital 1**	**Hospital 2**	**Hospital 1**	**Hospital 2**
	cfu/10mins	*cfu/10mins*	*cfu/10mins*	*cfu/10mins*	*cfu/10mins*	*cfu/10mins*
Escherichia coli	7±1.41	2.33±0.58	3.5±2.12	2.33±0.58	3±1.41	2±0
Proteus penneri	6±1.41	1.67±0.58	3.5±0.71	2.33±0.58	4±1.41	3.33±1.53

Providencia stuartii	2.5±0.71	1.67±1.15	1.5±0.71	1.67±0.58	1.5±0.71	2.33±0.58
Micrococcus luteus	2.5±0.71	1.33±0.58	1.5±0.71	1.33±0.58	2.5±0.71	3.67±1.53
Pragia fontium	3.5±0.71	1.67±0.58	2±1.41	2.33±1.15	1.5±0.71	1.67±0.58
Providencia rustigianii	1.5±0.71	2.67±1.15	2±1.41	1.33±0.58	2±1.41	4±1
Shimwellia blattae	5.5±0.71	2.33±0.58	3±0	2.33±0.58	5±1.41	2±0
Photorhabdus luminescens	2±1.41	1.67±0.58	2.5±2.12	2.33±0.58	3±1.41	3.33±1.53
Tatumella punctata	1.5±0.71	1.67±1.15	1.5±0.71	1.67±0.58	1.5±0.71	2.33±0.58

Table 2: Bacterial isolates frequency ($x10^5$cfu/24h) from Indirect Sedimentation

Indirect Sedimentation method	**Reception**		**Toilet**		**Laboratory**	
Bacterial Isolates	**Hospital 1**	**Hospital 2**	**Hospital 1**	**Hospital 2**	**Hospital 1**	**Hospital 2**
	$x10^5$cfu/24h	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*
Escherichia coli	9±0	8.67±2.08	2.67±0.58	2.67±1.15	12.67±2.08	9±2.65
Proteus penneri	25.33±1.53	20±2.65	8.33±1.15	7.67±2.08	14±4.58	9.67±1.15
Providencia stuartii	10.67±2.08	8±3	3.67±1.53	4.67±1.53	12±1	8.33±1.53
Micrococcus luteus	10±1	9.33±2.52	1.33±0.58	2±1	4.33±1.53	3.67±0.58
Pragia fontium	7±1.73	5.33±2.31	3.33±1.15	4.67±1.15	9±1.73	8±1.73
Providencia rustigianii	6±2	14.33±0.58	3.67±1.53	5±1.73	5.67±2.89	6±1
Shimwellia blattae	9±3.61	5±2	1±0	2.33±1.15	4±1.73	4.33±3.21
Photorhabdus luminescens	16.33±4.04	5.67±2.08	7±2.65	2.33±0.58	9±6	3±1.73
Tatumella punctata	9.33±3.06	6.67±3.51	1.33±0.58	1.33±0.58	6±3	5±3

Table 3: Fungal isolates freq. (cfu/10mins) direct Sedimentation

Direct Sedimentation method	**Reception**		**Toilet**		**Laboratory**	
Fungal isolates	**Hospital 1**	**Hospital 2**	**Hospital 1**	**Hospital 2**	**Hospital 1**	**Hospital 2**
	cfu/10mins	*cfu/10mins*	*cfu/10mins*	*cfu/10mins*	*cfu/10mins*	*cfu/10mins*
Penicillium sp	1±0	1.5±0.71	1.5±0.71	1.5±0.71	2±0	2.5±0.71
Chrysosporium spp	1±0	1±0	1.5±0.71	1.5±0.71	1.5±0.71	1.5±0.71
Mortierella spp	1.5±0.71	1.5±0.71	2±1.41	2±1.41	2±1.41	2±1.41
Mucor sp.	1±0	1.5±0.71	1.5±0.71	1.5±0.71	2.5±0.71	2.5±0.71
Aspergillus fumigatus	1.5±0.71	1.5±0.71	1.5±0.71	1.5±0.71	1.5±0.71	1.5±0.71
Cladosporium	1±0	1±0	1.5±0.71	1.5±0.71	2.5±0.71	3.5±0.71

Table 4: Fungal isolates freq. ($x10^5$cfu/24h) Indirect Sedimentation

	Reception		**Toilet**		**Laboratory**	
	Hospital 1	**Hospital 2**	**Hospital 1**	**Hospital 2**	**Hospital 1**	**Hospital 2**
	$x10^5$cfu/24h	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*	*$x10^5$cfu/24h*
Penicillium sp	1.67±0.58	2.33±0.58	1.33±0.58	2.33±0.58	1.67±0.58	2±0
Chrysosporium spp	1.33±0.58	1.67±0.58	2.33±1.53	2.33±0.58	3±2	3.33±1.53
Mortierella spp	1.33±0.58	1.67±1.15	2.33±1.15	1.67±0.58	1.67±1.15	2.33±0.58
Mucor sp.	2±1.73	1.33±0.58	1.67±0.58	1.33±0.58	2.33±0.58	3.67±1.53
Aspergillus fumigatus	1.67±0.58	1.67±0.58	2±1	2.33±1.15	1.33±0.58	1.67±0.58
Cladosporium	1.33±0.58	2.67±1.15	2±1	1.33±0.58	2±1	4±1

The sedimentation rates per colony (*Øcfu/min*) of the isolates were; bacteria: *Proteus penneri* 3.422 > *Escherichia coli* = *Shimwellia blattae* 3.360 > *Photorhabdus luminescens* 2.472 > *Providencia rustigianii* 2.250 > *Micrococcus luteus* 2.138 > *Pragia fontium* 2.112 > *Providencia stuartii* 1.862 > *Tatumella punctata* 1.696; while fungal species were *Cladosporium* spp = *Mortierella* spp 1.833 > *Mucor* spp 1.750 > *Penicillium* spp. 1.667 > *Aspergillus* spp. 1.500 > *Chrysosporium* spp. 1.333 (Fig 2-3). The differences in their sedimentation rate on the media plates could be due to size, shape, buoyancy structures, air flow direction/source, prevalent types of patients' diseases and exposure.

The United States, the Centers for Disease Control and Prevention estimated roughly 1.7 million hospital-associated infections, from all types of microorganisms, including bacteria and fungi combined, cause or contribute to 99,000 deaths each year (Klevens. *et al.,*2007). Types of nosocomial infections include: Hospital-acquired pneumonia (Ventilator-associated

pneumonia), Urinary tract infection, Gastroenteritis, Puerperal fever; Tuberculosis, Legionnaires' disease caused by *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Clostridium difficile*, *Escherichia coli,* Vancomycin-resistant *Enterococcus*. Nrior and Adiele (2015) report similar cases of isolating pathogens in laboratory, restaurant and toilet of university campus environment.

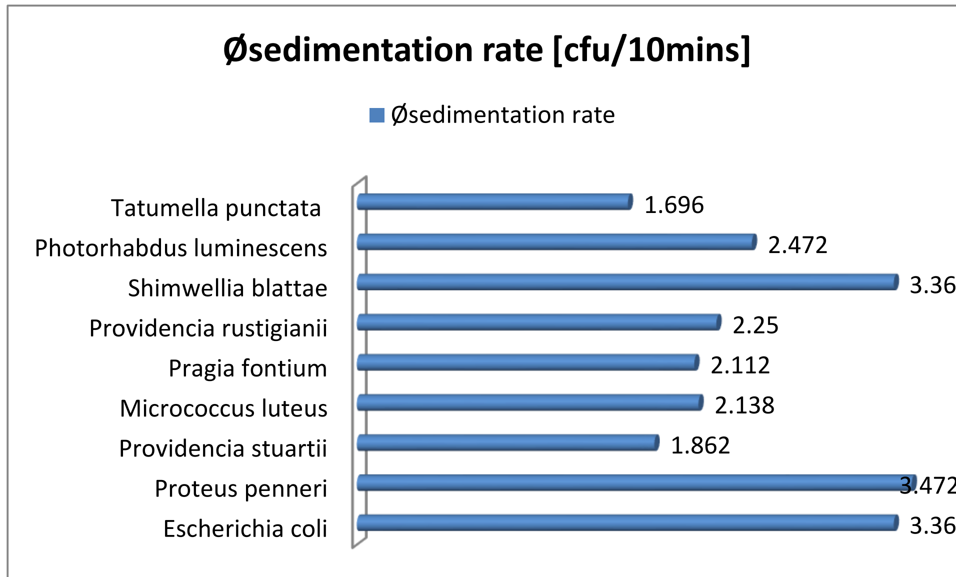

Fig 2: The average sedimentation rate (Øcfu/min) of bacterial isolates.

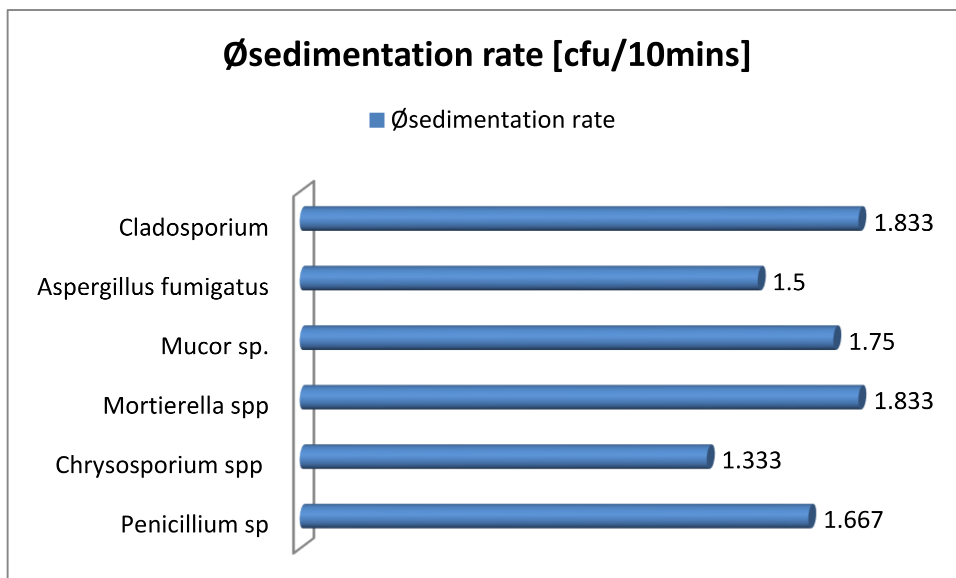

Fig. 3: The average sedimentation rate (Øcfu/min) of fungal isolates.

The percentage (%) frequency of first three most occurring bacterial isolates were *Proteus penneri* (15.28), *Escherchia coli* (14.79), *Shimweillia blattae* (14.79) (Fig. 4) while fungi *Cladosporium* spp = *Mortierella* spp (18.49), *Mucor* spp (17.65). The least occurring bacterium *Tatumella punctata* (7.46) while fungus *Chrysosporium* spp. (13.44) (Fig. 5). The

occurrence of pathogenic microorganisms like *Aspergillus fumigatus, Proteus penneri and Escherichia coli* in the air of a hospital possess a great health threat and should be given strict attention. Cases of pathogenic bioaerosols associated with black soot in indoor air was reported by Nrior and Chioma (2017)

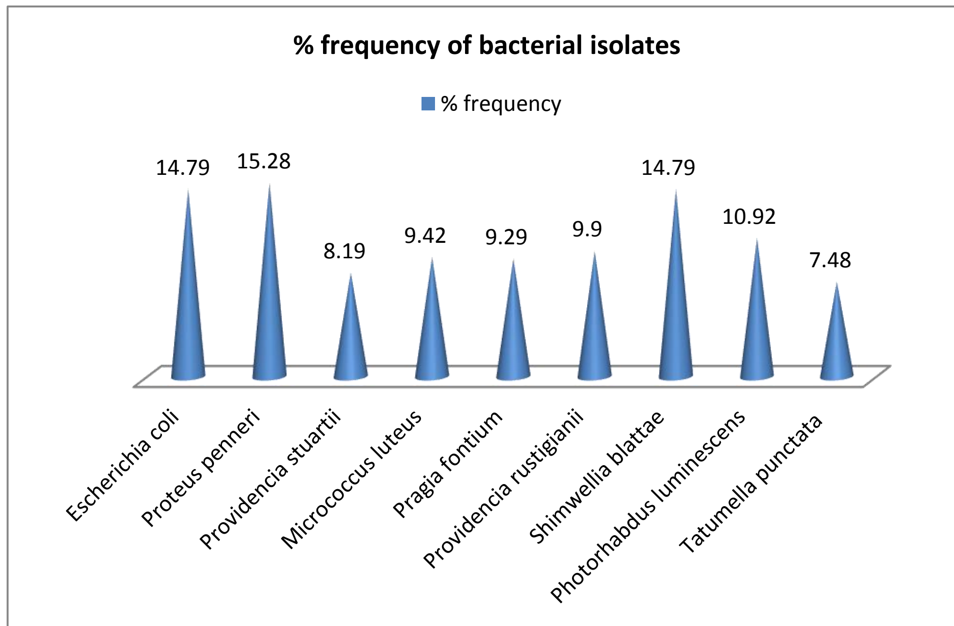

Fig 4: Percentage (%) frequency of bacterial isolates from hospital aerosols

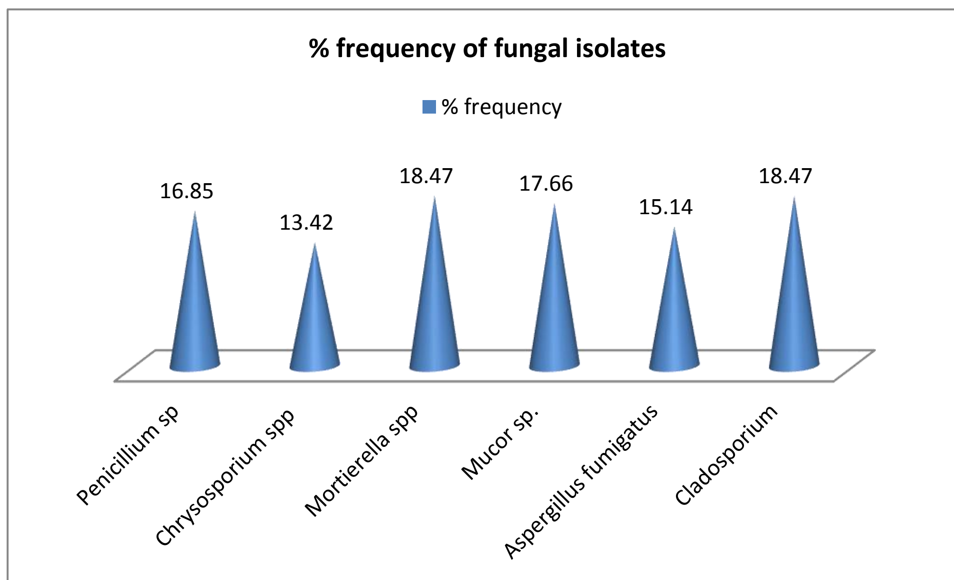

Fig 5: Percentage (%) frequency of fungal isolates from hospital aerosols

The occurrence of Proteus penneri-a gram negative facultative anaerobic rod-shaped bacterium which is an invasive pathogen to patients, poses a high health threat in the hospital. As it is the cause of nosocomial infection of the urinary tract and open wounds. Nosocomial infections call for a very stringent control because of immune-incompetent persons. Fungal spores and metabolites possess great detrimental effect not only to the immunocompromised

persons (Ortiz, 2009). The occurrence of pathogenic aerosols in health centres cannot be over emphasized, these pathogens possess a very high health treats to patients in this health centres. Most aerosols in the health center are opportunistic and are non-pathogenic, but they become pathogenic when they are found in a usual habitat in the human system. The survival of aeropathogens in the health centres depends greatly on the environmental condition of the health centre, so strict caution should be taken when running test in these health centres, because most of the air microorganism can alter the result of the test.

4. Conclusion

The occurrence of pathogenic microorganisms like *Aspergillus fumigatus, Proteus penneri and Escherichia coli* in the air of a hospital possess a great health threat and should be given strict attention.

It is therefore recommended that; Hospital worker should desist from using unsterilized materials on patients; the government should make provision for air dehumidifier to help keep the environment free from pathogenic aerosols; Patients that are immune-compromised should be given special attention to avoid the infections caused by opportunistic microorganisms.

Competing Interest

Authors have declared that no competing interests exist.

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