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EFFECT OF SOME COMMON COOKING METHODS ON GENTARYL D AND ANICILLIN RESIDUES IN EDIBLE TISSUE OF POULTRY USING MICROBIAL INHIBITION OF ISOLATES FROM POULTRY DROPPINGS

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ABSTRACT

The effect of frying, roasting, boiling and microwaving on Gentaryl D and Anicillin residues in tissues of cockerels and broilers using microbial inhibition method was investigated. Four 3 weeks old chicks each of broilers and cockerels were fed with water free antibiotics for 3 weeks while the antibiotics were excluded from the feed of the other set which served as the control. Gizzard, thigh, head and liver tissue from each cockerel and broiler subjected to the different cooking methods were placed on nutrient agar plates seeded with *Escherichia coli, staphylococcus aureus* and *salmonella typhi* isolated from the poultry droppings. The diameter of zones of inhibition of the antibiotic residues in each tissue was measured. The zone of inhibition for raw, boiled, roasted, microwaved and fried head tissues of the cockerels ranged from 3.00±0.58mm to 10.00± 1.15, 2.00± 0.00 to 7.33±0.67, 0.00 to 5.33± 0.67, 0.33±0.33 to 4.67±1.15mm and 1.67±0.33 to 4.00± 0.00mm respectively. The zone of inhibition for microwaved thigh tissue for the cockerels and broilers were the least which ranged from 1.33± 0.00 to 3.00± 1.00 and 0.33 to 0.67±0.67 respectively while microwaved and roasted Gizzard tissue of cockerels had the same values ranging from 0.67± 0.67 to 1.00± 0.00mm. Those for broilers ranged from 1.33±0.33 to 3.00± 1.00mm. The microwaved liver tissue had the least zone of inhibition of 0.00±0.00 to 0.67± 0.33mm for cockerels, while the fried liver tissue of broiler

had the least zone of inhibition of 0.00 to 0.67± 0.33mm. Microwaving proved to be the most effective of all the cooking methods on the basis of antibiotic residue reduction. Edible poultry tissues should be microwaved for some few minutes regardless of other methods employed in cooking.

Keywords: Gentaryl D, Anicillin Residues, Microbial Inhibition

INTRODUCTION

Antibiotics are chemical substances produced by microorganism that kill or inhibit the growth of another microorganism. They are used both in human and veterinary medicine and this has resulted to the increasing prevalence of antibiotic resistant bacterial infections seen in clinical practice [**1]**. Any use of antibiotics can increase selective pressure in a population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria die off. The method of microbial inhibition by antibiotics has been used in the detection of antibiotic residues in poultry as a quick and cost- effective alternative to detection of antibiotic concentration levels [**2]**. Gentaryl D is broad spectrum antibiotic used in poultry farming as a growth promoter prophylaxis and in the treatment of bacterial diseases. Anicillin is an antibiotic narrowed to be effective against *Staphylococcus aureus.*

The two antibiotics are usually combined in veterinary to exert bactericidal activities against gram positive and gram negative infections. They have been found to be composed of heavy metals in trace amounts [**3]**. Their relatively long half-life allows the antibiotics to be deposited in tissues if the proper withdrawal periods are not observed **[4, 5]**. This leads to the development of resistant strains of microorganism in birds likely transmitted to human when consumed [**6]**. The residues in food have carcinogenic potentials due to accumulation of the toxic heavy metals [**7**]. Most food-producing animals are always cooked before consumption by one method or another. The variations in the antibiotics levels in the tissue are dependent on type of cooking [**8]**.

It is therefore imperative that studies should be carried out on the effect of different methods of cooking on the residual antibiotics so as to determine consumer exposure to these drugs using easily assessable, quick and cost-effective methods. In view of these this study aims at determining the zones of inhibition of

GentarylD and Anicillin residue in some Cockerels and broilers tissue, determining the effects of roasting, boiling, frying and microwaving on the antibiotic residues using microbial inhibition method and determining the cooking method that best eliminates the antibiotic residues.

MATERIALS AND METHODS

Sample Collection

Eight 3-weeks old chicks were purchased from Running Poultry Farm, Abraka, Nigeria. The samples (chicks) consisted of 4 broilers and 4 cockerels. These were raised in a cage in Microbiology pent house.

Drug Administration

This was done using the method of Javadi*et al* **[3]**. The chicks were fed with feed (Livestock feed) and water free of antibiotics for 3 weeks after purchase (to get rid of probable antibiotics they had been fed with previously). They were then divided into two groups (control and test) containing two chicks from each breed. Each chick in the test group was fed with feed and water containing the antibiotics (Gentaryl D and Anicillin) for 15 days, observing appropriate 3 days withdrawal period (time duration the antibiotics are stopped beforeslaughtering). Chicks in the control group were fed with feed and water free of antibiotics for 15 days.

Preparation of Tissue Sample

The chickens were slaughtered aseptically with sterile knife. The head, thigh, gizzard and liver tissue were aseptically removed from each chicken carcass, sliced into ten equal parts of about 10 mm^2 and wrapped separately in sterile aluminum foil. They were labeled appropriately according to the cooking method to be employed.

Cooking Trials

A modified method of Javadi *et al*. was employed **[3]**.

Boiling

Two pieces of each tissue sample from each of cockerels and broiler carcass were placed in a strainer and immersed into water preheated to 100°C, cooked for a period of minutes for liver, 24 minutes for lap and head, and 35 minutes for gizzard.

Roasting

Two pieces from each tissue sample were placed on a metal baking tray and cooked in an oven at 180°C for 20mins for liver; 40mins for head and lap, and 60mins for gizzard.

Microwaving

Two pieces of each tissue sample was placed on a turn-table. The samples were cooked under full power (900W) for a period of four minutes for all the samples.

Frying

Two pieces of each sample were placed in a strainer and immersed into preheated vegetable oil and deep fried for 5minutes.

Identification of Test Organisms

Poultry droppings were collected in a sterile bottle from overnight droppings of chickens. They were transported to the laboratory in ice-packs, and cultured in both nutrient and MacConkey agar using streak plate method. The plates were incubated at 28±2°C and 37°C for 24hours. The isolates were identified using morphological characterization reaction and biochernical tests. *Escherichia coli Salmonella* and *Staphylococcus aureus* were used as the test organisms.

Preparation of Test Organisms

Distinct colonies from the culture of each test organism were picked and inoculated into sterile peptone water and incubated at 37°C for 24hours. Each was then seeded unto nutrient agar plates using the spread plate method. The plates were incubated at 37°C for 24hours.

Microbial inhibition of the Antibiotic Residues in the Cooked Tissues

Each plate was divided into five equal sections, and well labeled to each of the four cooking methods, and the raw sample. This was done for each tissue. Each tissue was placed directly on the surface of each corresponding section using sterile forceps. The plates were incubated at 37°C for 24hours.

RESULTS AND DISCUSSION

The identity of the organisms isolated from the poultry droppings were *Micrococcus* sp., *Pseudomonas, Escherichia coli, Proteus* sp., *Bacillus* sp, *Staphylococcus* sp. and *Salmonella* as shown in **Table 1**. This is in conformity with the results of a study **[3]**, that similar organisms were isolated from chicken and pigeon droppings. *S. aureus, S. typhi* and *E. coil* were subjected to microbial inhibition test **[3]**.

Table 2 shows the effects of the cooking methods on broiler tissue. The diameter of the zones of inhibition of the raw tissue samples were quite high compared with those subjected to the cooking methods. Tissues subjected to microwaving had the least

diameter of the zones of inhibition. This is in agreement with the observations in a study that there was significant reduction in antibiotic residues of animal tissues subjected to microwaving [**10**].

The effect of the cooking methods on the cockerel tissue samples **(Table 3)** had smaller diameters of the zones of inhibition on raw samples compared with the broiler tissue. The effect of the cooking methods had higher zones of inhibition in the broilers than in the cockerels. This might be due to the fact that cockerels retain more antibiotic residue than broilers, in that cockerels have less muscle tissues so the antibiotics can easily penetrate and be retained in the tissues.

In the microbial inhibition test, observation of the inhibition zones is possible when antibiotic residue is present and is of importance when it exceeds the maximum inhibition limit (2mm for raw samples and 1mm for cooked samples). All the results of the raw tissues still had antibiotic residue of significant importance. Microwaving proved to be the most effective, in that it had more results than had zones of inhibition within the maximum limit than the other cooking methods. The reduction of the antibiotics in muscles cooked by microwaving was rapid and was reduced from35 to 41% in l min [**8]**. Frying and roasting also proved to be effective in reducing the antibiotic residue in broiler tissues. The effect of boiling in reducing the antibiotic residue in both broilers and cockerels was not significant in that most of the results on boiling exceeded the maximum inhibition limit (1mm for cooked samples). The result contradicts the study, that showed that boiling brings about reduction effect on some antibiotics **[3]**. The results on the effect of microwaving might be attributed to the fact that microwaving might be attributed to the fact that microwaving uses electromagnetic waves alongside the high temperature in cooking which have aided the decomposition of the antibiotics.

Frying and roasting, which showed reasonable reduction levels might be attributed to the very high temperature of frying and roasting which might have caused denaturation of the antibiotics?

A study demonstrated that the most significant reasons for reduction of residual level and increase of loss are temperature and time effects [**9]**. Lap tissues showed relatively lower zones of inhibition than the maximum inhibition limit (11mm). This is in agreement with the report of the study that showed that raw thigh muscles hold more antibiotics residues in their raw state, but less antibiotic

residue in their cooked state than other cooked state than other cooked tissue part [**9]**. This can be attributed to the degradation possibility of the antibiotics binding with proteins of tissues **[9, 10]**.

Table 1: Identification of Isolates Used as Test Organisms

Cultural Characteristics/ Biochemical *Tests*	*Salmonella*	*E. coli*	*Staphylococcus aureus*
Gram Reaction	-	-	-
Shape	**Rod**	**Rod**	**Rod**
Endospore	-	-	-
Motility	+	+	+
Catalase	+	+	+
Oxidase	-	-	-
Indole		+	+
Citrate		-	-
Glucose		+	-
Lactose	-	+	-
H_2S	+	-	.-

Key: - Negative; + Positive

Table 2: Zones of Inhibition of Antibiotic Residue in Raw and Cooked Tissue Samples of Cockerels

Tissue Samples	Raw	Boiling	Roasting	Microwaving	Frying	Control
EcU	**7.33 ±0.68**	**3.33±0.67**	**4.00± 1.15**	**l.67±0.67**	**l.67±0.67**	**0.00**
StH	**3.00 ± 0.58**	**2.00 ± 0.00**	**0.00 ± 0.00**	**0.33 ±0.33**	**1.67 ± 0.33**	**0.00**
SaH	**10.00 ± 1.15**	**7.33 ± 0.67**	**5.33 ± 0.67**	**4.67 ± 1.15**	**4.00 ± 0.00**	**0.00**
EcT	**9.33±2.40**	**5.33±1.76**	**5.33±2.66**	**3.00± 1.00**	**5.33±0.67**	**0.00**
StT	**5.33±1.33**	**3.33±0.33**	**1.33±0.38**	**2.00±0.00**	**2.67± 1.33**	**0.00**
SaT	**13.33 ± 1.16**	**6.67 ± 0.67**	**2.67 ± 0.67**	**1.33 ± 0.33**	**2.67± 0.67**	**0.00**
EcG	**3.33 ± 1:33**	**2.00± 1.00**	**0.67±0.67**	**0.67±0.67**	**2.00±1.00**	**0:00**

StG	**2.67± 0.67**	**0.67±0.67**	**0.67±0.67**	**0.67±0.67**	**0.67± 0.67**	**0.00**
SaG	**3.33±1.33**	**2.00±0.00**	**1.00±0.58**	**1.00±0.00**	**1.33±0.67**	**0.00**
EtL	**5.33±0.67**	**2.67±1.33**	**1.33±0.67**	**0.00±0.00**	**0.00± 0.00**	**0.00**
StL	**4.67 ±0.67**	**1.33 ± 0.67**	**1.33 ± 0.67**	**0.67 ± 0.33**	**0.67 ±0.33**	**000**
SaL	**5.33±0.67**	**2.67±0.67**	**1.67±0.33**	**0.67±0.33**	**1.67±0.33**	**0.00**

Key: Ec: *Escherichia coli*; St: *Staphylococcus aureus* Sa: *Salmonella typhi*; H- Head; T- Thigh; L- Liver

Table 3: Diameter of Zones of Inhibition of Antibiotic Residue In Raw and Cooked Samples of Broiler

Tissue Sample	Raw	Boiling	Roasting	Microwaving	Frying	Control
EcH	**6.00± 1.16**	**3.33±0.67**	**L33± 0.67**	**1.33±0.67**	**1.33± 0.33**	**0.00**
StH	**2.00 ± 0.00**	**1.00 ± 0.00**	**0.33 ± 0.33**	**0.00 ± 0.00**	**0.00 ± 0.00**	**0.00**
SaH	**2.67 ± 0.67**	**2.00 ± 0.00**	**1.33 ± 0.67**	**1.67 ± 0.33**	**3.33 ± 0.67**	**0.00**
EcT	**4.00 ± 0.00**	**2.67 ± 0.67**	**0.00 ± 0.00**	**0.33 ± 0.33**	**3.33 ± 0.67**	**0.00**
StT	**4.46 ± 1.33**	**2.67 ± 1.33**	**0.67 ± 0.67**	**0.67 ± 0.67**	**0.00 ± 0.00**	**0.00**
SaT	**4.00 ± 0.00**	**2.67 ± 0.67**	**0.00± 0.00**	**0.33 ± 0.33**	**3.33 ± 0.67**	**0.00**
EcG	**4.33±0.33**	**1.33±0.67**	**2.67±0.33**	**1.00±0.00**	**1.33±0.33**	**0.00**
StG	**2.33 ± 0.88**	**0.00 ± 0.00**	**0.67 ± 0.67**	**0.33 ± 0.33**	**0.67 ± 0.33**	**0.00**
SaG	**6.00±1.15**	**4.67±0.67**	**4.00±0.00**	**1.67±0.33**	**2.67±0.67**	**0.00**
EtL	**7.33 ± 0.67**	**3.33 ± 0.67**	**2.00 ± 0.00**	**0.00 ± 0.00**	**0.67 ± 0.67**	**0.00**
StL	**4.00±0.00**	**2.00± 0.00**	**1.33±0.67**	**0.67 ± 0.67**	**0.67 ± 0.33**	**0.00**
SaL	**7.33 ± 0.67**	**2.67 ± 0.67**	**0.67 ± 0.67**	**1.33 ± 0.67**	**0.00 ± 0.00**	**0.00**

Key: Ec- *Escherichia coli*; St- *Staphylococcus aureus* H- Head; T- Thigh; L- Liver

CONCLUSION

From the results of this study, we can conclude that no cooking method guarantees a full breakdown of Gentaryl D and Anicillin residue in the edible tissue of poultry birds. Microwaving proved to be the most effective cooking method with respect to antibiotic residual removal. It is therefore advisable to cook edible poultry tissue by microwaving for at least 3 minutes regardless of other cooking methods employed. Alternatives to the use of antibiotics in poultry production should be

looked into to prevent the problem of drug resistance. Where the facilities are available, other methods of checking the antibiotic residue level can be done in addition to microbial inhibition method where there are uncertainties.

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