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Research paper

Comparative study of antibiotic resistance in bacteria isolated from dog and chicken

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Abstract

The emergence of antibiotic resistance has caused a threat to both human and animal population. This research was designed to investigate and compare the antibiotic resistance of bacteria isolated from chicken and dogs. A hundred and twelve samples of freshly dead chicken and eighty nine blood samples of sick dogs were analyzed. Pure culture of isolates were identified using cultural, morphological and biochemical characteristic. In vitro, susceptibility of the identified isolates against antimicrobial agents were determined by the standard disk diffusion procedure. One hundred and six isolates were recovered from chicken while 27 isolates were recovered from dogs. The organisms isolated include *E. coli*, *Haemophilus* sp, *Pasturella* sp, *Klebsiella* sp, *Enterobacter* sp, *Salmonella* sp, *Staphylococcus* sp., *Micrococcus* sp., *Pseudomonas* sp, *Proteus* sp, and *Listeria* sp. The antibiotic resistance showed that, gram-negative bacteria showed more resistance to the antibiotics used in this research compare to the gram-positive bacteria. This trend was found in isolates from both dog and chicken. In like manner, the bacteria isolates recovered from chicken showed a greater resistance when compare with the bacteria isolates recovered from dog. The increased resistance found in poultry makes poultry a suspect of residual resistance gene and probably reservoir for transmission.

Keywords: *Antibiotics; Dog, Feed; Poultry.*

1. Introduction

Antibiotics resistance in poultry and other animal has been of great importance to public health: they can serve as a source of recontamination for human or even reservoir of such as resistance gene. *Escherichia coli* and *Salmonella* from poultry have been found to be zoonotic, this makes antibiotic resistance in bacteria isolates from veterinary sources a matter of public health concern. Antibiotics are used in livestock feed sub-therapeutically to promote growth and increase feed efficiency. The use of antibiotics in animals is not totally safe and can lead to the development of antibiotic-resistant bacteria [9]. Misuse of antibiotics in poultry and pig production resulted in the development of antibiotic-resistant bacteria and eventually served as a reservoir of some of this resistant bacterium [8]. The emergence and spread of resistant bacterial strains like *Campylobacter* sp., *Escherichia coli* and *Enterococcus* sp in the intestinal tracts of these animals, making them reservoirs [5].

Dogs are often and generally used in security and as pets in households, to some, dog serves as a source of meat. This makes them to have a direct contact with humans who usually serve as the host in many communities. The health of this companion can also have a great toll on the human population. Since some of them harbor some organisms, which might be potentially zoonotic and are often shelled in their facial and urine. All of these, therefore, makes the well-being of these animals of public health concern.

Antibiotic resistant in chicken has been documented by many researchers [2], [3], [5], [14], [15]. Feed was reported to be a major vector for transmission of pathogens to farms and processing plants [4]. Both pre-harvest and post-harvest biological contaminants can be transmitted via feed ingredients to the mixed feed and finally to animals. It has generally been observed that the quality of animal feed is of public health importance because it affects the quality of animal, and the wholesomeness of meat consumed by man [10]. This research therefore, center on comparing the antibiotic resistance of bacteria isolates recovered from dogs with respect to those recovered from chicken.

2. Methodology

Hundred and twelve samples of chicken with different clinical signs were collected from thirty farms in Ekiti and Ondo States (south western Nigeria) between January and December 2016. Samples were transported to the microbiology laboratory within two hours of collection. The samples (one hundred and twelve freshly dead chicken) were necropsied; swabs were collected aseptically from the trachea, the spleen and the liver for bacteria isolation.

Blood samples were collected from eighty nine sick dogs brought to Metrovet veterinary hospital, Ado Ekiti.

2.1. Bacteriology

The liver, the spleen and the trachea were samples collected from the chicken. The samples were activated in buffered peptone water for 5 hours at 37°C. A loop full of the activated organisms in the buffered peptone water as well as the blood samples from the dogs were inoculated onto MacConkey agar, sheep blood agar, Eosin Methylene blue Agar, Nutrient Agar and Salmonella-Shigella agar by streaking. The plates were incubated at 37°C for 24 hours in an incubator (Royalcare England. DNP 9022A).

2.2. Cultural and biochemical characterization

The pure culture of each isolate was selected based on the cultural characteristics on agar. The morphological appearances under the light microscope after gram staining and biochemical reaction of the isolates were other characteristics used in the identification. Some of the biochemical parameters considered include: motility, catalase, oxidase, H_2S production, nitrate, urease, indole, methyl red, Voges-Proskauer and citrate use tests.

2.3. Antimicrobial drug sensitivity test

The antibiogram was carried out using the invitro standard disk diffusion procedure. The pure culture of each isolate was standardized using McFarland standard at the absorbance of 450nm. The isolates were inoculated on Muller-Hinton agar, after inoculation, the following antimicrobial agents were tested: Ceftazidime (CAZ 30 µg), Cefuroxime (CRX 30 µg), Gentamicin (GEN 10 µg), Ciprofloxacin (CPR 5 µg), Ofloxacin (OFL 5 µg), Nitrofurantoin (NIT 300 µg), Ampicillin (AMP 10 µg), Amoxicillin (AMOX 30 µg), Enrofloxacin (ENR10µg), Furasol (FUR 10 µg), and Tylosin (TLY 10 µg). Following the application of antimicrobial discs, the plates were inverted and incubated at 37°C for 24 h in an incubator (Royalcare England. DNP 9022A). The diameters of the zones of inhibition were measured (millimetres) and compared to internationally accepted standard to determine the susceptibility or resistance of the isolated.

3. Results

A total of one hundred and six isolates were recovered from the chicken over the period of this research. This comprised of 29 isolates of *E coli*, 4 isolates of *Haemophilus sp*; 13 isolates of *Pasturella* sp; 22 isolates of *Klebsiella* sp; 6 isolates of *Enterobacter* sp; 11 isolates of *Salmonella* sp; 15 isolates of *Staphylococcus* sp. and 6 isolates of *Micrococcus* sp.

Twenty seven bacteria isolates were recovered from the dog samples; this included 5 isolates of *Staphylococcus* sp; 8 isolates of *Bacillus* sp.; 4 isolates of *E. coli*; 4 isolates of *Klebsiella* sp; 1 isolate of *Pseudomonas* sp, 1 isolate of *Proteus* sp; 2 isolates of *listeria* sp, and 2 isolates of *Enterobacter* sp.

The antibiotic-resistant profile showed that gram negative bacteria isolates showed more resistance in bacteria isolates from chicken with the exemption of ciprofloxacin and furasol where higher resistance was found among the gram positive (Table 1).The result further showed that antibiotic resistance is higher among the gram-negative organisms from both chicken and dog except for cefuroxime where the gram positive had a higher percentage (46.7%) compare to the gram negatives (33.3%) as shown in table 2. The overall resistance of bacteria isolates was found to be higher in the isolates recovered from chicken compared with the isolates from dog samples (Table 1 and 2)

The antibiotic resistance pattern often encounter in this study was AMP, AMOX; TLY with 71.7 % of the bacteria isolates from chicken showing the pattern while 22.2% of the bacterial isolates from dog display the same resistance pattern (Table 3).

Table 1: Percentage Antibiotic Resistance and Susceptibility among 106 Bacteria Isolated to Recover from Chicken

Antibiotics	Resistance among G +ev	Susceptibility G +ve	Resistance among G -ev	Susceptibility G -ve	Total resistance	Total susceptibility
Cefuroxime	10 (47.6)	11 (52.4)	44 (51.8)	41 (48.2)	54 (50.9)	52 (49.1)
Ceftazidime	9 (42.9)	12 (47.1)	53 (62.4)	32 (37.6)	62 (58.5)	44 (41.5)
Amoxicillin	11 (52.4)	10 (47.6)	72 (84.7)	13 (15.3)	83 (78.3)	23 (21.7)
Ofloxacin	10 (47.6)	11 (52.4)	52(61.2)	33 (38.8)	62 (58.5)	44 (41.5)
Tylosin	20 (95.2)	1 (4.8)	75 (88.2)	10 (11.8)	95 (89.6)	11 (10.4)
Ciprofloxacin	15 (71.4)	6 (28.6)	57 (67.1)	28 (32.9)	72 (67.9)	34 (32.1)
Enrofloxacin	12 (47.1)	9 (42.9)	49 (57.6)	36 (42.4)	61 (57.5)	45 (42.5)
Nitrofurantoin	7 (33.3)	14 (66.7)	31 (36.5)	54 (63.5)	38 (35.8)	68 (64.2)
Furasol	11 (52.4)	10 (47.6)	38 (44.7)	47 (55.3)	49 (46.2)	57 (53.8)
Gentamicin	11 (52.4)	10 (47.6)	55 (64.7)	30 (35.3)	66 (62.3)	40 (37.7)
Ampicillin	16 (76.2)	5 (23.8)	80 (94.1)	5 (5.9)	96(90.6)	10 (9.4)

Table 2: Percentage Antibiotic Resistant and Susceptibility of 27 Bacteria Isolates Recovered from Dog

Antibiotic	Resistance among G +ev	Susceptibility G +ve	Resistance among G -ev	Susceptibility G -ve	Total resistance	Total susceptibility
Cefuroxime	7 (46.7)	8 (53.3)	4 (33.3)	8 (66.7)	11 (40.7)	16 (59.3)
Ceftazidime	6 (40.0)	9 (60.0)	6 (50.0)	6 (50.0)	12 (44.4)	15 (55.6)
Amoxicillin	9 (60.0)	6 (40.0)	10 (83.3)	2 (16.7)	19 (70.4)	8 (29.6)
Ofloxacin	2 (13.3)	13 (86.7)	3 (25.0)	9 (75.0)	5 (18.5)	22 (81.5)
Tylosin	6 (40.0)	9 (60.0)	5 (41.7)	7 (58.2)	11 (40.7)	16 (59.3)
Ciprofloxacin	4 (26.7)	11 (73.3)	7 (58.2)	5 (41.7)	11 (40.7)	16 (59.3)
Enrofloxacin	3 (20.0)	12 (80.0)	3 (25.0)	9 (75.0)	6 (22.2)	21 (77.8)
Nitrofurantoin	4 (26.7)	11 (73.2)	3 (25.0)	7 (58.2)	7 (25.9)	20 (74.1)
Furasol	6 (40.0)	9 (60.0)	1 (8.3)	11 (91.7)	7 (25.9)	20 (74.1)
Gentamicin	1 (6.7)	14 (93.3)	1 (8.3)	11 (91.7)	2 (7.4)	25 (92.6)
Ampicillin	10 (66.7)	5 (33.3)	10(83.3)	2 (16.7)	20 (74.1)	7 (25.9)

Table 3: Resistance Pattern in Bacteria Isolates from Dog and Chicken

Resistant pattern	Dog G +ve (n=15)	Dog G -ve (n=12)	TOTAL (n=27)	CHICKEN G+VE (n=21)	CHICKEN G-VE (n=85)	TOTAL (n=106)
AMP, AMOX, TLY	3 (20.0)	3 (23.1)	6 (22.2)	9 (42.8)	67 (78.8)	76 (71.7)
CEF, CAZ, AMOX	4 (26.7)	4 (33.3)	8 (29.6)	6 (28.6)	37 (43.5)	43 (40.6)
OFL, TLY, ENRO, AMOX	0 (0)	2 (16.7)	2 (7.4)	6 (28.6)	37 (43.5)	43 (40.6)
AMP, ENRO, FURA	0 (0)	0 (0)	0 (0)	7 (33.3)	27 (31.7)	34 (32.1)
GEN, NIT, CIP, FURA	0 (0)	0 (0)	0 (0)	1 (4.8)	6 (7.1)	7 (6.6)
GEN, OFL, CIP	0 (0)	0 (0)	0 (0)	7 (33.3)	62 (72.9)	69 (65.1)

CAZ, CEF, AMOX, OFL, TLY, CIP	0 (0)	0 (0)	0 (0)	2 (9.5)	16 (18.8)	18 (17.0)
ENRO, NIT, FURA, GEN, AMP	0 (0)	0 (0)	0 (0)	2 (9.5)	6(7.1)	8 (7.5)

4. Discussion

Antibiotic resistance remained one of the major concerns in the public health sector. Antibiotic resistance in bacteria isolated from chicken was reportedly linked to the ability of some of the bacteria to take up plasmid from other resistant bacteria [7]. The coexistence and spread of these small plasmids have resulted in most isolates showing multi-resistant [11]. The source of these bacteria in chicken may have resulted from the feed. Atere *et al*., [4] reported that antibiotics were often added to feed samples to boost the yield of the birds. The major organisms often encounter in chicken as presented in this research include *E. coli, Pasturella, Staphylococcus, Salmonella* and *Klebsiella*. This is comparatively related to what Uwaezuoke and Ogbulie, [12] reportedly recovered from poultry feeds, where *Bacillus, Pseudomonas, Escherichia coli, Staphylococcus aureus* and *Salmonella* were major bacteria often found in poultry feeds, this result was complemented by observation of Atere *et al*.[4] where *Escherichia coli, Klebsiella* sp, *Salmonella* sp, *Pseudomonas* sp, *Bacillus* sp, and *Staphylococcus aureus* were reportedly isolated. This is an indication that the source of the pathogen's encounter in this poultry may have resulted from the feed adopted.

The antibiotic resistance in this study is related to what was reported by Atere [2] in a study that centered on isolates of *E. coli* recovered from chicken. It was reported that 93. 8%% of the isolates were resistant to Ampicillin (AMP) while 100% of the isolates were resistant to Amoxicillin (AMOX). A similar high resistance is found in this research. The reason for such high and multiple resistance in chicken was attributed to misuse of antibiotics before clinical reports.

It is important to also note that, some of this organism may have acquired the resistant gene, even before they get to their host, in a report on the antibiotic resistance of organisms isolated from poultry feeds, it was observed that the organisms isolated showed resistance as high as 89.3% to Cloxicillin and 59.4% to Amoxicillin [4]. This is an indication that the bacterial often isolated may have acquired the resistance as a result of the sub-therapeutic dose often introduced in the poultry feed. In this study, nitrofurantoin showed a very high sensitivity. In previous studies, this was attributed to the seldom usage of the antibiotics in veterinary.

The antibiotic resistance in bacteria isolated from dogs are lower compared to isolate from chicken. This is evident in the antibiotic pattern observed in isolates from chicken compared to isolate from dog. Out of the eight antibiotic-resistant pattern, only three of them are evident in bacteria isolates from dogs while the whole patterns are visible in isolates from chicken. This may have resulted from the antibiotics often added to the feeds of poultry in other to improve the yield as earlier reported [6]. Arora *et al*, [1] also recorded that injudicious use of antibiotics in poultry has contributed remarkably in the resistance of bacterial isolates recovered from chicken. Up to date, there is no information on the bacteria associated with dog food as well as antibiotic resistance of organisms associated. There are no reports on the addition of antibiotics to the feeds of dogs, all of these factors combined may have resulted to the comparatively high susceptibility found in bacteria isolates recovered from dogs.

Dashe *et al*.,[6] reported that the antibiotic resistance often encounter in poultry could be attributed to the proliferation of fake or substandard drug in Nigeria while Van-den *et al*. [13] reported that the major factors responsible for antimicrobial resistance in bacteria are misuse of antibiotic, crowding and poor sanitation. These three factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in bacteria of poultry origin compared to bacteria from other sources [13].

5. Conclusion

There is a need to create more awareness among the population on proper hygiene when handling pets, this is because some of the bacteria isolated from dogs could easily be transmitted through contact, though the antibiotic resistance is low compare to what was observed for isolates from poultry, yet the effect this can have on the public health should not be under estimated. The poultry farmers in Nigeria should also be enlightened on the proper use of antibiotics, importance of clinical and laboratory test before administration of antibiotics, proper hygiene as well as the effect the type of feed adopted can have on their birds. Since some of these bacteria can easily be transmitted to human, it is of public health importance because these pets and birds can serve as the reservoir for the resistance gene.

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