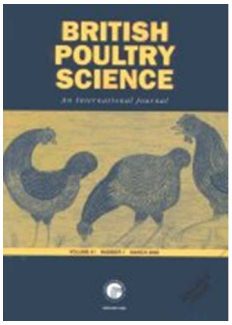

ABUSIVE USE OF ANTIBIOTICS IN POULTRY FARMING IN CAMEROON AND THEIR PUBLIC HEALTH IMPLICATIONS

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ABUSIVE USE OF ANTIBIOTICS IN POULTRY FARMING IN CAMEROON AND THEIR PUBLIC HEALTH IMPLICATIONS

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ABSTRACT

1 This study aimed to investigate the types and way of usages of antibiotics in poultry farms, their residual levels and the potential microbial resistances.

2 A questionnaire-based survey identified the different antibiotics used and High Performance Liquid Chromatography (HPLC) was used to determine antibiotics residual levels.

3 Pathogens were isolated, identified by use of API kits and Minimum inhibition Concentration (MIC) was determined.

4 Oxytetraxyclin, Tylocip and TCN were the most frequently used antibiotics. The antibiotics screened during HPLC were Chloramphenicol, Tetraxyclin and Vancomycin. All of them except Vancomycin were detected, and the concentration of these antibiotics was higher than the limit set by regulatory authorities Maximum Residual Limit (MRL).

5 However, no residues of various antibiotics were found in egg albumen or yolk. Furthermore, the concentration of Tetraxyclin was significantly high ($p<0.05$) in liver (150~~.030~~ ± 30.8~~780~~ µg/g) than in other tissues.

6 Foodborne pathogens including *Salmonella sp., Staphylococcus sp., Listeria sp., Clostridium sp.,* and *Escherichia species* were identified. Most of the pathogens were resistant to various antibiotics tested.

7 These findings imply a better management of antibiotics to control sources of food contamination and reduce health risks associated with the presence of residues and the development of resistant pathogens.

8 It is suggested that relevant stakeholders like Veterinary Services, Food and Drugs Board, the Ministry of Livestock, Fisheries and Animal Industries, the Ministry of Public Health, Cameroon Poultry Farmers Association such as IPAVIC ("Interprofession Avicole du Cameroun") and consumers associations make advocacy for enacting and enforcing regulations on food hygiene and use of antibiotics.

1. INTRODUCTION

The growth promoter effect of antibiotics was discovered in the 1940s, when it was observed that animals fed dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues improved their growth. Their mechanism of action when used as growth promoters was early related to their interactions with intestinal microbial population (Dibner and Richards, 2005; Niewold, 2007).

Nowadays, the use of antibiotics as growth promoter in developing counties such as Cameroon has facilitated the efficient production of poultry allowing Cameroonians to purchase, at a reasonable cost, high quality meat and eggs. Although these uses benefit all involved, unfortunately, the edible poultry tissues may have harmful concentrations of drug residues.

In fact, antibiotics are substances either produced naturally by living organisms or produced synthetically in the laboratory, and they are able to kill or inhibit the growth of microorganisms. Also, they can be classified according to their effects as either bactericidal or bacteriostatic and according to their range of efficacy as narrow or broad in spectrum. Theireir use in animals shortly followed their use in humans for the purpose of disease prevention and treatment (Gustafson, 1993). It have been also demonstrated that, the major antibiotics used for humans either belong to the same general classes or have the same mode of action as those used for animals (Joshi, 2002 Gelband *et al.*, 2015).

Today, antimicrobial drugs are used to control, prevent, and treat infection and to enhance animal growth and feed efficiency (Haihong *et al.*, 2014Tollefson and Miller, 2000). Currently, approximately 80% of all food-producing animals receive medication for part or most of their lives. The most commonly used antimicrobials in food-producing animals are the β-lactams, tetracyclines, aminoglycosides, lincosamides, macrolides, pleuromutilins, and sulfonamides (De BrayneLee *et al.*, 20141401). Nevertheless, the use of these antibiotics in food-producing animals canmay leave residues in foodstuffs of animal origin like meat, milk, and eggs.

A chemical residue is either the parent compound or its metabolites that may deposit accumulate or otherwise be stored within the cells, tissues, organs or edible products of animals following its use to prevent, control or treat animal disease or to enhance

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production (Riviere and Sundlof, 2001). Antibiotic residues in foods from animal origin may be the cause of numerous health concerns in humans. They range from direct toxicity on consumers exhibiting allergy reactions, immunopathological diseases, carcinogenicity effects (e.g., sulphamethazine, Oxytetraxyclin, and furazolidone), mutagenicity, nephropathy (e.g., Gentamycin), hepatotoxicity, reproductive disorders, bone marrow toxicity (e.g., Chloramphenicol), allergy (e.g., penicillin) and the destruction of useful microflora present in the gastro-intestinal tract especially of children leading to indigestion (Nisha, 2008; Nonga *et al.*, 2010); to indirect hazard through the generation of resistant strains of pathogenic bacteria which can be transfer to human and the residual contamination of manures used in crop productions (Dubois *et al.*, 2001; Kaitlin, 2013). Grote *et al.* (2007) showed in model farming experiments that even plants can take up antibiotics from manure present in soil. This raised concern as antibiotic residues might be transferred into plants in amounts that could pose a health risk for consumers (~~BfR~~Bundesinstitut für Risikobewertung, 2001).

These various health risks led to withdraw approval for antibiotics as growth promoters in the European Union since January 1, 2006. However, in other to ensure consumer safety, worldwide regulatory authorities have set MRL's (Maximum Residual Limit) for several veterinary drugs (European Union ~~EEC~~, 1990; Codex Alimentarius Commission ~~CAC~~, 2012). These MRL's, are expected to regulate the maximum permitted levels of the drug residue for each antibiotic which is considered safely acceptable in food of animal origin (Woodward, 1993).

Moreover, the development of antimicrobial resistant bacteria strains of animal origin associated with antibiotic residues and its consequent effect on human health regarding the efficacy of antimicrobial therapy ~~(Casadevall, 1996; Threlfall, 2002; Phillips *et al.*, 2004)~~ have become a worldwide public concern (Akbar and Anal, 2014). According to Prescott and Baggot (1993), microbial resistance to antibiotics, particularly aminoglycosides (Streptomycin, Neomycin, and Kanamycin) is very common and pathogens present in foodstuffs of animal origin mainly *S. aureus*, *E. coli* O157:H7 and *L. monocytogenes* may easily develop antimicrobial resistance (Tanih *et al.*, ~~Griffin and Tauxe~~, ~~1991~~2015).

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Therefore, monitoring antibiotics residues and the presence of pathogenic bacteria in animal derived food for human consumption has to be one of the most important duties for public health agencies (Samanidou *et al.*, 2008). Despite this recommendation, there is no clear regulation for control of such residues and pathogens in animal products for human consumption in many African countries particularly in Cameroon.

The aim of this study was to investigate on the use of antibiotics by poultry farmers in one of Cameroon's important agro-pastoral region (Western Highlands), determine the residual levels of some antibiotics by High Performance Liquid Chromatography (HPLC) and establish the resistance profile of isolated pathogenic bacteria in other to demonstrate the public health hazards.

2. MATERIALS AND METHODS

2.1 Localization of the study

The study was conducted in the Western Highland of Cameroon which is an important agro-pastoral area of the country. The geographical references of the Western Highlands of Cameroon are latitude 5° 20' and 7° North and longitude 9°40' and 11°10' East of the Equator (Nchinda and Mendi, 2008). This area includes two administrative Regions namely: the North-west Region with the town of Bamenda being the headquarters and the West Region with the town of Bafoussam as headquarters. Elevations reach as high as 3011 m and as low as 500 m above sea level, with the highest points being Mt. Bamboutos 2740 m in the West Region and Mt. Oku 3011 m in the North West Region. The climate is marked by a short dry season from November to mid March and a long rainy season from mid March to October. Rainfall ranges between 1300-3000 mm with a mean of 2000 mm. Minimum and maximum temperatures have means of 15.50°C and 24.5°C, respectively; although temperatures can go above 30°C. Three types of soils exist in the western highlands: volcanic, hydromorphic and ferralitic soils. The human population is estimated at 1.82 million inhabitants, being one of the highest population densities in the country, with at least 79 inhabitants per km^2 and a population growth rate of 3.1% (Nchinda and Mendi, 2008). This agro-pastoral area was purposively chosen, because he has the largest number of small and large scale poultry farms in Cameroon and contributing to about 56% of poultry production in Cameroon (Ngatchou and Teleu, 2006; Keambou, 2013).

2.2 Questionnaire-Based Survey on Major Farms

A Questionnaire-based survey in English and French was conducted on one hundred and thirty one (131) poultry farms to identify the most commonly used antibiotics, their dosage, timing of use and the practiced withholding times prior to dispatch. Between February and October 2012, several farms chosen randomly were contacted; only 131 agreed and participated between December 2012 and June 2013 to the survey. The georeference of each poultry farms was collected by the use of a Global Positioning System (GPS) receiver (GPSmap 76CSx, Garmin) and the softwares Google Earth, Global Mapper, Map Source and Adobe Illustrator CS4 were used to generate the map of the site.

2.3 Public health hazard

2.3.1 Identification and quantification of antibiotic in edible tissues and eggs by HPLC

2.3.1.1 Ethics statement

Animal experiments were performed according to the guidelines set for the care and use of laboratory animals and with the rules formulated under the Animal Welfare Act by the United States Department of Agriculture (USDA) and by adopting ARRIVE guidelines (Kilkenny *et al.*, 2011).

2.3.1.2 Preparation of samples

Eighty five Chickens (35 Layers and 50 Broilers) were randomly collected in various poultry farms without prior information to the farmers, killed by section of the jugular vein and muscle, liver, heart, kidney and gizzards were sampled aseptically from each carcass. The randomization process was performed in laying Hen farms by selecting an equal number of animals in each corner of the pen without showing any preference while in broiler farms, ~~:~~ an equal number of animals were collected in each corner of the pen with consideration to have an equal amount of sex. Furthermore~~Also~~, 20 samples of each tissue were collected from commercial barbecued sale points. At the same time, eggs samples (35

from poultry farms and 20 from commercial sale points) were randomly collected and placed in sterile polyethylene containers.

Prior to High Performance Liquid Chromatography (HPLC) analysis, a qualitative evaluation was performed through microbiological inhibition assay ("data not shown") as describe by Javadi *et al.* (2011), with the difference that the test organisms used were *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25922) and *Escherichia coli* (ATCC 13706) and also due to the fact that samples supernatant were used rather than tissues. Positive samples were selected for HPLC analysis.

2.3.1.3 Extraction and Quantitative Evaluation

The positive samples obtained (T= 41: 5 samples of each tissue, 8 albumen and 8 yolk) were dissolved in ultrapure water according to the ratio 0.3 g of sample in 10 mL and centrifuged at 2647 g for 10 min. The supernatant was filtered through a 0.20 µm cellulose acetate membrane filter (Schleicher & Schuell, Roma, Italy) and used for analysis. A portion of 25 µl of the filtrate was injected into the HPLC system for analysis. This analysis was performed on an Agilent Technologies 1200 HPLC system fitted with a SUPELCOSIL LC-18 column (length 250 mm, diameter 4.6 mm, packaging size 5 mm, TK mediterranea™ Sea 18, Roma, Italy) with ultra violet (UV) detector. The column temperature was settled to 20°C. The mobile phase consists of an aqueous solution of 0.5% volume acetic acid ("A") and acetic nitrile ("B"). Elution was performed as follows: At the beginning and during the first 2 min of run, 100% of "A"; from 2 min to 40 min after the beginning, a linear ramp was used, targeting 40% of "A" and 60% of "B". The flow rate was settled to 1 ml/min and antibiotics were detected by a UV detector (280 nm, TK mediterranea™ Sea 18, Roma, Italy). Beforehand, the retention times of the interest antibiotics compounds (Tetraxyclin, Chloramphenicol and Vancomycin purchased from Oxoid) were measured by using single antibiotic standard solutions at a concentration of 100 mg/l. These antibiotics were selected due to the high percentage of use by poultry farmers as reveal by the survey. The Detection Limit (DL) was defined as the concentration of antimicrobial that produces an analytical signal equal to thrice the standard deviation of the background signal and calculated as 8 ng/g.

2.3.2 Susceptibility to antibiotics of isolated poultry pathogens

2.3.2.1 Isolation and Identification

The collection of faeces was carried out on living birds localized at different geographical area according to the swab method as described by the International Organization for Animal Health (OIE) in the Terrestrial Manual (~~OIE,~~ 2005). After sampling, pathogenic bacteria were isolated from 45 swab samples following the procedure describe by Aly *et al.* (2004). The selective growth media Manitol salt agar (Biolife®, Milano, Italy), Listeria agar (Biolife®, Milano, Italy), Pseudomonas cetrimide agar (Oxoid, UK), Reinforce clostridia agar (Oxoid, UK) were used to isolate respectively *Staphylococci sp., Listeria sp., Pseudomonas sp.* and *Clostridia species*. Also, the semi-selective growth media Salmonella and Shigella agar (Merck, Darmstadt, Germany), XLD agar (Biolife®, Milano, Italy) were used to isolate respectively *Shigella sp.*, and *Salmonella species*. Finally, Mac Conkey agar (Conda, Madrid, Spain) was used to isolate other Enterobacteriaceae. All media and agar were prepared according to manufacturer's recommendations and were inoculated then incubated at 37°C for 24–48 h. After incubation, colonies were examined for cultural and morphological properties on growth media. The selected isolates were identified by using API systems (API 20 E, API Staph and API 20 NE) galleries (Biomérieux, Marcy l'Etoile, France). Interpretations of the fermentation profiles were facilitated by systematically comparing all results obtained for the isolates studied with information from the computer-aided database API LAB Plus V3.2.2. (). All cultures were maintained as stocks in specific broth at -20°C with 15% glycerol.

2.3.2.2 Determination of resistance profile of isolated pathogenic Bacteria

The microdilution method was adopted and performed in a 96 wells microplate and MICs (µg/ml) were determined. The results of susceptibility status were interpreted according to the recent FEEDAP (Panel on Additives and Products or substances used in Animal Feed) document of the European Food Safety Authority (EFSA) on the update of the criteria used in the assessment of antibiotics bacterial resistance of human or veterinary importance (~~EFSA,~~ 2008) and by the standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals approved by CLSI (Clinical Laboratory Standards

Institute), formerly National Committee for Clinical Laboratory Standards (~~NCCLS,~~ 2002). Strains showing MICs less than CLSI's breakpoints were considered sensitive; otherwise, they were resistant. The antibiotics including Ampicilin, Tetracyclin, Erythromycin, Amoxicillin-clavulanic acid, Chloramphenicol, Enrofloxacin, Gentamycin, Kanamycin, Vancomycin, Ceftiofur, and Trimethoprim-sulfamethoxazole obtained from Oxoid and Fluka were tested. The selection of these antibiotics was based on the CLSI's comprehensive list of antimicrobial agents that could be considered for routine testing by veterinary microbiology laboratories (National Committee for Clinical Laboratory Standards~~NCCLS~~, 2002).

2.4 Statistical Analyses

The computer program GraphPad InStat version 3.10 was used for the one-way analysis of variance (ANOVA). Student-Newman Keels means comparison test were use at a statistical significance pre-set at $P<0.05$.

3. Results and Discussion

One hundred and thirty one (131) poultry farms were enrolled and participated in the present investigation. They were mainly large scale semi-intensive or intensive production units without inclusion of backyard production units. The questionnaire used in the present study was written in English and French since Cameroon is a bilingual country and also in consideration that the Western Highlands of Cameroon covers English and the French region. Furthermore, the investigators were bilingual, were coming from various tribe of the region and were able to explain the questionnaire to farmers through culture mediated channels. ~~Between~~ Among the poultry farms~~them~~, 60.60% are localized in the West Region and 39.40% in the North West region (Figure). This proportion corroborate with the findings presented in the Food and Agriculture Organization (FAO) report establishing the aviculture situation in Cameroon (FAOSTAT, 2006).

Since the majority of farms managers and their farm hands had been generally formally educated, some with tertiary education and have had training in poultry production, they should be able to understand the necessity for enforcing farm hygiene and

making informed decisions on choice, administration, storage and withdrawal periods of antibiotics upon veterinary advice and prescriptions (Table 1). However, is obvious that these farms managers didn't implement farm hygiene and good antibiotic management ~~have concern~~given their education level. ~~to implement farm hygiene and good antibiotic management~~. Similar findings on farm staff educational backgrounds and their implications have been described by Turkson (2008). Moreover, the finding that as much as 89% of the farm staff had never been medically examined before in relation to their jobs, gave the impression that they did not care for being possible agents for transmission of zoonotic diseases.

It is evident ~~from~~ that majority of farmers constantly used antibiotics as prophylaxis and more intensively during disease outbreaks for treatments. Although minority of the farmers purchased medicines on prescription, it was noticeable that 80% of farmers, in spite of their formal education, made their own diagnosis and prognoses of diseases that were occurring or about to occur and formed their own opinions on what antibiotics to buy (Table 2). ~~Liberalization of antibiotic imports in Cameroon has made antibiotics easily available (reference).~~ It seemed that veterinary drug sellers did not insist on certified veterinary prescriptions before sales. They could even suggest the diagnoses of diseases to farmers so that they could sell their drugs. The situation could lead to unnecessary use and overuse of antibiotics, their wrong combinations, quick changeover to other drugs and improper dosage (Annan-Prah *et al.*, 2012~~Khan, 1975~~). The result would be the production of antibiotic resistant strains of bacteria ~~(Khachatourians, 1998)~~ and cross resistance with other bacteria (~~Baker-Austin *et al.*, 2006;~~ World Health Organization, 2014~~3~~).

From Table 3, it is apparent that the 26 drugs used in investigated farms could be grouped into antibiotics, formulations with low doses of antibiotics to be used as growth promoters, coccidiostats and ~~an~~ antihelminthic. Our results recorded that some of the antibiotics that were used neither gave information about their active ingredients nor their withdrawal periods. This usually occurred with imitated antibiotic products which could enter the country by unapproved routes to escape Veterinary Services, Food and Drugs Board and Standards Board's approval and customs duties (Annan-Prah *et al.*, 2012).

These results also indicate that Tylocip, TCN, Oxytetraxyclin and Amprolium powder were mostly used (Table 3). Tylosin is a macrolides antibiotic and the active ingredient of Tylocip. The soluble salt Tylosin tartrate is approved for poultry as a drinking water medication because Tylosin has a wide spectrum of activity against gram positive bacteria including *Staphylococci* and *Streptococci*, but narrow against gram negative bacteria like *Campylobacter* and *Pasteurella multocida* and against *Mycoplasma gallisepticum,* the causative agent of Chronic Respiratory Disease in poultry (Annan-Prah *et al.*, 2012). ~~However, resistance to Tylosin has been observed (ref)~~. Cross-resistance to other members of the macrolides group has been reported especially to erythromycin, which is used extensively in human treatments (~~BAM~~Bio Agri Mix, 2014). Although Tylosin is added to feed to promote increased rate of weight gain and improved feed efficiency, it is not approved for use as a feed medication for poultry in Canada and European countries (BAM, 2014; Phillips, 1999). It has been suggested that there are no or minimal benefits using antibiotics as growth promoters (Emborg *et al.*, 2001; Engster *et al.*, 2002; World Health Organization~~WHO~~, 2014~~2003~~). Further, USDA (2009) asserts that the assumed economic and production benefits of antibiotics in animal feed can largely be improved by improved cleanliness of animal houses and improved testing for diseases. However, World Health Organization~~WHO~~ (2000) advises that under no circumstances should antibiotics be used as an alternative to high-quality animal hygiene because overuse and abuse of antibiotics lead to the emergence of resistant strains in both the birds and man. The use of TNC powder presents two problems. The first is that it is a mixture of oxytetracycline, Chloramphenicol and Neomycin. The use of Chloramphenicol in veterinary medicine has been restricted to non-food animals (Annan-Prah *et al.*, 2012). The United States has banned nitrofurans, Chloramphenicol and Ampicilin in animal feed. Germany and the Netherlands have forbidden penicillin and tetracycline in feed. Neomycin can worsen kidney disease in man (Wongtavatchai *et al.*, 2004). The second issue is that TCN and Tylosin have withdrawal periods of 21 days and 10 days respectively, that makes it difficult for farmers who use them to wait for withdrawal periods before the sale of eggs or meat. Since 49.6% of investigated farms sold their products within the withdrawal periods, they is a high possibility for antibiotics residues to be present in these products reason while it is

important to monitor the concentration of these residues in other to be sure that they do not exceed the MRL.

In order to assess the occurrence of antibiotics in chicken edible tissues and eggs, the HPLC method was used after preliminary qualitative microbiological screening ("data not shown"). HPLC was applied to quantitatively determine antibiotics residues in samples (Table 4). The antibiotics screened were Chloramphenicol, Tetraxyclin and Vancomycin. All the compounds except Vancomycin were detected, and the concentration of these antibiotics was higher than the limit set by regulatory authorities Maximum Residual Limit (~~EU~~European ~~,~~Union, 2010). However, no residues of various antibiotics were found in egg albumen or yolk. This absence indicate that, the antimicrobial activities of selected eggs observed during preliminary qualitative microbiological screening maybe due to the presence of other antibiotics different from those use during HPLC. Kan and Petz (2000) had noted that drug residues will appear in both egg white and yolk after administration of drugs although poultry eggs contain a natural antibiotic substance, lysozyme, against most gram positive bacteria (Beuchat and Golden, 1989).

The levels of Tetraxyclin residues in all the tested samples were greater than the recommended MRL as set by the European Union (~~EU,~~ 2010) regulation commission (Table 4). Furthermore, the concentration of Tetraxyclin was significantly high ($p<0.05$) in liver (150~~.030~~ ± 30.8~~780~~ µg/g) than in other tissues. This result may indicate that the application doses used by the investigated farmers are exceeding the recommendations or the farmers are not observing the withdrawal period. These findings are similar to that obtained in a study from Taiwan (Su-Ching *et al.*, 2016) and come as confirmation of results presented earlier (Table 2) indicating that more than 49.6% of farmers sale their product within the withdrawal period. In addition, Chloramphenicol and Vancomycin is not approved for use as a medication for poultry in Canada and European countries (~~EU~~European Union, 2009; ~~BAM~~Bio Agri Mix, 2014; ~~Phillips, 1999~~). Mohammad *et al.* (1997) suggest that among the factors responsible for the occurrence of antibiotic residues in food are: failure to observe withdrawal periods, extended usage or excessive dosages, poor records of treatment, off-label use of antibiotics, lack of consumer awareness of hazards of antibiotic residues in food and lack of enforcement of legislation.

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The unnecessary use of therapeutic doses of antibiotics or as growth promoters in producing animals may be a main cause for the selection of multiple resistant strains of bacterial pathogens which can result in serious human and animal infections (World Health Organization, 2014~~Barber *et al.*, 2003~~). The microbiological analyses of swab samples from healthy chicken (Broilers and Layers) allowed in this study for the selection of the most common foodborne pathogens responsible of zoonoses diseases. These include among other *Salmonella sp*., *Staphylococcus sp*., *Listeria sp.*, and *Escherichia species* (Table 5). Proietti *et al.* (2007) isolated *salmonella* strains in conventional broiler chickens gastro-intestinal tract in central Italy. Neff *et al.* (2006) during a reference study on the prevalence of *salmonella* in flocks in Switzerland also isolated *Salmonella* strains. Furthermore, *salmonella* has been known to be the most prevalent pathogen to cause intramammary infections in poultry leading to major economic losses (Pengov *et al.*, 2005) and *Staphylococci* may produce a heat stable toxin in contaminated meat, eggs or milk (Normanno *et al.*, 2007). ~~Another~~Other serious pathogens such as ~~,~~ *Listeria* was also isolated from samples. *Listeria species* have been linked with numerous outbreaks associated with animal derived products (Lyytikainen *et al.*, 2000). Indeed, *Proteus sp.* are opportunistic diarrhea causes pathogens in poultry. Sambyal and Baxi (1980) had already detected occasional presence of bacteria of the genus Proteus in the digestive tract of chickens in Punjab in 1980. The other germs identified, namely *Clostridium sp.*, are frequent cause of foodborne disease and are also associated with necrotic enteritis in chickens (Seyed *et al.*, 2010). In addition, *Pseudomonas aeruginosa* infections are responsible of heavy losses in poultry farms. Furthermore, poor environmental sanitation noticed during the farms visits may be the cause of the presence of *Shigella sp., Providencia rettgevi and Escherichia species* in the analyzed samples. They are generally responsible of intestinal infections with more or less diarrhea. Recently, Tatsadjieu *et al.* (2009) isolated *Salmonella choleraesuis, Salmonella arizonae, Citrobacter diverticus, Aeromonas salmonicida, Bordetella sp., Cedecea lapagei, Vibrio damsel, Proteus mirabilis* and *Pseudomonas cepacia* in Broilers and Layers from poultry farms in North Cameroon (Ngaoundéré).

Studies have shown that *E. coli*, a normal habitat of human and animal intestines, when constantly gets exposed to antibiotics; it develops resistance in order to survive. When these resistant isolates are excreted to the environment by faeces, they tend to spread resistance genes by vertical gene transfer to pathogens (Sorum and Sunde, 2001; Richard and Yitzhak, 2014). Thus, this will result in resistance to antimicrobial drugs used in treating infectious diseases leading to serious health implications in both humans and animals.

The above risks are reflected in the results that showed most of all isolated microorganisms from samples to be resistant to various classes of antibiotics tested (Table 6). Interestingly, when comparing the MIC values (in µg/ml) of the pathogenic isolates with CLSI's Minimal Inhibitory Concentration breakpoints for veterinary pathogens, we can clearly establish that these microorganisms are resistant. In fact, it is generally noticeable that most of the dangerous foodborne pathogens that are *Listeria sp., Staphylococcus sp., Salmonella sp., Clostridium sp. and Escherichia species* are resistant. 63.64% of all pathogens were resistant to Tetracycline, 45.46% to Kanamycin and 63.64% to Amoxicillin-clavulanic acid. Moreover, the resistance percentage for Ampicilin was 54.55%, for Trimethoprim-sulfamethoxazole was 36.36% and 81.82% for Erythromycin. Finally, 45.46% of pathogens were resistant to Ceftiofur as well as 36.36%, 45.46%, 54.56% and 63.64% of them were resistant respectively to Chloramphenicol, Enrofloxacin, Gentamycin and Vancomycin. Similar result was reported by Tatsadjieu *et al.* (2009) indicating that the bacteria identified, presented multiresistance to the 11 antibiotics tested. Also, our results are in agreement with investigations showing a high prevalence of multidrug-resistant bacteria in poultry carcasses (Abdel-Maksoud *et al.*, 2015~~Ojeniyi, 1989; Manie *et al.*, 1998~~).

This may indicate that a high percentage of the chicken meat and eggs supply in Western Highlands market and in Cameroon in general may contain resistant strains of major foodborne pathogens against the mains drugs commonly used in therapeutic treatments; thus, incurring a major public health concern. Following the consumption of contaminated poultry meat or eggs, resistant bacterial strains may spread to ~~the~~ human population, which will lead to the transfer of genes coding for resistance (Bogaard and Stobberingh, 2000; Olatoye *et al.*, 2012; Richard and Yitzhak, 2014). The dissemination pathways of bacterial

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resistance from animals to humans were described earlier by Hummel *et al*. (1996). Levey *et al.* (1976) also confirmed that in chickens fed Tetracycline, the transfer rate of Tetracycline resistance genes between *Escherichia coli* strains from chicken to chicken and from chicken to human was higher.

In conclusion, antibiotics flood ~~the~~ Cameroonian market as medications and growth promoters and their purchase is often without prescription. The general organization of poultry production in one of Cameroon's important agro-pastoral region (Western Highlands) seems to rely on heavy doses of antibiotics to cover up hygiene deficiencies in their farm operations. Dosage and administration of antibiotics were often subjective and withdrawal periods were not observed in many cases. The direct consequence was firstly the quantification by HPLC of elevated amount of antibiotics residues in edible tissues greater than the recommended MRL and secondly by the identification of various resistance pathogens to the mains classes of antibiotics used. However, in order to reduce emergency of these resistant's pathogenic bacteria and subsequent contamination of poultry meat and egg, it is critical that risk reduction strategies are used throughout the food chain. Also, it is suggested that the relevant government agencies like the Veterinary Services, Food and Drugs Board, Ministry of Livestock, Fisheries and Animal Industries, Ministry of Public Health, Cameroon Poultry Farmers Association such as IPAVIC ("Interprofession Avicole du Cameroun") and consumers associations make advocacy for enacting and enforcing regulations on food hygiene and use of antibiotics.

RECOMMENDATIONS

- Cameroon's veterinary s~~S~~takeholders must come together to enact ~~guidelines regulating~~good farming practices ~~the presence of antibiotic residues in food~~ and enforce them to promote hygiene compliance in poultry farms.

- Furthermore, farmers should consult veterinarians and veterinary pharmacists or trained auxiliaries for a better advice on the type and quantity of antibiotics to be use as well as the respect of withdrawal period.
- Consumer associations should be more aware of the public health concern related to the presence of antibiotics residues in animal derived food and the generation of multiresistants pathogenic bacteria.
- Finally, the use of alternatives to antibiotics such as Probiotics, Prebiotics and Synbiotics as well as plant-derived antimicrobial substances and Charcoals may represent a promising option in the near future.

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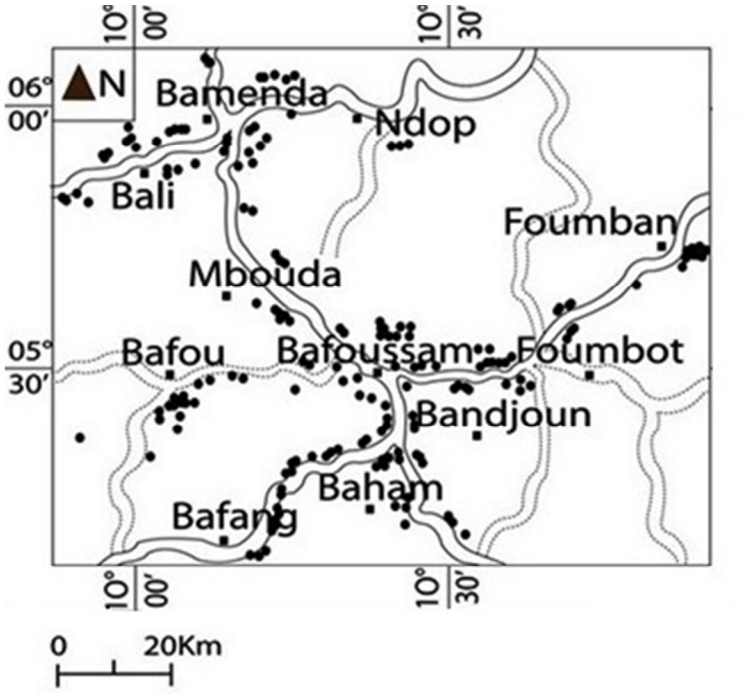

Figure: Georeference of investigated poultry farms in the Western Highlands of Cameroon. The georeference of each poultry farms was collected by the use of a Global Positioning System (GPS) receiver (GPSmap 76CSx, Garmin). Each point spot (•) represents a poultry farm. Each square spot (■) represents a town. The following symbols (————) and (•••••••••) indicate primary and secondary route respectively.
98x92mm (96 x 96 DPI)

Table 1: Percentage of poultry farmers whom have received an appropriate training, are regularly medically examined and their education level~~Educational status of staff of farms~~*

Factors	Frequency (n=131)
Education level	
Illiterate	0 (0)
Basic Education	20 (15)
Secondary/Vocational	90 (68)
Tertiary	20 (15)
No answer	1(1)
Training on poultry farming	
Trained	70 (53)
Untrained	61(47)
Medical examination	
Medically examined	15 (11)
Medically unexamined	116 (89)

**Percentages are in parenthesis*

Table 2: Knowledge of farmers on withdrawal period and it application as well as the rationale of usage and the factors they based on to select antibiotics ~~Antibiotic usage and handling~~*

Factors	Frequency (n=131)
Rationale for usage	
In disease outbreak	40 (31)
Prophylactic use	05 (4)
Prophylactic and curative	86 (66)
Reasons for choice	
Cost	117 (89)
Availability	96 (73)
Potency	26 (20)
Veterinary prescription	24 (20)
Farmer prescription	98 (80)
Cost	117 (89)
Knowledge and respect of withdrawal period	
Aware of withdrawal period	61 (46.6)
Respect of withdrawal	55 (42.0)
Sales of products within antibiotic withdrawal period	65 (49.6)
No sales of produce within antibiotic withdrawal period for eating	55 (42.0)
Aware of withdrawal period	61 (46.6)
Respect of withdrawal	55 (42.0)

**Percentages are in parenthesis*

Table 3: Percentage of antimicrobials used in investigated farms in the Western Highlands of Cameroon. The informations were collected by the use of a well structure questionnaire written in English and French ~~**Antimicrobials used in investigated farms**~~

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Antimicrobials used	Active ingredients	Withdrawal period	Total	Percentage (N= 131)
Hipralona Nor-S	Norfloxacin 200mg	NI*	49	37.4%
Enrofloxacin & Bromhexin HCl solution	Enrofloxacin 200mg	NI	35	26.7%
Amprolium	NI	NI	3	2.29%
Norfloxan 20%	Norfloxacin 200mg	4 days	40	30.53%
Anticoc super	Sodium sulfadimerazin 860g and diaveridin 105g	NI	18	13.74%
Enroveto – 20	Enrofloxacin 200mg	7days for meat and do not use in layers	38	29.00%
Oxyveto -50S	Oxytetraxyclin 500mg	7 days	121	93%
Vetacox S	Sodium Sulfadimidin 80g & diaveridin 8g	14 days	84	64%
TCN powder	Oxytetraxyclin HCL 50mg Chloramphenicol 50mg Neomycin sulphate 25mg	21 days	88	67.18%
T.T.S	Trimethoprim 4g sodium sulfadiazine 18.88g	12days	20	15.3%
BioPHA-FF	Flumequin 40g and Furaltadon 45g	NI	64	49%
Doxylin 200 wsp	Doxyciclin 200mg	7days	65	49.62%
Vet – colis 200 wsp	Colistin Sulphate 200mg	7days	53	40.5%

Oxytetraxyclin 50%	Oxytetraxyclin 500mg	7days	100	76.34%
Tylocip 20%	Tylosin 200mg	NI	115	87.8%
Ganadexil Enrofloxacina	Enrofloxacin 100mg	4 days for broiler and do not use in layers	35	26.7%
Anticox	Sodium Sulfadimidin 80g +& diaveridin 8g + vitamin K	12 days for both broilers and layers	79	60.3%
Diclacox	Diclazuril 1000mg	5 days	33	25%
Trisulmycin	NI	NI	46	35%
Colidox Forte	Colistin 5000I and Doxycyclin 200mg	7 days for both broilers and layers	76	58%
Tetracolivit	Oxytetracyclin 100mg + Colistin 7000I + vitamins	7 days for broilers and nil for layers	69	52.7%
Oxyvancovit	Oxytetracyclin 150mg + Vancomycin 125mg + vitamins	NI	100	76.34%
LEVA-200wsp	Levamisole 200mg	2 days for both broilers and layers	70	3.44%
Amprolium 300ws	Amprolium 200mg	3 days for both broilers and layers	94	72%
Oxydavit	NI	NI	18	13.74%
Levalap	Levamisole 200mg	2 days for both broilers and layers	60	45.8%

***NI**=No Indication about the withdrawal period or about the active compounds*

Table 4: Concentration of Chloramphenicol, Tetracyclin and Vancomycin in edible tissues as quantified by HPLC with comparison to MRL (Maximum Residue Limits) defined by the European Union (EU) regulation commission No 37/2010 ~~Concentration of antibiotics residues in various tissues~~

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Antibiotic	Sample	Residues level (µg/g)	MRLs* (µg/g)	Judgment
Chloramphenicol	muscle	1.4366 ± 0.3216[a]	Prohibited substance (MRL cannot be established)	Rejected
	gizzards	Not detectable [b]		
	heart	Not detectable 0.000 ± 0.000[b]		
	kidney	Not detectable 0.000 ± 0.000[b]		
	liver	Not detectable 0.000 ± 0.000[b]		
	Egg white	Not detectable 0.000 ± 0.000[b]		
	Egg yolk	Not detectable 0.000 ± 0.000[b]		
Tetracyclin	muscle	62.4380 ± 15.3261[b]	0.1	Rejected
	gizzards	21.3290 ± 4.3278[c]	ND**	Rejected
	heart	1615.950 ± 9.7629[c]	ND	Rejected
	kidney	8.9780 ± 4.9878[d]	0.6	Rejected
	liver	150.030 ± 30.8780[a]	0.3	Rejected
	Egg white	Not detectable0.000 ± 0.000[e]	0.2	Pass
	Egg yolk	Not detectable0.000 ± 0.000[e]	0.2	Pass
Vancomycin	muscle	Not detectable 0.000 ± 0.000[a]	Prohibited substance (MRL cannot be established)	Rejected
	gizzards	Not detectable 0.000 ± 0.000[a]		
	heart	Not detectable 0.000 ± 0.000[a]		
	kidney	Not detectable 0.000		

		~~± 0.000~~*		
	liver	Not detectable ~~0.000 ± 0.000~~*		
	Egg white	Not detectable ~~0.000 ± 0.000~~*		
	Egg yolk	Not detectable ~~0.000 ± 0.000~~*		

MRLs: Maximum Residue Limits, according to European Union (EU) regulation commission No 37/2010 [45]

***ND: Not defined; Number having the same letter are not significantly different (p>0.05).*

Table 5: Percentage of pathogenic strains isolated from chicken faeces using selective and semi-selective growth media and identified by the use of API 20E, API Staph and API 20NE systems~~Pathogenic strains isolated and identified~~

Name of strains	Percentage (%) of isolates (N= 28)
Clostridium sp.	7.14
Escherichia vulneris	10.71
Proteus vulgaris	7.14
Proteus mirabilis	10.74
Providencia rettgevi	10.71
Pseudomonas aeruginosa	3.57
Staphylococcus sciuri	7.14
Staphylococcus epidermidis	7.14
Salmonella sp.	17.86
Listeria sp.	10.71
Shigella sp.	7.14

Table 6: Percentage of antibiotic susceptibility of pathogenic strains isolated from chicken faeces as interpreted according to the FEEDAP (Panel on Additives and Products or substances used in Animal Feed) document of the EFSA (European Food Safety Authority) and the standards set by the CLSI (Clinical Laboratory Standards Institute), formerly National Committee for Clinical Laboratory Standards ~~Resistance percentage of pathogenic bacteria isolated from poultry~~

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Resistant percentage of isolated pathogenic strains											
	Antibiotics tested										
pathogenic strains	**GEN**	**KAN**	**AMC**	**AMP**	**ENR**	**ERY**	**XNL**	**CHL**	**SXT**	**TET**	**VAN**
Clostridium sp.	0	100	100	ND*	100	0	100	0	0	100	0
Escherichia vulneris	100	0	0	100	100	100	0	0	0	0	0
Proteus vulgaris	0	0	100	100	0	0	0	0	100	100	100
Proteus mirabilis	0	0	0	0	0	100	0	0	100	0	100
Providencia rettgevi	100	0	0	0	0	100	100	100	0	100	100
Pseudomonas aeruginosa	0	100	100	100	0	100	0	0	100	100	0
Staphylococcus sciuri	100	100	100	100	100	100	0	100	0	0	100
Staphylococcus epidermidis	100	100	100	100	100	100	0	0	0	0	100
Salmonella sp.	100	100	100	100	100	100	100	100	100	100	100
Listeria sp.	100	0	0	0	0	100	100	0	0	100	0
Shigella sp.	0	0	100	0	0	100	100	100	0	100	100
Percentage of resistant isolates/antibiotics	54.56%	45.46%	63.64%	54.55%	45.46%	81.82%	45.46%	36.36%	36.36%	63.64%	63.64%

***ND:** Not Defined; **GEN**= Gentamycin; **KAN**= Kanamycin; **AMC**=Amoxicillin-clavulanic acid; **AMP**= Ampicilin; **ENR**=Enrofloxacin; **ERY**=Erythromycin; **XNL**= Ceftiofur; **CHL**=Chloramphenicol; **SXT**=Trimethoprim-sulfamethoxazole; **TET**= Tetracycline; **VAN**= Vancomycin

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REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie

REPUBLIC OF CAMEROON
Peace-Work-Fatherland

Date:.....

GPS:.....

ACADEMIC INQUIRY FOR A DOCTORAL THESIS/PhD

IDENTIFICATION	*REGION.....	*DEPARTMENT.....	
	*DISTRICT.....	*QUARTER.....	
	*NAME OF THE FARM.....	*TYPE OF OPERATION	Poultry □
	* EDUCATION.....		Mixed Farming □

-Dear brother / sister:
-This questionnaire was developed in order to collect data on the use of antibiotics in poultry farms.
- On the last page, you can add information and comments that you consider useful in the practice of antibiotic therapy in this type of farming.
- With your valuable cooperation. Please accept dear brother, / sister, best regards.

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1. What is the importance of poultry activity in your life (check one)?

- Main activity [ ] - Secondary activity [ ]

2. What kind of speculation you generally follow?

- Broiler [.....] - Local chicks [.....] - Laying Hen [.....] - started [.....] - Broiler- Laying Hen [.....]

3. What is the herd size of animals in the current production?

.....

4. What are the main pathologies encountered?

	Major Diseases				
Speculation	Digestive	Breathing	Nervous	Locomotor App.	Nutritional
Broiler					
Laying Hen					
Local chicks					

5. Which antibiotic molecules do you use?

Furaltadon [.....] Flumequin [.....] Amoxicillin [.....] Céfixime [.....] Oxytetracyclin [.....] Streptomycin [.....] Colistin [.....] Nitrofurantoïn [.....] Neomycin [.....] Norfloxacin [.....] Vetpro-E [.....] Vetacox [.....] Aliseryl [.....] Fumesol [.....] Erythromycin [.....] Penicillin [.....] Ampicilin [.....] Tetracyclin [.....] T.T.S [.....] Chloramphenicol [.....] Doxycyclin [.....] Ciprofloxacin [.....] Bactrim (Cotrimodazole) [.....] Sulphamides [.....] Trimethoprim [.....] Flagyl (Metronidazole) [.....] Vermox (Mebendazole) [.....] Sulfadiazin [.....] Tylosin [.....]
Other ...../ ...../ ...../ ...../ ...../ ...../ ...../ ...../ ...../ ...../ ...../

6. For what purpose do you use antibiotics?

- Curative (in disease outbreak) [ ] - Prophylactic [ ] - Prophylactic and Curative [ ]

7. How do you choose antibiotics to be given to animals?

Personal selection [ ] - Cost [ ] – Availability [ ] – Efficacy (Potency) [ ] - Veterinary prescription [ ] - Drug dealer prescription [ ] - Other ...../ ...../

8. Where do you purchase the antibiotics?

-Veterinary Pharmacy [ ] - Farm Pharmacy [ ] - Local market [ ] - Other ...../ ...../

9. Who generally administer the antibiotic?

- Yourself [ ] - The Veterinary doctor [ ] - Other ...../ ...../

10. How do you administer the antibiotic?

- Water [ ] - Food [ ] - Gavage [ ] - Other ...../ ...../

11. When do you stop the antibiotic treatment?

- Disappearance of symptoms (even before the end of the specified time) [ ]
- End of the recommended amount of the drug [ ]

12. Practically, how do you establish the dosage?

- Count the animals [ ] - Estimation [ ] - Weighing (with scale) [ ] - Following Sheet [ ] - Estimation [ ] -Vet instructions [ ]

13. What is the frequency of administration of antibiotics by production cycle?

- 1 time [ ] -2 times [ ] -3 times [ ] - continuously [ ] - Depending on outbreak of diseases [ ] - Other ...../

14. What quantity of antibiotics do you use per production cycle of 100 chickens?

- 50g [ ] - 100g [ ] - 150g [ ] - 200g [ ] - 250g [ ] - 300g [ ] - 350g [ ] - 400g [ ] - 450g [ ] - 500g [ ] - Other...../

15. Do you know the concept of « withdrawal period»?

- Yes [ ] - No [ ]

16. If yes, do you observe these deadlines?

- Yes [ ] - No [ ]

17. What is the duration of the « withdrawal period» you observe?

- 0 day [ ] - 2 days [ ] - 4 days [ ] - 6 days [ ] - 7 days [ ] - 8 days [ ] - 10 days [ ] - 12 days [ ] - 14 days [ ]
- 15 days [ ] - 16 days [ ] - 17 days [ ] - 18 days [ ] - 19 days [ ] - 20 days [ ] - Other ...../ ...../ ...../ ...../

18. Do you sale the animals during this withdrawal period?

- Yes [ ] - No [ ]

19. Have you received training on poultry farming?

- Yes [ ] - No [ ]

20. Are you often medically examined?

- Yes [ ] - No [ ]

INFORMATION AND/OR NOTES

Thanks for your collaboration and time spent completing this questionnaire