Nigerian Veterinary Journal
Vol 36 (2) 1203-1209
ARTICLE

Antimicrobial Profiles of Bacteria Isolated from Lizards Encountered in Poultry in Ibadan, Oyo State, Nigeria.

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SUMMARY

Lizards have been implicated as reservoirs in the spread and emergence of drug resistant bacteria.Antibiotic resistance in pathogenic bacteria constitutes a great threat to human existence. However most studies on drug resistance from pathogenic bacteriaisolated from lizards focussed more onmicrobial agents such as *Salmonella enterica* but less commonly on some other bacteria agents associated with lizards whose pathogenic roles may not have been clearly elucidated. This study reports the antimicrobial susceptibly/ resistance patterns of sixteen bacteria isolates including: *Salmonella enterica, Esherichia coli, Acinetobacter haemolyticus, Acinetobacter baumanni, Morganellamorgani biotype 1, Morganellamorgani subspecies siboni, Xenorrhabdusnematophilus, Edwardsiellaiclari, Trabusiellaguamensis, Hafniaalveibiogroup 1, Citrobacterwerkmanni, Citrobacteralmalonaticus, Pseudomonas aeruginosa, Klebsiella species, Proteus mirabilis and Enterobacter cloaca,* recovered from mouth/anal swabs of lizards co-habitating with poultry in Ibadan.

Most of the bacteria displayed a high occurrence of drug resistance to the antibiotics such as cefepime, tetracycline, Kanamycin, nalidixic acid, ampicillin, streptomycin, chloramphenicol and ciprofloxacin used for the study. The drug resistant bacteria from lizards co-habitating with poultry poses a potential public health hazard due to the possibility their spreading the drug resistance traits to more pathogenic bacteria strains in poultry and human associated with poultry production.

Keywords: Antimicrobial profiles; lizard co-habitating with poultry

INTRODUCTION

Antibiotic resistance is of a major public health concern due to ever increasing numbers of resistant strains of pathogenic bacteria;encountered compared to the relativelylengthy and laborious period required fordevelopments of new antibiotics (Cohen, 1992; Hawkey, 2008). More Effortshave been directed towards surveillance for the usage of antibiotics and resistance in humans and food animals in most parts of the world, Nigeria inclusive(Hasman *et al.,* 2005; Vlieghe et al., 2010; Shahada *et al.,* 2010; Ogunleye and Carlson, 2011; Ogunleye *et al.,* 2013).

However, other wildlife animals as well as lizards, have been noted to play the roles of reservoirs in the spread and emergence of drug resistant bacteria, but very limited information is available on antibiotic use and resistant bacteria of this group of animals (Oboegbulem and Iseghohemhen, 1985; Gugnani *et al.,* 1986; Lloyd, 2007; Wesse, 2008; Blackburn *et al.,* 2010).

Also, there appears to be more records on drug resistant *Salmonella serotypes* in lizards related studies than for some other enteric bacteria associated with lizards which could also playimportant roles in the epidemiology of spread of drug resistance in human and animals (Böhme *et al.,* 2009). For example all the 18 *Salmonella species* isolated from cloaca swabs from 14 tegu lizards (*Tupinambis* species) screened in Reggio Calabra, Italy were resistant to at least 6 of the antimicrobial tested (Giacopello *et al.,* 2012). In Nigeria, *Salmonella pullorum* carrying transferable 3rd generation cephalosporin resistant genes was isolated from the intestine of an *Agama agama* lizard co-habitating with poultry and was characterized (Ogunleye *et al.,* 2010; 2013).

This current work focussed on screening different types of bacteria isolated from the oral and anal swabs of lizards' co- habitating with poultry in some commercial poultry farms located in Ibadan for their antibiotic susceptibility, in order to access their potential roles in the transmission of antibiotic resistance in poultry.

MATERIALS and METHODS

Bacteria isolates studied for antimicrobial susceptibility were earlier isolated and identified from 193 mouth swabs, and 193 cloaca swabs sampled from 193 *Agama agama* lizards co-habitating 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 MICROBACT® identification kit.The bacteria isolates used for this susceptibility study included: 64/82(78%) *Escherichia coli*, 38/42(90.5%) *Salmonella enterica,* 9/9(100%) *Acinetobacter haemolyticus*, 2/2(100%) *Acinetobacter baumanni*, 1/1(100%) *Morganella morgani biotype 1*,1/1(100%) *Morganella morgani subspecies siboni*, 2/2(100%) *Xenorhabdus nematophilus*, 2/2(100%) *Edwardsiella i-clari,* 1/1(100%)*Trabusiella guamensis*,1/1(100%) *Hafniaalvei biogroup 1*,1/1(100%) *Citrobacter werkmanni*, 1/1(100%) *Citrobacteral malonaticus*, 3/5(60%) *Pseudomonas aeruginosa,* 10/14(71.4%) *Klebsiella species,* 18/22(82%) *Proteus mirabilis* and 96/111(86.5%) *Enterobacter cloaca*. The bacteria isolates were recovered from the 366 oral/anal swabs obtained from 183 Agama agama lizards co habitating with poultry captured from eight commercial poultry farms in Ibadan Oyo state Nigeria. The bacteria were isolated and identified based on standard bacteriological procedures described byBarrow and Felthams, (1993); Garcia and Isenberg, (2007) with the aid of MICROBACT® identification kit accordingto the manufacturer's protocol. The kit software was used to identify the various bacteria isolates.

Antibiotics susceptibility testing

The bacteria isolates were grown aerobically in breakpoint concentrations of 32µg/mL of cefepime, tetracycline, kanamycin, nalidixic acid, ampicillin, chloramphenicol, streptomycin and 8µg/mL for ciprofloxacin(SIGMA-ALDRICH, inc, 3050 Spruce street, St Louis MO63103, USA) based on standard method(CLSI 2009). Resistance was allotted where flocculent growths were observed after 16 hours of aerobic incubation at 37^{0}C.

RESULTS

The highest resistance for ampicillin was shown by *Escherichia coli* 85.9% (55/64), *Acnetobacter haemolyticus* showed 80% (8/10) resistance for ampicillin and ciprofloxacin respectively. However, *Salmonella enterica* showed 89.6% (31/38) resistance for nalidixic

acid, followed by *Enterobacter cloaca*. with 81.3% (78/96) resistance for nalidixic acid and *Klebsiella* species with 80% (8/10) for nalidixic acid. While *Proteus mirabilis* showed 89.9% (16/18) resistance for chloramphenicol were recorded from bacteria recovered from oral /anal swabs of lizards' co-habitating with poultry Table 1.

Based on antibiotic susceptibility patterns, *Esherichia coli* isolates with an occurrence of at 71.9% (46/64) was most susceptible to streptomycin. This was followed by *Salmonella enterica* with 65.89% (25/38) susceptibility to ciprofloxacin while *Klebsiell a*species showed 70% (7/10) susceptibility to ciprofloxacin; *Proteus mirabilis* showed 61.1% (11/18) susceptibility to cefepime and *Enterobacter cloaca* showed 72.9% (70/96) susceptibility to cefepime. However, *Acnetobacter haemolyticus* showed 55.6% (5/9) susceptibility to cefepime, nalidixic acid and streptomycin respectively Table 2.
For *Escherichia coli*, 67.2% displayed resistance to tetracycline, 70.3% to nalidixic acid; 85.9%, ampicillin; 56.9%, chloramphenicol and 59.4% for ciprofloxacin. Likewise for the *Salmonella enterica* isolates, there were 65.8% resistance to teteracycline; 63.2%, kanamycin; 81.9%, nalidixic acid; 78.9%, ampicillin and 76.3% to chloramphenicol.

DISCUSSION

Most of the bacteria isolated from the lizards co-habitating with poultry studied exhibited a relatively high percentage of resistanceand this finding is of public health concern.
This is because a number of them, such as *Salmonella enterica, Citrobacter species, Edwardsiella species, Klebsiella species* among others have been associated with one form of infections or the other as well as beingcapable of transmitting drug resistance to other more virulent organisms in man and animals (Gugnani et al., 1986; Gardam et al., 2002). For instance *Salmonella enterica* isolatesfrom lizards, *Esherichia coli* and *Pseudomonas aeruginosa*; carrying mobile genetic elements like plasmid, transposoon and integron, have been linked with epidemiology of drug resistance,as potential reservoirs of antimicrobial resistant genes(Guerra *et al.*, 2003; Agerso and Sandvang, 2005).Hence the potential public health risk of antibiotic resistance transmission of the otherbacteria isolated from lizards during investigation among which are: *Citrobacter* species*, Proteus mirabilis, Enterobacter* species*, Klebsiella* species and *Acnetobacter* species can be explained based on their various associations with transmissible drug resistant mechanism. *Proteus mirabilis* for instance have been noted for production of extended-spectrum B-lactamase(ESBLS) or the AmpC-types cephalosporinase(Cohen *et al.,* 2010). *Enterobacter species* and *Klebsiella species*; apart from their acknowledged ability to cause occasional food-borne diseases, have also been reported to be involved with the spread of antibiotic resistance (Cooney *et al.,* 2014).

TABLE I: ANTIBIOTIC RESISTANCE PATTERN FOR ORAL/ANAL BACTERIA ISOLATES FROM LIZARDS CO-HABITATING WITH POULTRY

S/N	Bacteria Isolates	Antibiotics resistance patterns							
		CEF	TET	KAN	NAL	AMP	STREP	CHLORAM	CIP
1	*Escherichia coli*	21/64 (32.8%)	43/64 (67.2%)	42/64 (65.6%)	45/64 (70.3%)	55/64 (85.9%)	18/64 (28.1%)	36/64 (56.2%)	38/64 (59.4%)
2	*Salmonella enteric*	15/38 (39.5%)	25/38 (65.8%)	24/38 (63.2%)	31/38 (89.6%)	30/38 (78.9%)	18/38 (47.4%)	29/38 (76.3%)	13/38 (34.2%)
3	*Acinetabacterhaemolyticus*	4/9 (44.4%)	5/9 (55.6%)	6/9 (66.7%)	4/9 (44.4%)	7/9 (77.8%)	4/9 (44.4%)	5/9 (55.6%)	7/9 (77.8%)
4	*Acinetabacterbaumanni*	1/2(50.0%)	1/2(50.0%)	0(0%)	1/2(50.0%)	2/2(100%)	1/2(50.0%)	1/2(50.0%)	1/2(50.0%)
5	*Morganella morganibiogrp 1*	1/1(100%)	1/1(100%)	0(0%)	1/1(100%)	0(0%)	0(0%)	1/1(100%)	0(0%)
6	*Morganella morganisubspsiboni*	0(0%)	1/1(100%)	0(100%)	1/1(100%)	0(100%)	0(100%)	1/1(100%)	1/1(100%)
7	*Xenorhabdusnematophilis*	1/2(50.0%)	2/2(100%)	2/2(100%)	2/2(100%)	1/2(50.0%)	1/2(50.0%)	1/2(50.0%)	1/2(50.0%)
8	*Edwardsiellaictalari*	2/2(100%)	1/2(50.0%)	1/2(50.0%)	2/2(100%)	1/2(50.0%)	1/2(50.0%)	1/2(50.0%)	0(0%)
9	*Trabusiella guamensis*	0(0%)	1/1(100%)	1/1(100%)	0(0%)	1/1(100%)	0(0%)	0(0%)	0(0%)
10	*Hafniaalveibiogrp 1*	1/1(100%)	0(0%)	0(0%)	1/1(100%)	1/1(100%)	1/1(100%)	0(0%)	1/1(100%)
11	*Citrobacterwerkmanni*	0(0%)	1/1(100%)	0(0%)	1/1(100%)	0(0%)	0(0%)	1/1(100%)	0(0%)
12	*Citrobacteralmalonaticus*	0(0%)	1/1(100%)	1/1(100%)	1/1(100%)	0(0%)	0(0%)	1/1(100%)	0(0%)
13	*Pseudomonas aeruginosa*	1/3(33.3%)	2/3(66.7%)	2/3(66.7%)	1/3(33.3%)	3/3(100%)	1/3(33.3%)	2/3(66.7%)	1/3(33.3%)
14	*Klebsiella species*	6/10(60.0%)	7/10(70%)	6/10(60%)	8/10(80%)	5/10(50%)	7/10(70%)	7/10(70%)	3/10(30%)
15	Proteus mirabilis	7/18(38.9%)	10/18(55.6%)	13/18(72.2%)	14/18(77.8%)	15/18(83.3%)	9/18(50.0%)	16/18(88.9%)	9/18(50.0%)
16	*Enterobacter cloaca*	26/96(27.1%)	73/96(76.1%)	65/96(67.7%)	78/96(81.3%)	72/96(75.0%)	37/96(38.5%)	66/96(68.8%)	41/96(42.7%

Cef= cefepime; TET= tetracycline; Kan= Kanamycin; Nal= Nalidicic acid; Amp= ampicilin; Strep= streptomycin; chloram= chloramphenicol; cip= ciprofloxacin.

TABLE II: ANTIBIOTIC SUSCEPTIBILITY PATTERNS FOR ORAL/ANAL BACTERIA ISOLATES FROM LIZARDS' CO- HABITATING WITH POULTRY

S/N	Bacteria isolates	Antibiotics susceptibility patterns							
		CEF	TET	KAN	NAL	AMP	STREP	CHLORAM	CiP
1	*Escherichia coli*	43/64 (67.2%)	21/64 (32.8%)	22/64 (34.4%)	19/64 (29.7%)	9/64 (14.1%)	46/64 (71.9%)	28/64 (43.8%)	26/64 (40.6%)
2	*Salmonella enterica*	23/38 (60.5%)	13/38 (33.2%)	14/38 (36.8%)	7/38 (18.4%)	8/38 (21.1)	20/38 (52.6%)	9/38 (23.7%)	25/38 (65.89%)
3	*Acinetabacter haemolyticus*	5/9(55.6%)	4/9(44.4%)	3/9(33.3%)	5/9(55.6%)	2/9(22.2%)	5/9(55.6%)	4/9(44.4%)	2/9(22.2%)
4	*Acinetabacterbaumanni*	1/2 (50%)	1/2 (50%)	2/2(100%)	1/2 (50%)	0(0%)	1/2 (50%)	1/2(50%)	1/2 (50%)
5	*Morganella morganibiogrp 1*	0(0%)	0(0%)	1/1(100%)	0(0%)	0(0%)	1/1(100%)	0(0%)	1/1(100%)
6	*Morganella morganisubspsiboni*	1/1(100%)	0(0%)	1/1(100%)	0(0%)	1/1(100%)	1/1(100%)	0(0%)	0(0%)
7	*Xenorhabdus nematophilis*	1/2 (50%)	0(0%)	0(0%)	0(0%)	1/2 (50%)	1/2 (50%)	1/2 (50%)	1/2 (50%)
8	*Edwardsiellaictalari*	0(0%)	1/2 (50%)	1/2(50%)	0(0%)	1/2(50%)	1/2(50%)	1/2(50%)	2/2(100%)
9	*Trabusiella guamensis*	1/1(100%)	0(0%)	0(0%)	1/1(100%)	0(0%)	1/1(100%)	1/1(100%)	1/1(100%)
10	*Hafniaalveibiogrp 1*	0(0%)	1/1(100%)	1/1(100%)	0(0%)	0(0%)	0(0%)	1/1(100%)	0(0%)
11	*Citrobacterwerkmanni*	1/1(100%)	0(0%)	1/1(100%)	0(0%)	1/1(100%)	1/1(100%)	0(0%)	1/1(100%)
12	*Citrobacteralmalonaticus*	1/1(100%)	0(0%)	0(0%)	0(0%)	1/1(100%)	1/1(100%)	0(0%)	1/1(100%)
13	*Pseudomonas aeruginosa*	2/3(66.7%)	1/3(33.3%)	1/3(33.3%)	2/3(66.7%)	0(0%)	2/3(66.7%)	1/3(33.3%)	2/3(66.7%)
14	*Klebsiella species*	4/10(40%)	3/10(30%)	4/10(40%)	2/10(20%)	5/10(50%)	3/10(30%)	3/10(30%)	7/10(70%)
15	Proteus mirabilis	11/18(61.1%)	8/18(44.4%)	5/18(27.8%)	4/18(22.2%)	3/18(11.7%)	9/18(50%)	2/18(11.1%)	9/18(50%)
16	Enterobacter cloaca	70/96(72.9%)	23/96(23.9%)	31/96(32.3%)	18/96(18.7%)	24/96(25.0%)	59/96(61.5%)	30/96(31.2%)	55/9657.3%)

Cef= cefepime; TET= tetracycline; Kan= Kanamycin; Nal= Nalidicic acid; Amp= ampicilin; Strep= streptomycin; chloram= chloramphenicol; cip= ciprofloxacin.

Likewise, the emergence and spread of drug resistant *Acinetobacter* species that were resistant to most of the available antimicrobial agents have been reported from health care facilities(Manchanda *et al.,* 2010). The treatments of such multidrug resistant pathogensis usually challenging for physicians and clinical microbiologist because the organisms can persist and survive in the environment for a long time (Jawad, 1996; Fournier and Richet, 2006).

These drug resistant bacteria isolated from

lizards' co-habitating with poultry in this study constitutes a potentialsourceof transmission of drug resistance to other poultry pathogens as well as to humans in close associations with poultry like the poultry attendants or along the food chain. There is also the need for further molecular studies of the underlying mechanisms associated with the phenotypic resistance patterns observed in the bacteria isolates from the lizards from poultry houses in order to gain better insight into the possible mode of drug resistance transfer by these organisms andthe best control measures to prevent the possible transfer.

CONCLUSION

The antimicrobials resistant bacteria pathogens isolated from the lizards co- habituating with poultry in this study could pose public health risk by serving as sources of transmission of drug resistance to other poultry pathogens as well as to humans in close contact with poultry. There is therefore the need for a concerted control measurein the poultry industry in Nigeria to control access of lizards to the poultry houses, feeds and water sources.

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