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Isolation and Characterization of Microorganisms from Chicken Droppings

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Abstract
Humans have continued to research into the role of organic manure as alternative to inorganic manure. Poultry droppings have served as alternative source of nitrogen, phosphorous and potassium to crops. It is therefore important to investigate into the possibility of these droppings to serve as vector to zoonotic pathogens which may be detrimental to the health of the farmer, if not well managed. Isolation and characterization of microorganisms from some poultry houses were carried out. Poultry droppings were collected from poultry owners around Federal Polytechnic Nekede, Owerri, Imo State. About 100g of the sample was collected into a sterile container using sterile spatula and immediately taken to the laboratory in cold chain for analysis. Different media were used for the analysis and they were prepared based on manufacturers' specification. Tenfold serial dilution of the samples was made and spread plate technique was used in the isolation. Both morphological and biochemical characteristics were used in the identification of the isolates. The results obtained showed that microbial load of chicken droppings ranged from 1.0 x 10^3 to 2.0 x 10^5cfu/ml. Four bacteria genera- Staphylococcus, *Escherichia coli* Bacillus and Salmonella and four fungal genera - *Penicillium, Rhizopus, Alternaria and Sporotricum* were identified to be present on the chicken droppings. It can be concluded therefore that chicken droppings are potential vector of zoonotic pathogens hence should be handled with care when being evacuated and used as organic manure.

Keywords: Characterization, Droppings, Isolation, Poultry, zoonotic

Introduction

Poultry production has occupied a leading role in the agriculture industry worldwide in recent years. Poultry housing design plays a vital role in the determination of the internal climatic conditions of the house for optimum health, growth and productive performance of the birds (Oloyo, 2018). Microbial concentrations in poultry houses increase over time and contribute to the sick building syndrome. Very high and often logarithmic growth rates are reported for aerobic mesophilic bacteria, which account for the majority of known pathogenic bacteria. In addition to Gram-positive cocci (*Staphylococcus, Enterococcus*) and bacilli (*Bacillus*), other aerobic mesophilic bacteria include Gram-negative bacteria of the family *Enterobacteriaceae*, including *Escherichia coli, Salmonella* sp., *Shigella* sp., *Enterobacter* sp., *Proteus* sp., and *Klebsiella* sp., as well as *Pseudomonas* sp., *Acinetobacter* sp., *Flavobacterium* sp. (Dutkiewicz*et al.*, 2014) [2].

Some microbial species and serotypes, such as *Staphylococcus aureus, Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis, Trichophyton mentagrophytes*, and *Aspergillus fumigatus*, are pathogenic for animals and humans. Many bacteria and fungi are opportunists which are particularly dangerous for organisms with compromised immunity. Low hygiene standards and high levels of microbial and gaseous contamination may synergistically contribute to lower immunity and susceptibility to infections. The presence of microorganisms such as *Brevibacterium, Alternaria*, and *Cladosporium* in poultry houses indicates that microbes from the external environment, including soil, can spread to farm buildings (Vučemilo*et al.*, 2017) [8]. The prevalence of pathogenic microorganisms outside poultry buildings, even several kilometers away from the site, also indicates that ventilation air may contaminate the external environment. Broiler houses are particularly infested by fungi of the genera *Penicillium, Aspergillus*, and *Fusarium*, which are the main fungi producing pathogenic mycotoxins such as T-2 toxin, aflatoxin, ochratoxin, and zearalenone. Other toxigenic fungi species, for example, *Alternaria, Cladosporium, Trichoderma, Rhizopus, Stachybotrys*, have also been identified in poultry buildings. Even low concentration of mycotoxins is known to cause immunosuppression, allergies, inflammation of the respiratory tract, and they may have impact on growth parameters of birds (Vučemilo*et al.*, 2017) [8].

Several studies have demonstrated that the sources of microbial infections in poultry include contaminated feeds, drinking water, utensils, personnel, human wastes, rodents and hatchery related unhygienic activities (Okonko*et al.*, 2010) [5]. Recently, poultry feeds have been implicated in several poultry diseases of viral, bacterial and fungal origin, suggesting that such feeds can potentially act as carriers for human as well as animal pathogens (Musa *et al.*, 2014) [4]. Moreover, poultry housing and its environment like soil and drinking water, faeces, litters and wastes, live, moribund and dead chickens, meat, carcass, viscera, eggs, and poultry by-products could also carry microbes of public and veterinary health importance.

Sample Collection

The sample of the poultry waste was collected from poultry farmers around, Nekede, Owerri, Imo state. About 1 gram of the sample was collected in a sterile container using sterile spatula and immediately transported to the laboratory for analysis.

Sterilization of Glassware and Media

All glass wares needed for the experiment was sterilized so as to avoid contamination /alteration from other unwanted

isolates. The media used was autoclaved at 121 ^{0}C for 15 minutes.

Preparation of Media

All the media used for this project was prepared according to the manufacturers specifications and was sterilized at 121 ^{0}C for 15 minutes.

Viable Count of Bacteria and Fungi

Nine (9) ml of the diluent (distilled water) was dispensed into test tubes and 1 g of the dissolved poultry waste sample to be analyzed was taken with the aid of 5 ml sterile syringe and transferred to the first set of test tubes (dilution 10^{-1}). The 10^{-2} dilution was prepared by transferring 1 ml of dilution 10^{-1} into test tubes containing 9 ml diluent. Similarly, 1 ml of dilution 10^{-2} was dispensed into 9 ml diluent to obtain dilution 10^{-3}. The same procedure was used to make serial dilution of 10^{-4} to 10^{-6}. The spread plate was used in determining the viable count of the various samples of poultry waste.

Bacteria Culture

One milliliter (1ml) of the various dilutions was placed or transferred in the center of sterile Petri dishes using 5 ml sterile syringe. Molten cool agar that is (Nutrient agar for bacteria count) 20 ml was poured into the Petri dishes containing the inoculums (samples) and mixed well. After the solidification of the agar, the plates was inverted and incubated at 37^{0}C for 24-48 hours. Different bacterial colonies present on the plates was counted and multiplied by the reciprocal of the appropriate dilution factor to obtain the viable count.

Fungi Culture

The method stated above was used in determining the fungal count of the various samples but molten cooled Sabouraud Dextrose Agar (SDA) was used instead of Nutrient Agar (NA) as it is a selective medium primarily used for the isolation of dermatophytes, other fungi and yeasts. After the solidification of the agar, the plates were incubated at room temperature for three days. Different fungal colonies present on the plates was counted and multiplied by the reciprocal of the appropriate dilution factor to obtain the viable count.

Isolation of Bacteria and Fungi and Maintenance of Pure Culture

The spread plate method was used in isolating pure culture. Colonies that appeared on agar plate (Nutrient agar plates and Sabouraud Dextrose Agar plates) was sub-cultured aseptically by streaking into newly prepared agar plates using sterile wire loop. The streaked plates for bacteria was incubated for 24 hrs at 37^{0}C while the fungi colony morphology was identified directly from the plate after incubating for 3 days at room temperature. Pure colonies of bacteria was inoculated into slants and incubated for 24 hrs at 37^{0}C and kept in a refrigerator to keep the culture viable for further use.

Identification of Bacteria and Fungi from Poultry Waste Samples

Different biochemical tests were carried out to identify the isolates. These ranged morphological characteristics, Gram staining technique, motility, catalase test, coagulase test, oxidase, indole, methylene blue test, vogesproskauer, citrate, spore test, sugar fermentation and lactophenol cotton blue stain test.

Results

Result in Table 1 show the microbial load of the chicken droppings, from the result, TVC ranged from $2.0x10^3$ to 2.0×10^5 and TFC ranged from $1.0x10^3$ to $4.0x10^3$ (cfu/g)

Table 1: Total microbial load of the chicken dropping

Sample	TVC (cfu/g)	TFC (cfu/g)
A	$2.0X10^5$	$1.0X10^3$
B	$3.0X10^4$	$1.0X10^3$
C	$6.0X10^3$	$4.0X10^3$
D	$2.0X10^3$	$1.0X10^3$
E	$5.0X10^3$	$2.0X10^3$

Key: TVC= Total Viable Count, TFC= Total Fungal Count, cfu/g = colony forming unit per gram

Sample $_{A-E}$ = poultry droppings (waste) from farmers around Nekede, Owerri, Imo state.

The results obtained in Table 2 show the isolated bacteria present in poultry droppings include *Staphylococcus aureus, Escherichia coli, Bacillus* spp. and *Salmonella* spp.

Table 2: Morphological and biochemical Characteristics of Bacteria isolates from Chicken dropping

Samples	Media	Morphology	Gram Reaction	Oxidase	Motility	Indole	Spore test	Catalase.	Citrate	Coagulase	SFT S B GH$_2$S	Possible bacteria
Chicken dropping	N. A	Milikish raised non-mucoid colonies	Gram +cocci	-	-	-	-	+	-	+	No reaction	*Staphylococcus aureus*
Chicken dropping	N. A	Pinkish raised mucoid colonies	Gram -ve rod	-	-	+	-	-	+	-	Y Y+ -	*Escherichia coli*
Chicken dropping	N. A	Milikish flat non-mucoid colonies with rod rough edges	Gram - rod	-	-	-	+	+	-	-	R Y - -	*Bacillus* spp.
Chicken dropping	SSA	Milikish flat mucoid separated colonies	Gram - rod	-	-	-	-	-	+	-	Y Y +^{2} +	*Salmonella* spp.

KEY: N.A = Nutrient Agar, Morph= Morphological characteristics, Gram RXN = Gram reaction, Oxi = Oxidase test, Mot. = Motility Cat= Catalase test, Coag= coagulase test, Cit=Citrate test, SFT= Sugar fermentation test, +=positive, - =negative, S= Slope colouration, B=Butt colouration, G=Gas production, H_2S=Hydrogen sulphate production, Y=Yellowish colouration (acidic), R= Reddish pinkish colouration (alkaline production). SSA= Salmonella-Shigella agar.

From Table 3, the result obtained shows the presence of *Penicillium* spp., *Rhizopus* spp., *Alternaria* spp. and *Sporotrichum*spp. as the fungi present in the poultry droppings.

Table 4: Identification of fungal isolates

Sample	Macroscopic appearance on SDA	Microscopic Characteristics	Possible fungi
Chicken dropping	Whitish broom-like cottony colony with greenish center	Septate hyphae with conidia bearing sterigmaa	*Penicillium* spp.
Chicken dropping	Whitish broom-like cottony colony	Non-septate hyphae with terminal spore	*Rhizopus* spp.
Chicken dropping	Front pink reverse black capitates cottony colonies	Branched conidia	*Alternaria* spp.
Chicken dropping	Front cream reverses brown cerebriform and fluffy	Branched aseptate hyphae	*Sporotrichum*spp.

Discussion

The result obtained in this work is in line with the work of Wójcik*et al.,* (2010) [9], who reported the microbial composition of chicken dropping in the range of 10^9cfu/g.

According to Plewa-Tutaj*et al.,* (2014) [7], from their finding's poultry houses contain Gram-positive bacteria, including *Streptococcus, Staphylococcus, Micrococcus, Enterococcus, Aerococcus, Corynebacterium, Brevibacterium, Cellulomonas* and *Bacillus*, as well as Gram-negative bacteria, including *Escherichia, Enterobacter, Klebsiella, Proteus, Citrobacter, Pasteurella, Pantoea, Moraxella,* and *Pseudomonas*.

According to Abdalla *et al.,* (2013) [1], the levels of microbial contamination in broiler chicken farms may reflect the hygienic status of poultry meat production. Bacterial contamination on processed broiler carcasses may originate from environment, plant equipments and employees. Therefore, hygiene is an important factor to be considered in intensive poultry farms as it has considerable impacts on the health of both animals and humans working in the industry (Abdalla *et al.,* 2013) [1].

Abdalla *et al.,* (2013) [1], also reported that the microbial load of 10^{10}cfu/g poultry filter. However, the value obtained in this study was lower than those reported by the authors. The difference in the microbial load obtained in this study may be attributed to the age and nutrition of the birds used in this study. The bacteria isolates obtained included *Staphylococcus aureus, Escherichia coli, bacillus spp.* and *salmonella* spp. These pathogens can readily contaminate food produce if they are used as organic manure.

Wójcik *et al.,* (2010) [9], studied on the microbial air contamination in poultry houses in the summer and winter, from their result the following genera were isolated most frequently: *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., and *Scopulariopsis* sp. as well as, in substantially lower numbers, *Alternaria* sp., *Botrytis* sp., *Rhizophus* sp., *Rhizomucor* sp., and *Mucor* sp. Greater diversity of fungal flora was observed in the summer than in the winter. This most probably resulted from higher relative humidity levels during summer, which accompanied by high temperatures (22.9-24.5ºC) provided favorable conditions for microbial growth. As demonstrated by Karwowska, (2015) [3], fungi of the genera *Asperillus* sp., *Penicillium* sp., *Cladosporium* sp., and *Alternaria* sp. are most commonly encountered in poultry houses. The presence of isolated bacteria and fungi such as *Staphylococcus aureus, Escherichia coli, Bacillus* spp., *Salmonella* spp. *Penicillium* spp., *Rhizopus* spp., *Alternaria* spp. and *Sporotrichum*spp. in the poultry droppings have been reported in causing disease such as gasterointeristis, urinary tract infections, pneumonia, bacteraemia, secondary meningitis and wound infections, Bacteremia, eye-piercing, burn-out, skin, wounds and food poisoning (Vučemilo *et al.,* 2017) [8].

Conclusion and Recommendation

The outcome of this study has shown that chicken dropping contain pathogenic bacteria and fungi. The bacteria isolates was *staphylococcus aureus, Escherichia coli, Bacillus spp.* and *Salmonella spp.*, while the fungal species obtained were *Penicillium spp., Rhizopus spp., Alternaria* spp.and *sporotrichum*spp. The isolation of these bacteria and fungi is of public health concern. Chicken droppings should be treated before being used as an organic fertilizer to produce the microbial load and also prevent disease occurrences in consumers of these agricultural products. People who rear birds should ensure that the wash their hands thoroughly after contact with the chicken droppings before they touch foods to avoid zoonotic infections.

Conflict of interest

The authors declared that there is no conflict of interest regarding the publication of this manuscript

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