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Fluoroquinolone residues in raw meat from open markets in Ibadan, Southwest, Nigeria.

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ABSTRACT.

Misuse of fluoroquinolones in livestock production may lead to the presence of their residues in tissues of meat animals after slaughter, constituting health hazards to consumers. The present study was designed to screen for residues of three fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin) in raw meat. Microbiological assay, followed by High Performance Liquid Chromatography (HPLC) was used to screen three hundred and twenty samples of beef, chicken, pork and chevon purchased from open markets. Initial screening by microbiological assay revealed that 50%, 55%, 40% and 40% of beef, chicken, pork and chevon, respectively were positive for residues of antibiotics. Further analysis by HPLC with UV detection revealed the presence of ciprofloxacin, norfloxacin and ofloxacin at varying concentrations in the meat samples. Ofloxacin was the least in frequency and abundance in all meat types. Results obtained in this study have implications for public health and will lead to steps that will further enhance the safety of animal foods in order to protect consumers and the animal production industry.

KEYWORDS

Fluoroquinolone residues; Ciprofloxacin; Norfloxacin; Ofloxacin; Consumer protection; Meat safety.

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1 Introduction

Fluoroquinolones are synthetic antimicrobials used in the treatment of severe and invasive infections in humans and animals. The presence of fluoroquinolone residues in meat has attracted extensive attention from national and international public health agencies. This is because of the risks associated with the consumption of food contaminated with fluoroquinolone residues as they may be directly toxic or be a source of resistant human pathogens, representing a possible risk to human health (Juan-García *et al.* 2006). Conditions such as phototoxic skin reactions in humans (Klecak *et al.*, 1997) and chondrotoxic effects in young animals (Stahlmann *et al.*, 2000) can be induced by fluoroquinolones. Probably the most important threat to human health as a result of fluoroquinolone use in animal production is the development of fluoroquinolone-resistant bacteria as it has been discovered that a major route of transmission of resistant micro-organisms from animals to humans is through the food chain (Hernández-Serrano, 2005).

The abuse of veterinary drugs is one of the causes of drug residues in animal products (Salehzadeh et al, 2006, Fagbamila *et al.*, 2010). Drugs belonging to the fluoroquinolone class such as enrofloxacin, norfloxacin, pefloxacin, ciprofloxacin and ofloxacin are often used in livestock production to control diseases, leading to the production of healthy animals, thereby reducing farmers' losses.

Ofloxacin is a fluoroquinolone of interest due to the resistance of a broad spectrum of microbes to it, especially as a result of its use as growth promoter (Naeem *et al.*, 2006). Low levels of ciprofloxacin, another fluoroquinolone, can strongly suppress facultative anaerobic human intestinal microflora (Cerniglia and Kotarski, 1999); it also leads to the development of resistant bacteria. Similarly, exposure to norfloxacin has been shown to induce resistance to other fluoroquinolones and even to the structurally unrelated aminoglycosides in coagulase-negative staphylococci (Deshmukh *et al.*, 1997).

Detection and control of fluoroquinolone residues in meat is important due to market globalization and food safety issues. Methods developed for detecting fluoroquinolone residues include microbiological assays, immunoassays, liquid chromatography, among others. Microbiological inhibitory tests, which rely on inhibition of growth of the test organism by the residual drug, are inexpensive and simple; however, most lack specificity and few are quantitative. Microbiological assays are generally used for residue screening as part of an integrated system with follow-up confirmatory analysis of suspicious samples (Choi *et al.*, 1999). High performance liquid chromatography (HPLC) is used to separate, identify and quantify different chemical components. It has been successfully applied to the detection of different drug residues in foods of animal origin (Reig *et al.*, 2006).

In order to protect human health, regulatory bodies such as the European Union and Codex Alimentarius Commission, have established maximum residue limits (MRLs) for veterinary drugs in different matrices of different animal species. The EU MRL for the sum of enrofloxacin and ciprofloxacin is adopted for comparison in this study.

The World Health Organization has been campaigning for antibiotic resistance to be tackled from a food safety perspective (WHO, 2011). There is therefore a need to establish a comprehensive database for residues of relevant antibiotics in the meat production industry as a step towards controlling the rise in antimicrobial resistance in Africa. Presently, several countries cannot assure consumers of the safety of the meat products they consume with

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respect to drug residue concentration. This study was set up to investigate the presence of ciprofloxacin, norfloxacin and ofloxacin residues in four commonly consumed meat types in Nigeria.

2 Materials and Methods

A total of three hundred and twenty raw meat samples (comprising 80 each of beef, chicken, chevon and pork) were collected from four major markets in Ibadan, Nigeria. They were collected on ice and kept frozen at -3°C until analyzed. The analyses were carried out at the Meat Science Laboratory of the University of Ibadan and National Agency for Food and Drug Administration and Control (NAFDAC) Laboratory, Agulu, Anambra State, Nigeria. The samples were screened for three fluoroquinolones, ciprofloxacin, norfloxacin and ofloxacin.

2.1 Qualitative analysis: Microbiological screening

Initial screening was done using the One Plate Test (OPT) as described by Alla *et al.* (2011). The screening procedure has been previously reported in detail in a similar experiment by Omotoso and Omojola (2014). The test organism was *Escherichia coli* (ATCC 10536). Zone of inhibition was measured with mm graduated ruler. Negative samples were discarded while suspicious and positive samples were subjected to further analysis for identification and quantification of residual ciprofloxacin, norfloxacin and ofloxacin.

2.2 Identification and quantitative analysis

Following residue extraction as described by Ovando *et al.* (2004), the fluoroquinolones, ciprofloxacin, norfloxacin and ofloxacin were identified using HPLC with UV detection.

The HPLC equipment was Elite Lachrom VWR, Hitachi 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. Details of the extraction and subsequent analysis have been reported by Omotoso and Omojola (2014).

2.3 Statistical analysis

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

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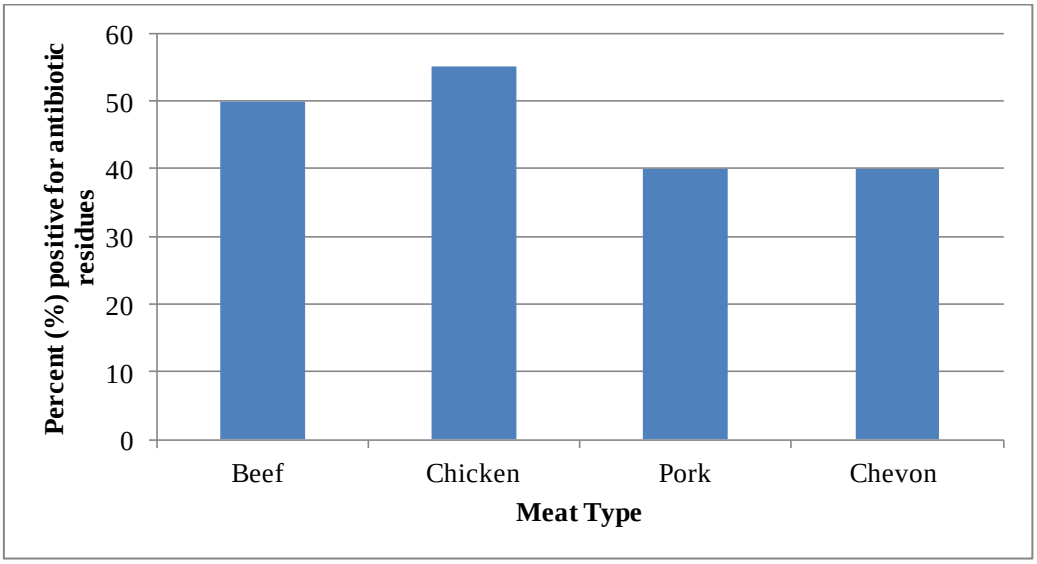
3 Results

Frequency of antibiotic contamination in the different meat types is shown in Figure 1. Initial microbiological screening revealed that more than half of the chicken samples tested positive for antimicrobial residues. This was followed by beef with 50% positive samples. Similarly, 40% of pork and chevon samples tested positive for antimicrobial residues.

Figure 1. Percentage of raw meat samples positive for antibiotic residues.



Percentages of meat samples positive for ciprofloxacin, norfloxacin and ofloxacin residues are shown in Figure 2. Most chicken and beef samples had detectable levels of ciprofloxacin; however, less than half of pork and chevon contained residues of this popular fluoroquinolone. The meat type with the highest frequency of norfloxacin residue was chicken, followed by beef. Ofloxacin occurred more frequently in beef (48.75%) than other meat types. It is worthy of note that pork, followed by chevon, had the least number of samples with detectable levels of any of the three fluoroquinolones.

Levels of ciprofloxacin, norfloxacin and ofloxacin residues in the tested raw meat samples are shown in Table 1. In beef, mean residual ciprofloxacin (231.08 ± 564.30) and norfloxacin (173.40 ± 154.57) were above the adopted MRL of 100µg/kg, while ofloxacin value (79.28 ± 183.70) was below the limit. In chicken, however, norfloxacin was the most abundant fluoroquinolone, with a mean value that was slightly above 100µg/kg and significantly different from the levels of ciprofloxacin and ofloxacin. Although ciprofloxacin in pork was very high, levels of norfloxacin and ofloxacin in this meat-type were very low. High level of ciprofloxacin was also recorded in chevon (345.62 ± 796.35) but this had lower norfloxacin and ofloxacin residues. Percentages of meat samples containing residues of ciprofloxacin, norfloxacin and ofloxacin above adopted MRL are shown in Table 2.

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Figure 2. Detection of ciprofloxacin, norfloxacin and ofloxacin in raw meat.

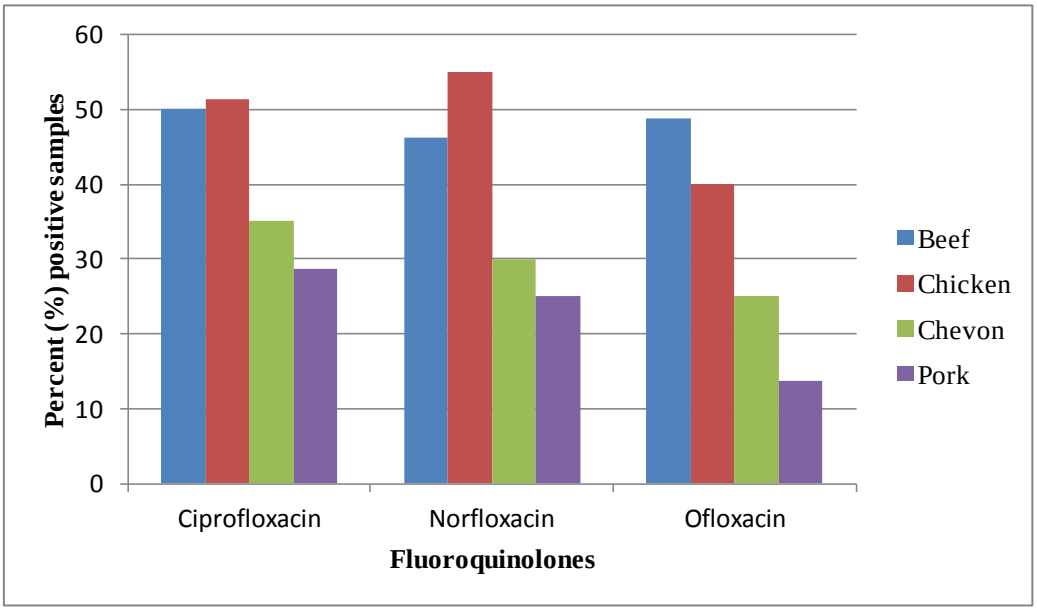

Table 1. Mean concentration of fluoroquinolones in beef, chicken, pork and chevon.

Meat type	Mean concentrations (µg/kg) of fluoroquinolones			P value
	Ciprofloxacin	Norfloxacin	Ofloxacin	
Beef	231.08 ± 564.30	173.40 ± 154.57	79.28 ± 183.70	0.536
Chicken	67.85 ± 125.42^{b}	113.59 ± 158.32^{a}	13.55 ± 22.46^{c}	0.022
Pork	315.30 ± 834.35^{a}	11.39 ± 28.31^{b}	27.02 ± 64.48^{b}	0.091
Chevon	345.62 ± 796.35^{a}	30.93 ± 129.18^{b}	12.37 ± 30.64^{b}	0.047

$^{a, b, c}$: means in the same row with different superscripts are significantly different (P ≤ 0.05)

4 Discussion

Several reports have shown that the presence of fluoroquinolone residues in human food obtained from animal sources has reduced the effectiveness of these agents in human medicine. In addition, direct toxicity and other side effects of fluoroquinolones are well established in animals and humans (Gruchalla and Pirmohamed, 2006; Khadra *et al.*, 2012). Thus it is important to monitor residues of fluoroquinolones in frequently consumed meat and meat products.

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Table 2. Percentage of raw meat samples with fluoroquinolone residues above maximum residue limit.

Fluoroquinolones	**Meat type**	**n/N**	**% above MRL***
Ciprofloxacin	Beef	40/80	23.75
	Chicken	41/80	15.00
	Pork	28/80	20.00
	Chevon	23/80	38.75
Norfloxacin	Beef	37/80	25.00
	Chicken	44/80	35.00
	Pork	24/80	6.25
	Chevon	20/80	5.00
Ofloxacin	Beef	39/80	15.00
	Chicken	32/80	0.00
	Pork	20/80	10.00
	Chevon	11/80	0.00

n=number of positive samples; N = number of examined samples

*100µg/kg- European Union MRL (Commission Regulation (EU) 37/2010) for the sum of ciprofloxacin and enrofloxacin in poultry muscle.

In the present study, raw samples of four meat types commonly consumed in Nigeria were tested for the presence of fluoroquinolones. Approximately 50% of the three hundred and twenty samples were positive for residues of at least one antibiotic active against the test organism, *Escherichia coli*. Fifty-five percent of chicken meat and fifty percent of beef were positive for antibiotic residues. In a related study using samples obtained from open markets in Sokoto, Nigeria, 44% of slaughtered cattle were found to contain residues of antibiotics (Ibrahim *et al.*, 2009). Residues have been reported in 21% of meat samples in Ghana (Novais *et al.*, 2010), and 70% in Tanzania (Kurwijila *et al.*, 2006). Tajick and Shohreh (2006) also found that more than 50% of poultry meat tissues tested in Iran had residues of antimicrobials. In contrast, Alla *et al.* (2011) analyzed beef samples in Sudan and reported that only 3% of the muscles contained antibiotic residue.

Findings in this study revealed that fluoroquinolones are frequently administered in meat-animal production in Nigeria. Results of microbiological screening were similar to findings in the screening of broiler meat and beef sold in the markets of Ankara, Turkey by Er *et al.*, (2013) who discovered that 45.75% of chicken and 57.7% of beef were positive for quinolone residues; however, mean concentrations obtained in their study were below the 100µg/kg mark (the

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mean levels of quinolone antibiotic residue were found to be 30.81 ± 0.45 µg/kg in positive chicken samples and as low as 6.64±1.11µg/kg in positive beef samples). In a similar experiment to test for the presence of fluoroquinolones in chicken, Omotoso and Omojola (2014) reported ciprofloxacin level of (354.83 ± 716.43 µg/kg) in imported chicken.

The frequency of occurrence and high concentration of ciprofloxacin in the present study may be due to the fact that ciprofloxacin is also a marker residue for enrofloxacin. Enrofloxacin is approved for use as a veterinary drug and when it is metabolized, its pharmacologically active metabolite, ciprofloxacin, is produced. Hence detection of ciprofloxacin during market screening often reflects the use of both enrofloxacin and ciprofloxacin (Navrátilová *et al*., 2011; Botsoglou and Fletouris 2001).

Mean norfloxacin residues in beef and chicken were higher than the MRL. Ofloxacin concentration was the lowest in all tested meat types except pork, which had higher ofloxacin than norfloxacin. Generally, ciprofloxacin was the most abundant in all except for chicken meat which had the highest mean norfloxacin (173.40 ± 154.57). The level of abundance of the individual agents in each meat type is as follows

Beef: Ciprofloxacin > Norfloxacin > Ofloxacin

Chicken: Norfloxacin > Ciprofloxacin > Ofloxacin

Pork: Ciprofloxacin > Ofloxacin > Norfloxacin

Chevon: Ciprofloxacin > Norfloxacin > Ofloxacin

The study shows that ofloxacin is not administered as frequently as the other two antimicrobial agents in livestock production in the study area. No individual chicken or chevon sample had ofloxacin levels above MRL (Table 2). However, 15% and 10% of beef and pork respectively contained ofloxacin at levels above MRL. This shows that ofloxacin may not necessarily be a drug of concern in chicken meat and chevon. Detection of fluoroquinolone residues in meat samples from the city of Ibadan, Nigeria, corroborates the findings of other researchers in other countries where residue monitoring is absent or inadequate.

5 Conclusion

In the absence of official monitoring programme on drug residues in developing countries, misuse of antimicrobials, including fluoroquinolones, is inevitable. Detection of residues of fluoroquinolones in half of tested meat samples shows the widespread use of these agents in cattle, poultry, hog and goat production in Nigeria during the period of the study. The present situation can be controlled as less frequent contamination and reduced concentration in contaminated samples may result when steps are taken towards encouraging prudent use of fluoroquinolones. Consumer protection can be ensured by screening food animals for residues of antibiotics before slaughter. Data collected over a period of time will provide basis for legislations that will reduce the risk to consumers.

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