# Original Research Article

# BACTERIOLOGICAL ASSESSMENT OF AIR SAMPLE IN POULTRY ENVIRONMENT

ABSTRACT

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| **Aims:** The aim of this study is to assess the bacterial load of air samples collected from Kwara State University poultry, Malete.**Study design:** The study utilized a cross-sectional design to assess microbial contamination in a poultry environment by exposing nutrient and MacConkey agar plates for one minute at different times (8am, 2pm, 4pm) and distances (2ft, 4ft, 8ft). Total bacterial and coliform counts were measured, and isolates identified through morphological and biochemical tests.**Methodology:** The plates of solidify nutrient agar and MacConkey agar were exposed in poultry environment for 1minute. The air sample were collected at different period of time 8am, 2pm, 4pm and at distance 2feet, 4feet, 8feet. Total bacterial counts and total coliform counts were evaluated. The isolates were identified through morphological observation and biochemical characteristics. The total coliform counts (CFU/M3) / Distance (feet) range from 34 ± 14.1 – 5 ± 14.8 whereas; total bacterial counts (CFU/M3) / Distance (feet) range from 79 ± 8.4 – 39 ± 9.1 on nutrient agar and MacConkey agar respectively.**Results:** The result showed a total of five bacterial genera were isolated which include: *Staphylococcus aureus, Salmonella species, Streptococcus species, Bacillus* and *Escherisia coli*. Since microorganisms found in the atmosphere are non-indigenous and usually introduced through human activities thereby, the presence of these potential pathogens could constitute health hazard not only to the workers but to the people around the poultry environment, thereby endangering the lives of community member through the spread of infectious diseases.**Conclusion:** Using the natural sedimentation technique, four genera of the organisms have been isolated comprising of *Staphylococcus aureus, Streptococcus spp, E. coli, Salmonella species* and *Bacillus*. And these organisms can cause several infections to the workers as well as inhabitants of this environment. In order to develop the quality of the poultry air in these farms, good ventilation system has to be designed and good hygiene practice must be observed by the workers. |

*Keywords: Bacteria, MacConkey agar, Nutrient agar, Infectious diseases, Public health, Total bacteria counts, Total coliform counts, Poultry environment, Air sampling.*

1. INTRODUCTION

*(1)* The level of microbial contamination in poultry houses is one of the most important sanitary and hygienic indicators. The main sources of microorganisms in poultry houses are birds, their excrements, feed, litter, ventilation air, and even employees. Microbes carried by dust, water vapor and secretions from the respiratory tract form bioaerosol. Birds breathe air which acts as a major vector for microorganisms. Most microbes are saprophytes, but some airborne microorganisms may be pathogenic. Pathogens that enter the respiratory system with liquid droplets and dust may cause infections. The smallest particles measuring <50 nm pose the greatest epizootic risk because they are slowly deposited and spread even at low air flow rates. The flock is constantly exposed to pathogenic bioaerosols when sick or infected birds are present in the poultry house *(1)*. Microbial survival is determined by temperature, humidity and other environmental parameters. Relative humidity in poultry houses generally does not support bacterial proliferation (the 50–80% range is lethal for bacteria), and microbial contamination of air, litter, and surfaces in poultry farm buildings can be attributed mainly to high flock density and the continued presence of microbial sources. Poultry farms are significant pollutants of the external environment, and they could pose an epidemiological risk if biosecurity principles are not observed. The microbial concentrations reported inside and outside poultry farms differ considerably in the literature *(2).* Microbial contamination levels are influenced by various factors, including bird species, stocking density, season, and ventilation system, microclimate, and litter quality.

An insignificant increase in litter pH was also noted throughout the experiment, which combined with increasing excreta amounts and fermentation processes in fresh litter could promote microbial growth. Despite a gradual decrease in indoor temperature accompanied by an increase in humidity, microbial air contamination did not follow the same pattern as litter contamination. Bacterial counts varied between weeks of the rearing period, most likely due to changes in dust levels and ventilation efficiency.

According to *(3),* who observed a significant increase in the concentrations of bacterial aerosols and endotoxins in chicken houses in successive stages of production. They also reported seasonal correlations in the size of bacterial populations. The concentrations of airborne bacteria were significantly higher in summer than in winter.

Numerous studies *(2)* also revealed that bioaerosols from poultry houses contain Gram-positive bacteria, including *Streptococcus, Staphylococcus, Micrococcus, Enterococcus* and *Bacillus*, as well as Gram-negative bacteria, including *Escherichia, Enterobacter, Klebsiella and Pasteurella.*

The increased need for poultry products and the exposure of poultry workers and passerbys to the bioareosol of poultry origin for an extended period of time during management constitute the need for this study to asertain the air quality of these areas. The interest in bioareosol exposure has increased over the last few decades, both due to the emerging understanding of it's association with a wide range of adverse health effects and due to the fear of bioterrorism. It is established that long term exposure to high concentration of air borne microorganisms can cause a number of respiratory damage, allergenic and immune toxic effects.

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2. material and methods

.**2.1 Study area**

The study was carried out in Kwara State University’s Poultry farm Malete, Moro Local Government Area, Kwara State, Nigeria. Further analysis and processing of samples was carried out in microbiology laboratory, Kwara State University, Malete.

**2. 2** **Sterilization of Materials**

The glassware including test-tubes, conical flasks, measuring cylinders, McCartney bottles; which were used in the course of study were washed with detergent, rinsed with water, wrapped with aluminum foil and sterilized in the hot air oven at a temperature of 170oC for 1hour. Inoculating needles and wire loops were heated to redness under the flame of the Bunsen burner. All media were sterilized by autoclaving at 121oC for 15 minutes

***2.3*****Culture Media Preparation**

The workbench was disinfected using ethanol and cotton wool. The culture media used were nutrient Agar and Macconkey Agar respectively were prepared according to the manufacturer’s specification by weighing a known gram of the agar medium and dissolving in accurate medium of diluents, then well corked and agitated to mix. Media was later sterilized by autoclaving at 121oC for 15 minutes.

**2.4 Sample collections**

Using sedimentation method of sample collection, the samples were collected on agar plate. The sampling period were Morning (8am), Afternoon (2pm) and Evening (4pm). The samples were taken to the laboratory for incubation and further analysis.

**2.5. Microbiological Analysis**

**2.5.1. Isolation and Microbial Identification**

The culture media used were MacConkey agar and nutrient agar. Isolation was performed by pour plate method. All plates were incubated at 37˚C for 24hrs.

**2.5.2. Isolation of isolate**

The plates were then inverted and incubated at 37oc for 24hours. After incubation period, the plates were removed from the incubator, the colonies observed were counted and recorded, distinct colonies thereafter were aseptically picked and streaked on agar plate respectively to obtain pure isolates. The plates were then, incubated at 37oc for 24hours.

**2.5.3. Colony and Cellular Morphology**

The appearance of the colonies on each agar plate respectively, their relative size, color, texture, opacity, surface elevation, edge and shape was observed and then used to clarify their own growth pattern and to identify the bacterial types.

**2.5.3.1. Morphology of isolates**

Morphology refers to the arrangement and shape of bacterial cells and it is important for the identification of species. Several procedures were undertaken to determine the morphological characteristics of the isolates.

**2.5.3.2. Preparation of Isolates on Slants**

Slants were prepared by dispensing 20ml of molten nutrient agar into properly washed and sterilized Flavour bottles and allowed to solidify in a slanting position. The distinct pure isolates were then aseptically inoculated into the bottles and then incubated at 37oc for 24hours. The bottles containing isolates were then kept in refrigerator for further use.

**2.6. Gram’s staining**

The principle is to determine the cell morphology and the Gram’s reaction of the isolates. A thin smear was made using a 18-24hours old culture of the test organism on a grease free microscope slide. The smear was heat fixed using Bunsen burner flame. The heat fixed smear is then flooded with crystal violet for 60 seconds and then rinsed off with water. Iodine was then added for 1 minute and then decolourised with 70% ethanol for 3-5secs and rinsed with water. It was then counter stained with Safranin for 60seconds and rinsed off with water. The resulting stained smear was allowed to air dry and later viewed under the microscope. Gram positive organisms retained the colour of the primary stain, crystal violet i.e. appear purple. While Gram negatives organisms retained the colour of the secondary stain. i.e appears red when viewed under microscope. The principle behind the test is to know the ability of the isolates to absorb the primary stain due to the presence or absence of thick peptidoglycan layer of the bacteria cell.

**2.7. Biochemical Test**

Pure cultures of the test organisms as determined by their Gram reaction were used for the various biochemical tests. The presumptive isolates that are isolated from above method would be identified based on morphology, Catalase, oxidase test and other biochemical tests as recommended by using Bergey’s manual of systematic Bacteriology.

**2.7.1. Citrate Utilization Test**

The Citrate test uses a medium in which Sodium citrate is the only source of carbon and energy. If an organism can use citrate as the sole source of Carbon and energy, it will need to use ammonium salts for Nitrogen. This will result in the release of ammonia, causing a colour change in the medium from green to blue. Tubes of Simon’s citrate agar were each inoculated with a test organism and incubated at 350C for 48 hours. A change in the medium from green to royal blue was recorded as a positive test while a negative reaction indicate no colour change in the medium.

**2.7.2. Indole Test**

Indole, a nitrogen containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme Tryptophanase, was tested by inoculating tubes of peptone water with each of the test organisms. The inoculated tubes were incubated at 350oC for 24hour. After incubation, I ml of Kovac’s reagent was added to each tube, shake gently, and allowed to settle. A red colour on the surface layer of the broth indicates positive result while yellow appearance indicates negative result.

**2.7.3. Catalase test**

A loopful of the broth culture was transfered to the surface of a clean glass slide. A drop of 3%hydrogen peroxide was added on to the medium and was observed for the evolution of production of gas bubbles (effervescence) indicates a positive Catalase test i.e. the organisms has the ability to produce Catalase enzyme which causes a rapid break down of hydrogen peroxide to liberate water and oxygen. When no bubbles is produced, it indicates a negative result.

**2.7.4. Oxidase test**

The oxidase test detects the presence of a cytochrome oxidase system that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye-tetramethyl-p-phenylene-diamine. A strip of filter paper is soaked with a little freshly made 1% solution of the reagent. A speck of culture is rubbed on it with a platinum loop. A positive reaction is indicated by an intense deep-purple colour, appearing within 5-10 seconds, a “delayed positive” reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds.

**2.7.5 Sugar Fermentation Test**

The production of acid by fermentation of different sugar in Nutrient broth (10g), methyl red indicator (0.2g), distilled water. Exactly 9ml was dispensed into test tube with an inverted Durham`s tube. The broth was then sterilized by autoclaving at 121oC for 15 minutes. 10g of the sugar; glucose, galactose, maltose, fructose, were prepared and sterilized. 1ml of each of the sugar was then added to the methyl-red broth and was incubated for 5 days at room temperature. The broth was observed for acid production which was indicated by a colour change from red to yellow while gas production was indicated by collection of gas in the Durham`s tube.

3. results and discussion

**3.1** **Coliform counts from Air Samples (CFU/M3)**

The value of coliform counts (CFU/M3) from air sample are presented in Table 1. At 8am, the coliform counts (CFU/M3) per distance (feet) range from 34, 9 and 7 for 2feet, 4feet and 8feet respectively. At 2pm, the coliform counts (CFU/M3) per distance (feet) range from 8,6 and 4 for 2feet, 4feet and 8feet respectively. At 4pm, the coliform counts (CFU/M3) per distance (feet) range from 5, 3 and 2 for 2feet, 4feet and 8feet respectively.

**TABLE 1: Coliform counts from Air Sample (CFU/M3)**

|  |  |
| --- | --- |
| Period |  Coliform counts (CFU/M3) / Distance (feet) |
| 8AM | 34a ± 14.1 | 9a ± 0.7 | 7a ± 7.7 |
| 2PM | 8a ± 24.7 | 6a ± 4.2 | 4a ± 2.1 |
| 4PM | 5b ± 14.8 | 3b ± 10.6 | 2b ± 6.3 |

Values are mean of duplicates $\pm standard deviation$

**3.2 Total Bacterial Counts of Air Sample (CFU/M3)**

The value of total bacterial counts (CFU/M3) from air sample are presented in Table 2. At 8am, the total bacterial counts (CFU/M3) per distance (feet) range from 79, 66 and 54 for 2feet, 4feet and 8feet respectively. At 2pm, the total bacterial counts (CFU/M3) per distance (feet) range from 55,43 and 39 for 2feet, 4feet and 8feet respectively. At 4pm, the total bacterial counts (CFU/M3) per distance (feet) range from 39, 28 and 15 for 2feet, 4feet and 8feet respectively.

**TABLE 2 : Heterotrophic Bacterial Counts of Air Samples from poultry environment (CFU/M3)**

|  |  |
| --- | --- |
| Period |  Bacterial counts (CFU/M3/ Distance) |
| 8AM | 79a ± 8.4 | 66a ± 14.8 | 54a ± 12.0 |
| 2PM | 55b ± 16.9 | 43b± 41.0 | 39b ± 13.1 |
| 4PM | 39c ± 9.1 | 28c ± 2.8 | 15c ± 23.3 |

Values are mean of duplicate $\pm standard deviation$

**3.3 Bacterial Isolated From Air Samples**

The morphological and biochemical characteristics of bacterial isolates from Air Samples are presented in Table 3. A total of 5 bacterial genera were isolated (Table 3). They are:

**Isolate A1:** (+) Cocci, Probable Organisms: (*staphylococcus aureus*), Catalse: (+), Oxidase: (-), Indole: (-), Maltase: (+), Glucose: (+), Fructose: (+), Galatose: (+), Citrate: (+).

**Isolate A2:**(+) Rod, Probable Organisms: (*Bacillus*), Catalse: (+), Oxidase: (+), Indole: (-), Maltase: (+), Glucose: (+), Fructose: (+), Galatose: (+), Citrate: (+).

**Isolate A3:**(-) Rod, Probable Organisms: (*Escherisia coli*), Catalse: (+), Oxidase: (-), Indole: (-), Maltase: (+), Glucose: (+), Fructose: (+), Galatose: (+), Citrate: (-).

**Isolate B1:**(+) Cocci, Probable Organisms: (*Streptococcus species*), Catalse: (-), Oxidase: (-), Indole: (-), Maltase: (-), Glucose: (+), Fructose: (-), Galatose: (+), Citrate: (+).

**Isolate B2**: (-) Rod, Probable Organisms: (*Salmonella species*), Catalse: (+), Oxidase: (-), Indole: (-), Maltase: (+), Glucose: (+), Fructose: (+), Galatose: (-), Citrate: (-).

**TABLE 3:** Morphological and biochemical characteristics of bacterial isolated from Air samples.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **COLONIES** | **GRAMS REACTION** | **CELL MORPHOLOGY** | **CATALASE** | **OXIDASE** | **INDOLE** | **MALTASE** | **GLUCOSE** | **FRUCTOSE** | **GALATOSE** | **CITRATE** | **PROBABLE ORGANISM** |
| A1 | + | Cocci | + |  - | - | + | + |  + |  + |  + | *Staphylococcus aureus* |
| A2 | + | Rod | + |  + | - | + |  + |  + |  + |  + | *Bacillus spp* |
| A3 | - | Rod | + |  - | + |  + |  + |  + |  + |  - | *Escherisia coli* |
| A4 | + | Cocci | + | - | - | + |  + |  + |  - |  - | *Salmonella species* |
| B1 | + | Cocci | - | - | - | - |  + |  - |  + |  + | *Streptococcus species* |

Keys: +=Positive, -=negative**.**

**3. 4 DISCUSSION**

A total of five bacterial genera isolates were obtained from air samples. The microbial isolates include: *Staphylococcus aureus, Bacillus, Escherisia coli, Streptococcus species and Salmonella species*. The frequency of occurrence of bacteria isolated showed that *Staphylococcus aureus, Bacillus, Escherisia coli, Streptococcus species* and *Salmonella species.* Have the highest percentage frequency of occurrence and constituted the most dominant bacteria isolated from air sample in poultry environment, this is supported by *(4).*

*Staphylococcus aureus* belong to normal flora of the human skin and nose; it is likely that this organism may be originated from the nose and skin flora of the workers at Agric poultry farm and School poultry farm. However, this higher incidence of *Staphylococcus aureus* obtained from this study correlate with several and similar findings of the studies conducted by several researchers. A study conducted by *(5)* at Omdurman and El-Rhibat hospital Sudan found that *Staphylococcus aureus* was the predominant air bacteria isolated from these hospitals. This study also support the finding of *(6),* in which the occurrence was reported to be 38% in a research conducted to detect the airborne microorganism from a college in Saudi Arabia. This result is also inconformity with the result obtained by *(7)*, who reported *Staphylococcus aureus* as the highest bacteria isolated from their study. In the present study *Staphylococcus aureus* was the dominant isolated organism and this bacterium is a common causative agent of various human diseases, it is responsible for many gastrointestinal tract infections, respiratory tract infections and skin disorders *(5)*. Another pathogen *E. coli* (25%) which was also isolated is of medical concern. It is one of the most commonly examined Gram-negative bacteria in microbiology. Though it is well known that *E. coli* inhabits the human bowel as part of normal microbiota, some strains are capable of causing significant intestinal/diarrheal and extraintestinal infections *(8).* *E. coli* is a leading cause of urinary tract infections and intra abdominal infections in which the extent of the disease can range from cystitis to life threatening sepsis *(9).* It is well known that *E. coli* is the most common etiologic agent of urinary tract infections *(10).*

Also the isolation of *Streptococcus species* 21% is of great concern due to the fact that these bacteria are responsible for many cases of meningitis, endocarditis, bacterial pneumonia and necrotizing fasciilitis. The reasons for high percentage frequency of occurrence of bacteria in this study could be due to low minimal usage of disinfection procedures against airborne pathogens, more number of students attending lecture classes and low degree of hygiene practices.

4. Conclusion

Using the natural sedimentation technique, four genera of the organisms have been isolated comprising of *Staphylococcus aureus, Streptococcus spp, E. coli, Salmonella species* and *Bacillus*. And these organisms can cause several infections to the workers as well as inhabitants of this environment. In order to develop the quality of the poultry air in these farms, good ventilation systems has to be designed and good hygiene practice must be observed by the workers.

References

[1] Kołacz, R and Dobrzański, Z., (2006). Livestock hygiene and welfare. *Agricultural University* *in Wroclaw: Wroclaw*. p. 76–81; 85–90.

[2] Witkowska, D., Chorąży, Ł., Mituniewicz, T and Makowski, T., (2010). Microbial contaminations of litter and air during broiler chickens rearing. *Woda-Środowisko-Obszary Wiejskie*. 10:201–210.

[3] Lawniczek-Walczyk, A., Gorny, R.L., Golofit-Szymczak, M., Niesler, A and Wlazlo, A. Occupational exposure to airborne microorganisms, endotoxins and β-glucans in poultry houses at different stages of the production cycle. *Annals of Agricultural and* *Environmental Medicine*. 20:259–268.

[4] ] Prussin, A.J II., Garcia, E.B and Marr, L.C. (2015). Total concentrations of virus and bacteria in indoor and outdoor air. *Environ Sci Technol Lett*. 2:84–8.

[5] Yagoub, S.O and Elagbashi, A., (2010). Isolation of potential pathogenic bacteria from the air of hospital Delivery and nursing rooms. *Int J Appl Sci*, 10 (11): p 1011-1014.

[6] Sheik, G.B., Abd, A.l., Rheam, A.I., A.l., Shehri, Z.S and A.l Otaibi, O.M. (2015). Assessment of Bacteria and Fungi in air from College of Applied Medical Sciences (Male) at AD-Dawadmi, *Saudi Arabia. Int Res J Biol Sci*, 4(9): p48-53

[7] Badri, M.R., Alani, R.R and Hassan, S.S. (2016). Identification and characterization of air bacteria from some school of Baghdad city, *Mesop. environ. j.* 2(4): p 9-13

[8] ] Ferro, A.R., Kopperud, R.j and Hildemann, L.M. (2004). Source strengths for indoor human activities that resuspend particulate matter. *Environ Sci Technol*. 38: 1759–64.

[9] Ejrnaes, K., (2011). Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*. *Dan Med Bull*. **58**(4): p. B4187.

[10] Alós, J.I. (2005). Epidemiology and etiology of urinary tract infections in the community. Antimicrobial susceptibility of the main pathogens and clinical significance of resistance. *Enfermedades Infecciosas Microbiología Clínica*; 4:3-8.