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Vibrio vulnificus and Proteus vulgaris Co-infection Associated with High Mortality in a Flock of Turkey in Ado Ekiti, Nigeria

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Abstract
The study reports bacteriological investigations carried out on a case of high mortality (10%) reported over a period of one week in a flock of turkey, 450 in number, aged 16 weeks old, in a poultry farm in Ado-Ekiti, Nigeria. Clinical signs were those of weakness, recumbence, diarrhoea (yellowish to greenish in colour) and weight loss. Post morterm examination was that of enteritis and mild liver enlargement, with no major pathognomonic lesions. Bacteriological investigations were carried out on clinical (Blood, Liver, Heart Muscles and Bile) and environmental (feed, drinking water and litter) samples. Bacteriological studies of the clinical samples obtained from dead birds revealed a co-infection of *Vibrio vulnificus* and *Proteus vulgaris*. The clinical bacterial isolates showed highest susceptibility to Ofloxacin (75%), with varied levels of susceptibility to other antibiotics tested. Mortality on the farm was curtailed following an oral medication with norfloxacin. A variety of bacteria, including *Vibrio vulnificus* and *Proteus vulgaris,* were isolated from the environmental samples. The bacterial isolates from the environmental samples were resistant to multiple drugs. Since the bacteria implicated in the clinical infection were isolated from environmental samples, an adequate biosecurity measures is needful in poultry farm for profitability.
Keywords: Antibiotic-resistance, Bacteria, Co-infection, Mortality and Turkey

Introduction

Poultry are domesticated birds reared by humans for eggs, meat, feathers, and sometimes as pets. Poultry also includes other birds that are killed for their meat such as the pigeons. The word 'poultry' comes from the French/Norman word poule, itself derived from the latin word pullus, which means small animal. (Crawford, 1990)

Poultry and farm animals are large reservoir of several bacterial species (Ojo, 1993; Olson and Bark, 1996; Cole *et al.,* 2000; Opplinger, 2008). Several pathogenic bacteria species cause various diseases of poultry resulting in heavy mortalities and some of these bacteria are zoonotic (Ojo, 1993; Olson and Bark, 1996; Okiki and Ogbimi, 2010). The evolution of resistance to common antibiotics by bacteria as a result of heavy usage of antibiotics to enhance livestock production performances has been reported (Ojeniyi, 1989; Tenover, 1996; Mculin, 2001; Okiki and Ogbimi 2010).

Over the past half century, food- animal production has changed from a largely entrepreneurial system run by independent farmers to an industrial mode of production in which a small number of companies control all aspects of production, from breeding and feed formulation to slaughter and distribution of consumer products. This shift in both the organization and methods of production has allowed for the reliable, high-throughput production of animals on a scale not seen in human history (Lance *et al*., 2007).

A 2011 study by the Translational Genomics Research Institute showed that 47% of the meat and poultry sold in United States grocery stores was contaminated with *Staphylococcus aureus*, and 52% of the bacteria concerned showed resistance to at least three groups of antibiotics. Thorough cooking of the product would kill these bacteria, but a risk of cross-contamination from improper handling of the raw product is still present (Waters *et al*., 2011). Also, some risk is present for consumers of poultry meat and eggs to bacterial infections such as *Salmonella* and *Campylobacter*. Poultry products may become contaminated by these bacteria during handling, processing, marketing, or storage, resulting in food-borne illness if the product is improperly cooked or handled (FAO, 2013).

The aim of the study was to investigate the cause of mortality in a flock of turkey in a poultry farm in Ado Ekiti, Nigeria, by Isolating and characterizing pathogenic bacteria from organs of dead turkey, as well as from feed, litter, faeces, and drinking water in the pen so as to proffer solution and prevent such future occurrence on other farms.

Materials and Methods

Study area and period

The research was carried out on a flock of turkey with high mortalities in the poultry unit of a farm in Ado-Ekiti, Nigeria. The turkeys were 450 in number and aged 16weeks before the outbreak of disease leading to high mortalities in November 2015.

Sample collection

Fifteen randomly selected freshly dead turkey birds, five per day, were brought from the turkey farm to the Microbiology Laboratory, Afe Babalola University Ado-Ekiti, Nigeria. Following autopsy, samples were obtained from the blood, liver, kidney, bile, heart and spleen of birds into sterile bottles for bacteriological investigations. Also from the turkey pens were collected samples of feed, drinking water, and litter for bacteriological studies. Samples that were unable to be processed immediately were stored in the refrigerator at 4ºC.

Isolation, characterization and identification of organisms

Weighed samples were inoculated into sterile Nutrient broth and incubated at 37ºC for 24 hours. After incubation for 24 hours, a loop- full from the Nutrient broth was transferred and inoculated on blood agar, MacConkey agar, MacConkey broth, Sorbitol MacConkey agar, Eosin methylene agar and Deoxycholate agar. All isolates were subcultured to obtain pure cultures. The bacterial isolates characterised and identified based on microscopy and standard biochemical tests as described by Barrow and Feltham (1993) and Cheesbrough (2010). Bacterial isolates were identified with the help of online Gideon informatics (1994-2016) with reference to Garrity *et al.* (2005).

Antibiotics Susceptibility Test

All the isolated organisms were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method on Muller-Hinton agar. The plates were incubated aerobically at 37ºC for 24 hours after which the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards institute (2013). Antibiotics used were Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Amoxycillin/ Clavulanate (30 µg), Nitrofurantoin (300 µg), Ampicilin (10 µg).

Results

Mortality (10%) over a period of 1 week was reported among a flock 450 turkeys, aged 16 weeks, in poultry farm in Ado Ekiti, Nigeria, in November 2015. Clinical signs were those of weakness, recumbence, diarrhoea (yellowish to greenish in colour) and weight loss. Post morterm examination was that of enteritis and mild liver enlargement, with no major pathognomonic lesions.

Bacteriological investigation of the clinical samples (Blood, Liver, Heart Muscles and Bile) revealed a co-infection of *Vibrio vulnificus* and *Proteus vulgaris* (Table 1). The clinical bacteria isolates showed high resistance to the antibiotics tested; 100% resistance to Ceftazidime with Ofloxacin recording the highest susceptibility value (75%) (Table 2).

Bacterial isolated from water samples collected from the drinkers were *Vibrio natiensis, Luteococcus sanguinis, Corynebacterium accolens, and Propinobacterium acnes*. The Feed samples collected directly from the feeders were found to have bacterial load of 3.61×10^4 CFU/g and bacteria isolated were *Arthrobacter woluwensis, Klebsiella granulomatis, Klebsiella pneumonia, and Vibrio vulnificus* . The Litter was found to have a bacterial load of 5.0×10^4 CFU/g. The bacteria isolated from the litter and faecal samples were *Vibrio vulnificus, Proteus vulgaris, Arthrobacter woluwensis, Branchiibius cervicis, Corynebacterium accolens, Corynebacterium argentoratense. Dermatophilus congolensis, Arthrobacter cumminsii, Propinobacterium acnes* and *Brachiibius cervcis.* The bacteria isolated from feed, drinking water and litter were resistant to multiple drugs (Table 3).

Discussion

Turkeys are large birds, their nearest relatives being the pheasant and the guinea fowls (Smith, 2006). Many different breeds have been developed, but the majority of commercial turkey birds are white, with improved dressed carcass (Pond *et al.,* 2010).

Mortality (10%) occurred in a 16 week old flock of turkey in a poultry farm, during the outbreak reported in this study. In turkey rearing, mortality is usually high in young birds but could be much higher in older birds (Kahn *et al*., 2010). Bacteriological investigation of the clinical samples (Blood, Liver, Heart Muscles and Bile) revealed a co-infection of *Vibrio vulnificus* and *Proteus vulgaris.* These bacterial isolates associated with the mortality were equally isolated from the poultry farm environment i.e. their feed, faeces, litter and drinking water. This could be an indication of lack of adequate biosecurity on the farm.

The high level of resistance to antibacterial agents reported in this study is food for taught, because antibacterial agents are commonly used as either growth promoter or for prophylaxis. The quinolones and penicillins were worst affected, while Gentamicin and Nitrofurantoin recorded high effectiveness. This resistance patterns could be due to drug misuse and abuse by farmers. Resistance was equally obtained against antibacterial agents that are not commonly used in Nigeria poultry industry, such as Cefuroxime and Ceftazidime.

Multidrug resistant gram-negative bacteria have been emerging worldwide (Yamane *et al.,* 2005) and resistance to antibiotics could be plasmid or chromosomal mediated (Okiki *et al.,* 2013). Diagnostic uncertainty has however been reported as key driver of drug misuse and overuse, which can lead to antimicrobial selection pressure and increased rates of resistant microbes. It has been reported that the risks associated with untreated

microbial infections and the lack of accurate clinical and laboratory prediction methods on results in low thresh hold for initiating empirical antimicrobial drug therapy (Nuermberger and Bishal, 2004; Fischer *et al.,* 2004).
Vibrio vulnificus isolated from clinical samples in this study are pathogenic bacteria of the genus *Vibrio*, and are related to *V. cholerea*, the causative agent of cholera (Oliver, and Kaper 2001; and Oliver 2005). It has been reported that Infection with *V. vulnificus* could lead to rapid expanding cellulitis or septicaemia. (James *et al*., 2006). McMullin (2004) reported vibrio hepatitis in chickens, transmission was said to be by faecal contamination; the birds remained carrier for months and disease precipitated by stress. The clinical signs reported were those of dejection, diarrhoea, loss of condition, inappetence, pale comb, wattles and jaundice among others.

Proteus vulgaris was implicated in mortality in this study. *Proteus* species are found in soil, water and are opportunistic pathogens of humans and animals. *Proteus vulgaris* is known to cause wound infections and other species of the genus are known to cause urinary tract infections (Struble, 2009 and O'Hara, 2000). *Proteus* species occasionally cause embryonic death, yolk sac infections, and mortality in young chickens, turkeys and ducks (Baruah *et al.,* 2001). Yolk sac infection is the main infectious cause of chick mortality during the first week of the post-hatching period (Rai *et al.,* 2005 and Yarssin *et al*., 2009); accounting for large economic losses to the poultry industry (Ulmer, 2011). It can cause mortality rate of about 5-10%; however the condition has also been associated with much higher mortality especially in chicks during first week of age (Rahman *et al.,* 2007). Contamination of unhealed navels has been suggested as a cause of yolk sac infection in newly hatched chicks (Fasenko and O'Dea, 2008).

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Table 1: Bacteria isolated from clinical samples and environmental samples in the turkey pen

Sample	**Bacteria isolated**
Clinical samples (Internal organs)	*Vibrio vulnificus, Proteus vulgaris*
Environmental samples:	
Water from drinkers	*Vibrio natiensis, Luteococcus sanguinis, Corynebacterium accolens, Propinobacterium acne.*
Feed from the Feeder	*Arthrobacter woluwensis, Klebsiella granulomatis, Klebsiella pneumonia, Vibrio vulnificus.*
Litters	*Vibrio vulnificus, Proteus vulgaris, Dermatophilus congolensis, Arthrobacter woluwensis, Arthrobacter cumminsii, Propinobacterium acnes, Brachiibius cervcis, Corynebacterium accolens, Corynebacterium argentoratense.*

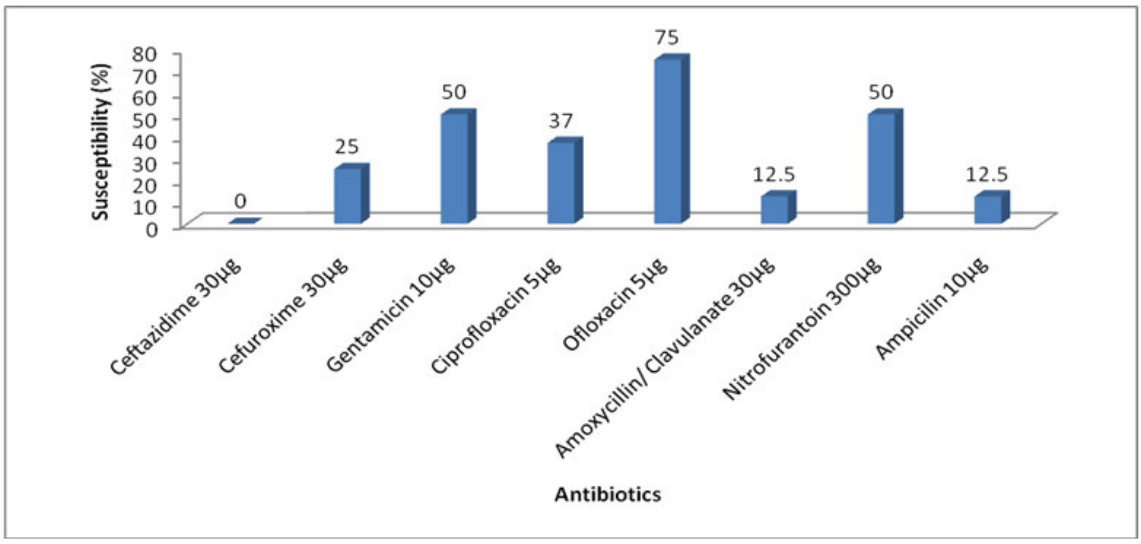

Figure 1: Antimicrobial susceptibility patterns of bacteria isolated from internal organs

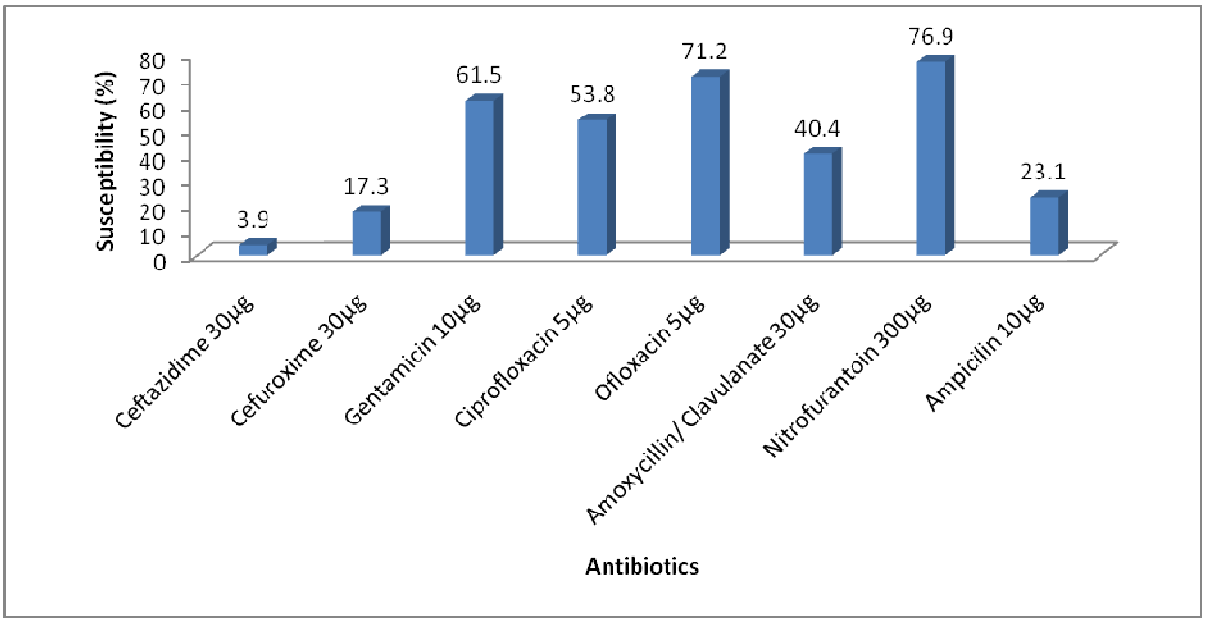

Figure 2: Antimicrobial susceptibility pattern of bacteria isolated from feed, water and litter.