

Isolation And Identification Of Air Microflora In Federal Polytechnic Offa Microbiology Laboratory

Balogun, Olubunmi D.

Department of SLT, Federal Polytechnic Offa, Kwara State

Owa, Stephen O.

Irokanulo, Emenike O.

Department of Microbiology, Landmark University, Omu-aran, Kwara State

Abstract: A total of 4 samples were collected during the course of this study out of which four were confirmed to be bacterial growth. The isolated bacterial species were identified as *Staphylococcus aureus*, *E. coli*, *Streptococcus species* and *Bacillus subtilis*. *Staphylococcus aureus* had the highest percentage occurrence of 51% followed by *E. coli* (25%) and *Streptococcus species* (21%) while *Bacillus subtilis* recorded the least (3%). These pathogens could be linked with several infections such as gastrointestinal tract infections, respiratory tract infections, urinary tract infections and skin disorders. These findings would alert the populace to the existence of air microflora in their environment.

I. INTRODUCTION

Air is a mixture of many gases and tiny dust particles. It is the clear gas in which living things or living organisms live and breathe. It has an indefinite shape and volume. It has a mass and weight, because it is matter. Air is a mixture of Nitrogen, Oxygen, Argon, Carbon dioxide, and moisture. There is an average of about 1% water vapor. Air is however invisible to man's natural eye though a shimmering in hot air can be seen. There are also cells and spores of bacteria, fungi, algae, viruses and protozoa in the air. If air is exposed to sunlight, it has a higher temperature and less moisture. So, if not protected from desiccation most of its microbial forms will die. Air serves as transport or dispersal medium for microorganism, they occur in relatively small number in air when compared with soil or water.

Microorganisms are found almost everywhere, and their presence in the air was established by Lazzaro Splallanzani in 1768 and Louis Pasteur at the end of the 19th century (Meraj-ul-Haque *et al.*, 2016)..

Though microorganisms are found in both indoor and outdoor environments, people spend most of their lives indoors: in houses, industries, offices, colleges, schools, hospitals etc., where they are exposed to many bio aerosols (biological air borne contaminants such as bacteria, viruses,

fungi or their by-products). Exposure to these airborne particles can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions (Naruka and Gaur 2019). In addition, long-term contact of people with bio aerosols can influence a person's mental power and learning ability (Naruka and Gaur, 2019). Different environmental conditions such as temperature, UV light, dryness and humidity, play a role in controlling the growth of airborne particles. Nevertheless, the microbes manage to reach new hosts through the air for its survival (Sheik *et al.*, 2019). Poor ventilation, crowded conditions and increase in number of air conditions inside building nowadays can facilitate the spread and the survival rates of airborne particles and also can increase the chance of people at risk of airborne infections (Yaghoub and Elagbash, 2020)

The microflora of air can be studied under two headings outdoor and indoor microflora.

OUTDOOR MICROFLORA

The air in the atmosphere, which is found outside the buildings, is referred to as outside air. The dominant microflora of outside air are fungi. The two common genus of fungi are *Cladosporium* and *Sporobolomyces*, besides this two

general, other genera found in air are *Aspergillus*, *Alternaria*, *Phytophthora* and *Erysiphe*. The outdoor air also contains *Besidi spores*, *Ascorpres* of yeast, fragments of mycelium and *Canadida* of molds. Among the bacterial genus *Bacillus* and *Clostridium*, *Sarcina*, *Mirococcus*, *Corynebacterium* and *Achromabacter* are widely found in the outside air, the number and kind of microorganism may vary from place to place, depending upon the human population and density.

INDOOR MICROFLORA

The air found inside the building is referred to as indoor air. The most common genus of fungi in indoor air are *Penicillium*, *Aspergillus*, the most common general of bacteria found in indoor air are *Staphylococci*, *Bacillus* and *Clostridium*. In case of occupants being infected, the composition shows slight variation with altitude (Shiva,2009).

More microbes are found in air over land masses than on sea. Spores of fungi especially *Alterneria*, *cladsporium*, *Penicillium* and *Aspergillus* are more numerous than other forms over sea with about 400 miles from land in both polar and tropical region. The dust and air of schools and hospital wards or the rooms of persons suffering from infectious disease usually contains microbes such as *Bacilli*, *streptococci*, *pneumococci* and *staphylococci*. These respiratory bacteria are dispersed in air in the droplets of saliva and mucus produced from coughing, sneezing, talking and laughing.

Viruses of respiratory tract and some enteric organisms are also transmitted from objects contaminated with infectious secretions that become infectious. Droplets are usually formed from sneezing, coughing and talking. Many plant pathogens are also transported from one field to another through air and the spread of many fungal diseases of plants can be predicted by measuring the concentration of airborne fungal spores. Bacterial pathogens from humans can cause airborne disease such as diphtheria, meningitis, pneumonia, tuberculosis and whooping cough are described.

One of the most common sources of air microflora are in the soil. Soil micro-organisms when dispersed by the wind goes in to the air and remain suspended for a long period of time. Man-made actions like digging or ploughing the soil may release soil borne microbes in the air. Similarly microorganisms can be released into the air in the form of water droplets or aerosols, splashing of water by wind action, a tidal action may also produce droplets (Jacob, 2021).

II. MATERIALS AND METHODS

COLLECTION OF AIR SAMPLES

Air samples from two different laboratories were analyzed. . The plated were labeled LAB A while the other two were labeled LAB B. The plates were exposed to the atmosphere in the target laboratories according to how they were labeled for 10 minutes. They were finally transferred into an incubator for 48 hours at a temperature of 38 degrees Celsius.

ENUMERATION OF BACTERIA COLONIES

Bacteria enumeration involves the counting of bacterial cells. Viable cell count of living cells, and total cells counts of all cells in a sample. Standard plate count involves diluting cultures onto agar plates and counting the number of colonies.

After 48 hours of incubation, there were growth of microorganisms on the petri dish (plate), the plates were held to a light source and the colonies were counted by marking their position on the back of the petri dish with a marking pen. This aids in keeping track of those colonies previously counted and avoids recounts. If plate has more than 300 colonies, record it as TNTC (too numerous to count). The cfu/ml was calculated using the formular:

$$\text{Cfu/ml} = \frac{\text{mean} \times \frac{1}{\text{dilution series}}}{\text{Inoculum}}$$

MORPHOLOGICAL CULTURAL CHARACTERISTICS

Colony morphology observations formed a major identifying criterion for bacteria. The characteristics observed included; Size (pinpoint, small, medium, large), Shape (circular, irregular, rhizoid, rod), Surface (smooth, rough), Elevation (flat, slightly raised or markedly raised), Edge (entire, rhizoid, unbonnate, undulate), Pigmentation (white, green, yellow, creamy), Optical characteristics (transparent, opaque, translucent) and Consistency (viscid, butyrous)

III. RESULTS

Sample	Dilution series	Plate 1	Plate 2	Mean	(Cfu/ml)
Lab A	10 ⁰	30	38	34	3.4×10 ¹
Lab B	10 ⁰	66	47	56	5.6×10 ¹

Table 1: Enumeration Of Bacterial Colonies Isolated From Lab A And Lab B

LABORATORY A

Cultural Characteristics	Isolate A ₁	Isolate A ₂	Isolate A ₃
Size	Small	Medium	Small
Shape	Circular	Circular	Rhizoid
Surface	Smooth	Smooth	Rough
Elevation	Flat	Flat	Raised
Edge	Entire	Entire	Unbonnate
Pigmentation	Creamy	Creamy	Creamy
Optical	Translucent	Translucent	Transparent
Consistency	Butyrous	Butyrous	Butyrous

Table 2: Cultural Characteristics Of Bacteria Colonies Isolated From Lab A And Lab B

Cultural Characteristics	Isolate B ₁	Isolate B ₂	Isolate B ₃
Size	Small	Small	Small
Shape	Rod	Circular	Rod
Surface	Smooth	Smooth	Rough
Elevation	Flat	Flat	Flat
Edge	Unbonnate	Entire	Entire
Pigmentation	Creamy	Creamy	Creamy
Optical	Translucent	Translucent	Opaque

Consistency	Butyrous	Viscid	Butyrous
-------------	----------	--------	----------

Table 3: Laboratory B

Biochemical test	Isolate A ₁	Isolate A ₂	Isolate A ₃	Isolate B ₁	Isolate B ₂	Isolate B ₃
Catalase	+	+	-	+	+	+
Coagulase	-	+	-	-	-	+
Indole	-	-	-	-	-	-
Methyl red	+	+	-	+	+	+
Motility	+	-	+	+	+	-
Gram staining reaction	+	+	+	+	+	+
Probable bacteria	<i>Bacillus subtilis</i>	<i>Staphylococcus cereus</i>	<i>Micrococcus</i>	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus cereus</i>

IV. DISCUSSION

The extent of microbial contamination in the laboratories by pathogenic gram negative bacteria was high. *Escherichia coli* a gram negative intestinal bacterial were frequency isolated. This bacterium is an indication of faecal contamination in the laboratories. The possible source includes bags, mobile phones and the laptops carried by the laboratory personnel. A gram stain procedure revealed that 57% of the samples were gram negative bacteria which are pathogenic and are majorly associated with faecal contamination (Mendoza et al., 2019). The gram positive were 43%. This include *Bacillus subtilis* and *Staphylococcus aureus*. Gram negative bacteria were *Escherichia coli*, *Shigella sp*, *Pseudomonas aeruginosa* and *Salmonella sp*. The laboratory walls and tables contained most of the contaminating microbes. These living biological contaminants can be transmitted by infected people, animals and they can also travels through the air and get inside buildings (Cheng et al., 2020). Bacteria species like *Staphylococcus sp* are found on human skin (Del et al., 2020). *Pseudomonas sp* has been reportedly associated with wet surfaces of air conditioning systems, cooling coils and drain pans (Tokuyasu, et al., 2021).

Result showed that the microbial population is higher in the preparation rooms than the incubating rooms. This might be attributed to the fact that more people enter the preparation room. Mitsuko et al. (2018) discovered that the presence of bacteria in a room indicates the presence of people and their levels may get high when the building is heavily populated. Fungal contaminants were also found associated with the tables, walls and human gloves. Typically fungi makes up two-thirds of air microflora (Saylani et al., 2019). Regularly used furniture has been reported as a major source of fungi spores (Zoumat et al, 2020). The study is consistent with that of Miller et al. (2017) who isolated *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* in an indoor environment.

V. CONCLUSION

The bacteria isolated and identified were infectious strains that can pose danger to the wellbeing of both staff and students. It is therefore necessary that safe laboratory ethics should be practiced and enforced such as not eating and drinking in the laboratories, use of protective wears such as hand gloves, laboratory coats, nose mask.

REFERENCES

- [1] Cheng, C., Sun, J., Zheng, F., Wu, K. and Rui, Y. (2020). Molecular identification of clinical "difficult-to-identify" microbes from sequencing 16S ribosomal DNA and internal transcribed spacer 2. *Journal of Clinical Microbiology*, 13: 1-7.
- [2] Del, L., Jaramillo, M., Talledo, M., Pons, M. and Flores, L. (2020). Development of a 16S rRNA PCR-RFLP assay for *Bartonella* identification. Applicability in the

Table 4: Result of biochemical characterization of bacteria Isolated from Lab A and Lab B

Sample	Dilution Series	Plate 1	Plate 2	Mean	(Cfu/ml)
Lab A	10 ⁰	14	12	13	1.3×10 ¹
Lab B	10 ⁰	06	08	7	7.0×10 ¹

Table 5: Result of Enumeration of fungi Isolated in Lab A and Lab B

Sample	Fungi Isolate A1	Frequenc y	% occurrence
Lab A	A1	06	46.15%
	A2	07	53.85%
Lab B	B1	07	100%

Table 6: Result of percentage occurrence of fungi Isolate from Lab A and Lab B

Sample	Fungi isolate	Morphology characteristic	Microscopic characteristic	Suspected fungus
Lab A	A ₁	White colony with a raised center, having a black dot at the center slow glowing with flat periphery	Branched septate hyphae with hyaline conidioare globuse	<i>Penicillium spp</i>
	A ₂	Filamentous brown colony with yellow periphery and white egde	Dark sporangia with numerous sporagio spores non septate hyphae	<i>Aspergillus fumigates</i>
	B ₁	Filamentous brown colony with yellow peripehery and white edge	Dark sporangia with numerous sporagio spores non septate hypae	<i>Aspergillus fumigates</i>

Table 7: Result of cultural and microscopic Characteristic of fungi isolated from Lab A and Lab B

- identification of species involved in human infections. *Universal Journal of Microbiology*, 2(1): 15–22.
- [3] Jacob H.J, Irshaid F.I, Alhalib A.M. Estimation and Identification of Airborne Bacteria and Fungi in the Outdoor Atmosphere of Al-Mafraq Area, Jordan. *Jordan J Biol Sci*, 2021. 9 (1): p3-4
- [4] Mendoza, J., Caso, W., Valdez, C., Pons, M., Valle, L., Ore, V., Michelena, D., Mayra, J., Gavidea, V., Vargas, M. and Ruiz, J. (2019). Diagnosis of carrion's disease by direct blood PCR in thin blood smear negative samples. *Journal of Clinical Microbiology*, 9: 92-183.
- [5] Mera-ul-Haque, Bhowal M., and A Patil. Diversity of aeromycoflora in indoor and outdoor environment. *Imp J of interdiscipl Res*, 2016. 2(8): p 240-248
- [6] Miller, J. D. (2017). Fungi and fungal products in some Canadians homes. *International Biodeteriation*, 24: 103-120.
- [7] Mitsuko, S., Yoshihisa, Y., Hirotaka, T., Hiromasa, T., Setsuko, S. and Masao, M. (2018). Loop-mediated isothermal amplification method targeting the *lytA* gene for detection of *Streptococcus pneumoniae*. *Journal of Clinical Microbiology*, 43(4): 1581–1586.
- [8] Naruka K. and Gaur J. Distribution Pattern of Airborne Bacteria and Fungi at Market Area. *American-Eurasian J Sci Res*, 2019. 9 (6): P186-192
- [9] Oduyayo, E. Ch.; Opuene, K., (2020). Preliminary assessment of trace metals and polycyclic aromatic hydrocarbons in the sediments. *Int. J. Environ. Sci. Tech.*, 4 (2), 233-240
- [10] Saylani M., A. Piotraszewska-Pajak, A. Szyszka, M. Nowicki and Filipiak M. Microbiological quality of indoor air in university rooms. *Polish J. of Environ. Stud.* 16(4): 623-632.2019
- [11] Sheik G.B., Abd Al Rheam A.I., Al Shehri Z.S., and Al Otaibi, O.M. Assessment of Bacteria and Fungi in air from College of Applied Medical Sciences (Male) at AD-Dawadmi, Saudi Arabia. *Int Res J Biol Sci*, 2019.4(9): p48-53
- [12] Tokuyasu, L. D., (2021). Introduction to aerobiology. Hurst, C. J.; Crawford, R. L.; Garland, J. L.; Lipson, D. A.; Mills, A. L.; Stetzenbach, L. D., (Eds.). *Manual of environmental microbiology*, ASM Press, Washington D.C., 925–938. Velmurugan, N.; Chun, S. S.; Han, S. S.; Lee, Y. S.
- [13] Yagboub S.O. and Elagbashi A. Isolation of potential pathogenic bacteria from the air of hospital Delivery and nursing rooms. *Int J Appl Sci*, 2010. 10 (11): p 1011-1014.
- [14] Zoumat M. F. and Almouqat S. (2010). Assessment of airborne bacteria and fungi in an indoor and Outdoor environment. *Int. J. Environ. Sci. Tech.*, 7 (3), 535-544