

**Short Communication****Open Access****Prevalence of antibiotic residues in body organs of pigs slaughtered in Jos, Nigeria***¹Anueyiagu, K. N., ¹Nandi, S., ¹Uzochukwu, I. A., and ²Sule, S. O.¹Federal College of Animal Health and Production Technology, NVRI Vom, Nigeria²Federal College of Veterinary and Medical Laboratory Technology, NVRI Vom, Nigeria*Correspondence to: anueyiagunnamdi@yahoo.com; +2348035841582;ORCID: <https://orcid.org/0000-0002-2566-7982>**Abstract:**

Background: Drug residue is a serious issue for the food chain when antimicrobial substances are inappropriately used or when the withdrawal times before slaughtering the treated animals are not respected. The aim of this study is to determine the prevalence of antibiotic residues in pork slaughtered for human consumption in Jos South Local Government Area (LGA), Plateau State, Nigeria.

Methodology: A total of 96 samples which included muscles (n=32), liver (n=32) and kidneys (n=32), were randomly collected from Gyel, Kuru, Du, and Vwang slaughter slabs in the LGA. The three-plate-test (TPT) technique was conducted where three batches of nutrient agar plates were aseptically prepared and adjusted to pH 6.0, 7.2 and 8.0. Each plate was seeded with isolated *Bacillus subtilis* and 5 holes were made on the plate with a sterile cork borer. The holes were inoculated with 80 µl of each organ extract, 10 µg/ml gentamicin (positive control) and distilled water (negative control), and incubated aerobically at 37°C for 18-24 hours. Positive antibiotic residue was shown by a clear zone of inhibition (annular diameter of ≥ 2 mm) around the holes.

Results: The result showed that 55.2% (53/96) of the organs tested positive for antibiotic residues while 44.8% (43/96) tested negative. The liver had the highest prevalence of antibiotic residues (68.8%, 22/32), followed by the kidneys (56.3%, 18/32) and the muscles (40.6%, 13/32). The difference in the prevalence of antibiotic residues between the organs was not statistically significant ($\chi^2=5.1391$, $p=0.0765$). Detection of tetracycline at pH 6.0 was highest in the organs while detection of sulphonamides was lowest at pH 7.2.

Conclusion: The determination of antibiotic residues in pork slaughtered for consumption in Jos South LGA of Plateau State, Nigeria posed potential public health risks. This situation indicates a wide spread uncontrolled use of antimicrobials in pig production, and requires urgent attention of government to enforce regulations guiding antibiotic use in veterinary settings in Nigeria.

Keywords: Antibiotic residues, antimicrobials, pigs, three plate test, Jos, Nigeria

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Prévalence des résidus d'antibiotiques dans les organes corporels des porcs abattus à Jos, Nigeria*¹Anueyiagu, K. N., ¹Nandi, S., ¹Uzochukwu, I. A., et ²Sule, S. O.¹Collège fédéral de santé animale et de technologie de production, NVRI Vom, Nigeria²Collège Fédéral de Technologie de Laboratoire Vétérinaire et Médical, NVRI Vom, Nigeria*Correspondance à: anueyiagunnamdi@yahoo.com; +2348035841582;ORCID: <https://orcid.org/0000-0002-2566-7982>**Résumé:**

Contexte: Les résidus de médicaments constituent un grave problème pour la chaîne alimentaire lorsque les substances antimicrobiennes sont utilisées de manière inappropriée ou lorsque les délais d'attente avant l'abattage des animaux traités ne sont pas respectés. Le but de cette étude est de déterminer la prévalence des résidus d'antibiotiques dans le porc abattu pour la consommation humaine dans la zone de gouvernement local (LGA) de Jos South, dans l'État du Plateau, au Nigeria.

Méthodologie: Un total de 96 échantillons comprenant des muscles (n=32), du foie (n=32) et des reins (n=32) ont été prélevés au hasard dans les dalles d'abattage de Gyel, Kuru, Du et Vwang dans la LGA. La technique du test à trois plaques (TPT) a été réalisée dans laquelle trois lots de plaques de gélose nutritive ont été préparés de manière aseptique et ajustés à pH 6,0, 7,2 et 8,0. Chaque plaque a étéensemencée avec *Bacillus subtilis* isolé

et 5 trous ont été faits sur la plaque avec un perce-bouchon stérile. Les trous ont été inoculés avec 80 µl de chaque extrait d'organe, 10 µg/ml de gentamicine (témoin positif) et de l'eau distillée (témoin négatif), et incubés en aérobiose à 37°C pendant 18 à 24 heures. Un résidu d'antibiotique positif a été mis en évidence par une zone claire d'inhibition (diamètre annulaire ≥ 2 mm) autour des trous.

Résultats: Le résultat a montré que 55,2% (53/96) des organes étaient positifs pour les résidus d'antibiotiques tandis que 44,8 % (43/96) étaient négatifs. Le foie présentait la plus forte prévalence de résidus d'antibiotiques (68,8%, 22/32), suivi des reins (56,3%, 18/32) et des muscles (40,6%, 13/32). La différence de prévalence des résidus d'antibiotiques entre les organes n'était pas statistiquement significative ($\chi^2=5,1391$, $p=0,0765$). La détection de la tétracycline à pH 6,0 était la plus élevée dans les organes tandis que la détection des sulfamides était la plus faible à pH 7,2.

Conclusion: La détermination des résidus d'antibiotiques dans la viande de porc abattue pour la consommation à Jos South LGA de l'État du Plateau, au Nigeria, pose des risques potentiels pour la santé publique. Cette situation indique une large utilisation incontrôlée des antimicrobiens dans la production porcine et nécessite une attention urgente du gouvernement pour faire appliquer les réglementations régissant l'utilisation des antibiotiques dans les établissements vétérinaires au Nigeria.

Mots-clés: Résidus d'antibiotiques, antimicrobiens, porcs, test à trois plaques, Jos, Nigeria

Introduction:

Antibiotics have been known to be incorporated in animal feeds to boost growth or to prevent and treat animal diseases. Antimicrobial residues are metabolites of antibiotics found in trace quantities found in edible portion of livestock products after antibiotics administration. When these residues are in excess of the acceptable limit they may lead to the development of antibiotic resistance in man or animals. The antimicrobial molecules are metabolized into other metabolites in blood, liver, and muscles and partially eliminated through kidneys or other routes (1). Consequently, these drugs and metabolites are accumulated in body cells and are known as drug residue. Drug residues may be detected in meat (2), eggs (3), milk or swine and poultry slurries (4). Drug residues are serious issues for the food chain when the antimicrobial substances are inappropriately used or when the withdrawal times before slaughtering the treated animals are not respected (5).

Antibiotic residues in meat and other foods are suspected to be responsible for drug allergy and other public health issues (6). Although there are scarce epidemiological data on the adverse effect of antibiotics residues, research studies indicate that animal food products may be a significant vehicle for the development and distribution of antimicrobial resistant bacteria (7,8). Many developed countries have banned the use of toxic antibiotics like chloramphenicol or avoparcin in animal food production, but they are still being used in developing countries (9).

Food safety legislation in Europe is known to be given a distinct attention with regards to checking for antibiotic residues in animal food products (10,11), and developing countries targeting to export products to Europe are mandated to keep strictly to specified guideline (12). Furthermore, consumers and retailers in non-industrialized nations are progressively mindful of the ill health conditions caused by drug residues in food of animal origin (13,14). Therefore, misuse of antimicro-

bial drugs in farms and residues in animal products becomes a major issue for the veterinary sector and public health services in Africa (2,15). The aim of this study is to determine the antibiotic residues in pork slaughtered for human consumption in Jos.

Materials and method:

Study area

Jos South is a Local Government Area (LGA) in Plateau State, Nigeria, located between latitude 9° 48'00N and Longitude 8° 52'00" E, with an area of 510 km² and a population of 306,716 at the 2006 census.

Sample collection

A total of 96 samples which included muscles (n=32), liver (n=32) and kidneys (n=32), were randomly collected from Gyel, Kuru, Du, and Vwang slaughter slabs located in Jos South LGA. Each slab was visited for 4 weeks and 32 samples were collected from each slab.

Sample preparation

Five grams of each organ were macerated using sterile pistol and mortar, and emulsified with 5mls of distilled water and then centrifuged at 5000rpm for 10 minutes. The supernatant was decanted into dark colored Eppendorf tubes and stored for analysis.

Isolation of *Bacillus subtilis* from soil sample

Five grams (5g) of the soil sample from waste dump site was weighed into 45 ml of Ringer's solution to form a stock mix and heated at 90°C for one hour to encourage the formation of spores of *Bacillus subtilis* if present as well as eliminate other unwanted microorganisms in the sample. One-tenth of the mixture was inoculated into already prepared 0.4% dextrose nutrient agar and incubated at 37°C for 24 hours. Identification of *Bacillus subtilis* was done according to method described by Cheesebrough (16).

Three-plate-test for detection of antibiotic residues in organ samples.

The 3-plate test (TPT) technique (17)

was used to detect residual antibiotics in the pork samples. Three batches of nutrient agar plates were aseptically prepared in Petri dishes and the pH adjusted to 6.0, 7.2 and 8.0. Each plate was seeded with *B. subtilis*. On each agar plate, 5 holes were made with a sterile cork borer and 80 µl of each organ extract (muscle, liver and kidney) was placed in the holes, while the remaining 2 holes were inoculated with 80 µl each of distill water and gentamicin (10µg/ml) serving as negative and positive control respectively. The plates were inoculated aerobically at 37°C for 18-24 hours, after which a positive antibiotic residue was shown by a clear zone of inhibition around the holes with an annular diameter of ≥ 2 mm (17).

Data analysis

The Chi-square test of independence, using R commander (18) software, was performed to test for the association between the prevalence of antibiotic residue and the type of organs sampled, and between the prevalence of antibiotic residues and slaughter slabs sampled.

Results:

Out of 96 organ samples, 53 (55.2%) tested positive while 43 (44.8%) tested negative for antibiotic residues (Table 1). The proportion of organs positive for antimicrobial

residues in descending order of frequency was liver (68.8%, 22/32), kidneys (56.3%, 18/32) and muscles (40.6%, 13/32). Test statistic showed that there was no association between the type of organs and the prevalence of antibiotic residues ($\chi^2=5.1391$, $p=0.0765$).

From Table 2, distribution of antibiotic residues by the slaughter slabs showed that of the 96 samples, 12 (22.6%) from Du slab were positive, 14 (26.4%) from Kuru slab were positive, 12 (22.6%) from Vwang slab were positive, and 14 (26.4%) from Gyel slab were positive. Test statistics showed that there was no association between antibiotic residues and the slaughter slabs ($\chi^2=3.699$, $p=0.7173$).

Of the 53 positive organ samples, 6 (11.3%) were detected at pH 6.0 (best detects oxytetracyclines and β -lactams), 4 (7.5%) were detected at pH 7.2 (best detects sulphoamides), 6 (11.3%) were detected at pH 8.0 (best detects aminoglycosides), 37 (69.8%) were detected at multiple pH i. e. 12 (22.6%) at pH 6.0 and 7.2; 7 (13.2%) at pH 6.0 and 8.0; 7 (13.2%) at pH 7.2 and 8.0, and 11 (20.8%) at the three levels of pH. There was no statistical association between antibiotic residues detection in the organs at a single pH level ($\chi^2=2$, $p=0.7358$) or at multiple pH levels ($\chi^2=1.5401$, $p=0.9568$).

Table 1: Prevalence of antimicrobial residues in organ samples of pig

Organs	No examined	No positive (%)	χ^2	<i>p</i>
Liver	32	22 (68.8)	5.1391	0.0765
Kidney	32	18 (56.3)		
Muscle	32	13 (40.6)		
Total	96	53 (55.2)		

χ^2 = Chi square

Table 2: Prevalence of antimicrobial residue in organ samples of pig according to location

Organs	No examined	No positive (%)				χ^2	<i>p</i>
		Du	Kuru	Vwang	Gyel		
Muscle	32	3 (7.4)	2 (4.9)	3 (7.4)	5 (12.3)	3.699	0.7173
Liver	32	6 (27.3)	5 (22.7)	6 (27.3)	5 (22.7)		
Kidney	32	3 (16.7)	7 (38.9)	3 (16.7)	4 (22.2)		
Total	96	12 (22.6)	14 (26.4)	12 (22.6)	14 (26.4)		

χ^2 = Chi square

Table 3: Organ distribution of antibiotic residues according to pH

Organ	No Positive	pH						
		Single			Multiple			
		6.0	7.2	8.0	6.0/7.2	6.0/8.0	7.2/8.0	6.0/7.2/8.0
Muscle	13	1	1	0	3	2	2	4
Liver	22	3	2	2	5	2	3	5
Kidney	18	3	1	3	4	3	2	2
Total	53	7	4	5	12	7	7	11
χ^2		2.0			1.5401			
<i>p</i>		0.7358			0.9568			

χ^2 = Chi square

Discussion:

In this present study, the results showed that out of 96 organ samples examined for antibiotic residues using the three-plate-test (TPT) method, 55.2% tested positive for the presence of antimicrobial residues. This high prevalence of antimicrobial residues in the meat samples may be a reflection of excessive antimicrobial prescription, overuse, and non-observance of the withdrawal period in veterinary practice that commonly occur in developing countries. However, it is believed that treatment of pigs with antimicrobial drugs is an important source of antibacterial residue in pigs (19). This problem in turn is due to unrestricted availability of antimicrobial drugs and the practice of self-medication by pig farmers. This unauthorized and unprofessional exposure of pigs to veterinary drugs without adherence to recommended dosages promotes accumulation of violative residues in tissues.

Despite the detection of antibiotic residues from all the organs, the liver stood out as the organ with the highest detection level of 68.75%, while the kidney level was 56.23% and muscle 40.62%. This could be due to the role the liver plays in humans and animals, which include protein synthesis, metabolism and detoxification processes. Most of the toxic substance and residues are metabolized in the liver. Our findings agree with that of Muriuki (20) in Nairobi, Kenya who found tetracycline residues in animal tissues with 24% in liver, 14% in kidney, and 7.6% in muscle samples. Additionally, in Nigeria, Olatoye and Ehinmowo (21) also reported that liver had the highest residue levels of 80% compared to the kidney and muscle with 55.0% and 28.3% respectively. However, the finding of our study contradicted the report of Hind (22) who reported higher antibiotic residues in the muscle (29.0%) than in the liver (28.0%) and kidney (21.4%). This contrasted situation was reported for pork meat in Nigeria, where 30.0% of sampled pork carcasses tested positive for antimicrobial residues (3), whereas in Vietnam, a study reported antimicrobial residues prevalence of 41.24% for pork meat samples analyzed (23).

Detection of antimicrobials at different pH in the same organ implies that different classes of antimicrobials are being administered to pigs at the same time. Sekyere (24) confirmed in their study, that farmers use different drugs at the same time to either treat or prevent diseases in their pigs. The farmers' attempts to control the problem of concurrent infections on their fields may be linked to the usage of various antibiotic classes, as was also reported by Riviere and Sundlof (25). *Bacillus subtilis* at pH 6.0 is said to best detect tetracycline (OTC) which is the most widely used

antibiotic in pig production in Nigeria (26). The detection of tetracycline was highest (n=7) in this study compared to sulphonamide (n=4) detected at pH 7.2 and aminoglycosides (n=5) detected at pH 8.0. The presence of antibiotic residues in some of the meat samples calls for concern, as this could pose serious public health risks to humans and animals, such as toxicity and resistance development.

The indiscriminate use of antimicrobial drugs as well as the failure to observe the withdrawal time, have been identified as the leading cause of violative residues in animal feeding items (25,27), which have led to availability of unsafe pork meats. The absence of enforcement of regulations of veterinary drug usage in Nigeria has resulted in drug abuse and misuse in food animals. The result of this study and those of others that detected residues in food animals in Nigeria should create awareness to the stakeholders (government and consumers) that should necessitate the enforcement of adherence to withdrawal periods, effective monitoring and surveillance of drug residues in meat animals and control of drug use in food animals, to prevent the occurrence of violative drug residues.

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Contribution of authors:

KNA was the principal investigator who conceived and designed the study. KNA and SN carried out sampling and bacteriological assays. KNA, SN, IAU and SOS wrote the manuscript and were responsible for the final editing of the manuscript.

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Conflict of interest:

Authors declare no conflict of interest

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