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Antiretroviral drugs found in pork on Ugandan market: Implications for HIV/AIDS treatment

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1. Background

Access to antiretroviral drugs (ARVs) has increased over time as a result of global initiatives such as the 90–90-90 strategy [1]. Increased access to ARVs could be associated with ARVs off-label use including using them as immune boosters for domestic and farm animals. In Uganda pig feeds have reportedly been adulterated with ARVs for similar reasons [2]. Efavirenz, nevirapine and tenofovir are among the ARVs used as pig immune boosters in Uganda. Efavirenz and nevirapine are largely metabolized through the cytochrome P-450 (CYP) pathways. Farm pigs and humans have been reported to have equivalent amounts of CYPs within the liver [3,4]. For instance there is 81.1% DNA sequence homology between the major human efavirenz metabolizing enzyme CYP2B6 and the equivalent porcine CYP2B22 [5]. Although phosphorylation, the primary metabolic pathway for tenofovir, has not been well characterized in pigs, other phase ii pathways such as sulfonation are slower in pigs than in humans [5]. Given the prolonged half-life ($t_{1/2}$) of all three drugs [6,7], there is potential for significant residual amounts of any or all the three drugs in pork at slaughter as well as sell points.

This off-label use of ARVs could lead to emergence of drug–resistant HIV strains. Indeed, pretreatment HIV-1 drug resistance (PDR) has increased during the last decade in African communities. In South Africa, the pooled annual PDR prevalence rose from 5% in 2009, to 11·9% in 2015 while the overall PDR of non-nucleoside reverse-transcriptase inhibitor PDR prevalence increased from 5% in 2011, to 10.0% by 2014 [8]. Sub-optimal exposure to anti–microbial agents is a well-known cause for emergence of microbial resistance [9]. We determined prevalence of residual ARVs in pigs slaughtered for consumption and established knowledge of, and potential reasons for the same among key stakeholders in Uganda.

2. Methods

Using a cross-sectional design, quantitative methods and focus group discussions (FGDs) were applied to determine the prevalence of detectable residues of ARVs in pork and explore causative practices respectively. The study was approved by Institutional Review Board (IRB) from College of Health Science (CHS), Makerere University, School of Biomedical Sciences Research and Ethics Committee (SBS-HDREC-590). Two study sites Teso and Wambizzi abattoirs in Lira and Kampala districts were purposively selected to represent peri-urban and urban settings of Uganda respectively. Blood sample were collected from all pigs brought to the slaughter houses during the study period (August to November 2018) consecutively. A total of 361 plasma samples (257 Wambizzi and 105 Teso) were extracted by centrifugation at 3000rpms for 10 min and stored at $^{-}20$ °C until analysis for efavirenz, nevirapine and tenofovir was performed using high performance liquid chromatography (HPLC) at Department of Pharmacology and Therapeutics Makerere University, Kampala.

3. HPLC determination of efavirenz, nevirapine and tenofovir

Plasma efavirenz, nevirapine and tenofovir were determined by reverse-phase HPLC with ultraviolet (UV) detection as previously described [10,11]. The HPLC machine used consisted of a system controller (model SCL-10AVP), a pump (model LC-10ATVP), auto injector (model SIL-10ADVP), and a spectrophotometric UV–vis detector (model SPD-10AVP) all supplied by Shimadzu co-operation Kyoto Japan.

For efavirenz determination, the column used: Eclipse 7.5 cm × 4.6 mm 3 μm (Agilent Technologies, USA). Mobile phase consisted of 30% acetonitrile, 30% methanol, 4 mmol l^{-1} potassium hydroxide and 10 mmol l^{-1} acetic acid (pH 4.3). Plasma proteins were precipitated with acetonitrile. Injection volume was 20 μl. The retention time approximately 7.6 min, run time: 10 min, detected at UV-VIS 1, 210 nm, UV-VIS 2220 nm and temperature 25 °C. This method is

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linear, with a within-day coefficient of variation of 3.2, 3.3 and 5.1% at concentrations of 0.63 mg/L (n = 17), 2.53 mg/L (n = 17), and 6.31 mg/L (n = 16), respectively, with a between-day coefficient of variation of 4.1% (n = 50). The limit of quantification was set at 0.2 mg/L, flow rate: 1.0 ml/min.

The column used for determination of plasma nevirapine was zorbax eclipse XBD-phenyl 5 μm C18, 4.6 mm × 150 mm (Agilent Technologies, USA). The mobile phase consisted of 75% phosphate buffer and 25% acetonitrile. Plasma precipitation used 0.55 M perchloric acid. The supernatant 200 μl) was eluted at 50 μl min^{-1} for 15 min. The retention time for nevirapine was 7.5 min as detected at UV-VIS 280 nm, flow rate was 0.6 ml/min. The method was linear, with a within-day coefficient of variation of 3.0, 2.3 and 4.2% at concentrations of 1.0 μg/ml (n = 20), 5.0 μg/ml (n = 17), and 16.0 μg/ml (n = 16), respectively, and a between-day coefficient of variation of 3.7% (n = 50). The limit of quantification was set at 0.05 mg/L.

The column used for determination of plasma tenofovir was Atlantis C18, 150 × 3.0 mm, 3 μl. Separation was performed at 25 °C with gradient elution, flow rate at 0.6 ml/min for tenofovir mobile phase A which consisted of 5 mM ammonium acetate buffer pH 2.5/methanol:99/1:v/v. Mobile Phase B and C contained 10 mM phosphate buffer pH 3.3/ Methanol: 99/1:v/v and 100% acetonitrile respectively. The plasma proteins were precipitated using the solid phase extraction method. After evaporation, samples were each reconstituted with 100ul of double distilled deionised water. Injection volume of 5ul was administered. UV-VIS detection was performed at 260 nm, acquisition delay 5 min, retention time approximately 6 min and total run time 25 min. Quantification was done using the peak areas of the analytes normalized to peak area of the internal standard; limit of quantification 0.01 mg/L. The method was linear, with a within-day coefficient of variation of 0.0, 2.8 and 3.2% and between day coefficient of variation of at concentrations of 0.08 mg/L (n = 5), 0.86 mg/L (n = 5), and 8.95 mg/L (n = 5), respectively.

Focus group discussions (n = 4) each consisting of eight participants were conducted. Participants included farmers, abattoir owners and agricultural extension officers. Descriptive statistics were conducted to summarize the data in form of frequencies and percentages using STATA/IC version 15 (Stata Corp, Texas, 77,845, USA).

4. Results

A total of 361 plasma samples from Kampala district (n = 256) and Lira district (n = 105) were analyzed for efavirenz and nevirapine residues, while 124 from Kampala district (n = 19) and Lira district (n = 105) were tested for tenofovir. Thirty-two (32) participants were involved in the FGDs. Most of the participants were famers 87.5% (n = 28). Agricultural officers and abattoir owners constituted only 12. 5% (n = 4).

ARV residues were detected in 27.4% (95%CI: 22–32.3) samples. Prevalence of ARV residues by the two study sites was 19.1% (CI: 14.5 to 24.5) and 47.6% (CI: 37.8 to 57.6) in Wambizzi and Teso respectively. Efavirenz and nevirapine detection rates were higher in Lira 33.3% (CI: 24.4 to 43.20) and 18.1% (CI: 11.3 to 26.8) compared to Kampala 5.5% (CI: 3.0 to 9.0) and 12. 1% (CI: 8.4 to 16.8), Table 1. The median (IQR) detectable ARV drug residual concentration were; EFV 0.26 (0.23–0.31) mg/L and NVP 0.08(0.06–0.11) mg/L. The median (IQR) residual EFV concentrations were 0.26 (0.24–0.28) mg/L and 0.26 L (0.22–0.33) mg/L in Wambizzi and Teso respectively whereas median (IQR) residual NVP concentrations were 0.08 (0.06–0.13) mg/L and 0.07 (0.05–0.1) mg/L for samples collected from Kampala and Lira districts respectively (Fig. 1).

The major possible cause of detectable residual ARVs in pigs slaughtered in Uganda is reflected in some quotes from FGDs below:

"*You see, when these pigs are given ARVs, they grow faster and fatter and are sold off quickly. I know someone who uses ARVs for their pigs, his pigs eat a lot, grow so fast and are sold off real fast, and they also take long to fall sick*".

"It works for human so the pigs will be healthy like humans".

5. Discussion

Significant residual efavirenz and nevirapine were detected in pork sold to Ugandan consumers. Although laboratory analysis for tenofovir was conducted, no residues were detected. However, since tenofovir is given as a constituent of a fixed dose combination with lamivudine and either efavirenz or nevirapine, its relatively shorter half-life [7] and low sensitivity of HPLC_UV method used [11] are possible explanations for this negative result. The FGDs confirmed the findings with similar reports previously made in Kenya [12].

Overall access to ARVs is restricted as a way of minimized un-controlled use of the same. While Community Health Workers (CHWs) contribute to, and strengthen HIV services in sub-Saharan Africa, this, and the rapidly increasing access to ARV drugs might facilitate their off-label use. The central ARV distribution to dispensing units including CHWs is guided by the number of registered patients and their re-fill schedules. Off-label use therefore culminates into poor adherence. Indeed, it was noted from FGDs that farmers access ARVs from ART recipients for about US$ 0.05 per pill. Poor adherence and direct exposure to sub-therapeutic ARV drug levels in animals and humans are major causes of acquired HIV drug resistance [13]. Ordinarily, sub-therapeutic exposure to antimicrobial agents result into selective killing of susceptible strains which reduces reproductive competition thus allowing the more resistant strain to multiply rapidly and gain dominance. More complex modes of HIVDR transmission are also possible. For instance, according to Barza et al., resistance traits could be acquired through plasmids in commensal flora of pigs in this case, which then may find their way through the food chain to commensals and pathogens of humans [14]. Although, human exposures to residual ARVs in pigs would be by eating pork, this study measured residual ARV concentrations in blood, an integral part of pork. Additionally, for most drug there is an equilibrium between blood and tissue at steady state conditions, rendering plasma concentration a reliable proxy for tissue drug concentrations. Using LOQs rather than LOD for categorization of plasma samples as positive or negative for residual ARVs might have led to more false negatives thus a possible underestimation of the problem. While empowering communities with knowledge may constitute a long-term strategy to mitigate off-label use of ARVs, deliberate efforts to promote effective accountability for ARVs by dispensing CHWs and patient adherence are paramount. Findings from the present study provide a solid benchmark for a more comprehensive study to guide policy on off–label use of ARVs and other anti-microbial agents.

Table 1
Prevalence of residual EFV and NVP in pigs from selected abattoirs in Uganda.

Variables	No. of positive samples EFV(n)	HPLC % EFV prevalence (95%CI)	No. of positive samples (NVP)	HPLC % NVP prevalence (95%CI)
Abattoirs		EFV		NVP
Lira	35(105)	33.33(24.43 to 43.20)	19(105)	18.09(11.26 to 26.81)
Kampala	14(256)	5.47 (3.022 to 9.005)	31(256)	12.12(8.378 to 16.746)

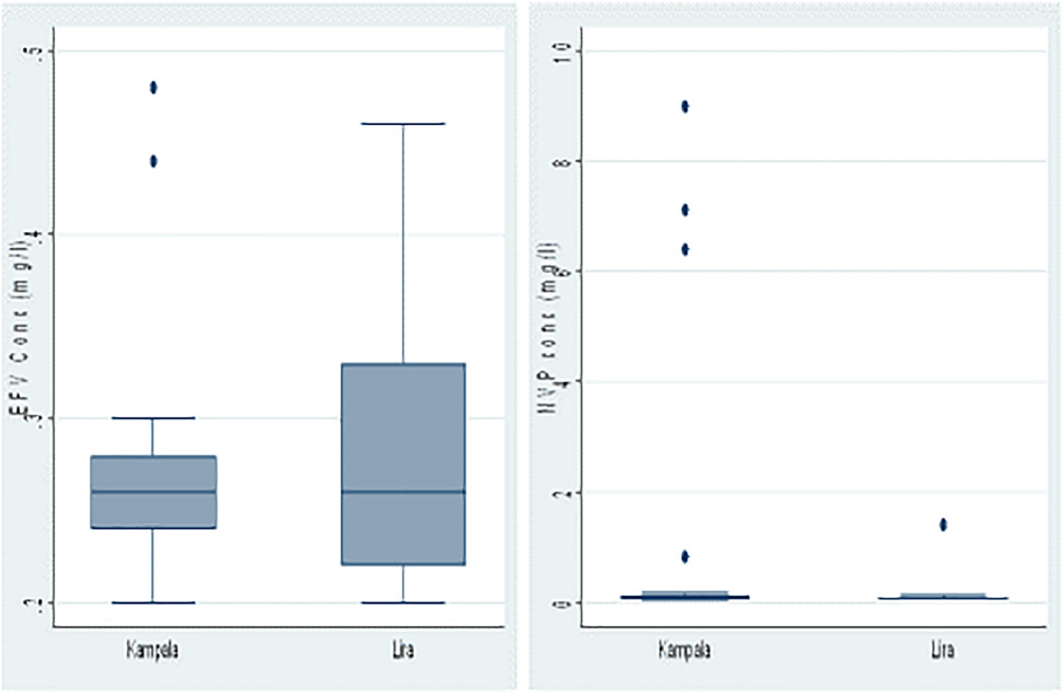

Fig. 1. Median efavirenz and nevirapine concentrations in ARVs positive samples.

6. Conclusion

Pork consumers in Uganda are substantially exposed to ARVs. ARVs are intentional adulterants of farm pig feeds and are a source of residues in pork that is consumed by communities in Uganda.

Authors' contribution

RN, JM, JKT, SN, were involved in the design of the study. JM supervised the laboratory work. JKT and JM read through the manuscript.

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Declaration of Competing Interest

The authors declare no conflict of interest.

References

[1] F. Bajunirwe, F. Tumwebaze, D. Akakimpa, C. Kityo, P. Mugyenyi, G. Abongomera, Towards 90-90-90 target: factors influencing availability, access, and utilization of HIV services—a qualitative study in 19 Ugandan districts, Biomed. Res. Int. 2018 (2018).
[2] B. Oketch, Lira farmers share ARVs with their pigs, Monitor Newsp. (115) (2017) 6.
[3] L. Dalgaard, Comparison of minipig, dog, monkey and human drug metabolism and disposition, J. Pharmacol. Toxicol. Methods 74 (2015) 80–92.
[4] E. Van Peer, E. Verbueken, M. Saad, C. Casteleyn, C. Van Ginneken, S. Van Cruchten, Ontogeny of CYP 3 a and P-glycoprotein in the liver and the small intestine of the Göttingen minipig: an immunohistochemical evaluation, Basic Clin. Pharmacol. Toxicol. 114 (5) (2014) 387–394.
[5] M. Kojima, T. Morozumi, Cloning of six full-length cDNAs encoding pig cytochrome P450 enzymes and gene expression of these enzymes in the liver and kidney, J. Health Sci. 50 (5) (2004) 518–529.
[6] J.M. Custodio, M. Fordyce, W. Garner, M. Vimal, K.H.J. Ling, B.P. Kearney, et al., Pharmacokinetics and safety of tenofovir alafenamide in HIV-uninfected subjects with severe renal impairment, Antimicrob. Agents Chemother. 60 (9) (2016) 5135–5140.
[7] D. Blumenthal, L.L. Brunton, Buxton IL, Parker KL, Goodman & Gilman's manual of pharmacology and therapeutics, (2008).
[8] B. Chimukangara, R.J. Lessells, S.-Y. Rhee, J. Giandhari, A.B. Kharsany, K. Naidoo, et al., Trends in pretreatment HIV-1 drug resistance in antiretroviral therapy-naive adults in South Africa, 2000–2016: a pooled sequence analysis, EClinicalMed. 9 (2019) 26–34.
[9] B. Shankar, B. Manjunatha Prabhu, S. Chandan, D. Ranjith, V. Shivakumar, Rapid methods for detection of veterinary drug residues in meat, Vet. World 3 (5) (2010).
[10] J.K. Mukonzo, D. Roshammar, P. Waako, M. Andersson, T. Fukasawa, L. Milani, et al., A novel polymorphism in ABCB1 gene, CYP2B6*6 and sex predict single-dose efavirenz population pharmacokinetics in Ugandans, Br. J. Clin. Pharmacol. 68 (5) (2009) 690–699.
[11] O.M. Minzi, E. Ngaimisi, Bioanalytical method for determination of nevirapine in-vivo in resource constrained laboratories, J. Chem. Pharm. Res. 2 (2010) 431–439.
[12] K. Valentine, How Kenyans Are Misusing Antiretroviral Drugs (ARVs), [Internet]. 2013 [cited 23/may/2019]. Available from, 2013. https://www.kenyaplex.com/resources/10130-how-kenyans-are-misusing-antiretroviral-drugs-arvs.aspx.
[13] B. Nachega, J.C. Marconi, V.U. van Zyl GM, E. Gardner, W.Y. Preiser, S. Hong, et al., HIV treatment adherence, drug resistance, virologic failure: evolving concepts, Infect. Disord. Drug Target. 11 (2) (2011) 167–174 (Formerly Current Drug Targets-Infectious Disorders).
[14] M. Barza, Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals, Clin. Infect. Dis. 34 (Suppl. 3) (2002) S123-S5.