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Dietary exposure to antibiotics residue in honey and the potential health risks to consumers in Adamawa state, Nigeria

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Abstract

Antibiotics are often used by beekeepers as growth enhancers and to treat bee-related diseases. Due to the important role of honey in Nigeria business enterprises, this study was conducted toward evaluating the dietary risk exposure of antibiotic residues in honey to public health. Raw Honey Samples (RHS) and samples sold in the markets; Commercial Honey Samples (CHS) were obtained from Gombi, Hong and Mubi North local Government Areas of Adamawa State. The pH values of the CHS were observed to be significantly ($p < 0.05$) acidic compared to the RHS. The acidity levels in the RHS and CHS were observed to follow same trend with the pH values. There was a significant ($p < 0.05$) difference in the moisture content of the honey samples between the RHS and the CHS. However, the moisture content for all the study areas were found to be within the international standard (20%). The ash content were found to be in the range of $0.72 \pm 0.01\%$ - $0.65 \pm 0.05\%$ for the RHS and $0.3 \pm 0.01\%$ - $0.22 \pm 0.07\%$ for the CHS. Tetracycline, streptomycin, chloramphenicol, and sulfonamides residues were all detected in the RHS from all the sample points. However, only the samples from Gombi/Garkida shows the presence of all the antibiotics in CHS. Streptomycin were Below the Detection Level (BDL) in the CHS from Uba/Uvu. Similarly, tetracycline, streptomycin and chloramphenicol were all found to be BDL in the CHS from Mubi/Vintim. With the exception of chloramphenicol which has no defined Acceptable Daily Intake (ADI) values, all the Estimated Daily Intake (EDI) values calculated for each antibiotics were observed to be significantly ($p < 0.05$) below their recommended ADI, suggesting low potential risk to the consumer, having $< 1\%$ of acceptable daily intake. The Target Hazard Quotient (THQ) estimated for the individual antibiotics were observed to be < 1 . The potential cocktail effects arising from consumers exposed to different antibiotic residues at the same time through the consumption of the honey were observed to also fall below the level of concern, showing a Health Index (HI) of < 1 .

Keywords: Honey, antibiotics, health risk assessment, dietary exposure

Introduction

Dietary food requirements or preference of a society are often influence by their sociocultural identity and in some instances, their religious believes. However, amidst all sociocultural/or religious diversity, honey and other bee products stands out as universal foods that encompassed all divides. It assumed a trinity concept of a nutrient, medicine and an ointment. An ancient sweetener, widely deployed as active ingredients in apitherapy (Bogdanov *et al.*, 2008) [1]. Though, honey production is less than 1% of global sugar production (Bogdanov *et al.*, 2008) [1] is however, becoming a perfect replacement for sugar-based products. Thus, making honey, a truly global commodity. Considering its myriad of applications, the global market value of honey from 2007 to 2016 experienced an annual growth of +4.3% reaching 1,847K tonnes in 2016 (The Guardian news, 2018) [2]. The trend was forecast to continue on this moderate upward trend with a projected GAGR of +1.6% from 2015 to 2025 which according to the market analysis could results to estimated volume of 2.1M tonnes by 2025 (The Guardian news, 2018) [2]. This development as at 2013, put the global foreign exchange earnings from honey production estimated at the international market at \$1,539 to a barrel of honey (Agriculture Nigeria) [3]. In Nigeria, domestic consumption rate of honey was estimated at 380,000 tonnes, with a global price of about 4.5 billion dollars (Vanguard News, 2017) [4]. Though, the country has the potential to produce about 800,000 tonnes, and can generate over 10 billion dollars from local and international trade, her current potential honey output is less than 3% (Punch News, 2017) [5]. The current production of honey liquid in Nigeria is 2 m liters but has potential to produce 20 m liters of honey annually (The Guardian news, 2018) [2].

Across Nigeria, Net return analysis showed that honey bee production is indeed profitable, posting encouraging gross margin and net income (Duruson, 2011; Igbokwe and Mbanaso, 2006; Uduma and Uдах, 2015, Olatubosun and Oluwale, 2016, Abdullahi *et al.*, 2014, Folayan and Bifarin, 2014) ^[6-11]. Though, the returns generated from the businesses have an encouraging outlook, this figures are considered far beyond the estimated market potential endowed within Nigeria and her young populace. The demand for honey in Nigeria is soaring up, prices increasing momentarily and production capacity below the market demands; thus leading the key players in the business to be motivated towards financial advantage. Based on market price of honey in Nigeria (Naira) culled from the web, a liter is between N1950-2520, while 500ml is between N1620-1800 respectively (<https://www.jumia.com.ng/honey>) ^[12]. These high value attached to honey visa vice the low production output puts it at risk for strong economic incentive that could translate into economically-motivated adulteration (Strayer *et al.*, 2014) ^[13].

In addition to the business and economic risk factors, honey business suffered from activity such as use of additives or extenders, growth busters, and indiscriminate use of antibiotics (Strayer *et al.*, 2014; MAREC, 2005) ^[13, 14]. Antibiotics, particularly streptomycin, sulfonamide, and chloramphenicol are often used by beekeepers to treat bee-related diseases and as growth enhancers. The relatively long shelf-life of antibiotic residues in foods could indirectly leads to the emergence of bacteria strain that can resist the antibiotics overtime. Could also induces allergic reactions in hypersensitive individuals, and could leads to the disorder of the haemopoietic system (Tillotson *et al.*, 2006) ^[15]. Due to the important role of honey in Nigeria business enterprises, several of research efforts were channeled toward evaluating honey quality for human consumption. These effort tends toward evaluating the physicochemical and biochemical components of the honey (Ndife *et al.*, 2014; Buba *et al.*, 2013; Lullah-Deh *et al.*, 2018; Adebisi *et al.*, 2004; Lawal *et al.*, 2009. Oladipupo and Isah, 2009, James *et al.*, 2009) ^[16-22], and microbial properties (Ndife *et al.*, 2014, Lawal *et al.*, 2010) ^[16, 23] with no available data relating the dietary risk exposure of the antibiotic residues in honey to public health.

Considering these negative effects of antibiotic, the residual level in foods from plant and animal origin are regulated in developed economy (Vragović *et al.*, 2012) ^[24], however, this limits have received poor recognition in developing economy like Nigeria, thus posed a serious public health risk burden (Mensah *et al.*, 2014) ^[25]. Therefore, the continual monitoring of antibiotic residues in honey and related products will undoubtedly help to assess the potential risk to human health and proffer remedial action that could checkmate adulteration and remediate treatment processes for beehive and crops around the bee colonies. Therefore, the objective of this study is to answer the underlining question relating to the potential risk associated to antibiotics residue in honey on consumption by human. The quality of the honey samples obtained within the study area will be assess by relating the changes in the physicochemical components of the honey. Similarly, dietary risk assessments of the honey sample for antibiotic residue will be investigated as well to relate the hazards associated with its indiscriminate use in beehive and the risk to consumer via the food chain.

Materials and Methods

Standard of tetracycline, streptomycin, sulfonamides and chloromphenicol were obtained from Adamawa State University clinic. The honey samples were collected from Adamawa State in three different local Government Areas (Gombi, Hong and Mubi North). Raw honey samples (RHS) were collected from bee's farmers in Garkida (Gombi), Uba/Uvu (UBA) and Vimtim (Mubi north) respectively. The sealed honey comb harvested directly from the hive were transported to the laboratory in labelled sealed containers. From the respective locations, commercially obtained samples (CHS) were purchased directly from local vendors in the respective markets. At the laboratory, the samples were homogenized thoroughly following the procedure described by IHC, (2002) ^[26]. For the physicochemical analysis, the moisture and ash content were determined using the method of IHC (2002) ^[26] and AOAC (1990) ^[27], while the pH was read and recorded using pH meter. The Total acidity results was carried out using the methods described by IHC, (2002) ^[26].

For the determination of the antibiotic residue using HPLC, the deproteinization of the honey samples was carried out in 3ml of acetonitrile (ACN) under agitation for 1 minute and centrifuged for 15 minutes at 5000 rpm. The obtained supernatant was dried under nitrogen flow at 40°C. Before, the HPLC analysis, the resulting residue are re-dissolved in methanol and filtered through 0.45 µm filter paper. Based on the methods described by Pagliuca *et al.*, (2002) ^[28], the presence of antibiotic residue in the honey samples was carried using different mixture of an aqueous mobile phase (A) Acidified water and organic mobile phase (B) methanol / ACN with a flow rate of 1ml /minute. The respective antibiotic were quantified by a modified method described by Albino *et al.*, (2005) ^[29], detected at 40-24 nm. The data obtained from all the analysis were statistically integrated and presented as mean ± S.D of three replicate analysis using Graph phard-prism (version 6.0), One-way ANOVA and students T-test. The level of significance was sets at P<0.05.

The health risk assessment and hazard characterization were carried by first estimating the daily intake (EDI) of the respective antibiotics in the honey. This was achieved by integrating the average concentration of the antibiotics in the honey, the average consumption rate of the honey and the average body weight per person as described in equation 1 (Forkuoh *et al.*, 2018; USEPA, 1997) ^[30, 31].

$$ED = \frac{C_h \times H_{IR}}{BW} \quad (1)$$

The C_h is the antibiotic concentration (µg/kg) in the honey, H_{IR} represents the average honey consumption rate or intake rate for an average child and adults. The BW is the average body weight of children (15 kg) and adults (60 kg) (USEPA, 2000; Akbari *et al.*, 2012) ^[32, 33]. The potential non-carcinogenic risk from the consumption of the antibiotics were estimated using Target Hazard Quotient (THQ) and the health index (HI) as described by the United State Environmental Protection Agency (USEPA, 1997) ^[31].

The THQ were estimated by integrating the ratio of the EDI to the acceptable daily intake (ADI) values for each antibiotic (FAO/WHO, 2002 and 2010; USEPA, 1996; Bwatanglang *et al.*, 2019; Bwatanglang, 2019) ^[34-38]. The expression for estimating the THQ are described in equation 2.

$$THQ = \frac{EDI}{ADI} \quad (2)$$

The HI, expressed as the sum of the THQ as described in equation 3 is the cumulative effect pose by the combination of the individual antibiotics presents in the honey (Forkuoh *et al.*, 2018; Reffstrup *et al.*, 2010) [30, 39]

$$HI = \frac{EDI_1}{ADI_1} + \frac{EDI_2}{ADI_2} + \frac{EDI_3}{ADI_3} + \dots + \frac{EDI_n}{ADI_n} = \sum_{i=1}^n \frac{EDI_i}{ADI_i} \quad (3)$$

Were the EDI_i represents the estimated daily intake dose of the individual antibiotics (1, 2, 3....) in the honey and the ADI_i is the acceptable daily intake dose for the individual antibiotics (1, 2, 3.....).

Results and Discussion

Physico-Chemical Analysis of Honey Samples

The pH analysis shows both the RHS and the CHS falling within the acidic range (Table 1). A range of 4.95±0.01-5.20±0.02 and 3.3±0.11 – 3.17±0.03 were determined in the RHS and CHS respectively. The pH values from the CHS were observed to be significantly ($p < 0.05$) acidic compared to the RHS. The values obtained from this study aggress with the pH range of 4.31 -6.02, 4.65 -5.14, and 3.22–5.00 reported by Adebisi *et al.*, (2004) [19], Lawal *et al.*, (2009) [20], and Lullah-Deh *et al.*, (2018) [18] respectively for Nigerian honey. And the pH recommended range of 3.5 and 5.5 set in Codex Alimentarius Commission, (2001a) [40] for honey. Low pH has the ability to influence the texture, stability and shelf life of honey (Buba *et al.*, 2013; Boussaid *et al.*, 2018; El-Metwally, 2015) [17, 41-42]. The acidity levels in the RHS and CHS were observed to follow same trend with the pH values. The acid content in the CHS were significantly ($p < 0.05$) higher to the values obtained from RHS. The RHS from Uba/Uvu and Gombi/Garkida show comparable free acidity content (0.45±0.01-0.42±0.02 Meq/kg), thus affirming their suitability as potent antibacterial agent, increasing the stability of honey against microbial spoilage. The flavour and the availability of minerals in honey are also linked to high acidity content (El-Metwally, 2015) [42]

There was a significant ($p < 0.05$) deference in the moisture content of the honey samples between the RHS and the CHS. The range recorded in this study for RHS is 10.20±0.01-10.45±0.02, while 16.85±0.01-18.32±0.03 were determined in the CHS. These values falls in the same range reported by Omafuvbe and Akanbi, (2009) [43] (11.47-19.62%), and Buba

et al., (2013) [17] (16.00 ± 2.19) for some Nigerian honeys. Even though the values were found to be lower than the values reported by Adebisi *et al.*, (2004) [19] (16.38-30.82%), and Lullah-Deh *et al.*, (2018) [18] (16.4 – 34.0%), for some Nigerian honeys. The moisture content for all the study areas were found to be within the international standard (20%) (Codex Alimentarius Commission, 2001a) [40], and further observed to be within the range reported in some countries. Reporting a moisture contents of 18.32 ± 0.67% in Egyptian honey, 16.28 ± 0.22% for Yemeni honey, 15.64 ± 0.30% for Saudian honey, and 14.73 ± 0.3% in honey from Kashmiri respectively (El Sohaimy *et al.*, 2015.) [44]. The high moisture contents in the CHS could be attributed to the composition and floral origin of honey. Could also be from a mixture of honey from different locations and compositions (Nanda *et al.*, 2003; Malika *et al.*, 2005) [45, 46]. The significance of low moisture in honey lies in its ability to increase the stability of the honey from fermentation and granulation processes. Impaired microbial growth and elongate the storage shelf life of the honey (Buba *et al.*, 2013; El-Metwally, 2015; Akhtar *et al.*, 2014; Bogdanov, 2009a) [17, 41, 47-48].

In this study, ash content of 0.72±0.01%, 0.70±0.03%, and 0.65± 0.05% were determined in the RHS. Similarly, 0.3±0.01%, 0.22±0.07%, and 0.25±0.02% were respectively determined in CHS from Uba/Mubi and Gombi/Garkida. Honey samples from Gombi/Garkida had the lowest ash content, this implies that the source of honey from Gombi/Garkida could be from floral plants origin since their ash contents falls within the recommend values of ≤0.6% (Codex 2001a and 2001b) [40,49]. Though, the honey from Gombi/Garkida could be nectar based (blossom honeys), relatively low level of mineral and trace element contents is expected. Studies shows that blossom honeys derived from nectar have a mineral content mostly between 0.1 and 0.3% while that of honeydew honeys can reach 1.0% of the total (Bogdanov, 2009b) [50]. The ash content determined in this study were found to fall in the range (0.095 – 0.518%) recorded by Adebisi *et al.*, (2004) [19] and 0.19 – 0.36% by Omafuvbe and Akanbi (2009) [43]. And lower than that obtained by Lawal *et al.*, (2009) [20] (0.60 – 0.84 %) for other Nigerian honey. From the results so far, the honey with high stability and relative quality is Gombi/Garkida follow by Uba/Uvu then Mubi/Vintim.

Table 1: Physicochemical Analysis of Honey Samples

Sample Locations	Samples	pH	Acidity (Meq/kg)	Moisture (%)	Ash (%)
Uba/Uvu	RHS	4.95±0.01	0.45±0.02	10.45±0.03	0.72±0.01
	CHS	3.30±0.11	0.60±0.01	16.85±0.01	0.30±0.01
	RHS	5.20±0.02	0.55±0.01	10.20±0.01	0.70±0.03
Mubi/Vintim	CHS	3.16±0.02	0.65±0.03	18.12±0.02	0.22±0.07
	RHS	5.00±0.00	0.42±0.02	10.35±0.05	0.65±0.05
Gombi/Garkida	CHS	3.17 ±0.03	0.64±0.04	18.32±0.03	0.25±0.02

Quantification of Antibiotic Residues in Honey Samples using HPLC

Maximum residue limits (MRLs) are yet to be establish for honey and other bee products (Al-Waili *et al.*, 2012) [51]. The idea is to ensure no residual level of antibiotic in any honey products for human consumption. Since, bee farmers has to control and treat bee-related disease using antibiotics, it will be very difficult to completely eliminate possible residual level in the harvested honey even if the withdrawal time frame before harvesting are fully observed. As a fallout from

this observation, the European Union (EU), under the Council Directive 2001/110/EC has set a Reference Points for Action (RPAs) for antibiotic residues in honey which is also used as provisional MRL (Forsgren, 2010; Johnson *et al.*, 2010; EFSA, 2013) [52-54]. The RPAs are residue concentrations which are technically feasible for analytical considerations. Place as a benchmark to reject any bee product exceeding these limits (Johnson *et al.*, 2010; EFSA, 2013; Mutinelli, 2003) [53, 44-55]. Provisional MRL in parts per billion (ppb) for oxytetracycline (25 ppb), chloramphenicol (0.3 ppb) and

nitrofurans (1.0 ppb) were established by EU for honey (Johnson *et al.*, 2010) [53]. While a range of 0.01 to 0.05 mg/kg were considered as a RPAs for antibiotic residues in honey in Switzerland, UK and Belgium, (Al-Waili *et al.*, 2012; Reybroeck, 2003; Diserens, 2007; EFSA, 2014) [51, 56-58]. Similarly, Export Inspection Council (EIC) of India placed the Level of Action for Chloramphenicol at 0.3 ppb, Nitrofurans 1 ppb, Tetracyclines (group) 10 ppb, Streptomycin 10 ppb and Sulphonamides (group) 20 ppb (Johnson *et al.*, 2010) [53]. Though this value were purposely established for analytical considerations, the reports by the CONTAM Panel concluded that, the RPAs when applied to feeds and other food from animal origin could in the absence of defined MRL are considered relatively sufficient to protect both animal and public health (EFSA, 2014) [58].

As shown in Table 2, tetracycline, streptomycin, chloramphenicol, and sulfonamides residues were all detected in the RHS from all the sample points. However, for the CHS, only the samples from Gombi/Garkida shows the presence of all the antibiotics. Streptomycin were below the detection level (BDL) in the CHS from Uba/Uvu. Similarly, tetracycline, streptomycin and chloramphenicol were all found to be BDL in the CHS from Mubi/Vimtim. In the class of antibiotic analyzed, only sulfonamides were detected in virtually all the samples. The antibiotics with the highest concentration detected in the honey samples are sulfonamides and chloramphenicol. The highest concentration of tetracycline were found in the RHS from Mubi/Vimtim (1.35±0.07 µg/kg), and Uba/Uvu (1.34±0.05 µg/kg). The RHS from Uba/Uvu and Gombi/Garkida were observed to contain the highest concentration of streptomycin, with a mean concentration of 1.75±0.07 µg/kg and 1.58±0.02 µg/kg

respectively. High concentration of 5.32±0.03 µg/kg and 5.15±0.02 µg/kg were detected for chloramphenicol in the RHS from Uba/Uvu and Gombi/Garkida respectively. Furthermore, highest concentration of sulfonamides were detected in RHS from Uba/Uvu (4.52±0.04 µg/kg) and Gombi/Garkida (4.33±0.05 µg/kg). These values were found to be significantly ($p < 0.05$) higher to the values obtained from the CHS for all the sample points.

Studies conducted in other countries also reported the presence of antibiotics in honey samples. About 13 honey samples out of 34 samples imported from Asian countries into Switzerland were found to contain 0.4 and 9.0 µgkg⁻¹ of chloramphenicol, with at least two samples containing up to 5 µgkg⁻¹ (Ortelli *et al.*, 2004) [59]. Twenty nine percent of 251 honey samples produced across Greece were observed to contain residual level of tetracycline from 0.018-0.100 mg/kg (Saridaki-Papakonstadinou *et al.*, 2006) [60]. In another study, analysis of nectar and honey samples obtained from southern part of Tamil Nadu, India were observed to contain 4-17 and 11-29 µg/kg of streptomycin, 2-29 and 3-44 µg/kg of ampicillin and 17-34 and 26-48 µg/kg of kanamycin respectively (Solomon *et al.*, 2006) [61]. Similarly, Streptomycin 3 – 10,820 µg/kg, Sulfonamides 5 – 4,592 µg/kg, Tetracyclines 5 – 2,076 µg/kg, Chloramphenicol 0.1 – 169µg/kg, were detected in honey samples from EU (Diserens, 2007) [57].

Even though antibiotics at varying concentrations were found in both the RHS and CHS from the respective sample points in this study, the concentration detected were all found to be below the RPAs of 0.01-0.05 for antibiotic residue in honey set by EU, or the RPAs set by EIC (Johnson *et al.*, 2010; Reybroeck, 2003; Diserens, 2007) [53, 56-57].

Table 2: Concentration of Antibiotic Residues (µg/kg) in Honey Samples

Sample Locations	Samples:	Tetracycline	Streptomycin	Chloramphenicol	Sulfonamides
Uba/Uvu	RHS	1.34±0.05	1.75±0.07	3.20±0.05	4.52±0.04
	CHS	1.03±0.04	ND	1.63±0.05	1.50±0.01
	RHS	1.35±0.07	1.45±0.05	5.32±0.03	2.75±0.01
Mubi/Vimtim	CHS	ND	ND	ND	0.95±0.05
	RHS	0.95±0.07	1.58±0.02	5.15±0.02	4.33±0.05
Gombi/Garkida	CHS	0.48±0.03	0.55±0.02	1.14±0.05	0.75±0.05
RAPs					

Health risk characterization

The level of exposure to the antibiotics through the consumption of the honey from the respective sample points were assess based on the mathematical model described in equation 1 and thus compared with the recommended acceptable daily intake level (ADI) for each of the antibiotics allowed in foods. The dietary exposure assessments conducted is critical toward quantifying the risk associated with antibiotics in the honey. Helping to determining whether a residual level of the antibiotics in the honey is above or below the level of concerns to pose a potential risk to public health. The results are presented in Table 3. Among the class of antibiotics detected in the study, sulfonamides was found to possess the highest exposure risk to both the adults (7.5E-05 µg/kg/bw) and children (3.0E-04 µg/kg/bw) consuming RHS from Uba/Uvu. While chloramphenicol presents the highest EDI for the adults (2.7E-05 µg/kg/bw) and children (1.1E-04 µg/kg/bw) consuming the CHS from the same sample points. The highest EDI of 8.9E-05 µg/kg/bw and 3.5E-04 µg/kg/bw for chloramphenicol were detected in RHS for both the adults and children respectively. Furthermore, chloramphenicol were observed to possess the highest EDI in the RHS (8.6E-05

µg/kg/bw) and CHS (1.9E-05 µg/kg/bw) from Gombi/Garkida in Adults. Similar trend were observed in children, showing and EDI of 3.4E-04 µg/kg/bw and 7.9E-05 µg/kg/bw for the RHS and CHS respectively. From the results so far, due to the smaller body weight and physiological susceptibility in the children (Bwatanglang and Magili, 2019; Bwatanglang *et al.*, 2019) [37, 62], the EDI were observed to be higher than that of the adults. With the exception of chloramphenicol which has no defined ADI, all the EDI calculated for each antibiotics were observed to be significantly ($p < 0.05$) below their recommended ADI (Johnson *et al.*, 2010; FAO/WHO, 2008) [53, 63], suggesting low potential risk to the consumer, having <1% of acceptable daily intake.

Other related study reported various exposure indices due to dietary intake of antibiotics by human. Residues of quinolones and sulfonamides were found to be widely distributed in in cultured fish samples from the Pearl River Delta, South China. The EDI results showed that the consumption of the fishes to dietary intakes of quinolones and sulfonamides were far below the acceptable daily intake (ADI) and poses no risk to the public health (He *et al.*, 2016)

[64]. Similarly, EDI of 2.09 ng/kg body weight (BW)/day and 1.83 ng/kg BW/day for tetracycline and penicillin residues were determined in dairy products resulting in 0.007% and 0.006% of the ADI respectively (Kabrite *et al.*, 2019) [65]. Furthermore, dietary exposure assessment of streptomycin and tetracycline following the consumption of food of animal origin from Croatian market shows that meats contributes to 41% EDI to streptomycin and milk contributes 46% of the dietary intake of tetracycline (Vragović *et al.*, 2012) [24]. Same trend were reported by Vragovic *et al.*, (2011) [66]

Further assessment based on the THQ and HI shows that exposure to the antibiotics through the consumption of the honey possess no immediate danger to non-carcinogenic related risk. The THQ estimated for the individual antibiotics (except chloramphenicol which has no defined ADI) were observed to be <1. The potential cocktail effects arising from consumers exposed to different antibiotic residues at the same time through the consumption of honey were observed to also fall below the level of concern, showing a HI of <1. The risk characterization processes based on THQ and HI analysis is a health-based statistical probability expressed as a function of the quantified level of concern; a process developed to estimate the potential health risk associated with long-term exposure to environmental pollutants (Bwatanglang, 2019) [38]. The THQ values of <1 observed in the study suggest no health risk associated with the level of tetracycline, streptomycin, and sulfonamide in the honey, thus the population consuming these honey are in no immediate danger for non-carcinogenic risk. In a related study, risk assessment due to dietary exposure to oxytetracycline, tetracycline, and chlortetracycline through milk consumption in India showed HQ of <1 (Chauhan *et al.*, 2018) [67]. Similar trend were also observed for oxytetracycline and tetracycline in pooled raw milk (Gaurav *et al.*, 2014) [68]. Tetracycline

residue intake via the consumption of Yugoslavian milk were also observed to show a HQ of <1 (Gradinaru *et al.*, 2011) [68], arising from the low EDI recorded for the antibiotics (Prado *et al.*, 2014) [70].

Even though ADI was not defined for chloramphenicol and THQ/or HI could not be established in this study, the consumption of the honey samples analyzed in this study points toward potential health risk to chloramphenicol. The non-availability of ADI for chloramphenicol further suggest zero tolerance level to this antibiotics. An ADI could not be establish for chloramphenicol due to lack of genotoxic and toxicological data, in addition to lack of definable NOAEL (No Observed Adverse Effect Level) or LOAEL (Lowest Observed Adverse Effect Level) (EFSA, 2013) [54]. For these reasons, residual level of chloramphenicol are not allowed in the animal food-production chain. And thus, reported to constitute threat to public health (EFSA 2013; Commission Regulation (EU) No 37/2010; and No 165/2010) [54, 71-72]. The RPA designated for chloramphenicol (0.03µg/kg), though solely related to analytical considerations and to enables detection at ever-lower level (Hanekamp and Bast, 2015) [73], were also considered sufficiently protective for public health (EFSA, 2014). But further recommend zero tolerance level in foods (EFSA 2013; Commission Regulation (EU) No 37/2010; and No 165/2010) [54, 71-72]. Tissue bioavailability of chloramphenicol upon oral exposure were observed to readily transverse across the placental and mammary barriers. Following through some bioreactive chemistry enter reductive and/or oxidative pathways yielding toxic/reactive metabolites leading to the onset of genotoxic-related complications (EFSA, 2014) [58].

Estimated Daily Intake, Target hazard quotient (THQ) and Health index (HI) in Adults and Children Exposed to the antibiotics through Honey Consumption:

Estimated Daily Intake (EDI) in mg/kg/bw													
	Adults						Children						
	Uba/ Uvu		Mubi/ Vimtim		Gombi/ Garkida		Uba/ Uvu		Mubi/ Vimtim		Gombi/ Garkida		
Antibiotics	RHS	CHS	RHS	CHS	RHS	CHS	RHS	CHS	RHS	CHS	RHS	CHS	ADIs
Tetracycline	2.3E-05	1.7E-05	2.3E-05	-	1.6E-05	8.0E-06	9.0E-05	6.9E-05	9.0E-05	-	6.3E-05	3.2E-05	3.0E+01
Streptomycin	2.9E-05	-	2.4E-05	-	2.6E-05	9.2E-06	1.2E-04	-	9.7E-05	-	1.1E-04	3.7E-05	5.0E+01
Chloramphenicol	5.3E-05	2.7E-05	8.9E-05	-	8.6E-05	1.9E-05	2.1E-04	1.1E-04	3.5E-04	-	3.4E-04	7.6E-05	NA
Sulfonamides	7.5E-05	2.5E-05	4.6E-05	1.6E-05	7.2E-05	1.3E-05	3.0E-04	1.0E-04	1.8E-04	6.3E-05	2.9E-04	5.0E-05	5.0E+01
Target Hazard Quotient (THQ)													
	Adults						Children						
	Uba/ Uvu		Mubi/ Vimtim		Gombi/ Garkida		Uba/ Uvu		Mubi/ Vimtim		Gombi/ Garkida		
	RHS	CHS	RHS	CHS	RHS	CHS	RHS	CHS	RHS	CHS	RHS	CHS	
Tetracycline	7.5E-07	5.7E-07	7.5E-07	-	5.3E-07	2.7E-07	3.0E-06	2.3E-06	3.0E-06	-	2.1E-06	1.1E-06	
Streptomycin	5.8E-07	-	4.8E-07	-	5.3E-07	1.8E-07	2.3E-06	-	1.9E-06	-	2.1E-06	7.3E-07	
Chloramphenicol	-	-	-	-	-	-	-	-	-	-	-	-	
Sulfonamides	1.5E-06	5.0E-07	9.2E-07	3.2E-07	1.4E-06	2.5E-07	6.0E-06	2.0E-06	3.7E-06	1.3E-06	5.8E-06	1.0E-06	
HI	2.8E-06	1.1E-06	2.2E-06	3.2E-07	2.5E-06	7.0E-07	1.1E-05	4.3E-06	8.6E-06	1.3E-06	1.0E-05	2.8E-06	

Conclusion

The values obtained from the physicochemical analysis (pH, acidity level, moisture and ash content) for all the honey samples studied falls within recommend values specified by the international honey regulations. However, from the results of the study, it will suffice to say that the control use of antibiotic and observance of antibiotic withdrawal timeframe in bee-farming are not fully observed. The use and application of approved antibiotics in bee-farming should include a required withdrawal time, which refers to the period of time during and after treatment in which honey from the treated hive should not be collected for consumption. Withdrawal period required to decrease the possibility of antibiotic residues entering the food supply. The EDI values and THQ conducted for each of the antibiotics however, significantly

($p < 0.05$) below their recommended ADI values and <1, the residual level detected in both the RHS and the CHS collected from the sample sites further calls for continual monitoring and evaluation of bee products for human consumption. The continual monitoring of antibiotic residues in honey and related products will undoubtedly help to assess the potential risk to human health and proffer remedial action that could checkmate adulteration and remediate treatment processes for beehive and crops around the bee colonies.

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