

# Multiple antibiotic residues in meat from slaughtered cattle in Nigeria

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## Abstract

Drug residues in animal derived products are of public health and economic importance. A microbial inhibition test using *Bacillus stearothermophilus* and *Staphylococcus aureus* ATCC25923 was employed to screen 50 slaughtered cattle at Sokoto metropolitan abattoir for antibiotic residues in meat. 44% of the slaughtered cattle were positive. Penicillin (14%) was the drug with the highest rate of occurrence followed by tetracycline (8%) and streptomycin (4%) in samples positive on a single plate. Multiple antibiotic residues were found from 9(18%) slaughtered cattle. There is the need to prevent veterinary drug residues in food animals, and animal products containing residues from getting into the Nigerian food chain. Observance of withdrawal period, effective surveillance, monitoring and control on the use of veterinary drugs are recommended among others.

## INTRODUCTION

The primary purpose of veterinary drugs, biologics and pesticide chemicals is to safeguard the health and welfare of animals<sup>4</sup>. Antibiotics used for these purposes can occur as residues for some time in these animals before they are excreted. A chemical residue is either the parent compound or its metabolites that may deposit accumulate or otherwise be stored within the cells, tissues, organs or edible products of animals following its use to prevent, control or treat animal disease or to enhance production<sup>14</sup>. Drugs are the most frequently detected chemical residues, the overwhelming majority of which are antimicrobials<sup>15</sup> and, are commonly used drugs in veterinary practice in Nigeria<sup>1</sup>. The concerns over drug residues are public health and economically related<sup>15</sup>. Drug and pesticide residue concerns are among the reasons adduced for Africa's denial into European and American livestock markets<sup>1</sup>. The palatability, aroma and quality of meat could be affected by drug residues. Residues of food animal drugs threaten human health by been acutely or cumulatively allergenic, organotoxic, mutagenic, teratogenic or carcinogenic<sup>1</sup>. Violative residues of penicillin are the most frequently cited causes of allergic reaction in persons that consume animal products containing residues. Many other drugs including tetracyclines, sulphonamides and aminoglycosides can also cause allergic reaction<sup>15</sup>. Aminoglycosides (e.g. streptomycin) can cause varying degree of nephrotoxicity and ototoxicity, a potential to cause muscular paralysis, a tubacurare like activity on respiratory

muscles and death. Antimicrobial residues were detected in slaughtered cattle<sup>6,9,15</sup> goat and pig<sup>5</sup> and in chicken<sup>7</sup> in some different parts of Nigeria. Drug resistance appears to be the most important hazard of drug residues in the country<sup>6</sup>. The resistant bacteria could then cause disease that is difficult to treat in humans and may also transfer the resistant gene to some other human pathogens<sup>2,8</sup>. Hitherto, withdrawal period are not observed and there is no programme for monitoring/surveillance of veterinary drug residues as such, a number of slaughtered cattle may contain an undetermined magnitude of residue<sup>1,6,12</sup>. Sokoto state is a major source of beef in Nigeria. The risk to adverse effects of drug residue and drug resistance could be higher if the trend of misuse and abuse of drugs is left unchecked. Hence, the present study attempted to determine the presence of antibiotic residue in meat from slaughtered cattle in Sokoto, Nigeria.

## MATERIALS AND METHODS

### STUDY AREA

The study was conducted in Sokoto, northwestern part of Nigeria. With a land area of 28, 232, 37 sq kilometer, Sokoto state is located between longitude 11 30" to 13 50" east and latitude 4 to 6 north. It is bordered in the north by the Niger republic, Zamfara state to the east and Kebbi state to the south and east. Agriculture is the major means of livelihood.

**SAMPLING**

Fifty slaughtered cattle were randomly selected over a period of four weeks, from 23<sup>rd</sup> June to 18<sup>th</sup> July 2008. Samples transported in an ice pack to the Veterinary Public Health Laboratory of Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto. A total of 100 samples (50 liver and 50 kidneys) were screened for antibiotic residues.

**PREPARATION OF INOCULUMS AND MEDIA**

Pure cultures of *Bacillus stearothermophilus* and *Staphylococcus aureus* ATCC25923 in nutrient and blood agar respectively were gotten from bacteriology research laboratory of NVRI Vom, Jos, Nigeria. Primary cultures were sub cultured into a nutrient or brain heart infusion broth for about 8 to 12 hours before inoculating into a nutrient agar under an aseptic condition. Nutrient agar was prepared according to producers' guidelines. The pH was adjusted using a pH meter (pHS-25 model) by adding drops of NaOH or HCL solutions. 6mLs of the adjusted media was then introduced into a universal (bijou) bottle and sterilized using an autoclave at 121C for 15min. The sterilized media poured onto a Petri dish and allowed to solidify. It was then incubated for 18-24hour at 37°C before inoculating the test bacteria.

**TECHNIQUE**

The European Four Plate Test as described by Oboegbulem and Fedelis (1996) was employed. A piece of meat (1x2.5mm) was applied to four plates of agar media, three of which were inoculated with *Bacillus stearothermophilus* at pH6 (Plate 1), pH7 (Plate 2), pH8 (Plate 3) and the fourth plate (Plate 4) at pH8 inoculated with *Staphylococcus aureus* ATCC25923 using a sterile swab. The plates incubated at optimal temperature for growth of the test bacteria. Positive sample was indicated by a complete inhibition of growth in an annular zone not less than 2mm around the piece of meat. Less than 2mm of inhibitory zone indicate negative result.

Test bacterium has known susceptibility to antibiotics. Presumptively, positive result at pH6 indicates the presence of tetracycline antibiotics, streptomycines at pH7 and penicillines at pH8. These antibiotics readily diffuse at the respective pH inhibiting bacterial growth around zone of meat. At significantly high concentration (MRL)\* zone of inhibition is  $\geq 2$ mm.

The European FPT is essentially a multiresidue technique for screening animal products. Despite limitations, the use of bacteria in detecting residues has since been useful in the

meat industry<sup>3,13</sup>. *Bacillus stearothermophilus* is the test bacterium use in Premi® Test, Charm Farm Test (CFT) and Brilliant Black Reductase Test Kit (BR Test) for screening antimicrobial residues in meat<sup>10</sup>.

\*Maximum residue limit (MRL) or violative residue is the residue above tolerance level.

**RESULTS**

Table 1 shows a total of 22(44%) slaughtered cattle positive for the presence of antibacterial substances. Out of these, the liver of 16(32%) slaughtered cattle was found positive while, both liver and kidneys of 6(12%) slaughtered cattle tested positive. However, base on the pH to which meat sample was found positive, presumptively, penicillin (14%) is the leading antibiotic followed by tetracycline (8%) and streptomycin (4%) in samples positive on a single plate. For samples with the multiple antibiotic residues: 8% contain tetracycline, streptomycin and penicillin, 4% tetracycline and streptomycin, 1% tetracycline and penicillin and, 4% penicillin and streptomycin.

**Figure 1**

Meat Sample	Tetracyclines		Streptomycin		Penicillins		Penicillins	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
MS1	--	--	--	--	--	--	6mm	3mm
MS2	--	--	--	--	6mm	--	4mm	4mm
MS3	--	--	--	--	5mm	--	4mm	--
MS5	8mm	--	--	--	5mm	--	--	--
MS6	--	--	--	--	--	--	--	--
MS8	6mm	--	--	--	--	--	--	--
MS9	6mm	--	--	--	--	--	--	--
MS15	--	--	--	--	--	--	2mm	2mm
MS16	--	--	4mm	--	--	--	--	--
MS17	3mm	--	--	--	--	--	--	--
MS18	5mm	--	--	--	--	--	--	--
MS19	4mm	4mm	--	--	--	--	--	--
MS21	5mm	--	4mm	--	--	--	--	--
MS22	4mm	--	6mm	--	5mm	--	--	--
MS23	6mm	--	--	--	4mm	--	--	--
MS25	6mm	--	4mm	--	3mm	--	--	--
MS26	--	--	--	--	5mm	--	--	--
MS27	--	--	--	--	3mm	--	--	--
MS28	--	--	4mm	--	3mm	--	--	--
MS29	4mm	--	4mm	--	--	--	--	--
MS41	--	--	--	--	--	--	3mm	--
MS47	--	--	--	3mm	--	--	3mm	3mm

\*Note that a sample consist of a liver and kidney from a slaughtered cattle

**DISCUSSION**

The issue of veterinary drug residues in animal derived food has become increasingly important in many developing countries<sup>4</sup>. The presence of antibiotic residues in liver and kidney is similar to other findings in Nigeria<sup>5, 6, 7, 9, 12</sup>. However, these researches detected single antibiotic. The result of this study indicates the presence of multiple antibiotic residues and that penicillin, tetracycline and

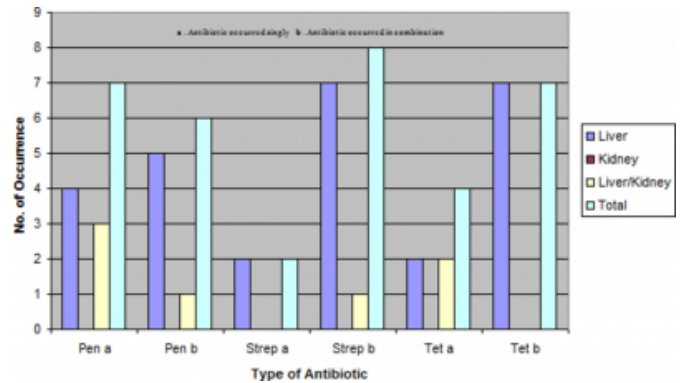
streptomycin in order frequency are commonly use or misuse drugs in cattle in Sokoto. Antibiotic residues were found more in the liver (44%) than in the kidney (12%). This could suggest that most samples were taken at the time when drugs were been metabolised in the liver not yet at the stage of clearance by the kidney. Invariably, antibiotics are most often administered close to the time of slaughter. The result also shows higher rate (44%) of antimicrobials than previous works done by Oboegbulem and Fedelis (1996), Kabir et al, (2001) and, Dipeolu and Alonge (2002) with prevalence of 0.24%, 7.4%, and 16.7% in slaughtered cattle respectively. Therefore, in Sokoto, antibiotics are used but often more misused than areas were other works were conducted in Nigeria (southwest: Dipeolu, 2002; Dipeolu and Alonge, 2002; east: Oboegbulem and Fedelis, 1996 and north central: Kabir et al, 2001) with resultant higher antibiotic residues. There might be a general increase in the abuse of veterinary drugs in the country. Withdrawal period is likely being observed more in those areas than in Sokoto or that these drugs are more commonly used here singly or in combination. It is likely that some cattle treated in Sokoto excrete the pharmacologically active compound during transit to, with resultant less antibiotic residues in southwest, eastern and north central Nigeria.

The zone of inhibition found between two pieces of liver on one of the plates could be due to synergism (from synergistic drugs e.g penicillin and streptomycin) or probably a reflection of high concentration of single inhibitory substance. Synergism occurs when the pharmacologic effect of interaction between two drugs exceeds additive effect of concurrent administration<sup>11</sup>.

The presence of antibiotic residues in meat is a serious problem that is yet to be addressed in developing countries like Nigeria where safety of food regarding drug residue is highly questionable. The multi antibiotic residues detected in meat from slaughtered cattle intended for sale to the human populace in Sokoto could be attributed to irrational use of drugs in food animals consequent to lack of regulatory system for veterinary drug use and control as observed previously<sup>1</sup>. In addition stakeholders involved do not adhere to withdrawal period. Non adherence to withdrawal periods is the major cause of chemical residues<sup>14</sup>. To date there have been only few studies to determine the lowest dose of antibiotic required to perturb the normal human flora<sup>15</sup>. It is therefore safer to consider any amount of drug residue significant. This necessitates that all effort including awareness creation, observance of withdrawal period,

effective surveillance, monitoring and control on the use of veterinary drugs to prevent drug residues in animal derived products be employed.

**Figure 2**



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