Comparative assessment of indoor and outdoor air environment of poultry farms in Edo State, Nigeria

Rahmatulai Adams¹, Blessing Iyore Idemudia¹, Emmanuel Esosa Imarhiagbe², Beckley Ikhajiagbe^{3⊠}, Emmanuel Ukpebor⁴ and Frederick Osaro Ekhaise¹

¹Department of Microbiology, University of Benin, Benin City, Nigeria; ²Department of Environmental Management and Toxicology, University of Benin, Nigeria; ³Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria; ⁴Department of Chemistry, University of Benin, Benin City, Nigeria; **Corresponding author, E-mail: beckley.ikhajiagbe@uniben.edu**

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Abstract. Intensive poultry farming creates the ideal environment for pathogen concentration and transmission. The presence of thousands of birds in an enclosed, warm, and dusty atmosphere is ideal for the transmission of infectious diseases from birds to humans. This study was conducted to assess the indoor and outdoor air quality of different poultry types in Edo State, Nigeria. The physicochemical conditions of the air around the poultry environments differed with location and poultry types. The concentrations of carbon dioxide (CO₂), nitrous oxide (N₂O), hydrogen sulphide (H_2S) as well as particulate matter (PM_{10}) were all within recommended limits established by the World Health Organization. However, significant elevations in Ammonia (NH₃) and sulphur dioxide (SO₂) levels were observed in substandard poultry farms across the locations. Total bacterial counts ranged from 1.38CFU/m⁵ - 90.35 x 10⁵CFU/m³ irrespective of location and poultry type. Within the poultry types, bacteria count inside the poultry environment $(3.11 \times 10^5 \text{CFU}/\text{m}^3)$ significantly differed from concentrations outside the poultry environment $(22.58 \text{ x}10^5 \text{CFU/m}^3, \text{ p} < 0.05)$. The Lowest microbial counts were obtained in the standard poultry farms. Molecular identifications revealed the presence of Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus, R. ADAMS, B. I. IDEMUDIA, E. E. IMARHIAGBE, B. IKHAJIAGBE, E. UKPEBOR, F. O. EKHAISE

Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis as the bacterial isolates whereas *Fusarium oxysporum, Aspergillus niger, Rhizopus stolonifer, Trichoderma polysporum, Aspergillus fumigatus* were the fungal isolates. *Staphylococcusaureus* was the most predominant bacterial species (25%) while *Aspergillus niger* was the most predominant fungal species (30%).

Keywords: air quality, bacteria, chicken, fungi, microflora poultry.

Introduction

The importance of good air quality for health, quality of life, and the environment cannot be overstated. When pollutants that are hazardous to human, animal, and plant health are present in the air, it is considered contaminated. Air contaminants, both within and outside, are widely regarded as a major cause of medical problems in both urban and national settings (WHO, 2005). Poultry is a significant and rapidly growing source of meat on the planet today, accounting for a quarter of all meat produced in the year 2000. This is accomplished by genetic selection, modified feeding, and strict health-monitoring procedures that include the use of antibiotics as therapeutic agents for bacterial diseases in intensive farming systems (Apata, 2009).

The following components are common to all poultry systems: (a) an enclosed structure that can hold a large number of animals in a small space; (b) a ventilation system; (c) a system for watering the livestock; (d) a system for feeding the livestock; and (e) a system for handling animal waste (Aromolaran *et al.*, 2013, Ohajianya *et al.*, 2013 and Cole *et al.*, 2000). Nigeria has an estimated poultry population of over 140 million birds mostly concentrated in the south western part of the country. Approximately 60 percent of the poultry production takes place in small backyard farms distributed throughout the rural areas (FAO, 2006).

In the last few years, Nigerian poultry production has increased. In the years 2001, 2002, 2003, and 2004, the estimated number of fowls raised in millions was 117.3 126.7, 142.7, 143.1, and 144.3. Between 2001 and 2004, the production of poultry meat increased from 88,000 to 108,000 tonnes (CBN, 2004, Akinola *et al.*, 2008). Nigeria's "140-160 million poultry account for 10% of its gross domestic product and a large proportion of the protein eaten by its 132 million citizens," according to the Food and Agricultural Organization, FAO. However, the country's H5NI avian influenza outbreaks, which began on February 8, 2006 and have resulted in the death of a large number of birds, might have caused a temporary setback (World Organization for Animal Health, 2006).

Although ambient air pollution is a major concern, indoor air can be more contaminated than outdoor air because indoor natural quality can be affected by a variety of factors, including organic compounds, and particulate pollutants (O'Connor *et al.*, 2004).

Infectious microbial spores, chemical pollutants, irritants and allergens may be found in both indoor and outside air which can reduce the quality of life and trigger diseases (Killebrew *et al.*, 2010, Kusi *et al.*, 2015), much like the inward breath of these airborne contaminants. The dangers of existing residue in enclosed environments acting as a source of synthetic concoctions and resuspended elements, triggering inhabitant's presentation through both inward breath and incidental ingestion, are becoming more widely recognized. Increased exposure to indoor contaminants in residue may have negative consequences on children's health, including slowed growth and learning inabilities, hypersensitivities, malignancy, sensory system harm and different ailments (Oyeyinka *et al.*, 2011, Purnomo *et al.*, 2014).

Gases in poultry confinements are a product of the degradation of droppings, animal respiration and building operations. Some of these gases include ammonia, carbon dioxide, carbon monoxide and hydrogen sulphide. Each of these gases may affect respiratory health (Cole *et al.*, 2000, Pickrel, 1991, Okoli *et al.*, 2006). They can be a respiratory toxicant or irritant such as ammonia (Smith *et al.*, 2003, Wafi *et al.*, 2011) which cause asphyxiation, blood poisoning, anoxia, pulmonary oedema or sudden death like carbon dioxide and carbon monoxide (Okoli *et al.*, 2006, Sainsbury, 1993) and pulmonary oedema or sudden death, like hydrogen sulphide poisoning (Donham and Rylander, 1986).

In communities surrounded by a high concentration of livestock, complaints of odour nuisance, environmental pollution, and ill health have become more common (Pinkerton *et al.*, 2000). Studies have shown that the physical and conceptual health of residents existing close to a high concentration of livestock is compromised (Cole *et al.*, 2000, Kusi *et al.*, 2015). Poultry and farm animals have also been reported to be a large reservoir of several bacteria species (Cole *et al.*, 2000). Poultry birds have remained connected with higher airborne dust, microorganisms and endotoxin absorptions in confinements (Oyeyinka *et al.*, 2011). Also, older litter, which is basically dried manure, is dustier owing to an escalation in friability when associated with fresher wood chip litter (Cole *et al.*, 2000). The aim of the study, therefore, was to evaluate the indoor as well as the outdoor air-borne quality and microflora assessment of standard, semi-standard and substandard poultry farms in Edo State, Nigeria.

Materials and methods

Study Area/ Study locations

The Data for this study were collected from 9 selected poultry farms in Edo State. The State is separated into three Agricultural regions, namely: Edo Central, Edo South and Edo North. The State lies between longitudes 05º 041 E and 06° 431E and latitudes 05° 441N and 07° 341N of the equator. The populace of the study consists of nine commercial poultry farms housing domestic chickens in confinements identified by the Poultry Association of Nigeria, Edo State Branch and the Ministry of Agriculture and Natural Resources, Benin City, Edo State (Figure 1). Three types of poultry systems (standard, semi-standard and substandard) were taken into consideration with respect to Mechanization and Automation. Commercial flocks of rearing and laving birds with a capacity of 2,000-6,500 were considered for this study; Standard Poultry farms with mechanical ventilation, automated feeding and drinking systems and Standard practices (Figure 2a). The semi-standard-Poultry farms with mechanical ventilation, automated drinking system and traditional feeding methods (Figure 2b). The third category was the sub-standard- Poultry farms without mechanical ventilation systems, only natural ventilation with traditional feeding and drinking methods (Figure 2c).

Sample Collection Air sampling

For a period of 12 months, the air on all poultry farms was sampled at various points in the field between December 2017 and November 2018, the study was conducted in nine (9) poultry houses in Edo State's three senatorial districts (Edo Central, Edo North, and Edo South). The sampling was conducted at three different locations, Ekpoma (Edo Central), Auchi (Edo North), and Benin were the study sites (Edo South). The sampling times and dates were chosen based on high activity levels in the sampled poultry farms. Poultry air was sampled at 9 a.m. at each sampling period. Over 2,000 birds were housed in each of the poultry samples. Casella cell 712 air sampler (Casella incorporated) was used to collect air samples. (Casella incorporated, U.S.A). Depending on the predicted pollution level, measurements were made in triplicates, each time collecting 0.1m³ of air in 1 minute. During the measurement, the sampler was placed 1.5 meters above the human inhalation zone. Each sampling site took 4 hours to complete. Throughout the fattening season, indoor and outdoor samples were collected in the poultry bio field. Airborne bacteria and fungi were used to continuously assess bio pollutants. In the poultry houses, samples of bacteria and fungi were taken at a central location. The discharge level outside the poultry farms was determined correspondingly.

Determination of Microclimatic Parameters.

The physicochemical parameters, and relative humidity in addition to temperature, were measured with Testo Device 400 (Testo GmbH & Co., Lenzkirch, Germany).

Media preparation

The media used in this study to isolate bacteria and fungi were Nutrient Agar (NA), MacConkay Agar, Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA) and Blood Agar. All media preparations were carried out in accord with the Manufacturer's guidelines. (The composition and preparation of media are in Appendix). Antibiotics (Streptomycin and Chloramphenicol – 50mg/L each) were introduced into the dissolved media after sterilization was carried out for the inhibition of bacteria. Sterilization of media was by autoclaving for 15 mins at 121° C and 15 pounds pressure.

Measurement of dust concentration

The concentration of dust of aerodynamic diameter of <10mn was determined electronically with the aid of a direct reading active personal sampler, Casella Cell Dust (Environmental Device co-operation, USA). An active sampler uses a pump as well as a power source to transport air over a collector (WHO, 2000). The sampler has a sampling flow rate of 1.0 l/minutes and the instrument software allows direct reading of dust concentration. The sampler was placed 1.5m above the floor, the device switched on and dust concentration was determined after 1 minute and measurements were taken on a monthly basis in each of the poultry houses investigated. The results were expressed in mg/m³.

Enumeration and isolation of airborne bacterial and fungal isolates

Qualitative and quantitative bacterial and fungal investigations were carried out in the poultry buildings.

A filtration method was used in this study due to the predicted high concentration of microflora in poultry facilities. Indoor and outdoor air samples were obtained using measuring sets that included a Casella Personal Air Sampling Pump (Model: Apex2, IndiaMART, New Delhi) and mixed cellulose ester filter paper (ME Range ME 24, 3.1 mm white/black grid for membrane-butler, 0.2 μ m pore size, 47 mm circle (400 pcs), manufactured by Cytiva, USA) to determine concentrations of airborne Microflora. The evaluating sets were calibrated with a Gillibrator 2 calibrator before each sampling process. After sampling, sterile tweezers were used to pick the filters containing the biological material into a tightly sealed container Stuart-Ringertz medium

(Sigma-Aldrich chemie GMLH Munich, Germany) was used, and the samples were transported to the lab for microbiological analysis. The filters in the transport medium containers were submerged in 5ml of phosphate buffer solution (BTL, Lodz, Poland) and the biological material on the filters was eluded by shaking on a shaker at 420 revolutions per minute for 50 minutes. From the elutes, a series of threefold dilutions were made.



Figure 1. Map of Edo State Displaying the Location of Study Sites Marked According to Poultry Standards



Figure 2. Different standards of poultry environment with birds in confinements (a) standard Poultry in Auchi, (b) semi-standard Poultry in Benin, and (c) substandard Poultry in Ekpoma

Isolation of airborne bacteria from the air:

All media were aseptically prepared according to the manufacturers' instructions. Thereafter 0.1ml of the 10³ dilution were inoculated onto sterile plates of mannitol salt agar, nutrient agar, blood agar and MacConkay agar (Merck, Dermstadt, Germany). The plates, which were prepared in triplicates were covered, and incubated for 18-24 hours at 37^oC for the isolation of pathogenic bacteria. One set of plates was incubated under aerobic conditions, while the other set was incubated under anaerobic conditions. The airborne bacterial isolates were enumerated using the formula:

(Number of colonies x Dilution factor x Elute volume)/ (Serial dilution material plated x Volume of air sampled)

The resultant concentration was expressed in terms of the number of colony forming unit per cubic meter (CFU/m³). Thereafter, discrete colonies were sub-cultured for preliminary identification subjected to biochemical tests and characterized on the basis of their cultural, morphological and biochemical characteristics, as described by Cheesebrough, 2006.

Isolation of airborne fungi from the air:

Sterile dishes of PDA and SDA (Oxoid Ltd., England) incorporated with penicillin and streptomycin were used for the enumeration and isolation of airborne fungal isolates. The plates were incubated for 3-5 days at room temperature (28±2°C), discrete colonies were sub cultured and the airborne fungal isolates were characterized based on their morphological appearances. The fungal colonies were counted using the same formula in the section above and subcultured to obtain pure cultures which were identified according to Barnett and Hunter (Barnett and Hunter, 1972).

Measurement of gases in poultry housings

The concentrations of ammonia (NH₃), hydrogen sulphide (H₂S), carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) in poultry houses were determined with the aid of a portable direct-reading instrument, the procedure involved taking a representable reading at different locations in the poultry farm. The measurements of gases were taken in triplicates inside the poultry houses at the three locations (near the entrance, at the center and the end wall). The representative evaluations from every confinement were assembled to obtain the mean for every poultry. The concentration of ammonia, hydrogen sulphide, carbon monoxide and nitrous oxide were measured in Parts Per Million (ppm) while methane was measured as Lower Emissible Limits (LEL) as a flammable gas with the aid of a Gasman Hand-held Personal Gas detector

(Crowcon Instruments Ltd., England), which employs electrochemical sensors and catalytic bead sensors for flammable gas measurements. All through the gas measurements, the handheld equipment was held at approximately one foot directly above the litter level and the evaluations were recorded within 20 secs. All analyses were calibrated for zero and span before and after reading.

Extraction and amplification of DNA

The isolated colonies were transferred from the surface of a single agar plate into a pre-cooled (20°C) sterile ceramic mortar, liquid nitrogen was added, and the mixture was ground into a fine powder with a sterile ceramic pestle. To suspend the powder, two ml of buffer G-2 (Genomic DNA buffer set; manufactured by Qiagen, Valencia, California, USA) with RNase (200 g/ml; manufactured by Sigma Aldrich Chemical Company, Germany) was applied, and the suspension was transferred to a clean test tube. A total of 45 microliters of proteinase K solution (20 mg/ml stock solution; Sigma Aldrich Chemical Company, Germany) was applied to the suspension, which was then incubated at 55°C for 3 hours with intermittent agitation. The suspension was centrifuged for 10 minutes at 21,500 g. The supernatant was transferred to a clean test tube, and DNA was extracted and purified using Qiagen Genomic-tip 20/G columns, as directed by the manufacturer. The eluted DNA was treated with two and a half microliters of glycogen solution (20 mg/ml; Gentra Systems, Minneapolis, USA), which was then precipitated using standard isopropanol and ethanol methods. DNA was resuspended in 60 l of DNA rehydration buffer (Gentra Systems' PureGene kit, QIAGEN, USA) and held at 20°C until required. After a pure PCR product of the 16S gene was obtained, sequenced, and aligned against bacterial or fungal DNA data base, the species were identified.

Statistical analyses

The Data obtained from this research were expressed as either mean \pm SEM (standard error or mean) or percentages. The t-test statistics was used to test for statistical difference between the treatment and control groups studied. The statistical package used for data analyses was SPSS version 21.0. Values in triplicates were evaluated using measures of central tendency (mean \pm standard deviation). One-Way ANOVA was used to compare multiple variables while Duncan's multiple range test was used to check for significant differences between means of values determined. *P*-values less than 0.05 were considered statistically significant. Diversity indices of microbial isolates were computed using PAST software (version 2. 17c).

Results

Mean indoor temperature levels ranged from 24.60°C - 32.21°C, 25.5°C - 32.20°C and 25.10°C - 32.40°C in Standard, Semi-standard and Sub-standard Poultry farms respectively. The highest temperature reading of 33.60°C was in the standard poultry farm in Auchi while the lowest temperature reading 24.60°C was recorded in the standard poultry farm in Ekpoma. The mean indoor relative humidity results ranged from 50 – 88%, 52- 93% and 51 - 91% in Standard, Semi-standard and Sub-standard poultry farms respectively with the highest reading of 95% recorded in Semi-standard poultry farm in Ekpoma while the lowest 50% was recorded in the standard poultry farm in Auchi (Table 1). The airborne monitoring of gaseous pollutants, fungi and bacteria was carried out in indoor and outdoor environments of the poultry farms. The idea of monitoring these variables in the outdoor environment was to examine for the existence of any significant variation exist in aerial pollutants levels between both extremes in the poultry houses.

Mean hydrogen sulphide concentrations in the environment were recorded between 0.02 ppm and 13.10 ppm (Table 1). There was a statistically significant difference in concentrations in indoor and outdoor environments in all poultry farms studied (P<0.05). The H₂S levels were generally higher in the indoor environment for the poultry houses studied. Hydrogen sulphide concentrations in Sub-standard poultry across all locations exceeded the WHO permissible limit (7.00ppm). A significant difference in H₂S concentrations between poultry types in all locations(P<0.05) was also observed with the exception of Standard and Semi-standard poultry in Auchi and Ekpoma indoor and outdoor as well as semi-standard and sub-standard poultry outdoor in Benin City. The concentration of ammonia (NH₃) ranged from 0.004ppm to 9.14 ppm. The concentration was recorded to also be above the W.H.O set limit of 7ppm in Sub-standard poultry in all sampled locations. Indoor and outdoor air had significant differences in mean values, with outdoor air being generally higher.

While there was a statistically significant difference between semistandard and sub-standard poultry in Auchi (indoor and outdoor) and Ekpoma, the levels between semi-standard and sub-standard poultry in Auchi (indoor and outdoor) and Ekpoma were statistically comparable (P>0.05). The recorded levels of methane were higher in the indoor air compared to the outdoor air with concentrations ranging from 0.22 LEL in Standard poultry (Auchi outdoor) to 7.54 LEL (Sub-standard Poultry Ekpoma indoor) (Table 1). Significant difference in methane concentrations was also observed among poultry types and was higher in semi-standard poultry than standard and substandard poultry in the indoor and outdoor poultry farms in Auchi as well as the outdoor environment in the substandard poultry farms in all three locations.

 PM_{10} levels in the indoor, as well as the outdoor environment of poultry houses, were similarly measured, concentrations were significantly different statistically up to 89% in all poultry types, and ranged between 0.01 ± 0.00 to 1.75 ± 0.01 mg/m³ (Table 1). The highest reading 1.75 mg/m³ was recorded in the Semi-standard poultry farm in Auchi while the lowest reading of 0.01 mg/m³ was recorded in the Standard poultry farm in Ekpoma. However, levels in Semi-standard poultry in Auchi were similar statistically, (P>0.05). Indoor CO_2 concentrations were significantly high in semi-standard and sub-standard poultry farms in Ekpoma and Benin, there was however no significant difference in CO_2 concentrations among poultry farms in Auchi. While outdoor concentrations of CO_2 were higher in semi-standard poultry than sub-standard in Auchi, there was however no difference in outdoor CO_2 levels in the three poultry farms in Ekpoma and Benin.

Poultry	Temp.	Humidity	CO ₂	NH ₃	CH4	N ₂ O	H ₂ S	SO ₂	Dust
A3I	29.28 ^{ab}	73.5ª	28.5 ^{de}	0.1ª	2.02 ^{bcd}	0.2bc	1.34 ^{bc}	1.02 ^{de}	0.11 ^{ab}
A30	30.79 ^b	77.92 ^a	13.1ª	0.04 ^a	0.84 ^a	0.09 ^a	0.56 ^{ab}	0.41^{ab}	0.03 ^a
A2I	29.09 ^{ab}	74.33ª	27.1 ^d	39.1 ^d	4.66 ^h	0.42e	2.36 ^{de}	0.22 ^{ab}	0.87^{fg}
A20	30.73 ^b	77.92ª	12.4ª	18.1 ^b	2.3 ^{de}	0.2^{bc}	1.18 ^{bc}	0.05ª	0.59 ^{de}
A1I	29.3 ^{ab}	73.33ª	30.5 ^e	40.5 ^d	6.12 ^j	0.49 ^{ef}	9.71 ^h	0.11ª	0.86 ^{fg}
A10	30.73 ^b	76.33ª	16 ^b	18 ^b	2.81 ^f	0.22c	4.1 ^f	0.04 ^a	0.44 ^{cd}
E3I	28.59ª	77.58ª	16.5 ^b	2.24 ^a	2.17 ^{cde}	0.32 ^d	1.65 ^{cd}	1.19 ^{de}	0.21 ^{ab}
E30	29.23 ^{ab}	80.08 ^a	11.5ª	0.2ª	1.04 ^a	0.13 ^{ab}	0.64 ^{ab}	0.51 ^{bc}	0.05 ^a
E2I	29.18 ^{ab}	76.58ª	20.5c	51.3e	3.41g	0.6 ^g	2.33 ^{de}	0.511 ^{bc}	0.99 ^{fg}
E20	29.5 ^{ab}	77.75ª	10.5ª	22.8 ^{bc}	1.56 ^b	0.23c	1.09 ^{bc}	0.19 ^{ab}	0.47 ^{cd}
E1I	28.73ª	77.92 ^a	21.1 ^c	61 ^f	6.15 ^j	0.45 ^e	11 ⁱ	1.61 ^f	1.06 ^g
E10	29.76 ^{ab}	80.08 ^a	10.7ª	23.2 ^{bc}	2.93 ^f	0.2^{bc}	5.16 ^g	0.86 ^{cd}	0.47 ^{cd}
B3I	28.18 ^a	74.83 ^a	22.7c	3.38 ^a	1.93 ^{bcd}	0.41e	0.21ª	1.38 ^{ef}	0.29 ^{bc}
B30	28.91ª	79.17ª	10.9ª	1.65ª	0.93 ^a	0.18 ^{bc}	0.05 ^a	0.5 ^{1bc}	0.09 ^{ab}
B2I	28.55ª	74.5ª	29.2 ^{de}	47.7e	3.78 ^g	0.56^{fg}	2.49 ^e	1.21 ^{de}	0.64 ^{de}
B20	29.57 ^{ab}	79.25ª	12.5ª	22.8 ^{bc}	1.75 ^{bc}	0.25 ^{cd}	1.23 ^{bc}	0.34 ^{ab}	0.17 ^{ab}
B1I	28.03 ^a	72.5ª	30.6 ^e	57.3 ^f	5.36 ⁱ	0.6 ^g	9.36 ^h	1.99g	0.78 ^{ef}
B10	28.69 ^a	76.58ª	12.2ª	25.7¢	2.56 ^{ef}	0.27 ^{cd}	4.3 ^f	1.03 ^{de}	0.16 ^{ab}
p-values	0.854	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.002

Table 1. Comparative mean annual physicochemical composition of airin and around poetry environments in Edo State

Means with similar alphabetic superscripts within the same columns do not differ from each other (p>0.05)

A is Auchi, *B* is Benin City, *E* is Ekpoma, 1 is substandard, 2 semi-standard, 3 standard poultry, *I* is inside and 0 is outside poultry environment

Table 2 shows the airborne fungal counts which range between $9.33 \pm 0.78 \text{ CFU/m}^3$ to $285.62 \pm 16.76 \text{ CFU/m}^3$, with the highest count recorded in Semi-standard poultry in Ekpoma (indoor) in July 2017 and the lowest count at $9.33 \pm 0.78 \text{ CFU/m}^3$ recorded in the standard poultry farm in Benin in February. Statistical significance in the difference between indoor and outdoor counts was only seen among Semi-standard and Sub-standard poultry farms in Auchi and Benin. Counts in indoor environments of poultry houses were significantly higher in standard poultry than semi-standard and sub-standard poultry in standard poultry than semi-standard and sub-standard poultry in standard and semi-standard poultry while there were no significant differences in fungal counts among poultry houses in Benin City. The fungal counts in all poultry farms sampled were however below the 3000-5000 CFU/m³ standard set by the Polish authority for occupational exposure to airborne fungi (Lonc and Plewa, 2010).

Table 2 also shows the indoor and outdoor airborne bacterial counts in Poultry houses which ranged between 90 \pm 44.48 CFU/m³ to 690.30 \pm 0.08 CFU/m³. The highest counts of 690.30 \pm 0.08 CFU/m³ was recorded in the Substandard poultry in Auchi indoor environment in September 2017 while the lowest count of 90 \pm 44.48 CFU/m³ was recorded in the Standard poultry farm in Ekpoma February. The bacterial counts between indoor and outdoor air varied significantly only in Standard and Sub-standard poultry farms in Benin as well as in Substandard poultry in Auchi. The bacterial counts were higher in all indoor environments than in the outdoor environment. A comparism between poultry types revealed that bacteria counts in indoor air varied significantly

		Bacterial		Fungal					
	Indoor	Outdoor	p- value	Indoor	Outdoor	p-value			
Auchi									
1	296.65±48.38 ^b	266.80±58.92 ^b	0.184	42.98±9.11 ^b	45.24±8.71 ^{bc}	0.633			
2	308.23±87.41 ^d	272.96±87.17 ^d	0.142	34.57 ± 11.42^{ad}	25.82±8.74 ^{acd}	0.139			
3	481.29±148.99 ^{abd}	351.75 ± 69.28^{abd}	0.094	71.87 ± 15.09^{abd}	65.98 ± 9.97^{abd}	0.322			
Ekpoma									
1	148.05±46.24	165.34±51.28 ^b	0.633	39.68±14.64 ^b	40.66 ± 7.96 ab d	0.732			
2	226.55±70.95	207.48±54.61 ^d	0.354	159.31 ± 60.17 d	147.32±41.79 °	0.138			
3	388.10±183.17	304.91±168.49bd	0.535	150.32±39.89 ^{bd}	173.50±43.32 ^b	0.244			
Benin city	7								
1	214.24±71.36 ^{ab}	165.13±45.02 ^{abc}	0.132	48.28±20.00	57.70±13.51 ^c	0.214			
2	302.8967.80 ^d	277.00±75.46	0.325	50.99±8.49 a	42.27±8.23 ^{acd}	0.093			
3	441.30±130.46 ^{abd}	332.86±108.01 ^{ab}	0.174	39.97±7.22 ª	32.86 ± 108.01^{ab}	0.112			

Table 2. Mean airborne microbial counts Dec. 2016 - Nov. 2017.

Keys: 1-Standard Poultry, 2-Semi-standard Poultry, 3-Substandard Poultry

between standard and Substandard poultry farms as well as between Semistandard and Sub-standard poultry farms in all three locations with Substandard having the higher count. The outdoor bacterial counts were different between standard and sub-standard poultry farms in all three locations as well as between semi-standard and sub-standard in Auchi with Substandard having highest counts. Counts in Benin were also significantly different between Standard and Semi-standard poultry with the Semi-standard poultry recording the highest concentration.

Results of diversity indices showed more individual bacterial and fungal isolates in Ekpoma than in Benin City (Table 3); however, no differences in dominance indices were obtained either for bacterial isolates or fungi irrespective of location or type of poultry. The diversity and statistical association between gaseous pollutants and microbial load showed a positive correlation between hydrogen sulphide and bacterial loads, an indication that an increase in hydrogen concentration in the Poultry farm could influence the increase in the bacterial loads (Table 4). The results of the high concentration of the hydrogen sulphide were traced to the anaerobic decomposition of the accumulated poultry litters in the environment. A regression model was projected to justify the relationship between the bacterial loads and the concentration of hydrogen sulphide. Figure 3 shows a significant positive relationship between bacterial loads and Hydrogen sulphide (Figure 3a) as well as between bacterial Loads and Ammonia (Figure 3b).

With repagard to the diversity indices of bacterial and fungal isolates presented on Table 3, there was a minimum of 4 bacterial taxa and 6 fungal taxa. All isolated organisms were present in Auchi. In terms of diversity, Auchi was also the highest. In terms of the surplus of the isolates, the Auchi location was also the highest. The individuals represent the species and the number of times they appeared. The highest individual species was recorded in the Ekpoma location. The Dominance index ranges from 0 to 1. The closer it is to 1, the more dominant it is. A location is considered to have a higher dominance over another if the isolates are not evenly distributed meaning that there is more of one type of organism in a particular location than others. The dominance index indicated that no single organism showed dominance over the others. The evenness index also ranges from 0 to 1 indicating the even distribution of the individual species. The lowest evenness ratio was in Auchi locations but there was a general indication that the isolates were evenly distributed. The Menhinick or Margalef indices are called specie richness indices indicating which location had more specie richness. The highest Margalef indices was in Auchi location (Table 3).

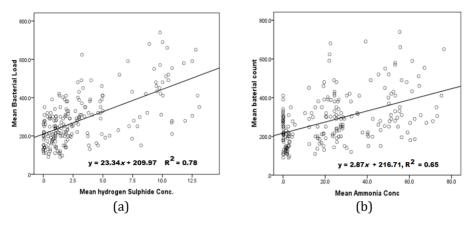


Figure 3. Regression Model of Bivariate relationship between (a) Bacterial loads and Hydrogen sulphide and between (b) bacterial Loads and Ammonia

	nom the designated sampling areas																	
	A3I	A30	A2I	A20	A1I	A10	E3I	E30	E2I	E20	E1I	E10	B3I	B30	B2I	B20	B1I	B10
Bacteria																		
Taxa_S	5	4	5	5	5	4	5	4	5	5	5	5	5	5	4	4	5	4
Individuals	26	26	28	22	26	22	45	38	47	42	46	41	39	38	39	40	43	36
Dominance	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.3
Simpson	0.8	0.7	0.8	0.7	0.7	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.7
Shannon	1.5	1.3	1.5	1.4	1.4	1.3	1.5	1.4	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.4	1.5	1.4
Evenness	0.9	0.9	0.9	0.8	0.8	1	0.9	1	0.9	0.9	0.9	0.9	0.9	0.9	1	1	0.9	1
Menhinick	1	0.8	0.9	1.1	1	0.9	0.8	0.7	0.7	0.8	0.7	0.8	0.8	0.8	0.6	0.6	0.8	0.7
Margalef	1.2	0.9	1.2	1.3	1.2	1	1.1	0.8	1	1.1	1.1	1.1	1.1	1.1	0.8	0.8	1.1	0.8
Fungi																		
Taxa_S	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Individuals	27	34	27	29	32	33	61	58	53	57	60	57	51	49	50	50	44	47
Dominance	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Simpson	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Shannon	1.6	1.7	1.6	1.7	1.7	1.7	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.7	1.8	1.7	1.7
Evenness	0.9	0.9	0.8	0.9	0.9	0.9	1	1	1	1	1	1	1	1	1	1	1	0.9
Menhinick	1.2	1	1.2	1.1	1.1	1	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.9	0.9	0.9	0.9	0.9
Margalef	1.5	1.4	1.5	1.5	1.4	1.4	1.2	1.2	1.3	1.2	1.2	1.2	1.3	1.3	1.3	1.3	1.3	1.3

Table 3. Diversity indices of (a) fungal (b) bacterial isolates obtainedfrom the designated sampling areas

Keys: A is Auchi, B is Benin City, E is Ekpoma, 1 is substandard, 2 semi-standard, 3 standard poultry, I is inside and 0 is outside poultry environment

	Bacterial count	Fungal count
CO ₂	0.442**	-0.098
NH ₃	0.476**	0.264**
CH ₄	0.576**	0.155*
N20	0.391**	0.211**
H ₂ S	0.621**	0.219**
SO ₂	0.239**	0.135*
Dust	0.219**	0.230**
Temp	-0.253**	-0.124
Humidity	0.107	0.059

Table 4. Bivariate correlation between Selected Gases and Microbial Load

*. Correlation is significant at the 0.05 level (2-tailed).

Keys; CO₂ . Carbon dioxide, NH₃ – Ammonia,CH₄ – Methane, N₂O - Nitrous oxide, H_2S – Hydrogen sulphide, SO₂ – Sulphur dioxide.

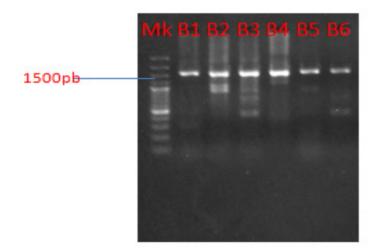


Figure 4. Agarose gel electrophoresis of the extracted DNA from six bacteria samples isolated from poultry houses. Gel labelled B1-B6 correspond to *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis* respectively while Mk represents the molecular marker.

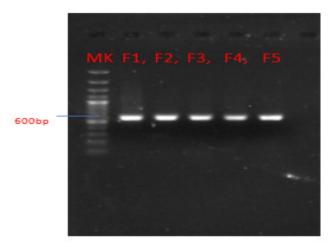


Figure 5. Agarose gel electrophoresis of the PCR products of five fungi samples isolated from poultry houses. Band size of approximately 600bp confirms positive amplification. Samples isolated from, Ekpoma (F1, F2) and Benin (F3, F4, F5,) were identified to *be Fusarium oxysporum, Aspergillus niger, Rhizopusstolonifer Trichoderma polysporum and Aspergillus fumigatus* respectively.

BacteriaB1Escherichia coliMK271753strain RB1B2Streptococcus pyogenesMK271754Strain RB2B3Staphylococcus aureusMK271755Strain RB3B4Pseudomonas aeruginosaMK271756Strain RB4B5Klebsiella pneumoniaeMK271757Strain RB5B6Bacillus subtilisMK271758Strain RB6	Code	Organism	NCBI accession number	Strain
B2Streptococcus pyogenesMK271754Strain RB2B3Staphylococcus aureusMK271755Strain RB3B4Pseudomonas aeruginosaMK271756Strain RB4B5Klebsiella pneumoniaeMK271757Strain RB5	Bacteria			
B3Staphylococcus aureusMK271755Strain RB3B4Pseudomonas aeruginosaMK271756Strain RB4B5Klebsiella pneumoniaeMK271757Strain RB5	B1	Escherichia coli	MK271753	strain RB1
B4Pseudomonas aeruginosaMK271756Strain RB4B5Klebsiella pneumoniaeMK271757Strain RB5	B2	Streptococcus pyogenes	MK271754	Strain RB2
B5 <i>Klebsiella pneumoniae</i> MK271757 Strain RB5	B3	Staphylococcus aureus	MK271755	Strain RB3
	B4	Pseudomonas aeruginosa	MK271756	Strain RB4
B6 Bacillus subtilis MK271758 Strain RB6	B5	Klebsiella pneumoniae	MK271757	Strain RB5
	B6	Bacillus subtilis	MK271758	Strain RB6
Fungi	Fungi			
F1 Fusarium oxysporum MK271759 strain RF1	F1	Fusa <i>rium oxysporum</i>	MK271759	strain RF1
F2 Aspergillus niger MK271760 strain RF2	F2	Aspergillus niger	MK271760	strain RF2
F3 Rhizopusstolonifer MK271761 strain RF3	F3	Rhizopusstolonifer	MK271761	strain RF3
F4 Trichoderma polysporum MK271762 strain RF4	F4	Trichoderma polysporum	MK271762	strain RF4
F5 Aspergillus fumigatus MK2717639 strain RF5	F5	Aspergillus fumigatus	MK2717639	strain RF5

Table 5. Molecular identification of bacterial and fungal isolates

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Airborne Bacterial Isolates	Percentage frequency (%)	
Bacteria		
Staphylococcus aureus	40 (25.3)	
Enterococcus faecalis	20 (12.5)	
Bacillus subtilis	22(13.8)	
Escherichia coli	24 (15.0)	
Klebsiella pneumonia	30(19.0)	
Pseudomonas aeruginosa	22 (14.4)	
Total	158 (100)	
Fungi		
Fusarium oxysporium	8(19.6)	
Aspergillus niger	13 (29.8)	
Rhizopusstolonifer	9 (21.1)	
Trichoderma polysporium	1 (2.6)	
Aspergillus fumigatus	11 (26.9)	
Total	42 (100)	

Table 6. Frequency of Occurrence of Airborne Bacterial and Fungal Isolates in Poultry Farms

Phenotypically, six airborne bacterial and five airborne fungal isolates were isolated and characterized. The airborne bacterial and fungal isolates were further characterized using culture-dependent molecular characterization and identification technique to reveal the presence of the following: *Escherichia* coli, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruainosa, Enterococcus faecalis, Klebsiella pneumonia and Bacillus subtilis, Fusarium oxysporum, Aspergillus niger, Rhizopusstolonifer, Trichoderma polysporum and Aspergillus fumigatus (Table 5, Figures 4 and 5). The highest frequency of occurrence of the airborne bacterial isolates was recorded for Staphylococcus aureus (25.34%) while the least was recorded for *Enterococcus faecalis* (13.25%) (Table 6) Among the airborne fungal isolates, *Aspergillus niger* (30.21%) recorded the highest frequency of occurrence while the least was recorded for Trichoderma *polysporum* (2.34%)(Table 6). A comparison of types of poultry with respect to bacterial and fungal loads has been presented (Table 7). Bacterial and fungal loads were significantly higher in the substandard poultry compared to the standard ones. Similarly, with respect to weather, bacterial and fungal loads were higher throughout the rainy period collections as likened to dry season collections (Table 8). The dendrogram from hierarchical cluster analysis showing a bivariate association between any two poultry environments on the basis of bacterial (Figure 6a) and fungal (Figure 6b) composition revealed that the Auchi groups were statistically separated from the others and this can be attributed to the weather condition peculiar to that environment.

COMPARATIVE ASSESSMENT OF INDOOR AND OUTDOOR AIR IN A POULTRY

Poultry types	Bacterial load	Fungal load
Sub-standard Poultry	383.22a	93.94a
Semi-standard Poultry	266.01b	76.71b
Standard Poultry	209.02c	59.09c
P-value	< 0.001	< 0.001

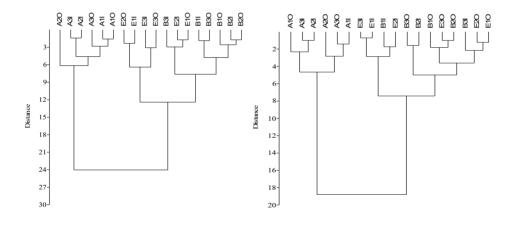
Table 7. Comparison of types of Poultry with respect to Bacterial and Fungal loads.

Values within similar columns with the same alphabets do not differ from each other (p>0.05)

	2017 Season	Mean counts (x 10 ² CFU/m ³)	SD	SEM	t-value	p-value
Bacterial loads	Rainy Dry	3.62 2.72	92.07 104.47	27.76 33.03	2.103	0.049*
Fungal loads	Rainy Dry	0.68 0.8	21.88 23.71	6.59 7.49	-1.148	0.265

Table 8. Differences in Bacterial and Fungal composition in the Sampled Season.

Mean difference is significant, p<0.05



(a) (b)
 Figure 6. Dendrogram from hierarchical cluster analysis showing a bivariate association between any two poultry environments based on (a) bacterial composition and (b) fungal composition.
 Keys: A is Auchi, B is Benin City, E is Ekpoma, 1 is substandard, 2 semi-standard,

3 standard poultry, I is inside and O is outside poultry environment

Discussion

This research showed that gaseous pollutants, as well as airborne fungi and bacteria, were relatively higher in the indoor environment than in outdoor areas, this is similar to findings of other authors (Lonc and Plewa 2010), the sources of these variations may be farm objects. There were however contrary findings in all poultry farms in Ekpoma and Benin as well as standard poultries in Auchi and Benin, where the fungi load in indoor and outdoor areas were not significantly different, this can be attributed to a lack of good ventilation system. A Similar trend was also observed for bacterial load in all poultry types in Ekpoma and standard poultry in Auchi as well as semi-standard poultry in Auchi and Benin, a key reason for this uncommon occurrence in standard and semi-standard poultry farms may be a result of non or improperly cleaned ventilation system, mechanical ventilation systems not properly cleaned can be a source of microbial proliferation and spending of microorganisms as reported by Collins (Collins, 2007).

Results from this study indicate that hydrogen sulphide concentrations were relatively higher in sub-standard poultry farms across all locations, however, the high concentrations recorded in some standard poultries are an indication of an unhygienic state and hydrogen sulphide is released from manure decay. The Highest indoor level in this study recorded in sub-standard poultry was far above the > 1 ppm concentration in poultry confinement (Jones *et al.*, 2000).

Nitrous oxide concentrations in the indoor areas of poultry houses were generally higher in sub-standard poultry farms, however, concentrations in semi-standard and sub-standard poultry farms in Auchi and Benin were similar statistically, this may be a result of improper heating systems coupled with the relatively higher levels recorded in these areas during May through July which are the peak wet seasons in Nigeria, as higher concentrations of N_2O were recorded by Calvet *et al.*, 2011 during winter periods when compared with values record in summer. Another possible reason may be the bird feed compositions in the poultries as well as the stage of maturity of the birds.

Indoor levels of methane in this study though below the 25 LEL permissible limit set by the World Health Organization similar to findings of previous authors (Calvet *et al.*, 2011) who also stated that the amount of methane emitted from poultry houses depends on management and condition of the poultry. Concentrations were, however, higher in sub-standard poultry farms in all locations except that levels in standard and semi-standard poultry farms in Benin were not significantly different, this may be attributed to the number of birds in the poultry (Calvet *et al.*, 2011).

Sulphur dioxide concentrations inside poultry facilities were higher in sub-standard poultry in Benin City, relatively higher concentrations were recorded in standard poultry farms in Ekpoma and Benin than in semi-standard and sub-standard poultry farms. This result thus gives a clue into the fact that the ventilation system has little role to play in the amount of SO_2 in poultry confinement.

Ammonia concentrations in semi-standard poultry in Ekpoma were above the W.H.O permissible limit, this may be attributed to the feed sources as previously reported by (Nahm, 2000), undigested proteins in poultry manure are potential sources of ammonia polarization.

Considering poultry types, the concentrations of ammonia were significantly higher in sub-standard poultry farms than in other types of poultry farms, there was, however, no significant difference between concentrations in semi-standard and sub-standard poultries in Auchi and Ekpoma. The feed types used in these poultry farms maybe a probable explanation for this.

Carbon dioxide levels observed indoors during this research were higher in semi and sub-standard poultry with no difference between concentrations in all three poultry farms in Auchi. As reported in earlier studies high CO_2 levels may be a result of type of heating system used (Knížatová *et al.*, 2010, reported that the use of natural gas as source of heating system could contribute to the amount of CO_2 emitted in an animal farm. These authors also suggested that the CO_2 amount in the indoor air of poultry should be considered in the operation of ventilation systems.

Dust levels in the poultry houses were the highest in semi and substandard poultries with similar levels statistically recorded. Dust in the range of PM_{10} was, however, the only form sampled in this study, as this is the maximum level that is respirable and is capable of lodging in the lungs. Statistically similar levels were recorded in the indoor and outdoor areas of the semi-standard poultry in Auchi, this can be attributed to the presence of several quarries around the sampling areas and thus the poultry facility may not be the only contributor to the outdoor levels of the PM_{10} observed.

The Highest PM_{10} level recorded in this study (1.75mg/m³ in sub-standard poultry) was higher than the 0.02 mg/m³ level recorded by Jones *et al.* (2000), though the poultry they studied was not defined. In poultry facilities PM_{10} originate from feed particles, bedding material, manure particles and feather particles blown from poultry fans (Nahm, 2000), thus ventilation system may be a major factor in the distribution of PM_{10} inside a poultry building.

Fungal loads in Ekpoma were not significantly different in the indoor and outdoor environments, this was contrary to the studies by Lonc and Plewa 2010, the indoor fungal load was higher than the outdoor fungal load, this may be a result of the relatively high humidity in the outdoor environment of these poultry farms, which is capable of supporting the proliferation of fungi (Lonc and Plewa, 2010 and Knížatová *et al.*, 2010). Indoor fungi load was the highest in substandard poultry farms, this may be a confirmation of the assumption that poultry types play vital roles in determining fungal count as previous studies by Sowiak *et al.*, showed that mechanical ventilation systems coupled with increase air flow rate contribute significantly to reducing fungal loads inside poultry facilities, however, no significant difference was recorded among poultry types in Benin-City.

High fungal loads recorded in standard and semi-standard poultry farms in Benin- City may be as a result of improper cleaning of the ventilation systems in these facilities, as suggested by Collins, 2007, who reported high fungal counts in poultry with mechanical ventilators when compared to those adopting gravity ventilation.

Performed analysis of variance demonstrated a significant impact of poultry types on indoor airborne bacteria loads, owing to the fact that high bacterial loads were recorded in all sub-standard poultry farms sampled, but similar to fungal, there was no significant difference between indoor and outdoor bacterial loads in all poultry farms in Ekpoma as well as in standard and semi-standard poultry farms in Auchi and Benin City. This result did, however, not agree with the findings by Lonc and Plewa, 2010, and may be an outcome of improper hygiene and practices that encourage the growth and abundance of bacterial outside poultry environment.

The isolated bacteria in this study are of both veterinary and public health importance. Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Klebsiella pnemoniae and Pseudomonas aeruginosa were identified culturally and biochemically, with *S. aureus* being the most frequently isolated. E. coli and other enteric bacteria such as Klebsiella and Pseudomonas species isolated from the sampled poultry houses in this study are members of normal intestinal flora. These bacteria become pathogenic when they reach tissues outside their normal intestinal or other normal flora sites. The anatomic sites of clinical importance in humans are urinary tracts, biliary tract, lung, bone, meninges, prostate gland and blood (bacteraemia). The presence of these bacteria in poultry facilities is in conformity with previous studies (Collins, 2007, Jones et al., 2000). Two of the bacteria, E. coli and K. pnuemoniae isolated during this study belong to the risk group 2 bacterial according to the Polish Ordinance, which is a risk classification for occupational exposure to bioaerosols (Lonc and Plewa, 2010). E. coli is an opportunistic pathogen, which can cause urinary tract infections, K. pnuemoniae on the other hand is also an opportunistic pathogen capable of causing respiratory tract infections.

S. aureus, while not being a spore-producing bacteria, has been shown to live longer in the air than any other bacteria, implying that it has a high capacity for airborne dissemination and infection. Its high frequency in aerosols, combined with this, makes it a likely candidate for bioaerosol airborne emissions. Given its high pathogenicity and virulence, the high prevalence of *Staphylococcus aureus* in the sampled poultry farms may be cause for concern. Several human diseases have been linked to the organism, including cellulitis, local abscess formation (furuncles and carbuncles), and lymphadenitis. Primary osteomyelitis and septic arthritis may occur when the infection spreads to the bones and joints (Brodka *et al.*, 2012) Inhalation of *Pseudomonas aeruginosa* may cause necrotizing pneumonia and the involvement of the ear and eye may result in otitis externa and rapid destruction of the eye respectively (Jones *et al.*, 2000).

All fungi isolated in this study were in the mould group and include *Fusarium oxysporium, Trichoderma polysporum, Aspergillus niger, Aspergillus fumigatus* and *Rhizopus stolonifer.* They are referred to as opportunistic fungi. They do not usually induce diseases, but do so when the body's host defense is compromised (Brooks *et al.*, 2007). Similar results were obtained in the study by Sowiak *et al.*, 2012 who isolated moulds as the major group of fungi from poultry facilities. Moulds are associated with humid environments and are capable of causing respiratory tract infections, as well as allergic effects, more worrisome, is that *A. fumigatus* which among moulds isolated during this study is classified as a risk group two biological agent. *A. fumigatus* is closely associated with humid environment and is frequently isolated from the surface of ventilators and settled dust (Sowiak *et al.*, 2012).

Conclusions

Poor standardization of poultry farming processes implied poor air quality as well as the worsened microbial quality of the air within and around the poultry farm. As evident from this research, poultry farms are substantial reservoirs and emitters of microbiological and gaseous contaminants into the environment. The growth of the Nigerian poultry industry needs a holistic approach that provides the best environment, nutrition and health for birds as well as minimizes occupational and environmental health risks. Strict biosecurity will reduce bacteria and other infectious microbes in the poultry environment.

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