



Bacteria Isolated from the Oropharynxes of Apparently Healthy Chickens in Jos, Nigeria

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Received 10 August 2015; accepted 26 August 2015; published 1 September 2015

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Abstract

There are concerns that apparently healthy chickens may transmit potentially pathogenic pathogens that could threaten not only poultry industry, but also public health. This investigative study was carried out in Jos, Nigeria to determine the prevalent bacterial agents in the oropharynxes of apparently healthy chickens. Four hundred (400) oro-pharyngeal swabs were collected from 400 apparently healthy chickens for bacteriological analysis. Swab from each sample was cultured on 7% defibrinated sheep blood, Casein Sucrose Yeast (CSY) agar and MacConkey. Oro-pharyngeal swabs were cultured indirectly by inoculating into 5 ml of brain heart infusion broth (BHI), incubated at 37°C for 24 h and then streaked unto Casein Sucrose Yeast (CSY) agar, MacConkey agar and Blood agar. Presumptive colonies of bacterial agents were subjected to conventional biochemical characterization. The result of biochemical test identified the following bacteria: *Staphylococcus aureus* 82 (20.5%), *Escherichia coli* 53 (13.3%), *Klebsiella pneumonia* 35 (8.8%) and *Proteus species* 26 (6.6%), *Pasteurella multocida* 5 (1.3%) among others. *Staphylococcus aureus* (20.5%) was the highest followed by *Escherichia coli* (13.3%), *Klebsiella pneumoniae* (8.8%) and *Pasteurella multocida* (1.3%) was the least. It was concluded that the oropharynxes of apparently healthy chickens harbored a lot of bacterial agents which could cause infections when the immune system was compromised.

Keywords

Aerobic, Bacteria, Chicken, Jos, Nigeria

Subject Areas: Microbiology, Veterinary Medicine

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1. Introduction

Bacteria are known to be associated with a variety of poultry diseases. *Escherichia coli* is a common avian pathogen which contributes to enormous losses in poultry industry. *Bacillus subtilis* produces serine proteinase (trypsin and chymotrypsin) which contributes to causing mortality in affected birds [1]. *Staphylococcus* species cause acute death in laying birds and seem to be prevalent in tropical environment. *E. coli* and *Staphylococcus* species can also act as secondary bacteria to exacerbate clinical conditions leading to high mortality [2]. *Klebsiella pneumoniae* can occasionally cause embryonic mortality and severe losses in young chickens and turkeys [3]. *Aeromonas hydrophila* alone or concurrently with other organisms can cause localized and systemic infections in avian species including poultry [4]. *Proteus* species occasionally cause embryonic death, yolk sac infections, and mortality in young chickens, turkeys and ducks [5].

Despite the established roles of bacteria in causing opportunistic infections in poultry, there is a paucity of information on the prevalent bacteria in the oropharynx of chickens in Jos, Nigeria. This study is therefore aimed at determining the prevalent bacterial agents in the oropharynx of apparently healthy chickens in Jos, Nigeria.

2. Materials and Methods

2.1. Study Area

The study was conducted in Jos North and South Local Government Areas of Plateau state, which is located between latitudes 8.50°N - 10.46°N and longitudes 8.20°E - 10.36°E in the North-Central geopolitical zone of Nigeria. Plateau state shares common borders with four of the 36 states of the country. To the South-West is Nasarawa, while to the North-West and North-East are Kaduna and Bauchi respectively and to the South-East is Taraba state. The Jos terrain consists of a flat land resembling a table top, from which the name "Plateau" of the state is derived. According to the report of [6], at Jos, the average monthly temperature ranges between 21°C and 25°C (69°F and 77°F), the average humidity is 60% and the average annual rainfall is 1400 mm (56"). Owing to its high altitude placement, Jos has a much cooler weather when compared with most other parts of Nigeria. The vegetation of the area is extensive highland savannah with short grasses interspersed with very few trees. There are two major seasons on the Jos Plateau: A long dry season that commences at about the middle of October and ends in March. Jos receives an average of 1351 mm of precipitation annually, and up to 96 percent of the rainfall occurs between April and September of each year [7]. According to 2006 national Census of Nigeria Report [8], Jos North LGA has a land area of 291 km² and a population of 429,300, while Jos South LGA has a land area of 510 km² and a population of 306,716. The study area was purposively chosen for this study because of the availability of veterinary hospitals and clinics, high concentration of poultry farms and willingness of poultry owners and clinicians to cooperate with the researchers.

2.2. Collection of Samples

Poultry clinics, poultry farms and live birds markets were identified in Jos North (Railway-terminus and Yan Kaji) and South Local Government Areas (National Veterinary Research Institute canteen and Poultry Division Vom, Kugiya in Bukuru) for sample collection.

2.3. Sampling Method

Systematic random sampling method (one in five; every 5th bird on each visit) was applied for the selection of 400 apparently healthy chickens between November, 2010 and October, 2011 (8 chickens/week).

2.4. Sampling Locations

Sampling of apparently healthy chickens

Notable areas in Jos South such as National Veterinary Research Institute canteen and Poultry Division Vom and Jos North (Kugiya in Bukuru, Railway-terminus and Yan Kaji) were used for the collection of four hundred oro-pharyngeal swabs from apparently healthy chickens. Eighty oro-pharyngeal swabs were collected at each of the five locations.

2.5. Transportation of Samples

The samples collected were transported on ice to the Bacteriology Unit of the Central Diagnostic Laboratory, NVRI, Vom for culture and microbiological examination as described by [9].

2.6. Culture and Isolation of Organism

Oro-pharyngeal swabs were cultured indirectly by first inoculating each sample into 5 ml of brain heart infusion broth (BHI), followed by incubation of the broth mixture at 37°C for 24 h and then streaking loop samples of the broth culture onto media such as Casein Sucrose Yeast (CSY) agar, Blood and MacConkey agar. Colonies representing each bacteria species were identified and characterized according to the methods described by [10], while organisms belonging to the *Enterobacteriaceae* were identified using standard biochemical methods described by [10]. Presumptive *Pasteurella multocida* colonies were subjected to Gram and methylene blue staining for cellular morphology. Cultural and morphological examinations were conducted as described by [10]. Capsular and bipolar organisms were further confirmed as *Pasteurella multocida* by biochemical tests according to [9]. The biochemical reagents and tests used included: Triple sugar iron agar, urease, Simmons citrate, nitrate, indole, motility, methyl red and Voges Proskauer. Catalase, and coagulase tests were performed on presumed *Staphylococcus aureus* isolates.

2.7. Microbact Test

All *Pasteurella multocida*, *Escherichia coli*, *Aeromonas hydrophila* isolates recovered by biochemical tests were further subjected to additional analytical profile test using Oxoid™ Microbact GNB 24E kit (a commercially available biochemical test kit in microplate format for identifying *Enterobacteriaceae* and miscellaneous Gram negative bacilli) and this was done according to the manufacturer's instruction.

2.8. Statistical Analysis

Data generated was entered and processed using Microsoft Excel 2010. Descriptive statistical analysis was conducted using [11] and the results were summarized as percentages in a table.

3. Results

From the 400 oro-pharyngeal swabs collected, a total of 7 aerobic bacterial species were isolated from 250 (62.5%) samples, while the remaining 150 (37.5%) yielded no bacteria. Of the bacteria isolated, 3 (42.8%) were gram positive, while 4 (57.1%) gram negative. Bacteria isolated from the apparently healthy chickens indicated that *Staphylococcus aureus* 82 (20.5%) was the highest followed by *Escherichia coli* 53 (13.3%), *Klebsiella pneumoniae* 35 (8.8%) and *Pasteurella multocida* 5 (1.3%) was the least (**Table 1**). Microbact test identified *Pasteurella multocida* (1.0%) and *Escherichia coli* (7.5%) from the oro-pharyngeal swabs apparently healthy chickens.

Table 1. Bacteria isolated from oropharynx of apparently healthy chickens in Jos, Nigeria.

Bacteria	Number of bacteria isolated	Percentage (%)
<i>Staphylococcus aureus</i>	82	20.5
<i>Escherichia coli</i>	53	13.3
<i>Klebsiella pneumoniae</i>	35	8.8
<i>E. coli</i> and <i>Staphylococcus aureus</i>	16	4
<i>Pasteurella multocida</i>	5	1.3
<i>Streptococcus pneumoniae</i>	18	4.5
<i>Bacillus species</i>	15	3.8
<i>Proteus species</i>	26	6.5
No bacterial growth	150	37.5
Total	400	100

4. Discussion

Bacterial agents have been reported to contribute immensely to economic losses incurred in poultry production in Nigeria [12]. Some of the bacterial species isolated in the present study such as *S. aureus* and *Proteus* species are considered to be opportunistic invaders from environmental sources, while others (*E. coli*, *Klebsiella* species) are normal intestinal flora of poultry, but could cause infections whenever the immune system of affected bird is compromised [13]. It was observed that none of the chickens from which oro-pharyngeal swab was taken showed any clinical signs of disease, suggesting that the pathogens were present as subclinical infections, or as commensals in the chickens. Though the chickens appeared to be apparently healthy, the high prevalence of potentially pathogenic bacteria species isolated from their oro-pharynx could signify that they could possibly act as reservoirs for the bacterial species detected, with the potential risk of transmitting these pathogens to other poultry. The preponderance of these bacterial agents in apparently healthy poultry could possibly be responsible for their worldwide association with various disease conditions in a broad range of host including domestic and wild birds. This study also indicated that there were predominantly gram negative bacteria compared to gram positive. This finding is concurred to the report of [14]. The reflection of bacteria type identified in this study could have a significant impact on the chickens based on the severity of diseases they caused when the immune system was compromised. [2] reported that the activities of some aerobic bacteria, such as *E. coli*, *Staphylococcus* species and others could exacerbate clinical conditions leading to high mortality. The high isolation rate of *E. coli* in the oropharynx could probably be attributed to the ability of the organism to be acquired from the environment within the few days after hatching. This bacterium has also been reported to be one of the commonest bacteria that complicate both bacterial and viral diseases of poultry [15].

Despite the fact that the isolation rate of *P. multocida* was low, the implication of the isolation of this organism was that it could act as secondary invader during other diseases. These findings have also revealed that apparently healthy chickens can be carriers of *P. multocida*. [16] reported that *P. multocida* had been consistently recovered in the upper respiratory tract of apparently and clinically sick birds. This suggests that this bacterium might have played a significant role in the fulminating and high mortality encountered during fowl cholera outbreaks. The finding of this study conformed to the previous report of [17] who opined that in their study on relationship of *Pasteurella* isolated from free ranging chickens and contact animals that healthy chickens could be carriers of *P. multocida* which caused clinical disease when the immune system was compromised. In a similar study conducted by [18] also indicated that carriers of *P. multocida* might exist within poultry flocks with no history of previous outbreaks of fowl cholera and they might play a dominant role in the transmission of the disease. [19] asserted that the pathogenicity *P. multocida* increased by bird-to-bird passage over a short period of time; this may indicate that natural passage of *P. multocida* between the birds may enhance the virulence of a normally avirulent strain, leading to clinical disease in a flock of carriers. The isolation of these micro-organisms in the present study suggests their probable role in the epizootiology and source of infections to susceptible domestic birds, since oro-pharyngeal carriage contribute to the shedding of these bacteria, which may add to the rapid spread of infections for other domestic poultry.

The finding of this study has shown that aerobic bacterial agents were present in the oro-pharynx of apparently healthy chickens in Jos metropolis. Further study to elucidate the role and associated economic impact of these organisms during outbreaks is recommended.

Acknowledgements

The authors acknowledge the assistance of the Executive Director Veterinary Research, Vom, staff of Molecular Biology Department, National Veterinary Research Institute, Vom and Central Diagnostic Laboratory.

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