Assessment of antimicrobial drug residues in beef in Abuja, the Federal Capital Territory, Nigeria

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Summary
Drugs administered to food-producing animals close to the time of slaughter often result in prohibited antimicrobial residues in the animal tissues at slaughter. Evidence based on the Premi® test confirmed the occurrence of antimicrobial drug residues in 89.3% of kidney and urine samples from cattle slaughtered within Abuja town where the residents rely heavily on beef as a source of protein. The administration of antibiotics close to the time of slaughter by marketers/herd owners and transporters was found to be significantly (p<0.05) higher when compared with butchers and abattoir workers. The practice of administering antibiotics to animals close to the time of slaughter was believed to be profit-motivated. The research suggests that awareness campaigns amongst the stakeholders, the enactment of appropriate laws for the control of antibiotic use and the empowerment of veterinary public health practitioners in food regulatory agencies as some of the strategies which may positively reduce the risk of antimicrobial drug residues in food animals in Nigeria.

Keywords

La valutazione dei residui di farmaci antimicrobici in carni bovine ad Abuja, capitale del Territorio Federale, Nigeria

Riassunto
I farmaci somministrati ad animali da reddito in tempi ravvicinati alla macellazione spesso inducono residui vietati di antimicrobici nei tessuti degli animali al momento dell’abbattimento. Prove effettuate con il Premi® test hanno confermato la presenza di residui di farmaci antimicrobici nell’89,3% dei campioni di rene e di urina in bovini macellati nel territorio di Abuja dove i residenti utilizzano in maniera rilevante la carne bovina come fonte di proteine. La somministrazione di antibiotici in tempi ravvicinati alla macellazione è risultata significativamente più alta da parte dei venditori / proprietari dell’allevamento e trasportatori (p<0.05) rispetto a quella effettuata dai macellai e dai lavoratori del mattatoio. Si ritiene che la prassi di somministrare antibiotici prima della macellazione sia motivata da ragioni economiche. La ricerca suggerisce che le campagne di sensibilizzazione presso i portatori di interesse, la promulgazione di leggi adeguate per il controllo dell’uso di antibiotici, la responsabilizzazione dei veterinari di sanità pubblica che operano nelle agenzie di controllo degli alimenti e l’attivazione di specifiche strategie possono positivamente ridurre il rischio di residui di farmaci antimicrobici negli animali da reddito in Nigeria.

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**Introduction**

Beef is becoming the cheapest available source of animal protein in Nigeria. In most developing countries of the world with low per capita income and uncontrolled population growth, beef is increasingly becoming a vital component of a balanced diet (28). The contribution of the beef sector to both the well-being of consumers and the economy of the country is important. It is therefore imperative that the sector be well managed by experts who ensure that animals for human consumption are healthy. Skilled manpower is also needed to meet the challenges of relative protein deficiencies which are commonly experienced in most developing nations of the world, including Nigeria. Unfortunately, in this part of the world, serious attention is not given to the sector but rather, it is left in the hands of individuals and businessmen whose interest is to maximise profits even when consumers’ health may be compromised.

In Nigeria, there is no strict legislation that governs antibiotic residues in animal tissues (14). The National Agency for Food, Drug Administration and Control (NAFDAC) is charged with the responsibilities of regulating drug use but the emphasis is limited to human drug preparations only and there is no fully established veterinary department that regulates veterinary drug use based on human and animal health safety, as well as the effectiveness of antibacterial drugs (16, 29).

Proper abattoir practices are essential to ensure an uncontaminated meat supply. However, in Nigeria, abattoir systems are substandard. There is no monitoring by official veterinarians, as occurs in other jurisdictions, and there are no proper ante-mortem and post-mortem inspections by veterinary officers.

Detection of antibiotic residues in food poses no risk to consumers’ health if the level falls below the maximum residue limit stipulated by the world regulatory bodies (29). However, risks to human health may arise when antibiotic residues with no safe limits are found in foods of animal origin destined for human consumption. Mercer (18) and Santos and Ramos (21) reported that under no circumstance should chloramphenicol be found as residues in food. In addition, NAFDAC banned the use of nitrofurans in food animals in Nigeria (19).

Antibiotics are essential in the control of bacterial infections and improved performance in herds of cattle (13, 17) but should not be used as a substitute for good husbandry practices. The problem arises when treated animals are slaughtered without recourse to withdrawal periods for the specific drug (9). This situation is more likely to occur with prolonged administration and over dosage of antimicrobial agents (26). Toxic levels of antimicrobial residues in food animals are known to cause diverse health hazards to susceptible human consumers (24). For this reason, a ‘zero tolerance’ was specified by the Federal Drug Agency in the United States, for antibiotic residues in meat and meat products intended for human consumption (12).

Abuja town is currently faced with the problem of a population explosion, with an increasing percentage of the population consuming beef daily as a delicacy. The occurrence of antimicrobial residues has previously been reported in some other locations within Nigeria (10, 14). This study sought to assess the magnitude of antimicrobial residue contamination of beef in abattoirs within the town, with a view to providing evidence to support proactive measures to protect consumer health.

**Materials and methods**

**Investigation of antimicrobial use in beef animals prior to slaughter**

A questionnaire was designed to collect information from four slaughterhouses in different areas of the Abuja metropolis. A total of 210 respondents among abattoir workers, butchers, transporters and marketers/herd owners of livestock were required to provide
information on their respective profiles, types of drugs, frequency of use, purpose, the time of administration of drugs to animals prior to slaughter and their respect of recommended withdrawal periods. The replies were collated and the data subjected to Chi-square statistical analysis. Mean differences at p<0.05 were considered significant.

**Determination of the presence of antimicrobial drug residues in urine, kidney, liver and muscle samples at slaughter**

The study was performed using the Premi® test kit obtained from DSM Nutritional products: version 0505, Geleen, The Netherlands. The kit consists of agar ampoules containing spores of the thermophilic bacterium, *Bacillus stearothermophilus*. The spores germinate when heated and produce carbonic acid. This acid causes the bromocresol purple indicator in the ampoule to change from purple to yellow. The presence of antimicrobials in test samples inhibits the growth of the bacteria and the ampoule remains purple, indicating a positive result (7). A 5 ml sample of urine was aseptically collected directly from the bladders of four randomly selected slaughtered animals in each of the four different abattoirs (a total of 16 animals) in Abuja. Tissue samples (50 g) of kidney, liver and muscle were also collected from the same slaughtered animals. This exercise was repeated twice a week for 14 weeks between October 2008 and January 2009. All samples were placed in separate polythene bags and transported on ice packs to the laboratory for immediate analysis. Prior to the test, the kit was pre-heated at 80°C for 10 min and 64°C for 2 h 45 min, consecutively. The high temperature was adequate to eliminate other bacterial contaminants in the test medium. Tissue extracts were obtained from the samples with an extractor incorporated in the kit.

The Premi® test was performed in accordance with the manufacturer’s instructions. A volume (100 μl) of each test sample was pipetted onto the surface of the test ampoule. The ampoule was kept at room temperature for 20 min for a pre-diffusion stage before covering with aluminium foil and incubating at 64°C. Distilled water was used as a negative control and 100 μg/ml-1 amoxicillin as a positive control. During the incubation period (2 h 45 min), the ampoules were examined and then re-examined at intervals until the negative control had turned from purple to yellow. When this occurred, the ampoules were incubated for a further 10 min to negate variation between ampoules and the results were then read from the lower two-thirds of the agar. A purple colour indicated a positive result, whereas a change to yellow indicated a negative result.

**Results**

**Investigation of antimicrobial use in beef animals prior to slaughter**

The results from the investigation of antimicrobial use in local beef production in Abuja are presented in Table I. A total of 210 respondents were interviewed. Transporters and marketers/herd owners were those

<table>
<thead>
<tr>
<th>Stakeholders</th>
<th>Routine drug use</th>
<th>Prevent zoonoses</th>
<th>Prevent losses due to deaths</th>
<th>Prevent weight loss due to transportation stress</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abattoir workers</td>
<td>8</td>
<td>13</td>
<td>5</td>
<td>0</td>
<td>26 (12.4%)</td>
</tr>
<tr>
<td>Butchers</td>
<td>13</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>31 (14.8%)</td>
</tr>
<tr>
<td>Transporters</td>
<td>31</td>
<td>0</td>
<td>16</td>
<td>25</td>
<td>72 (34.3%)</td>
</tr>
<tr>
<td>Marketers/herd owners</td>
<td>38</td>
<td>0</td>
<td>28</td>
<td>15</td>
<td>81 (38.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>90 (42.9%)</td>
<td>13 (6.2%)</td>
<td>57 (27.1%)</td>
<td>50 (23.8%)</td>
<td>210(100.0%)</td>
</tr>
</tbody>
</table>
who used antimicrobials most in the pre-slaughter treatment of animals with 34.3% and 38.6%, respectively. There were a variety of reasons reported for administering antimicrobials with those who reported the administration of drugs to animals ‘as a routine’, accounted for 42.9%, followed by those who administered drugs ‘to prevent losses due to death’ 27.1%, those ‘to prevent weight loss due to transportation stress’ (23.8%) and then those ‘treating animals to prevent zoonoses’ at 6.2%. The activities of marketers/herd owners and transporters with respect to pre-slaughter administration of drugs were significantly (p<0.05) higher compared to those of butchers and abattoir workers.

**Antimicrobial drug and time interval of administration prior to slaughter**

Results from this study showed that a large proportion of stakeholders (53.2%) administered drugs to cattle designated for meat at 24 h prior to slaughter and many others (26.4%) at 48 h prior to slaughter (Table II). Long-acting oxytetracycline and a penicillin/streptomycin combination were the drugs used most frequently. Procaine penicillin and oxytetracycline/penicillin antibiotics were also popular amongst the drugs administered to cattle within less than 48 h prior to slaughter.

**Antimicrobial drug residues in urine, kidney, liver and muscle samples of cattle at slaughter**

An insignificant number of animals studied were found to have antimicrobial drug residues in both liver and muscle tissues. There was, however, a statistically significant difference (p<0.05) between the occurrence of residues in the kidney and urine on one hand, and liver and muscle tissues on the other. In the kidney and urine samples tested, 50 out of 56 (representing 89.3%) in each case were positive for antimicrobial drug residues (Table III).

**Discussion**

The study revealed that antibiotics were being inappropriately administered to cattle prior to slaughter in Abuja. The withdrawal period for oxytetracycline injection is 28 days for beef cattle (3) yet this was the most common antibiotic administered in the pre-slaughter period.

In our study, there were variations in the time of administration of the drugs, most of the treatments were given within 48 h prior to slaughter. This suggests the increased likelihood of antimicrobial drug residues in the meat subsequently offered for public consumption. Situations of antimicrobial drug residues in food of animal origin are well documented when drugs are given for prolonged periods, at extra label doses and/or outside prescription (26). Similar studies have revealed the presence of antimicrobial drug residues in laying birds and eggs 6-12 days post treatment (1, 15).

Tetracycline residues have caused excessive flatulence in humans (4). Degraded tetracycline has been incriminated in Fanconi syndrome in predisposed humans (11).
Penicillin can cause hypersensitivity reactions and even death in highly susceptible human subjects (24); its degradation products are known to combine with host proteins and become antigenic (20). Risks of cancer, bacterial resistance and continuous changes in susceptibility patterns to anti-microbial agents (8) are some of the realities that cannot be avoided with consumption of antimicrobial drug residues in animal tissues. Inappropriate use of antibiotics in both humans and animals can lead to the emergence of multidrug-resistant (MDR) bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamases-producing enteric bacteria (5, 29) and multiple resistant *Escherichia coli* (6). The presence of MDR pathogens reduces the therapeutic options available to treat those human cases requiring antibiotic therapy. In the context of developing countries, where laboratory capacity for their identification and the range of available antibiotics may be limited, these multi-drug resistant pathogens are problematic.

A disease outbreak caused by pathogenic bacteria which are resistant to most antibiotics is a serious public health concern in a community (15).

The tolerance limits established for the sum of residues of tetracyclines including chlortetra- cycline, oxytetracycline in tissues of cattle, dairy calves, swine, sheep, chickens, turkeys, catfish, lobsters and salmonids are 2 parts per million (ppm) in muscle, 6 ppm in liver and 12 ppm in fat and kidney (7). In our study, high proportions (89.3%) of kidney and urine samples tested positive for antimicrobial residues but muscle and liver tissues revealed insignificant numbers. The different profiles of antimicrobial residues observed in the various animal tissues could, amongst other factors, be due to the time of drug administration prior to slaughter, route of administration, nature of drug relative to its protein-binding ability, the route of excretion, degree of tissue perfusion and even the age of the animal (23, 25).

The high occurrences of antimicrobial residues in both kidney and urine were supported by earlier reports (30). Some antibiotics, for instance, aminoglycosides, are known to be sequestered in the kidneys even long after detectable concentrations have disappeared from other organs or tissues (2, 27). Non-specific inhibitory substances against the growth of the test micro-organisms have occasionally been detected in kidney and urine of slaughtered animals (22). The Premi® test used is an automated screening method that enables the qualitative detection of antimicrobial residues in tissues of meat, fish and eggs (7). The sensitivity of the test procedure for aqueous solutions of various antimicrobials was determined; penicillin G was detected at 0.0025 ppm, streptomycin at 2.0 ppm, oxytetracycline and tetracycline were both detected at 0.3 ppm (7).

**Conclusions**

This study highlights that the presence of antimicrobial drug residues in beef consumed in Abuja poses a potential public health risk that needs to be addressed. There should be massive public enlightenment of all

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**Table III**

Results of samples tested in the four abattoirs in the study

<table>
<thead>
<tr>
<th>Organ</th>
<th>Abattoir A</th>
<th>Abattoir B</th>
<th>Abattoir C</th>
<th>Abattoir D</th>
<th>Tests</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>11</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>12</td>
<td>0</td>
<td>15</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Muscle</td>
<td>1</td>
<td>17</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Urine</td>
<td>13</td>
<td>1</td>
<td>11</td>
<td>2</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>32</td>
<td>21</td>
<td>28</td>
<td>30</td>
<td>33</td>
</tr>
</tbody>
</table>

+ positive
- negative
stakeholders in the beef industry in regard to the potential public health risks associated with the inappropriate use of antimicrobial drugs in food-producing animals. The World Health Organization has advocated the need to promote awareness of the adverse health consequences associated with drug residues amongst the developing nations of the world (13). Proper legislation to restrict the inappropriate treatment of animals should be enacted. The veterinary profession should be strengthened and empowered to take on the responsibility of overseeing the safety of the beef supply in Nigeria. A total overhaul of drug dispensing systems in the country is advocated. Drugs should not be purchased without a prescription from a qualified veterinary doctor. NAFDAC should be encouraged to create a veterinary department to effectively regulate the use of all animal drugs. Proper regulation and policing of antibiotic use in live animals, together with effective surveillance in slaughtered animals to detect events of non-compliance, are essential components to ensure a safe beef supply.

Our research has provided evidence of the widespread occurrence of antimicrobial drug residues in beef consumed by most inhabitants of Abuja, the Federal Capital Territory of Nigeria. The suggestions put forward in this study to tackle the problem should be implemented to avert adverse human health effects associated with the inappropriate use of antibiotics in animals.

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**References**