INTERNATIONAL JOURNAL OF HEALTH & ANIMAL SCIENCE & FOOD SAFETY

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Screening of fluoroquinolone residues in imported and locally produced broiler chicken meat in Ibadan, Nigeria.

Omotoso Adekunbi B.[1*] and Omojola Andrew B.[1]

[1] Animal Products and Processing Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria

ABSTRACT.

The study was conducted to investigate residues of three fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin) in chicken meat sold in Ibadan, Nigeria. Two hundred and ninety-seven (297) samples of imported frozen (99) locally produced frozen (99) and freshly slaughtered (99) broiler chicken meat products were screened for antibiotic residues by microbiological assay using *Escherichia coli* as test organism. High performance liquid chromatography (HPLC) with ultraviolet (UV) detection was used for the determination of ciprofloxacin, norfloxacin and ofloxacin in the positive samples. One hundred and sixty (160) samples, constituting more than half (53.87%) of total sample size tested positive for *Escherichia coli*–sensitive antibiotic residues. Positive samples were 54.55%, 56.57% and 52.53%, of freshly slaughtered, locally produced frozen chicken and imported frozen chicken meat respectively. Residues of investigated fluoroquinolones occurred more frequently in locally produced frozen chicken than in imported frozen chicken. The concentrations were however consistently higher in imported chicken. Among the three fluoroquinolones examined, the most abundant in imported frozen chicken was ciprofloxacin with mean 354.83±716.43µg/kg. Norfloxacin was the most abundant in freshly slaughtered and locally produced frozen chicken meat, having mean values of 107.70±138.36µg/kg and 120.96±162.83µg/kg respectively while ofloxacin was the lowest in all categories. Most frozen chicken products imported into Nigeria at the time of this study contain higher levels of residual fluoroquinolones than the locally produced chicken. In order to tackle fluoroquinolone resistance from a food safety perspective, proper usage and monitoring of fluoroquinolones in meat animals should be encouraged in developing countries.

KEYWORDS

Fluoroquinolones, Residues, Chicken, Antibiotic resistance, Nigeria.

PAGES

25 – 34

REFERENCES

Vol. 1 No. 2 (2014)

ARTICLE HISTORY

Submitted: September 26, 2014
Revised: November 29, 2014
Accepted: December 02, 2014
Published: December 04, 2014

CORRESPONDING AUTHOR

Omotoso Adekunbi B.,
Animal Products and Processing Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria

e-mail: kunbiadeshiyan@yahoo.com
phone: +234 08033900828

JOURNAL HOME PAGE

riviste.unimi.it/index.php/haf

1 Introduction

The World Health Organization (WHO) recognises that while antibiotics can play a critical role in food production, there is need to balance their use to ensure they remain a valuable tool for both human and animal health (WHO, 2011b). Antibiotics used in meat animal production do not pose a health hazard provided they are used in accordance with the recommendations for their use, proper dosage, proper route of administration, proper species of animal and adequate withdrawal period before slaughter (Dipeolu, 2010).

Quinolones are effective chemotherapeutic agents with very good antibacterial activity that target DNA synthesis. Fluoroquinolones are derivatives of quinolones which are typically fluorinated at C-6 or C-7 position of the quinolone ring. Norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin, enrofloxacin are second generation quinolones, exhibiting a broader activity against gram negative and gram positive bacteria, less protein binding, higher drug tolerance, lower toxicity and longer half life than the first generation (Emami *et al.*, 2010; Somasundaram and Manivannan, 2013).

Drug residues in foods have been said to cause allergic reactions, toxicity, technological problems in fermented products and the development of antibiotic resistance in human pathogens. It has been documented that a major route of transmission of resistant micro-organisms from animals to humans is through the food chain (Hernandez-Serrano, 2005; Canada-Canada, 2012). Quite unfortunately, poultry production has been implicated in the emergence of quinolone resistant bacteria.

Resistance to fluoroquinolones in *Escherichia coli* and *Salmonella typhi* is an increasing problem in Nigeria and many other countries (Daini *et al.*, 2005). Researchers have demonstrated the prevalence of bacteria which are resistant to various fluoroquinolones in different parts of Nigeria, including Lagos (Aibinu *et al.*, 2004), Benin City (Enabulele *et al.*, 2006), Oshogbo (Olowe *et al.*, 2008) and Ibadan (Makanjuola *et al.*, 2012).

Antibiotic resistance has long been recognised as a food safety issue and all stakeholders in food production are responsible for the prevention and control of antibiotic resistance through the food chain. The rising prevalence of antibiotic resistance is a particularly important problem in developing countries where there is limited control of the quality, distribution and use of antibiotics in human medicine, veterinary medicine and food animal agriculture (Okeke *et al.*, 1999). It is important for countries to monitor residues of antibiotics in their foods because the use of antibiotics in one sector, setting or country affects the spread of resistance in others. The WHO has noted that since animal products are traded worldwide, they contribute to antibiotic resistance in countries far from where the problem originates. Hence the need to monitor both imported and locally produced animal products for residues of antimicrobials, especially highest priority critically important antimicrobials such as the fluoroquinolones (WHO, 2011a).

Ciprofloxacin and norfloxacin are among the most commonly used fluoroquinolones in poultry production in Ibadan metropolis and ofloxacin is the common drug for treating tuberculosis in Nigeria (Daniel *et al.*, 2011). This study seeks to examine samples of locally produced and imported chicken products for residues of three fluoroquinolone compounds (ciprofloxacin, norfloxacin and ofloxacin) because of their importance in both animal and human medicine.

ISSN: 2283-3927

2 Materials and Methods

2.1 Study area

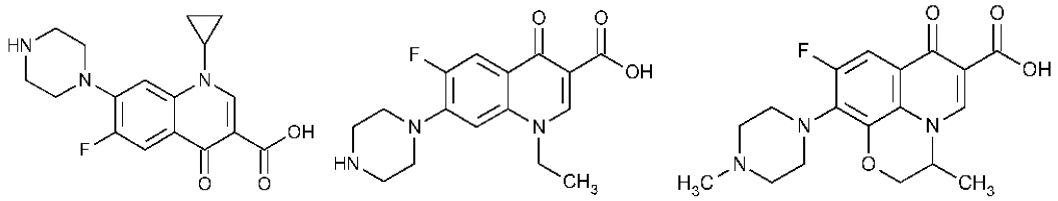
The study was carried out in Ibadan, Oyo State, Nigeria. Samples were collected from small, medium and large scale farms as well as open frozen chicken market spread across the eleven (11) local government areas that make up Ibadan, the capital of Oyo State, Nigeria.

2.2 Sample collection

Sample size was two hundred and ninety seven (297) comprising 99 samples of freshly slaughtered chicken (from 3 small scale, 3 medium scale and 3 large scale farms in each of eleven local government areas), 99 samples of locally produced frozen chicken and 99 samples of imported frozen chicken thigh muscles. The samples were collected on ice and kept frozen at -3°C at the Meat Science Unit of the Department of Animal Science, University of Ibadan, until they were further transported in padded coolers with ice packs to the Agulu Laboratory of the National Agency for Food and Drug Administration and Control (NAFDAC) where the analysis was done. The samples were screened for three fluoroquinolones, ciprofloxacin, norfloxacin and ofloxacin with structural formulae as shown in Figure 1.

Figure 1. Structural formulae of ciprofloxacin, norfloxacin and ofloxacin.

Source: http://www.newdruginfo.com/pharmacopeia/usp28/v28230/usp28nf23s0.htm



Ciprofloxacin (b) Norfloxacin (c) Ofloxacin

2.3 Microbiological screening

One Plate Test (OPT) as described by Alla *et al.* (2011) was adapted for this study. Microbiological screening using the plate test depends on bacterial growth inhibition. The test organism was *Escherichia coli* (American Type Tissue Culture 11303). *Escherichia coli* has been indicated as a reliable test organism for the detection of fluoroquinolones in animal products. To prepare the sub-culture, wire loop chrome passed through Bunsen burner flame was allowed to cool and used to seed *Escherichia coli* in the nutrient broth which was then incubated at 37°C for 24 hours. The culture medium was prepared by suspending 34g of Mueller Hinton Agar (Merck KGaA, Damstadt, Germany) in 1 litre of demineralised water and heating in a boiling water bath. It was then autoclaved for 15 minutes at 120 °C. The culture medium was sterilized in an autoclave at 121 °C for 20 minutes.

ISSN: 2283-3927

Sterilized stainless steel scissors, forceps and cork borers were used in handling meat samples. The work table was wiped down intermittently with 70% ethanol.

20ml of the culture medium was poured into each 90mm Petri dish and left on the work-bench for about 15 minutes to solidify. With the aid of sterile swab sticks, *Escherichia coli* was seeded in the plates. Holes were punched into each agar plate with a cork borer. Sterile forceps were used to place meat discs inside the hole. The plates were then incubated at 37°C±2 for 24 hours. Zone of inhibition was measured with a mm-graduated ruler. Samples with no clear zone or with clear zones less than 1mm were taken as negative. Clear zones between 1mm and 2mm were considered doubtful while zones from 2mm upwards were positive.

2.4 Confirmation and quantification of fluoroquinolones by HPLC with UV detection

Negative samples were discarded while chicken samples that were either doubtful or positive for residues of antibiotics were subjected to HPLC-UV for identification and quantification.

Residue extraction was done as described by (Ovando *et al.*, 2004). 0.2g thigh muscle was homogenized with 2mL phosphate buffer (pH 7.2) prepared in the laboratory. 8mL Dicholoromethane (Sigma Aldrich, St. Louis, Mo, USA) was added to the homogenate, which was mixed on a Stuart Scientific SA8 vortex mixer (Sigma Aldrich, St. Louis, Mo, USA) at 1000rpm for 1 minute and centrifuged at 4000 rpm for 20 minutes. The upper aqueous layer was discarded, the organic phase was transferred to a clean tube and the tissue was again extracted with 6mL of dichloromethane. Organic layers were combined and evaporated at 30°C under nitrogen stream. The extract was re-dissolved with 200µL of mobile phase and 100µL was used for HPLC analysis.

The HPLC equipment was Elite Lachrom VWR, Hitachi HPLC chromatograph (Hitachi, Tokyo, Japan) comprising L2200 autosampler, L2130 pump and L2350 column oven, equipped with L2400 UV VIS detector and Ezchrom Elite software. The separating column was Elite C18 (250mmx4.6mmx5µm) (Hitachi, Tokyo, Japan).

Reference standards of ciprofloxacin (99.6%), norfloxacin (99.5%) and ofloxacin (99.9) were provided by NAFDAC. Buffer phosphate solution 0.1M, pH 7.2 was prepared in the laboratory. Deionised water, passed through 0.45µm Whatman filter was used throughout. The solvents, dichloromethane, acetonitrile and triethylamine were HPLC grade. The mobile phase was water: acetonitrile: triethylamine (80:19:1), adjusted to pH 3.0 with phosphoric acid, filtered through 0.45µm nylon membrane.

Samples and separate standard solutions were identified using the HPLC chromatograph with UV detector. Ciprofloxacin, norfloxacin and ofloxacin were identified individually by comparing the retention time, area and spectra of peaks of unknown substance with respective standard substances. The quantity of identified substances was calculated using the formula adapted from Naeem *et al.* (2006).

$$Amount\ of\ quinolone\ in\ sample\ (\mu g) = \frac{Area\ under\ curve\ of\ sample\ X\ purity\ of\ standard}{Area\ under\ curve\ of\ standard} \times 100$$

ISSN: 2283-3927

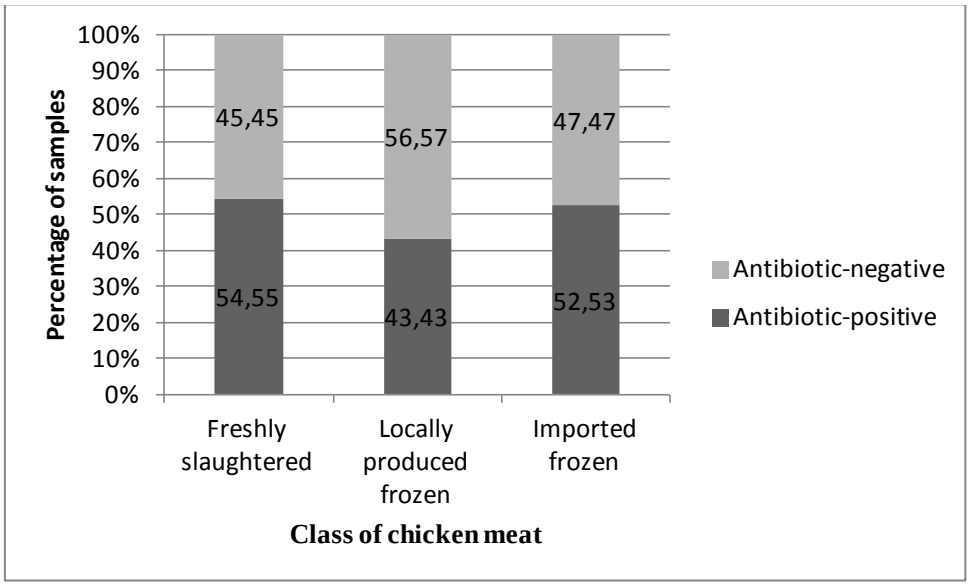
2.5 Statistical analysis

Statistical analysis was accomplished by analysis of variance using SPSS (2005). Treatment means were separated by Duncan Multiple Range Test and statistical significance was set at a probability of $P \leq 0.05$.

3 Results

Results of microbiological screening show that out of a total sample size of 297 (two hundred and ninety seven), 162 (54.55%) were positive for at least one antibiotic to which *Escherichia coli* was susceptible. Samples were categorised as negative and positive based on the zones of inhibition around meat samples and confirmation with HPLC (Figure 2).

Figure 2. Result of antibiotic residue screening in chicken meat purchased from different markets in Ibadan.



Ciprofloxacin, norfloxacin and ofloxacin residues are present together in most freshly slaughtered and locally produced frozen chicken samples. Fewer samples contained either ciprofloxacin alone or ofloxacin alone. However, norfloxacin residues did not exist in isolation in any of the samples. Distribution of identified fluoroquinolones in the three sample groups is shown in Table 1.

Mean residual concentration of ciprofloxacin in imported frozen chicken samples (354.83 ± 716.43 µg/kg) was significantly ($P \leq 0.05$) higher than the concentration in locally produced frozen chicken (64.63 ± 120.77 µg/kg). Ofloxacin follows the same trend but no significant difference was observed in concentration of norfloxacin residue in the three categories.

ISSN: 2283-3927

Table 1: Occurrence of three fluoroquinolones in chicken meat sold in Ibadan as confirmed with HPLC

	Type of chicken meat		
	Freshly slaughtered	**Locally produced frozen**	**Imported frozen**
Samples with one of the fluoroquinolones			
Ciprofloxacin only	-	-	5
Norfloxacin only	-	-	-
Ofloxacin only	-	3	3
Samples with 2 of 3 fluoroquinolones			
Ciprofloxacin & norfloxacin	5	18	10
Ciprofloxacin & ofloxacin	-	-	5
Norfloxacin & ofloxacin	5	3	9
Samples with all three fluoroquinolones			
Ciprofloxacin, norfloxacin & ofloxacin	44	32	20
Total positive samples	54	56	52
Total no of samples	99	99	80
% of total positive with at least one fluoroquinolone	54.55	56.57	52.53

4 Discussion

Investigating residues of fluoroquinolones in broiler chicken is of great public health concern (AL-Mustafa and Al-Ghamdi, 2000; WHO, 2011b). It has been argued that small doses of fluoroquinolones ingested from consumption of meat products with residues of fluoroquinolones weakens their effectiveness leading to drug resistance by bacteria.

The National Policy on Food Hygiene and Safety (NPFHS) seeks to ensure that all foods consumed in Nigeria, whether imported or locally produced, are wholesome, nutritious and free from contaminants (Omotayo and Denloye, 2002). There is currently a ban on the importation of poultry products in Nigeria but imported chicken parts are still freely sold in open markets, especially in the south western parts of the country. Since the products are illegally imported, not much attention is given to their inspection and quality control (Dipeolu, 2010).

ISSN: 2283-3927

Table 2: Fluoroquinolone residues in chicken meat purchased from different markets in Ibadan.

Fluoroquinolones	Mean concentration ± SD of fluoroquinolones (µg/kg)			P value
	Freshly slaughtered	Locally produced frozen	Imported frozen	
Ciprofloxacin	78.02 ± 202.38^{by}	64.63 ± 120.77^{by}	354.83 ± 716.43^{ax}	0.000
Norfloxacin	107.70 ± 138.36^{x}	120.96 ± 162.83^{x}	127.99 ± 279.63^{y}	0.931
Ofloxacin	15.18 ± 24.35^{bz}	13.55 ± 22.46^{bz}	42.33 ± 102.53^{ay}	0.019
P value	0.007	0.000	0.049	

$^{a, b}$ Means in the same row with different superscripts are significantly different (P ≤ 0.05)
^{x,y} Means in the same column with different superscripts are significantly different (P ≤ 0.05)

Table 3: Range of concentration of three fluoroquinolones in chicken meat purchased from different markets in Ibadan, Nigeria

Fluoroquinolones	Type of chicken meat	Range of concentration in positive samples (µg/kg)	% above 100 (µg/kg)*
Ciprofloxacin	Freshly slaughtered	2.45 to 888.90	15.15
	Locally produced frozen	2.45 to 591.50	24.24
	Imported frozen	14.00 to 2767.00	32.32
Norfloxacin	Freshly slaughtered	3.98 to 393.17	40.40
	Locally produced frozen	20.50 to 466.65	33.33
	Imported frozen	6.18 to 1081.27	20.20
Ofloxacin	Freshly slaughtered	4.36 to 77.51	0.00
	Locally produced frozen	8.76 to 67.13	0.00
	Imported frozen	14.53 to 431.66	0.00

***** European Union MRL (Commission Regulation (EU) 37/2010) for the sum of enrofloxacin and ciprofloxacin in poultry muscle

Maximum residue limits (MRL) have been set for different antimicrobials in different food matrices by different organizations and countries. If the residue of a chemical exceeds the MRL in meat, it is considered unsafe (Petrovic *et al.*, 2006). MRL for antibiotics in foods of animal origin have not been set in Nigeria. Thus the EU MRL for the sum of enrofloxacin and ciprofloxacin is adopted for the purpose of this study.

ISSN: 2283-3927

Microbial inhibition assay was employed as a first qualitative screening step to sift out large number of compliant samples. Overall, one hundred and thirty seven (137) representing 46.13% of total sample size contained no residues of *Escherichia coli* - sensitive antimicrobials. Fluoroquinolone residues occurred more frequently in locally produced frozen broiler chicken samples. Positive samples were 54.55%, 56.57% and 52.53%, of freshly slaughtered, locally produced frozen chicken and imported frozen chicken respectively.

Norfloxacin residues are present in higher concentration in chicken produced within Nigeria and are thus of more concern than ciprofloxacin and ofloxacin. However, ciprofloxacin is more abundant than norfloxacin and ofloxacin in imported products (Table 2).

In a similar experiment, Al-Mustafa and Al-Ghamdi (2000) detected norfloxacin in raw chicken tissues from the eastern province of Saudi Arabia at levels that were 2.7 to 34.3 folds higher than the MRL. Naeem *et al.* (2006) observed residues of ciprofloxacin, norfloxacin and ofloxacin in liver samples purchased from various markets in Lahore, Pakistan, to be 2.45 to 245.00 µg/kg, 2 20 to 31.00 µg/kg and 2.05 to 22 µg/kg respectively in summer. In the present study, there is wide variation in concentration (Table 3) of fluoroquinolones in chicken samples.

5 Conclusions

If antibiotic resistance must be tackled from a food safety perspective, chicken meat, whether imported or locally produced, must be monitored for residues of antimicrobials, especially those that have been classified as critically important. The present ban on importation of poultry meat in Nigeria should be properly implemented to reduce the exposure of consumers to fluoroquinolone residues. As a matter of public health, it is important to control the use of these antimicrobials in the local production of chicken so as to maintain their potency for use in human medicine.

6 Acknowledgements

This study was technically supported by the National Agency for Food and Drug Administration and Control (NAFDAC), Nigeria. The authors are thankful for the assistance of the staff of NAFDAC regional laboratory, Agulu.

References

Aibinu, I., Adenipekun, E. and Odugbemi, T., 2004. Emergence of quinolone resistance amongst Escherichia coli. Nigerian Journal of Health and Biomedical Science. 3(2), 73-78.

ISSN: 2283-3927

Alla, M.B.W., Mohamed, T.E and Abdelgadir, A.E., 2011. Detection of antibiotics residues in beef in Ghanawa Slaughterhouse, Khartoum State, Sudan African Journal of Food Science Vol. 5(10), 574-58.

Al-Mustafa Z.H. and Al-Ghamdi M. S., 2000. Use of norfloxacin in poultry production in the eastern province of Saudi Arabia and its possible impact on public health. International Journal of Environmental Health Research. 10, 291–299.

Barrow, G. I, Feltham, R.K.A., 1993. Cowan and Steel's manual for the identification of medical bacteria. London: Cambridge University press.

Cañada-Cañada, F., Espinosa-Mansilla, A., Jiménez, Girón A., Muñoz de la Peña A., 2012. Simultaneous determination of the residues of fourteen quinolones and fluoroquinolones in fish samples using liquid chromatography with photometric and fluorescence detection. Czechoslovakia Journal of Food Science. 30, 74–82.

Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

Daini, A., Ogbolu, O. D. and Ogunledun, A., 2005. Quinolone resistance and R plasmids of some Gram negative enteric bacilli. African Journal of Clinical Experimental Microbiology. 6 (1), 14-20.

Daniel, O., Osman, E., Bakare, R., Adebiyi, P., Ige, O., Ogiri, S., Awe, E., Kabir, M., Ogundahunsi, O., Mourad, G. and Declarq, E., 2011. Ofloxacin resistance among Mycobacterium tuberculosis isolates in two states of south-west Nigeria. African Journal of Respiratory Medicine. 23, 18-20.

Dipeolu, M. A., (2010). Healthy meat for wealth. UNAAB Inaugural Lecture Series. 29, 12-49.

Emami, S., Shafiee, A., Foroumadi, A., 2010. Quinolones: recent structural and clinical developments. Iran Journal of Pharmaceutical Research. 4(3), 123-136.

Enabulele, I.O., Yah, S.C., Yusuf, E.O., Eghafona, N.O., 2006. Emerging quinolone resistant transfer genes among gram-negative bacteria, isolated from faeces of HIV/AIDS patients attending some clinics and hospitals in the city of Benin, Edo State, Nigeria. Online Journal of Health and Allied Sciences. 3, 3.

Hernández Serrano P., 2005: Responsible Use of Antibiotics in Aquaculture. FAO, Rome.

Makanjuola, B.O, Bakare, R.A and Fayemiwo, S.A., 2012. Quinolone and Multidrug Resistant Salmonella typhi in Ibadan, Nigeria. International Journal of Tropical Medicine. 7, 103-107.

Naeem, M., Khan, K. and Rafiq, S., 2006. Determination of Residues of Quinolones in Poultry Products by High Pressure Liquid Chromatography . Journal of Applied Sciences. 6(2), 373-379.

Okeke, I.N., Lamikanra, A., Edelman, R. 1999. Socioeconomic and behavioural factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerging Infectious Diseases. 5, 18-27.

Olowe, O.A., Ogungbamigbe, T.O., Kolawole, S.O., Olowe, R.A., and Olayemi, A.B., 2008. Hemolysis production and resistance to fluoroquinolones among clinical isolates of

Escherichia coli in Osogbo metropolis, Southwest Nigeria. New York Science Journal. 1(1), 13-16.

Omotayo, R.K. and Denloye, S.A., 2002. The Nigerian Experience On Food Safety Regulations. FAO/WHO Global Forum of Food Safety Regulators Marrakesh, Morocco; GF/CRD Nigeria-1, Agenda Item 4.1a and 4.1b.

Ovando, H.G., Gorla, N., Weyers, A., ugnia, L., magnoli, A., 2004. Simultaneous quantification of ciprofloxacin, enrofloxacin and balofloxacin in broiler chicken muscle. Archivos de Medicina Veterinaria. 36(1), 93-97.

Oyinloye, J.M., Adedeji, O.O., Akeredolu, A.A., Ezekiel, C.N., Babalola, B.T., Obebe, O.O., Nwadike, F.U. and Alatise, F.A., 2014. Comparative in vitro potency of four fluoroquinolones on clinical isolates over a year period. Journal of Clinical Medicine and Research. Academic Journals. 6(1), 5-10.

Petrovic J., Baltic M., Jupic V., Stefanovic S. and Stojanovic D., 2006. Residues of enrofloxacin and its main metabolite ciprofloxacin in broiler chickens. Acta Veterinaria (Beograd). 56 (5-6), 497-506.

Pikkemaat, M.G., Mulder, P.P.J., Elferink, J.W.A., De Cocq, A., Nielen, M.W.F., Van Egmond, H.J., 2007. Food Additives and Contaminants. 24, 8.

Somasundaram, S. and Manivannan, K., 2013. An Overview of Fluoroquinolones. Annual Review & Research in Biology 3(3), 296-313.

Stanier, R.Y., Ingraham, J.L, Eelis, M.L., Painter, P.P., 1986. The Microbial World. 5th ed. New Jersey: Pristice Hall.

World Health Organization, 2009. Report of the First Meeting of the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), 15-19 June Copenhagen Geneva.

World Health Organization, 2011a. Critically important antimicrobials for human medicine. WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), 3rd Revision, 5-31.

World Health Organization, 2011b. Tackling antibiotic resistance from a food safety perspective in Europe WHO Regional Office for Europe, Copenhagen, Denmark.

ISSN: 2283-3927