# DETECTION OF ANTIMICROBIAL DRUG RESIDUES IN EDIBLE TISSUES FROM BUNAJI CATTLE SLAUGHTERED IN MINNA ABATTOIR

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**MTech/SAAT/2017/7530**

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## ABSTRACT

Occurrence of antimicrobial drug residues in edible tissues from Bunaji cattle slaughtered in Minna abattoir was investigated during this study. 168 edible tissue samples comprising of meat, liver and kidney were collected from 56 Bunaji cattle slaughtered in Minna abattoir at 2 weeks intervals for a period of 4 months. Antimicrobial inhibition test was used to screened tissue samples for evidence of Antimicrobial drug residues with zone of inhibition measured in millimetres (mm). ELISA test kit was further employed for confirmatory and quantification of oxytetracycline residues from the screened positive samples obtained from the inhibition test. The data obtained were analysed using descriptive percentages and presented as tables for both antimicrobial screening and oxytetracycline residues. Chi-square test was used to test the associations between occurrence of drug residues and other variables. Out of 168 samples screened, 89 (52.97 %) of the samples were confirmed positive for antimicrobial residues at various inhibition zones with values of 29 (51.79 %), 37 (66.07 %) and 23 (41.07 %) obtained for meat, liver and kidney, respectively. There was significant association ($P<0.05$) between tissue samples screened and antimicrobial residues analysed. Sex variation showed female animals had highest values of 68 (70.78 %) antimicrobial residues distributed at 20 (69.96 %), 29 (78.37 %) and 14 (60.86 %) for meat, liver and kidney respectively, when compared to males with values of 9 (31.03 %), 8 (25.80 %) and 9 (39.13 %) for meat, liver and kidney, respectively. Animals between the ages of 6-10 years had the highest antimicrobial residue values of 56 (62.92 %) above other age groups. The 89 (52.97 %) obtained from screened samples were subjected to quantitative analysis using Elisa kit test, thereafter, overall values of 37 (41.57 %) oxytetracycline residues were detected above maximum residue limits (MRLs), as well as values of 25 (28.08 %), 10 (11.23 %) and 2 (2.24 %) were obtained for muscle, liver and kidney, respectively. There was significant association ($P<0.05$) between screened tissue samples and oxytetracycline residue analysed. Female animals had 29 (32.98 %) oxytetracycline residues above set limits as against males that had 8 (8.99 %). Age variation indicated 22 (24.71 %) values of oxytetracycline residues above limit for animals within 6-10 years age groups, which was higher than other age groups. From the results, it is clear that consumers of cattle meat in Minna metropolitan are predisposed to health hazards due to consumption of high levels of antimicrobials and oxytetracycline residues in cattle tissues slaughtered in the study area. This emphasised the need for effective ante-mortem inspection of animals in the lairage before slaughtered, general enlightenment on the withdrawal period before the animals are slaughtered and possible establishment of antimicrobial residues monitoring unit in the study area.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Antimicrobial drugs administration to domestic animal need careful attention of its effect not only to the animal but also to the public that consumed the product from such animals (Kabir *et al.*, 2004). Antimicrobials are natural product of microorganism either synthetic or semi-synthetic that prevent the growth of microorganisms or destroy the microbes (Kirbis, 2007). In animal health practice, antimicrobials are used as therapeutic, prophylactic or as growth promoters to domestic animals (Donoghue, 2003). Antimicrobial are of different types with various pharmacokinetic activities that may become a potential hazard to animals or human. Presence of antimicrobials in edible tissues of food animals is one of the most important concerns for their safety to human consumers (Kozarova *et al.*, 2001).

Many livestock farmers administered antibiotics indiscriminately for purposes of treating or preventing an ailment or as growth promoters without cognisance of the need to consult an expert for necessary advice. Such animals are often slaughtered without observing withdrawal period of the drugs. They have little knowledge of the pharmacokinetic activities of most of the antimicrobials drugs used. Indiscriminate administration of antimicrobials in livestock has raised serious public health concern globally and especially in sub-sahara Africa and particularly Nigeria (Shehu, 2018).Wesangula (2018) pointed the looming danger to public health due to indiscriminate use of antimicrobial drugs in foods animals.

Drug residue occurred as a result of slaughtering treated animals without observing waiting period of the drug used. The situation usually occurred due to prolonged administration and or over dosage of antimicrobial agent as reported by (Omeiza *et. al.*, 2012).

Toxic levels of antimicrobial residues in edible tissues can cause different health problems to unsuspecting consumers (Omeiza *et al*., 2012).Concern has been shown about the possible harmful effect on the consumers, through ingestion of drug residues contaminated animal tissues, which may include microbial drug resistance, allergy, toxicity, and sensitisation to antimicrobial (Ahendi *et al*, 2000).

Cattle playedan important role in animal protein supply which constitute of about 45% of meat industry (Alphonsus *et al*., 2012). Cattle population in Nigeria was estimated to 15.3million as reported by (Kubkomawa, (2017)). Bunaji are the most widely spread across northern part of the country, among all Nigeria cattle breeds (Umar, 2007). More than 1000 cattle, sheep and goat are slaughtered daily across Niger state (Dukku, 2018). About 50-60 heads of cattle are slaughtered daily in Minna abattoir and sixty percent (60 %) are Bunaji cattle. The abattoir where these animals are usually slaughtered and processed for public consumption, contributed immensely to the economic development of states. Nigeria as many other countries of the world, there are no good structure regarding animal drugs usage. Most antibiotics are readily available and obtainable over the counter as well as every rural cattle market, which make it easily accessible to different categories of livestock handlers including the nomadic pastoralist and the middle men. Therefore, it means that proper dosage and withdrawal period of antimicrobial drugs are not usually observed hence, meat safety and quality assurance cannot be granted (Dina and Arowolo1991, Olatoye and Ogundipe, 2009 and Fagbamila *et al*., 2010). Therefore this study aimed at determining the occurrence of antimicrobial residues, as well as quantifying levels of oxytetracycline drug residues in edible tissues of Bunaji cattle slaughtered in Minna abattoir.

## 1.1 Statement of Research Problem

Federal Government of Nigeria warned animal health personnel's against the indiscriminate use of antimicrobial drugs to treat livestock, stressing the effect on animal and human health, which could lead to antimicrobial residue (FMARD, 2018). Another problem envisaged by the Niger state government is the existence of poisonous meat as result of massive used of steroid by farmers for fattening animals (Dukku, 2018). Olaleye (2018) also reported that, National Institute of Pharmaceutical Research and Development (NIPRD) has found the existence of heavy metals and antibiotic residues in meat produced in the Federal Capital Territory, Abuja, which could lead to kidney disease to human consumers. The possible harmful effects the residues could caused to human include, microbial drug resistance, drug residues in meat, allergic reaction and drug toxicity (Alhendi *et al.,* 2000)

Another problem is the improper dosage of oxtetracycline antibiotic to animals which can results into residue in meat. Human health problems related to consumption of unacceptable levels of oxtetracycline residues in edible tissues that could arise, include gastrointestinal, disturbances, hypersensitivity, bone and teeth problems in children and development of resistance to antibiotics reported by (Olatoye and Ehinmowo, 2009 and Larkin *et al.*, 2004).

## 1.2 Justification

The paucity of information on antimicrobial and oxytetracycline residues in meat in Niger state is a justification for the current study. However, there are scanty reports on veterinary drug residues on the extent of the residue problem in Nigeria (Yaqub *et al*., 2009)

The research would provide more information on the occurrence or otherwise of antimicrobial and existence of oxytetracycline drug residues in meat, liver and kidney from Bunaji cattle consumed Minna as claimed by the state government. .

**1.3 Aim and Objectives of the Study**

The study sought to detect occurrence of antimicrobial drugs residues and also confirm/quantify levels of oxytetracycline residues in Bunaji Cattle Slaughtered in Minna abattoir.

The objectives of the study are to

- Determine the occurrence of antimicrobial residues in meat, liver and kidney from Bunaji cattle slaughtered in Minna abattoir using microbial inhibition test.
- Determine presence and levels of oxytetracycline in meat, liver and kidney from Bunaji cattle slaughtered in Minna abattoir, using Enzyme Link Immuno Solvent Assay (ELISA) test kit.
- Determine occurrence of antimicrobial and oxytetracycline drug residues as it affect age and sex of the cattle slaughtered.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Antimicrobial Drugs

Antimicrobial agents are naturally occurring substances either synthetic or semi-synthetic, that either kills or prevent the growth of microorganisms and eventually destroyed them (OIE, 2011). Antimicrobial agents include drugs active against all disease causing micro organisms such as bacteria, protozoa, viruses and fungi. Antimicrobial drugs are used for decades to improve both human and animal health (Flynn, 2012). The discovery of antimicrobial agents brought about changes in the management of infectious diseases that kill human and animals during the pre-antimicrobials era (Clardy *et al*., 2009). Godfrey and Norah (2013) also define antimicrobials drugs as chemical substances naturally produced by different species of microorganisms such as bacteria and fungi which include antibiotics, antiprotozoa, antifungi and antiseptics. In animal health practice, antimicrobials are used for the treatment and prevention of diseases or as growth promoting agents and also for nutritive purposes in livestock production (Riveire and Sundluf, 2001).Antibiotics are drugs used for the treatment of different forms of bacterial diseases which include drugs such as oxytetracycline, penicillin, streptomycin and sulphonamides. Various antibiotics take different time intervals to be excreted out of the body of animals. Meat from such animals may become hazard to human health (Normanno *et al.*, 2007), presence of antimicrobials drugs, especially antibiotic residues in animal edible tissues are among the most important factors for their safety. Many livestock farmers administered antimicrobials drugs to animals by themselves, with little understanding of the situation and quantity to administer or the withdrawal time, in most cases, there

are veterinary drugs intended for other species of animals been used on ruminants by those farmers.

### 2.2 Antimicrobial Drugs use for Food Producing Animals

Several antimicrobial drugs are used in the treatment of different diseases of livestock especially in food-producing animals worldwide. Most of these agents are also used in microbial infections in humans (FDA, 2010). However, most of these drugs are abused by herdsmen and other livestock farmers. Many farmers treat their suspected sick animals with antimicrobial drugs themselves without seeking professional advice. The problem is worse in developing countries that have no standard veterinary regulations, making the cost of treatments to be very expensive for the farmers (Godfrey and Norah, 2013). Many farmers access these drugs easily form the market and treat their animals even in cases where use of antimicrobials agent is not necessary, as a result of this act, unquantify levels of antimicrobial drugs used remain in the tissues of the animals or are released in the environment, thus increasing selection of the antimicrobials resistant organisms that can spread from the animals, hence increasing the cost of treatments in both animals and humans (Martinez, 2009).

### 2.3 Authorised Veterinary Antimicrobials Drugs

Products containing antimicrobial agents authorised for veterinary use are those that have passed through marketing authorisation process of the national or European authority. After the evaluation of the scientific data proving the efficacy of such product and its safety for humans, animals and the environment they are authorise for importation, distribution and usage However, there might always be problems in its implementation because the technical evaluation of a marketing application is limited to an administrative procedure alone (Mensah *et al.*, 2014).

### 2.3.1 Oxytetracycline

Oxytetracycline can be defined as broad spectrum antibiotic obtained from a soil *actinomycete*and used against various forms of *bacteria*l and *rickettsia*l infections. Chopra and Roberts (2001) reported that, oxytetracycline antibiotics works by inhibiting bacterial protein synthesis and binding on the*aminoacycl-tRNA* with the bacterial ribosome (Schnappinger and Hillen, 1996). The inhibition is done by the drug through penetrating into the outer membrane of gram negative enteric bacteria as positively charged action complexes. The resistance has been attributed to the use ofoxytetracycline in livestock for treatment and preventing purpose without proper observation of the withdrawal period (Singer *et al.*, 2006).Oxytetracycline is one of the most common antibiotics relatively easy and inexpensive to obtained (Zakeri and Wright, 2008). The acceptable maximum residues limit (MRLs) for oxytetracyclines as recommended by the Joint Food and Agriculture Organization and World Health Organization (FAO)/WHO Expert Committee on Food Additives are 0.2mg, 0.6mg, and 1.2mg for muscles, liver and kidney respectively (Abbasi *et al.*, 2012).

### 2.3.2. Quinolones or fluoroquinolones

Quinolones are among the antibiotics group, which include, ciprofloxacin, enrofloxacin, flumequine, gatofloxacin, and grepafloxacin, the drugs are widely used in livestock production for the treatment and prevention of diseases (Pyun *et al.,* 2008). Using ciprofloxacin for prophylaxis and curative means forthe treatment of cattle diseases is authorised in the European Union (EU) countries and have established a Maximum Residue Level (MRL) of 0.2, 0.1, and 0.3mg/kg for liver, muscle and kidney tissues, respectively.

### 2.3.3 Streptomycin

Streptomycin is a narrow spectrum antibiotic produced by some *Streptomyces griseus* strains it is used on respiratory tract infection such as pneumonia and tuberculosis, also used in veterinary medicine on the treatment of gram-negative bacterial infections because of its effectiveness (Huet *et al*, 2006). It action is based on the inhibition of ribosomal protein that leads to death of cells. Susceptible strains include *Escherichia coli, Salmonella spp.* (Bogialli and Corcia, 2009).

## 2.4 Microbial Growth Inhibition Test Method

Microbial growth inhibition method, provide the use of a standard culture of a tested microorganism in a liquid or solid medium (Heeschen and Bluthgen, 1991) *Bacillus subtilis, or Bacillus megaterium.* The analysed sample is placed on the agar surface either directly or with a paper disc (disc assay plate methods). At the process of incubation, diffusion of the sample into the medium takes place it is called agar diffusion principle, and if the sample contains inhibitor agents, reduction or total inhibition occurs on the tested microorganism growth. Depending on the method used, the presence of inhibitor agents in the tested sample is indicated by the formation of a clear zone of inhibition around the disc (disc assay plate methods) or a change in the medium colour (Botsoglou and Fletouris, 2001).

## 2.5 Immunoassays

Nonisotopic immunoassays test such as Enzyme linked immune sorbent assay (ELISA), Fluorescence polarisation immunoassay (FPIA), Particle-concentration immunoassay (PCIA), particle-concentration fluorescence immunoassay (PCFIA),and monoclonal-based immunoassays (MBIA) all play an important role in antimicrobial screening and specificity (Roeder and Roeder, 2000).

## 2.6 Antimicrobial Residue

Drug residues are defined as active ingredients or metabolites that remain in meat or other foodstuffs from animal to which the antimicrobial agent has been administered. (EC, 1981).Regulation No. 470/2009 of the European Parliament and of the Council defines residues as all pharmacologically active substances, whether active ingredients or degradable products, and their metabolites, which remain in animal-derived food. The concept of drug residues in food was developed over the second half of the 20th Century, resulting in the definition of an observed effect, level and acceptable daily intake (ADI) and a Maximum residue limit (MRL) in food (Codex Alimenterius Commission, 2011).

The use of antibiotics in food-producing animals may leave residues in tissues of animal such as meat, liver and kidney. The occurrence of these residues may be due to a failure to observe the withdrawal periods of the drug, extra-label dosages for animals, contamination of animal feed with the excreta of treated animals or medicated feed or the use of unlicensed antibiotics (Zeenatudeen, 2015).

Antibiotic residues in edible tissues may be the cause of various health related problems in humans. These may be toxicological, transfer of antibiotic resistant bacteria to humans, immune pathological effects, carcinogenicity, mutagenicity, nephropathy and allergy as reported by (Nisha, 2008).

## 2.7 Occurrence of Antimicrobial Residues in Meat

In Nigeria various research have been carried out on drug residues existence in edible tissues of cattle and other domestic animals Dipeolu and Alonge (2002), Kabir *et al.*,(2004) and Omeiza *et al*. (2012), have all demonstrated the presence of antimicrobial residues in meat.

Omeiza *et al*. (2012) reported that, antimicrobials drugs given to domestic animals close to the period of slaughter usually result into antimicrobial residues in animal tissues. Research conducted confirmed the occurrence of antimicrobial residues at 83.9% in animal tissue, kidney and liver in cattle slaughtered within Abuja town, they further stated that, use of antimicrobials on animals close to the time of slaughter between herd owners and transporters found to be significantly higher compared with antibiotics administration by other animal health personnel.

Olatoye and Ogundipe (2009), in a research conducted on meat consumed in Ibadan, Nigeria, they confirmed the existence of antimicrobials residues at 69.74 % out of the total edible beef samples screened.

Olatoye, and Elinmowo (2010) also reported that, out of one hundred and eighty (180) meat and other edible portion of slaughter cattle from Akure metropolitan abattoir that were analysed for evidence of oxytetracycline residue, 98 sample representing (54.44 %) had detectable levels of drug residues while 62 (34.44 %) 0f the sample had oxytetracycline residues at volatile level above the World Health Organization (WHO)/Food and Agricultural Organization (FAO) maximum residue limits (MRL)

Akinwumi *et al.* (2012) reported that twenty four (24) beef samples were randomly collected from four (4) agricultural zones in Oyo state. The samples were analysed for detection of the residues of antimicrobials and cooking loss. The study revealed 46.00 % samples to be positive for antimicrobial residue.

According to a report by Shehu (2018) antibiotics can be defined as powerful medicines that fight bacterial infection. When bacteria developed resistance to the antibiotics, it is termed Antibiotic Resistance (ABR), while Antimicrobial Resistance (AMR) occurs when disease-causing organisms (bacteria, viruses, and parasites) can no longer be killed by antimicrobial agents. Wesangula (2018) pointed out at the

possible danger on public health as a result of rampart used of antibiotics in animals. His position reflects the grave concern expressed in Nigeria by the Nigerian Veterinary Medical Association, he stressed that the use of antibiotics in livestock in present day is even greater than in humans. The health expert expressed worry that in many villages farmers used human antibiotics to treat animal against various diseases. He categorically stated that, by consuming meat, egg or milk with antibiotics residues, one under-dosed himself by exposing his microbes to non-lethal quantities of the drug, which will make them resistant to many antibiotic drugs when he is sick. Shehu (2018) also explained how humans become the victim of the antibiotic used in livestock: He also stated that the practice "is worse in poultry. What we have is people produced a cocktail of drugs.

Two, three, or four different antibiotics are put into one – whether the birds are sick or not, they keep putting it into the water. They think it will enhance their growth, make them healthier or may not come down with any disease. While this is happening, the birds are laying eggs, or they are maturing as broilers and we are eating them. So we now get exposed to very low concentration of antibiotics and when people go to hospitals when sick they give you drugs, it does not work because you have already been exposed to some sub-lethal concentration of the drugs and the organisms themselves have found a way to dodge the effect of the drugs so the drugs are no more effective. According to Wesangula (2018), about 4.2 million people in Africa are likely to die due to antimicrobial resistance. The World Bank estimates global healthcare cost of more than $32 trillion by 2050. National Institute for Pharmaceutical Research and Development (NIPRD) has discovered heavy metals and antimicrobial resistance in meat products in the Federal Capital Territory, Abuja. NIPRD said a high consumption of such metals in meat could damage the kidneys

(Olaleye, 2018). Dukku (2018) reported that, Niger state government raises alarm over existence of poisonous meat in the state. That it had discovered massive use of antimicrobials by livestock farmers and butchers which make most meat in circulation unsafe for consumption. It has also been discovered that some people are inducing livestock hence circulating poisonous meat. He also stated that, farmers are using antibiotics as a method for growth on their animals, the state statistics shows that between 800 – 1000 cattle sheep and goats are slaughter while over 5000 chickens are consumed daily across the state which put great percentage of consumers at risk of unwholesome practice he said, the inducement of those animals pose grave and dangerous health hazard to the consumer and increase the cost of human health.

## 2.8 Cattle

Cattle played an important role in meat supply and livestock industry. Cattle edible tissues are estimated to supply about 45 percent of total meat consumed in Nigeria. Cattle population in Nigeria has been estimated as 15.3 million (Kubkomawa, 2017).The most common breeds of cattle indigenous to Nigeria include Bunaji, Red Bororo, Sokoto Gudali, Adamawa Gudali, Muturu, and Keteku (Umar *et al* 2008). Over 90 percent of cattle in Nigeria are in the hands of traditional livestock farmers and mostly found in the Northern parts of the country (Umar, 2007). The growth rate in the national herd is estimated at 1.5 percent annually. It is interesting to note that, although developing countries contain about two-thirds of the World Cattle Population, about two-thirds of total beef production is accounted for by developed countries (Kubkomawa, 2017).

### 2.8.1 Bunaji cattle

Bunai are the most populated and widespread of all cattle breeds in Nigerian (Blench *et al.*, 1993). Alphonsus *et al*. (2012) estimated that, Bunaji represents 37 % of

Nigerian cattle breed and are found in almost all part of Nigeria but more numerous in the northern part of the country. The movement of cattle from the northern part to the southern part of Nigeria has largely been of Bunaji. Pastoralists generally agreed hat, they are better and superior than all other breeds in diseases resistance tolerance, and have the ability to withstand weather condition (Blench *et al*., 1993). The Bunaji cattle are, however, important for their genetic predisposition of hardiness, heat tolerance and adaptation to local environment Bunaji are triple-purpose animals it can be fattened for meat, keep for milk production and can be used as draught animal, especially the bull (Alphonsus *et al*., 2012).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Experimental Site

Samples were collected from Minna abattoir located along Tayi village in Minna, Niger state, Nigeria. Minna is situated on latitude $9^0$3223 N and longitude $6^0$ 288 E, with an annual rainfall of 1,200 mm -1,300 mm, with temperature of 21-36degree centigrade, the area has an altitude of 1,46m above sea level in the Southern-guinea savannah vegetation zone of Nigeria it is characterised by two season dry and wet season (April - October and dry season November –march. Niger State Agricultural and Mechanization Development Agency (NAMDA, 2019)

### 3.2 Sample Collection, Duration and Preservation

One hundred and sixty eight (168) samples from 3 edible parts of cattle (Liver, meat and kidney) were randomly collected from slaughtered Bunaji cattle in Minna abattoir for a period of 4 months at 2 weeks intervals, with special emphasis on age, sex and time intervals**.** The samples were properly packed and labelled in a poly bag and store under a refrigerator at a temperature of -$20^0$C until the time for the analysis.

### 3.3 Location of the Analysis

The analysis was carried out in the Department of Veterinary Public Health and preventive medicine, Faculty of veterinary medicine, Ahmadu Bello University, Zaria, Nigeria

### 3.4 Sample Analysis

The following laboratory techniques were used for sample analysis

- **Microbial inhibition test (Screening)**
- **Elisa kit test (confirmatory)**

### 3.5 Laboratory Procedure

Microbial inhibition test was used to screened samples for evidence of antimicrobial residues as described by (Huber *et. al*.1969). There after based on the positive results obtained from the screening test, quantitative measurement of Oxytetracycline residues was done using Oxytetracycline ELISA test kit which was purchased from BIOTUVA Life sciences, 4283 Express Lane, suite 728504 Sarasota, FL 34249, USA

#### 3.5.1 Microbial inhibition test (Screening)

Mueller Hinton Agar (MHA) was prepared in plates according to manufacturer`s instruction. *Bacillus subtilis*ATCC 6633 was used as test organism. An 18 hour culture of the test organism in 10 ml nutrient broth was done and used to inoculate plates. Plates were inoculated with the test organism by dipping sterile cotton swab sticks into the suspension of the test organism until saturated (kabir *et al.,* 2004). The MHA Plates were gently but thoroughly swabbed uniformly to achieve a lawn of confluent growth and was allowed to dry for 5 minutes. The extract from the sample were tested by dipping sterile 1.2 mm diameter *whatmann* filter paper disc into the juice extract, until saturated, excess samples were allowed to drained along the walls of the tubes, then with sterile thumb forceps, the impregnated disc was gently placed on the inoculated MHA plates and labelled accordingly. *Whatman* filter paper disc containing 0.3mg of oxytetracycline Hcl was used as controlled discs, placed at the centre of the plates, with test discs at the periphery of the plates and were incubated at 37 degree centigrade for 18 hours, after which presence or absence of zone of inhibition and that of the disc were calculated. Discs with a difference above 1mm were considered positive for antimicrobial residues.

### 3.5.2 ELISA test kit procedure (confirmatory)

The components of the kit were allowed to equilibrate to room temperature, 0.5mg of samples (based on the screened positive samples), were added to the bottom of each well. The side of the strip holder were slightly tapped to evenly distribute the sample and a 100mg of oxytetracycline antibody working solution was added to each well and incubated at room temperature for 40 minutes, after which the solution in the well was discarded by inverting and shaking. The microtiter well was washed 3 times to remove non-bound conjugate. 0.1 mg enzymes conjugate was added to each well and incubated at room temperature for 20 minutes. 0.05 mg of stop solution was added to each well and the strips tapped for proper mixture. The absorbance was read at 405 mm within 30mins using an ELISA reader, the results were calculated by obtaining optical density (OD) value.

### 3.6 Statistics Analysis

The data obtained were analysed using descriptive statistics and expressed in percentages and presented as tables for both antimicrobial screening and oxytetracycline residues. Statistical package for social sciences (IBM USA Version 16.0 2010) was used to analysed the data. Chi-square test was used to test the associations between occurrence of drug residues and other variables.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Antimicrobial Drug Residues in Bunaji Cattle Slaughtered in Minna using Microbial Inhibition Test.

The result of antimicrobial drug residues obtained from tissue samples using microbial inhibition test is presented in Table 4.1. Out of 168 samples analyzed for antimicrobial residues during this study; 89(52.97%) while the values of 29 (17.26 %), 37 (22.02 %) and 23 (13.69 %) for meat, liver and kidney respectively were found at different inhibition zones ranging from 10 mm- 20 mm, while the remaining 79 (47.03 %) were negative for anti-microbial residues. There was significant association ($P<0.05$) between the tissue samples screened and antimicrobial residues analysed.

### 4.2 Distribution of Antimicrobial Drug Residues from the Screened Tissues Samples based on sex of animals

The result of antimicrobial drug residues obtained from screened tissue samples based on sex is shown in Table 4.2. 26 (29.2 %) of the total positive samples were males with values of 9 (31.03 %), 8 (25.80 %) and 9 (39.13 %) obtained for meat, liver and kidney respectively. Female animals had the highest value of 63 (70.78 %), at 20 (68.96 %), 29 (78.37 %) and 14 (60.36 %) obtained for meat, liver and kidney respectively. There was no evidence of significant association between animal sex and the presence of antimicrobial residues in the tissues screened.

**Table 4.1**: Antimicrobial drug residues in Bunaji cattle slaughtered in Minna abattoir using microbial inhibition test (screening).

| SAMPLE | POSITIVE (%) | NEGETIVE (%) | % AMR ($V^+$) |
|---|---|---|---|
| **MEAT** | 29 (51.79)[b] | 27 (48.21)[c] | 51.79 % |
| **LIVER** | 37 (66.17)[a] | 19 (32.92)[b] | 66.17 % |
| **KIDNEY** | 23 (41.07)[c] | 33 (56.92)[a] | 41.97% |
| **TOTAL** | **89 (52.97)** | **79 (47.03)** | |

$X^2$= 7.072 with associated P-value = 0.0291, df = 2

% AMR = Percentage Antimicrobial Residue (Positive)

**Table 4.2**: Distribution of Antimicrobial drug residues from screened tissue samples based on sex

| | **Gender** | | |
|---|---|---|---|
| **Samples** | **Males** | **Females** | Total |
| **MEAT** | 9(31.03 %) | 20(68.96 %) | 29 |
| **LIVER** | 8(25.80 %) | 29(78.37 %) | 37 |
| **KIDNEY** | 9(39.13 %) | 14(60.86 %) | 23 |
| **TOTAL** | 26(29.21 %) | 63(70.78 %) | 89 |

$X^2$ = 2.171 with associated P- value = 0.3376, df= 2,

### 4.3 Distribution of Antimicrobial Drug Residues Obtained from Screened Tissue Samples based on Age

Result for age variation is showed in Table 4.3. From the screened samples it found that, 6-10 years had 56 (62.92 %), 0-5 years 21 (23.59 %) while above 10 years had 12 (13.48 %). There was no significant association (P>0.05) between age of the animals and presence of antimicrobial residues in the screened tissues.

### 4.4 Confirmatory and Quantifications of Oxytetracycline Drug residues from Screened Tissue Samples using ELISA Test Kit

The result of confirmatory and quantification of oxytetracycline drug residues obtained from screened tissue samples using Elisa test kit is presented in Table 4.4. Out of 89 tissue samples found positive for antimicrobial residues during screening, Oxytetrocycline residues was detected in all the samples(100%) at various optical density levels (0.24, 0.63 and 1.23mg/kg for meat, liver and kidney respectively). 37 (41.57 %) were above the set residue limit, at 25 (86.20 %), 10 (27.05 %) and 2 (8.70 %) for meat, liver and kidney respectively. There was significant association that yielded, x-squared = 49.642 and P-value = 4.288e-10 between oxytetracycline residue values and screened tissue samples.

**Table 4.3**: Distribution of Antimicrobial drug residues from screened tissue samples based on age

| | **Age** | | | |
|---|---|---|---|---|
| **Samples** | **0-5 (years)** | **6-10 (years)** | **>10 (years)** | Total |
| **MEAT** | 7(24.13 %) | 17(58.62 %) | 5(17.24 %) | 29 |
| **LIVER** | 9(24.32 %) | 24(64.86 %) | 4(10.81 %) | 37 |
| **KIDNEY** | 5(21.73 %) | 15(65.21 %) | 3(13.04 %) | 23 |
| **TOTAL** | 21 (23.59 %) | 56 (62.92 %) | 12 (13.48 %) | **89** |

$X^2$= 0.6753 with associated P-value = 0.9543, df = 4,

**Table 4.4**: Confirmatory and quantifications of Oxytetracycline drug residues from screened tissue samples using ELISA test kit

| Samples | MRL | Range of oxytetracycline residues | | |
|---|---|---|---|---|
| | | **Below limit** | **Within limit** | **Above limit** |
| **Meat** | 0.2mg/kg | 1 (3.44 %) | 3 (10.34 %) | 25 (86.20 %)[a] |
| **Liver** | 0.6mg/kg | 27 (72.97 %)[a] | 0 | 10 (27.05 %)[b] |
| **Kidney** | 1.2mg/kg | 21 (64.31 %) | 0 | 2 (8.70 %)[c] |
| **Total** | | **49 (55.05 %)** | | **37 (41.57 %)** |

$X^2$ 49.642 with associated P-value = 4.288e-10, df = 4

ELISA = Enzyme Linked Immune sorbent Assay

MRL = Maximum Residue Limit

### 4.5 Distribution of Oxtetracycline Drug Residues from Screened Tissue Samples Base on Sex and Meat

The result for Distribution of oxtetracycline drug residues obtained from the screened tissue samples of bunaji cattle slaughtered in the study area based on sex and meat from the animals is showed in Table 4.5. The analysis yielded, X-squared = 0.45242 and associated P- value = 0.5012. Therefore the test for independence was not significant, oxytetracycline residues presence is higher in female meat 20 (68.96 %) than expected.

### 4.6 Distribution of Oxtetracycline Drug Residues from Screened Tissue Samples Based on Sex and Liver

The result for Distribution of oxtetracycline drug residues obtained from the screened tissue samples of Bunaji cattle slaughtered in the study area based on sex and Liver from the animals is showed in Table 4.6. The analysis turned out, X-squared = 0.00002 and associated P- value = 1. This showed that there was no significant association in the oxytetracycline residue presence, between sex and maximum residue level in the animal liver. Female had 8 (21.62 %) at maximum residue limit than males 2 (5.40 %) oxytetracycline residue above maximum residue limit.

**Table 4.5**: Distribution of oxtetracycline drug residues from screened tissue samples cattle based on sex and Meat

| | **Gender (Meat)** | |
|---|---|---|
| **Residue limit** | **Male** | **Female** |
| Below limit | 2(6.89%) | 2(6.89%) |
| Above limit | 5(17.24%) | 20(68.96%) |

$X^2$= 0.452 with associated P-value =0.501, df= 2

**Table 4.6**: Distribution of oxtetracycline drug residues from the screened tissue samples based on sex and Liver

| | **Gender (Liver)** | |
|---|---|---|
| **Residue limit** | **Male** | **Female** |
| Below limit | 5 (13.52 %) | 22 (59.45 %) |
| Above limit | 2 (5.40 %) | 8 (21.62 %) |

$X^2$= 2.1867e-30 with associated P-value =1, df = 1

### 4.7 Distribution of Oxytetracycline Drug Residues from Screened Tissue Samples Based on Sex and kidney

The result for Distribution of oxtetracycline drug residues obtained from the screened tissue samples of Bunaji cattle slaughtered in the study area based on sex and Kidney from the animals is showed in Table 4.7. The analysis gave, X-squared = 0.01369 and associated P- value = 0.9069. This showed that there was no significant association in the oxytetracycline residue presence and between sex and maximum residue level in the animal Kidney. Female had 1(4.34%) at maximum residue limit than male 1(4.34 %) of oxytetracycline residue above maximum residue limit.

### 4.8 Distribution of Oxytetracycline drug residues from screened tissue samples based on age and Meat

The result for distribution of oxytetracycline residue obtained from screened tissue samples based on age and tissue type (Meat) of the animals using Elisa test kit for Oxytetracycline residues above maximum residue set limit irrespective of the tissue types confirmed within age groups showed that; 11 (12.36 %); 22 (24.71 %) and 4 (4.41 %) were obtained for ages 0-5; 6-10 and above 10 years respectively. Table 4.8 showed the analysis yielded X- squared = 0.5328 and P- value = 0.7661.This shows that there was no significant association (P>0.05) in the oxytetracycline residues distribution between maximum residue limit and age category.

**Table 4.7:** Distribution of oxtetracycline drug residues from screened tissue samples based on sex and Kidney

| **Residue limit** | **Gender (Kidney)** | |
|---|---|---|
| | **Male** | **Female** |
| Below limit | **4 (17.**39 %**)** | 17 (73.91 %) |
| Above limit | 1 (4.34 %) | 1 (4.34 %) |

$X^2$=0.1369 with associated P-value = 0.969, df = 1

**Table 4.8:** Distribution of oxtetracycline drug residues from screened tissue samples based on age and meat

| | **AGE (Meat)** | | |
|---|---|---|---|
| **Residue limit** | **0-5** | **6-10** | **>10** |
| Below limit | 1 (3.44 %) | 2 (6.89 %) | 1 (3.44 %) |
| Above limit | 6 (20.69 %) | 16 (55.17 %) | 3 (10.34 %) |

$X^2$ = 0.5328 with associated P-value = 0.7661, df = 2

### 4.9 Distribution of Oxytetracycline drug residues from screened tissue samples based on age and Liver.

The result for distribution of oxytetracycline residue obtained from screened tissue samples on age and tissue type (Meat) of the animals using Elisa test kit for Oxytetracycline residues above maximum residue set limit is shown in Table 4.9. The analysis yielded X- squared = 0.5O87 and P- value = 0.7754. This shows that there was no significant association (P>0.05) in the oxytetracycline residues distribution between maximum residue limit and age categories of the animals.

### 4.10 Distribution of Oxytetracycline drug residues from screened tissue samples based on age and Kidney.

The result for distribution of oxytetracycline residue obtained from screened tissue samples on age and tissue type (Kidney) of the animals using Elisa test kit for Oxytetracycline residues above maximum residue set limit is shown in Table 4.10. The analysis yielded X- squared = 7.8857 and P- value = 0.0194. This showed that there was significant association (P<0.05) in the oxytetracycline residues distribution between maximum residue limit and age categories of the animals. Pearson residuals analysis showed that the oxytetracycline residual load were higher in age category 6-10 for below limit 15 (65.21 %) than expected but higher in age 0-5 years for the above limit 2 (8.67 %)

**Table 4.9**: Distribution of oxtetracycline drug residues from screened tissue samples based on age and Liver.

| | AGE (Liver) | | |
|---|---|---|---|
| **Residue limit** | **0-5** | **6-10** | **>10** |
| Below limit | 6 (16.21 %) | 16 (43.24 %) | 5 (13.52 %) |
| Above limit | 3 (8.11 %) | 6 (16.21 %) | 1 (2.70 %) |

$X^2$=0.5087 with associated P-value = 7754, df = 2

**Table 4.10**: Distribution of oxtetracycline drug residues from screened tissue sample based on age and kidney.

| | AGE (Kidney) | | |
|---|---|---|---|
| **Residue limit** | **0-5** | **6-10** | **>10** |
| Below limit | 3 (13.04 %) | 15 (65.21 %)[a] | 3 (13.04 %) |
| Above limit | 2 (8.67 %)[a] | 0 | 0 |

$X^2$ = 7.8857 with associated P-value =0.0194, df = 2

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Discussion

### 5.1. Antimicrobial Drug Residues in Bunaji Cattle Slaughtered in Minna using Microbial Inhibition Test (Screening)

The overall results from this study when considered regardless of the tissue types, indicated 89 (52.97 %) incidence of antimicrobial drug residues from the screened tissue samples, the result is higher than 46% antimicrobial residue in beef in Oyo (Akinwumi *et al,* .2012). 30 % and 25 % antimicrobial residues in pig and goat tissues reported by Ezeduka and Chinyere (2012) and 25 % in milk of cattle (Yusuf *et al*., 2017). However the result is lower than 89.3% as reported by Omeiza *et al*,(2012) from meat of cattle slaughtered in Abuja and 69.74 % from preliminary screening of beef consumed in Ibadan, (Olatoye and Oguudipe, 2009).

There was significant association ($p<0.005$) obtained between the screened tissue samples and the antimicrobial drug residues which might be due to indiscriminate slaughter of sick animals, non adherence to strict ante-mortem inspection in the lairage and withdrawal period of treated cattle might have not been observed before slaughter. This is in line with the findings of Olatoye and Ogundipe (2009) that attributed their report to indiscriminate use of antimicrobials by the nomadic pastoralists and non adherence to ante- mortem inspection. Test for equality and proportion also indicated that, there are significant different ($p<0.05$) (not equality) in the antimicrobial presence (positives) from the tissues. The estimated proportion distributions of microbial presence are 51.79 %, 66.1 %, and 41.07 % in meat, liver and kidney respectively. Liver had the highest level of antimicrobial residues, as an organ responsible for drug metabolism.

### 5.1.2 Distribution of Antimicrobial Drug Residues from Screened Tissue Samples Based on Sex

Sexvariation showed that females had the highest values of 63 (70.78 %) antimicrobial drugs residues at 20 (68.96 %), 29 (78.37 %) and 14 (60.36 %) for meat, liver and kidney respectively than male, this may be attributed to physiological factors such as hormonal inter phase during pregnancy which might tend to affect drug absorption, metabolism and excretion,. This is in line with the findings of Nina and Kenneth (2013) that reported, an evidence of pregnancy altering the functions of drug metabolising enzymes and drug transporters at gastrointestinal stage.

Statistically there was no strong evidence of association in the presence of antimicrobials residue in the sampled tissues and sex. A further Pearson residuals test shows that antimicrobial residues presence was higher in male meat and kidney than expected, but higher in female liver than expected.

### 5.1.3 Distribution of Antimicrobial drug residues from screened tissue samples based on Age

The level of antimicrobial residues and determination on animal age factor showed 56 (62.92 %) to be of age 6-10years with 17 (58.62 %), 24 (64.86 %) and 15 (65.21 %) for meat, liver and kidney respectively. The result is higher than 14.7 % reported by Zeenatudeen (2015) on raw milk. The higher values obtained at (6-10 years of age) in this study might be attributed to decline in physiological processes at advanced age of the animals which might have an effect on the level of deposits seen on different tissues sampled. Mangoni and Jackson (2004) confirmed that advancing in age affect pharmacokinetic activities of drugs. There was no significant association ($P>0.05$) between age of the animals and presence of antimicrobial residues in the screened tissues sampled. Further Pearson residuals test shows that antimicrobial residue presence was higher than expected in meat for age above 10 , but higher in liver and

kidney for age 6-10 than expected, but least antimicrobial presence than expected in liver of age above 10 .

### 5.1.4 Confirmatory and Quantifications of Oxytetracycline Drug Residues from Screened Tissue Samples using ELISA Test Kit

Out of 89 tissue samples positive for antimicrobial residues analysed, this study found 37 samples, representing 41.57 % of oxytetracycline residue above maximum residue set limit, 25 (28.08 %), 10(11.23 %) and 2 (2.24 %) at 0.23mg/kg, 0.64mg/kg and 1.3mg/kg values were obtained for meat, liver and kidney respectively, from the sampled tissues. These values were above normal maximum residue limit and unacceptably high when compared to the maximum residue limits of 0.2mg, 0.6mg and 1.2mg for meat, liver and kidney respectively as set by (CAC, 2011). The results agreed with 29 (32.6 %), 5 (5 %) and 3 (3/.1 %) obtained for muscle, liver and kidney onoxytetracycline above maximum residues limit in tissues of cattle slaughtered for human consumption in Maiduguri (Yaqub *et al.,* 2009*).* The result also agreed with45.6% in muscle samples reported in a study from Kenya (Muiruki *et al.*2001). However the result is lower than54.44% oxytetracycline drug residue obtained in edible tissues of cattle from Akure Olatoye and Ehinmowo (2010), 71.3 % from cattle meat in Ethiopia Bedada and zewde, (2012), The prevalence of 41.57 % oxytetracycline obtained from this study might be due to non-adherence to ante-mortem inspection in the lairage, could also be attributed to cattle being sold for slaughtered whilst under a therapeutic or prophylactic regimen of oxytetracycline or non observance of withdrawal period of the drug. High level of Oxytetracycline residue was observed in meat and liver which might be attributed to slaughtering of cattle that were treated to mask clinical signs, hence these organs are responsible for absorption, distribution and metabolism. This is in agreement with Ezenduka and

Chinyere (2012) who attributed it to the practice of selling off sick animals after treatment to mask symptoms.37(41.57 %) were above the set residue limit, at 25 (86.20 %), 10 (27.05 %) and 2 (8.70 %) for meat, liver and kidney respectively.

There was significant association (P<0.05) between oxytetracycline residue values and screened tissue samples. Further analysis showed that, the residues was higher than expected on oxytetracycline above limit in meat than expected, while it was higher than expected on oxytetracycline below limit in both liver and kidney than expected count.

### 5.1.5 Distribution of Oxtetracycline Drug Residues from Screened Tissue Samples Based on Sex and Meat.

Based on the values obtained from Elisa test kit for oxytetracycline residues, female animals had the highest residue above set limit of 29 (32.98 %) from the total screened samples. Values of 20 (68.96 %) and 9 (39.03 %) was obtained for females and males animal. Physiological factors in female animals most especially during heat periods, the hormonal interphase tends to affect basic physiological functions which may tend to also affect drug metabolism and excretion. Nina and Kenneth (2013), reported an evidence of physiological factors altering the functions of drug metabolising enzymes and transporters in the gastrointestinal stage in female. The result obtained could be attributed to muscle been organ of absorption. There was no evidence of significant association (P<0.05) in the oxytetracycline distribution in the meat of male and female animals.

### 5.1.6 Distribution of Oxtetracycline Drug Residues from Screened Tissue Samples Based on Sex and Liver.

From the values obtained Liver it showed that Females had 8(21.62 %) of oxytetracycline residue than males 2 (5.40 %) above maximum residue limit. The

result agreed with Yaquob, *et al., (*2009) that reported 5 (5 %) of oxytetracycline in liver from slaughtered cattle in Maiduguri.

There was no significant association (P<0.05) in the oxytetracycline distribution in Liver and animal gender. Although, the oxytetracycline presence was higher at residue below limit of female liver than expected, while higher at residue above limit of male liver than expected.

### 5.1.7 Distribution of Oxtetracycline Drug Residues from Screened Tissue Samples based on Sex and kidney.

The result obtained for Kidney from Elisa test kit for oxytetracycline residues showed 17 (73.91 %) for below limit and 1 (4.34 %) on above limit for female animals, while males had 4 (17.39 %) for below limit while 1(4.34 %) on below limit for males. There was no significant association (P>0.05) of oxytetracycline residue between kidney and sex of animal.

### 5.1.8 Distribution of Oxytetracycline Drug Residues from the Screened Tissue Samples based on Age and Meat.

Based on the values obtained from Elisa test kit for oxytetracycline residues for age above set residue limits, 16 (55.17 %) were obtained for ages between 6-10 years and had highest residue than other age groups. The higher values obtained at this age (6-10 years) in this study might be attributed to decline in physiological processes at advanced age of the animals which might have an effect on the level of deposits seen on meat tissue sampled, the result agreed with Mangoni and Jackson (2004). There was no significant association (P> 0.05) in oxytetracycline residue in Meat between maximum residues limit and age categories.

### 5.1.9 Distribution of Oxytetracycline Drug Residues from the Screened Tissue Samples based on Age and Liver.

Based on the values obtained from Elisa test kit for oxytetracycline residues for liver tissue sampled on age categories above set residue limits, 6 (16.21 %) were obtained

for ages between 6-10 years and had highest residue than other age groups. The high residues obtained could be attributed to Liver been organ for drug metabolism. There was no significant association (P> 0.05) in theoxytetracycline residue in Liver between maximum residues limit and age categories. This might be attributed to Liver been organ of metabolism and therefore it is at high risk to exposure to residue. Olatoye and Ehinmowo (2010) attributed his result on high level of oxtetracycline on liver to been the organs of metabolisms and therefore is at risk to organ having residues.

### 5.1.10 Distribution of Oxytetracycline Drug Residues from the Screened Tissue Samples based on Age and Kidney.

Significant association ($p<0.005$) was observed between the kidney tissue and oxytetracycycline residues via age category of the sampled animals. This might be attributed to kidney been organ of excretion and therefore is at greater risk to exposure to residue. Olatoye and Ehinmowo (2010) attributed their result on high level of oxtetracycline to kidney been organs of excretion and therefore has tendency to residue accumulation.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATION

## 6.0 Conclusions

1. The Study revealed the level of contamination of antimicrobial drug residues in meat, liver and kidney samples from Bunaji cattle slaughtered in Minna abattoir that were screened, to be 29 (51.79 %), 37 (66.17 %) and 23 (41.07 %) for meat, liver and kidney respectively. The overall antimicrobial residue values found in all the samples translated to 89 (52.97 %).

2. The overall values obtained from tissues sampled using Elisa test kit was 37 (41.57 %), distributed at 25 (86.20 %), 10 (27.05 %) and 2 (8.70 %) for meat, liver and kidney respectively above maximum residue set limit.

3. The result also revealed the occurrence of residue to varied by tissue type, the highest occurrence rate of 86.2 % and 27.05 % Oxytetracycline residues above maximum limit where obtained for meat and liver samples respectively. This clearly indicated that consumers of Bunaji cattle tissues slaughtered in Mimma abattoir are predisposed to various health hazards through consumption of these products.

4. The result indicated that age and sex of animals also affect the presence of antimicrobial drug residue in meat of cattle with females becoming more exposed than males.

## 6.1 Recommendations

1. The results of antimicrobial residues obtained in the edible tissues (meat, liver and kidney) of Bunaji cattle slaughtered in the study area, signifies attendant health risk factors to the Meat consumers as a result of drug residue effects. There is need for strict ante-mortem inspection of animals at the lairage such that sick animals are not allowed to be slaughtered.

2. Niger state Government through her relevant agencies should create awareness to all stake holders on the effect of veterinary drug use in food animals and adherence to withdrawal periods at lairage before slaughter.

3. Further studies should be carried out involving other livestock species in major abattoirs in Niger state, such that the possibility of more residue detections could be established.

## REFERENCES


Abbasi, M.M.; Nemati, M.; Babaei, H.; Ansarin, M.& Nourdadgar, A. O. S. (2012).Solid-phase extraction and simultaneous determination of tetracycline residues in edible cattle tissues using an HPLC-FL method. Iran. *Journal of Pharmacological Research. 11, 781*

Akinwumi, A. O., Adunsi, A. A., Ojola, A. B. & Rafiu, T. A., (2012).Effect of Antibiotic Residue on the physical qualities of Beef in Oyo State Nigeria.*4th International Conference .on Agriculture and Animal Science 47-15*

Alhendi, *A B.,* Homeida A.M & Gaili E. (2000). Drug residue in broiler chicken fed with antibiotic in ration; *veterinarskiarhiv 70(4) 199-205*

Alphonsus, C., Akpa, G. N., Barje, P. P., Finangwai, H. I., & Adamu, B. D. (2012). Comparative evaluation of linear udder and body conformation traits of bunaji and friesian x bunaji cows. *World Journal of Life Science and Medical Research.*2 (4), 134 - 140. .

Bedada, A.H. & Zewde, B.M., (2012), 'Tetracycline residue levels in slaughtered beef cattle from three slaughterhouses in central Ethiopia.' *Journal of Global Veterinaria* (6), 546–554.

Blench, R. M., De Jode, A., Gherzi, E., & Di Domenico, C. (1998). Keteku and Ndama crossbred cattle in Nigeria: History, distribution and productivity. In C. Seignobos and E. Thys Paris (Ed.) *Des Taurins au Cameroun et Nigeria*.: ORSTOM/IEMVT, Maisons-Alfort, pp. 293-310.

Bogialli, S.; & Di Corcia, A. (2009).Recent applications of liquid chromatography–mass spectrometry to residue analysis of antimicrobials in food of animal origin. *Analatical/Bioanalatical Chemstry, 395, 947–966*

Botsoglou, N.A. & Fletouris, D.J. (2001): Drug Residues in Foods, Pharmacology, *Food Safety, and Analysis. Marcel Dekker, New York.* XV 1194 p i11

Chopra, I. & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews,* 65**,** 232-260.

Clardy, J., Fischbach, M. & Currie, C. (2009).The natural history of antibiotics..*Current Biology,* 437-441.

Codex Alimentarius Commission.(1998). *Reports of the sessions of the Codex Committees on Residues of Veterinary Drugs in Foods*, 1987-1995. Washington,

Codex Alimentarius Commission (CAC), (2011). Maximum residue limits for veterinary drugs in food. *Washington DC.* (4) 3

Dina, O.A. & Arowolo, R.O.A.(1991). The crisis in antibiotics resistance. considerations on veterinary drug use and supply in Nigeria. *Revue d'levageet de medecine vetetrinaire des pays tropicaux,* 44: 29-31.

Dipeolu, M.A. & Kanamar, U. Y. (1996): The establishment of a method of analysis for tetracycline residues in meat. *Research Bulletin Faculty of Agriculture, Gifu University*, **61**, 141-146.

Donoghue, D.J. (2003). Antibiotic residues in poultry tissues and eggs: Human health concerns. *Yozrlt. Science.,82: 618-621.*

Dukku, U. (2018). How antibiotics in livestock farming places all at risk, Daily trust Newspaper, published *December 27, 3:26 am.*

European Commission (EC) (2010).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. *Official Journal of European Union*.15:1–72.

Ezenduka, E. V & Chiyere, U. (2012); Antimicrobial residues screening in pigs and goats slaughtered in Nsuksa municipal abattoir south eastern, Nigeria.*African Journal of Biotechnology*, *11(57) 12138-12140*,

Fagbamila, I., Kabir, J., Abdu, P., Omeiza, G., Ankeli, P., Ngulukun, S. & Umoh, J. (2010).Antimicrobial screening of commercial eggs and determination of tetracycline residue using two microbiological methods. *International Journal of Poultry Science* 9(10):959-962

FAO (1999): Residues of some veterinary drugs in animals and foods. *Food and nutrition paper* 41/3, 97-119.

FAO/WHO (2003).Codex Alimentarius Commission; Food and Agriculture Organization of the United Nations; World Health Organization. Codex Alimentarius: Food Hygiene, Basic Texts; *Food and Agriculture Organization of the United Nations: Rome, Italy*,

Federal Ministry of Agriculture and Rural Development, (2018), Heavy metal discovered in Abuja meat; *The punch news paper; December* 22, 2018.

Flynn, W.T. (2012).The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals .*Center for Veterinary Medicine* (HFV-1), Food and Drug Administration. U.S. Department of Health and Human Services.http://www.fda.gov/downloads/animalveterinary/guidancecompliance enforcement/guidanceforindustry/ucm216936.pdf

Food and Drug Administration (FDA) (2010). Questions and Answers on FDA's Draft Guidance on the Judicious Use of Medically Important Antimicrobial Drugs in Food- Producing Animals. *June 2010. Food and Drug Administration* (FDA).

Godfrey, S. B.& Norah, M. (2013). Global irrational antibiotics/antibacterial drugs use: current and future health and environmental consequences. Microbial pathogens and strategies of combating them*: science technology and education* 1645-1655.

Heeschen, W.H. & Bluthgen A. (1991): Veterinary drugs and pharmacologically active compounds. Monograph on residues and contaminants in milk and milk products. *IDF, Brussel: 13–69.*

Huber, W. G., Caslon, T. M. B. & Lepper M. H. (1969).Pennicillin and antibiotic residues in domestic animals at slaughter. *Food microbiology, 154; 1590-1595*

Huet, A.C.; Charlier, C.; Tittlemier, S.A.; Singh, G.; Benrejeb & S.; Delahaut, P. (2006). Simultaneous determination of (fluoro) quinolone antibiotics in kidney, marine products, eggs, and muscle by enzyme-linked immunosorbent assay (ELISA). *Journal of Agriculture and Food Chemistry, 54, 2822–2827.*

Joint FAO/WHO (2004**)** Technical Workshop on Residues of Veterinary Drugs washout ADI/MRL - *Bangkok, 24 – 26 August 2004*

Kabir, J., Umoh,V. I., Audu-Okooh, E., Umoh, I. U. & Kwaga.J.K.P. (2004)**.** Veterinary drug use in poultry farms, and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State, Nigeria .*Food Control.15:3-10*

Kirbis, A. (2007). Microbiological screening method for Detection of aminoglycosides, B- lactomas, macidider, tetracyclines and quinolones in meat samples.*Slow veterinary research 44(2): 11-48.*

Kozarova, L., Mate, D. & Cabadaj, R., (2001). Methods for the determination of sulphonamides residues in milk and animal n products In: *Proceeding of Lectures and Posters of the International Conference Hygiena Alimentorium XXIII.*

Kubkomawa, H. I. (2017). Indigenous breeds of Cattle, their Productivity, Economic and Cultural Values in Sub-sahara Africa*. International Journal of Research Studies in Agricultural Sciences,*3*, issue* 1,27-43

Larkin, C., Poppe, C., McNab, B., McEwen, B., Madhi A. & Odumeru J. (2004). Antibiotic resistance of Salmonella isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario. *Journal of Food Production* 67:48-455.

Mangoni A.A & Jackson S.H.D. (2004) Age related changed in pharmacokinetics and pharmaco dynamics basic principles and practical application. *British journal of clinical pharmacology* British pharmacological society. 57 (1), pp 6-24

Martinez, J..L.(2009). Environmental pollution by antibiotics and by antibiotic resistance determinants.*EnvironmentalPollution*,157(11

Mensah, S.E.P., Koudande, O.D., Sanders, P., Laurentie, M., Mensah, G.A. & Abiola, F.A. (2014). Antimicrobial residues in foods of animal origin in Africa: public health risks. *Scientific and Technical Review.*33(3): 1-26

Mubito, E.P.; Shahada, F.; Kimanya, M.E. & Buza, J.J. (2014) Sulfonamide residues in commercial layer chicken eggs in Dar-es-Salaam, Tanzania. *Annual Journal Research Commun. 2, 124–132.*

Muriuki, F. K.,Ogara, W. O.Njeruh, & Mitema, E .S.,( 2001). Tetracycline residue levels in cattle meat from Nairobi slaughterhouse in Kenya. *Journal of veterinary sciences*, 2, 97 – 101

NAMDA (2019), Niger State Agricultural and Mechanization Development Agency *Annual report* pp iv

Nina I & Kenneth E.T (2013) Drug metabolism and Disposition. *American society of pharmacology and experimental Therapeutics. Volume* 41 (2) pp 256-262

Nisha, A.R. (2008). Antibiotic residues- a global health hazard. *Veterinary World*,1(12):375-377.

Normanno, G.; La Salandra, G.; Dambrosio, A.; Quaglia, N.C.; Corrente, M.; Parisi, A.; Santagada, G.; Firinu, A.; Crisetti, E. & Celano, G.V. (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic Staphylococcus aureus isolated from meat and dairy products. *International Journal of Food Microbiology*, 115,290–296.

Office International des Epizooties, (2011).Terrestrial Animal Health. Code. *Scientific and Technical Review of the Office International des Epizooties (OIE).*31(2): 585-590.

Olaleye, A. (2018), Heavy metal discovered in Meat in Abuja, Federal Ministry of Agriculture and Rural Development. The punch Newspaper; Friday, December, 22th. 2018

Olatoye, O I.& Ogundipe, G. A. T. ( 2009), Preliminary screening of beef consumed in Ibadan, Nigeria for antimicrobial residue. *Africa journal of biomedical research.* 12, no.3,pp 245-247

Olatoye, I. O & A.A. Ehinmowo. (2010). Oxytetracycline residue in edible tissues of cattle slaughtered in Akure, Nigeria. *International Journal of food safety,*11:62-66.

Olufemi, O.I. & Agboola, E.A., 2009, 'Oxytetracycline residues in edible tissues cattle slaughtered in Akure, Nigeria', *Internet Journal of Food Safety* 11, 62–66.

Omeiza, G. K., Ajayi, I. E & Ode, O. J., (2012). Assessment of antimicrobial drugs residue in beef in Abuja Federal Capital Territory, Nigeria, *Veterinaria italiana ,48(3) 283-289.*

Omotoso, A. B., & Omojola, A. (2015).Fluoroquinolone residues in raw meat from open markets in Ibadan, Southwest, Nigeria. *International Journal of Health and Animal Science Food Safety, **2***(1): 32-40.

Pyun, C.W.,Abd El-Aty, A.M.S; Hashim, M.M.M.; Shim, J.H.; Lee, S.K.; Choi, K.D.; Park, K.H.; Shin, H.C.;& Lee, C. (2008). Monitoring of streptomycin and dihydrostreptomycin residual levels in porcine meat press juice and muscle via solid-phase fluorescence immunoassay and confirmatory analysis by liquid

chromatography after post-column derivatization. *Biomedical Chromatography*., 22, 254–259.

Riviere, J. E, & Sundlof, S. F. (2001).Chemical residue in tissues of food animals. *International Veterinary Pharmacology and Therapeutics*. Ed. Adams HR: 8th Edition; *Blackwell Publishing Professional Lowa.*1166-1174

Roeder, A. M. & Roeder, M. (2000): Antibiotics in foods of animal origin. *Encyclopedia of Food Science and Technology*.*Volume 1, 2nd Edition, 54-53 .John Wiley & Sons Inc., New York.*

Schnappinger, D. & Hillen, W. (1996).Tetracyclines: antibiotic action, uptake, and resistance mechanisms. *Archives of microbiology,* 165**,** 359-369.

Shehu, (2018). How antibiotics in livestock farming places all at risk, *Daily trust Newspaper, published December 27, 3:26 am.*

Singer, R. S., Reid-Smith, R. &Sischo, W. M. (2006). Stakeholder Position Paper: Epidemiological perspectives on antibiotic use in animals. *Preventive veterinary medicine,* 73**,** 153-161.

SPSS.(2010). Statistical Package for the Social sciences, *Version 16.0 IBM USA.*

Umar, A. S. S. (2007). Financial analysis of small scale beef fattening enterprise, inbama local government area of Borno state. M.Sc. Thesis, Department of Agricultural Economics and Rural Sociology, Ahmadu Bello University, Zaria, Nigeria.

Umar, A. S., Alamu, J. F., & Adeniyi, O. B. (2008). Economic Analysis of Small-scale Cow Fattening Enterprise in Bama Local Government of Borno State, Nigeria PAT, 4(1), 1-10

Wasengula, A. (2018), A speech delivered at the Nigerian veterinary medical association conference Nigeria. *Punch Newspaper, 9th November, 2018*

World Health Organization (WHO) (2011).Tackling antibiotic resistance from a food safety perspective in Europe..*Retrieved from* http*://www.euro.who.int/_data/assets/pdf_file/0005/136454/e94889*

Yaqub, A.G., Hamidu, U., Hassan, I.N., Franca, A. & Yemisi, A. (2009).Oxytetracycline and procaine penicillin residues in tissues of slaughtered cattle in Maiduguri, Borno State, Nigeria. *Terrestrial and Aquatic Environment Toxicology, Global science book* 3(2):68-70

Yusuf, M. S., Kabir, J. & Babashani, M. (2017).Occurrence of Tetracycline Residue in Milk from Diary Farms in Kano state Nigeria. *Proceedings of 54th Annual Congress of the Nigerian Veterinary Medical Association (Kano, 2017)*pp 135-164

Zakeri, B. & Wright, G.D.(2008). Chemical biology of tetracycline antibiotics This paper is one of a selection of papers published in this Special Issue, entitled *CSBMCB—Systems and Chemical Biology*, and has undergone the Journal's usual peer review process. *Biochem.Cell Biology*. 86, 124–136.

Zeenatudeen, M. (2015). Dissertation submitted to the postgraduate school Department of Veterinary public Health and preventive medicine, Ahmadu Bello University, Zaria.

# APPENDIX

## ANALYSIS OF MICROBIAL SCEENING IN ANIMALS

```
> rm(list=ls()) #cleaning up
> ###Adama Screening###
> #load R to word package
> #install.packages("R2wd")
> library(R2wd)
> setwd("C:/Users/Adeyemi/Desktop/adama_sceen")
>
> # Table 4.1: antimicrobial drug
> AA<- as.table(cbind(c(29, 37, 23), c(27,19,33)))
> dimnames(AA) <- list(Tissue=c("Meat","Liver", "kidney"),
+                      OTC=c("Positive","Negative"))
> AA
        OTC
Tissue   Positive Negative
  Meat         29       27
  Liver        37       19
  kidney       23       33
> (Xsq <- chisq.test(AA))  # Prints test summary

        Pearson's Chi-squared test

data:  AA
X-squared = 7.0727, df = 2, p-value = 0.02912

> Xsq$observed   # observed counts (same as M)
        OTC
Tissue   Positive Negative
  Meat         29       27
  Liver        37       19
  kidney       23       33
> Xsq$expected   # expected counts under the null
        OTC
Tissue   Positive Negative
  Meat   29.66667 26.33333
  Liver  29.66667 26.33333
  kidney 29.66667 26.33333
> Xsq$residuals  # Pearson residuals
        OTC
Tissue    Positive  Negative
  Meat   -0.122398  0.129914
  Liver   1.346378 -1.429054
  kidney -1.223980  1.299140
> Xsq$stdres     # standardized residuals
        OTC
Tissue     Positive   Negative
  Meat   -0.2186055  0.2186055
  Liver   2.4046605 -2.4046605
  kidney -2.1860550  2.1860550
> fisher.test(AA)

        Fisher's Exact Test for Count Data

data:  AA
p-value = 0.03205
alternative hypothesis: two.sided
```

```
> ### TEST of Proportion of positivity distribution of microbial
presence
> y_obs <- c(29,37,23)
> n_sample <-c(56,56,56)
> #prop.test(c(y1,y2),c(n1,n2))
> prop.test(y_obs,n_sample) # without continuity correction

        3-sample test for equality of proportions without continuity
correction

data:  y_obs out of n_sample
X-squared = 7.0727, df = 2, p-value = 0.02912
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3
0.5178571 0.6607143 0.4107143

> prop.test(y_obs,n_sample, correct= FALSE) # Turn off the
continuity correction

        3-sample test for equality of proportions without continuity
correction

data:  y_obs out of n_sample
X-squared = 7.0727, df = 2, p-value = 0.02912
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3
0.5178571 0.6607143 0.4107143
#*********************************************************************
> ## From Agresti(2007) p.39
> setwd("C:/Users/Adeyemi/Desktop/adama_sceen")
> #Overall OTC Page 28
> A<- as.table(cbind(c(1, 27, 21), c(28,10,2)))
> dimnames(A) <- list(Tissue=c("Meat","Liver", "kidney"),
+                     OTC=c("Below Limit","Above Limit"))
> A
         OTC
Tissue    Below Limit Above Limit
  Meat              1          28
  Liver            27          10
  kidney           21           2
> (Xsq <- chisq.test(A))  # Prints test summary

        Pearson's Chi-squared test

data:  A
X-squared = 48.227, df = 2, p-value = 3.369e-11

> Xsq$observed   # observed counts (same as M)
         OTC
Tissue    Below Limit Above Limit
  Meat              1          28
  Liver            27          10
  kidney           21           2
> Xsq$expected   # expected counts under the null
        g OTC
Tissue    Below Limit Above Limit
  Meat       15.96629    13.03371
  Liver      20.37079    16.62921
  kidney     12.66292    10.33708
> Xsq$residuals  # Pearson residuals
```

```
          OTC
Tissue    Below Limit  Above Limit
  Meat      -3.745521    4.145532
  Liver      1.468785   -1.625647
  kidney     2.342863   -2.593074
> Xsq$stdres      # standardized residuals
          OTC
 Tissue    Below Limit Above Limit
   Meat      -6.804505    6.804505
   Liver      2.866266   -2.866266
   kidney     4.058214   -4.058214
> fisher.test(A)

        Fisher's Exact Test for Count Data

data:  A
p-value = 7.249e-13
alternative hypothesis: two.sided

>
> #### Antimocobial screening
> # Tissue X sex
> B<- as.table(cbind(c(9, 8, 9), c(20,29,14)))
> dimnames(B) <- list(Tissue=c("Meat","Liver", "kidney"),
+                     gender=c("Male","Female"))
> B
         gender
Tissue    Male Female
  Meat       9     20
  Liver      8     29
  kidney     9     14
> (Xsq <- chisq.test(B))  # Prints test summary

        Pearson's Chi-squared test

data:  B
X-squared = 2.1716, df = 2, p-value = 0.3376

> Xsq$observed    # observed counts (same as M)
         gender
Tissue    Male Female
  Meat       9     20
  Liver      8     29
  kidney     9     14
> Xsq$expected    # expected counts under the null
         gender
Tissue         Male   Female
  Meat     8.471910 20.52809
  Liver   10.808989 26.19101
  kidney   6.719101 16.28090
> Xsq$residuals  # Pearson residuals
         gender
Tissue           Male      Female
  Meat     0.1814334 -0.1165557
  Liver   -0.8543926  0.5488755
  kidney   0.8799347 -0.5652842
> Xsq$stdres      # standardized residuals
         gender
Tissue           Male      Female
  Meat     0.2626403 -0.2626403
  Liver   -1.3285427  1.3285427
  kidney   1.2145019 -1.2145019
```

```
> fisher.test(A)

        Fisher's Exact Test for Count Data

data:  A
p-value = 7.249e-13
alternative hypothesis: two.sided

>
> # Screening Tissue X AGE
> C<- as.table(cbind(c(7, 9, 5), c(17,24,15), c(5,4,3)))
> dimnames(C) <- list(Tissue=c("Meat","Liver", "kidney"),
+                     age=c("0-5","6-10", "Above 10"))
> C
        age
Tissue   0-5 6-10 Above 10
  Meat     7   17        5
  Liver    9   24        4
  kidney   5   15        3
> (Xsq <- chisq.test(C))  # Prints test summary

        Pearson's Chi-squared test

data:  C
X-squared = 0.67532, df = 4, p-value = 0.9543

Warning message:
In chisq.test(C) : Chi-squared approximation may be incorrect
> Xsq$observed   # observed counts (same as M)
        age
Tissue   0-5 6-10 Above 10
  Meat     7   17        5
  Liver    9   24        4
  kidney   5   15        3
> Xsq$expected   # expected counts under the null
        age
Tissue        0-5     6-10 Above 10
  Meat    6.842697 18.24719 3.910112
  Liver   8.730337 23.28090 4.988764
  kidney 5.426966 14.47191 3.101124
> Xsq$residuals  # Pearson residuals
        age
Tissue           0-5        6-10     Above 10
  Meat    0.06013459 -0.29196780  0.55117195
  Liver   0.09126531  0.14903563 -0.44268640
  kidney -0.18327998  0.13881773 -0.05742394
> Xsq$stdres     # standardized residuals
        age
Tissue           0-5        6-10     Above 10
  Meat    0.08378848 -0.58397247  0.72169904
  Liver   0.13659651  0.32020010 -0.62264307
  kidney -0.24348859  0.26473173 -0.07169117
> fisher.test(C)

        Fisher's Exact Test for Count Data

data:  C
p-value = 0.9688
alternative hypothesis: two.sided

>
>
```

```
> ### OTC Meat
> MAT<- as.table(rbind(c(2, 2), c(5,20)))
> dimnames(MAT) <- list(OTC=c("Below","Above"),
+                       gender=c("Male","Female"))
>
> (Xsq <- chisq.test(MAT))  # Prints test summary

        Pearson's Chi-squared test with Yates' continuity correction

data:  MAT
X-squared = 0.45242, df = 1, p-value = 0.5012

Warning message:
In chisq.test(MAT) : Chi-squared approximation may be incorrect
> Xsq$observed   # observed counts (same as M)
       gender
MRL     Male Female
  Below    2      2
  Above    5     20
> Xsq$expected   # expected counts under the null
       gender
MRL          Male    Female
  Below 0.9655172  3.034483
  Above 6.0344828 18.965517
> Xsq$residuals  # Pearson residuals
       gender
MRL           Male     Female
  Below  1.0527936 -0.5938557
  Above -0.4211174  0.2375423
> Xsq$stdres     # standardized residuals
       gender
MRL          Male    Female
  Below  1.301847 -1.301847
  Above -1.301847  1.301847
> MAT
       gender
MRL     Male Female
  Below    2      2
  Above    5     20
> fisher.test(MAT)

        Fisher's Exact Test for Count Data

data:  MAT
p-value = 0.2381
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.2226706 64.6533212
sample estimates:
odds ratio
  3.766528

>
> ### MRL LIVER
> LIV<- as.table(rbind(c(5, 22), c(2,6)))
> dimnames(LIV) <- list(OTC=c("Below","Above"),
+                       gender=c("Male","Female"))
> LIV
       gender
MRL     Male Female
  Below    5     22
  Above    2      6
```

```
> (Xsq <- chisq.test(LIV))  # Prints test summary

        Pearson's Chi-squared test with Yates' continuity correction

data:  LIV
X-squared = 2.1867e-30, df = 1, p-value = 1

Warning message:
In chisq.test(LIV) : Chi-squared approximation may be incorrect
> Xsq$observed   # observed counts (same as M)
        gender
MRL      Male Female
  Below     5     22
  Above     2      6
> Xsq$expected   # expected counts under the null
        gender
MRL      Male Female
  Below  5.4    21.6
  Above  1.6     6.4
> Xsq$residuals  # Pearson residuals
        gender
MRL            Male      Female
  Below -0.1721326  0.0860663
  Above  0.3162278 -0.1581139
> Xsq$stdres     # standardized residuals
        gender
MRL           Male      Female
  Below -0.4025382  0.4025382
  Above  0.4025382 -0.4025382
>
> fisher.test(LIV)

        Fisher's Exact Test for Count Data

data:  LIV
p-value = 0.6478
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.0822901 8.9624697
sample estimates:
odds ratio
  0.689805

>
> ### MRL KIDNEY
> KD<- as.table(rbind(c(4, 17), c(1,1)))
> dimnames(KD) <- list(OTC=c("Below","Above"),
+                      gender=c("Male","Female"))
> KD
        gender
OTC      Male Female
  Below     4     17
  Above     1      1
> (Xsq <- chisq.test(KD))  # Prints test summary

        Pearson's Chi-squared test with Yates' continuity correction

data:  KD
X-squared = 0.01369, df = 1, p-value = 0.9069

Warning message:
In chisq.test(KD) : Chi-squared approximation may be incorrect
```

```
> Xsq$observed   # observed counts (same as M)
       gender
MRL     Male Female
  Below    4     17
  Above    1      1
> Xsq$expected   # expected counts under the null
       gender
MRL          Male    Female
  Below 4.5652174 16.434783
  Above 0.4347826  1.565217
> Xsq$residuals  # Pearson residuals
       gender
MRL           Male      Female
  Below -0.2645360  0.1394227
  Above  0.8571946 -0.4517812
> Xsq$stdres     # standardized residuals
       gender
OTC          Male    Female
   Below -1.014055  1.014055
   Above  1.014055 -1.014055
>
> fisher.test(KD)

        Fisher's Exact Test for Count Data

data:  KD
p-value = 0.3953
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.002845001 23.051797489
sample estimates:
odds ratio
  0.255655

>
> ### Tests for Comparing Proportions from
> ##the Tissues above limit in respective gender
> y_obs <- c(25,10,2)
> n_sample <-c(29,37,23)
> #prop.test(c(y1,y2),c(n1,n2))
> prop.test(y_obs,n_sample) # without continuity correction

        3-sample test for equality of proportions without continuity
correction

data:  y_obs out of n_sample
X-squared = 37.243, df = 2, p-value = 8.18e-09
alternative hypothesis: two.sided
sample estimates:
    prop 1     prop 2     prop 3
0.86206897 0.27027027 0.08695652

> prop.test(y_obs,n_sample, correct= FALSE) # Turn off the
continuity correction

        3-sample test for equality of proportions without continuity
correction

data:  y_obs out of n_sample
X-squared = 37.243, df = 2, p-value = 8.18e-09
alternative hypothesis: two.sided
sample estimates:
```

```
    prop 1     prop 2     prop 3
0.86206897 0.27027027 0.08695652

>
> ### Tests for Comparing Proportions from
> ##the Tissues above limit in respective AGE
> y_obs <- c(25,10,2)
> n_sample <-c(29,37,23)
> #prop.test(c(y1,y2),c(n1,n2))
> prop.test(y_obs,n_sample) # without continuity correction

        3-sample test for equality of proportions without continuity
correction

data:  y_obs out of n_sample
X-squared = 37.243, df = 2, p-value = 8.18e-09
alternative hypothesis: two.sided
sample estimates:
    prop 1     prop 2     prop 3
0.86206897 0.27027027 0.08695652




> #**** AGE OTC*********************************
> ### OTC Meat
> MATA<- as.table(rbind(c(1, 2, 1), c(6,16,3)))
> dimnames(MATA) <- list(OTC=c("Below","Above"),
+                        age=c("0-5","6-10"," Above 10"))
>
> (Xsq <- chisq.test(MATA))  # Prints test summary

        Pearson's Chi-squared test

data:  MATA
X-squared = 0.53282, df = 2, p-value = 0.7661

Warning message:
In chisq.test(MATA) : Chi-squared approximation may be incorrect
> Xsq$observed   # observed counts (same as M)
       age
OTC     0-5 6-10  Above 10
  Below   1    2         1
  Above   6   16         3
> Xsq$expected   # expected counts under the null
       age
OTC           0-5      6-10  Above 10
  Below 0.9655172  2.482759 0.5517241
  Above 6.0344828 15.517241 3.4482759
> Xsq$residuals  # Pearson residuals
       age
OTC              0-5        6-10     Above 10
  Below   0.03509312 -0.30638168   0.60350985
  Above -0.01403725  0.12255267 -0.24140394
> Xsq$stdres     # standardized residuals
       age
OTC              0-5        6-10     Above 10
  Below   0.04339489 -0.53578980   0.70007142
  Above  -0.04339489  0.53578980 -0.70007142
>
```

```
> fisher.test(MATA)

        Fisher's Exact Test for Count Data

data:  MATA
p-value = 0.7595
alternative hypothesis: two.sided

>
> ### OTC LIVER AGE
> LIVA<- as.table(rbind(c(6, 16,5), c(3,6,1)))
> dimnames(LIVA) <- list(OTC2=c("Below","Above"),
+                        age=c("0-5","6-10"," Above 10"))
> LIVA
       age
OTC2    0-5 6-10  Above 10
  Below   6   16          5
  Above   3    6          1
> (Xsq <- chisq.t st(LIVA) )  # Prints test summary

        Pearson's Chi-squared test

data:  LIVA
X-squared = 0.5087, df = 2, p-value = 0.7754

Warning message:
In chisq.test(LIVA) : Chi-squared approximation may be incorrect
> Xsq$observed   # observed counts (same as M)
       age
OTC2    0-5 6-10  Above 10
  Below   6   16          5
  Above   3    6          1
> Xsq$expected   # expected counts under the null
       age
OTC2          0-5      6-10  Above 10
  Below 6.567568 16.054054  4.378378
  Above 2.432432  5.945946  1.621622
> Xsq$residuals  # Pearson residuals
       age
OTC2             0-5         6-10     Above 10
  Below -0.22147020 -0.01349074   0.29707730
  Above  0.36391267  0.02216755 -0.48814781
> Xsq$stdres     # standardized residuals
       age
OTC2             0-5         6-10     Above 10
  Below -0.48970891 -0.04075604  0.62429570
  Above  0.48970891  0.04075604 -0.62429570
>
> (Fish<-fisher.test(LIVA))

        Fisher's Exact Test for Count Data

data:  LIVA
p-value = 0.8843
alternative hypothesis: two.sided

>
> ### OTC KIDNEY
> KDA<- as.table(rbind(c(3, 15, 3), c(2,0,0)))
> dimnames(KDA) <- list(OTC2=c("Below","Above"),
+                        age=c("0-5","6-10"," Above 10"))
> KDA
```

```
       age
OTC2    0-5 6-10  Above 10
  Below   3   15         3
  Above   2    0         0
> (Xsq <- chisq.test(KDA))  # Prints test summary

        Pearson's Chi-squared test

data:  KDA
X-squared = 7.8857, df = 2, p-value = 0.01939

Warning message:
In chisq.test(KDA) : Chi-squared approximation may be incorrect
> Xsq$observed   # observed counts (same as M)
       age
OTC2    0-5 6-10  Above 10
  Below   3   15         3
  Above   2    0         0
> Xsq$expected   # expected counts under the null
       age
OTC2          0-5      6-10  Above 10
  Below 4.5652174 13.695652 2.7391304
  Above 0.4347826  1.304348 0.2608696
> Xsq$residuals  # Pearson residuals
       age
OTC2           0-5       6-10    Above 10
  Below -0.7325612  0.3524537   0.1576221
  Above  2.3737697 -1.1420805  -0.5107539
> Xsq$stdres     # standardized residuals
       age
OTC2           0-5       6-10    Above 10
  Below -2.8081514  2.0266087  0.5732115
  Above  2.8081514 -2.0266087 -0.5732115
>
> fisher.test(KDA)

        Fisher's Exact Test for Count Data

data:  KDA
p-value = 0.05138
alternative hypothesis: two.sided
```