

Distribution of Aerobic Bacteria in Visceral Organs of Sick and Apparently Health Chickens in Jos, Nigeria

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Abstract

A study was conducted to determine the distribution of aerobic bacteria in visceral organs of clinically sick and healthy chickens between November, 2010 and October, 2011. A total of 2000 samples consisting of bone marrow, heart, liver, lung and spleen (400 each) were aseptically collected from 400 clinically sick chickens for bacteriology. Four hundred (400) oropharyngeal swabs were also collected from 400 apparently healthy chickens for bacteriological analysis. Swab from each sample was cultured on 7% defibrinated sheep blood, MacConkey and casein sucrose yeast agar. Presumptive colonies of bacterial agents were subjected to conventional biochemical characterization. The result of biochemical test identified the following bacteria species from the samples of clinically sick chickens; *Escherichia coli* (7.5%), *Staphylococcus aureus* (2.3%) and *Proteus vulgaris* (0.7%) among others. *Escherichia coli* and *Pasteurella multocida* were the most frequently isolated bacteria from the heart, whereas, *Staphylococcus aureus* was isolated mostly from the spleen. The monthly frequency of isolation of *P. multocida* showed that the bacterium was isolated mostly between the months of July and October. Bacteria isolated from apparently healthy birds indicated that *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 aerobic bacterial agents were present in the oro-pharynx of apparently healthy chicken and also widely distributed in visceral organs of clinically sick chickens in Jos, Nigeria.

Keywords: Aerobic, bacteria, chicken, viscera, Jos, Nigeria.

INTRODUCTION

Fowl cholera is a contagious bacterial disease affecting both domesticated and wild avian species (Glisson et al., 2003; Kwon and Kang, 2003). The disease is caused by *Pasteurella multocida*. It typically occurs as a fulminating disease with massive bacteraemia and high morbidity and mortality. Chronic infections also occur with clinical signs and lesions related to localized infections. The pulmonary system and tissues associated with the musculoskeletal system are often the seats of chronic infection (OIE, 2008).

Bacteria are known to be associated with a variety of poultry diseases. Some of these bacteria can act as primary causal agents or secondary opportunists in immunocompromised birds. *Escherichia coli* is common avian pathogen which contributes to enormous losses in poultry industry. *Bacillus subtilis* produces serine proteinase (trypsin and chymotrypsin) which contributes in causing mortality in affected birds (Glisson et al., 2003). *Staphylococcus* species cause acute death in laying birds and seem to be prevalent in tropical environment. *Klebsiella pneumoniae* occasionally cause embryonic mortality and severe losses in young chickens and turkeys (Orajaka and Mohan, 1985). *Aeromonas hydrophila* alone or in concurrently with other organisms, can cause localized and systemic infections in avian species including poultry (Glunder and Siegmann, 1989).

Proteus species occasionally cause embryonic death, yolk sac infections, and mortality in young chickens, turkeys and ducks (Baruah et al., 2001).

Despite the established roles of bacteria in causing opportunistic infections in poultry, there is a paucity of information on the distribution of aerobic bacteria in visceral organs of birds affected by fowl cholera. This study was therefore aimed at determining the distribution of aerobic bacteria in visceral organs of clinically sick and apparently healthy chickens.

MATERIALS AND METHODS

Study Area

The study was conducted in Jos North and South Local Government Areas of Plateau state, which is located between Latitudes 8.50° -100.46° N and Longitudes 8.20°-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.

Collection of Samples

Poultry clinics, poultry farms and live birds markets were identified in Jos North and South Local Government Areas for sample collection.

Sampling Method

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

Sampling Locations

(a) Sampling of clinically sick chickens

Three sampling points such as Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom, Plateau State Veterinary Hospital, Jos and ECWA Veterinary Clinic, Bukuru, Jos were used for the collection of tissue samples from sick chickens submitted for diagnosis. Tissue samples collected were heart blood, femur, lungs, spleen and liver (400 each from clinically sick chickens, giving a total of 2000 tissue samples). One

hundred and thirty three clinically sick chickens were sampled at each point.

(b) Sampling of apparently healthy chickens

Notable areas such as National Veterinary Research Institute canteen and Poultry Division Vom, Kugiya in Bukuru, Railway-terminus and Yan Kaji in Jos North 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.

Transportation of Samples

The samples collected were transported on ice in to the Bacteriology Unit of the Central Diagnostic Laboratory, NVRI, Vom for culture and microbiological examination as described by CLSI (2009).

Culture and Isolation of Organism

Each sampled organ was seared with spatula and incised with a small sterile scapel blade. Swabs from these organs were inoculated directly on media such as Casein Sucrose Yeast (CSY) agar, blood agar and MacConkey agar and incubated aerobically at 37°C for 24 h. Oro-pharyngeal swabs were cultured indirectly by inoculating into 5ml of brain heart infusion broth (BHI), incubated at 37°C for 24 h and then streak unto Casein Sucrose Yeast (CSY) agar, MacConkey agar and Blood agar. Presumptive *Pasteurella multocida* colonies were subjected to Gram and methylene blue staining for cellular morphology. Cultural and morphological examinations was conducted as described by Barrow and Felthan (2004). Capsular and bipolar organisms were further confirmed as *Pasteurella multocida* by biochemical tests according to CLSI (2009). Colonies representing each bacteria specie were identified and characterized according to the methods described by Barrow and Felthan (2004), while organisms belonging to the *Enterobacteriaceae* were identified using standard biochemical methods described Barrow and Felthan (2004). 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.

Microbact

All *Pasteurella multocida*, *Escherichia coli*, *Aeromonas hydrophila* isolates recovered by biochemical test were

further subjected to Analytical profile test using commercially available kit (Macrobact GNB 24E kit) Oxoid according to the manufacturer's instruction.

Statistical Analysis

Data generated was entered into Microsoft excel, while descriptive statistical analysis was conducted using statistical package for social sciences SPSS (version 12.01)

RESULTS

From the 2000 tissue samples consisting of bone marrow, heart, liver, lungs and spleen (400 each) were examined, a total of 13 aerobic bacterial species including *Escherichia coli*, *Staphylococcus aureus*, *Pasteurella multocida*, *Proteus species*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Salmonella gallinarum* were isolated. Others were *Staphylococcus epidermidis*, *Micrococcus species*, *Streptococcus pneumoniae*, and *Streptococcus faecalis* (Table 1). The distribution of bacteria in tissues of clinically sick chickens were: bone marrow 38 (9.5%), heart 95 (23.8%), liver 63(15.8%), lungs 72(18.0%) and spleen 52(13%). Bacteria were isolated from 151 (25.2%) samples, while the remaining 449 (74.8%) yielded no bacteria. *Escherichia coli* (7.5%) and *Staphylococcus aureus* (2.3%) were isolated from all tissue samples. *Escherichia coli* were the most frequently isolated bacterium mostly from the heart, followed by *Staphylococcus aureus*. *Proteus species* (0.7%) and *Bacillus subtilis* (0.8%) were isolated from almost all samples. All the seven *Salmonella gallinarum* isolates (0.9%) were obtained from liver samples. Bacterial isolated from apparently healthy birds indicated that *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 and were not found to be statistically significant ($P>0.05$) (Table 3). Microbact test identified *Pasteurella multocida* (1.0%), *Escherichia coli* (7.5%) and *Aeromonas hydrophila* (0.5%) isolates from clinical sick chickens cases.

DISCUSSION

Economic losses are incurred in poultry production in Nigeria due to viral, bacterial and fungal agents (Oladele and Raji, 1997). Isolation of concurrent bacterial agents in clinically sick and apparently healthy chickens has been reported by Masdoq et al. (2008) and Dashe et al. (2012). The resultant effect arising from concurrent or secondary bacterial infections are usually towards

producing increase severity of the clinical picture observed in the infections primarily initiated by other agents like *Pasteurella multocida*. 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 (Anonymous, 2006). Alexander (2000) reported that the activities of some aerobic bacteria, such as *E. coli*, *Staphylococcus species* and others can exacerbate clinical conditions leading to high mortality. The wide distribution of *E. coli* in the bone marrow, heart, spleen, liver and lungs of clinically sick chickens could probably indicate concurrent extra-intestinal infections. *Escherichia coli* are common avian pathogens mainly associated with extra intestinal infections collectively known as colibacillosis (Dias de Silveira et al., 2002). *Escherichia coli* produces serine proteases (*EspP*), an accessory virulence factor that is plasmid mediated which can exacerbate some disease conditions (Schmidt et al., 2001). This bacterium has also been reported to complicate viral diseases. Lewis (1997), documented that he isolated mostly *E. coli* in a study conducted on 8,000 nine-week-old Frazer Valley turkeys affected by H5N1 virus. This suggests that *E. coli* is one of the commonest bacteria that complicate both bacterial and viral diseases of poultry (Oladele et al., 1999). The none isolation of bacteria from most of the tissue samples of clinically sick birds could probably be due to indiscriminate administration antibiotics by poultry farmers whenever they notice any sign of a disease.

Although, *Pasteurella multocida* is known to have tissue tropism (Glisson, 2003), however, the profound debilitation seen in poultry affected by fowl cholera might have been exacerbated by most of these bacteria such as *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella gallinarum*, *E. coli* among others. All the seven *Salmonella gallinarum* isolates were recovered from the liver/ gall bladder, which supports the report by Robert (1975) that these organs serve as reservoir for *Salmonella gallinarum*. This finding indicates that liver are good samples for the isolation of *Salmonella gallinarum*. The high isolation rate of *Klebsiella pneumoniae* and *Streptococcus pneumoniae* from the lungs could possibly be responsible for the respiratory distress encountered in poultry affected by fowl cholera during outbreak. The isolation of *E. coli*, *Staph. aureus*, *Proteus specie* and *Pasteurella multocida* among others from tissues of apparently healthy birds suggest that poultry species are important reservoirs for these pathogens. These findings have also revealed that apparently healthy chickens can be carriers of *P. multocida*. This conforms with the previous report of Muhairwa and(2000) who pointed out in his study on relationship of *Pasteurella* isolated from free ranging chickens and contact animals that healthy

Table 1. Frequency of isolation and distribution of bacteria in tissues of clinically sick chickens in Jos, Nigeria.

Tissue samples							
Bacteria	Bone marrow	Heart	Liver	Lungs	Spleen	*Total	**Percentage (%)
<i>Escherichia coli</i>	24	53	20	19	20	156	7.5
<i>Staphylococcus aureus</i>	2	12	3	13	15	45	2.3
<i>Pasteurella multocida</i>	4	8	4	3	1	20	1.0
<i>Proteus species</i>	-	4	-	3	7	14	0.7
<i>Klebsiella pneumoniae</i>	-	1	2	14	-	17	0.8
<i>Bacillus subtilis</i>	5	-	2	2	8	17	0.8
<i>Salmonella gallinarum</i>	-	-	18	-	-	18	0.9
<i>Staphylococcus epidermidis</i>	1	1	1	1	-	4	0.2
<i>E. coli and Staph. aureus</i>	-	2	1	1	-	4	0.2
<i>Aeromonas hydrophila</i>	1	4	6	-	-	11	0.5
<i>Streptococcus pneumoniae</i>	-	3	2	12	1	18	0.9
<i>Pseudomonas aeruginosa</i>	-	2	1	4	-	7	0.3
<i>Micrococcus species</i>	1	3	1	-	-	5	0.25
Multiple bacterial isolates	-	2	2	-	-	4	1.6
No bacterial growth	362	305	337	328	348	1680	83
***Total	400	400	400	400	400	2000	100

* Total number of organs that yielded bacterial species.

** Percentage of organs that yielded various bacterial species.

*** Total number of each organ that yielded bacteria out of 400 each examined

Table 2. Distribution of bacteria in tissues of clinically sick chickens in Jos, Nigeria

Tissue	Num. of bacteria Isolated	Percentage (%)
Bone marrow	38	9.5
Heart	95	23.7
Liver	63	15.7
Lung	72	18.0
Spleen	52	13.0
Total	320	80.1

Table 3. Bacteria isolated from oropharynx of apparently healthy chickens in Jos, Nigeria

Bacteria	Number of bacteria	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 and 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 growth	150	37.5
Total	400	100

chickens can be carriers of *P. multocida* which cause clinical disease when the immune system is compromised.

This study has shown that aerobic bacterial agents were widely distributed in visceral organs of clinically sick chickens in Jos metropolis. Further study to elucidate the virulence factors and associated economic impact of these organisms during fowl cholera 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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