Full Length Research Paper

# Preliminary Report on Isolation of *Morganella Morganii* from Fatal Infections of Chickens in Kaduna State, Nigeria

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Abstract

A two-year study was carried out between February 2011 and January, 2013 to isolate and characterize *Salmonella* from cases of suspected fowl typhoid and Pullorum disease in chickens in Kaduna, Nigeria. *Morganella morganii* was isolated in pure culture from many of the fatal cases suspected to be fowl typhoid or Pullorum disease. *Morganella morganii*, a Gram-negative rod commonly found in the intestines of humans and other animals, was isolated in fatal infections in chickens by culture, isolation and biochemical characterization using conventional means and commercial kits (Microbact<sup>®</sup> GNB 24E and api<sup>®</sup>NH). Biochemical tests revealed that the isolates were positive for: catalase, nitrate, citrate, urease, methyl red, and indole; and negative for oxidase and Voges Proskauer; they were fermenters on Oxidative-fermentative test. Some important pathologic lesions noticed in birds at post mortem included: enlarged and congested spleen, enlarged congested and friable liver with areas of necrosis, congested lungs; congested, mis-shapened and regressed ovaries. Experimental infection of chickens with the organism is hereby recommended. There is also the need for further characterization of isolates to determine the strains and antibiotic resistance genes.

Keywords: Morganella morganii, Chicken, Kits, Biochemical tests.

# INTRODUCTION

Within the family *Enterobacteriaceae*, the genus *Morganella* is a member of the tribe *Proteeae* which also includes the genera *Proteus* and *Providencia*. The genus *Morganella* consists of one species, *Morganella morganii*, with two subspecies, *morganii* and *sibonii* (Stock and Wiedemann, 1998; O'Hara et al., 2000). In humans, *M. morganii* has been reported to be mostly found in opportunistic and nosocomial infections (Cetin et al., 2008; Ghosh et al., 2009; Lee et al., 2009; Singla et al., 2010; Nakao et al., 2013). The bacterium has been reported in some mammals, reptiles and seals to

cause a wide range of symptoms including pneumonia, peritonitis, empyema, pericarditis, arthropathy, endophthalmitis, meningitis and wound infections (Heard et al., 1988; Thornton et al., 1998; Ono et al., 2001; Choi et al., 2002; Roels et al., 2007; Moyaert et al., 2008; Vandenberge et al., 2013). Generally there have been very scanty reports on Morganella morganii isolated from birds. It has, for instance, been reported to be isolated among the bacterial microbiota of the ocular surface of microbats (Leigue Dos Santos et al., 2013); in poultry, it has been reported to cause fatal infection in chickens (Zhao et al., 2012) and has been isolated from chicken carcasses (Kilonzo-Nthenge et al., 2013). In 2012, there was a report on the sequencing of the whole genome and identification of the pathogenicity-related genes of the KT strain of Morganella morganii isolated from a

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human patient in Taiwan (Chen et al., 2012). To the best of our knowledge, this will be the first report of the isolation of Morganella morganii from infections of poultry in the study area. This preliminary report isolation documents the and biochemical characterization of *M. morganii* from chickens that were dying of an infection that presented with clinical signs similar to those of fowl typhoid in Kaduna, Nigeria. Avian host salmonellae include: Salmonella Gallinarum and Salmonella Pullorum which cause fowl typhoid and Pullorum disease, respectively. These salmonellae can cause severe mortality among chicken resulting in huge economic losses. Infected chickens have a high mortality rate, succumbing to septicemia, enteritis, and hemolytic anemia (Shivaprasad, 2003). Fowl typhoid can occur in older as well as young birds. The clinical signs may include decreased appetite, depression, dehydration, weight loss, ruffled feathers, and watery to mucoid diarrhoea. A progressive loss of condition can lead to anemia with pale, shrunken combs. The lesions in young birds may include unabsorbed yolk sacs, peritonitis and signs of septicemia. The subcutaneous blood vessels may be dilated, the liver, spleen and kidneys are often enlarged and congested, and the spleen may be mottled (FAO, 2009). Pullorum disease also called "Bacillary White Diarrhoea" caused by Salmonella Pullorum is accompanied by clinical signs such as inappetance, depression, ruffled feathers, closed eyes, loud chirping, white diarrhoea, vent pasting, gasping, lameness and death (mortality could be as high as 100 %). Here, young birds are mostly affected but can affect older birds too (OIE, 2009).

# MATERIALS AND METHODS

This study was carried out in the course of an investigation primarily for purposes of isolation and identification of Salmonella from the liver, spleen and faecal samples obtained from cases of suspected fowl typhoid in chickens in Kaduna State, Nigeria. Kaduna State is located between Latitude 10° and 11° N and Longitude 7 ° and 8 ° E. Biochemical characterization of the strains was carried out using both conventional biochemical tests and commercially available kits (Microbact<sup>®</sup> GNB 24E and api<sup>®</sup>NH). Affected flocks showed mortality rates ranging between 40 and 50 %. The liver and kidneys were enlarged and showed diffuse hemorrhages. Samples like the spleen, liver, and gall bladder from sick birds (those that showed pathological lesions such as bronze-coloured and enlarged liver with necrotic foci; enlarged, friable and congested spleen and kidneys) were cultured directly on MacConkey and Brilliant Green Agar in some instances, while in most instances, Salmonella-Shigella agar and Xylose Lysine Deoxycholate agar (Oxoid<sup>®</sup>) were used. Cloacal swabs

from clinically healthy birds were enriched in selenite broth, incubated at 37  $^{\circ}$ C for 24 h before being subcultured onto the afore-mentioned selective media. Enrichment step was included to enhance isolation rate. Further differentiation was carried out by catalase and oxidase tests, determination of H<sub>2</sub>S production on TSI, fermentation of lactose, sucrose, sorbitol, rhamnose and dulcitol; acid and/or gas production from glucose, fructose, xylose and arabinose; incubated as previously described (Trabulsi and Edwards, 1962; OIE, 2009).

# RESULTS

Over a 2-year period (February, 2011 to January, 2013), samples were collected from both clinically healthy and sick birds which presented with gross pathologic lesions typical of fowl typhoid (Shivaprasad, 2003) from poultry farms in Kaduna. Such pathologic lesions included: enlarged and congested spleen, enlarged congested and friable liver with areas of necrosis, congested lungs, congested and mis-shapened ovarian follicles which were regressed. Of the 534 samples (organs and cloacal swabs) tested, 17 (3.18 %) isolates of Morganella morganii were recovered. All the isolates were from birds that had pathologic lesions typical of fowl typhoid as listed above. No isolates of Morganella morganii were recovered from the apparently healthy birds. All isolates were confirmed as *M. morganii* by biochemical characterization using both Microbact® GNB 24E at an average probability of 99 % and api<sup>®</sup>NH at an average probability of 95 %; while 70 (13.1 %) isolates, which were recovered from sick birds, were confirmed positive as S. Gallinarum.

The isolates of *M. morganii* were tested for susceptibility to a panel of 10 antimicrobial agents by the disk diffusion method (Bauer et al., 1966) and were found to be generally resistant to erythromycin and nitrofurantoin; intermediately sensitive to ciprofloxacin and susceptible to amoxicillin, chloramphenicol, tetracycline, sulphamethoxazole, neomycin, enrofloxacin and gentamicin (Table 1).

# DISCUSSION

Epidemiological studies have revealed that *M. morganii* is frequently isolated from human clinical specimens collected from patients with nosocomial bacterial infections (Lee and Liu, 2006; Dutta and Narang, 2004; Gebhart-Moueller et al., 1998). Although *Morganella and Salmonella* belong to different tribes of the same family *Enterobacteriaceae*, they share many common characteristics such as: both being facultative anaerobes that are commonly found in the environment and in the intestinal tracts of humans and animals as normal flora.

Antibiotics	Abbreviations	Concentrations (µg/disc)	No. (%) of isolates susceptible	Av. Zone diameter inhibition (mm) and Interpretation by CLSI, 2010
Amoxicillin	AMC	30	16 (94.1 %)	29 (S)
Chloramphenicol	С	30	15 (88.2 %)	27 (S)
Erythromycin	E	5	0 (0 %)	0 (R)
Tetracycline	TE	30	16 (94.1 %)	23 (S)
Sulphamethoxazole	SXT	25	14 (82.3 %)	23 (S)
Neomycin	N	10	15 (88.2 %)	17 (S)
Nitrofurantoin	F	50	3 (17.6 %)	9 (R)
Enrofloxacin	ENR	30	12 (70.6 %)	16 (S)
Ciprofloxacin	CIP	30	2 (11.8 %)	18 (I)
Gentamicin	CN	30	15 (88.2 %)	22 (S)

Table 1. Antibiotic susceptibility testing of Morganella morganii strains isolated from poultry in Kaduna, Nigeria

S=Sensitive, I=Intermediate, R=Resistant (According to CLSI - Clinical Laboratory Standards Institute, 2010).

They are also Gram-negative non-lactose fermenters; and give similar reactions in biochemical tests for oxidase and TSI reaction. They however differ in some others such as indole, Voges Proskauer and urea reactions where *Salmonella* is usually negative while *Morganella* is positive (O'Hara et al., 2000). All *Morganella morganii* isolates in this study were recovered from birds that had clinical signs and pathological lesions as listed earlier; and were isolated in pure culture which suggest that the bacterium was likely responsible for the lesions seen in the affected birds. The pathological findings observed in the dead poultry in this study have been seen in mortality caused by both *Salmonella* spp. (Shivaprasad, 2003) and *M. morganii* (Lee and Liu, 2006; Chen et al., 2012).

The susceptibility of isolates to 10 different antibiotics is shown and based on the data, the isolates were generally found to be resistant to two antibiotics which were erythromycin and nitrofurantoin; and sensitive to 7 of the 10 antibiotics tested. The low level of resistance to the tested antimicrobials is an indication that the bacteria had not been particularly targeted during treatments in the study area, owing most probably to some misdiagnoses that might have been occurring. Besides, the two antimicrobials, erythromycin and nitrofurantoin are known to be more effective against Gram positive bacteria; meanwhile, M. morganii is a Gram negative bacterium. This finding on the resistance of *M. morganii* to erythromycin agrees with the report by Zhao et al. (2012) who also reported resistance of Morganella morganii to erythromycin; however, in their study, the authors reported resistance to a higher number of antibiotics.

#### CONCLUSIONS AND RECOMMENDATIONS

The present study has established the presence of Morganella morganii isolated from clinically sick

chickens. The possibility for misdiagnosis exists due to reported similarity in clinical and pathological presentations of diseases caused by Morganella morganii and those of some other microorganisms. There is therefore, the need for laboratory-based diagnosis in order to identify all the bacterial species responsible for infections during outbreaks. Experimental infection of chickens with Morganella morganii is hereby recommended; which will enable the documentation of the specific clinical signs and pathologic lesions associated with Morganella in poultry so as to differentiate them from those of Salmonella and perhaps other species. There is also the need for further characterization of isolates to determine the phenotypes and genotypes of the strains and antibiotic resistance genes encoded.

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