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Determination of gentamicin use in poultry farms in Enugu state, Nigeria, and detection of its residue in slaughter commercial broilers

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Abstract

Background and Aim: Gentamicin is a broad-spectrum antimicrobial used in the treatment of a variety of bacterial infections in poultry, its residue in tissues is known to have a deleterious effect in human when consumed. The study aimed to determine the use of gentamicin by poultry farmers and the presence and occurrence of gentamicin residue in tissues of commercial broilers in Ikpa slaughterhouse Nsukka.

Materials and Methods: The study adopted a cross-sectional survey using questionnaire and immunological screening. A total of 108 broilers were sampled, testing 108 each of muscle, liver, and kidney tissues for gentamicin residue using enzyme-linked immunosorbent assay.

Results: The questionnaire survey revealed that in the management practices, 55.2% of the farms used intensive deep litter, 21% intensive battery cage, and 23.6% extensive farming system. Twenty-seven (71%) farms reared only broilers, 10.5% layers, and 18.4% both. The feeding management showed that 21.1% of farmers produced their own feed (medicated) while 78.8% used commercial feed, of which 11.5% incorporated antibiotics. Hundred (100%) of the respondents used gentamicin and only 47.3% were aware of the consequences of antimicrobial residue in foods of animal origin, with knowledge of the legislation on the prudent use of antimicrobials in food animals. The occurrence of gentamicin residue in surveyed birds was 65% with tissue distribution as follows: Muscle (44.4%), liver (51.9%), and kidney (59.3%).

Conclusion: Poultry farms in the study area make constant use of gentamicin without observing its withdrawal period before sale, and a high percentage of the commercial birds meant for human consumption contains gentamicin residue. Therefore, consumers might be at risk of consuming gentamicin as a parent drug or its metabolites in commercial poultry.

Keywords: enzyme-linked immunosorbent assay, farm management, gentamicin, poultry, residue.

Introduction

The intensification in the agricultural sector in modern times showed that an increase in livestock production and crop yield has been aided using veterinary medicinal products, especially, anti-infective drugs and chemicals such as herbicides and pesticides [1]. This is consequent on trying to meet up with the demand of the growing populace for animal protein [2,3]. As at 2010, Biswas *et al*., 2010 [4], stated that about 42% of all veterinary pharmaceuticals used in poultry and livestock industry are used as feed additives, 19% as anti-infectives, 13% as parasiticides, 11% as biologic, and 15% as other pharmaceuticals. Recently, it has been reported that about 80% of all the antibiotics administered in veterinary practice are used as growth promoters and in most cases, exceeding the total antibiotics used for human health care [5]. In developed countries such as the USA, Food and Drug Administration (FDA) [6] reported that about 80% of total antimicrobial consumption in some counties are used in food animals, which supports the observation made by Aarestrup [7] that global consumption of antibiotic in animals is twice that of humans. Van Boeckel *et al*. [8] also found that about 45, 148, and 172 mg/kg of antimicrobial per animal are used annually for cattle, chicken, and pig production globally, respectively, with the prediction that consumption of antimicrobial by livestock will increase at the rate of 67% from 63,151 tons in 2010 to 105,596 tons by 2030.

These veterinary products are used either as chemotherapy, applied individually or collectively to animals with microbial infections, or as a preventive measure against the onset of certain diseases [9,10]. Preventive measures (using anti-infectives as a medicine) are a more recent development and seen as big breakthroughs in medicine because the incidence of morbidity and mortality caused by many infectious diseases can be reduced by it [11]. Following the administration of drugs, particularly antimicrobials, to animals and subsequent non-observance of withdrawal periods, drugs remain in the tissues of the animals and food products derived from them [12]. The presence of antibiotic residue in food above the maximum level has

been become a global issue and recognized worldwide by various public and government authorities [9]. Among the most widely used antimicrobials in livestock and poultry production is gentamicin [13]. It is an antimicrobial with bactericidal activity against most Gram-negative bacteria. It has problems of toxicity and resistance when misused. Gentamicin residue in foods of animal origin is of great public health importance worldwide, mainly due to the health risk associated with it, especially its nephrotoxic and ototoxic effect, and it is also known to deleteriously affect the immune system leading to the development of drug-resistant microorganisms in animals and humans [14-17].

The study aimed to determine the use of gentamicin by poultry farmers and the presence and occurrence of gentamicin residue in tissues of commercial broilers in Ikpa slaughterhouse Nsukka.

Materials and Methods

Ethical approval and informed consent

Consent was sought and was granted by the owners and management of the poultry farms and retail outlets. Consent was also sought and willingly granted by the owners of the slaughter birds at the abattoir where samples were collected.

Study area

The study was done in Enugu state which covers approximately 7617.82 km^2 and is located between latitude 6°45' and 7°N and longitude 7°12.5' and 7°36' W in the Southeast geopolitical zone of Nigeria. It has three agricultural zones: Awgu, Nsukka and Enugu. Nsukka and Enugu (major cities in Enugu state) metropolises representing two of the three agricultural zones purposively selected for the study. Samples for gentamicin residue detection were collected from Ikpa slaughter (Figure-1), which is the major slaughterhouse within the Nsukka local government area, serving the whole of Nsukka and environs.

Study design

The study adopted a cross-sectional survey using a questionnaire to extract information on poultry farm management and gentamicin drug use, and immunological screening for the presence of gentamicin residue in commercial birds using enzyme-linked immunosorbent assay (ELISA).

Sample source, size, and sampling technique

Forty of the listed farms from both metropolises were selected for the questionnaire survey using a systematic random sampling technique, 15 from Nsukka and 25 from Enugu. Samples for screening were collected from the poultry processing unit at Ikpa slaughterhouse. The slaughter was visited weekly for a period of 8 weeks and on the first 6 weeks, systematic random sampling technique was used to select 14 birds from a daily slaughter capacity of about 30 birds. Twelve birds were selected on the last 2 weeks. A total of 108 birds were sampled. Three major organs (liver, muscle, and kidney) serving as post-slaughter matrix were harvested from each bird making a total of 324 organ specimens. The sample size of 324 was determined using Thrusfield, 2007 [18], as follows: $n=(Z^2\times P(1-P))/d^2$. Where Z (1.96) and d (0.05) are constants and P was an initial prevalence of 30% (0.3). The calculated sample size was 322, but 324 tissue samples were harvested from 108 birds.

Sample preparation and extraction of gentamicin

ELISA kit used for screening was procured from Shenzhen Lvshiyuan Biotechnology Company Limited. Juices from the organs were extracted following the instruction of the ELISA kit manufacturer. One gram (1±0.5 g) of each organ was macerated using sterile pestle and mortar, emulsified with 5 ml of diluted extraction solution and after shaking for 3 min, centrifuged at 715.5 g at room temperature for 10 min, 50 µl of the supernatant was decanted into Eppendorf tubes and stored for analysis. The test kit is based on competitive

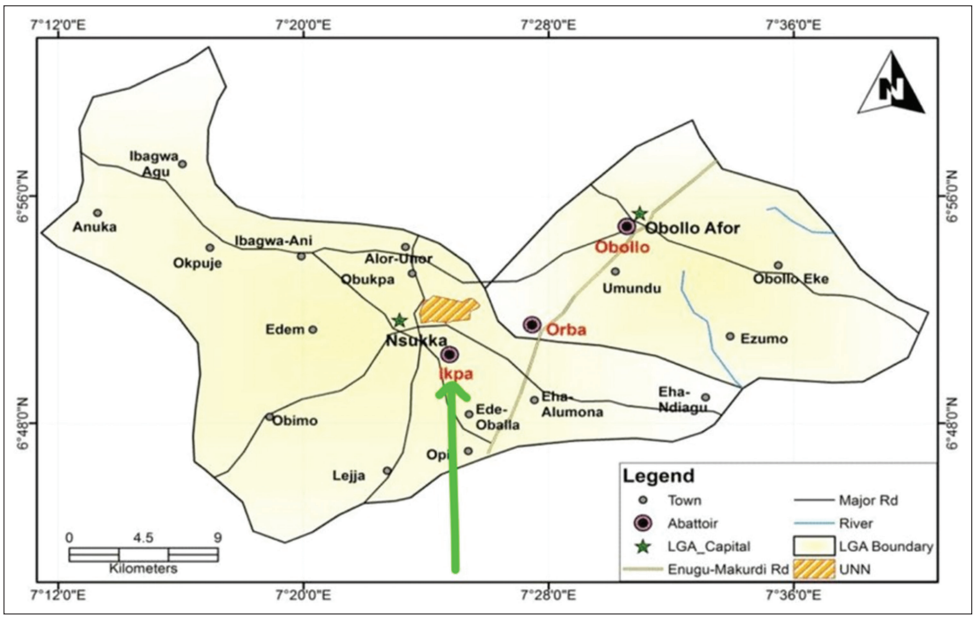

Figure-1: Map of Nsukka urban showing the location of the abattoir.

enzyme immunoassay for the detection of gentamicin in the sample. The coupling antigen is pre-coated on the microwell strips. The gentamicin in the sample and pre-coated coupling antigen on the microwell strips compete for the anti-gentamicin antibody. After the addition of the enzyme conjugate, the 3,3',5,5'-tetramethylbenzsidine substrate is added for coloration. The optical density value of the sample has a negative correlation with the gentamicin in it. The value is compared to the standard curve and the gentamicin concentration is subsequently obtained (manufacturer's guide).

Statistical analysis

Data from the study were analyzed usingGraph-Pad Prism Statistical Software Version 5.02 [La Jolla, California, USA, (www.graphpad.com)]. Chi-square test (p-value) was used for statistical significance and inference. The alpha value of significance was set at the probability level of <0.05.

Results

Questionnaire survey (farm management and use of gentamicin)

Out of the 40 farms evaluated, 38 (95%) responded while 2 (5%) did not. In the management practice, 55.2% of the farms use intensive deep litter, 21% intensive battery cage, and 23.6% extensive farming system. Twenty-seven (71%) farms rear only broilers, 10.5% layers, and 18.4% both. The feeding management showed that 21.1% of farmers produce their own feed with inclusion of antibiotics while 78.8% use commercial feed, of which 11.5% incorporate antibiotics (Figure-2).

Table-1 shows that all (100%) the farms used gentamicin in the past 6 months either as single formulation (Intergen and Gentreat genta) or in combination with other antibiotics (Doxygen, Biodoxygen, Intergendox, Gentaryl D, and Centre-Gentatylo). Table - shows that 54.6% were aware of antimicrobial residues; 65.8% know about withdrawal periods and 0% observed withdrawal periods prior to sale of the birds and eggs.

Screening for gentamicin residue in commercial chickens

The detection limit of the kit is 0.05; therefore, concentrations equal or <0.05 are termed negative while those above 0.05 are positive for antimicrobial residues. Figure-3 shows that 70 (65%) out of the 108

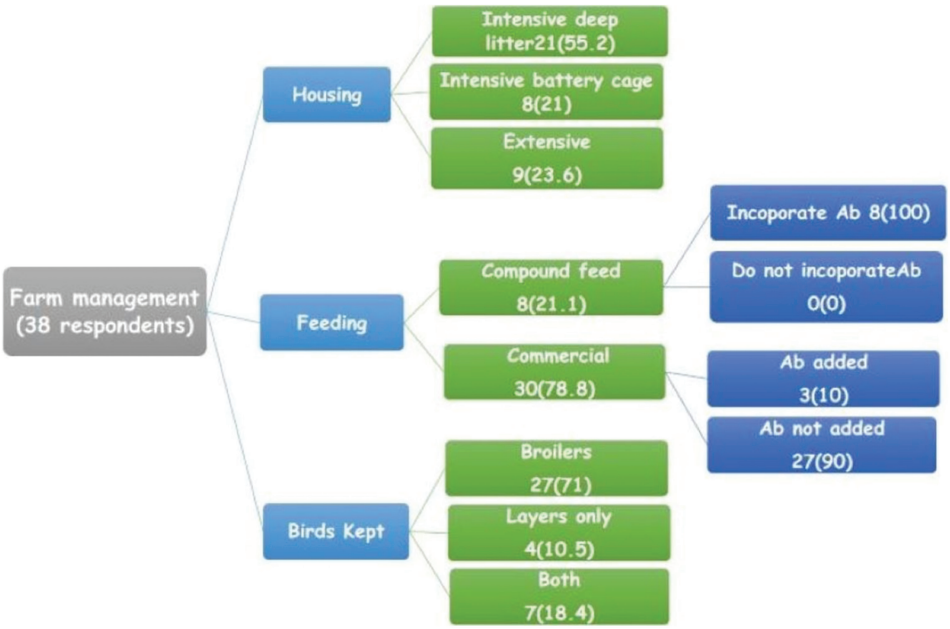

Figure-2: Poultry farm management practices in the two major cities (Nsukka and Enugu) of the two agricultural zones.

Table-1: Gentamicin drug use by farms in Nsukka and Enugu metropolis, Enugu state, Southeast Nigeria.

Drug	Frequency		Total (n=38)	Proportion (%)
	Nsukka (14)	Enugu (24)		
Doxygen 20/20 WSP	15	23	38	100
Gentaryl D	10	24	34	90
Biodoxygen	9	25	34	90
Intergendox WS	10	20	30	80
Intergen-200 WS	12	18	30	80
Gentreat genta	14	15	29	75
Centre-Gentatylo	4	15	19	50

Doxygen, Biodoxygen, Intergendox=doxycycline and gentamicin, Gentaryl D=Gentamicin, doxycycline and multivitamins, Intergen and Gentreat genta=Gentamicin sulfate, Centre-Gentatylo=Gentamicin and tylosin

Table-2: Awareness to prudent use of antimicrobial in poultry and livestock production and observance of withdrawal period.

Awareness	Yes (%)	No (%)
Awareness to antimicrobial residues	20 (54.6)	18 (45.4)
Awareness to legislation on the prudent use of antimicrobials	12 (31.5)	26 (68.5)
Awareness to antimicrobial withdrawal periods	25 (65.8)	13 (34.2)
Observance of withdrawal period	0 (0)	38 (100)

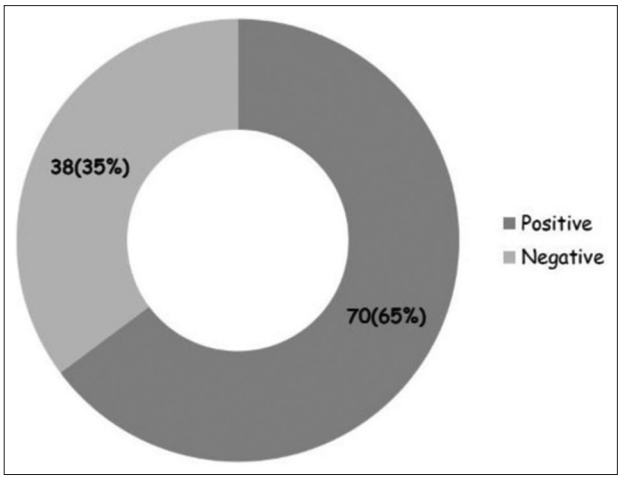

Figure-3: Occurrence of gentamicin residue in commercial birds sold at Ikpa, Nsukka.

screened commercial birds were positive for gentamicin residue while 38 (35%) were negative. Among the tissues tested, liver and kidney had higher prevalence of gentamicin residue at 51.85% and 59.26%, respectively, while the muscle had the least at 44.4% (Table-3). The difference in their proportions was not statistically significant $\chi^2(2) = 1.82$; $p = 0.4$.

Discussion

The use of gentamicin in poultry industry was surveyed and its presence in commercial birds was determined using ELISA immunoassay. Almost half of the farms surveyed use intensive deep litter which allows for cross infection among birds within and across pens, this management system may make the use of antibiotics for prevention and treatment inevitable. Some farmers that produce their feeds as well as some commercial feeds incorporate antibiotics not only for prophylactic purposes but also for growth promotion, as maximum growth for the shortest amount of time is desirable for economic gains in poultry business [13]. The incorporation of antibiotics in feed causes gradual accumulation of the drug in the tissues and since the feed is taken on daily basis, observing the withdrawal period becomes difficult. In the light of this, the US FDA revised the Veterinary Feed Directive regulations with a view to reduce the use of antimicrobials in animals specifically, the use of any antimicrobial drug medically important to humans for growth or to improve feed efficiency. Therefore, the use of antibiotics for such purpose became illegal [19].

More than half of the respondents (54.6%) are aware of antimicrobial residues and know about withdrawal period (65.8%), yet none of them (0%) observed withdrawal periods before sale of the birds and eggs when drugs are used for treatment. Non-observance of drug withdrawal period is the major cause of antimicrobial residues in foods of animal origin [20]. The withdrawal period of every antimicrobial is boldly stated on the drug as part of manufacturer's instructions for use; therefore, it is either that the farm management do not read the manufactures instructions or they read but not aware of the consequences of consuming chicken or eggs that contain violative level of antimicrobial residues. This is largely due to non-implementation of available legislation on the prudent use of antimicrobials in food animals and may also be a possible cause of low (31.5%) awareness to prudent use of antimicrobials.

Sixty-five (65%) of commercial broilers investigated were positive for gentamicin residue. They had detectable levels of gentamicin residues at the point of purchase, indicating that consumers sourcing broilers from Ikpa abattoir may be exposed to violative levels of the drug residue. A similar work done in Gaza by Albayoumi and Elmanama [21], recorded 27.36% prevalence of aminoglycosides in slaughtered broilers. The disparity in the findings may be attributed to a stricter regulation for antimicrobial use in Iraq. The high prevalence of gentamicin residue detected is at par with the findings of Adebowale *et al*., 2015 [22], who recorded that gentamicin is the most widely used drug in poultry production in Ogun state, Nigeria, and 100% usage of gentamicin either alone or in combination with other antibiotics from this study. This is not only due to failure to adhere to withdrawal periods [20] but also overuse as well as the practice of self-medication by poultry farmers. The frequent and wide use of this drug could be attributed to its recommendation for the prevention of early mortality associated with *Escherichia coli*, *Salmonella* Typhimurium, and *Pseudomonas aeruginosa*; farmers, therefore, use it in different forms as the last resort for infections caused by *Salmonellae* (Pullorum, etc.). Furthermore, the ready availability of these drugs over the counter makes them easy reach for farmers. Although it is warned that due to the potential toxic side effects, this drug should only be given to poultry with life-threatening infections which are resistant to less toxic antibiotics [23], they are given at will in the study area.

The high occurrence of gentamicin residue observed in liver and kidney may be attributed to the fact that the liver and kidney are organs of drug detoxification and excretion of gentamicin and other drugs [24], respectively. Although the occurrence of gentamicin residue in tissues appears to be dependent on the type of tissue as presented in the

Table-3: Frequency of occurrence of gentamicin residue in different organs (undetected).

Sample type	No.	Frequency of occurrence		Proportion detected (%)
		<0.05 (µg/kg) (−)	>0.05 (µg/kg) (+)	
Muscle	108	60	48	44.4
Kidney	108	44	64	59.26
Liver	108	52	56	51.85
Total	324	156	168	51.85

χ^2 (2)=1.82; p=0.4

result, there is no significant association existing between occurrence of gentamicin and the type of tissue, χ^2 (2)=1.82; p=0.4.

Conclusion and Recommendation

There is constant use of gentamicin without observance of withdrawal period in the study area, thereby causing a high prevalence of gentamicin residue in tissues of commercial birds meant for human consumption. It is then recommended that an integrated drug-resistant surveillance and control program in order to protect the public from health hazards associated with drug residue should be established, in addition, enforcement of laws guarding the prudent use of antimicrobials in food animals should be done.

Authors' Contributions

EVE and AOA conceived and designed the study. CTO carried out the fieldwork of questionnaire administration, collected samples, and did the laboratory analysis with EVE. EVE did the statistical analysis. All the authors wrote, reviewed, edited, and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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