Distribution of Aerobic Bacteria in Visceral Organs of Poultry Affected By Highly Pathogenic Avian Influenza (H_5N_1) in Nigeria

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Abstract: A study was conducted to determine the distribution of aerobic bacteria in visceral organs of poultry affected by outbreaks of highly pathogenic avian influenza (HPAI) that occurred in Nigeria between December, 2006 and July, 2007. A total of 100 poultry from 114 commercial, backyard and free range flocks infected with Haemgglutinin neuramidase (H5N1) virus within the study period were sampled. The heart, liver/gall bladder, lungs, spleen, trachea and intestine from each poultry were aseptically collected for bacteriology. Collated data from the results were put on Microsoft excel and descriptive statistical analysis was carried out using statistical package for social sciences (SPSS) version 12.0. A total of 600 tissues were cultured for aerobic bacteria. Swabs from each tissue sample were cultured directly in Selenite F broth, MacConkey agar, 7% defibrinated Sheep Blood agar, and Eosin Methylene Blue agar. Biochemical tests were performed on presumed isolates for further confirmation. The number of birds in the affected flocks was 244,990. A total of 11 aerobic bacterial species were isolated. The frequency of bacteria by types of tissue was heart 48(8%), intestine 13(2.2%), liver 18(3%), lungs 32(5.3%), spleen 15(2.5%) and trachea 23(3.8%).

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1. Introduction

Highly pathogenic avian influenza (HPAI) is a viral disease affecting almost all domestic and wild birds (Easterday *et al.,* 1997; Alexander, 1999). The species of animals affected by AI include birds, seals, whales, humans, horses and swine (Webster *et al.,* 1992). Avian influenza virus belongs to the Family *Orthomyxoviridae* which include the genera *influenza A, B* and *C*. AI virus codes for 10 proteins including haemagglutinin (HA), neuraminidase (NA), protein matrix, (RNP) among others (Alexander, 1999; Swayne, 2003). There are 16 HA and 9 NA subtypes (Fouchier *et al.,* 2005). Avian influenza depresses the host immune system thereby paving way for opportunistic microbes to invade and exert an exacerbative effect resulting in high mortality in affected flocks (Aleksandr *et al*., 2004). 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 immuno-compromised birds. *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*). Bacillus subtilis* produces serine proteinase (trypsin and chymotrypsin). *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). Despite the established roles of bacteria as opportunistic infection in avian influenza, there is a paucity of information on the distribution of aerobic bacteria in visceral organs of poultry affected by HPAI outbreaks in Nigeria.

This study was therefore aimed at determining the distribution of aerobic bacteria in visceral organs of poultry affected by HPAI outbreaks in Nigeria.

2.0 Material and Methods

One hundred poultry were collected using simple random sampling from 114 commercial, backyard and free range flocks affected by HPAI in different parts of Nigeria. A total of 244,992 poultry were sampled.

Six samples consisting of heart, intestine, liver/gall bladder, lungs, spleen and trachea were collected from each of 100 HPAI affected birds, giving a total of 600 specimens. Samples were collected over a period of eight months from December, 2006 and July, 2007. The presence of H5N1 subtype virus was confirmed by the Viral Research Department of the National Veterinary Research Institute (NVRI), Vom,

Plateau State, Nigeria, using rapid antigen test, agar gel immuno-diffusion test, viral isolation in embryonated eggs, haemagglutination inhibition and reverse transcriptase polymerase chain reaction. All tissues were kept in double transparent polythene bags, labeled and preserved at -70^{0}C at the Central Diagnostic Department, NVRI. The tissues were later transported in a leak proof insulated box packed with ice to the Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria for bacterial isolation and identification.

2.1 **Bacterial Isolation**

Swabs aseptically collected from the heart, trachea, lungs, spleen and liver were cultured directly on 7% defibrinated sheep blood agar (BA) and MacConkey agar (MCA), while swabs from the intestine were enriched in Selenite F broth prior to plating on MCA. Isolates presumed to be *E. coli* were subcultured on Eosin Methylene Blue (EMB). All cultures were incubated aerobically at 37^{0}C for 24 h.

2.2 **Identification of Organisms**

Colonies representing each bacteria specie were identified and characterized according to the methods described by Barrow and Felthan (2004), while organisms belonging to the *Enterobacteriaceace* were identified using standard biochemical methods described Edwards and Ewings (1986).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.

2.3 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).

3.0 Results

From the 600 tissue samples (heart, intestine, liver/gall bladder, lungs, spleen and trachea) examined, a total of 11 aerobic bacterial species including *Escherichia coli*, *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Bacillus subtilis and Salmonella* Gallinarum were isolated. Others were *Staphylococcus epidermidis, Corynebacterium species, Streptococcus pneumoniae, Streptococcus faecalis and Citrobacter freundii* (Table 1). The distribution of bacteria by types of tissue was heart 48 (8%), intestine 13(2.2%), liver/gall bladder 18(3%), lungs 32(5.3%), spleen 15(2.5%) and trachea 23(3.8%). Bacteria were isolated from 151 (25.2%) samples, while the remaining 449 (74.8%) yielded no bacteria. *Escherichia coli* (8.3%) and *Staphylococcus aureus* (5.2%) were isolated from all tissue samples. *Escherichia coli* was the most frequently isolated bacterium mostly from the heart, followed by *Staphylococcus aureus*. *Proteus vulgaris* (1.8%) and *Bacillus subtilis* (1.3%) were isolated from almost all samples. All the seven *Salmonella* Gallinarum isolates (1.2%) were obtained from liver/gall bladder samples. Liver affected by *S.* Gallinarum were observed to be dark green in colour (bronze colour) (Figure 1).

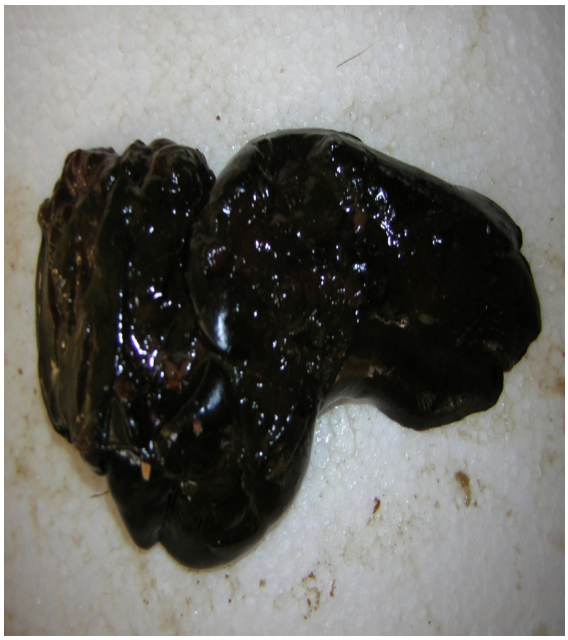

Figure 1: Bronze coloured liver due to *Salmonella gallinarum*; sample from chicken affected by HPAI Virus

4.0 Discussion

Economic losses are incurred in poultry production in Nigeria due to viral, bacterial and fungal agents (Oladele and Raji, 1997). Complications of avian influenza by bacteria has been reported by some workers (Lewis, 1997; Alexander, 2000). Some of the bacterial species isolated in the present study (such as *S. aureus, P. vulgaris, Corynebacterium 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).

Table 1: Frequency of isolation and distribution of bacteria in tissues of birds affected by highly pathogenic avian influenza virus (H5N1).

	Tissue samples							
Bacteria	Heart	Intestine	Liver/gall bladder	Lungs	Spleen	Trachea	Total	**Percentage (%)
Escherichia coli	36	1	1	4	1	6	49	8.3
Staphylococcus aureus	4	5	5	8	6	3	31	5.2
Proteus vulgaris	2	-	-	3	2	4	11	1.8
Klebsiella pneumoniae	-	-	1	6	-	2	9	1.5
Bacillus subtilis	-	1	-	3	3	1	8	1.3
*Salmonella G*allinarum	-	-	7	-	-	-	7	1.2
Staphylococcus epidermidis	4	-	-	-	1	2	7	1.2
E. coli and Staph. aureus	1	-	1	3	-	1	6	0.8
Corynebacterium species	-	1	1	1	-	1	4	0.7
Streptococcus pneumoniae	-	-	-	3	-	1	4	0.7
Streptococcus faecalis	1	1	-	-	-	1	3	0.5
Citrobacter freundii	-	2	-	-	-	-	2	0.3
Multiple bacterial isolates	-	2	2	1	2	3	10	1.6
***Total	**48**	**13**	**18**	**32**	**15**	**25**	**151**	**25.2%**

* 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 100 each examined

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 during HPAI outbreaks. The wide distribution of *E. coli* in the heart, trachea, spleen, liver and lungs of birds affected by H5NI could probably indicate concurrent extra–intestinal infections. The result of this study as well as that of Lewis (1997), who isolated mostly *E. coli* in a similar study conducted on 8,000 nine-week-old Frazer Valley turkeys affected by H5N1 virus, suggest that *E. coli* is one of the commonest bacteria that complicates avian influenza (H5N1) during outbreaks. A low isolation rate of *E. coli* was observed from the intestine. This could probably be due to indiscriminate administration of gut active antibiotics by poultry farmers whenever they notice any sign of a disease. Although HPAI virus is known to have tissue tropism (Rott, 1992; Shinya *et al.,* 2004), however, the profound debilitation seen in poultry affected by HPAI might have been exacerbated by most of these bacteria such as *Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae*, *Corynebacterium Specie*, *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*. The isolation of *Klebsiella pneumoniae* and *Streptococcus pneumoniae* (from the lungs and trachea) could possibly be responsible for the respiratory distress encountered in poultry affected by HPAI during the outbreak. This study has shown that aerobic bacterial agents were widely distributed in visceral organs of poultry affected by HPAI outbreak between December, 2006 and July, 2007. Further study to elucidate the virulence factors and associated economic impact of these organisms during HPAI outbreaks is recommended.

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