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The Pattern of Environmental Conditions and Genomic Typing of Airborne Bacteria and Fungi in selected Farm Settlements in Ogun State, Nigeria

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Abstract

Low attention has been given to environmental health and the impacts of agricultural farming activities on the microbial quality of the surrounding air. This study assessed the bacteria and fungi in the indoor and outdoor air of seven farm settlements in Ogun State. The study used the settle-plate technique characterized by phenotypic and genomic methods through the isolation of genomic DNA, PCR amplification and sequencing of 16S rRNA and ITS genes. The nucleotide sequences of the isolates were assembled, aligned, and compared with the GenBank database at NCBI using the BLAST search tool. Results showed a total of 39 distinct bacteria colonies with 29 (74.4%) gram-positive and 10 (25.6%) gramnegative and 17 fungal isolates belonging to different genera and species. The indoor air of Ikenne had the most diverse bacteria in the morning (21) and evening (22), while the outdoor of Ado-Odo displayed the highest number of diverse bacteria both in the morning (21) and evening (19) during the wet season. However, during the dry season, the indoor air of Ibiade and Ado-Odo had the most diverse bacteria in the morning (16) and evening (18), respectively while the outdoor air revealed highest in Ado-Odo (17) in the morning and at Ado-Odo and Ikenne (13) in the evening. Farm settlements may be a potential source of pathogenic organisms to communities, especially where personal hygiene and sanitation are not adequately maintained. This study recommends that environmental conditions of farm settlements should be given utmost attention to prevent the proliferation of pathogenic organisms and outbreak of diseases.

Le modèle des conditions environnementales et le typage génomique des bactéries et champignons dans des établissements agricoles sélectionnés dans l'état d'Ogun au Nigeria

Résumé

Peu d'attention a été accordée à la santé environnementale et aux impacts des activités agricoles sur la qualité microbienne de l'air ambiant. Cette étude a évalué les bactéries et les champignons dans l'air intérieur et extérieur de sept établissements agricoles de l'État d'Ogun à l'aide de la technique de la plaque de sédimentation et caractérisés par des méthodes phénotypiques et génomiques grâce à l'isolement de l'ADN génomique, à

© African Journal of Environmental Health Sciences Volume 9, November, 2022 l'amplification par PCR et au séquençage de l'ARNr 16S et des gènes ITS. Les séquences nucléotidiques des isolats ont été assemblées, alignées et comparées avec la base de données GenBank du NCBI à l'aide de l'outil de recherche BLAST. Les résultats ont montré un total de 39 colonies de bactéries distinctes avec 29 (74,4%) gram-positifs et 10 (25,6%) gramnégatifs et 17 isolats fongiques appartenant à différents genres et espèces. L'air intérieur d'Ikenne avait les bactéries les plus diverses le matin (21) et le soir (22), tandis que l'air extérieur d'Ado-Odo affichait le plus grand nombre de bactéries diverses le matin (21) et le soir (19) pendant la saison des pluies. Cependant, l'air intérieur d'Ibiade et d'Ado-Odo avait les bactéries les plus diverses le matin (16) et le soir (18), respectivement tandis que l'air extérieur était le plus élevé à Ado-Odo (17) le matin et à Ado-Odo et Ikenne (13) le soir. Les établissements agricoles peuvent être une source potentielle d'organismes pathogènes pour les communautés, en particulier là où l'hygiène personnelle et l'assainissement ne sont pas correctement entretenus. Cette étude recommande d'accorder la plus grande attention aux conditions environnementales des établissements agricoles afin de prévenir la prolifération d'organismes pathogènes et l'apparition de maladies.

Introduction

Human activities in the environment contribute to airborne microbial ecology (Adams *et al.*, 2015). Numerous studies have established that the plethora of activities before and during the habitation in a facility and the prevailing environment serve as the factors that determine the quality and quantity of gaseous pollutants, aerosol, and bioaerosol concentrates found in such spaces (Fabian *et al.*, 2005; Chen and Hildemann, 2009; Shiaka and Yakubu, 2013; Hoseinzadeh *et al.*, 2013; Pearson *et al.*, 2015).

The significant public health concern from the burden of bio-aerosol discharged from animal buildings play critical roles in respiratory disorders in individuals living around animal undertakings (Seedorf, 2004), and could impact the air of the surrounding environment (Schulz *et al.*, 2005). Dungan (2010) and Samadi *et al.* (2013) studied various agricultural activities, particularly animal husbandry, with specific reference to atmospheric pollutants, including transport of micro-organisms through the air, and affirmed related hazards to residents around domesticated animal buildings for pigs, cattle, and poultry (Eisenberg *et al.* (2010); Sowiak *et al.* (2012); Brodka *et al.* (2012). The investigation by Manninen *et al.* (2014) reported the direct connection between airborne dust and different biological particles, particularly Particulate Matter (PM).

Most environmentally-related research in agricultural settings, mainly where the significant activity is crop production, has concentrated on soil fertility, the impacts of fertilizer utilization on soil properties, and hydrology (surface and underground), including the economic benefits of crop yield. Public attention and spotlight are on the noticeable indications of the farming impact. In contrast, air contamination's more subtle or less obvious effects may introduce the worst economic loss (Pretty et al., 2001). Micro-organisms and endotoxins related to particles are aerial pollutants in livestock buildings that have been connected with several ailments (respiratory system diseases, and allergic airway responses in susceptible groups (Jaber, 2002). They are assumed to represent a hazard for the healthy farmers and labourers in the farms and the neighbouring local locations around intensive domesticated animal structures (Grimm and Eckhof, 2002).

Bio-aerosol pollutants in restricted animal houses are detrimental to the respiratory health of animals kept in these facilities. Opportunistic microbial pathogens may directly cause infectious and allergic diseases in farm animals. Chronic

exposure to some types of aerial pollutants may exacerbate multi-factorial environmental disorders. There are few international surveys paying attention to the health of farmers, their support staff, and the spread of pathogens from farm buildings. Hamid *et al.* (2018) reported Organic Dust Toxic Syndrome (ODTS), asthma, and other respiratory infections such as coughing, sputum, and wheezing in farmers and farm workers. Besides, research evidence revealed that various pathogens can survive in ambient air for several minutes and may travel over long distances, for example, foot-and-mouth disease (FMD) virus for more than 50 km, and *Staphylococcus* up to 500 m) (Fernandez *et al.*, 2019).

Bio-aerosols range within aerodynamic diameters of 0.5 to 100 μ m in size, affecting living things through infectivity, allergenicity, toxicity, pharmacological or other processes (Theisinger & de Smidt, 2017). Elevated exposure to fungi found in animal production facilities presents an increased hazard for work-related respiratory diseases. Sabino *et al.* (2012) detected *Aspergillus* species in Portuguese swine and poultry farms; furthermore, they affirmed that these organisms induced hypersensitive reactions in exposed humans. In the investigation of Nowak (1998), including 1,861 farmers in northern Germany, around 22% of the pig farmers, 17% of the steers ranchers, and 13% of the poultry farmers were

admitted to have airway-related health problems. Also, the indoor and outdoor studies of farm settlements by Oyebanji *et al.* (2019a,b) have shown the very high concentrations of PM and bioaerosol load. Hence, this study was aimed at (i) assessing the building and environmental conditions influencing the bioaerosol load, and (ii) determining seasonal variations in the quality of bacteria and fungi present indoor and outdoor of selected sites in the farm settlements. The knowledge will bridge the gap between agricultural activities, diversity, and seasonal variations in bio-aerosol ecology. It would help prevent specific allergic responses caused by these organisms and experienced by farmers.

Materials and Methods

Sampling sites

Samples were collected from 390 sites (195 indoor and 195 outdoor) across the seven farm settlements within Ogun State, southwest Nigeria (Fig. 1). Ogun is one of the states in Southwestern Nigeria, created in 1976. The 2006 evaluation recorded a populace of 3,751,140 inhabitants containing 1,864,907 males and 1,886,233 females with a land territory of 16,409.26 km². The State is endowed with a favourable climate and great vegetation to cultivate cash and food crops and raise livestock (OGSG, 2017).

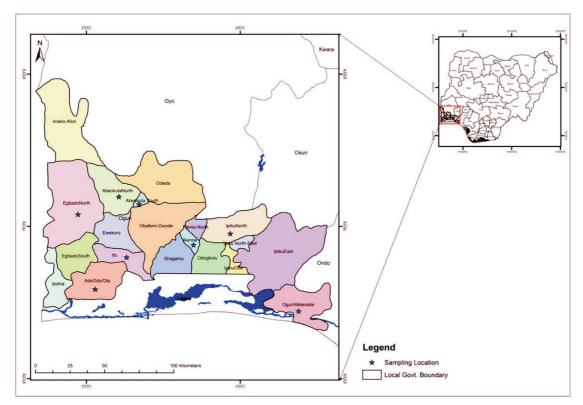


Figure 1: Map of Ogun State showing locations of selected farm settlements.

Sampling procedure

Walkthrough inspection

This study carried out a walkthrough and on-site physical observation of the selected households using an observational checklist. The items observed included general aesthetics (wall, building, and floor concreting), the storage systems for farm produce, mould growth, wall dampness, presence and adequacy of ventilation, animal pets, and occupants' population and housekeeping. The checklists were analysed using the scoring system for each section.

Bio-aerosol sampling

Samples were collected at each of the selected indoor and outdoor sites once within three months window based on two factors: season (December – February for the dry, and May – July for the wet season) and time of the day (morning: 8 am - 10

am, evening: 5 pm - 7 pm). The isolation of bacteria and fungi from aerosol samples was performed on nutrient agar and potato dextrose agar, respectively, using the Settle plate technique described by Stryjakowska-Sekulska *et al.* (2007); Mayowa *et al.* (2015).

The Petri dishes containing solidified nutrient agar (NA) and potato dextrose agar (PDA) were exposed at each sampling site for 30 minutes for gravitational settling/collection (sedimentation /open plate) of bio-aerosol at the height of 1 m from the floor and at a distance of about 1m from the wall or any object as described by Napoli et al. (2012). After exposure, the Petri dishes and the control plates (plates which were not exposed) were incubated at 37°C for 18-24 hrs for bacteria and 27°C for 48-72 hrs for fungi (Wemedo et al., 2012) to allow proper growth of the bacterial and fungal colonies for identification. The number of colonies is counted and recorded. Pure bacterial isolates was obtained by a series of sub-culturing on nutrient agar plates incubated at 37 °C for 24

hrs. In contrast, those fungi were obtained on potato dextrose agar plates incubated at 25 ± 2 °C. All pure bacteria and fungi isolates were maintained on nutrient agar and potato dextrose agar slants, respectively, and stored at 4°C.

Phenotypic identification of bacterial and fungal isolates

Bacterial isolates were characterised by observing their colonial characteristics (size, shape, pigmentation, texture, elevation, margin), Gram staining, motility, and were subjected series of biochemical tests using Microbact 24 E Biochemical test kit (Oxoid, UK) following the manufacturer's instructions. Fungal isolates were identified based on colonial characteristics (surface appearance, texture, and colour of the colonies from upper and lower sides) and microscopic features. The morphology of spores and mycelia of the fungal isolates were examined under the microscope at \times 40 magnifications after staining with lactophenol cotton blue. The authors compared the characteristics with mycological identification keys and taxonomic descriptions already documented in the literature (Watanabe, 2002).

Molecular characterisation of bacterial and fungal colonies

The molecular characterisation of the bacteria and fungal isolated was done using the gene sequencing method. Genomic DNA of the bacteria and fungal isolate was extracted using single colonies of 24- hr old culture of bacteria, and 72hr old fungi by picking and suspended in PBS (Phosphate Buffer Saline). The suspending colony was applied into a Whatman FTA cards as described by Borman et al. (2008) and allowed to dry for two hours at room temperature. The DNA was amplified with the PCR assay using primers targeting the V1 – V3 regions of the prokaryotic 16S rRNA gene described by Schoch et al. (2012) for bacteria and ITS for fungi. The primers used were universal 16S primers, forward primer 27F and reverse primer 907R (Torok, 2008). The sequences of these primers are 5'-AGAGTTTGATCMTGGCTCAG-3' and 5'-CCGTCAATTCMTTTRAGTTT-3' ("16S Ribosomal DNA"). In addition, using a set of forwarding primers, ITS1F (5'-TCCGTAGGTGAACCTGCGG-3') and reverse primers ITS4R (5'-TCCTCCGCTTATTGATATGC-3') (Gautam and Bhadauria, 2012; Schoch et al., (2012) for Fungi. DNA purification, amplification, and sequencing were done at the STAB Vida, Monte de Caparica, 2825-182 Caparica, Portugal. The resulting DNA sequences were edited and aligned. Genetic similarity searches were performed by aligning the sequences using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) to identify the genus and species of the isolates.

Phylogenetic analysis

The phylogenetic relationship among the identified bacteria and fungi was determined using Molecular Evaluation and Genetic Analysis (MEGA 6) software (Tamura *et al.*, 2013). The phylogenetic tree was inferred using the neighbour-joining method, while the reliability of the inferred phylogenetic tree was evaluated using bootstrap analysis of 1000 replicates.

Results

Environmental conditions

Table 1 shows the on-site indoor environment characteristics and the scores for each location based on the average individual household sanitation and hygiene. The highest score, 60%, was measured at Coker, while the least 31.11% was at Ikenne based on the checklist. The order is Ikenne < Ago-Iwoye < Ibiade < Sawonjo < Ado-Odo < Ajegunle < Coker. The embedded pictures also buttress the scores allocated. The residences are highly characterised by many structural defects, including broken walls, un-concreted floors, poor

ventilation, overcrowding, and unhygienic domestication of animal pets. Concerning the availability of farm structures and general housing conditions, the scores followed the order Ajegunle>Ibiade=Sawonjo>Ago-Iwoye>Ado-Odo=Ikenne. Ago-Iwoye, Ado-Odo, and Ikenne.

However, concerning the availability and functionality of other environmental, hygiene, and sanitation facilities, the order is Ikenne < Coker = Ajegunle = Ado-Odo < Ago-Iwoye < Sawonjo < Ibiade.

Phenotypic characteristics of isolated bacteria and fungi and distribution across the farm settlements

Table 2 shows the morphological and biochemical characteristics of the bacteria isolated from indoor and outdoor environments of farm settlements in Ogun State. Thirty-nine (39) bacterial isolates were obtained, of which 29 (74.4%) were Grampositive, and 10 (25.6%) were Gram-negative. The majority (59%) of the bacteria were non-motile. Only 10 (25.6%) are oxidase-positive, and 10 (25.6%) produced H₂S. About 67% of the

isolates were rod-shaped, belonging to the *Bacillus* group, 11 (28.2%) belonged to the *Cocci* group, and 2 (5.13%) belonged to *Coccobacilli* group. Table 3 shows the colonial and microscopic features of fungi isolated from farm settlements' indoor and outdoor environments. A total of 17 fungal isolates belonging to different genera and species were obtained. The isolated fungi were predominantly with irregular forms, rough edges, dry surface, and raised. Additionally, there was various front and reverse colours with varieties of pigmentation.

Table 4 shows the distribution of isolated bacteria across locations, sites, and monitoring time during the wet season. It was discovered that the indoor air of Ikenne during the evening had the most diverse bacteria with 22 colonies (5.73% of 384), while the outdoor atmosphere of Ibiade during the evening displayed the least diversity with six different bacterial colonies (1.56% of 384). Also, isolate code ten showed the highest relative abundance occurring 19 times (4.92%), while isolate code 14 displayed a minor relative abundance of 4 times (1.04%).

 Table 1: Characteristics and score for walkthrough inspection

Indicators	Ado-odo	Ikenne	Ago-Iwoye	Sawonjo	Ibiade	Ajegunle	Coker
Unconcreted floors/walls	HP	HP	MP	MP	MP	MP	ABS
Indoor storage of farm materials and produce	e MP	HP	MP	MP	HP	MP	ABS
Visible mould growth and spider webs	MP	HP	MP	MP	MP	ABS	ABS
Moist air	MP	HP	MP	MP	MP	MP	ABS
Damp walls	MP	MP	MP	MP	MP	ABS	ABS
Indoor animal pets	MP	MP	MP	MP	MP	MP	MP
Overcrowding	MP	MP	ABS	MP	MP	ABS	ABS
Electricity supply	ABS	MP	ABS	ABS	MP	ABS	ABS
Window provision	MP	MP	HP	HP	MP	MP	HP
Cross/through ventilation	MP	MP	HP	HP	MP	MP	HP
Score (%)	53.33	31.11	48.88	51.11	48.89	57.78	60

HP (Highly present); MP (Moderately present); ABS (Absent)

Code	Gram	Shape	Motility	H_2S	Oxidase	Query Genera	Microbact Code	Percentage Similarity	Organism
-	+	Slender short rods	+			Bacillus spp			
7		Slender short rods with polar ends	+	+		Bacillus spp	7552622	80.64%	S. arizonae subsp.3A
б	ı	Rods	+	+		Bacillus spp	67436327	57.58%	S. liquefaciens complex
4	ı	Rods	+	ı	+	Bacillus spp	57773364	83.27%	E. agglomerans complex
5	+	Rods in clustered spiral form		ı		Spirillia spp			
9	+	Rods in chains	+	ı	+	Streptobacilli spp			
7		Rod	+	+		Bacillus spp	75674767	64.88%	S. odorifera biogp 1
8	+	Cocci in chains and twos	ı			Diplococci/Staphylococci spp			
6	+	Cocci in clusters, twos and single	ı	,	,	Cocci/Diplococci/Staphylococci spp			
10		Rods	+		,	Bacillus spp	7552622	80.64%	S. arizonae subsp.3A
11	+	Cocci	ı	+	+	Coccei spp			
12	+	Cocci	ı	ı	,	Cocci spp			
13	+	Rods in chains		+		Streptobacilli spp			
14	+	Rod	+	+	+	Bacillus spp			
15	+	Cocci in clusters and twos	ı	,	,	Diplococci/Staphylococci spp			
16	+	Cocci in clusters	ı	ı	ı	Staphyloccoci spp			
17	ı	Rods	ı	ı	ı	Bacillus spp	7077256	97.79%	E. cloacae
18	+	Cocci	+	ı	+	Cocci spp			
19	+	Rods	+	+	+	Bacillus spp			
20	ī	Rods	+	+	+	Bacillus spp	7757636	40.49%	E.gergoviae
21	+	Short rods	1	ı		Bacillus spp			
22	+	Cocci in clusters	+		ı	Staphyloccoci spp			
23	ı	Short rods	ı	+	ı	Bacillus spp	5777376	99.89%	K.oxytoca
24	+	Coccobacillus	ı	,	,	Coccobacillus spp			
25	+	Rods	ı	ı	ı	Bacillus spp			
26	+	Cocci in cluster	ı	ı	ı	Staphyloccoci spp			
27	·	Short rods				Bacillus spp	77630567	99.96%	E.coli
28	+	Rods	ı	ı	ı	Bacillus spp			
29	+	Short rods	+	ı	,	Bacillus spp			
30	+	Short rods	ı	ı	+	Bacillus spp			
31	+	Rods	ı	,		Bacillus spp			
32	+	Cocci		ı		Cocci spp			
33	+	Cocci				Cocci spp			
34	+	Short rods				Bacillus spp			
35	+	Coccobacillus	+		ı	Coccobacillus			
36		Short rods		ı	+	Bacillus spp	55776220	98.06	E.agglomerans complex
37	+	Short rods	+			Bacillus spp			
38	+	Short rods	+	+		Bacillus spp			
39	+	Short rods			+	Bacillus spp			

Table 2: Biochemical status of isolated bacteria

Key: + organism present, - organism absent

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		Iable J. Ollalactelisitos of isolated fullgal colorites	0			
S/N	S/N Form	Colour	Edges	Surface	Elevation	Reverse side
	Irregular	yellow with greenish pigment	Rough	dry	raised	yellow
7	Round	orange with greenish pigment	Rough	dry, hairy	raised	orange
З	Circular	Black	Rough	dry, wool-like	raised	white, black patches
4	Irregular	white, white wool-like pigment	Smooth	wet	flat	white
5	Irregular	Green	Rough	dry, filamentous	slightly raised	greenish yellow
9	Circular	dark green		dry	flat	green
٢	Irregular	Pink	Rough	dry	raised	greenish yellow
8	Irregular	light grey suede	Rough	dry	raised	dark
6	Irregular	light brown deep brown patches	Rough	dry	flat	golden yellow
10	Irregular	chocolate brown	Smooth	dry, filamentous	raised	cream
11	Irregular	brown suede-like	Rough	dry	flat	brown with white
12	Irregular	light suede-like	Smooth	dry, wool-like strands	flat	light brown with white patches
13	Irregular	suede like lemon	non-filamentous	dry	raised	cream
14	Irregular	suede like blackish brown	Smooth	dry, filamentous	raised	cream
15	Irregular	chalky white	Smooth	dry	flat	brown
16	Irregular	black with greenish pigment	Rough	dry	raised	yellow
17	Circular	whitish light green dots	Smooth	dry	raised	pink

Table 3: Characteristics of isolated fungal colonies

Code	Indoor M E	or E	Ado-odo Outdoor M E	E E	Indoor M E		Ikenne Outdoor M E	00r	Indoor M	Ago-iwoye Out E M	op		Indoor M E	awoi	njo Outdoor M E		loor E	Ibiade Outdoor M F	00r E	Indoor M]	Ajegı	oop		Indoor M E	Coker Ou M	ker Outdoor M E	Total
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	+	+	+	,	,	+	,	+					, ,	'	+	'	'	,	,	,	+	+		+	+	,	10
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61	+	,	,	+	+	,	,		+	+		+	+	'	'	+	+		+	+	+			+	'	,	14
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22	,	+	,	+	+	,	+	,	+		+	+	'	'	'	+	'	,	,	,	+	+	+	'	'	+	12
23	+	,	,	,	+	,	+	+	+		+			'	'	'					+			•	'		7
24	,	+	+	,	+	+	,	+	+	,	+		+		+	'	,	,	,	,	+			+	'	,	11
25	,	,	,	,	,	,	+	+	,	+	,		+		'	'	,	,	,	,	,			+	+	,	9
26	,	,			,	+	+			+		+	+	•	+	'	'	+	,					'	'	'	7
27	,	+	+	,	,	,	,	,	+	,			++	+	'	+	,	+	,	,	,			' +	'	,	6
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30	+	+	+	+	+	,	+	+	,	,	+	+	'	'	'	+	+	,	+	,	+			'	'	,	13
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32	,	,	,	,	+	+	,	,	,	+	,		' +	'	+	'	+	,	,	+	+	+	+	+	'	,	12
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36	+	,	,	+	+	,		+			+		'	'	'	+	•	+	,			+		'	'	,	8
37	+	+	+	+	+	+	,	,	,	,	,		'	'	'	+	,	+	,	,	,			'	'	,	~
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Table 5 shows the distribution of isolated bacteria across locations, sites, and monitoring time during the dry season. It was discovered that the indoor air of Ado-Odo during the evening had the most diverse bacteria with 18 (6% of 300) different bacteria, while the outdoor atmosphere of Ibiade during the evening displayed the least diversity of 5 colonies (1.67% of 300). Also, isolate code displayed the highest relative abundance occurring 13 times (4.30% of 302), while isolate code 14 showed the most negligible relative abundance of 4 times (1.33% of 302).

Table 6 shows the distribution of isolated fungal colonies during the wet season. It can be deduced that isolate code 8 had the highest relative abundance occurring 19 times across the sites and settlements. In comparison, isolate code 12 recorded the least abundance occurring only four times. Similarly, the indoor air of Ikenne during the

evening period displayed the most diverse fungal isolates having a total of 12 of the 17 recorded in this study, while the outdoor of Coker and Sawonjo during the evening period displayed the least diversity of 4.

Table 7 shows the distribution of isolated fungal colonies during the dry season. It can be deduced that isolate code 8 had the highest relative abundance occurring 13 times across the site and settlements. In comparison, code 11 recorded the least abundance occurring only two times each. Similarly, the indoor air of Ikenne and Ibiade during the evening period, indoor of Ibiade in the morning, and Ibiade outdoor in the evening displayed the most diverse fungal isolates having a total of 7 of the 17 recorded in this study. The indoor air during the evening the evening displayed the most diverse fungal isolates having a total of 7 of the 17 recorded in this study. The indoor air during the evening the evening displayed the least diversity of 2 of the total 17 isolated fungi.

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Oyebanji, *et al.*: The Pattern of Environmental Conditions and Genomic Typing of Airborne Bacteria and Fungi in selected Farm Settlements in Ogun State, Nigeria

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Molecular characteristics of isolated bacteria and fungi

The results of molecular characterisation of the bacterial isolates by 16S rRNA gene sequencing are presented in Table 8. The sequences of the bacterial isolates showed a percentage identity between 83% to 99%. However, it was observed that isolate code 10 was similar to three different *Enterobacter species*, namely *Enterobacter cloacae*, *Enterobacter cancerogenous*, and *Enterobacter ludwigii*, having the same maximum score, total score, query cover, E value, and identity percentage. Similarly, isolates codes 4 and 7 with an identity percentage of 94 and 99 are the same organisms.

Table 9 displayed the features of the five (5) successfully sequenced fungal species. The list of fungal species identified percentage identity, and the accession numbers are presented

corresponding to the blast results with a percentage identity range between 92% and 99%. It was, however, observed that isolate codes 6 and 8 were identified to be from the genus *Aspergillus*.

Phylogenetic tree relationship among identified bacteria and fungi

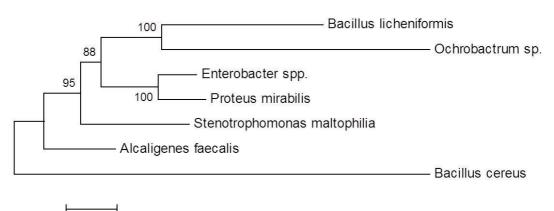
The phylogenetic relationship among the identified bacteria is shown in Figure 2. Five clusters were formed with *Bacillus licheniformis* and *Ochrobactrum* spp, forming a sister taxon, while *Enterobacter* spp. and *Proteus mirabilis* included another sister taxa. The phylogenetic relationship among the identified fungi is shown in Figure 3. Two clades were formed with *Aspergillus tamari* and *Aspergillus orycae* forming sister taxa, while *Trichoderma longibrachrum* formed the ancestral lineage.

Table 8: Genomic features of isolated bacte	ria
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S/N	Code	Name	Maximum score	Total score	Query cover %	E value	Identity %	Accession no.
1	6	Bacillus cereus	1506	1506	77	0.0	93	MG892776.1
2	20	Alcaligenes faecalis	1703	1703	83	0.0	94	KU937382.1
3	14	Bacillus licheniformis	1437	1437	97	0.0	98	MG892780.1
4	19	Bacillus licheniformis	1576	1576	86	0.0	95	KX553847.1
5	2	Stenotrophomonas maltophilia	1816	1816	82	0.0	97	KR149597.1
6	3	Ochrobactrum sp.	909	909	71	0.0	83	EU668002.1
7	4	Proteus mirabilis	1716	1716	84	0.0	94	KY027141.1
8	10	Enterobacter cloacae	1847	1847	83	0.0	98	MG274270.1
		Enterobacter cancerogenous						KM502221.1
		Enterobacter ludwigii						KF835774.1
9	7	Proteus mirabilis	1929	1929	85	0.0	99	MK430917.1

		ated fungi

S/N	Code	Name	Maximum Score	Total score	Query cover %	E value	Identity %	Accession no
1	6	Aspergillus tamari	1733	1733	98	0.0	99	JX110981.1
2	8	Aspergillus oryzae	1192	1192	97	0.0	99	KJ650340.1
3.	15	Trichoderma longibrachiatum	774	774	44	0.0	92	KM100791.1
4.	16	Gibberella moniliformis	1663	1663	86	0.0	98	GU480956.1
5.	22	Corynespora cassiicola	1206	1206	67	0.0	98	MG865981.1



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Figure 2: Phylogenetic tree among the bacteria identified

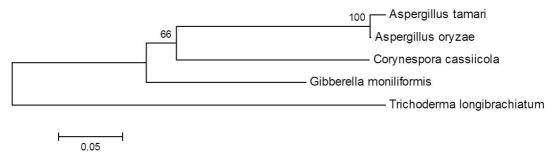


Figure 3: Phylogenetic relationship among the identified fungi species

Discussion

Environmental conditions are critical in determining the quality of indoor and outdoor air. This study revealed that across the farm settlements, there was generally poor natural ventilation which is a risk factor that affects the concentration of indoor pollutants, affecting both the health of building and occupants (Graudenz *et al.*, 2005; Chuaybamroong *et al.*, 2008; McCarthy *et al.*, 2000; Ayanbimpe *et al.*, 2010). Leaky roof results in high moisture content and is characterised by a high percentage of humidity identified as significant risk factors for the increased population of indoor bacteria and fungi (Oyebanji *et al.*, 2019) and sick building syndrome (Burge, 2004). Indoor dampness is also associated with asthma exacerbation and other associated respiratory disorders (Fisk *et al.*, 2007; Sahakian *et al.*, 2008; Mendell *et al.*, 2011).

The key determining factors for the overall behaviour of air pollutants in terms of stability and dispersion, according to Camuffo (2014), are indoor temperature and humidity levels. The observational checklist also corroborates the observations summarised in the inventory and trend reported by (CCOHS 2009). Poor housekeeping can result in accidental ingestion of airborne dust (Greenberg, 2003) due to indoor storage of farm produce which allows accumulation of dust particles and an increase in indoor PM concentration. Similarly, Majd *et al.* (2019) highlighted that the physical conditions of mid-Atlantic schools contributed to the indoor air quality of the schools. Such a condition provides a conducive and thriving

atmosphere for both pest infestation and the spread of infections.

Bacillus cereus (one of the identified bacteria) is widespread in decaying organic matter, fresh and marine waters, vegetables, and fomites and is primarily related to soil and growing plants. The bacteria grow in the intestinal tract of insects and mammals as favourable habitats (Stenfors-Arnesen et al., 2008). To support this, Cui et al. (2016) reported the prevalence of B. cereus isolated in bedding, faeces, feed, liquid manure, and in raw milk was 93.3%, 78.9%, 41.2%, 100.0%, and 9.8%, respectively. This adaptability makes it spread easily to foods causing various foodassociated illnesses, diarrhea, and B. cereus been a Gram-positive, aerobic-to-facultative, sporeforming rod widely distributed environmentally. There were close phenotypic and genetic (16S rRNA) semblance and relationships with several other Bacillus species, especially Bacillus anthracis (Bottone, 2010). B.anthracis causes anthrax, a rapidly lethal infectious disease, primarily herbivores, but all mammals are susceptible (Mock and Fouet, 2001).

Furthermore, in terms of virulence, there are similar determinants with *Bacillus thuringiensis* and *Bacillus anthracis* (Stenfors-Arnesen *et al.*, 2008). Although this study isolated *B. cereus* from the air around farm settlements, a similar survey by Kassa *et al.* (2017) investigated the associated risk factors in bovine raw milk in Debre Zeit town, Ethiopia. *B. cereus* and its spores have diverse contamination sources like soil, bedding, feed, dust, air, faeces, dirty teats, and milking equipment. This serves as a possible explanation for the possible sources of the aerosolized *B. cereus* and risk of the contaminated milk from both zero-grazing and semi-intensive farm management systems.

Stenotrophomonas maltophilia was also identified in this study. The study of Deredjian *et al.* (2016) identified the possible sources of this organism. Their study assessed the occurrence of *S. maltophilia* from organic amendments and agricultural soils from various sites in France and Tunisia, where the organism was recovered from about 84% of the tested soil samples. *S. maltophilia* was recovered from all the trapped air in hospital rooms (Di Bonaventura *et al.*, 2004) and in clean spacecraft assemblage rooms (Moissl *et al.*, 2008). However, *S. maltophilia* may be helpful as biocontrol of potato brown rot in the area where it was initially isolated (Messiha *et al.*, 2007). This organism has been confirmed as an emerging multidrug-resistant global opportunistic pathogen of the environment, mainly of plant-associated origin (Berg and Martinez, 2015). Although, distinguishing between the beneficial and harmful strains of *S. maltophilia* is yet to be ascertained. This is due to the complexities surrounding the species' ecology, evolution, and pathogenicity (Berg and Martinez, 2015).

Proteus spp. were first described in 1885 by Gustav Hauser; the in-depth revelation of their feature regarding intensive swarming growth has been extensively discussed. This bacterium is identified in this study. It can be hosted in many wild and domestic animals, acting as a parasite (Drzewiecka, 2016), the principal practice in the area under study. However, the pathogenicity of P. mirabilis bacteria is yet to be established. Nahar et al. (2014) isolated P. mirabilis from chicken droppings on commercial farms in Bangladesh. Shoket et al. (2014) noted in their study on the occurrence of human pathogens in spinach and tomato detected its presence in non-composted or improperly composted manure contaminate fruits and vegetables through using a fertilizer soil amendment/ or in irrigation water and Tambekar and Wate (2007) isolated them from the air in the hospitals in Amravati.

Similarly, Ogunleye and Carlson (2016) isolated *P. mirabilis* from poultry house rats in Nigeria. According to Nahar *et al.* (2014), *P. mirabilis* is a human zoonotic pathogen of urinary tract infection (UTI), nosocomial disease, and wound infection; therefore, a potential threat to public health. Roque *et al.*, (2016) have already established the prevalence of gram-positive bacteria such as Bacillus (B.) cereus, *B. licheniformis*, and *Enterococcus faecalis* in indoor air from porcine chicken bovine husbandry confinement buildings in Korea. However, the environmental implication identified is that *Proteus* spp. can tolerate

or utilize compounds such as high concentrations of heavy metals or toxic substances by exploiting them as sources of energy and nutrition providing the possibility of employing these microorganisms in bioremediation and environmental protection. Ochrobactrum spp. occurrence was investigated and isolated from agricultural soils (Bathe, et al., 2006); this explains its presence in the air around farm settlements. Meanwhile, Vogel, et al., (2008) confirmed that B. licheniformis isolated from animal sheds possess allergy-protective and inflammatory properties. B. licheniformis was reduced when open-air incineration of livestock carcasses and manure was carried out, Nakano, et al., (2013) also isolated B. licheniformis in an agricultural environment (Reuter, et al., 2011).

Enterobacter spp. in the air around farms corroborates the study of Sans *et al.* (2018). Apart from the presence of these bacteria around animal houses, they were also found in animal houses, either confined or otherwise. *Enterobacter* spp. can contaminate farm produce consumed raw, as revealed (ols tor pe *et al.*, 2012).

Conclusion

This study was carried out across farm settlements in Ogun State, and it revealed the concentration of different bacterial and fungal species in this environment. The highest and lowest scores for general housing conditions observed using the checklist were at Coker and Ikenne, respectively. In contrast, the availability of general environmental hygiene and sanitation facilities was highest at Ikenne and lowest at Ibiade. Many farm settlements are characterized by broken or unconcreted floors, dilapidated walls, poor indoor ventilation, and indoor storage of farm produce. The environmental conditions of the residences of the settlers are moderately poor, with structural defects and unhygienic conditions. Such a condition implies that a conducive and thriving atmosphere is available for pest infestation and the spread of infection. The lack of smooth accessibility to a sequencer within the country limited this study. This affected the viability and purity of the extracted DNA samples, many of which were contaminated. This study recommends that adequate care be taken to prevent the inhalation of fungal spores as prolonged exposure to vast numbers of spores, often due to occupational circumstances, can develop allergic alveolitis.

Acknowledgments

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APPENDIX







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