

# Analysis of Indoor Air Microflora of Some Daycare Centres in Ilorin South Local Government Area, Nigeria

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## Abstract

The microflora of air in ten daycare centres within Ilorin South Local Government Area of Kwara State, Nigeria was investigated by settling plate technique. The plates were exposed for a period of 10 minutes at each sampling point. The counts of bacteria, total coliform and fungi ranged from 10 to 200, 10 to 170 and 6 to 110 cfu/10 minutes respectively. A total of 23 microorganisms comprising 12 bacteria and 11 fungi were isolated in this study. These bacteria were *Micrococcus* sp., *Staphylococcus arlettae*, *Corynebacterium pseudotuberculosis*, *Staphylococcus haemolyticus*, *Corynebacterium kutscheri*, *Staphylococcus warneri*, *Staphylococcus aureus*, *Corynebacterium diphtheriae*., *Corynebacterium amycolatum*, *Bacillus pumilus*, *Corynebacterium matruchotii* and *Listeria grayi* while the fungi were *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium* sp., *Aspergillus flavus*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Alternaria alternata*, *Aspergillus glaucus* and *Polyporus* sp. The predominant bacterium and fungus in this study were *Micrococcus* sp. and *Aspergillus fumigatus* respectively. It can be concluded that the indoor air of the daycare centres used for this study had diverse species of Gram positive bacteria, high occurrence of non-pathogenic bacteria and fungi. Moulds dominate the fungal isolates and some of the bacterial species are commensals found on human body whereas the fungi are environmental in origin. A few pathogenic species were isolated among the bacteria and fungi.

**Keywords:** Microbial, Flora, Air, Daycare, Hygiene

## 1.0 Introduction

Aeromicrobiology is the study of living microbes (bioaerosols) which are suspended in the air. Microorganisms in the air are far less than those in soil and water but their populations are still large enough in number that they can affect the atmosphere. These aerosols are ecologically significant because they are associated with diseases in humans, animals and plants [1]. There are many factors within the physical environment that affect the launching, transport and depositions of bioaerosols. Particles which become suspended in the air column arise mainly from terrestrial and aquatic environment and they are typically launched by air turbulence. Bioaerosols are primarily transported by wind and they can be deposited by a number of mechanisms including force of gravity, contact with surfaces, or combining with rain which pulls the particles back down to earth's surface [2].

About 90% of the world's population stay many hours indoors in houses, offices, schools and daycare centres where they are affected by indoor environmental factors which influence their health and physical conditions [3]. Various layers can be recognized in the atmosphere including troposphere which is the layer directly above the earth extending between 11–16 km in temperate regions [4]. The troposphere is characterized by a heavy load of microorganisms including vegetative cells and spores of bacteria, fungi, algae, viruses and protozoa cysts in air.

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Microorganisms are discharged into the air as infectious droplets, droplet nuclei and dust. Droplets are usually generated by sneezing, coughing or talking, which involve saliva and mucus while droplet nuclei are formed when small liquid droplets evaporate. Dusts are released into the air during activities such as sweeping, tillage of the soil, movement of heavy vehicles, blasting of stone and air turbulence [4]. Air currents may also bring microorganisms from plant, animal and other solid surfaces into the air [5]. Microorganisms suspended in air are only rarely found in free state [6].

Air current influence the time which either the microorganisms or the particles laden with microorganisms remain suspended in the air. In still air, the particles tend to settle down. Air current is important in the dispersal of microorganisms as it carries them over a long distance. Air current produces turbulence which causes vertical distribution of air flora. No microbes are indigenous to the atmosphere rather they represent allochthonous populations transported from aquatic and terrestrial habitats into the atmosphere [7].

Nowadays, many women are engaged in economic activities which necessitate keeping their babies in daycare centres during working hours. More often, such babies come home with episode of diseases which are environmental in origin. Hence, it is necessary to determine the level of air hygiene of these centres. Therefore, the objectives of this research was to determine the number, species and occurrence of bacteria and fungi in the air of some daycare centres in Ilorin South Local Government Area of Kwara State, Nigeria and provide recommendation on ways of minimizing contamination of the air in daycare centres.

## 2.0 Materials and Methods

### 2.1 Sampling Sites

The sampling points for this study were ten different daycare centres within Ilorin South Local Government Area of Kwara State, Nigeria. These daycare centres are located in 4 locations namely Tanke, Pipeline, Gaa-Akanbi and a Tertiary Institution.

### 2.2 Isolation and Preservation of Pure Cultures of Microorganisms

The settling plate technique was employed to sample air in the daycare centres. This was done by exposing solidified plate of nutrient agar (NA), MacConkey agar (MA) and potato dextrose agar (PDA) to air for 10 minutes. The NA plates were incubated at 37°C for 48 hours while the PDA plates were incubated at room temperature (25°C) for 72 hours [8]. The microorganisms were subcultured repeatedly until pure cultures were obtained using nutrient agar and potato dextrose agar for bacteria and fungi respectively. The pure cultures obtained were then stocked as agar slants and kept in a refrigerator until needed [9].

### 2.3 Characterization and Identification of Isolates

The characterization and identification of bacterial isolates were based on their colonial morphology, cellular morphology and biochemical characteristics [10]. The biochemical tests conducted include testing for production of enzymes such as catalase, oxidase and urease as well as the ability of the bacteria to utilize biological molecules such as starch, tryptophan to produce indole, nitrate, citrate and sugars [9]. The fungal isolates were characterized and identified based on their macroscopic and microscopic characteristics [11].

## 3.0 Results

### 3.1 Counts of Indoor Air Microflora of Daycare Centres

The bacteria and total coliform counts ranged from 10 to 200 and 10 to 170 cfu/10 minutes respectively. The fungal counts of air of the daycares ranged from 6 to 110 cfu/10 minutes (Table 1).

### 3.2 Characterization, Identification and Occurrence of Isolates

The bacterial isolates identified were *Micrococcus* sp., *Staphylococcus arlettae*, *Corynebacterium pseudotuberculosis*, *Staphylococcus haemolyticus*, *Corynebacterium kutscheri*, *Staphylococcus warneri*, *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Corynebacterium amycolatum*, *Bacillus pumilus*, *Corynebacterium matruchotii* and *Listeria grayi* (Table 2). The occurrence of the bacterial isolates is presented in Table 3.

Table 1: Microbial Counts of Indoor Air of Selected Daycare Centres in Ilorin

Day care codes	Microbial counts (cfu/10 minutes)		
	Bacteria	Total coliform	Fungi
TK I	160	60	52
TK II	49	85	10
TK III	121	10	110
TK IV	189	10	105
TK V	24	60	16
TK VI	10	26	6
PL I	200	170	54
PL II	32	17	42
GA	83	40	6
TI	104	32	15

TK = Tanke; PL = Pipeline; GA = Gaa-Akanbi; TI = Tertiary Institution

Table 2: Characterization and Identification of Bacterial Isolates

Bacterial isolates	Colonial colour	Colonial edge	Gram staining	Shape of Cell	Arrangement of cell	Spore staining	Motility	Catalase	Coagulase	H <sub>2</sub> S on TSI	Oxidase	Hhaemolysis	Methyl red	Voges proskauer	Citrate	Urease	Starch hydrolysis	Nitrate reduction	Maltose	Sucrose	Lactose	Mannitol	Glucose	Fructose	OF	Probable identity
1	Y	en	+	c	ch	-	-	+	-	-	+	γ	+	+	-	+	-	-	-	A	-	A	A	A	O	<i>Micrococcus</i> Sp.
2	or	en	+	c	cl	-	-	+	-	-	-	γ	-	-	+	-	+	-	A	A	A	A	A	A	F	<i>Staphylococcus</i> <i>arlettae</i>
3	cr	ir	+	r	ch	-	-	+	-	-	+	β	+	+	+	+	+	-	A	A	-	-	A	A	F	<i>Corynebacterium</i> <i>pseudotuberculosis</i>
4	W	en	+	c	cl	-	-	+	-	-	-	β	+	+	+	-	-	+	A	A	A	A	A	A	F	<i>Staphylococcus</i> <i>haemolyticus</i>
5	cr	ir	+	r	cl	-	-	+	-	-	+	γ	-	-	+	+	+	+	A	A	-	-	A	A	F	<i>Corynebacterium</i> <i>kutscheri</i>
6	cr	en	+	c	cl	-	-	+	-	-	-	β	-	+	+	+	+	-	A	A	-	-	A	A	F	<i>Staphylococcus</i> <i>warneri</i>
7	Y	en	+	c	cl	-	-	+	+	-	-	γ	+	+	+	+	+	+	A	A	A	A	A	A	F	<i>Staphylococcus</i> <i>aureus</i>
8	cr	ir	+	r	ch	-	-	+	-	-	-	β	+	-	+	-	-	+	A	-	-	A	A	A	F	<i>Corynebacterium</i> <i>diphtheriae</i>
9	cr	ir	+	r	cl	-	-	+	-	-	+	γ	+	+	+	-	-	-	A	-	-	-	A	A	F	<i>Corynebacterium</i> <i>amycolatium</i>
10	cr	en	+	r	cl	+	-	+	-	-	+	β	+	+	+	-	-	-	A	A	-	A	A	A	F	<i>Bacillus</i> <i>pumilus</i>
11	cr	en	+	r	ch	-	-	+	-	-	+	γ	+	-	+	+	-	+	A	A	-	-	A	A	F	<i>Corynebacterium</i> <i>matruchotii</i>
12	cr	en	+	r	cl	-	+	+	-	-	-	α	+	+	-	-	-	-	A	A	A	A	A	A	F	<i>Listeria</i> <i>grayi</i>

Key: - = Negative reaction; + = Positive reaction; y = Yellow; cr = Cream; or = Orange; w = White; en = entire; Ir = Irregular; O = Opaque; c = Cocci; r = Rod; cl = Cluster; Ch = chain; F = Fermentative; O = Oxidative; OF = Oxidation-Fermentation reaction; TSI = Triple sugar iron agar; AG = Acid and gas; A = Acid only

**Table 3: Occurrence of Bacterial Isolates in the Indoor Air of Selected Daycare Centres in Ilorin**

Daycare codes	Bacterial isolates											
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
TK I	+	-	+	+	+	-	+	-	+	+	+	+
TK II	+	-	+	+	-	+	+	+	-	-	-	-
TK III	+	+	+	-	-	-	-	-	-	-	-	-
TK IV	+	+	+	-	-	-	-	-	-	-	-	+
TK V	+	+	-	-	-	-	-	-	-	+	-	-
TK VI	+	-	-	-	-	-	-	-	-	-	-	-
PL I	+	+	+	+	-	-	-	-	-	-	-	-
PL II	+	+	+	-	+	-	-	-	-	-	-	-
GA	+	+	-	+	+	+	-	-	+	-	-	-
TI	+	+	+	-	-	-	-	-	-	-	+	-

+ = isolated, - = not isolated, B1 = *Micrococcus* sp., B2 = *Staphylococcus arlettae*., B3 = *Corynebacterium pseudotuberculosis*, B4 = *Staphylococcus haemolyticus*, B5 = *Corynebacterium kutscheri*, B6 = *Staphylococcus warneri*, B7 = *Staphylococcus aureus*, B8 = *Corynebacterium diphtheriae*, B9 = *Corynebacterium amycolatum*, B10 = *Bacillus pumilus*, B11 = *Corynebacterium matruchotii*, B12 = *Listeria grayi*, TK = Tanke, PL = Pipeline, GA = Gaa-Akanbi, TI = Tertiary Institution.

The fungal species isolated were *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium*. sp., *Aspergillus flavus*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Alternaria alternata*, *Aspergillus glaucus* and *Polyporus* sp. The occurrence of the fungal isolates is indicated in Table 4.

**Table 4: Occurrence of Fungal Isolates in the Air of Selected Daycare Centres in Ilorin**

Day care codes	Fungal isolates										
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
TK I	+	-	+	-	-	-	-	-	-	-	-
TK II	-	-	+	+	-	+	+	+	-	-	-
TK III	+	-	+	-	-	-	-	+	-	-	-
TK IV	+	+	+	-	-	-	-	-	-	-	-
TK V	+	+	-	-	-	-	-	-	+	-	-
TK VI	-	-	-	-	-	+	-	-	-	-	-
PL I	+	+	-	+	-	+	-	+	-	+	-
PL II	-	-	+	-	+	-	-	-	-	-	+
GA	-	+	-	+	+	+	-	-	+	-	-
TI	-	+	+	-	-	-	-	-	-	-	-

F1 = *Aspergillus fumigatus*, F2 = *Aspergillus niger*, F3 = *Penicillium chrysogenum*, F4 = *Penicillium* sp., F5 = *Aspergillus flavus*, F6 = *Rhizopus stolonifer*, F7 = *Fusarium oxysporum*, F8 = *Saccharomyces cerevisiae*, F9 = *Alternaria alternata*, F10 = *Aspergillus glaucus*, F11 = *Polyporus* sp. TK = Tanke, PL = Pipeline, GA = Gaa-Akanbi, TI = Tertiary Institution

#### 4.0 Discussion

The children group comprises about 45% of the population in developing countries and infection rate is high especially in the age range under 5 years. Some of the diseases suffered by infants are environmental in origin which can be contained and prevented by relevant basic hygiene skills [12]. Dirty environment may harbour infectious agents. A good daycare is however able to keep its environment in good working order freed from all preventable epidemics. The care of the infants required greater skills and attention because the immune system of infants is not well developed. Mend *et al.* [4] has reported that daycare centres have been implicated in the spread of communicable diseases, especially diarrhoea and respiratory diseases among young susceptible children (1-5 years) due to poor hygiene. Some of the factors which could increase the level of microorganisms in the air are ventilation rate, health status, number of children, and human activities.

The present study revealed a remarkable diversity of bacteria present in the air of daycare facilities. The bacteria isolated belong to the genera: *Micrococcus*, *Staphylococcus*, *Corynebacterium*, *Bacillus* and *Listeria*. In a related study, *Micrococcus*, *Paracoccus*, *Staphylococcus* and *Enhydrobacter* were isolated in the study of indoor and outdoor environments of child care facilities [13] while *Staphylococcus*, *Proteus*, *Streptococcus* and *Micrococcus* were isolated from indoor air of laboratories [14]. All the bacteria isolated in this study were Gram positive cocci and rods; similar to those isolated in the air of hospital lobbies [12]. This is not surprising since Gram positive bacteria have thick layer of peptidoglycan in their cell wall than Gram negative bacteria. Hence, they dominated the bacterial flora in this study.

Based on the relationship of the isolates with their host (humans) the organisms isolated in this study can be classified as being commensals or pathogens. These microbes are derived either from humans or soil environment. The commensals are normal body flora of humans which can be found on the skin, nose and mouth. Under normal circumstances, they do not cause infection in their host except when the immune system is compromised [7]. The bacteria which are non-pathogenic in this study were 10 out of 12 representing 83.3% of the bacterial isolates while the pathogens, *S. aureus* and *C. diphtheriae* represent 16.7% of the bacterial isolates. These bacterial pathogens were isolated from 2 daycare centres out of 10 (20%). Most of the bacteria isolated in this study are environmental in origin. Moulds dominated the fungi isolated in this study. The only yeast isolated was *S. cerevisiae*. Moulds are environmental in origin while *S. cerevisiae* could originate from humans (biological). Three out of eleven fungi (27.2%) isolated in this study are known to be pathogenic. These are *A. niger* which can cause aspergillosis while *A. flavus* and *A. fumigatus* are known to produce mycotoxins [11].

*Micrococcus* sp. was common in almost all the daycare centres, and this is because the organism can be found in many places such as skin surface, dust, water and soil. *Corynebacterium* species occurs commonly in nature in the soil, plants and food products. It can also be found in the mucosa and skin flora of humans and animals. The five species of *Corynebacterium* isolated in this study namely *C. amycolatum*, *C. Striatum*, *C. jeikeium*, *C. urealyticum* and *C. Xerosis* have been isolated as pathogens in immunosuppressed patients [15].

Four species of *Staphylococcus* were isolated in this study. Most Staphylococci reside on the skin and mucous membranes of human and other organisms. They are found worldwide and are component of soil microbial flora. The most important human pathogens are *S. aureus*, *S. epidermidis* and *S. saprophyticus* [7]. *Bacillus* species are saprophytic organisms prevalent in air and some other habitats. Several of them cause diseases in humans. The endospores of bacilli are more resistant than vegetative cells to heat, drying and disinfectants. They are present in soil, dust and plant products and are able to survive better in air [6].

In this study, *Aspergillus* spp. dominated the genera of fungi isolated since they have capacity to sporulate. Out of the four species isolated, *Aspergillus flavus* has been reported to produce aflatoxin [16]. Previous studies also reported the presence of *Aspergillus niger*, *Aspergillus flavus*, *A. glaucus*, *A. versicolor*, *Alternaria alternata*, *Geotrichum candidum* and *Rhizopus* in some offices at University of Ilorin, Ilorin, Nigeria [17]. *Saccharomyces cerevisiae* is a yeast whose natural strains have been found on the surface of plants, gastrointestinal tract of humans, body surface of insects and warm-blooded animals, as well as in the soils. It is linked with diseases in immunosuppressed patients such as those with Crohn's disease; it also causes diseases like vaginitis and flu-like infection [18].

*Rhizopus stolonifer* is a very common fungus that is involved in spoilage of many foods, fruits, vegetables and bread. They may be derived from outdoor sources such as grains, damp vegetation and compost heaps etc when particles harbouring them might be blown into the daycare building [11]. *Alternaria* spp. are ubiquitous in the environment. *Alternaria* species are known as major plant pathogens. They are also common allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometime led to asthma [19].

## 5.0 Recommendation and Conclusion

The amount of bioburden in the daycare centres can be minimized by preventing any activities that can generate dust such as sweeping. The floor should be mopped rather than being swept. The parents are advised to vaccinate their children against respiratory diseases such as diphtheria, whooping cough etc. Sick infants with visible signs of respiratory disorders such as rhinitis, frequent sneezing etc should not be allowed into the daycare so as to prevent spread of contaminated droplets to other children. The daycare room should be fumigated with chemical disinfectant monthly in order to decontaminate the room. Ultraviolet lamp can be installed and used only when there is no occupant in the room. Also natural ventilation of rooms and the use of air conditioning system can ensure reduction of air contamination.

It can be concluded that the indoor air of the daycare centres used for this study had diverse species of Gram positive bacteria, high occurrence of non-pathogenic bacteria and fungi, moulds dominate the fungal isolates and some of the bacterial species are commensals found on human body whereas the fungi are environmental in origin. A few pathogenic species were isolated among the bacteria and fungi.

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