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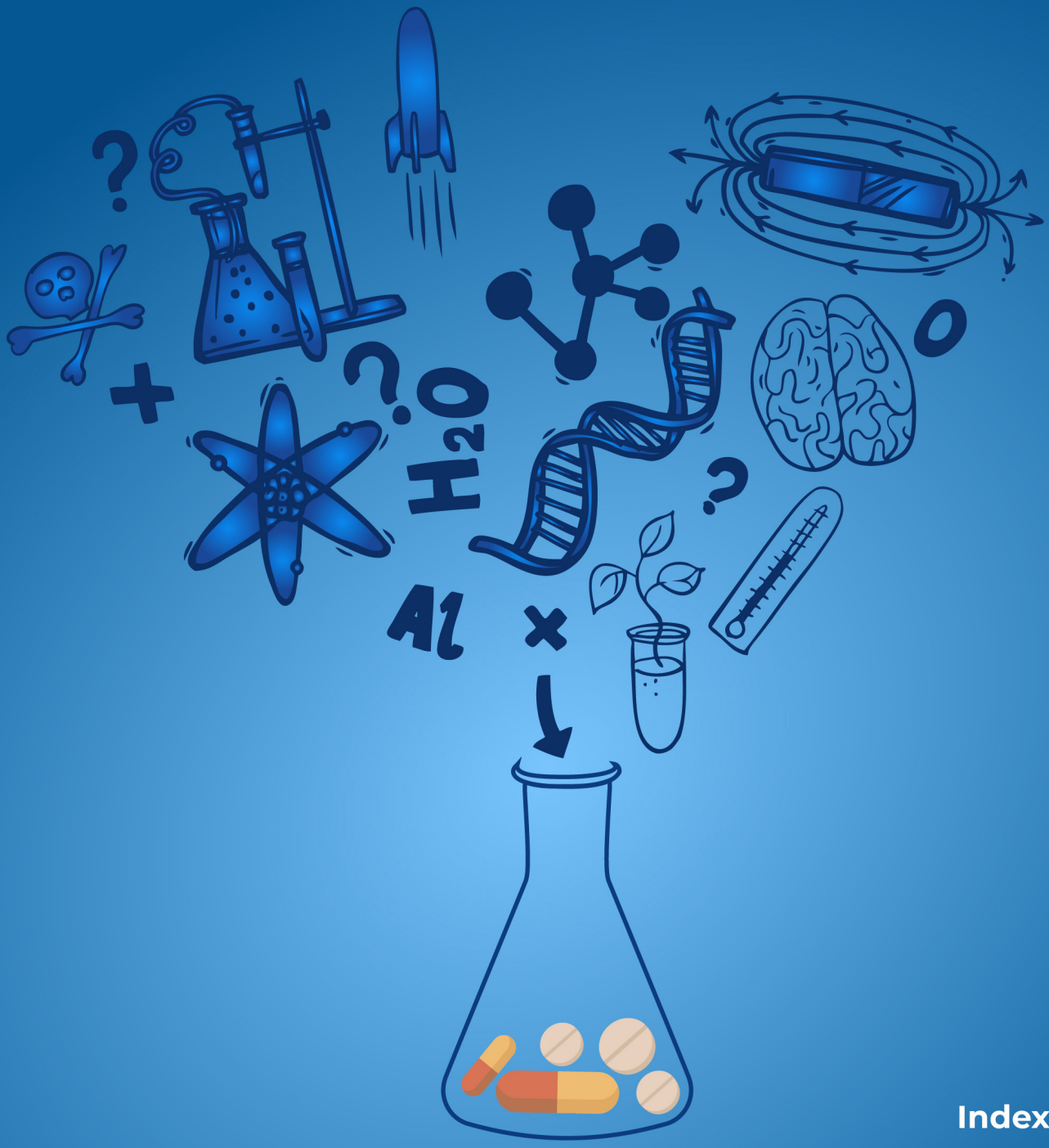

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Dietary Risks Assessment of Antibiotic Residues and Microbial Safety of Local Honey in Adamawa State

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ABSTRACT

This study investigated the microbial safety and dietary risk assessments of antibiotic residues in honey. The determination of antibiotic residues in this study were carried out using HPLC (LC 1200 series Agilent Tech). The results of the microbial analysis of the honey samples showed that, some of the honey samples were contaminated with micro-organisms. The antibiotics with highest mean concentration in the study were chloramphenicol (6.468±0.03 µg/kg) and sulfonamide (5.553±0.04 µg/kg) from sample HS1 and HG2 respectively. Estimated daily intake (EDIs) of detected antibiotic residues which were compared with the recommended acceptable daily intake (ADIs) for each antibiotic allowed in food. Chloramphenicol was observed to possess the highest risk for adults and children (1.08E-04 µg/kg/bw) and (4.31E-04 µg/kg/bw) respectively in sample HS1. All the EDIs evaluated for antibiotic residues falls below acceptable daily intake (ADI). The target hazard quotient (THQ) and health index (HI) for antibiotic residues in all the samples were < 1, this suggested no risks associated with the level of antibiotic residues risks thus having less danger to human health through its consumption.

INTRODUCTION

Honey is the natural sweet, viscous substance produced by honeybees from the nectar of flowers or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature (Abeshu and Geleta, 2016).

It is one of the most widely sought products because of its unique nutritional and medicinal properties (James *et al*., 2009). The variety produced by honey bees (the genus Apis) is the most commonly sought for because it is collected by most beekeepers and consumed by people. Honey is also produced by bumble bees, stingless bees and other hymenopteran insects such as honey wasps, though the quantity is generally lower and they have slightly different properties compared with honey from the honey bees. Honey bees convert nectar into honey by a process of regurgitation and evaporation: they store it as a primary food source in wax honeycombs inside the beehive (Satarupa and Subha, 2014). Honey is composed primarily of the sugar: glucose and fructose; its third greatest component is water (Singh *et al*., 2012). Honey also is composed of a complex mixture of carbohydrates and other less frequent substances, such as organic acids, amino acids, proteins, minerals, vitamins, lipids (Blasa *et al*., 2006; Ball, 2007; Zerrouk *et al*., 2011), aromatic compounds, flavonoids, vitamins, pigments, waxes, pollen grains, several enzymes and other phytochemicals (Gomes *et al*., 2010).

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). 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). The current production of honey liquid in Nigeria is 2 million liters but has potential to produce 20 million liters of honey annually (The Guardian news, 2018). Across Nigeria, Net return analysis showed that honey bee production is profitable, encouraging gross margin and net income (Duruson, 2011; Igbokwe and Mbanaso, 2006; Uduma and Udah, 2015, Abdullahi *et al*., 2014, Folayan and Bifarin, 2014). Though, the returns generated from the businesses have an encouraging outlook, these figures are considered far beyond the estimated market potential endowed within Nigeria and her young populace.

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 hemopoietin system (Tillotson *et al*., 2006). Antibiotic residues in honey have become a major consumer concern. Some drugs have the potential to produce toxic reactions in consumers directly while some other is able to produce allergic or hypersensitivity reactions (Velicer *et al*., 2004). 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), however, these limits have received poor recognition in developing economy like Nigeria, thus posed a serious public health risk burden (Mensah *et al*., 2014). The determination of antibiotic residues and microbial safety

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in honey and other bee products has become a growing concern considering the growing popularity of honey in human daily diets. Its ingestion without knowing its source and safety might carry significant health hazards. Residual level of contamination cannot be changed through various production techniques, hence the need to study the microbial safety and dietary risks of antibiotics residues in the samples of local honey.

METHODOLOGY

Materials and Chemicals/Reagents

Standard of tetracycline, Streptomycin, Sulfonamides, chloramphenicol, distilled water, deionized water, acidified water, sodium acetate, anhydrous magnesium sulphate, sodium hydroxide, plastic bottles, methanol, acetonitrile, volumetric volumes, electric centrifuge, beaker, glass tubes, polyethylene bottles, petri dish, sterile pipette, Potato Dextrose agar, tryptone Neomycin agar, potassium chloride, HPLC (LC 1200 series Agilent Tech). All are analytical grade.

Study area and Geographical Location

Adamawa is a state in North-eastern Nigeria, with its capital at Yola. It is located between latitude 9°20'N and longitude 12°30'E (Fig 3.1). It occupied an area of 36,917 square kilometers. The state has population of 3,178,950 (NPC, 2006). Adamawa is one of the largest States of Nigeria, the State of Borno borders it to the Northwest, Gombe to the West and Taraba to the Southwest. It's eastern bordered with Cameroon. It comprises of 21 local Government area.

Sample collection, Storage and Preservation

The honey samples were collected from three (3) local government areas of Adamawa state (Ganye, Song and Mubi North from the Southern, Central and Northern senatorial zones respectively). The samples were mixed to make a composite sample that represents each sampling location (Table 3.1). Six (6) composite samples were collected purposively from the bee-keepers farms namely: Sugu, Ganye I, Dirma, Shimba, Lokuwa and Yewa Hosere at different locations. Similarly, three (3) composite samples were purchased from retailers at different point (Ganye II, Song and Mubi) in the same geographical area. The samples were collected during dry season (January-March 2021). The collected samples were properly stored at room temperature and preserved to avoid loss due to poor storage, was timely analyzed to minimize loss due to prolonged storage. Samples were stored in polyethylene bottles preserved for analysis (Japhet *et al*., 2018).

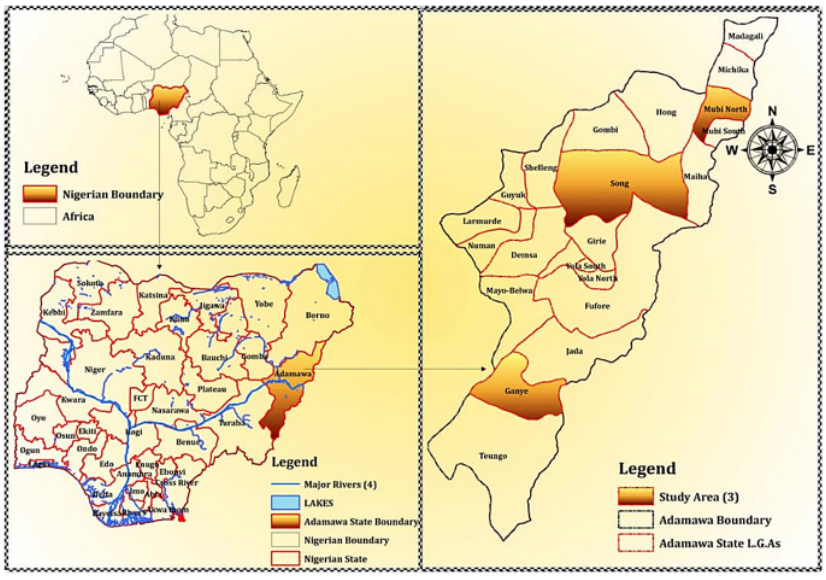

Figure 1: Map of the study Area (Google map 2020).

Table 1: Sample locations of honey and coding

L.G.A of the senatorial zones in Adamawa State	Sample Locations	Code
Ganye (Southern Senatorial zone)	Sugu	HG1
	Ganye I	HG2
	Ganye II	HG3
Song (Central Senatorial zone)	Dirma	HS1
	Shimba	HS2
	Song	HS3
Mubi North (Northern Senatorial zone)	Lokuwa	HM1
	Yewa Hosere	HM2
	Mubi	HM3

Figure 2: Samples collected for analysis

Microbial Contaminants analysis

Standard plate count

Ten grams of honey was suspended in 90 ml of 0.1% phosphate buffer solution. A series of dilutions was then carried out and 0.1 ml spread on Plate Count Agar (PCA) (OXOID). The culture was incubated for 72 h at 37°C.

Yeast and mold count

Isolation of microorganisms

One ml of each sample was picked with the aid of a sterile pipette. A plastic rack was arranged with 9 sterile test tubes containing 9 milliliters of sterile distilled water. A ten-fold dilution adopted by (Eleazu *et al*., 2013) was done by dispersing 1 ml of the sample into the first test tube (10-1) which will be well shaken. One milliliter was then taken again from 10-1 dilution and transferred to the next test tube (10-2). The dilution continued to 10-9. Each test tube was shaken vigorously before each transfer.

Inoculation

Shree & Arlis (2003) used the pour plate method to plate all the samples. 0.1 ml from dilution 10-2 was dispersed into sterile petri dish with the aid of a sterile pipette. Potato dextrose agars was poured into the plates (10 ml) and isolation of fungi were carried out using the potato dextrose agar. The plates were swirled gently for easy mixing of the samples and the media. All plates were allowed to solidify on the bench and each plated sample was duplicated.

Incubation

The potato dextrose agar (PDA) plates were transferred to an incubator at 25°C for 3-5 days. All incubated plates were examined daily for mycelia and colony growth.

Subculture and purification

After the incubation period, a flamed surgical knife was used to subculture different color of mycelia growth from potato dextrose agar (PDA) plates on newly prepared PDA plates. All while the PDA plates were incubated at 25°C for 3-5 days.

Counting of colonies

After incubation of all the plates, counts of the number of colonies in each plate was done with a hand tally counter (Fawole *et al*., 1988). The mean counts were obtained and multiplied by the appropriate dilution factor. The mean count was calculated as:

Mean = (Total viable count)/(Number of plates)

The estimation of the viable counts in each sample was made in colony forming unit (CFU) and Total viable count = (Number of colonies X Dilution Factor)/ (Amount Plated (ml))

Sulfite-Reducing Anaerobic Bacteria (clostridia)

The technique of enumeration carried out the sulfite-reducing anaerobic bacteria analysis in a solid medium in tubes. To 1 ml decimal dilution (10−2), contained in sterile tubes, 20 ml of the melted Sulfite Tryptone Neomycin agar was added and cooled in a water bath at 45°C. After homogenization and solidification, the tubes were incubated anaerobically at 37°C for 24–48 hours. Colonies characteristics of sulfite-reducing anaerobic bacteria appear black in the tubes (Francois *et al*., 2018)

Total Coliform and fecal coliform (ISO 4831: 2006)

The total coliform search was carried out according to ISO 4831: 2006. 1 ml of decimal dilution (10−2) was poured aseptically into sterile plates. Purple crystal, bile-lactose neutral red agar (VRBL), melted and cooled in a water bath at 45°C, was added to the inoculum at a rate of 15 ml per dish. The mixture was then homogenized by rotary movements. After solidification of the first layer, a second 5 ml layer of VBRL was added. Control of the sterility of the medium was carried out in a Petri dish

with approximately 15 ml of VBRL. The total coliform count was done directly after incubation at 30°C for 24–48 hours. Fecal coliforms are characterized by a small mass of fluorescent colonies with a diameter of 0.5 mm (Francois *et al*., 2018).

Extraction and determination of Antibiotic residues using HPLC

The extraction of honey samples was carryout subjecting to deproteinizing chemical procedure using Acetonitrile (ACN). 2 g of the honey sample was place into 10 ml test tubes and shake intensively with 3 ml acetonitrile (ACN) for 1minute. The mixture was centrifuge for 15 minutes at 5000 rpm. The supernatant was collected and dried under Nitrogen stream at 40°C. The residue will be re-dissolved in methanol and filtered through 0.45µm filter paper describe by Pagliuca *et al*., 2002).

The presence of antibiotic residues in the honey samples was carried out using different mixture of aqueous mobile phase (A) Acidified water and organic mobile phase (B) methanol/ACN with a flow rate of 1 ml/min. The respective antibiotic residues were quantified by a modified method described by (Albino *et al*., 2005 and Shafqat *et al*., 2012), detected at 210-240 nm.

The health risk assessment and hazard characterization of antibiotic residue

The health risk assessment and hazard characterization were carryout 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).

EDI=(Ch×Hir)/BW…… equation 1

The Ch is the antibiotic concentration (µg/kg) in the honey, Hir represents the average honey consumption rate or intake rate for an average child and adults (0.001). The BW is the average body weight of children (15 kg) and adults (60 kg) (USEPA, 2000; Akbari *et al*., 2012).

The potential non-carcinogenic risk from the consumption of the antibiotics

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). The THQ was 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). The expression for estimating the THQ are described in equation 2.

THQ=EDI/ADI…….....…equation 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).

HI = (EDI1/ADI1) +(EDI2/ADI2) +(EDI3/ADI)+….(EDIi/ADIi)……..equation 3

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

Statistical analysis

The mean and standard deviation of the results were done using Minitab 19 and data was generated in triplicate. The results were express as Mean± SD

RESULTS AND DISCUSSION

Microbial analysis

The results of the microbial analysis of the honey samples showed that, some of the honey samples are contaminated with micro-organisms. Microbial analysis results are shown in Table 2 and 3 The SPC were found in low numbers in all samples of honey with a mean count which varies within 1.0 x 104 – 3.3 x 104 CFU/ml. The yeast and mold count of honey samples were less than 10 CFU/ml in sample in all the samples.

Table 2: Mean results of microbial analysis

Sample Location	Sample	SPC in CFU/ml	Mold and Yeast (CFU/ml) x 10^2	Sulfite-reducing Clostridia	Total coliform and fecal Coliform
GANYE	HG1	1.7 x 10^4	2	ND	ND
	HG2	2.0 x 10^4	ND	ND	ND
	HG3	3.2 x 10^4	1.8	ND	ND
SONG	HS1	1.0 x 10^4	ND	ND	ND
	HS2	ND	ND	ND	ND
	HS3	3.3 x 10^4	1.6	ND	ND
MUBI-NORTH	HM1	1.3 x10^4	ND	ND	ND
	HM2	ND	1	ND	ND
	HM3	ND	1.5	ND	ND

SPC = Standard Plate Count
ND = Not detected
CFU = Colony forming units

Table 3: Isolated micro-organisms PDA (Fungi) from local honey samples in Adamawa State

Sample Location	Sample	Aspergillus fumigatus	Aspergillus flavus	Aspergillus niger	penicillum sp	Trichophyton rumbrum
GANYE	HG1	+	-	-	-	+
	HG2	-	-	-	-	-
	HG3	-	+	-	+	-
SONG	HS1	-	-	-	-	-
	HS2	-	-	-	-	-
	HS3	+	-	+	-	-
MUBI-NORTH	HM1	-	-	-	-	-
	HM2	-	-	+	-	+
	HM3	-	+	-	-	-

Key: + = Present
- = Absent
PDA = Potato Dextrose Agar

Microbial Contamination

The mean level of microbial contamination of different honey samples were presented in Table 2. The contamination with standard plate count (SPC) for Aerobic Mesophilic Bacteria varies within 1.0 x 104 – 3.3 x 104 CFU/ml. This result conforms with the result of Ndife *et al*., 2014. According to Tchoumboue *et al*., 2008, the contamination with fungi and bacteria indicate inadequate hygienic conditions during collecting, manipulating, processing and storing. Microbial contamination during and post processing can also result in spoilage or persistence of some bacteria in honey.

The yeast and mold count of honey samples were less than 10 CFU/ml in samples HG1, HG3, HS3, HM2 and HM3 but was not detected in Sampling points HG2, HS1, HS2 and HM1 (Table 3). There was total absence of sulfite-reducing clostridia and fecal coliforms in all the samples, indicating that honeys were produced in accordance with good hygiene practices during extraction, packaging and storage (Guiraud, 2003). The low microbial loads of honey samples could be attributed to their low pH values and high amount of total soluble sugars and possibly phenolic compounds and their synergistic interaction (Alvarez-Suarez *et al*., 2010).

Yeast, mold and spore-forming bacteria (coliform) have been implicated to survive in honey and are indicative of the sanitary quality of the honey (Ezeama, 2007; Eleazu *et al*., 2013). In a study conducted by Omafuvbe and Okanbi 2009, observed that no mold contamination was detected. The micro-organisms that were isolated in the honey samples from all sampling points are fungi (Table 3). The presence of micro-organisms in honey can sometimes influence the stability of the product and its hygienic quality. Normal honey must lack pathogenic micro-organisms or micro-organisms that produce enteric illnesses (Popa *et al*., 2009).

Determination of Antibiotic Residues

The determination of antibiotic residues in all the sampling points was carried out using HPLC (LC 1200 Series, Agilent Tech). The Health Risks Assessment, Target Hazard Quotient and Health Index was estimated using various mathematical expressions and results presented in Tables.

Maximum residues level (MRLs) are yet to be established for honey and other bee products (Al- Waili *et al*., 2012). The EU (European Union) under the council Directive 2001/110/EC has set a reference point for action (RPAs) for antibiotic residues in honey which is also used as provisional MRL (Forsgen, 2010; Johnson *et al*., 2010; EFSA, 2013). The RPAs are residue concentrations which are technically feasible for analytical considerations place as a bench mark to reject any bee products exceeding these limits (Johnson *et al*., 2010; ESFA, 2013; Mutinelli, 2003). 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).

Mean concentration of antibiotic residues

The samples' average mean± Sd concentration of antibiotic residues were analyzed and the results presented in Table 4. All the samples contained at least two or more antibiotic residues detected. Streptomycin were below the detection level (BDL) in samples HG1, HG2, HG3, HS1, HS2 and HM2. Only samples from Song (HS3) and Mubi North (HM1 and HM3) contains the level of streptomycin antibiotic residues. Similarly, tetracycline was below the detection limit (BDL) in samples from HG1, HS1, HS2, HM2 and HM3 as can be seen in Table 4.

Among the antibiotics analyzed, sulfonamide was detected in virtually all the samples except sample HS3 and HM1 from Song and Mubi-North respectively. The antibiotic with the highest concentration detected in the honey samples from all locations are: Chloramphenicol and Sulfonamide (Table 4). The highest mean concentration of tetracycline and streptomycin were found in sampling point HG3 from Ganye (1.513± 0.01 µg/kg) and sampling point HS3 from Song (1.513± 0.05 µg/kg) respectively. The samples HG2 and HS1 from Ganye and Song were observed to contain the highest concentration of sulfonamide with a mean concentration of 5.553±0.04 µg/kg and 5.386±0.07 µg/kg respectively. Similarly, highest mean concentration of 6.468± 0.03 µg/kg and 6.447± 0.03 µg/kg were detected for chloramphenicol in sampling point HS1 and HG2 from Song and Ganye respectively.

In general, the consumption of honey containing antibiotic residues represents a real danger to human health, considering that the indiscriminate consumption of these compounds is the main cause of bacteria resistance to antibiotics, especially in the case of aminoglycosides (streptomycin). Also, antibiotics can cause allergic reactions in people sensitive to certain classes of compounds and can cause hypersensitivity in up to 5% of individuals exposed to these substances (Brum, 2018; Ford, 2017).

Aminoglycosides are antibiotics that act by interfering with the replication of bacterial DNA by blocking protein synthesis. This class of antibiotics causes serious toxic adverse effects on the kidneys and the auditory system and, when used in high concentrations, can cause a serious risk of neuromuscular paralysis. Regarding tetracyclines, these cause adverse effects to the gastrointestinal system, causing irritation and may lead to deficiency disorders in the absorption of some vitamins. Besides, they cause kidney damage and bone marrow disorders, and can also cross the placental barrier and deposit in the bones (Brum, 2018; Ford, 2017).

Sulfonamides, better known as sulfas, are antibiotics that act by inhibiting the metabolism of bacterial cells by inhibiting folic acid, making the cells unable to grow and multiply. Regarding the adverse effects caused by these substances, these can range from itchy skin, hypersensitivity reactions, liver problems to problems related to blood cells. (Brum, 2018; Ford, 2017; Omidi *et al*., 2016).

Bwatanglang *et al*., (2019) reported present of antibiotics residues from Uba/Uvu, Mubi/Vimtim and Gombi/Garkida for both Raw Honey Sample (RHS) and Commercial Honey sample (CHS). It was found that tetracycline, chloramphenicol, sulfonamide and streptomycin were all detected in the RHS from all the sampling points.

Studies conducted in other countries also reported the presence of antibiotic residues in honey samples. Twenty nine percent of 251 honey samples produces across Greece were observed to contain residual level of tetracycline from 0.018- 0.100 mg/kg (Saridaki-Papakonstadinou *et al*., 2006). About 13 honey samples out of 34 imported from Asian countries into Switzerland were found to 0.4 and 9.0 µg/kg of chloramphenicol with at least two samples containing up to 5.0 µg/kg (Orteli *et al*., 2004). In another study, streptomycin 3- 10,820 µg/kg, sulfonamide 5- 4,592 µg/kg, chloramphenicol 0.1-169 µg/kg were detected in honey samples from EU (Diserens, 2007).

Table 4: Mean Concentrations of Antibiotic Residues (µg/kg) in Honey samples

Sample Location	Sampling Points	Chloramphenicol	Sulfonamide	Tetracycline	Streptomycin
GANYE	HG1	0.102±0.05	2.232±0.02	ND	ND
	HG2	6.447±0.03	5.553±0.04	0.031±0.03	ND
	HG3	ND	0.576±0.01	1.513±0.01	ND
SONG	HS1	6.468±0.03	5.386±0.07	ND	ND
	HS2	0.194±0.02	0.150±0.01	ND	ND
	HS3	1.743±0.05	ND	1.292±0.03	1.513±0.05
MUBI-NORTH	HM1	ND	ND	0.060±0.01	0.126±0.07
	HM2	1.042±0.04	0.687±0.01	ND	ND
	HM3	ND	5.042±0.03	ND	0.215±0.02

ND = Not Detected

Potential health risk of antibiotic residues associated with consumption of honey samples

The results of human health risk assessments were presented in Table 5. The Estimated Daily Intake (EDIs) of detected antibiotic residues for both adults and children in all the samples were compared with the recommended acceptable daily intake (ADIs) for each antibiotic in all food. The dietary exposure assessment is very critical towards evaluating the risks associated with antibiotics in honey. Helping to determining whether a residual of concerns to pose a potential risk to public health.

Based on the antibiotic residues detected in all the sampling points, chloramphenicol was found to possessed the highest exposure risks to both adults and children (1.08E-04 µg/kg/bw) and (4.31E-04 µg/kg/bw) respectively in sample HS1 (see Table 5). Sulfonamide presents the highest EDIs for adults (9.26E-05 µg/kg/bw) and children (3.70E-04 µg/kg/bw) from sample HG2 (Table 5). Similarly, the highest EDIs for tetracycline was found in sampling point HG3 (2.52E-05 µg/kg/bw) and (1.00E-04 µg/kg/bw) for adults and children respectively while EDIs for streptomycin was only obtained in sample points HS3, HM1 and HM3 from Ganye and Mubi-North for both adults and children (Table 5)

Due to the smaller body weight (15 kg) and physiological susceptibility in children, the EDI were observed to be higher in children than that of the adults. With the exception of chloramphenicol which has no define ADI, all the EDIs calculated for each antibiotic residue were found to be below their recommended ADIs (Johnson *et al*., 2010). This shows that the consumption has a negligible risk to human health having < 1% ADI in all the samples.

Estimated Daily Intake (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). 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).

Table 5: Estimated Daily Intake (EDI) in µg/kg/bw for Antibiotic Residues

ADULTS										
	GANYE			SONG			MUBI NORTH			
ANTIBIOTICS	HG1	HG2	HG3	HS1	HS2	HS3	HM1	HM2	HM3	ADI
Chloramphenicol	1.70E-06	1.07E-04	0.00E+0	1.08E-04	3.23E-06	2.91E-05	0.00E+0	1.74E-05	0.00E+0	NA
Sulfonamide	3.72E-05	9.26E-05	9.60E-06	8.98E-05	2.50E-06	0.00E+0	0.00E+0	1.15E-05	8.40E-05	5.00E+01
Tetracycline	0.00E+0	5.17E-07	2.52E-05	0.00E+0	0.00E+0	2.15E-05	1.00E-06	0.00E+0	0.00E+0	3.00E+01
Streptomycin	0.00E+0	0.00E+0	0.00E+0	0.00E+0	0.00E+0	2.52E-05	2.10E-06	0.00E+0	3.58E-06	5.00E+01
CHILDREN										
	GANYE			SONG			MUBI NORTH			
ANTIBIOTICS	HG1	HG2	HG3	HS1	HS2	HS3	HM1	HM2	HM3	ADI
Chloramphenicol	6.80E-06	4.30E-04	0.00E+0	4.31E-04	1.29E-05	1.16E-04	0.00E+0	6.95E-05	0.00E+0	NA
Sulfonamide	1.49E-04	3.70E-04	3.84E-05	3.59E-04	1.00E-05	0.00E+0	0.00E+0	4.58E-05	3.36E-04	5.00E+01
Tetracycline	0.00E+0	2.07E-06	1.00E-04	0.00E+0	0.00E+0	8.61E-05	4.00E-06	0.00E+0	0.00E+0	3.00E+01
Streptomycin	0.00E+0	0.00E+0	0.00E+0	0.00E+0	0.00E+0	1.01E-04	8.40E-06	0.00E+0	1.43E-05	5.00E+01

Table 6: Target Hazard Quotient (THQ) and Health Index (HI) for Antibiotic Residues

ADULTS									
	GANYE			SONG			MUBI NORTH		
ANTIBIOTICS	HG1	HG2	HG3	HS1	HS2	HS3	HM1	HM2	HM3
Chloramphenicol	-	-	-	-	-	-	-	-	-
Sulfonamide	7.44E-07	1.86E-06	1.92E-07	1.80E-06	5.00E-08	0.00E+0	0.00E+0	2.30E-07	1.68E-06
Tetracycline	0.00E+0	1.72E-08	8.40E-07	0.00E+0	0.00E+0	7.17E-07	3.33E-08	0.00E+0	0.00E+0
Streptomycin	0.00E+0	0.00E+0	0.00E+0	0.00E+0	0.00E+0	5.04E-07	4.20E-08	0.00E+0	7.20E-08
HI	7.44E-07	1.88E-06	1.03E-06	1.80E-06	5.00E-08	1.22E-06	7.53E-08	2.30E-07	1.75E-06
CHILDREN									
	GANYE			SONG			MUBI NORTH		
ANTIBIOTICS	HG1	HG2	HG3	HS1	HS2	HS3	HM1	HM2	HM3
Chloramphenicol	-	-	-	-	-	-	-	-	-
Sulfonamide	3.00E-06	7.40E-06	7.68E-07	7.18E-06	2.00E-07	0.00E+0	0.00E+0	9.16E-07	6.72E-06
Tetracycline	0.00E+0	6.90E-08	3.33E-06	0.00E+0	0.00E+0	2.87E-06	1.33E-07	0.00E+0	0.00E+0
Streptomycin	0.00E+0	0.00E+0	0.00E+0	0.00E+0	0.00E+0	2.02E-06	1.68E-07	0.00E+0	2.86E-07
HI	3.00E-06	7.47E-06	4.10E-06	7.18E-06	2.00E-07	4.89E-06	3.01E-07	9.16E-07	7.01E-06

Target Hazard Quotient (THQ) and Health Index (HI) for antibiotic residues

The results of THQ and HI for potential non-Carcinogenic risks were evaluated and presented in Table 6. Based on the results obtained for THQ and HI, shows that the exposure to the antibiotics through the dietary consumption of honey possess no immediate effect to human health as a result of non- carcinogenic related risks (Table 6). The Health Index (HI) for all the samples analyzed falls below the level of concern to human health, shows HI < 1 in all the samples as can be seen in Table 6. The THQ and HI analysis are health-based statistical probability expressed as a function of quantified level of concern; a process developed to exposure to environmental pollutants (Bwatanglang, 2019). The target hazard quotient (THQ) and health index (HI) obtained < 1 in all the sampling points also suggest no risks associated with the level of chloramphenicol, sulfonamide, tetracycline and streptomycin in honey, thus having less danger to human health through its consumption. But children are more adaptable to encountered the risks associated with their health due to the high value of THQ and HI observed more than the adults. Similar study on the risk assessment due to dietary exposure to oxytetracycline, tetracycline and chlortetracycline through milk consumption in India showed HQ < 1 (Chauhan *et al*., 2018).

Though ADI was not defined for chloramphenicol and the THQ and HI could not be evaluated in this study, the consumption of the honey samples analyzed in this study points toward potential health risk to chloramphenicol. Lack of availability of ADI for chloramphenicol further suggest a zero-tolerance level to the antibiotics. An ADI could not be established for chloramphenicol due to lack of genotoxic and toxicological data, in addition to lack of definable NOAEL (No Observe Adverse Effect Level) or LOAEL (Lowest Observed Adverse Effect Level) (EFSA, 2013). These makes residual level of chloramphenicol 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).

CONCLUSION

Honey is consumed on a large scale throughout the world and becomes mandatory to carryout microbial analysis, monitoring and evaluation of the risk to the health of

the consumers. The result of the microbial safety show that the samples studied are of very good microbiological and hygienic quality, though there were isolated micro-organisms in the honey samples from some sampling points. This maybe as a result of improper handling during the production process and also air dust from the environment. The presence of micro-organisms in honey can sometimes influence the stability of the product and its hygienic quality. The potential human health risks associated with exposure to antibiotic residues were found to be lower than each acceptable daily intake (ADI) but the presence of antibiotic and pesticide residues in honey is a concern to health. Due to the smaller body weight (15 kg) and physiological susceptibility in children, the EDI were observed to be higher in children than that of the adults.

RECOMMENDATIONS

The following recommendations have been suggested to enable better understanding and improve the results obtained from such research:

I. There should be educational programs to the bee keepers within the state on beehive management, this will create awareness on the safety of consumers and public health.

II. The presence of some pathogenic fungus in the samples necessitates an urgent need to always monitor microbial status of honey.

III. Microbial testing should guarantee both good hygienic and good marketable qualities of this products and good production efficiency.

IV. There should be continual monitoring and evaluation of antibiotic and residues in honey, this will help to assess the potential risks to human health.

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