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Phenotypic and molecular identification of antimicrobial resistance in *Escherichia coli* and *Salmonella* species isolated from apparently healthy broilers and zoo birds in Cameroon

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Abstract:

Background: Knowledge of antimicrobial resistance patterns of bacteria in food and pet birds in our environment is a prerequisite to effective control of bacterial diseases in humans and other food animals. Particularly, there is a dearth of information on the prevalence of resistant bacteria in pet and zoo birds in Cameroon. This study was carried out to determine the antibiotic resistance profiles of *Escherichia coli* and *Salmonella* spp isolates in apparently healthy poultry and zoo birds in Cameroon and to phenotypically and genotypically identify extended-spectrum β-lactamases (ESBLs) isolates in the poultry and aviary birds.
Methodology: This was a cross-sectional study of 320 randomly selected birds, which included 172 poultry and 148 zoo birds over a period of nine months, from which a total of 320 different non-repetitive samples were collected. The specimens were processed by standard microbiological culture methods at the National Veterinary Laboratory (LANAVET), Yaoundé annex, Cameroon. All isolated bacteria from cultures were identified as *E. coli* and *Salmonella* spp by conventional biochemical test scheme and confirmed with API®20E gallery. Antibiotic susceptibility test (AST) of confirmed isolates was done using the Kirby–Bauer disc diffusion technique, with AST results interpreted according to CLSI guidelines. Isolates with phenotypic characteristics of extended-spectrum beta-lactamase were subjected to molecular identification for $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV} genes. Data obtained were analyzed using descriptive statistics.
Results: Out of the 320 samples, a total of 88 *E. coli* and 17 *Salmonella* species were isolated from both broilers and zoo birds with an overall isolation prevalence of 27.5% and 5.3% respectively. High resistance of *E. coli* was observed among isolates from broiler, especially to trimethoprim-sulfamethoxazole (96.7%), ampicillin and ticarcillin (88.3%), norfloxacin (81.7%), piperacillin (78.3%) and ceftriaxone (63.3%). However, the resistance pattern among isolates from aviary birds was low with the highest resistance observed for imipenem (39.28%). The isolates had multiple antibiotic resistance indices (MARI) between 0.18-0.75 with an average of 0.3. A striking MAR index of 0.94 was observed in an ESBL isolate. Detection of β-lactamase genes in 16 phenotypic ESBL-producing *E. coli* and *Salmonella* isolates showed the presence of 75.0%, 6.3% and 12.5% for $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes respectively.
Conclusion: ESBL isolates were widespread among apparently healthy broilers in live-bird markets in Cameroon with ESBL-producing *E. coli* and *Salmonella* species showing high resistance to penicillin, quinolones and sulphonamides. In addition, there is evidence of antibiotic-resistant bacteria in wild birds which can be transmitted to humans through fecal droppings or by being in close contact with them.

Keywords: Aviary birds, faecal colonization; ESBL, Cameroon

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Identification phénotypique et moléculaire de la résistance aux antimicrobiens chez les espèces d'*Escherichia coli* et de

Salmonella isolées en portage chez des poulets de chair et d'oiseaux de zoo au Cameroun

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Resumé:

Contexte: La résistance aux antimicrobiens est un problème croissant dans le monde entier, avec des implications majeures pour la santé humaine et animale. Au Cameroun, l'étude de la prévalence des bactéries résistantes chez les oiseaux d'abattages et les oiseaux de zoo revêt une importance capitale pour comprendre et combattre efficacement les maladies bactériennes. Cependant, il existe un manque d'informations sur ce sujet dans ce pays. C'est dans ce contexte qu'une étude a été menée pour évaluer les profils de résistance aux antibiotiques des souches *d'Escherichia coli* et de *Salmonella* spp chez les volailles et les oiseaux de zoo sains et la caractérisation moléculaire des gènes de résistance chez les isolats de phénotype BLSE positifs.
Méthodologie: Il s'agissait d'une étude transversale portant sur 320 espèces aviaires sélectionnées au hasard qui comprenait 172 volailles et 148 oiseaux de zoo sur une période de 9 mois. Les écouvillons cloacaux ont été effectués chez ses espèces aviaires selon les procédures standards. La culture a été faite selon les techniques usuelles au Laboratoire vétérinaire national (LANAVET) annexe de Yaoundé, au Cameroun. Les isolats d'*E. coli* et *Salmonella* spp ont été confirmées a l'aide de la gallérie API20E, l'antibiogramme par la méthode de diffusion en milieu gélosé de Bauer-Kirby et la présence des gènes bla_{CTX-M}, bla_{TEM} et bla_{SHV} par PCR.
Résultats: Sur les 320 échantillons, un total de 88 souches d'*E. coli* et 17 souches d'espèces de *Salmonella* ont été isolées chez des poulets de chair et des oiseaux de zoo, avec une prévalence globale d'isolement de 27,5% et 5,3% respectivement. Une résistance élevée à *E. coli* a été observée parmi les isolats de poulets de chair, en particulier au triméthoprime-sulfaméthoxazole (96,7%), à l'ampicilline et à la ticarcilline (88,3%), à la norfloxacine (81,7%), à la pipéracilline (78,3%) et à la ceftriaxone (63,3%). Cependant, le profil de résistance parmi les isolats d'oiseaux de zoo était faible, la résistance la plus élevée ayant été observée pour l'imipénème, dont la résistance était de 39,28%. Les isolats présentaient plusieurs indices d'indice de résistance aux antibiotiques (MARI) compris entre 0,18 et 0,94. Un indice MAR frappant de 0,94 a été observé dans un isolat de BLSE. La détection de gènes de BLSE chez 15 isolats d'*E. coli* et un isolat de *Salmonella* producteurs de phénotypes positifs a montré la présence de 75.0%, 6,3% et 12,5% des gènes bla_{CTX-M}, bla_{TEM} et bla_{SHV} respectivement.
Conclusion: Les isolats de BLSE étaient répandus parmi les poulets de chair commercialise dans les marches au Cameroun. Les espèces *d'E. coli* et de *Salmonella* productrices de BLSE présentaient une résistance élevée à la pénicilline, aux quinolones et aux sulfamides. De plus, il existe des preuves de bactéries résistantes aux antibiotiques chez les oiseaux sauvages qui peuvent être transmises à l'homme par les excréments fécaux ou par contact étroit avec eux. Le séquençage des génomes complets permettra de mieux étudier les différents gènes de résistance circulant chez la volaille domestique et les oiseaux de la faune sauvage afin de faire une comparaison avec les isolats humains.

Mots clés: Oiseaux de zoo, Colonization fecale, BLSE, Cameroun

Introduction:

Antimicrobial resistance (AMR) is an international danger to development and health. To fulfill the objectives of the Sustainable Development Goals (SDGs), urgent multi-sectoral actions are needed. According to the WHO, among the top ten global public health hazards to humanity is AMR (1). In effect, a wide range of microorganisms exists in nature, both pathogens and commensals. They include bacteria, fungi, archaea, and protists. Commensal and pathogenic bacteria include *Escherichia coli* and *Salmonella* species (2). Salmonellosis and avian colibacillosis are regarded as the two most common bacteria diseases in the poultry industry globally and they are the most prevalent avian illnesses that can infect humans (3,4).

Antibiotics are widely used in poultry production to control infectious diseases, which consequently enhances high growth rate. This practice is reported to have caused high resistance to antibiotics by pathogenic microorganisms in poultry (5). Concerns about harmful bacteria developing high levels of antibiotic resistance due to overuse, misuse and abse of antibiotics in chicken production are spreading around the globe (6). Sub-therapeutic application of antibiotics in the form of feed

additives has been cited as one of the selective forces for emergence of antibiotic resistance (2,7). Resistant genes find their way into the environment hence may be transferred to other livestock, human and even get into wild animals which end up serving as reservoirs of such resistant genes (8).

Zoo birds are wild/captive birds that are kept in conservational centers, which serve as a protected habitat to protect them or preserve the endangered species from extinction. Environment is one of the most significant owner factors on the health of a zoo bird (9). Wild birds are important with regard to antibiotic resistance because they can move across great distances quickly. They may act as potential carriers of antibiotic resistance and they can also act as a store house and melting pot for genes and microorganisms resistant to antibiotics (10). These resistant bacteria may serve as a potential source of resistant genes that are subsequently transmitted to human pathogens by the process of conjugation (8,11). This study was carried out to identify antibiotic resistance in microbial reservoirs in birds so as to be able to address the increasing problem of antibiotic resistance in human, wildlife and livestock pathogens.

Materials and method:

Study area:

This study was carried out in the Center, Southwest and North regions of Cameroon. The choice of study area was based on the fact that these regions represent the hub of poultry production in Cameroon and hosts the three zoological centers in the country.

Samples from live bird markets were collected in poultry markets in Yaoundé in the Center region while samples from zoo birds were collected in Mvog-betsi botanical and zoological garden in the Center region, Limbe wildlife center in the Southwest region and Garoua zoological garden in the North region.

Study design and period:

This study was a cross-sectional design involving 320 avian species; 172 broilers and 148 zoo birds. The study was conducted over a period of nine months (14 January 2023 to 19 September 2023).

Ethical approval and authorization for study:

The Regional delegation of livestock, Fisheries and Animal industries (DREPIA) for the Centre region, authorized the collection of samples from poultry markets (N^0 54/2023/L /MINEPIA/DREPIA-CE/DDEPIA-MFD) and the Ministry of Forestry and Wildlife gave authorization for samples to be collected from the three zoos in Cameroon (N^0 1783/L/MINFOF/ SETAT/SG/DFAP/SDVEF/SC/ENJ).

Sample size:

The sample size was calculated using the Thrusfield formula (12), which gave a calculated minimum sample size of 257. Nevertheless, a total number of 320 avian bird species were sampled in our study.

Data and sample collection:

Rectal swabs were taken from the selected avian species by holding the bird with its head down and its posterior end facing up for easy location of the cloaca. The swab was then removed carefully and placed in sterile tubes containing 0.5ml Normal Saline to keep the content moist. The tubes were placed in cooler containing ice packs and then transported to National Veterinary Laboratory (LANAVET) for microbiological analysis.

Culture and isolation of *Escherichia coli* and *Salmonella* species:

Samples were pre-enriched in peptone water broth and the solution was spread using a sterile bacteriological loop onto MacConkey and *Salmonella-Shigella* (SS) agar plates, and incubated at 37^0C for 18 to 24 hours. *E. coli* grew on MacConkey agar as non-lactose fermenter (pink color colonies) while *Salmonella* spp grew on *Salmonella-Shigella* agar as transparent colonies with dark centers.

Colonies were identified as Gram-negative bacilli or coccobacilli on Gram staining while preliminary identification was done by conventional biochemical tests (13). Presumptively identified isolates were confirmed using API®20 E gallery and the identification validated by its analytic catalogue. *Escherichia coli* ATCC 352218 and *Salmonella* spp ATCC 14028 were used as controls for each test protocol.

Antimicrobial sensitivity testing:

The antimicrobial susceptibility (AST) of each isolate was done by the disk diffusion method of Kirby-Bauer (14). Overnight colony suspension of each isolate was prepared in nutritional broth and compared to the turbidity of 0.5 McFarland standards. Mueller-Hinton agar plates were inoculated with the organism suspension using a sterile swab stick, and prediffusion was allowed to occur for 30 minutes. The following antibiotics were placed on the inoculated MH agar surface; ampicillin (AMP 10µg), ticarcillin (TIC 75µg), piperacillin (PIP 30µg), piperacillin-tazobactam (TZP 10-100µg, ceftazidime (CAZ 10µg), amoxicillin-clavulanic acid (AMC 30µg), cefotaxime (CTX 5µg) aztreonam (ATM 30 µg), cefepime (FEP 30µg), imipenem (IPM 10µg), ceftriaxone (CRO 30µg), norfloxacin (NOR 10µg), cefoxitin (FOX 30µg), ciprofloxacin (CIP 5µg) and sulfamethoxazole-trimethoprim (SXT 23.75/1.25µg). The isolates were categorized as susceptible or resistant in accordance with the Clinical and Laboratory Standards Institute guidelines after the

inhibition zone diameters were measured with a vernier caliper (15).

Multi-drug resistance (MDR) of *E. coli* and *Salmonella* spp was taken as simultaneous resistance to three or more classes of antibiotics (16). The formula for calculating and interpreting the multiple antibiotic resistance index (MARI) was MARI=a/b (17), where 'a' represents the number of antibiotics to which a specific isolate was resistant to, and 'b' represents the total number of antibiotics tested against the isolate.

Double disk synergy test:

ESBL-producing isolate was identified by the double disk synergy test using a combination of amoxicillin-clavulanic acid disc and a third or fourth generation cephalosporin (15). A standardized inoculum of each isolate was used to inoculate MH agar plate. Amoxicillin-clavulanic acid disc was placed at the center and ceftriaxone (30µg) or ceftazidime (30µg) or cefotaxime (30µg) or aztreonam (30µg) disc was placed around the disc. The plate was incubated for 16 to 24 hours at 35± 2°C (18). The result was considered positive if the zone of inhibition of the cephalosporin disc increased towards the amoxicillin-clavulanic acid disc, producing a characteristic "Champagne cork" or "keyhole" effect.

Molecular identification of ESBL isolates

Molecular identification of phenotypically positive ESBL isolates was done by the quantitative real time PCR assay. The DNA of the isolates was first extracted using commercial extraction kit (QIAGEN©, Germany) at the molecular biology unit of LANAVET in accordance with the manufacturer guideline. The different reagents were reconstituted before the extraction process using 95% alcohol. After extraction, the presence of DNA in the samples was verified by electrophoretic migration on 1% agarose gel.

Amplification by Real time PCR assy

Simplex real time PCR (rt PCR) was used to amplify the genes encoding extended spectrum beta lactamases (*bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$) using the primers shown in Table 1. With the use of readily accessible commercial qPCR mixes and SYBR Green universal master mix, we created a simplex real-time qPCR test combined with melt curve analysis in a thermo cycler connected to a computer. About 4 µL of the extracted DNA, 10 µL of Luna universal qPCR Mix, 1 µL of primer pairs, and 5 µL of nuclease-free water made up the PCR reaction mixture, which had a total volume of 20 µl. All the genes were amplified using the following PCR conditions; initial denaturation for one minute at 95°C, 40 cycles of denaturation for fifteen seconds at 95°C, extension for thirty seconds at 60°C, and a melt curve (Tm) for 60°C, with Tm separation of > 2°C deemed adequate (19).

Prior to the assay, research has produced high resolution melting curve tests for the typing and subtyping of microbiological species and beta lactamases which were typically isolated from specific medical environments (20). The amplicons with similar melting temperatures (Tm) clustered in a successive manner with each other. The melt curves successfully detected the genes from isolates containing ESBL genes, with *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ showing melting temperatures at 87.5-88^{0}c, 85^{0}c and 75^{0}c respectively (Figs 1, 2 & 3).

Table 1: Specific primers used for PCR assy

Target gene	Primer sequence (5'-3")	Amplicon size	Hybridization temperature	Reference
bla$_{SHV}$	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	768	58	19
bla$_{TEM}$	F: GCGGAACCCCTATTTG R: ACCAATGCTTAATCAGTGAG	964	55	19
bla$_{CTX-M}$	F: ATGTGCAGYACCAGAARGTKTGC R: TGGGTRAARTARGTSACCAGAAYSAGCGG	592	55	19
bla$_{CTX-M-1}$	F: GGTTAAAAAATCACTGCGTC R: TTGGTGACGATTTTAGCCGC	863	55	19
bla$_{CTX-M-2}$	F: GATGAGACCTTCCGTCTGGACAG AAA R: CCGTGGGTTACGAT	397	55	19

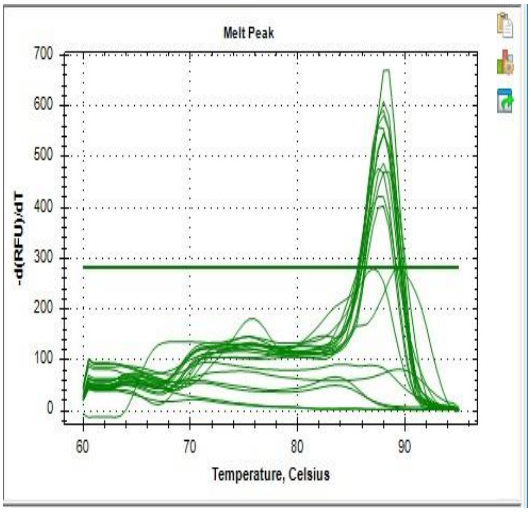

Fig 1: qRT-PCR result (melt curve analysis) of *bla*$_{CTX-M}$ (12 positive samples)

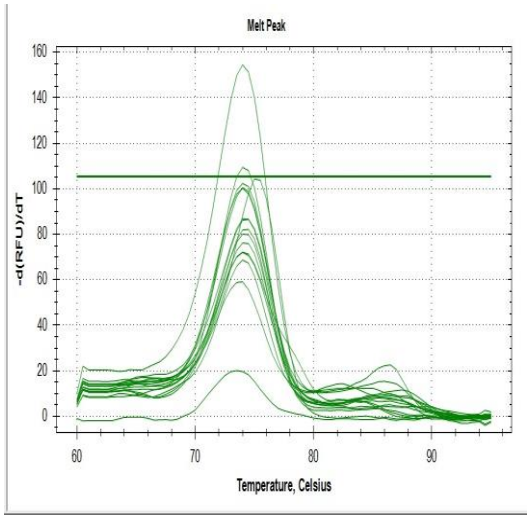

Fig 2: qRT-PCR result (melt curve analysis) of *bla*$_{SHV}$ (1 positive sample)

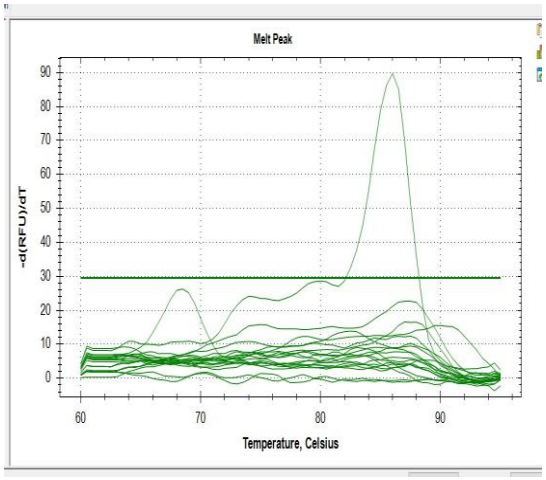

Fig 3: qRT-PCR result (melt curve analysis) of *bla*$_{TEM}$ (1 positive sample)

Results:

Out of the 320 avian species sampled, a total of 88 *E. coli* and 17 *Salmonella* spp were isolated from broilers and zoo birds, with an overall isolation prevalence of 27.5% and 5.3% respectively. Of the 88 *E. coli* isolates, 60 (68.2%) were from live bird markets (broilers) while 28 (31.8%) were isolated from the zoo birds. Of the 17 *Salmonella* spp isolates, 13 (76.5%) were from broilers while 4 (23.5 %) were from zoo birds.

The resistance profile of *E. coli* isolates with respect to animal species showed higher resistance rates among isolates from broilers, especially to trimethoprim-sulfamethoxazole (96.7%), ampicillin and ticarcillin (88.3%), norfloxacin (81.7%), piperacillin (78.3%) and ceftriaxone (63.3%), compared *to E. coli* isolates from aviary birds, in which resistance rates were low to these respective antibiotics (Fig 4), with the highest resistance rates observed against imipenem (39.28%) and piperacillin-tazobactam (38.0%). Isolates from zoo birds showed 100.0% susceptibility to ciprofloxacin, aztreonam and cefotaxime.

As shown in Fig 5, of the fifteen antibiotics tested, *Salmonella* spp from broilers were most resistant to trimethoprim-sulfamethoxazole and ciprofloxacin (84.6%), ticarcillin (69.2%), ampicillin (69.2%) and norfloxacin (69.2%) whereas in zoo birds, the isolates were 100.0% sensitive to most of the cephalosporins (ceftazidime, cefotaxime, cefoxitin), as well as to piperacillin, piperacillin-tazobactam, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole.

The prevalence of MDR in the isolates was 77.1% (81/105). The predominant MARI phenotype was in the following antibiotic classes; penicillin, quinolones and sulphonamides. Of the total of 81 MDR isolates, 11 (34.4%) were from zoo birds, while 70 (95.9%) were from broilers. Sixty-five (80.24%) *E. coli* isolates and 16 (19.75%) *Salmonella* species were MDR. The isolates had MARI indices between 0.18-0.94. A striking MARI index of 0.94 was observed in an ESBL isolate**.**

The phenotypic characteristics of the MDR ESBL revealed that 16 isolates were ESBL producers, out of which 15 were *E. coli* and 1 was *Salmonella* species. There were 75.0% (n=12), 6.3% (n=1) and 12.5% (n=2) of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes, respectively from the 16 phenotypic ESBL producing *E. coli* and *Salmonella* species isolates (Table 2).

The simultaneous presence of two (bla_{TEM}, bla_{SHV}) and three (bla_{CTX-M}, bla_{CTXM1}, bla_{CTX-M2}) ESBL genes in a single isolate occurred in 12.5% (n=2) and 62.5% (n=10) isolates, respectively (Table 2). One of the phenotypically ESBL positive isolate (6.3%) was negative for bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes.

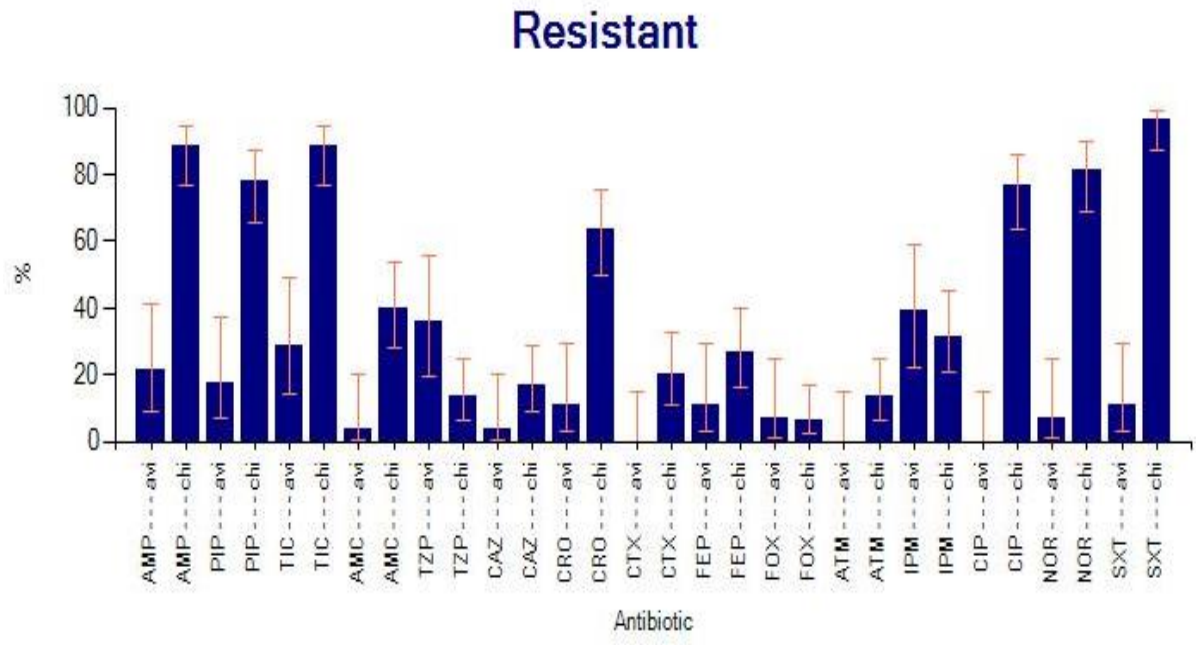

Fig 4: Comparative antibiotic resistance of *Escherichia coli* isolates from broilers (chi) and zoo (avi) birds

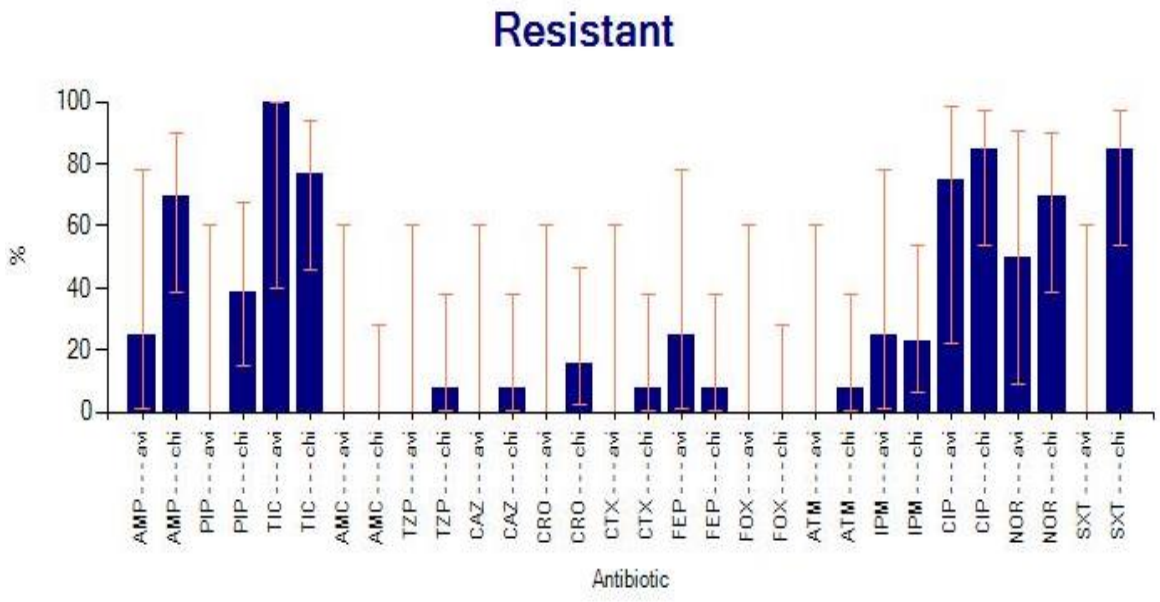

Fig 5: Comparative antibiotic resistance of *Salmonella* spp isolates from broilers (chi) and zoo (avi) birds

Table 2: Beta-lactamase gene pattern in the ESBL isolates

Beta lactamase gene pattern	No of isolates (n=16, %)
bla_{CTX-M}	12 (75.0)
bla_{CTX-M1}	12 (75.0)
bla_{CTX-M2}	12 (75.0)
bla_{TEM}	1 (6.3)
bla_{SHV}	2 (12.5)
$bla_{CTX-M, CTXM1, CTXM2}$	10 (62.5)
$bla_{TEM, SHV}$	2 (12.5)
No bla_{CTX-M}, bla_{TEM}, bla_{SHV}	1 (6.3)

Discussion:

Most of the antimicrobial agents tested in our study are frequently used in the poultry industry in Cameroon. *E. coli* isolates from chickens clearly demonstrated high resistance rates to almost all tested antibiotics used, in contrast to zoo birds, where relatively lower resistance rates were observed. This suggests that the degree of resistance to an antibiotic relates to the extent of its use. Isolation prevalence of 27.5% for *E. coli* and 5.3% for *Salmonella* spp in chickens in our study is lower than the prevalence reported in a study in Bangladesh (21) where the overall prevalence of *Salmonella* spp was 31.25%. However, 27.5% prevalence of *E. coli* in our study is similar to that of Leinyuy et al., (22) who reported *E. coli* isolation rate of 20.56%, followed by *Salmonella* isolation rate of 18.78% in Western region of Cameroon. The lower prevalence of *Salmonella* spp (5.3%) observed in our study could be due to differences in the types of samples collected, sampling period, locations, and types of production systems used.

In aviary birds, 31.8% of *E. coli* and 23.5% of *Salmonella* species were isolated from zoo birds. Isolation prevalence of *E. coli* (31.8%) in zoo birds in this study is similar to the findings in Messina in Italy, where 36.1% of *E. coli* was isolated from wild and zoo birds (23). The source of transmission of resistant bacteria of human and veterinary origin to wild birds seems to be via food acquisition and intake of water polluted with feces or human waste. However, it is important to conduct further epidemiological studies to understand the transmission of resistant bacteria to wild birds and back to the environment.

The susceptibility results of our study showed that *E. coli* isolates from commercial broilers were sensitive to cefoxitin (93.3%) and imipenem (63.3%). The high sensitivity to cefoxitin could be because this drug is not commonly used in chicken breeding in Cameroon. Nevertheless, a study conducted on broilers in the West region of Cameroon by Moffo et al., (7) in 2022 revealed a 100.0% sensitivity to imipenem. The low sensitivity to imipenem (63.3%) reported in our study may be an indication that farmers are resulting to the usage of this antibiotic, inspite of the fact this is an antibiotic of 'last resort' that must be used sparingly.

The results of our study showed that *E. coli* isolates from commercial chickens were resistant to trimethoprim-sulphamethoxazole, ampicillin, norfloxacin, piperacillin and ceftriaxone at the rate of 96.7%, 88.3%, 81.7%, 78.3%, and 73.3%, respectively. Higher resistance rates were seen to antibiotics of the penicillin, quinolone, and sulfonamide classes. Since these are the antibiotics reported to be frequently used by livestock caretakers in a study conducted in Cameroon (7), therefore, high resistance to antibiotics of these classes is not surprising. The result of our study is consistent with those of previous researchers, which raised concerns about the possibility that the use of antibiotics in food animals for growth or therapeutic purposes could select for antibiotic-resistant zoonotic enteric pathogens, which could subsequently be spread to humans through contaminated food or direct animal contact.

The most resisted antibiotics by *Salmonella* species isolated from broilers were trimethoprim-sulfamethoxazole (84.6%) and ciprofloxacin (84.6%), similar to the report in Bangladesh by Paul et al., (24), where 80.0% of *Salmonella* species isolates from broilers was resistant to ciprofloxacin. This high resistance rate is a major public health concern since fluoroquinolones are important antimicrobial compounds in the treatment of salmonellosis in humans.

The MDR prevalence in *E. coli* isola-

tes was 80.24% which is similar to 86.3% reported in broilers in Tanzania (25) but lower than 89.2% reported in China (26). Since MDR isolates could have a chance to contaminate food products and subsequently spread to humans, the high incidence of MDR in our study, particularly regarding isolates obtained from broiler chicken, is extremely substantial and needs to be considered a severe public health risk.

The information regarding the antibiotic resistance of the isolates from zoo birds particularly to carbapenems, will require the education of wildlife workers about the significance of using appropriate sanitation measures when handling zoo birds. As shown by a recent work, the role of wild birds as a reservoir for carbapenemase-encoding genes should be taken into account (27). Imipenem is a carbapenem, which is an antimicrobial agent that is used to treat a variety of serious infections when a microorganism is resistant to the primary agent of choice. Resistance to these antimicrobial agents is rare and limits therapeutic options. In our study resistance to imipenem occurred in 39.28% of isolates from zoo birds. It is unclear how microorganisms from these birds acquired imipenem resistance and, it is reason for concern.

Of 16 phenotypic ESBL identified, 15 were *E. coli* isolates while 1 was *Salmonella* specie. To the best of our knowledge, this is the first report of an ESBL in a *Salmonella* species isolate in Cameroon, similar to that of Mulvey et al., (28) where the first ESBL producing *Salmonella* species was discovered in an isolate in Canada. No ESBL producing bacteria was isolated in zoo birds in this study, which agrees with findings of studies carried out in other parts of the world (24).

The molecular analysis in our study showed that phenotypic ESBL *E. coli* isolates harbored various ESBL gene types. The frequency of β-lactamase genes has been reported to vary among nations, cities, and geographical areas (29). The prevalence of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} in the current study was 75.0%, 6.3% and 12.5%, respectively. This finding is similar to those reported by de Jong et al., (30) in 2014 and Saliu et al., (31) in 2017, who reported that bla_{CTX} was the most predominant ESBL gene. Interestingly, 1 (6.25%) of the phenotypically positive ESBL isolate was negative for bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes. This can be explained by the possible presence of other ESBL genes, which we did not investigate in this study. In other similar studies (29,32), the coexistence of different β-lactamase genes within the same isolates have been reported. Our results showed that 12.5% of the ESBL-producing isolates carry two β-lactamase genes. Many other researchers (33,34) have expressed significant concerns regarding the possibility of ESBL transmission from poultry to humans in Africa through zoonotic agents, based primarily on the existence of the same variants of the bla_{CTX-M} ESBL genes in birds and humans.

Conclusion:

In our study, ESBL isolates were wide spread among poultry that appeared to be apparently in good health. These isolates showed high resistance rates to penicillin, quinolones and sulphonamides antimicrobial groups that are commonly used on poultry farms in Cameroon. Our research identified major ESBL genes (bla_{CTX-M}, bla_{TEM} and bla_{SHV}) as the genetic basis for resistance in the ESBL isolates, with predominance of bla_{CTX-M}. There is ample evidence to support the idea that wild birds carry antibiotic-resistant bacteria and can possibly transmit it to humans through their droppings or by being in close contact with them.

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Contributions of authors:

ZZN, KDAN, OAO, and MMMM were involved in study conceptualization, methodology, data collection, curation, resource mobilization, original manuscript draft writing, review and editing; ZZN, KDAN, ID, OAO, MMMM and NMJC were involved in formal data analysis and software; KDAN, ID and OAO were involved in study supervision; ZZN and KDAN were involved in laboratory investigation; ZN and OAO were involved in funding acquisition; and ZN, DANK, OAO and MMMM were involved in data validation. All authors approved the submitted version of the manuscript.

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Conflict of interest:

The authors declare that the research was conducted in the absence of any

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