# Bacteriological Assessment of Air Sample in Poultry Environment

ABSTRACT

|  |
| --- |
| **Aims:** This study aims to assess the bacterial load of air samples collected from Kwara State University poultry, Malete on the 24th of May 2022.**Study design:** The study utilized a cross-sectional design to assess microbial contamination in a poultry environment. It exposed nutrient and MacConkey agar plates for one minute at different times (8 am, 2 pm, 4 pm) and distances (2ft, 4ft, 8ft). Total bacterial and coliform counts were measured, and isolates were identified through morphological and biochemical tests.**Methodology:** The plates of solidifying nutrient agar and MacConkey agar were exposed in a poultry environment for 1 minute. 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 *Escherichia coli*. Since microorganisms found in the atmosphere are non-indigenous and usually introduced through human activities, the presence of these potential pathogens could constitute a health hazard not only to the workers but to the people around the poultry environment, thereby endangering the lives of community members through the spread of infectious diseases.**Conclusion:** Using the natural sedimentation technique, four genera of the organisms have been isolated *Staphylococcus aureus, Streptococcus spp, E. coli, Salmonella species,* and *Bacillus*. These organisms can cause several infections to the workers as well as inhabitants of this environment. To develop the quality of the poultry air in these farms, a good ventilation system has to be designed and good hygiene practices 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 excrement, 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 airflow 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 the 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), (4)* 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 passerby to the bioaerosol of poultry origin for an extended period during management constitute the need for this study to ascertain the air quality of these areas. The interest in bioaerosol exposure has increased over the last few decades, both due to the emerging understanding of its 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 concentrations of airborne microorganisms can cause several respiratory damage, and allergenic and immune toxic effects.

.

2. material and methods

.**2.1 Study area**

The study was carried out on 24th of may 2022 in Kwara State University`s Poultry farm Malete, Moro Local Government Area, Kwara State, Nigeria. Further analysis and processing of samples were carried out in the microbiology laboratory, Kwara State University, Malete.

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

The glassware including test-tubes, conical flasks, measuring cylinders, and McCartney bottles; which were used in the course of the 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 the accurate medium of diluents, then well-corked and agitated to mix. The media was later sterilized by autoclaving at 121oC for 15 minutes.

**2.4 Sample collections**

Using the sedimentation method of sample collection, the samples were collected on an agar plate. The sampling periods were Morning (8 am), Afternoon (2 pm), and Evening (4 pm). 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 the pour plate method. All plates were incubated at 37˚C for 24 hours.

**2.5.2. Isolation of isolate**

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

**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 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 24 hours. The bottles containing isolates were then kept in the 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 an 18-24 hour old culture of the test organism on a grease-free microscope slide. The smear was heat-fixed using a 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 decolorized with 70% ethanol for 3-5secs and rinsed with water. It was then counter-stained with Safranin for 60 seconds 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 color of the primary stain, crystal violet i.e. appeared purple. While Gram-negative organisms retained the color of the secondary stain. i.e. appears red when viewed under a 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 a 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 the 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 color 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 indicated no color change in the medium.

**2.7.2. Indole Test**

Indole, a nitrogen-containing compound formed when the amino acid tryptophan is hydrolyzed 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 24 hours. After incubation, I ml of Kovac`s reagent was added to each tube, shaken gently, and allowed to settle. A red color on the surface layer of the broth indicates a positive result while a yellow appearance indicates a negative result.

**2.7.3. Catalase test**

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

**2.7.4. Oxidase test**

The oxidase test detects the presence of a cytochrome oxidase system that will catalyze 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 color, appearing within 5-10 seconds, a “delayed positive” reaction by coloration in 10-60 seconds, and a negative reaction by the absence of coloration or by coloration later than 60 seconds.

**2.7.5 Sugar Fermentation Test**

The production of acid by fermentation of different sugars in Nutrient broth (10g), methyl red indicator (0.2g), and distilled water. Exactly 9ml was dispensed into a 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, and fructose, were prepared and sterilized. 1 ml 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 color change from red to yellow while gas production was indicated by a collection of gas in 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 samples is presented in Table 1. At 8 am, the coliform counts (CFU/M3) per distance (feet) range from 34, 9, and 7 for 2 feet, 4 feet, and 8 feet respectively. At 2 pm, the coliform counts (CFU/M3) per distance (feet) range from 8, 6, and 4 for 2 feet, 4 feet, and 8 feet respectively. At 4 pm, the coliform counts (CFU/M3) per distance (feet) range from 5, 3, and 2 for 2 feet, 4 feet, and 8 feet respectively.

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

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

Values are the mean of duplicates $\pm standard deviation$

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

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

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

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

Values are the 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*), Catalase: (+), Oxidase: (-), Indole: (-), Maltase: (+), Glucose: (+), Fructose: (+), Galactose: (+), Citrate: (+).

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

**Isolate A3:**(-) Rod, Probable Organisms: (*Escherichia coli*), Catalase: (+), Oxidase: (-), Indole: (-), Maltase: (+), Glucose: (+), Fructose: (+), Galactose: (+), Citrate: (-).

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

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

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

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

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

A total of five bacterial genera isolates were obtained from air samples. The microbial isolates include: *Staphylococcus aureus, Bacillus, Escherichia coli, Streptococcus species and Salmonella species*. The frequency of occurrence of bacteria isolated showed that *Staphylococcus aureus, Bacillus, Escherichia coli, Streptococcus species* and *Salmonella species.* Have the highest percentage frequency of occurrence and constitute the most dominant bacteria isolated from air samples in poultry environments, this is supported by *(5).*

*Staphylococcus aureus* belong to normal flora of the human skin and nose; this organism may likely have 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 correlates with several similar findings of the studies conducted by several researchers. A study conducted by *(6)* at Omdurman and El-Rhibat Hospital Sudan found that “*Staphylococcus aureus* was the predominant air bacteria isolated from these hospitals. This study also supports the finding of” *(7),* in which “the occurrence was reported to be 38% in research conducted to detect the airborne microorganism from a college in Saudi Arabia. This result is also in conformity with the result obtained by” *(8)*, 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” *(6)*. “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” *(9).* “*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” *(10).* “It is well known that *E. coli* is the most common etiologic agent of urinary tract infections” *(11).*

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

4. Conclusion

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

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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 chicken 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] Jilenkerian, B.K., Nisafi, A., Kara Ali, A., AI-Eissa, B. (2022). First study of the impact of the Syrian natural zeolite on air biological contamination concentrations in broiler farms during spring and autumn. *Asian Journal of Advances in Research*, 17 (4), p 84-92. Web of science.

[5] 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.

[6] 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.

[7] 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 the air from College of Applied Medical Sciences (Male) at AD-Dawadmi, *Saudi Arabia. Int Res J Biol Sci*, 4(9): p48-53

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

[9] 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.

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

[11] 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.