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MICROBIAL AIR ASSESSMENT TO ASCERTAIN ITS HEALTH CHALLENGES

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Abstract

This study was carried out in a domestic and commercial pig farms in Nnewi North to assess the microbial air quality. The air sample was collected through passive or sedimentation method into a plate containing well prepared. Nutrient agar and sabouraud dextrose agar which was exposed to trap the air and was analysed at the laboratory .The bacterial and fungal samples were incubated at 24 - 48hrs and 120hrs and 37°c respectively. The samples and ere sub cultured, slide cultured, stain application, molecular identifications were carried out on the fungal isolates and biochemical tests were carried out on the bacterial isolates. The bacterial isolates. The bacterial isolates identifies were Staphylococcus Aureus (41.67%), Clostridum Perfringes (25%), Lactobacillus casei (33.3%). They were all gram positive. In spore test L. Casei was negative, S. Aureus was negative and C. Perfringes was positive. In catalase test S. Aureus was positive while C. Perfriges and L. Casei were negative. In mannitol salt agar L. Casei was positive, S. aureus was positive and C. Perfriges was negative. In Coagulase- test it give the same result with catalase test. In blood agar S.Aureus showed B-Haemolysis. In motility test it gave the same result with the spore test. .In oxygen requirement test, L. Casei was a facultative anaerobe, S. Aureus was facultative Anaerobe and C. Perfringes was anaerobic. The fungal isolates identified were Aspergillius species, penicillum digitatum, graphium eumorphum, trichophytum, mentagrophytes, sporothnx schenkii. Their percentage of occurrence were 30%, 20%, 10%, 20%, 20% respectively. The analytical statistic for the parameters used to assess the microbial air quantity showed the significance of the sample (bacterial and fungal) is greater than or equal to the site[P> 0.05]. Conclusively,

this study reveals that ensuring a good air quantity in pig farm is important for the health of the human workers. It also shows that airborne bacteria and fungi contamination is high as well as representing a risk factor for the health of swine and its care takers.

Keywords: Aurus, Casei, Oxygen, Microbial Air

Introduction

Pigs are unique amongst farm animals because of their environmental needs. They lack an external insulating coat and modern genotypes bear little insulating fat; that is why pigs are commonly group housed within a compact air space. They are sensitive to draughts and have limited powers of thermoregulation, so their environment must be managed for them to remain within their comfort zone and keep healthy and productive. In particular new born and early weaned pigs are environmentally sensitive. Since growth and seed utilization in the early stages has a big influence on production efficiency, ensuring that pigs have the very best environment in their early days is crucial. It provides a unique opportunity to establish the pigs and set them up for efficient production.

In addition to thermal control, environmental management also involves maintaining good air quality. This not only improves pig health, performance and welfare but also reduces emissions of greenhouse gases odours and dust to the environment beyond the form. Keeping many pigs in a single airspace creates a lot of microbes, dust and noxious gases, all of which must be removed by the verification system.

Their alimination method will also benefit those who work in pig buildings. The problem is that if the ventilation system removes all of these, it will also remove the heat produced by the pigs, which is needed to keep them warm in cold weather (chambers et al 1999) social problems related to environmental pollution and industrial hygiene caused by the operation of intensive pig production have been raised as a result most pig farm managers have difficulties in keeping up with much governmental regulation which are imposed to prevent environmental contamination,

Ventilation is essential to prevent a buildup of microbes, dust and exhaled air in any building. It is also required to remove excess heat produced by the pigs in warm weather in other to prevent overheating. The minimal ventilation in cold weather can be calculated by working out how

much cabondioxide (co2) is building up in the room. More is produced as pigs grow and eat more food. The maximum ventilation in hot weather is used to remove heat produced bu the pigs as well as maintain an acceptable level of microbes and dust in the inspired air. Most ventilation system are designed to keep the inside temperature down to either 3 or 4c above the outside temperature. Pigs tend to lay where they are comfortable and dung somewhere else. In pens where the pigs have solid floors and no showers, they overcome heat problems by creating a "wallow" (rollover) with dung and urine. It provides a way for diseases such as to spread from pig to pig. It also encourages the release of ammonia into the atmosphere of the house from the decomposing and drying. Dirty pigs can also be prone to skatole taint which impacts on meat eating quality and consumer acceptability. (Miurhead and Alexander. 1997)

Open pig buildings are operated unhygienically under poor economic conditions. As a result, airborne contaminates generated in pig buildings have a potential to adversely affect the health of farmers and pigs, cause environmental and nuianse such as odour and help transmit infectious diseases (Clark *et al.,* 1983, Aarmink *et al.,* 1999).

Airborne contaminants in the building can be classified into gases, panticubites and airborne microorganisms (wathes, 1994) suspended microbial pathogens combined with solid particles, bioqerosol can cause infections, allergic diseases such as pneumonia, asthema and rhinitis. In farmers and pigs ensuring good air quality is important for the health and welfare of animals and care takers as well as for external environment. [Douham et al, 1990, Duchaine et al 2001].

Controlling the microbiological quality of the air in pigs houses is extremely important and should be performed regularly. Urgent hygienic measures are required in those barns where the microbiological air quality is poor, having significant deviation from the recommended standards. [Banhazi et al 2008]. The indoor and outdoor quality was established based on the number of airborne bacteria (mesophilic, staphylococci, streptococci; gram negative) and fungi globally, livestock production accounts for 18% of anthropogenic emissions of is not well established and bioaerosols could play an important role [Masclaux etal,2013].

Objectives:

This work is aimed to assessing microbial air quality in pig farms. Effects of housing conditions in pig farms.

Use and effectiveness of dust and participate controls. To identify numbers and species of microorganisms comprising of Bioaerosol associated with agricultural operation especially in intensive pig production.

Methods used to detect, collect Bioaerosols in agricultural including culturally microorganism end toxins.

Materials and method

Description of the sampling site

The study area is Nnewi North local government area. Nnewi city lies on the geographical co-ordinates of 6° 1'0'N, 6° 55' 0" E. It is situated at 6.02° North and 10. 0 latitude; 6.9, East and 8.0 longitude. It is 149 meters elevation above the sea level. Nnewi is a big town in Anambra, it comprises of commercial centuries, urban and rural areas.

The sample collection sites are visited on, the 12^{U}, May 2016 which include God is great farm a commercial pig farm located in a sub- urban area of Nnewi. It is situated before summit hospital, off Erne court road Okpunoegbu Umudim. Secondly, John Akuye pig farm a domestic' pig farm located in a rural. Area of Nnewi, it is situated after Saint Cletus Catholic Church Obiofia Otolo Nnewi.

Collection Of Sample

The air sample was collect through passive or sedimentation method. It was done by exposing the labeled NA Nutrient Agar and Sabour Dextrose Agar (SDA) containing four sterile petridishes at different elevations (up and down) in the pigs pen for thirty minutes at each sampling site, after which the petridishes were aseptically covered and carefully sealed with a masking tape and foil and then transported to the laboratory for further analysis. The nutrient agar containing petridishes were stored in the incubator at 35-37°c for 24-48 hours while the SDA containing petridishes were incubated at room temperature for 120hours.

3.2.1 The nutrient agar containing the mixed culture was observed after twenty four hours at which sample A and B from site A gave at four different colours. Which were yellow white, milkfish and red; some appeared to be flat, raised, circular while in site B gave off different colours such as yellow, milkfish, white, red, some were clustered. Both site A and B both gave the same colour.

The four samples (two samples for each site) were later counted using a standard

colony counter, the plates containing colonies within the ranges of 30-300 were counted.

The number of colonies were calculated using the following equation; Colony forming units per ml (efulml) =

$$\text{Number of colonies} \times \frac{1}{\text{dilution}} \times \frac{1}{\text{Amount plated}}$$

3.2.2 The SDA containing the mixed culture was observed after *5* days at which sample A and B inside A gave colours like brown, green, orange, grey and white. Some of which were cotton-like (flusly) while in site B; sample A and B gave colours like yellow, green, brown, white, milkfish. The four samples {two sample for each site} were later counted using a standard colony counter, the plates containing colonies with the ranges of 30 to 300 were counted. The numbers of colonies were counted using the following;

Colony forming units per ml (cftilml)

$$\text{Number of colonies} \times \frac{xl}{\text{dilution}} \times \frac{1}{\text{Amount plated}}$$

Preparation Of Media

The media used during the course of analysis were nutrient agar, sabar and dextrose agar and manifold salt agar. Nutrient agar is a general purpose media containing 0.5% peptone, 0.3% beefextract yeast extract, 1.5% agar, 0.5% Nacl.

According to manufacturer's instruction, 28g of the agar is to be dissolved in 100ml of water.

Sabouraud dextrose agar is a selective media containing 40g|L dextrose, 10g|L peptone, 20gL agar, PH 5.6.

According to the manufacturer's instruction 65g of the agar is to be dissolved in 1000ml of water 250ml of ciprofloxin is dissolved in 20ml of water and added to the sabouraud dextrose agar.

Mannitol salt agar is a selective and differential media containing 50gk enzymatic digest of casein, 5.0 g|L enzymatic digest of animal tissue, 1.0g|L beefextract, 10.0g|L D. monritol, 75.0gL Nacl, 0.025g|L phenol red, 15.0g|L agar,PH7.4 = 02at25°c.

According to the manufacturer's instruction, lllg of the agar was dissolved in 1000ml of water.

Sterilization Of Materials

As stated in prescolt *et al.* (2005), conical flasks, prepared media and other plastic material were sterilized by autoclaving at 121°^ for 15 minutes at a pressure of 15Psi.

Glass waves such as petridishes microscopic slides, measuring cylinder and other glass materials were sterilized in the laboratory hot air oven at 160°c for 1 hour before use.

Isolation of Bacterial Culture

The mixed culture contained in the nutrient agar petridishes were siib-cutaced using eight sterile petridishes' Nutrient agar was freshly prepared; it was sterilizes by autoclaving at 121°c for ISinins. An inoculating 1000 was sterilized by flaming and the freshly prepared medium was inoculated aseptically under sterile condition using each colony as innoculum from the mixed culture plate.

The inoculated plates were carefully covered with masking tape and then incubated at 35 - 37°c for 24-48 hours.

Isolation Of Fungal Culture

The plate containing fungal mixed culture as subcultured. Sabouraud dextrose Agar containing ciprofloxacin was freshly prepared and was sterilized by autocl'aving at 12l°c for 15 minutes. The subculture was carried out in an agar slant using a well labeled bijou bottle for each number of colony. An inoculating needle was sterilized by flaming and each colony was picked and dropped in the bijou bottle under sterile condition. The inoculated bijou bottle was properly covered and then incubated at room temperature for 3-5days.

SLIDE CULTURE

This is carried out by using one plate of freshly prepared SDA. Aseptically, with a pair of sterile forceps, place a sheet of sterile filter paper in a petridish, place a sterile u-shape glass rod on the filter paper (rod can be sterilized by flaming it help by forceps). Pour enough sterile water (about 4ml) on the filter paper to completely moisten it. With forceps, place a sterile slide on the u-shaped rod gently flame a scalpel to sterilize and out a 5mm square block of the medium from the plate of SDA. Pick up the block of SDA by inserting the scalpel and carefully transfer the block aseptically to the centre of the slide. Inoculate four slides of the agar square with spores or mycelia fragments of the fungus to be examined. Be sure to flame and cool the loop prior to picking up spores. Aseptically place a sterile coverslip on the upper surface of the agar cube place the cover slip on the petridish and incubate at room temperature for 7-10 days before making the stained slides.

APPLICATION OF STAIN

Place a drop of lactophenol cotton blue stain on a clean microscopic slide.

Remove the coverslip from the slide culture and discard the block of agar place the coverslip, mold side down on the drop of lactophenol cotton blue stain on the slide. Examine the slide under microscope.

IDENTIFICATION OF SELECTED BACTERIAL ISOLATES. 3.6.1 GRAM STAINING

This technique divides bacteria into Gram positive and Gram negative groups. A smear of the isolate was made on clean, dry grease free slide, using a sterile wireldop. The smear was air dried and heat fixed by passing over flame quickly as three times. The slide was flood with crystal violet and allowed it for linin. after which it was washed off with distilled water. After cooling the sliJc v/as flooded with Gram's iodine (which served as a mordant that fixes the dye inside the cell). The iodine was washed off after one minute and 70% alcohol was used to decolonize the smear for 30 seconds. The smear was counter-stained with safranin dye for one minute. It was then washed off and slide air dried, and observed under the microscope using oil immersion objective gram positive and negative reactions were indicated by purple and red colours respectively (Bottger 1996).

SPORE STAINING

In this method, smears of the isolates were prepared and fixed on a slide. The underside was vapor heated and flooded with 5% malachite green solution. Heating would continue until visible water condensate forms under the slide with evaporation at the top. It was washed using distilled water. Smears were counter stained with 0.5% safranin for 10 seconds. Slides were washed, dried and observed under oil immersion lens. A green space with in the cells indicates the presence of spores.

BIOCHEMICAL TEST

CATALASE TEST

The test identifies organism that produce the enzyme catalyse [Chears Brough 2000]. It was used to differentiate staphylococcus organisms which do not processes catalyse enzyme. A drop of 30% freshly prepared *hyd*^{r}*^gcn* ponoxidc was placed on a clean slid; loop full of isolate was transferred into it and emulsified. The appearance of gas bubbles indicates positive reaction.

MANITOL FERMENTATION TEST

The purpose of the test is to see if the microbe can ferment the carbohydrate (sugar) inanitol as a carbon source. If manitol is fermented to produce acid end products, the PH of the medium will drop.

A PH indicator in the medium charges color to indicate acid production. An inoculum from a pure culture is transferred aseptically to a sterile plate of phenol red mannitol agar. The inoculated plate is incubated at 35-37 c for 24hrs and the results are determined. A positive test consist of a color change from red to yellow indicating a PH change to acidic or magenta or hot pink in the presence of bases alkali(indicating a negative test).

COAGULASE TEST

This test helps to differentiate **staphylococcus aures** from other coagulase positive staph species congulase is an enzyme that cloths blood plasma, this test is performed on gram positive, catalase positive species to identify the coagulase positive staphylococcus aureus. The procedure follows thus:

Place a drop of distilled water on a slide. Emulsify the test strain to obtain in a homogenous thick suspension:

Observe for auto agiilatination.

Dip a straight wire loop into the plasma.

Mix gently with the homogenous suspension.

Suspension result shows inside dumping within 10 seconds.

BLOOD AGAR PLATE

This is a differential medium. It is a rich complex medium that contains *5%* sheep red blood cells. Blood agar plates tests the ability of an organism to produce haemolysis, enzymes that damage or lyse red blood cells (erythrocytes). The degree of haemolysis by these haemolysis is helpful in differentiating members of the genera Staphylococcus, Streptococcus and Entrerococcus.

a) Beta- haemolysis: this is complete haemolysis and is characterized by a clear(zone surrounding the colonies)

b) Partial haemolysis: it is also known as alpha- haemolysis. Its colonies are typically surrounded by a green opaque zone.

c) Gamma haemolysis: this is usually when haemolysis does not occur and there are notable zones around the colonies.

MOTILITY TEST

This test was carried out using a semi solid agar. It was prepared by adding 4g of bacteriological agar of 15g of nutrient both in 1 liter of deozonized water. Heat was applied to dissolve the agar and 10ml amount were dispenses into the test tubes and sterilized by out clearing. The test tubes were allowed to set in a vertical position. Inoculation was done by making a single stab down the center of the

medium using a sterile stabbing read! The test tubes were inoculated at 44°c and growth examined after 24 hours. Motile bacteria swarm gave a diffused spreading growth of bacteria that was visible to the eyes.

OXYGEN REQUIREMENT

This process was carried out in order to determine those organisms that can grow in an aerobic or anaerobic condition. Nutrient agar was prepared and allowed to gel. After which the subculture[1] bacterium colonies was collected using a sterile wire loop and inoculated on the surface of the prepared nutrient agar. The plates was then put in a container and placed with candle and covered. The container was incubated at 37°c for 24hrs. Growth shows that the organism can grow in an aerobic condition and no growth indicates that the organism can grow in an anaerobic condition.

Table 5: prevalence of bacterial source in the area studied.

Isolate	**A%**	**I**
Lactobacillus casei	3(25.00)	2(16.67)
Staphylococcus aiireus	2(16.67)	2(16.67)
Clostridium perfringes	2(16.67)	1(8.33)
Total	7(58.34)	5(41,67)

BACTERIAL COUNT

Four plates were counted using a colony counter. Each sample yield the following result listed below.

Table 6: Bacteria count

Sample	Site A	Siteb
A	200	110
B	108	70

The number of colonies in a cultural plate is calculated using the formula

colony forming unit (cfu/ml) =

Total colony x 10^3

Airflow rate xcollection time

Table 7: Test for significant values using "T" test for bacteria

Sites	Bacterial count in cfulm^{j}	Mean ± SD	T-test	Critical values
A	2.67xl0^{3}	2.(		
B	1.44xlO^{J}		1.29	4.30
C	1.47 x 10^{J}	L15±0.3 x 10^{3}		
D	0.93 x 10^{3}			

The table above shows the significant levels between two samples and sites.

FUNGI ISOLATES

Table 8: Isolates with their macroscopy & microscopy

Isolates	Macroscopy	
Aspergillus Niger	Colonies consists of a com basal fent covered by a d brown to black conidal heads.	Graphium Eumuphum	Colonies appear to be grayish wh maturation becomes grayish black
		Trichophyton mentagrophytes	Colonies pigmentation appears to be brown in color.
		Pernicillium Digitatum	Showing greenish colonies
		Sporothrix schenxit	Colonies are most filaments are a leathery to relvety with a finely wrinkled Color is white initially later changes to milkfish color.
		AspergilmS Athaceus	Colonies consist of a compact white o basal felt covered by adense brown, conidial heads which become radiate, tending to split several loose columns. Candiospores are smooth walled, hyaline or turmine dark towards the vesicle conidial heads are biserial with the phialides borne on
		Explanation for table 8: shows the	

occurrence of fungi isolated from both commercial and domestic piggery air tested using he slide culture technique and microscopy.	
Aspergillus Nomius	Colonies consists of a compact v basal felt covered by a dense laye to black conidial heads.

Table 9: Types of fungi with frequency of isolation from domestic and commercial piggery air.

Types of fungi	Frequenc
Aspergillus niger	3
Graphium Eumorphum	1
Trichophyton mentagrophytes	2
Penicillmm Digifatum	2
Sporothrix Schenxii	2

Table 10: prevalence of fungal species in the studied area

Isolate	A%
Aspergillus Niger	2(20.00)
Graphium Eumorphum	1(10.00)
Trichophyton mentagrophytes	1(10.00)
Pemcillium Digifatum	1(10.00)
Sporotlirix Schenkii	1(10.00)

FUNGAL COUNT

Four plates were counted using a standard and colony counter. Each sample yielded the following result listed in the table below.

Table 11: Fungal Count

Sample	**Site A**
A	40
B	83

The number of colonies in a cultured plate was calculated using colony forming

unit (cfulml) =

Total *colonies*x 10^3 *Air flow rate ^collection time*

Table 12: Test for significant values using student "T" test for Fungi

Sites	Fungal count in cfulm	Mean ± S
A	53 x 10^2	3,2
B	1.1 x 10^3	
C	7.3 x IQ^2	6.15
D	5.0 x 10^2	

The table above shows there is a significant difference between the sample and the site inferring that null hypothesis is accepted.

Discussion

The microbial air quality in pig farms in Nnewi North Local Government was carried out using sedimentation or passive air sampling method. This was done by placing the petridish.es containing the sun table media (NA, SDA) at different elevations in the pig pen which was taken to the -laboratory for further analysis. The bacteria were incubated for 24-48 hours while the fungus is s incubated for 120 hours at form temperature. The both gave off colonies the bacteria was subciiltui ed in a petridish to give a pure culture while the fungi was subcultured in bijou bottles. Identification of the isolated bacteria was Darned out by Gram staining to identify the gram negative and gram positive groups and the sporeformers were noted in this method as stated by (Bottges 1996) the isolate were smeared and fixed on a slide. Biochemical tests were carried out The bacteria isolated were lactobacillus easel, staphylococcus, and cj^tridium perfringes and their percentage of occurrence are 33.3% 41.7% and 25% respectively in the slide. Staphvlococcus aureus is the predominant organism in bioaersol of swine barns, lactobacillus case are found in the production of probiotics used in pigs feed while clostridium perfringes is the major toxin produced and is also the most wide spread being found as part of the microbial of the pigs. the fungal isoalates were identified were Aspergillus species, graphum eumorphum, trichophyton mentagrophytes, penicillum digitatum and sporotlirix schenxii and their percentage occurrence are 30%, 10%, 20%, 2%, 20% respectively,

Aspergillus specie at high level of exposure in the environment of the piggery increase the risk of respiratory disease, penicillum

specie expose the animals to mycotoxins through the consumption of mouldly infected feeds. Fungi can be said to be a part of microflora in animal houses but some species like pencillum species, Aspergillus species can cause asthora, allergies and fungal infections. The concentration of fungi varied in the studied pig farm with averages from $lOxlO^3$ to $3.3xl0^2$ and from $7.3xl0^2$ to $S.QxlO^2$ $cfulin^3$. These results correspond with other studies which mean that the total number of $aiibc^{rrip}$ fungi ranges from 10^3 to 10^6 $cfulm^3$ (Donham 2006, Chang et al., 2001),

The airborne flora is of great importance because of its pathogenic effect on the health of the pig (Koffret et al., 2003). The predominant microorganism from other studies were positive bacteria, Gram negative bacteria and fungi (Hartungi 1992) shows that the air borne flora in animal houses consist mainly of Gram positive bacteria such as staphylococci and streptococci and it is could be mainly because of their high resistance in the environment, other studies shows that the most frequent bacterial isolates were Gram positive bacteria up to 90% (Zucker et al., 2000) the analytical statistics used to asses the microbial air quality in farm A and B showed the significance of the sample is greater than and equal to the site ($P > 0,05$).

Conclusion

This study shows that air borne fungi and bacteria contamination is high and poses a high risk factor for the health of animals and caretakers. The caretakers can reduce exposure by wearing an approved mask especially when there is high concentration of aerial emissions e.g. sorting, weighing ensuring the beddings are clean, the animal feeders and the environmental agency should regulate intensive pig activities which a. key part is managing potential exposure to the public most especially people living around the pig farms to bioaerosol emissions.

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