Microbial Evaluation of Air Inside Some Eateries Located in Ado-Ekiti, Nigeria

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Abstract: The microbial load of air in seven eateries within Ado-Ekiti was determined. Petri dishes with different media were left opened in the eateries for 20 minutes, covered afterwards and incubated in the laboratory. Biochemical tests were carried out to identify the isolates. Forty-seven bacterial isolates were obtained and included; *Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus luteus, Neisseria polysaccharea, Acinetobacter parvus, Arthrobacter agilis, Asaia bogorensis, Bordetella trematum, Pseudomonas stutzeri, Necropsobacter rosorum, Microvirga massiliensis, Pseudomonas oryzihabitans, Stenotrophomonas maltophilia, Staphylococcus petrasi,* and *Acinetobacter ursingii* amongst others. Most of the bacterial isolates were susceptible to Amoxycillin and Gentamicin and resistant to Ceftazidime. Twelve fungal isolates were obtained and included; *Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Penicillium chyrsogenum and Cladosporium* spp. *Micrococcus luteus* had the highest frequency of occurrence of 10% followed by *Staphylococcus petrasii* with 8%. *Stenotrophomonas maltophilia, Pseudomonas aeruginosa and Microvirga massiliensis* had frequency of occurrence of 6% each while *Pseudomonas oryzihabitans, Acinetobacter ursingii, Bordetella trematum, Pseudomonas stutzeri and Necropsobacter rosorum* had frequency of occurrence of 4% each. *Rhizopus stolonifer* had the highest frequency of occurrence of 25%. *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Aspergillus fumigatus* had frequency of occurrence of 17% each while *Cladosporium spp* had the lowest frequency of occurrence of 8%. The results observed in this study revealed that the micro flora of air in these eateries is dynamic and that some microorganisms are common to indoor environments such as eateries.

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1. Introduction

Healthy environment has a very strong connection with human health (Botkin and Keller, 2007). Bacteria, fungi, pollen, viruses and mites can be sources of biological air contamination (Nevalainen and Seuri, 2005; Khan and Karuppayil, 2010) and clean air is required by all living humans and animals for good health and wellbeing. However, due to urban development, the air is continuously polluted. Urban ambient air is more polluted than overall atmosphere, due to high density of human population and their activities in urban areas. (Ling *et al.,* 2011).

Some of the health effects of exposure to air pollution, such as the impact on the respiratory and cardiovascular systems, have been extensively studied, thus it is well-known that exposure to air pollutants leads to an increase in mortality and morbidity rates of the population (Kunzli *et al.,* 2004).

Indoor air quality can be defined as the air quality inside a building that will lead to occupant comfort and health. A poor indoor air quality can cause a variety of short-term and long-term health problems including allergic reactions, respiratory problems, eye irritation, sinusitis, bronchitis and pneumonia. (Marmot *et al.,* 2006).

Biological contamination of indoor air is mostly caused by bacteria, moulds and yeast.

Exposure to bio-aerosols, containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions (Gorny *et al*., 2002; Fracchia *et al*., 2006). The atmosphere is considered an oligotrophic and harsh environment as a result of low humidity, scarce nutrients, variable temperatures, and UV exposure, and therefore challenging, not only for the survival, but also growth of microorganisms. Despite this, bacteria continue to be ubiquitously present in ambient air and in significant cell concentrations (Maron *et al.*, 2005). The airborne communities even appear to show high diversities, comparable to soil and water communities (Maron *et al.*, 2005). This apparent contradiction can be explained by various bacterial adaptation mechanisms such as temperature tolerance, DNA-repair mechanisms, and other means for UV protection like embedding in particles (that can contain liquid) or producing a wide range of pigments (Polymenakou, 2012).

Airborne bacteria are playing an essential role in ecosystems: their presence (or colonization by wind transportation) or absence can influence the balance of ecosystems.

Fungi are common in indoor and outdoor environments and nearly 10 % of people worldwide have fungal allergy (Pasanen *et al.,* 1996). Fungal flora can be hazardous for health, particularly in rooms with heating, ventilation and air conditioning systems in place. (La Serna *et al.,* 2002).

The relative humidity and/ or the moisture content of the materials determines to what extent different micro-organisms can grow on indoor or outdoor materials (Dhanasekaran *et al.,* 2009). These may cause destruction, adverse health effects and unpleasant odours.

However, despite the dangers and health hazards associated with indoor environment due to microbial load, there is no known documentation on the microbial evaluation of air in eateries in Ado-Ekiti, where multitudes throng to have their breakfast, lunch and dinner. There is therefore a need for the microbial assessment and of air around these places. This work will form a baseline study on microbial load and create awareness on their health implications and suggest ways of preventing health hazards arising from such air pollution.

The aim of the study was to isolate and identify microorganism present in air around some eateries. in Ado Ekiti, Ekiti State using morphological and biochemical methods and to determine the antibiotic susceptibility of the bacterial isolates.

2. Materials and Methods

This study was carried out using air samples from the following eateries; Captain Cook (CE), Tantalizers (TA), Chicken Republic (CG), Portofino (PF), Danke Fast Foods (DC), Take Away (TB) and Tasty and spicy (TD) between January and March, 2018. Air samples were collected and nutrient agar, mannitol salt agar and MacConkey agar were usd for the isolation of bacteria and potato dextrose agar for fungi. The plates were exposed to the air for 20 minutes in these eateries, covered and taken to the Microbiology Laboratory at Afe Babalola University, Ado Ekiti. The plates for bacteria were incubated for 24 hours at 37^0C and for fungi at 28^0C for 72 hours.

Purification of bacterial isolates

After incubation, the bacterial isolates were sub cultured on Nutrient Agar plates and incubated at 37^0C for 24 hours and fungal isolates on potato dextrose agar for 72 hours at 28^0C until pure isolates were obtained. They were stored on slants and kept in the refrigerator at 4^0C until needed.

Identification of microorganisms was carried out using Gram staining technique according to the method of Davies *et al.*, (1983). The bacterial isolates were subjected to various biochemical tests. mannitol fermentation, spore staining (Cappuccino and Sherman, 2005); Triple sugar iron test, oxidase test (Cheesbrough, 2006); Citrate utilization, starch hydrolysis, indole test (Hemraj *et al*, 2013). The Global Infectious Diseases and Epidemiology Network (GIDEON) online software was used for identification of bacterial isolates.

Fungal Identification was done by adding two drops of lactophenol cotton blue on a clean slide, and using a sterile inoculating loop, the fungal mycelia was transferred onto the fluid on the slide and teased out on the stain. It was covered with a cover slip and observed under magnification of X4 of the microscope (Sekar *et al*., 2008).

Antibiotics susceptibility test was carried out to determine whether an etiological agent is sufficiently sensitive to an antimicrobial agent to permit its use for treatment. The test was carried out with discs containing known concentrations of antibiotics against Gram positive and Gram negative bacteria. Pure isolates were spread evenly on prepared Meuller-Hinton agar using sterile swab sticks aseptically on the agar plates for Gram positive and negative bacteria respectively and incubated at 37^0C for 24 hours. After incubation, the zone of inhibition of each antibiotic was measured and then compared to a set of standards. Depending on the measurement, the organism was categorized to be either susceptible (S), intermediately susceptible (I), or resistant (R) to the antibiotics used (World Health Organisation, 2017).

3. Results

Forty-seven bacterial and twelve fungal isolates were obtained from samples from seven eateries in Ado-Ekiti; twenty-four were Gram positive while twenty-three Gram negative. Table 1 shows the biochemical characteristics of the bacterial isolates and the organisms identified included the following: *Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus luteus, Neisseria polysaccharea, Acinetobacter parvus, Arthrobacter agilis* and *Asaia bogorensis*. Isolate A1 from Portofino was a Gram negative cocci, positive for catalase, citrate, urease tests and hydrolysed starch. It grew on Mannitol Salt Agar and did not produce hydrogen sulphide. Growth was observed on blood agar with no haemolysis and probable organism was *Neisseria polysaccharea.* Isolate E4 from Captain Cook was a Gram positive rod and tested positive for catalase, citrate test and hydrolysed starch. It grew on Mannitol salt agar and Blood agar and produced endospore. The probable organism was *Bacillus subtilis.* Isolate F4 from Take Away was a Gram negative rod. It was positive for catalase, oxidase, citrate and urease tests, hydrolysed starch and grew on Mannitol Salt Agar and Blood Agar with haemolysis. The probable organism was *Pseudomonas aeruginosa.*

Table 2 shows the frequency of occurrence of the

bacterial isolates. *Micrococcus luteus* had the highest frequency of occurrence of 10% followed by *Staphylococcus petrasii* with 8%. *Stenotrophomonas maltophilia, Pseudomonas aeruginosa, Bacillus subtilis and Microvirga massiliensis* had frequency of occurrence of 6% each.

Table 3 shows the antibiotic susceptibility of Gram positive bacterial isolates to antibiotics tested. The Gram positive antibiotics discs contained the following antibiotics; Ceftazidime, Cefuroxime, Gentamicin, Ceftriaxone, Erythomycin, Cloxicillin, Ofloxacin and Amoxycillin/ Clavulanate. The isolates showed high resistance to Ceftazidime at 94.7% and the least resistance to Gentamicin at 11%. The highest susceptibility of the isolates was to Gentamicin at 89% and least susceptible to Ceftazidime.

Table 4 shows the antibiotic susceptibility of the Gram negative bacteria to antibiotics. The Gram negative antibiotics disc contained Ciporfloxacin, Nitrofurantoin, Ampicillin, Ceftazidime, Cefuroxime, Gentamicin and Ofloxacin. The isolates showed high resistance to Amoxycillin/ Clavulanate, least resistance to Gentamicin and highest susceptibility to Gentamicin, followed by Ofloxacin.

Table 1: Biochemical identification of bacterial isolates of air samples from eateries in Ado-Ekiti

Sample Codes	Gram stain	Cell shape	Catalase	Oxidase	Citrate	Urease	Indole	Starch hydrolysis	Glucose	Lactose	Sucrose	Gas	H_2S	Butt	Slant	Mannitol (6.5% NaCl)	Growth on blood agar	Haemolysis	Endospore Staining	Probable organisms
Sample A																				
A1	-	Cocci	+	-	+	+	-	+	-	-	-	-	-	R	R	Growth/+	+	-	-	*Neisseria polysaccharea*
A2	+	Cocci	+	-	-	+	-	-	+	+	+	-	-	Y	Y	Growth/-	+	-	-	*Gordonia terrae*
A3	+	Rod	+	-	-	+	-	-	+	+	+	-	-	Y	Y	No growth	+	-	+	*Bacillus megaterium*
A4	-	Cocci	+	-	+		-	-	+	+	+	-	-	Y	Y	Growth/-	+	-	-	*Acinetobacter ursingii*
A5	-	Cocci	+	-	+	+	-	-	+	+	+		+	Y	Y	Growth/+	+	+	-	*Bordetella trematum*
A6	-	Rod	+		+	+		-	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Advenella incenata*
A7	+	Cocci	+	-	-	+	-	-	+	+	+	-	-	Y	Y	Growth/-	+	-	-	*Micrococcus luteus*
Sample B																				
B1	+	Cocci	+	-	+	-	-	-	+	-	-	-	-	Y	R	Growth/-	+	-	-	*Staphylococcus auricularis*
B2	-	Cocci	+	-	-	-	-	-	+	+	+	-	-	Y	Y	Growth/+	+	-	-	*Necropsobacter rosorum*
B3	-	Rod	+	-	+	-	-	+	+	-	-	-	-	Y	R	Growth/+	+	-	-	*Microvirga massiliensis*
B4	-	Cocci	-	-	-	-	-	+	+	-	-	-	-	Y	R	No growth	+	-	-	*Neiserria subflava*
B5	-	Cocci	+	-	-	-	-	-	-	+	+	-	-	R	Y	Growth/-	+	-	-	*Acinetobacter parvus*
B6	+	Cocci	+	-	+	-	-	+	+	+	+	-	-	Y	Y	Growth/-	+	-	-	*Staphylococcus petrasii*
Sample C																				
C1	+	Cocci	+	-	-	-	-	+	+	+	+	-	-	Y	Y	Growth/+	-	+	-	*Staphylococcus petrasii*
C2	-	Rod	+	-	+	+	-	+	+	-	-	-	-	Y	R	Growth/+	-	+	-	*Pseudomonas oryzihabitans*
C3	-	Rod	+	-	+	-	-	-	-	-	-	-	-	R	R	Growth/+	-	+	-	*Stenotrophomonas maltophilia*
C4	-	Rod	+	-	+	-	-	+	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Microvirga massiliensis*
C5	+	Cocci	+	-	-	-	-	-	+	-	-	-	-	Y	R	Growth/+	-	+	-	*Kocuria kristinae*
C6	-	Rod	+	-	+	+	-	-	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Pseudomonas aeruginosa*
Sample D																				
D1	-	Rod		+	+	-	-	+	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Pseudomonas stutzeri*
D2	-	Rod		-	-	-	-	+	+	+	+	+	+	B	Y	Growth/+	+	-	-	*Microbacterium imperial*
D3	+	Cocci	+	-	-	-	-	-	+	-	-	-	-	Y	Y	Growth/-	+	-	-	*Micrococcus luteus*
D4	-	Cocci		-	-	+	-	-	+	+	+	+	-	Y	Y	Growth/-	+	-	-	*Necropsobacter rosorum*
D5	-	Rod		-	-	-	-	-	-	-	-	-	-	R	R	No growth	+	-	-	*Moraxella lacunata*
D6	-	Rod		-	-	-	-	+	-	-	-	-	-	R	R	Growth/+	+	-		*Stenotrophomonas maltophilia*
Sample E																				
E1	+	Cocci	+	-	+	-	-	-	+	-	-	-	-	Y	R	Growth/+	+	-	-	*Micrococcus luteus*
E2	+	Cocci	+	-	-	-	-	+	+	-	-	-	-	Y	R		+	+	-	*Arthrobacter agilis*
E3	+	Cocci	+	-	+	-	-	-	+	+	+	-	-	Y	Y	Growth/+	+	-	-	*Staphylococcus petrasii*
E4	+	Rod	+	-	+	-	-	+	+	-	-	-	-	Y	R	Growth/+	+	-	+	*Bacillus subtilis*
E5	-	Cocci	+	-	+	-	-	+	+	+	+	+	+	B	Y	Growth/+	+	+	-	*Bordetella trematum*
E6	+	Cocci	-	-	-	-	-		+	-	-	-	-	Y	R	Growth/-	+	-	-	*Brevibacterium epidermidis*
Sample F																				
F1	+	Cocci	+	-	-	+	-	-	-	-	-	-	-	R	R	Growth/-	+	-	-	*Campylobacter ureolyticus*
F2	-	Coco bacilli	+	-	+	+	-	+	-	-	-	-	-	R	R	Growth/+	+	-	-	*Pseudomonas oryzihabitans*
F3	-	Rods	+	-	-	+	-	-	+	-	-	-	-	Y	R	Growth/+	+	-	-	*Stenotrophomonas maltophilia*
F4	-	Rods	+	+	+	+	-	-	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Pseudomonas aerugiinosa*
F5	+	Cocci	+	-	-	-	-	-	+	+	+	-	-	Y	Y	Growth/+	+	-	-	*Staphylococcus petrasii*
F6	+	Cocci	+	-	+	+	-	-	+	-	-	-	-	Y	R	Growth/+	+	-	-	*Micrococcus luteus*
F8	+	Rod	+	-	+	+	-	-	-	+	+	-	-	R	Y	Growth/+	+	-	+	*Bacillus subtilis*
Sample G																				
G1	-	Rod	-	+	+	+	-	-	-	-	-	-	-	R	R	Growth/-	+	+	-	*Pseudomonas aeruginosa*
G2	-	Rod	+	-	+	-	-	-	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Asaia bogorensis*
G3	+	Rod	+	-	+	-	-	-	+	-	-	-	-	Y	R	Growth/-	+	+	-	*Bacillus subtilis*

Sample Codes	Gram stain	Cell shape	Catalase	Oxidase	Citrate	Urease	Indole	Starch hydrolysis	Glucose	Lactose	Sucrose	Gas	H_2S	Butt	Slant	Mannitol (6.5% NaCl)	Growth on blood agar	Haemolysis	Endospore Staining	Probable organisms
G4	-	Rod	+	-	+	-	-	-	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Microvirga massiliensis*
G6	-	Rod	+	-	+	-	-	+	+	-	-	-	-	Y	R	Growth/+	+	+	-	*Pseudomonas stutzeri*
G7	-	Rod	+	-	+	-	-	+	+	-	-	-	-	Y	R	Growth/+	+	-	-	*Stenotrophomonas maltophilia*
G8	-	Cocci	+	-	+	-	-	-	-	+	+	-	-	R	Y	Growth/-	+	-	-	*Micrococcus luteus*
G9		Cocci	+	-	+	-	-	-	+	+	+	-	-	Y	Y	Growth/+	+	-	-	*Acinetobacter ursingii*
G10	-	Cocci	+	-	+	-	-	+	+	-	-	-	-	Y	R	Growth/+	+	-	-	*Neisseria meningitidis*

Key: R: Red Y: Yellow B: Black
Sample A: Isolates from Portofino Sample B: Isolates from Tantalizers
Sample C: Isolates from Danke Fast Foods Sample D: Isolates from Chicken Republic
Sample E: Isolates from captain Cook Sample F: Isolates from Take Away
Sample G: Isolates from Tasty and Spicy No Growth: Did not survive in salt conditions
Growth/+: Survived in salt conditions and fermented mannitol salt Growth/-: Survived in salt conditions but did not ferment mannitol salt

Table 2: Frequency of occurrence of bacterial isolates from air samples in eateries in Ado-Ekiti

S/N	Identified Isolates	Frequency	Percentage Occurrence %
1	*Pseudomonas oryzihabitans*	2	4
2	*Stenotrophomonas maltophilia*	3	6
3	*Pseudomonas aeruginosa*	3	6
4	*Staphylococcus petrasii*	4	8
5	*Micrococcus luteus*	5	10
6	*Bacillus subtilis*	3	6
7	*Acinetobacter ursingii*	2	4
8	*Bordetella trematum*	2	4
9	*Pseudomonas stutzeri*	2	4
10	*Necropsobacter rosorum*	2	4
11	*Microvirga massiliensis*	3	6

Table 3: Antibiotic susceptibility test for Gram positive bacteria from air samples in eateries in Ado-Ekiti

Organisms	Ceftazidime (30ug)	Cefuroxime (30ug)	Gentamicin (10ug)	Ceftriaxone (30ug)	Erythromycin (5ug)	Cloxicillin (5ug)	Ofloxacin (5ug)	Amoxycillin/ Clavulanate (30ug)
Gordonia terrae	11(R)	28(S)	28(S)	29(S)	26(S)	23(S)	25(S)	28(S)
Bacillus megaterium	0(R)	42(S)	24(S)	24(S)	21(S)	21(S)	18(S)	27(S)
Micrococcus luteus	0(R)	15(I)	23(S)	12(R)	0(R)	0(R)	21(S)	0(R)
Staphylococcus auricularis	0(R)	0(R)	20(S)	11(R)	0(R)	0(R)	25(S)	0(R)
Staphylococcus petrasii	0(R)	28(S)	27(S)	26(S)	29(S)	0(R)	16(I)	24(S)
Staphylococcus petrasii	13(R)	33(S)	24(S)	27(S)	24(S)	16(I)	17(I)	28(S)
Kocuria kristinae	0(R)	0(R)	0(R)	19(S)	0(R)	0(R)	16(I)	0(R)
Micrococcus luteus	0(R)	25(S)	21(S)	24(S)	26(S)	0(R)	18(S)	26(S)
Micrococcus luteus	17(I)	33(S)	28(S)	30(S)	30(S)	22(S)	15(I)	30(S)
Arthrobacter agilis	11(R)	20(S)	18(S)	11(I)	0(R)	0(R)	22(S)	25(S)
Staphylococcus petrasii	16(I)	26(S)	20(S)	26(S)	19(I)	21(S)	25(S)	25(S)
Bacillus subtilis	0(R)	22(S)	22(S)	24(S)	0(R)	0(R)	19(I)	0(R)
Brevibacterium epidermidis	11(R)	33(S)	21(S)	21(S)	23(S)	19(S)	25(S)	30(S)
Campylobacter ureolyticus	0(R)	28(S)	25(S)	28(S)	27(S)	0(R)	18(S)	28(S)
Staphylococcus petrasii	13(R)	26(S)	20(S)	22(S)	17(I)	15(I)	13(R)	26(S)
Micrococcus luteus	0(R)	23(S)	26(S)	23(S)	29(S)	0(R)	11(R)	23(S)
Bacillus subtilis	0(R)	27(S)	23(S)	13(R)	22(S)	17(I)	25(S)	23(S)
Bacillus subtilis	0(R)	29(S)	25(S)	30(S)	22(S)	0(R)	24(S)	24(S)
Micrococcus luteus	0(R)	30(S)	0(R)	26(S)	23(S)	30(S)	27(S)	13(R)
Susceptibility (%)	5.3	87	89	81	68	53	76	73
Resistance (%)	94.7	13	11	19	32	47	24	27

Key:
S: Susceptible R: Resistant
I: Intermediate

Table 4: Antibiotic Susceptibility test for Gram negative bacteria from air samples in eateries in Ado-Ekiti

Organisms	Ceftazidime (30ug)	Cefuroxime (30ug)	Gentamicin (10ug)	Ciprofloxacin (5ug)	Ofloxacin (5ug)	Amoxycillin/ Clavulanate (30ug)	Nitrofurantoin (300ug)	Ampicillin (10ug)
Neisseria subflava	27(S)	21(S)	20(S)	26(S)	22(S)	24(S)	0(R)	21(S)

Microvirga massiliensis	0(R)	16(I)	25(S)	25(S)	26(S)	18(S)	23(S)	0(R)
Acinetobacter parvus	29(S)	0(R)	29(S)	23(S)	21(S)	21(S)	29(S)	28(S)
Stenotrophomonas maltophilia	0(R)	22(S)	25(S)	24(S)	26(S)	25(S)	21(S)	0(R)
Pseudomonas oryzihabitans	34(S)	36(S)	31(S)	34(S)	24(S)	38(S)	22(S)	22(S)
Necropsobacter rosorum	30(S)	33(S)	0(R)	0(R)	0(R)	32(S)	33(S)	23(R)
Pseudomonas aeruginosa	26(S)	28(S)	27(S)	29(S)	29(S)	35(S)	26(S)	27(S)
Microvirga massiliensis	0(R)	19(S)	23(S)	27(S)	24(S)	27(S)	20(S)	14(I)
Neisseria meningitides	17(I)	27(S)	20(S)	24(R)	25(S)	29(S)	27(S)	28(S)
Acinetobacter ursingii	28(S)	30(S)	23(S)	17(I)	16(I)	20(S)	24(S)	0(R)
Stenotrophomonas maltophilia	0(R)	0(R)	19(S)	18(S)	21(S)	0(R)	0(R)	0(R)
Pseudomonas stutzeri	0(R)	0(R)	17(I)	20(S)	19(S)	0(R)	18(S)	0(R)
Necropsobacter rosorum	23(S)	20(S)	21(S)	23(S)	23(S)	24(S)	0(R)	22(S)
Pseudomonas aeruginosa	0(R)	21(S)	27(S)	24(S)	28(S)	26(S)	24(S)	17(I)
Stenotrophomonas maltophilia	16(I)	25(S)	26(S)	27(S)	27(S)	27(S)	23(S)	24(S)
Pseudomonas oryzihabitans	19(S)	24(S)	24(S)	23(S)	20(S)	18(S)	20(S)	16(I)
Microvirga massiliensis	20(S)	21(S)	24(S)	22(S)	23(S)	29(S)	24(S)	26(S)
Asaia bogorensis	25(S)	23(S)	28(S)	28(S)	26(S)	27(S)	28(S)	27(S)
Pseudomonas stutzeri	0(R)	0(R)	19(S)	19(S)	21(S)	10(R)	19(S)	0(R)
Bordetella trematum	25(S)	29(S)	23(S)	25(S)	24(S)	33(S)	23(S)	23(S)
Advenella incenata	21(S)	14(R)	22(S)	22(S)	22(S)	11(R)	26(S)	20(S)
Neisseria polysaccharea	21(S)	22(S)	18(S)	20(S)	21(S)	30(S)	26(S)	20(S)
Acinetobacter ursingii	30(S)	31(S)	29(S)	27(S)	28(S)	30(S)	33(S)	25(S)
Moraxella lacunata	29(S)	21(S)	17(I)	26(S)	25(S)	32(S)	26(S)	29(S)
Susceptibility (%)	66	77	92	88	48	83	88	65
Resistance (%)	34	23	8	12	52	17	12	35

Key:
S: Susceptible
R: Resistant
I: Intermediate

Table 5 shows the morphological characteristics of the fungal isolates and included the following: *Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Penicillium chrysogenum and Cladosporium spp*. Isolate a1 from Sample PF had white mycelia with black sporangia, branched hyphae which was non-septate. The probable organism was *Rhizopus stolonifer*. Isolate b2 from Sample TA had grey-green conidia with a narrow white border. The hyphae were septate with smooth conidiophores. The probable organism was *Apergillus fumigatus*. Isolate f1 from Sample TB had white septate hyphae developing with yellowish-green conidia formation which was radially arranged. The probable organism was *Aspergillus flavus*.

Table 6 shows the frequency of occurrence of the fungal isolates. *Rhizopus stolonifer* had the highest frequency of 25%. *Aspergillus niger, Aspergillus*

flavus, Penicillium chrysogenum and *Aspergillus fumigatus* had frequencies of occurrence of 17% while *Cladosporium* spp had the lowest frequency of occurrence of 8%.

Table 5: Morphological characteristics of fungal isolates from air samples in eateries in Ado-Ekiti

Sample Code	Description	Probable organism
SamplePF		
a1	Non-septate branching hypae with a white mycelia and black sporangia.	*Rhizopus stolonifer*
a2	White hyphae developing with black conidia with septate hyphae.	*Aspergillus niger*
SampleTA		
b1	Non-septate hyphae with a white mycelia and black sporangia.	*Rhizopus stolonifer*
b2	Grey-green conidia with a narrow white border, septate hyphae with smooth conidiophores.	*Aspergillus fumigatus*
SampleDC		
c1	White hyphae developing with yellowish green conidia formation arranged radially with septate hyphae.	*Aspergillus flavus*
c2	Blue-green conidia, dry chains of spores from brush-shaped conidiophores.	*Penicillium chrysogenum*
SampleCG		
d1	Grey-green conidia with a narrow white border, septate hyphae with smooth conidiophores.	*Aspergillus fumigatus*
SampleCE		
e1	White hyphae developing with black conidia with septate hyphae.	*Aspergillus niger*
e2	Blue-green conidia, dry chains of spores from brush-shaped conidiophores.	*Penicillium chrysogenum*
SampleTB		
f1	White hyphae developing with yellowish green conidia formation arranged radially with septate hyphae.	*Aspergillus flavus*
SampleTD		
g1	Non-septate hyphae with a white mycelia and black sporangia.	*Rhizopus stolonifer*
g2	Brown colonies with dark pigmented conidia.	*Cladosporium* spp

Key:
Sample PF: Isolates from Portofino
Sample TA: Isolates from Tantalizers
Sample DC: Isolates from Danke Fast Foods
Sample TB: Isolates from Take Away
Sample CG: Isolates from Chicken Republic
Sample CE: Isolates from captain Cook
SampleTD: Isolates from Tasty and Spicy

Table 6: Frequency of occurrence of fungal isolates from air samples in Ado-Ekiti

S/N	Identified isolates	No of times occurred	Frequency of occurrence (%)
1	*Rhizopus stolonifer*	3	25
2	*Aspergillus niger*	2	17
3	*Aspergillus flavus*	2	17
4	*Penicillium chrsogenum*	2	17
5	*Aspergillus fumigatus*	2	17
6	*Cladosporium* spp	1	8

4. Discussions

Since bacteria are ubiquitous, they are present in air and able to bring about its contamination. In this present study, results showed that air around eateries was contaminated with microorganisms. Microbial contamination of air around eateries has been reported by several researchers (Jensen and Schafer, 1998; Ahmed *et al.,* 2000; Jamriska, 2000; Gorny, 2004; Fleischer, 2006; Fracchia *et al.,* 2006; Dhanasekaran *et al.,* 2009; Howard *et al.,* 2013). *Acinetobacter sp., Bacillus sp., Stphylococcus and Micrococcus* were isolated from indoor air in these eateries and agrees with the studies carried out by Wanner *et al*, (1993); Ahmed *et al.,* (2000); Künzli *et al.,* (2000); La Serna *et al.,* (2002); Gorny, (2004); Fleischer, (2006); Fracchia *et al*, (2006) and Dhanasekaran *et al.,* (2009). *Cladosporium* spp and *Penicillium* spp. were isolated from this study and agrees with the work carried out

by Samet and Spengler, (2003). This study shows the presence of *Aspergillus niger* and *Aspergillus flavus* from four eateries and poses a health risk. The difference in the number of customers that visited these restaurants reflected in the microbial load of the individual eateries with the eateries with higher influx of people having higher microbial concentrations than those with lesser number of people.

Antibacterial activity of the antibiotics showed that almost all the organisms tested were susceptible to Ceftazidime, Cefuroxime, Gentamicin, Ceftriaxone, Cloxicillin, Ofloxacin and Amoxycillin/clavulanate for the Gram positive isolates while the Gram negative isolates were susceptible to Cefuroxime, Gentamicin, Ciprofloxacin, Oflovacin, Amoxycillin/ Clavulanate, Nitrofurantoin and Ampicillin. The antibiotics exhibited more antibacterial effect on *Pseudomonas oryzihabitans, Acinetobacter ursingii, Bordetella trematum, Pseudomonas stutzeri, Necropsobacter rosorum.* In this study, the antibiotics had more effects on *Acinetobacter ursingii*, *Stenotrophomonas maltophilia* and *Micrococcus luteus* and these observations contrast with those of Marmot *et al.* (2006) and Maron (2005). The disparity observed might be attributed to differences in geographical location and environmental factors like climate, sanitary practices and quality of staff and visitors to the eateries.

5. Conclusion

Air pollution poses a major threat to health and climate. Bacteria and fungi isolated from air within these eateries showed their presence could have health implication. Some of them were found to be susceptible to some antibiotics

6. Recommendation

Promoting awareness among the public, health care professionals and government officials about the relationship between air pollution and health should be encouraged and information of ways by which indoor air pollution can be reduced should be disseminated.

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