www.ajbrui.net

Afr. J. Biomed. Res. Vol.18 (September, 2015); 211- 215

Full Length Research Paper

Bacteria Isolated from the Oral and Cloaca Swabs of Lizards Co-habitating with Poultry in Some Poultry Farms in Ibadan, Oyo State, Nigeria.

Ajayi J. O[2], Ogunleye A.O[1*], Happi A. N[2] and Okunlade A. O[1]

Department of Veterinary Microbiology and Parasitology, University of Ibadan, Nigeria

Department of Veterinary Pathology, University of Ibadan, Nigeria

ABSTRACT

Reptiles like lizards can carry bacteria agents in their digestive tract without manifesting any associated symptom other than serving as sources of contaminating the environment and of infections to human and animal. In this study a total of 20 species of bacteria including *Salmonella enterica*, *Esherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* among some other *Enterobacteria* were isolated and identified from 193 mouth swabs, and 193 cloaca swabs sampled from 193 *Agama agama* lizards co habituating with poultry from 8 commercial poultry farms in Ibadan, Oyo State, Nigeria. The bacteria were isolated and identified based on standard cultural, morphological, biochemical methods and the use of MICOBACT^{R} identification kit. Based on the free access and close contacts of *Agama agama* lizards to the poultry environment, feed and water supply in many poultry houses in Nigeria and particularly in the studied area, they could be sources of contaminating the environment, poultry feed and water with the potential pathogenic bacteria identified from the mouth and cloaca swab sampled.

Key words: *Agama agama lizard, poultry, Co-habitating, Enterobacteria*

INTRODUCTION

A number of reptiles, lizards inclusive have been incriminated as the source of bacteria pathogens (Oboegbulem and Iseghohimhen, 1985; Austin and Wilkins, 1998). More than 1000 *Salmonella serovars* have been isolated from reptiles (Mitchell, 2006).

Different species of lizards have also been associated with various bacteria species. For example members of the family *Enterobacteriaceae* had been isolated from the intestines of Iguanid lizard's species from Texas (Mathewson, 1979). Two other lizard species namely: *Scleloporus oliveceus* and *Crotaphylus collaris* were also reported to have 100% prevalence for *Salmonella* (Mathewson, 1979). In Brazil, the prevalence of 26.9%(21/78) for *Salmonella enterica*; 15.4%(12/78) *Citrobacter freundii*; 11.5%(9/78) *Escherichia coli*; 10.2%(8/78) *Enterobacter sakaski*; among other *Enterobacteria* such as *Kluvera species*, *Citrobacter amalonaticus*, *Serratia marcescens*, *Citrobacter diversus*, *Serratia adorfera* and *Serratia liquefaciens* in lizards belonging to *Tupinambis merianae* were documented(Carvalho *et al.*, 2013).

*Corresponding author:
E-mail: peculiarj@yahoo.com
Tel: +2348054461821

Revised version Received: February, 2015
Date Accepted:, May, 2015

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

In Nigeria *Agama agama* lizards have been noted to have close association with human and particularly animal houses where they are commonly seen with free access in and out of animal pens as well as to animal feeds and water sources (Ogunleye *et al.,* 2013). The insight into the role of Agama agama lizards in disease transmission can be traced to the 1950s when *Salmonella* Agama was first characterized as a new serotype of *Salmonella enterica* from faeces of agama lizard (Agama agama) in Nigeria (Collard and Montefiore, 1957). This *Salmonella* serotypes was subsequently isolated from Gekos and mammals from Africa (Collard and Sen, 1960; Oboegbulem and Okoronkwo, 1990 ;Orji *et al.,* 2005), as well as in United Kingdom(Wilson *et al.,* 2003). More recently *Salmonella* Pullorum was isolated and characterized from the intestine of one *Agama agama* lizard co habitaing with poultry in a Teaching and research farm in Nigeria (Ogunleye *et al.,* 2013).

The role of lizards in disease transmission to man and animals is of interest due to their acknowledged close association to human and animal environments (Bélard *et al.,* 2007). In Gabon just like in Nigeria, it was reported that lizards are commonly seen around all habitations including terrace of houses and this observation was attributed and associated with a case of a 25years old man from Germany who visited Gabon and took ill 1month after returning (Bélard *et al.,* 2007). *Salmonella* Agama (*S. enterica* subspecies enterica serotype Agama 4,12:i:1,6) was isolated from the case (Bélard *et al.,* 2007). Reptiles generally are capable of eliminating bacteria pathogens through their digestive tract without any apparent clinical symptom, however they are potential sources of infections for human particularly children and immuno - compromised adults (Carvalho *et al.,* 2013). Studies on the enteropathogens of public health significance in lizards therefore at times involved euthanizing and collecting their intestinal contents to determine the kind of bacteria present (Singh *et al.,* 2013).

In this study, based on the observation of the close association of *Agama agama* lizards with poultry houses in Nigeria and the recent isolation of *Salmonella* Pullorum from the intestine of an *Agama agama* lizard co habitating with poultry in Nigeria, we screened the oral and anal from Agama agama lizards co-habitating with poultry in eight commercial poultry farms in Ibadan, Oyo State Nigeria for the kind of bacteria present to consider their possible role in poultry disease transmission.

MATERIALS AND METHODS

Sample Collection:

A total of 183 *Agama agama* co habitating with poultry were captured from eight commercial poultry farm in Ibadan Oyo State Nigeria. The lizards were captured in the night while sleeping with touch light and kept in cages that allowed for their proper ventilation. They were retrained with hands fortified with thick hand gloves. The oral and anal regions were sterilized with methylated spirit. For oral swab sample collections, using hand already gloved, the lizards held between the head and cranial cervical region with one hand, while the second hand was used to pull down the lower jaw gently, subsequently the sterile swab stick were inserted into the oral cavity by someone else for oral swab collections. The cloaca swabs were sampled by restraining the neck region as earlier described, and the lumber region with the second hand. The ventral region of the lizard under restrain was then turned upward to allow for the insertion of the sterile swab sticks into the cloaca region. The lizards were subsequently released following the sample collection. Oral swabs totalling 183 and 183 anal swabs were aseptically collected. The samples included 22, 22, 30, 24, 90, 76, 58 and 44 from farms 1, 2, 3, 4, 5, 6, 7, and 8 respectively.

Bacteriological Analysis:

The oral and anal swab samples were streaked on Blood agar and MaCconkey agar, incubated at 37°C for 24hours. All the discrete colonies of the various bacteria growth on both laboratory media were subjected to further morphological and biochemical screening using standard bacteriological methods (Barrow and Felthams, 1993; Garcia and Isenberg, 2007). Subsequently all the bacteria isolated were subjected to further identification by using MICROBACT^{R} identification kit based on the manufacturer protocols. The kit software was used to identify the various bacteria isolates.

Statistical Analysis

The data obtained from the isolated bacteria from the mouth and anal regions of lizard's co-habitating with poultry were subjected to descriptive and inferential statistics respectively. For the inferential statistics, chi-square test of association was used for isolates from the mouth and cloaca, while frequency table consisting of the number of isolates with their respective percentage and bar chart plots were used to describe the items. The null hypothesis was rejected when the level of significance was less than 0.05 ($\alpha < 0.05$).

RESULTS

Based on the MICROBACT^{R} identification kit analysis, 20 *species* of bacteria namely: *Escherichia coli, Salmonella enterica, Proteus mirabilis, Klebsiella species, Enterobacter cloaca, Enterobacter aerogenes, Enterobacter ictaluri, Enterobacter hormaechei, Pseudomonas aeruginosa, Pseudomonas stutzeri, Acinetobacter haemolyticus, Acinetobacter baumanni, Morganella morganii biogrp 1, Morganella morgnii subspecies siboni, Xenorhabdus nematophilus, Edwardsiella ictaluri, Trabusiella guamensis, Hafnia alvei biogrp 1, Citrobacter werkmanii and Citrobacter almalonatius*. Table 1 shows the number and total percentage of each of the bacteria species isolated from both the mouth and cloaca of the lizards sampled. Table 2 showed the numbers and percentage of each of the bacteria isolated from the mouth and cloaca sampled respectively.

Table 1:
Percentage of bacteria isolated from mouth and cloaca of lizards co-habitating with poultry

S/N	Bacteria isolates	Number isolated (%)
1	*Escherichia coli*	82/366(22.40)
2	*Salmonella enterica*	42/366(11.48)
3	*Proteus mirabilis*	22/366(6.01)
4	*Klebsiella species*	14/366(3.83)
5	*Enterobacter cloaca*	111/366(30.33)
6	*Enterobacter aerogenes*	6/366(1.64)
7	*Enterobacter ictaluri*	1/366(0.27)
8	*Enterobacter hormaechei*	1/366(0.27)
9	*Pseudomonas aeruginosa*	5/366(1.37)
10	*Pseudomonas stutzeri*	1/366(0.27)
11	*Acinetobacter haemolyticus*	9/366(2.46)
12	*Acinetobacterbaumanni*	2/366(0.55)
13	*Morganella morganii biogp 1*	1/366(0.27)
14	*Morganella morganii subspp*	1/366(0.27)
15	*Xenorrhabdus nematophilus*	2/366(0.55)
16	*Edwardsiella ictaluri*	2/366(0.55)
17	*Trabusiella guamensis*	1/366(0.27)
18	*Hafnia alvei biogrp 1*	1/366(0.27)
19	*Citrobacter werkmanii*	1/366(0.27)
20	*Citrobacter almanolaticus*	1/366(0.27)

Equal numbers of *Klebsiella species* were isolated from mouth and cloaca of the lizards sampled as shown in figure 1, whereas for *Pseudomonas species*, more were isolated from the mouth than in the cloaca figure 2. There were significant associations between isolates from the mouth and cloaca for; *Escherichia coli, Salmonella enterica, Proteus mirabilis, Enterobacter cloaca, Pseudomonas aeruginosa,* and *for Acinetobacter haemolyticus.* Whereas there was no association between *Klebsiella species* isolated from the mouth and cloaca.

Table 2:
Comparative percentage of bacteria isolated from mouth and cloaca of lizards co-habitating with poultry

S/N	Bacteria isolates	Number isolated(%) from mouth	Number isolated(%) from cloaca
1	*Escherichia coli*	32(39%)	50(61%)
2	*Salmonella enterica*	14(33.3%)	28(66.7%)
3	Proteus mirabilis	8(36.4%)	14(63.6%)
4	*Klebsiella species*	7(50%)	7(50%)
5	*Enterobacter species*	54(45.4%)	65(54.6%)
6	*Pseudomonas species*	4(66.7%)	2(33.3%)
7	*Acinetobacter baumanni*	-	2(100%)
8	*Morganella morganii biogp 1*	1(100%)	-
9	*Morganella morganii subspp siboni*	1(100%)	-
10	*Xenorrhabdus nematophilus*	-	2(100%)
11	*Edwardsiella ictaluri*	2(100%)	-
12	*Trabusiella guamensis*	1(100%)	-
13	*Hafnia alvei biogrp 1*	-	1(100%)
14	*Citrobacter werkmanii*	-	1(100%)
15	*Citrobacter almanolaticus*	-	1(100%)

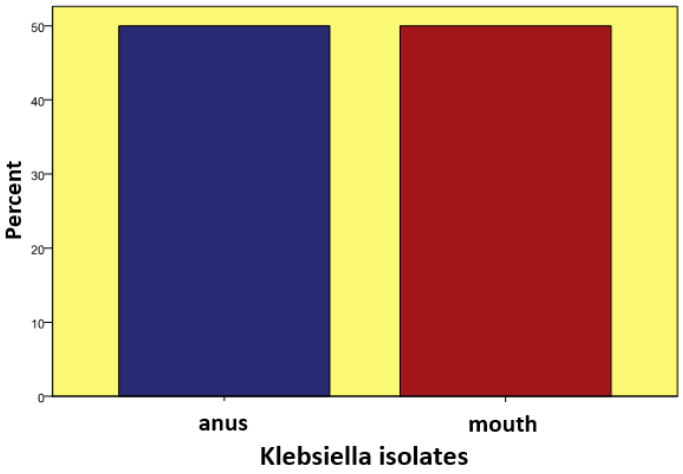

Figure 1
Equal numbers of *Klebsiella species* isolated from both mouth and cloaca swabs

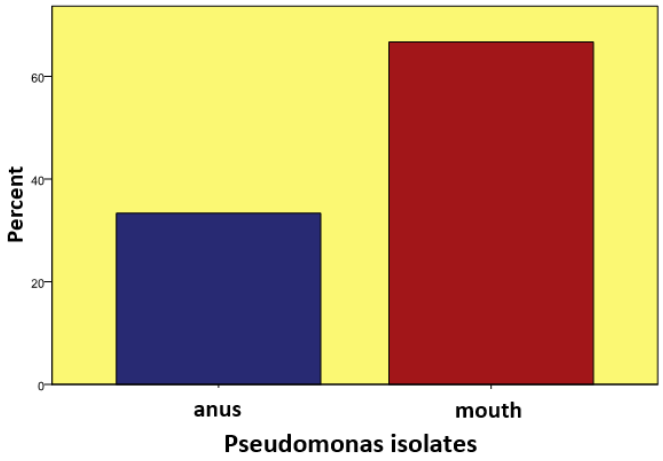

Figure 2:
More *Pseudomonas species* isolated from the mouth than cloaca

No test of association was established for *Acinetobacter baumanni, Morganella morganii biogroup 1, Morganella morganii subspp siboni, Xernorhabdus nematophilus, Edwardsiella ictaluri, Trabusiella guamensis, Hafnia alvei biogrp 1, Citrobacter werkminii* and *Citrobacter almalonaticus*.

DISCUSSION

Scientific focus had been on the role of lizards as possible carriers of potential pathogenic bacteria for humans and animals. Studies on *Geckos* in particular from some parts of the World like Nigeria and India have identified them as carrier/ reservoir of bacteria, including: Non typhoidal *Salmonella, Citrobacter freundi, Citrobacter intermedius, Erwinia herbicola, Enterobacter cloacae, Shigella sonnei, Edwardsilla tarda, Enterobacter species, Serratia marcescens, Proteus species, Klebsiella pneumoniae* and *Escherichia coli* (Kaura *et al.,* 1968; Oboegbulem and Iseghohemhen, 1985; Gigani *et al.,* 1986; Singh *et al.,* 2013). As far back as 1950s *Salmonella* Agama was first isolated and characterized from *Agama agama* lizard from Nigeria(Collard and Montefiore, 1957), and more recently a *Salmonella* Pullorum was identified from the intestine of a lizard co habitating with poultry in Nigeria(Ogunleye *et al.,* 2013). The current work identified up to 20 species of bacteria and established significant associations between some of them isolated from the mouth and cloaca of the *Agama agama* lizards co- habitating with poultry. Apart from bacteria like *Escherichia coli* and *Salmonella enterica* that are known to be pathogenic to poutry(Piercy and West, 1976; Dho-Moulin and Fairbrother, 1999; Ogunleye *et al.,* 2006), some other bacteria isolated like *Pseudomonas aeruginosa* and *Proteus mirabilis* have often been isolated from organs of chickens that died of septicaemic conditions sent for bacteria culture and sensitivity from post-mortem examinations of such carcasses in some Veterinary Teaching Hospitals in Nigeria(Unpublished data). Thus the need to investigate the pathogenic roles of these bacteria organisms and others isolated from these studies whose epidemiologic role in poultry is yet to be established.

Some of the bacteria isolated in this study, such as *Citrobacter amalonaticus, Esherichia coli and Salmonella enterica* have been identified also from Geckos (Singh *et al.,* 2013). Reptiles, lizards inclusive have been acknowledged to eliminate bacteria pathogens such as *Salmonella* through their digestive tract without any apparent clinical symptoms, but rather serving as potential sources of contamination as well as infections to the environment and man and animal (Shinohara *et al.,* 2008; Carvalho *et al.,* 2013). The isolation of the bacteria organisms from this study is therefore of epidemiologic importance to the possible disease transmission from lizards to poultry and possibly to humans, especially due to free access of these *Agama agama* lizards to the poultry houses, as well as their sources of food and water.

REFERENCES

Austin, C.C. and Wilkins, M.J.(1998): Reptile associated Salmonellosis. J. Am. Vet med Assoc. 212, 866-867.

Barrow, G.H., and Feltham, R.K.A. (1993): Cowan and Steel's manual for identification of medical bacteria, 3rd edition. Cambridge. UK. Cambridge University Press. 331.

Bélard, S., Kist, M., Ramharter, M. (2007): travel related Salmonella Agama, Gabon(letter). Emerg Infect Dis(serial on the internet). Available from http:// www.cdc.gov/eid/content 13/5/790.htm.

Carvalho, A.M., Junior, A.K.P., Andrade, M.A., and Jayme, V.(2013): Prevalence of *Enterobacteriaceae* in *Tupinambis merianae* (*Squamata Teidae*) from a captive facility in central Brazil, with a profile of antimicrobial drug resistance in Salmonella enteric. Phyllomedusa. 12(1), 57-67.

Collard, P., and Montefiore, D. (1956): *Agama agama* as a reservoir of *Salmonella* infection in Ibadan. West Afr. Med. J. 5, 54-56.

Collard, P., and Sen, R. (1960): Serotypes of Salmonella at Ibadan, Nigeria with special note of the new serotypes isolated in Nigeria. J. Infect Dis. 106, 270-275.

Dho-Moulin, M., and Fairbrother, J.M.(1999): Avian pathogenic Escherichia coli(APEC). Vet. Res 30(2-3), 299-316.

Garcia, L.S., and Isenberg, H.D.(2007): Clinical Microbiology Procedures Handbook Vol. 1, Second edn. update ASM Press American Society for Microbiology 1752 N St., N.W. Washington, DC 20036-290.

Gigani, H.C., Oguike, J.U., and Sakazaki, R.(1986): Salmonellae and other enteropathogenic bacteria in the intestines of wall gekos in Nigeria. Antonie Van Leewenhoek. 5, 117-120.

Kaura, Y.K., and Singh, I.P.(1968): Prevalence of Salmonellae in some of the common wall lizards, birds and rodents.Indian med. Res. 56, 1174-1179.

Mathewson, J.J.(1979): Enterobacteriaceae isolated from Iguanid lizards of West central Texas. Applied and Environmental Microbiology.38(3),402-405. Downloaded from http://aem.org/on December 4, 2014 by Guest.

Mitchell, M.A.(2006): Salmonella: diagnostic methods for reptiles. In Reptile Medicine and Surgery(D.M. Mader ed). St. Lous, Saunders Elsevier. Pp 900- 905.

Oboegbulem, S.I. and Iseghimhen, A.U.(1985): Wall gekos(Gekonidae as reservoirs of Salmonella in Nigeria: problem for epidemiology and public health. Int. J. Zoon. 12, 228-232.

Oboegbulem, S.I. and Okoronkwo, I. (1990): *Salmonellae* in African great cane rat (*Thryonomys swinderianus*). J Wildl Dis. 26, 119-121.

Ogunleye, A.O., Ajuwape, A.T.P., Alaka, O.O., and Adetosoye, A.I.(2013): Salmonella enterica Serotype Pullorum isolated from a lizard co-habitating with poultry. African Journal of Microbiology Research. 7(14), 1215-1221.

Ogunleye, A.O., Ajuwape, A.T.P., Adetosoye, A.I., and Ohore, O.G.(2006): Pathogenicity of *Salmonella* Paratyphi A in pullet. Revue Élev. Méd. Vét. Pays trop. 59(1-4), 5-9.

Orji, M.U., Onuigbo, H.C., Mbata, T.I.(2005): Isolation of Salmonella from poultry droppings and other environmental sources in Awka, Nigeria. Int J infect Dis. 9, 86-89.

Piercy, D.W.T., and West, B. (1976): Experimental Escherichia coli infections in broiler chickens: course of the disease induced by inoculation via the air sac route. J. Comp. Pathol. 86, 203-210.

Shinohara, N.K.S., Barros, V.B., Jimenez, S.M.C., Machado, E.C.L., Dutra., A.F., Lima, F.(2008): Salmonella species: importante agente pathogenic veiculedo em alimentos: Revistaciência and saúde coletiva. 13, 1675-1683.

Singh, B.R., Singh, V., Ebibeni, N., Singh, R.K.(2013): Antimicrobial and herbal drug resistance in enteric Bacteria isolated from faecal Droppings of common house Lizard/ geko(Hemidactylus fenatus). International Journal of Microbiology. http://dx.doi.org/10.1155/2013/340848.

Wilson, J.S., Hazel, S.M., Wilson, N.J., Phiri, A., French, N.P., Hart, C.A.(2003): Non typhoidal Salmonellae in United Kingdom badgers: prevalence and spartial distribution. Appl Environ Microbiol. 69, 4312-4315.