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Isolation of *Salmonella* species and some other gram negative bacteria from rats cohabitating with poultry in Ibadan, Oyo State, Nigeria

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The aim of this study was to isolate *Salmonella* species and other enteric bacteria in rats cohabiting with poultry; in order to consider the potential role of rats in their transmissions to poultry and humans. Four hundred samples comprising 200 oral swabs and 200 anal swabs were collected from rats cohabiting with poultry from 5 local government areas in Ibadan. The samples were subjected to standard bacteriological analysis. A total of 228 Gram negative bacteria including 21 different species of both Lactose fermenters and non-Lactose fermenters were isolated. The identified organisms include: *Salmonella* subspecies 1, *Salmonella* Arizonae, *Escherichia coli*, *Escherichia coli* inactive, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Enterobacter agglomerans*, *Enterobacter dissolvens*, *Enterobacter gergoviae*, and *Aeromonas hydrophila*, *Proteus mirabilis*, *Proteus vulgaris*, *Hafnia alvei*, *Morganella morganii* biogp 1, *Morganella morganii* subspecies Siboni 1, *Citrobacter diversus*, *Serratia liquefaciens*, *Pragia fontium* and *Providencia alcalifaciens*. The organisms were identified using Oxoid Microbact GNB 24E® (MB24E) and accompanying computer software package (Oxoid Microbact®) 2000 version 2.03. Some of the isolated bacteria from rats' cohabitating with poultry have been associated with diseases in poultry and humans. The findings therefore serves to create fresh awareness among poultry farmers and other stakeholders in the industry from the studied area, that rats do not only constitute physical threat in terms of destruction of infrastructures and feeding on poultry feeds, they also pose a great risk in terms of transmission of bacterial infection to poultry and men associated with poultry production. Possible measures to control rats' infestations within poultry houses are highlighted.

Key words: Rats, poultry, co-habiting, Gram negative Bacteria.

INTRODUCTION

Rodents are known to be so numerous and very diverse in nature; and they are well acknowledged as a major contributors to human disease, right from the middle age (Meerburg et al., 2009; Webster, 1996). Black rats for

example were associated with the transmission of plague long time ago; in the same way, more than 24 different infectious agents have been directly or indirectly reported as been transmitted by rodents to humans (Meerburg et

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al., 2009; Webster, 1996). Wild rodents serve as reservoirs and vectors for some pathogenic organisms like: *Leptospira* spp., *Salmonella* spp., *Campylobacter* spp., *Trichinella* spp., *Toxoplasma* spp. which are well known disease causing agents of food animals and humans (Gratz, 1994, Hiett et al., 2002). For instance, it had been noted that, rat population at a poultry farms can be a major reservoir of pathogenic bacteria and they are capable of transmitting the pathogens to the environment, food and animals (Rose et al., 2000). Henzler et al. (1998) demonstrated the direct association between the presence of rats and poultry diseases because they documented that effective control measure of rodents in the poultry house resulted in reduced bacterial infection of the associated poultry. It has also been shown that rodent control measures can effectively decrease *Salmonella* species in poultry houses (Rodenburg et al., 2004). Rats are common commensal pest (Meerburg et al., 2009), which can damage the food products, buildings, stored products, and also serve as potential vector of pathogenic diseases in human and animals. Rats may transmit the bacteria through feces, urine, and hair remnants (Meerburg et al., 2009).

Rodents are capable of perpetrating long existence of pathogens in the animal house and population. They can acquire infection through contact with faeces of infected livestock on the farm (Oosterom, 1991), from other wild animals such as wild birds or from their own family members as rodents. And since they tend to live close to each other, it encourages the infections to remain resident in the population for a long time (Singh et al., 1980; Hilton et al., 2002). Notably among the group of bacteria organisms carried and transmitted by rodents are enteric bacteria belonging to the Family enterobacteriaceae, of which *Salmonella* species; an important animal and human pathogen belongs.

Salmonella species are Gram-negative, non-spore forming rod-shaped bacteria, a member of the family Enterobacteriaceae (Jay et al., 2003). They are generally known to be actively motile with the exception of *Salmonella Pullorum* and *Gallinarum*; they grow at optimum temperature of 35 to 37°C and they are facultative anaerobic organisms (Jay et al., 2003). They are non-capsulated except *Salmonella typhi* (Cheesbrough, 2002). Persistence of *Salmonella* on farms is about two times higher for a farm where rodents are resident (Rose et al., 2000). According to Mehmood et al. (2011), bandicoot rat served as reservoir of bacterial infection of *Escherichia coli*, *Klebsiella* species, *Proteus* species and *Salmonella* species at poultry farms at Rawalpindi/Islamabad, Pakistan.

The Family Enterobacteriaceae to which *Salmonella* belong are a large family of Gram-negative bacteria that includes many of the more familiar pathogens, such as, *E. coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*, *Proteus*, *Enterobacter*, *Serratia* and *Citrobacter* (Engelkirk, 2007). The family belongs to the order Enterobacteriales, the

class Gammaproteobacteria and phylum Proteobacteria (Brenner et al., 2005). Members of the Enterobacteriaceae are rod-shaped, typically 0.6 to 6 µm in length and appear as small grey colonies on blood agar (Engelkirk, 2007). They are facultative anaerobes, they ferment sugars to produce lactic acid and various other end product (Ewing, 1986), most also reduce nitrate to nitrite (Mac Faddin, 2000). Bacteriological screening of rodents in close association with poultry houses for their carriage of pathogenic bacteria could be helpful to determine their role in bacterial disease transmission in poultry. The role of rodents, rat inclusive in destruction of poultry infrastructures is well appreciated among poultry farmers in Ibadan, Nigeria. There is however limited information on the types of bacteria carried by the rats, despite their often close interactions with poultry / poultry feeds and water sources. We therefore screened the oral and anal region of rats cohabitating with poultry from some poultry farms in Ibadan, Oyo State Nigeria to isolate and identify the kind of pathogen they carry as a mean of accessing their possible roles in disease transmission to poultry and human.

MATERIALS AND METHODS

Sample location

Purposive samples were collected from poultry farms (comprising of both commercial and breeder flocks) in Suburbs of Ibadan, Oyo State, South-Western Nigeria (Latitude 7° 23' N and Longitude 3° 56' E) with known problems of rat infestation. The south-western States including; Oyo, Osun, Ogun, Ondo and Lagos are the main centers of poultry industry in Nigeria and Ibadan is a major and largest central city in the south-western States playing strategic roles in the national poultry production. Samples were collected from 5/11 local government areas located in Ibadan suburbs namely: Akinyele, Ido, Ona ara, Lagelu and Egbeda, where most of the poultry farms are located. One farm each was chosen from each LGA for sample collection in rats cohabitating with chicken flocks.

Sample collection

Two hundred rats were captured alive using adhesive glue boards. They were subsequently retrained with hands fortified with hand gloves for oral and anal sample collections. The oral and anal regions were sterilized with methylated spirit for aseptic collection of samples and to reduce contamination. A sterile swab stick was then inserted into the anus and mouth and rolled gently for sample collection. A total of four hundred samples were collected including; two hundred oral swab and two hundred anal swabs from rats cohabitating with poultry. The samples were transported to the laboratory and bacteriologically processed under 24 h of collection to reduce contamination and to ensure viability of organisms.

Bacteriological analysis

The samples were processed for bacteria isolation and identification using standard morphological, biochemical screening and serological procedures (Barrow and Felthams, 1993; Garcia and Isenberg, 2007; Cheesbrough, 2002). The oral and anal samples

were streaked on MacConkey agar plates and inoculated into Selenite F Broth and subsequently incubated at 37°C for 24 h for primary isolation. Lactose fermenters were sub-cultured to Eosine Methylene Blue agar and incubated at 37°C for 24 h. Organisms that produced metallic sheen colonies on EMB were stored for further biochemical identification. Discrete colonies of Lactose and non-Lactose fermenters were also sub-cultured from MacConkey agar on to Tryptose agar slope for further identification. Isolates that showed brick red colouration on Selenite F Broth were sub-cultured onto Deoxycholate Citrate Agar and incubated at 37°C for 24 h. All the Gram-negative isolates were identified with Oxoid Microbact GNB 24E® (MB24E) and accompanying computer software package (Oxoid Microbact®) 2000 version 2.03 according to the manufacturers procedures. The list of the reagents included in the kits are: Lysine, Ornithine, H₂S, Glucose, Mannitol, Xylose, ONPG, Indole, Urease, Voges Proskauer, Citrate, Gelatine, TDA (Tryptophan deaminase), Malonate, Inositol, Sorbitol, Rhamnose, Sucrose, Lactose, Arabinose, Adonitol, Raffinose, Salicin and Arginine.

Serology

All the suspected *Salmonella* species were subjected to slide agglutination test by standard method using Polyvalent Salmonella Antisera (Difco^m Salmonella O Antiserum Poly A-I and V1) (Cheesbrough, 2002; Andrews et al., 2005). The *Salmonella* polyvalent antisera was reconstituted with 3mls of sterile 0.85% NaCl solution according to the manufacturer's instructions. From test culture on a non selective media (Mueller Hilton), a discrete colony of the suspected *Salmonella* species organism was emulsified in a drop of sterile saline on a glass slide. A drop of the antiserum was then mixed with the emulsified culture on the slide, rotated for one minute to observe for agglutination. Agglutination indicated a positive result while absence of agglutination indicated a negative result.

RESULTS

Colonial morphology

One hundred and nineteen lactose fermenters were isolated on MacConkey agar; colonies were circular, 0.5 to 5 mm, pink coloured, with sweet odour and glistening surface. Forty-one non-lactose fermenting organisms were isolated from primary isolation; the colonies were light yellow, circular, translucent with entire edges (some had serrated edges), they were low convex, 0.5 to 2 mm with bad odour and with glistening surface. Sixty four isolates had characteristic metallic sheen on EMB. One hundred and thirty three isolates exhibited brick red coloration in selenite F Broth, 68 isolates grew on Deoxycholate Citrate Agar, out of which 32 isolates appeared as cream transparent to translucent circular, convex colonies with black centers, whereas 36 isolates appeared as white opaque convex colonies without black centers.

Biochemical test

A total of 10 genera of Enterobacteriaceae family namely:

Citrobacter, *Escherichia*, *Enterobacter*, *Hafnia*, *Morganella*, *Pragia*, *Proteus*, *Providencia*, *Salmonella* and *Serratia* were identified to species level based on Microbact^R identification procedure. The other non-lactose fermenters identified include *Pseudomonas* species and *Aeromonas hydrophilia* (Table 1). Table 2 shows the various biochemical reactions of each of the isolated organism to the 24 biochemical tests included in the Microbact^R kit. Table 1 shows the details of the numbers and respective percentages of the identified organisms. *Enterobacter* species had the highest percentage (22.7%) while *A. hydrophilia* had the lowest (0.25%). Only 7(1.75%) *Salmonella* organisms belonging to two serovars: namely, *Salmonella* subspecies 1, 4(1%) and *Salmonella* Arizonae 3(0.75) were isolated and identified.

Serology

The identities of the 7 suspected *Salmonella* isolates were confirmed by slide agglutination with the Polyvalent Salmonella Antisera (Difco^m Salmonella O Antiserum Poly A-I and V1),

DISCUSSION

Members of the family Enterobacteriaceae have been associated with diseases in poultry and humans (Yusop et al., 1991; Tsolis et al., 2008; Velge et al., 2012). In this current study only 2 groups of *Salmonella* was identified namely *Salmonella* subspecies 1 with an isolation rate of 1% and *Salmonella* Arizonae with an isolation rate of 0.75% giving a total isolation rate of 1.75%. These two subspecies of *Salmonella* have been implicated in poultry diseases and human infection. Brenner, 1998 stated that *Salmonella enterica* subspecies I are the predominant pathogens associated with birds and mammals; Velge et al. (2012) also reported that strains of *S. enterica* subspecies 1 causes 99% of Salmonella infections in humans and other warm blooded animals. It produces cytotoxin and causes inflammatory diarrhea due to their ability to invade colonic epithelium, and they produce fever in humans (Tsolis et al., 2008; Guerrant and Steiner, 2005). On the other hand, *Salmonella* Arizonae subspecies have been associated with diseases in Turkey and Sheep (Grimont and Weill, 2007), although they are mostly isolated from reptiles and rarely isolated from man (Weiss et al., 1986). It was believed to be the cause of septiceamia, pneumonia, otitis media, brain abscess, meningitis, osteomyelitis, gastroenteritis and hepatic abscess in humans in many studies (McIntyre et al., 1982, Petru and Richman, 1981 and Yusop et al., 1991).

The other 9 organisms belonging to the Enterobacteriaceae family were isolated and identified. Five *Enterobacter* species with highest isolation rate of

Table 1. Types, number and the percentage of bacteria isolated from the mouth and anus of rats co-habitating with poultry.

Organism	No.	Percentage (%)
<i>Aeromonas hydrophila</i>	1	0.25
<i>Citrobacter diversus</i>	3	0.75
<i>Escherichia coli</i>	64	16
<i>E. coli</i> inactive	2	0.5
<i>Enterobacter agglomerans</i>	2	0.5
<i>Enterobacter cloacae</i>	73	18.25
<i>Enterobacter dissolvens</i>	3	0.75
<i>Enterobacter gergoviae</i>	2	0.5
<i>Enterobacter hormaechei</i>	11	2.75
<i>Hafnia alvei</i>	2	0.5
<i>Morganella morganii</i> biogroup 1	15	3.75
<i>M. morganii</i> subsp. <i>Siboni</i>	5	1.25
<i>Pragia fontium</i>	1	0.25
<i>Proteus mirabilis</i>	12	3
<i>Proteus vulgaris</i>	6	1.5
<i>Pseudomonas aeruginosa</i>	15	3.75
<i>Pseudomonas fluorescens</i>	1	0.25
<i>Providencia alcalifaciens</i>	1	1.25
<i>Salmonella</i> subspecies 1	4	1
<i>Salmonella</i> <i>Arizonae</i>	3	0.75
<i>Serratia liquefaciens</i>	2	0.5

Table 2. Biochemical reactions of the bacteria isolated from rats co-habitating with poultry.

Bacteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
<i>A. hydrophila</i>	+	-	-	+	+	-	+	-	+	+	+	+	+	+	-	-	-	+	-	+	-	-	-	+
<i>C. diversus</i>	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	-	-	+
<i>E. coli</i>	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	+
<i>E. coli</i> inactive	+	-	-	+	+	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+
<i>E. agglomerans</i>	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	-	+
<i>E. cloacae</i>	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+
<i>E. dissolvens</i>	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-	+	+	+	-	+	-	+	+	+
<i>E. gergoviae</i>	+	+	-	-	+	-	+	-	+	+	+	-	-	+	-	-	-	+	-	+	-	+	-	+
<i>E. hormaechei</i>	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+	-	+	-	-	-	+

Table 2. Contd.

<i>Hafnia alvei</i>	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	-	+
<i>Morganella morganii</i> biogroup 1	+	+	+	+	-	-	-	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+
<i>M. morganii</i> subsp <i>Siboni</i>	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+
<i>Pragia fontium</i>	+	-	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-
<i>Proteus mirabilis</i>	+	+	+	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+
<i>Proteus vulgaris</i>	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	-	-	-	-	+
<i>P. aeruginosa</i>	+	-	+	-	-	+	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+
<i>P. fluorescens</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>Providencia alcalifaciens</i>	+	-	-	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	+
<i>Samonella</i> subsp. 1	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	+
<i>Samonella</i> Arizonae	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	+
<i>Serratia liquefaciens</i>	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	-	+	-	+	-	-	-	+

1, Lysine; 2, Ornithine; 3, H₂S; 4, Glucose; 5, Mannitol; 6, Xylose; 7, ONPG; 8, Indole; 9, Urease; 10, Voges Proskauer; 11, Citrate; 12, TDA; 13, Gelatin; 14, Malonate; 15, Inositol; 16, Sorbitol; 17, Rhamnose; 18, Sucrose; 19, Lactose; 20, Arabinose; 21, Adonitol; 22, Raffinose; 23, Salicin; 24, Arginine; *P. aeruginosa* = *Pseudomonas aeruginosa*; *P. fluorescens* = *Pseudomonas fluorescens*; *E. hormaechei* = *Enterobacter hormaechei*; *E. gergoviae* = *Enterobacter gergoviae*; *E. agglomerans* = *Enterobacter agglomerans*; *E. cloacae* = *Enterobacter cloacae*; *E. dissolvens* = *Enterobacter dissolvens*; *A. hydrophila* = *Aeromonas hydrophila*; *C. diversus* = *Citrobacter diversus*; *E. coli* = *Escherichia coli*; *E. coli* inactive = *Escherichia coli* inactive.

22.75% included, *Enterobacter agglomerans* (0.5%), *Enterobacter cloacae* (18.25%), *Enterobacter dissolvens* (0.75%), *Enterobacter gergoviae* (0.5%) and *Enterobacter hormaechei* (2.75%). This was followed by *E. coli* with an isolation rate of 16.5%. *Morganella* species isolation rate was 5% comprising: *Morganella morganii* biogroup 1 (3.75%) and *M. morganii* subspecies *Siboni* (1.25%). *Proteus* species (4.5%) identified are; *Proteus mirabilis* (3%) and *Proteus vulgaris* (1.5%). The other bacteria isolates are *Citrobacter diversus* with isolation rate of 0.75%, *Serratia liquefaciens* and *Hafnia alvei* had the same isolation rate of 0.5% while *Providencia alcalifaciens* and *Pragia fontium* had the lowest isolation rate of 0.25% respectively. Most of these organisms have been reported to be pathogenic to poultry and humans. For example, *E. coli* according to Todar (2007), can

cause urinary tract infection (UTI), neonatal meningitis and intestinal disease (gastroenteritis) in humans. It is also the aetiology of avian colibacillosis which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry (Calnek et al., 1997). A group of *E. coli* called *E. coli* inactive was also isolated and identified, this was discovered to be non-lactose fermenters and also non-motile. This is in agreement with Le Minor and Richard (1993) and Holt et al. (1994) who discovered that a lactose-negative, non-motile organism thought to be *Shigella* species were inactive *E. coli* when identified serologically. *M. morganii* had been implicated in outbreaks of septicemia and bacteremia in humans and animals (Barragan Casas et al., 1999; Heard et al., 1988; Rowen and Lopez, 1998). *Serratia liquefaciens* had been

implicated in the contamination of chicken carcasses (Lahellec et al., 1975) and it is also occasionally isolated from clinical specimens, although its pathogenic role has not been established (Grimont and Grimont, 1984). The presence of *Proteus mirabilis* in poultry meat had also been documented (Kim et al., 2005; Wong et al., 2013), and the presence of *Proteus* species in chicken droppings had been acknowledged as one of the route of its transmission as a zoonotic pathogens to vulnerable workers while handling infected chicken directly (Tonkic et al., 2010) or through fecal-contaminated poultry products as other Enteric bacteria (Lima-Filho et al., 2013). A more current report also associated poultry-borne *Proteus* species with zoonotic urinary tract infection (Armbruster et al., 2014). It was also associated with rheumatoid arthritis that is common in developing countries (Ebringer and

Rashid, 2014).

Hafnia species has been recovered from mammals as well as from poultry where it has been identified as infectious agent (Farmer, 2003; Proietti et al., 2004). Additionally, it has been reported as agent of nosocomial infections and community acquired infections in humans (Günthard and Pennekamp, 1996), of urinary tract infection and sepsis in infants (Laupland et al., 2006). Likewise, *Enterobacter* species have been reported to cause disease in poultry (Nandi et al., 2013), it has also been isolated from egg shell of poultry together with other pathogenic Enterobacteriaceae (Musgrove et al., 2008). Some species of *Enterobacter* have also been isolated from patients' cerebrospinal fluid (Parodi et al., 2003).

Pseudomonas species and *Aeromonas hydrophila* which do not belong to the Enterobacteriaceae family were also isolated. Two species of *Pseudomonas* (4%) namely: *Pseudomonas aeruginosa* (3.75%) and *Pseudomonas fluorescens* (0.25%) were isolated and identified. The percentage isolation rate of 0.25% was obtained for *Aeromonas hydrophila*. These two organisms have been implicated in poultry diseases and are also of zoonotic importance. It has been reported that *Pseudomonas* can be highly virulent causing 50 to 100% mortality in experimentally inoculated 4-week-old chickens (Saif et al., 2003). Fekadu (2010) also reported high mortality in newly hatched chickens and death at later stage of embryos in poultry farms as a result of *Pseudomonas* infection. On the other hand, *A. hydrophila* has been isolated from organs of clinically sick commercial chickens in Jos, Nigeria (Dashe et al., 2014). According to Okpokwasili and Ogbulie (2001), disease caused by *A. hydrophila* is endemic in Nigeria and is the most important zoonotic disease of fish. It can be deduced from this study that rats cohabiting with poultry harbour pathogens of poultry and humans, these rats should be put into consideration whenever the sources of bacterial diseases aetiologic agents in poultry and humans is been traced. From this study, rat cohabiting with poultry poses public health threat to poultry and humans as possible agents of disease transmission.

Preventive measures to reduce rats' population in contact with poultry are highly recommended. This becomes imperative, since rat do not just constitute physical threat through their activities in destruction of poultry house infrastructures, they are also of great risk in the transmission of bacterial infection to poultry and man. Therefore adequate measures should be taken to prevent and eradicate rats from poultry houses and human abodes. Some of the recommended steps that could be taken to control rats population in and around poultry facilities include: Good sanitation; all feed bins must be kept in good repairs, and outside debris such as old equipments must be eliminated and grain of feed spillage which occurs during grain delivery must be cleaned immediately. Bushes around poultry houses should also be cleared regularly, as this act as breeding sites for

rodents.

Rodent-proof construction should be encouraged: A lasting form of rodent control is to keep them out of poultry houses by eliminating all openings through which rodents can enter a poultry houses, this is often cost prohibitive but it is an effective measure. Likewise, grain storage houses should be made rodent proof.

Farmers should be educated and enlightened on the role of rodents in the transmission of bacterial disease to poultry, this will enable them pay more attention to rodent control.

Conflict of interests

The authors have not declare any conflict of interest.

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