UNIVERSITY FOR DEVELOPMENT STUDIES

ANTIBIOTICS RESIDUE AND RESISTANCE PROFILE OF BACTERIAL ISOLATES IN IMPORTED AND LOCALLY PRODUCED HONEY FROM LOCATIONS WITHIN THE TAMALE METROPOLIS OF THE NORTHERN REGION OF GHANA

BY

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in the university or elsewhere. Research works that were consulted have been duly acknowledged by way of references.

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Supervisor's Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Honey remains a valued natural product and has been used by humans as an important food source and for disease treatment since ancient times. Honey is often considered a healthy substitute to sugar. However, recent reports on adulteration of honey, and honey polluted with contaminants like pesticides, antibiotics, heavy metals as well as microorganisms have gained public attention. This news has instigated public fear on the consumption of honey since it is perceived to be sterile and of medicinal use. There have been few works in Ghana on the physicochemical properties and microbial contamination of honey. However, there is no known studies on *Listeria*, *Campylobacter* and *Clostridium* contamination of honey. Neither is there any report of detection of antibiotic residues in honey. Thus, this study assessed the quality and safety of imported and locally produced honey collected from locations within the Tamale metropolis of Ghana by specifically examining the microbial quality and antibiotic residues. The procedures outlined by the Association of Official Analytical Chemist (AOAC) was employed in determining the physicochemical quality whilst that described by the Codex Alimentarius Commission was used to determine the microbial quality of the imported (n = 7) and the locally produced (n = 23) honey samples. Whereas the presence of antibiotics residue was determined using the Premi® test kit, antibiotics sensitivity testing was done according to the Kirby-Bauer disc diffusion method. Results on the physicochemical quality analysis showed that sampled honey (both imported and locally produced) were within acceptable set standards. However, results on the physicochemical quality of the honey samples did not reflect on its microbial quality as high incidence of bacterial contamination and resistances was recorded in the study. Furthermore, 27(90%) of the honey samples tested positive for the presence of antibiotics residue of which 6(85.7%) were samples from imported source whilst the remaining 21(91.3%) were locally produced honey samples. High

incidence of bacterial contamination and antibiotic resistance recorded in this study gives an indication that all is not well in the honey industry and therefore the need for serious concern to avert possible health issues associated with the consumption of honey found within the metropolis.

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DEDICATION

This work is dedicated to all researchers and scientist working to overcome the war against multidrug resistant bacteria.

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LIST OF ACRONYMS

ANOVA	Analysis of variance
APHA	American Public Health Association
CFU	Colony-forming unit
cP	Centipoise
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
kg	Kilogram
meq	milliequivalent
min	Minute
ml	Milliliter
TLC	Thin Layer Chromatography
UK	United Kingdom
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.0 Background

Honey is a sweetener produced naturally by bees from the secretions or nectar of flowering plants and is available all over the world (Rao et al., 2016). The nectar from the flower or secretions from plants are converted into honey by bees (particularly those in the genera, *Apis*) through the process of regurgitation and evaporation, and afterwards stored primarily as a food source inside the beehive. As a complex mixture, honey basically consist of water, sugars, vitamins, minerals, nitrogenous compounds, and some acids (Bogdanov, 2015; Mijanur et al., 2014).

The benefits of honey have been realized largely in the food, pharmaceutical and cosmetic industries as a natural sweetener, therapeutic substance and cosmetic agent respectively (Ediriweera & Premarathna, 2012). Honey has long been employed in modern folk medicine for treating sore throats and coughs, gastric ulcers, infected leg ulcers as well as for topical treatment of measles (Molan, 2001; Samarghandian et al., 2017).

Bee products, particularly honey have the characteristics of being natural, and free from contamination. Nevertheless, in present time, bee products are produced in surroundings contaminated with pollutants of different sources (Iurlina & Fritz, 2005). The various ways through which raw materials (nectar, honeydew, pollen, plant exudates) of bee products get polluted with contaminants are by air, water, soil as well as from flora sources. These contaminants are transported into the beehive by the bees (Bogdanov et al., 2006). Contaminants of bee products especially honey may arise from sources such as acaride treatments (Nasr & Wallner, 2003),

polyaromatic compounds (Ciemniak et al., 2013), pesticides used in agriculture (Ridding et al., 2018) as well as microbial contamination (Olaitan & Iyabo, 2007).

In recent times, news about antibiotic-contaminated honey has gain mass media attention and such developments counteract to the health benefits derived from honey. Residues of antibiotics in honey are those that are applied for the prevention of bacterial honeybee diseases such as European foulbrood, and American foulbrood (Spivak & Reuter, 2016). Antibiotics aside its use as a therapeutic agent for the treatment of bacterial brood diseases, is also use at a relatively low dose by beekeepers as 'growth promoters' (Obakpororo et al., 2017). Some beekeepers consider the use of antibiotics in beekeeping as an alternative way of maximizing profit at the same time ensuring a less labor-intensive production system.

Antibiotic residues found in human food as a result of indiscriminate use is a calls for public health concern to consumers worldwide, due to possible toxic, allergic reactions, and the possibility of pathogenic organisms developing resistant to these antimicrobial agents due to exposure (Mahmoudi et al., 2014). Other notable pathological effects of antibiotic residues include carcinogenicity, bone marrow toxicity, and autoimmunity (Nisha, 2008; Pavlov et al., 2008).

These documented effects of antimicrobial residues have led to the development of varying biological and chemical tests to assess the presence, type and level of antimicrobial residues in food of animal origin (Oboegbulem & Fidelis, 1996; Pennycott, 1987). Among these developments include the European Four Plate Test (FPT), *Bacillus stearothermophillus* Disc assay (BsDa), the German Three Plate Test (TPT), the Premi® test and other available commercial test kits.

The Premi test which is based on microbial inhibition is more rapid with development times estimated between 3 to 4 hours (Schneider & Lehotay, 2008). This test has been successfully used for the rapid determination of Aminoglycosides, β-lactams, Macrolides, and Sulfonamides

(Jayalakshmi et al., 2017; Reybroeck, 2000). It is said that laboratories facing difficulties of analyzing large samples should adopt the rapid screening method as it narrows the sample size to those detected present and upon found to be non-compliant, then a confirmatory and/or a quantitative method (HPLC or LC/MS) becomes the next available option (Barganska et al., 2011).

1.1 Problem Statement and Justification

In Ghana, there is a high market demand for honey which significantly exceed supply (Akangaamkum et al., 2010), as a result of the recent trend of the middle classes becoming more aware and concern of the health impacts of sugar consumption compared to the perceived health benefits of natural honey. In spite of the country's good potentials for honey production, the demand for honey in the country is met through importation (Abdul-Malik & Mohammed, 2012). Furthermore, concerns about the poor quality of locally produced honey, as a result of the crude method of harvesting and adulteration compounds have led to the quality of local honey becoming questionable (Burns et al., 2018; Dinu, 2018). To affirm that local honey are supplemented with imported ones, a survey by the Netherlands Development Organization (SNV, 2006) recorded 18 imported brands of honey as against 7 made-in-Ghana honey brands on the Ghanaian market in Accra.

Unlike many other global bee-keepers, most Ghanaian bee-keepers have little or no knowledge of the treatment of bees with antibiotics. This is because *Apis*, the predominant genera of bees in Africa displays resistance to the varroa mite (*Varroa destructor* syn. *V. jacobsoni*) as such does not suffer from colony collapse disorder (Santos et al., 2016). In contrast, is the intensive use of antibiotics in professional beekeeping in the developed countries (Carrillo, 2014) for the treatment of bacterial brood diseases (Mutinelli, 2003). There have been a number of international reports

about antibiotic residues above the maximum residue limits in honey sampled from different countries (Korkmaz et al., 2017; Ullah et al., 2013)

In addition to increasing bacteria resistances, residues of antibiotics consumed along with honey can cause modification of the intestinal flora, dermatitis, induce allergic reactions, gastrointestinal symptoms, cutaneous eruptions, and anaphylaxis even at low doses (Nisha, 2008; Prajwal et al., 2017). Al-Waili et al. (2012), opines that the long-term effects of antibiotic residues consumed along with food can lead to reproductive effects, carcinogenicity, microbiological hazards, and teratogenicity. Due to the increased in number of bacteria resistance to antibiotics, antibiotic resistance is currently one of the world's most pressing health problems (Bacanil, 2019; Newman & Opintan, 2015). The World Health Organization has identified the continuing exposure to antibiotics through their use as human medicines as well as veterinary use for food producing animals as a cause for public health concern.

Nonetheless, in Ghana, only microbial pathogens, pesticide residues, heavy metals, and aflatoxins are extensively studied as measures to evaluate the safety of food meant for consumption, because they are perceived as hazards which poses serious threat to public health (Darko et al., 2017; Magna et al., 2018). The issue of antimicrobial residues in honey from both imported and local sources has almost never been a serious issue for researchers, parallel to the situation in livestock. Notwithstanding that, little has been reported on the antibacterial resistance or susceptibility pattern of bacteria isolates in honey. Some of the few works done on the Ghanaian artisanal honey were on the assessment of bacteria quality of honey produced in Tamale metropolis (Adadi & Obeng, 2017) and the technical efficiency of beekeeping farmers in Tolon-Kumbungu district of northern region of Ghana (Abdul-Malik & Mohammed, 2012). Currently, there is no available record on the screening or antibiotic residue in honey found on the Ghanaian market and this

presents an avenue for potential research. The need to investigate the antibiotic residues and resistance profile of bacteria isolates from imported and locally produced honey is thus timely and necessary to provide basis for intervention policies on the minimum limits of antibiotic residues present in honey.

1.2 Study objectives

1.2.1 Main objective

The main objective of this research is to assess the microbial quality and safety as well as the antibiotic dynamics of honey from both local and foreign (imported) sources sold at different retail outlets in the Tamale metropolis.

1.2.2 Specific objectives

The specific objectives of the research are:

i. Assess the microbiological quality of both imported and locally produced honey

ii. Determine the antibiotic resistance profile of bacteria isolates in both the locally produced and imported honey

iii. Determine the antibiotic residues of the artisanal produced and imported honey

iv. Determine the physicochemical properties of imported and locally produced honey.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Distribution of Honey

Honey is a natural sweetener produced by honey bees particularly those of the genera *Apis* from the nectar or secretion of plants (Alvarez-Suarez et al., 2014). These materials are collected, converted and combined with other substances of their (bees) own; deposited, dehydrated, stored and left in honey combs to ripe and mature (Codex Alimentarius Commission, 2001). Honey is believed to be the first discovered natural sweetener and has since been an important food source for *Homo sapiens* (Gangwar, 2016). Nayik et al. (2014), mentioned that the origin and accurate date of the existent of this old natural sweetener remains unknown since the evolution of man. In an attempt to trace the origin of honey some researchers sought to narrate human's involvement with bees, which is beekeeping.

Human's relationship with bees can be traced back to the Stone age (Bogdanov et al., 2008). However, archeological evidence of the development of beekeeping was found in a cave painting in Cueva de la Araña, Spain, and honey use on Sumerian clay tablets (Yaghoobi et al., 2008). Mahmoudi and Pakbin (2015), believes that beekeeping which is the rearing or keeping of bees for the production and harvesting of honey have been practiced by human since 4000 BC. Notwithstanding, in terms of honey production, the honey bee, *Apis mellifera* is of interest as it is the only specie that produces edible honey for human consumption (Israili, 2014).

Honey was in great demand in India, Assyria, Persia, Greece, Arabia and in the Roman Empire as a therapeutic agent for internal and external use (Jones, 2010). The nutritional and medicinal value of honey has been documented in several religious books by ancient scribes and scholars. In the

Holy Bible, the word 'honey' is mentioned 61 times in the various books of the Old and New Testaments. In the Islamic book of the Muslims, there is a whole 'Sura' dedicated for bees and honey. The Holy prophet Muhammad (SAW) taught his followers about the significance of honey and strongly recommended for them to take honey against some ailments (Lee et al., 2011; Rosner, 2000). Gangwar (2016), opined that globally, there is no country with a longer tradition of beekeeping than Ethiopia. There are about three to five million bee colonies in Ethiopia due to high demand of wax for religious ceremonies. India also has a lucrative Bee keeping industry. According to the Indian's beekeeping development committee's report (2019), beekeeping is currently the most widespread agriculture activity in India with about 1.4 million colonies of bee and 52, 000 tonnes of honey produced per year. Beekeeping is an integral component of Portuguese agriculture. There are more than 26,000 beekeepers in Portugal that produces an average of 11, 000 tons of honey per year (Iglesias et al., 2012). In Chile, varieties of unifloral and polyfloral honey are produced by the different 14, 000 beekeepers. It is estimated that the country has over 335, 000 apiaries (Muñoz et al., 2007). Akangaamkum et al. (2010), revealed that even though average honey production per hive throughout the world is 20 kg, countries like China, Argentina, Mexico, Canada, Australia and Hungary have high production per hive. In Ghana, the main type of honey produced is the multifloral type of honey because beekeepers have no controlled of bee foraging.

2.2 Composition of Honey

Honey, an easily digestible foodstuff contains different important nutritional compounds (Kaakeh et al., 2005). However, the composition and properties varies greatly on the sort, location, environment, time of collection and climatic condition (Naab et al., 2008). The major components of honey includes; saccharides, water, amino acids, proteins, vitamins and some unstable

compounds such as enzymes (Bogdanov et al., 2008; Omafuvbe & Akanbi, 2009; Ullah et al., 2018). With respect to saccharides, honey is mainly glucose and fructose, 31.0% and 38.5%, respectively (National Honey Board, 2008). Sabatini (2007), mentioned that sugars forms the primary constituents of honey, consisting of about 95% of the dry weight of honey. The sugar composition of honey can be determined by varying chromatographic methods, with high performance liquid chromatography HPLC been the most widely used method (Bogdanov et al., 2008).

The next predominant constituent of honey is water. However, the overall water content of honey is dependent on several environmental factors like the weather and humidity inside the hive as well as treatment of honey during extraction and storage. The amount of water in honey is critical for storage because honey with less than 18% of water can have a longer shelf life with minimal or no issue of fermentation (Doner, 1977). Hermosín et al. (2003), mentioned that darker honeys are substantially richer in minerals (such as potassium, chlorine, sulfur, iron, manganese and magnesium) than clear honeys. Azeredo et al. (2003), reported high protein content in honey although, the fraction of such amount of protein is considered low for human protein intake. Furthermore, proline has been reported as the major contributor (50%) of the overall amino acids found in honey (Iglesias et al., 2004). Perez et al. (2007), identified the main amino acids present in honey from different geographical origin and botanical as proline, tyrosine, leucine, phenylalanine, tryptophan, isoleucine, methionine, arginine, glycine, aspartic acid, glutamic acid, cysteine, lysine, histidine, valine ammonium ion, α- alanine, g-amino butyric acid, β-alanine, asparagine+serine, threonine and ornithine.

2.3 Classification of Honey

The classification of honey may be done on the basis of its origin, how it was harvested and processed, and its intended use. However, apiary honey and forest honey have been stated as the two types of honey (Akpabli-Tsigbe, 2015). Honey produced by *Apis mellifera*, and *Apis cerana indica* in apiaries and harvested by modern extraction techniques is the apiary honey while honey produced by the rock bee, *Apis dorsata* or from wild nest bee, *A. cerana indica* in forests, and is collected by the crude method of squeezing the comb is the forest honey (Manyi-loh et al., 2011). Other classification may exist as blossom honey, honeydew honey, monofloral honey and polyfloral honey (Ouchemoukh et al., 2007). The National Honey Board opines that more than 300 types of honey exist in the United States, with each of them from a different floral source.

2.4 Uses of Honey

Dating back to the era of hunting and gathering, the primitive man made use of honey both as a food source and for medicinal purposes (Bogdanov et al., 2008). Honey when consumed at high doses of 50-80 g per intake, has a number of positive nutritional, healing and prophylactic properties (Samarghandian et al., 2017). The medicinal properties of honey as an antiseptic for wound dressing has long been recorded on clay tablets dating from 1900 to 1250 BC (Stomfay-Stitz & Kominos, 1960). Honey was used by ancient Egyptians for treating infections of the eyes and skin diseases as well as for embalming (Al-waili, 2003). In ancient Greek, a mixture of honey and water was always given to athletes to drink before major athletic events as a therapy for fatigue (Crittenden, 2011). In Ghana and among the Bambara people of Mali, honey was mixed with lime leaves and palm kernel as a traditional medicine for wound healing and as a treatment for measles respectively (Jeffrey & Echazarreta, 1996). Honey was found to exhibit anti-inflammatory properties. In some clinical trials, honey was found to effectively reduce oedema around wounds

(Subrahmanyam, 1998), exudation from wounds (Molan & Rhodes, 2015) as well as soothing pains associated with wounds (Subrahmanyam, 1993). Church (1954), reported a decrease in stiffness of inflamed wrist joints of guinea pigs when honey was applied directly to the affected part. An added advantage of the consumption of honey as a food source is its ability to boost the immune system. Honey has unequivocally been demonstrated to stimulate T-lymphocytes and B-lymphocytes in cell culture to multiply and activates neutrophils (Tonks et al., 2003). Similarly, Tonks et al. (2007), revealed how the world most pure honey, manuka, stimulates the immune system to produce inflammatory cytokines which necessitates wound healing. The antibacterial effect of honey has been on the spotlight of most scientific journals. A laboratory studies on manuka honey reveals its antibacterial action against a broad spectrum of bacteria; *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus (*MRSA), *Pseudomonas aeruginosa*, and vancomycin-sensitive and vancomycin-resistant enterococci (Grecka et al., 2018; Liu et al., 2018; Mandal et al., 2010). As a cosmetic agent, honey is used in hair conditioners and skin moisturizes (Ediriweera & Premarathna, 2012). There is also a long history of honey as an antimicrobial agent. Honey was reported to have an inhibitory effect on the activities of the rubella virus (Jeffrey & Echazarreta, 1996). In one study, honey was found to be comparatively effective and safe in managing genital herpes than acyclovir, a synthetic cream use in treating the disease (Al-Waili, 2004). Also, there are studies confirming the antifungal actions of honey. Honey was reported to have exhibited an antifungal action by inhibiting the growth of Aspergillus, Penicillium and *Candida albicans* (Bansal et al., 2005; Brady et al., 1997; Obaseiki-Ebor & Afonya, 1984; Sampath-Kumar et al., 2010).

Gangwar (2016), mentioned how the belief of honey as a nutrient source, as a drug and ointment has been carried into our present day. In modern day Ghana, honey as a commodity is used in

several ways; as a food source, natural sweetener and flavor in beverages and confectionaries, as a cosmetic agent and for medicinal purposes. Honey has often been recommended as a food source for infants, pupils or students for the purpose of retentive memory or developing their intellectual ability and for the prevention of diabetes in the aged. Generally, utilization of honey in the country has been on the rise particularly since it inclusion in most herbal medicines as well as in pharmaceutical products (Akangaamkum et al., 2010).

2.5 Adverse Effects of Honey

Honey in its pure state has no adverse effect upon consumption. However, few cases of anaphylaxis such as dysphagia, cough, pruritic cheilitis, bronchitis, urticarial and angioedema have been recorded in both adults and young as a result of honey consumption (Tuncel et al., 2011). In infants, a large number of botulism cases have been reported with some severe neurological manifestations. Babies or infants fed with raw honey exposed to soil or dust contaminated with *Clostridium botulinum* accounts for incidence of botulism in infants (Koepke et al., 2008; Mari Nevas, 2006). This is in line with the US Center for Disease Control and Prevention regulation that honey should not be given to infants less than 12 months. Honey may also be contaminated with toxins acquired from the nectar of flowers. Consumption of such honey causes poisoning (Ajibola et al., 2012). Nonetheless, consuming honey in excess can result in hyperglycemia and gastrointestinal problems due to the high levels of fructose (Israili, 2014).

2.6 Quality Indices of Honey

The increasing level of demand for honey both locally and internationally have necessitated various studies on the quality and/or safety of honey produced in different countries and the setting of standards for quality check. At the moment, the quality and/or safety of honey is determined by

its sensory, physical, chemical, and microbiological characteristics (Belay et al., 2014). The European Commission Directive 2001/110 (EU, 2001) specified the physicochemical quality criteria for honey with main criteria of interest on moisture content, ash content, electrical conductivity, free acidity, reducing and non-reducing sugars, diastase activity, and hydroxymethylfurfural (HMF) content. The Codex Alimentarius standard for the physicochemical parameters of honey include moisture content, acidity, mineral content, diastase activity, hydroxymethylfurfural (HMF) content, apparent sugar content, and water insoluble solids content. In contrast, specifications on microbial and hygienic quality of honey have not been made available yet. However, current purchase specifications for microbial contamination in honey are often based on specifications for other ready to eat foods. The quality index for microbial contamination include standard plate count and tests for coliforms, moulds, yeasts and pathogenic bacteria such as *Salmonella, Staphylococcus* and *Clostridium* species (Snowdon & Cliver, 1996). Also, quality control of honey is imperative to estimate its suitability for processing as well as to meet the demand of international markets. In most developing countries, quality control aspect of honey is often disregarded by many producers and processors (Tesfaye, 2016).

2.7 Methods for Assessing Quality of Honey

In general, the physical, chemical and organoleptic qualities of honey is dependent on the sugar content, maturity or ripen stage of the honey, presence of certain active compounds (Pucciarelli et al., 2014). These factors as well as presence of microorganisms have high influence on the hygienic quality and stability of honey.

Even though different methods have been described for assessing quality of honey, yet researchers and regulatory authorities have not been reluctant in the search for recent, easy, sensitive, and economical methods (Ruiz-Matute et al., 2010). Thermal analysis, isotopic, chromatographic, and

nuclear magnetic resonance are some of the analytic techniques that have been employed in determining honey quality assessment (Arida et al., 2012). High performance liquid chromatography (HPLC) has been the most extensive used method for assessing the quality of honey but the gas chromatograph/mass spectrometry (GC/MS) technique is said to give a more precise results in assessing honey quality (Padovan et al., 2003). Nonetheless, as opined by Bogdanov et al. (1999) that generally the chemical composition of honey reflects its many physicochemical properties.

2.8 Physical and Chemical Characteristics of Honey

The physical characteristic of honey forms the basis of its characterization (Gangwar et al., 2010). The parameters often used to characterize honey are pH, colour, viscosity, hygroscopicity, density, crystallization, surface tension and thermal properties. These are comparatively simple to measure and provide a good information value. In some studies (Abdulkhaliq & Swaileh, 2017; Sohaimy et al., 2015), the diversity of physical characteristics of honey was found to be dependent on the nectar, pollen, colour, flavour, moisture and contents of sugars and proteins.

Honey as a food of animal origin is of high acidity with pH values estimated from 3.6 to 6.5 for the different types of honey (Bogdanov, 2009). pH is an important quality of honey as it influences greatly microbial growth and thereby contributing to the longer shelf life of the honey product (Gomes et al., 2010). According to Manyi-loh et al. (2011), gluconic acid constitutes the predominant acid in honey, in addition to formic acid, lactic acid and oxalic acid. The pH of honey is an important index of honey in determining adulteration and the possibility of microbial contamination. This is because; most bacteria grow in a mildly alkaline and neutral environment. Gebremariam and Brhane (2014), articulated that the pH of an adulterated honey is often higher than that of the pure honey. Also, pH as an important physical characteristic has an influence on

the texture, stability and shelf-life of honey (Terrab et al., 2002). The codex Alimentarius Commission (2001), recommends 3.4-6.10 as the pH of honey from the different floral sources.

According to Mohammed and Babiker (2009), the pH of honey does not constitute its total acid content but an indicator of its buffering action of inorganic cation from the present organic acids. Therefore, titratable acidity, total acidity and free acidity are mostly used as the total acidic content of honey. Estevinho et al. (2013), mentioned that the free acidity content of honey is due to the presence of organic acids with its corresponding lactones and some other organic ions. Azonwade et al. (2018), added that the free acidity of honey is the total free acids present in the honey which is expressed in milliequivalent per kilogram (meq/kg). However, the Codex Alimentarius Commission (2001), recommends 50meq/kg as the maximum permissible free acidity of honey.

Another important physical and sensory characteristic of honey use as a measure of its quality is its viscosity (Yanniotis et al., 2006). Honey described to be of high quality is usually viscous. Mohamed & Mohamed (2015), articulated that viscosity of honey is linked to the composition of its sugars, water and colloid content. However, temperature, the presence of crystals or colloids and moisture content have a high influenced on the viscosity of honey (Yanniotis et al., 2006).

Moisture content of honey is important in assessing the quality of honey particularly its risk of spoilage due to fermentation (Prica et al., 2014). Therefore, the amount of water contained in honey becomes an important parameter for its preservation (Sohaimy et al., 2015). The amount of water in honey is also an indicator of its botanical origin, the condition of storage and the degree of maturity (Osman et al., 2007). Due to artificial alteration in water content of honey its reliance as an indicator of its botanical origin becomes questionable and insufficient (Felsner et al., 2004). Generally, the moisture content in honey ranges between 15.1 to 21% (Gangwar, 2016). Moisture

content of 18% and below inhibits the successful multiplication and survival of microorganisms of all kinds (Roslan et al., 2015). Water is usually the most common agent of adulterating honey in attempt of making higher profits (Felsner et al., 2004; Osman et al., 2007).

Another quality index of honey is determining its total soluble solids. Generally, total soluble solids for honey are the different sugars found in it which accounts for about 80% or more solids by weight (Nyau, Mwanza, & Moonga, 2013). Furthermore, total soluble solids is a reliable index of adulteration and a critical factor in considering the glycemic index which is of a great concern for diabetic patients (Viuda-Martos et al., 2010).

Also, the sugar content of honey such as its total sugars, reducing sugars and non-reducing sugars are determined to assess adulteration by sugars. Atikah and Nadia (2018), mentioned that the total sugar content of honey is the totality of all oligosaccharides, disaccharides and monosaccharide whereas non-reducing sugar content indicates its sucrose content. A higher sucrose content of honey indicates adulteration of the honey with sugars or the inability of bees to convert the sucrose content in the honey (Krishnasree & Ukkuru, 2017). The Codex Alimentarius Commission (2001), states that the reducing sugar content of honey should not be more than 5% whilst that of total sugars and reducing sugar should be greater than or equal to 50%.

In order to identify the floral source of honey, the ash content is determined. Ash content is often used to identify honeydew type of honey because of its relatively high mineral contents (1.0%) compared to the other types of honey (Silici, 2011). Ash content found for floral honeys are between 0.1 and 0.3%. Ash content is influenced by the chemical composition of the nectar and it is generally low in respect to dry matter weight of honey (Gangwar, 2016).

2.9 Diseases of Honey Bee

The reduction in colony number of bees since 1975 has led to an exponential increase in the number of publications on honeybee colony losses (Requier et al., 2015).To explain the decline in honeybee numbers, scientist came out with three (3) main causes: Genetic diversity and vitality, Environmental stress, Parasites and Pathogens. The different pathogenic organisms found to infect bees are bacteria, virus, fungi and protozoan organisms. In addition to pests and predators attack on bees, several surveys have revealed the presence of other bee diseases such as Nosema, Chalk brood and Amoeba (Desalegn, 2006; FAO, 1989). Chalk brood is a fungus infection caused by Ascosphaera. Chalk brood disease affects the honeybee larvae by mummifying sealed brood of honeybee with subsequent weakness of the colony and death (Root, 1990). This disease was first reported in Ethiopia around Holetta with an infection rate of 37.12%, 19.89%, and 17.93% in Amhara, Oromia and Benshangul- gumuz, respectively (Aster et al., 2010). Nosema, on the other hand is a fungal disease that affects the intestinal tract of adult bees. A prevalence rate of 53.3% of nosema infection was recorded in Addis Ababa by Desalegn and Yosef (2005). However, a higher prevalence rate of 58%, 60% and 47% were reported in Oromia, Benishangul-Gumuz and Amhara regions, respectively (Aster et al., 2007). A Gram-positive bacterium known as *Paenibacillus* has been found as the causative agent of the American foulbrood (AFB). This disease attacks honeybee at the larval stage (Heyndrickx et al., 1996). European foulbrood which is also a destructive and deadly disease is caused by the bacterium *Melissococcus plutonius* (Bailey, 1983). A parasitic associated disease of the honeybee is Amoeba and it is caused by the parasite *malpighamoeba mellificae.* This disease shortens the life cycle of bees by destroying the malpigian tubules (FAO, 1989).

2.10 Contamination of Honey

Honey like other foods is susceptible to contamination and adulteration. Contamination of honey occurs during collection from different floral sources and processing of honey by the honeybee (Ajibola et al., 2012). The different environmental sources of honey contamination includes microbial contamination and chemical contaminants such as antibiotics, heavy metals, pesticides as well as other materials from air pollution (Al-Waili et al., 2012).

Natural activities as well as anthropogenic activities like mining, indiscriminate use of pesticides in agriculture results in the production of heavy metals found in the environment (Tmava et al., 2013). It is true living organisms require some amounts of certain heavy metals such as cobalt, zinc, iron, copper and magnesium. However, excessive intakes of these metals are detrimental (Chourpagar & Kulkarni, 2011). Rahman et al. (2012), reported on chronic toxicity such as impaired kidney function caused by cadmium and kidney problems such as nephritis and anuria by zinc and copper.

Pesticides are used largely in agriculture to protect crop as a way of maximizing productivity. Extensive use of pesticides under uncontrolled application causes contamination of the environment thereby affecting animal species and human being (Al-Waili et al., 2012). Mullin et al. (2010), reported over 150 different pesticides in colony samples. Organic contaminants and polychlorinated biphenyl which are produced from lubricants, motor oil and coolants which are classified as persistent pollutants can contaminate bee products. Even though, their quantities in beeswax are higher than in honey (Jan & Cerne, 1993). Also, in Ghana, honey extraction is done by any of these four (4) extraction procedures: cold dripping, hand squeezing or crushing, honey

press and solar extraction. These extraction methods have high tendency of exposing honey to microbial contaminants such as yeast, fungi, bacteria and molds (Arnon, 2018).

2.10.1 Microorganisms in Honey

People are more aware that the quality of their health is closely associated to the food they consume (Román et al., 2017). The inability to adhere to stringent hygienic practices when handling honey can adversely compromise its quality (WHO/ FAO, 2003). Joseph et al. (2007), opined that microbial contamination of honey serves as an indication of inadequate hygienic practices during collection, processing and storage. Microorganisms found in honey include bacteria, molds and yeast and these may come from bees, nectar and other external sources. As every living organism have normal flora, the intestine of bees constitutes 1% of yeast, 27% of Gram-positive bacteria and 70% of Gram-negative bacteria (Rada et al., 1997). Human beings, equipment, dust, wind, animals, water and insects have been studied to constitute secondary sources of microorganisms in honey (Sereia et al., 2017). Though, the antimicrobial properties of honey coupled with the low water activity inhibit the growth of many microorganisms but some pathogenic microorganisms have been found to survive under such conditions (Snowdon, 1999). Sereia et al. (2011), confirmed that, higher fluid grade as oppose to transmission currents and oxygen dissolution as well as the minimal antibacterial activity favours the growth of both aerobic and anaerobic osmophilic yeast. However, honey contamination with spores forming microorganisms has been documented in many countries (Różańska, 2011). In Finland, 16% of imported honey samples were found to be contaminated with spores of *Clostridium botulinum* (Nevas et al., 2002). The case was worse in California where six (6) of nine (9) samples that were contaminated with *Clostridium botulinum* had already been fed to babies (Midura & Arnon, 1976). In Brazil, 7.06% of honey samples assessed for bacteriological quality were found to be contaminated with *Clostridium botulinum*

(Midura & Arnon, 1976). In the Tamale Metropolis of the northern region of Ghana, 66.7% of honey samples analyzed were found to be contaminated with *Escherichia coli*, *Bacillus* spp., *Shigella* spp., *Staphylococcus* and *Streptococcus* spp. (Adadi and Obeng, 2017). Investigation on fungi contamination of honey has also been carried out. In one of such studies, out of a total 80 samples, 71 were found to be contaminated with fungi species (Martins et al., 2003). Nasser et al. (2004), also recorded fungi contamination of forty (40) samples representing 88.9% of the forty-five (45) samples tested. The presence of microorganisms in honey has adverse effects on consumer health as well as a negative impact on a country's economy as a result of economic losses due to spoilage (Davidson, 2001).

2.10.2 Antibiotics Residues in Honey

The beekeeping industry faces numerous challenges. Among these challenges are the increasing changes in agricultural practices, bulk use of chemicals such as pesticides, and the ever increasing use of antibiotics to control and treat bee infections by pathogenic microorganisms (Forsgren, 2010). This is why honey among other rich foods in the world is monitored for antibiotic residues (Mahmoudi et al., 2014). The minimal amount or fewer concentrations of drugs, their derivatives or active metabolites in a food sample after treating the animal is what is considered as its residue (Codex Alimentarius Commision, 1998). Antibiotic contamination in honey is largely as a result of the extensive application for the treatment of bacterial diseases affecting bees and recently a parasitic disease that affects adult bees (Mahmoudi et al., 2014). Nonetheless, antibiotics sprayed on fruit trees to treat fire blight can contaminate honey (Henzelin et al., 2007). Amid all concerns, antibiotic residues in honey have not been of a major concerned for investigation in Ghana. The extensive use of antibiotics as growth promoters and as treatment for bacterial infections leads to an accumulation of antibiotic residues in honey (Tillotson et al., 2006). The presence of antibiotic

residue in honey is not only as a result of the application of antibiotics in beekeeping but also the application of some herbicides. Sulfanilamide residues were found in honey samples in Switzerland as a result of the disintegration of the herbicide asulam (Kaufmann & Kaenzig, 2004). The use of antibiotics in beekeeping affects the quality of honey and makes it difficult to trade at international markets. There are several scientific studies and reports of antibiotic residues in honey. Antibiotics used in treating animal diseases have been found to occur in both animal foods and their products (Wassenaar, 2005). Residues of oxytetracycline and chloramphenicol in honey were found to exceed the regulatory standards set for honey (Ortelli et al., 2004; Saridaki-Papakonstadinou et al., 2006). Five (5) antibiotics compounds; doxycycline, tetracycline, chlortetracycline, oxytetracycline and chloramphenicol were determined in honey samples in China (Chen et al., 2001). In India, the occurrence of antibiotic residues in honey is very alarming and calls for concern. The Agricultural Processed Food Product Export Development Agency, in 2005, reported high levels of antibiotics in honey that have been exported from India to the EU and US markets (Al-Waili et al., 2012). The issue was worst in 2009-2010, where 29.2% (of 362 samples) of the samples tested were above the prescribed limit of antibiotics. Also, in Switzerland, a study involving seventy-five (75) samples of which thirty-four (Blasco, Fernandez, & Pena, 2003) had their origin from Asian countries, revealed chloramphenicol residues contained in thirteen (13) of the samples (Ortelli et al., 2004). Antibiotic residues contamination of honey is no exemption in Greece, where 29% of 251 honey samples had residues of tetracycline (Saridaki-Papakonstadinou et al., 2006). Residues of sarafloxacin, sulfadimidine, tylosin and sulfachlorypyridazine have been detected in honey samples in Granada and Almeria (Galarini et al., 2013). In Belgium, a study was carried out to assess whether sulfonamide containing beeswax could result in contamination of honey. Data gathered revealed that the greater the concentration

of sulfamethazine in the wax, the higher the concentration of sulfamethazine in honey (W Reybroeck, 2003). Residues of antibiotics in honey is a cause for concern due to its relatively long half -life and its direct toxic effect on consumers (Tillotson et al., 2006). The escalating number of resistant pathogens associated to the exposure of bacteria to antibiotics poses major problems on the treatment of patients with acquired resistant bacterial infections (Putman, 2000). Also, the presence of residues of antibiotics in honey might be unfavourable to sensitive individuals with allergic reactions.

2.11 Antibiotics

Among the most commonly used drugs in the world are antibiotics. Antibiotic refers to any therapeutic agent with anti-fungal, anti-parasitic, anti-bacterial and anti-viral activity against microorganisms. Serrano (2005), defines antibiotic as a chemotherapeutic agent that are sufficiently not injurious to the host used to control and managed bacterial infections. Even though, some people are reported to be ignorant of the roles antibiotics play in managing some common infections (Wise et al., 1998). The available antibiotics in the many chemical shops are of natural sources and of synthetic derived (Walsh, 2003). In the late 19th century, Louis Pasteur and Robert Koch demonstrated conclusively that some living organisms were the cause of the many diseases including anthrax and cholera (Madigan et al., 2006). This discovery sparked the interest of research into identifying an agent strong enough to eliminate the discovered microorganisms. Paul Ehrlich, who is credited as the founder of chemotherapy initiated the search for the 'magic bullet,' an agent that would kill the microbe without necessarily harming the patient (Strebhardt & Ullrich, 2008). It is believed that sulphonamide, penicillin, streptomycin and cephalosporin constitute the first discovered antibiotics. Until the 1940's, the most popular antibiotic in used was the organoarsenic compound which was later named as salvarsan (Schwartz, 2004). Arsphenamine

was discovered by Sahachiro Hata when working on several arsenic compounds in Paul Ehrlich's laboratory. Gerhard Domagk's ability to have proved the antibiotic property of sulphonamides as against infections of *Streptococcus pyrogenes* led to the acceptance of sulfa as the first antibiotic in 1932 (Zaffiri et al., 2012). However, Alexander Fleming is said to have revolutionized the concept of bacterial infection treatment with antibiotic (Fleming, 1922). Though, Fleming could not demonstrate the therapeutic value of penicillin, but he did evaluate the antimicrobial activity of moulds (Fleming, 1929). Mass production of penicillin began in the United States, United Kingdom and Australia after the successful treatment of wounded soldiers during World War II (Florey et al., 1943). Streptomycin, a popular antibiotic for treating pulmonary tuberculosis was discovered in the 19th century. Selman Waksman, a soil microbiologist is associated with the discovery of streptomycin (Pfuetze et al., 1955). Investigation of penicillin N led to the discovery of cephalosporin in 1961 (Hamilton-Miller, 2000). The year 1950 was termed as the 'golden age' of antibiotic discovery among the scientific community. It is believed it was in that year that half of the antibiotics known and use today were discovered (Wright, 2007).

2.11.1 Misuse of Antibiotics

The underlying factors influencing the misuse of antibiotics are really complex as several factors are associated with the overuse of antibiotics. Lack of health education seems to have compounded the overuse and misuse of antibiotics (Cebotarenco & Bush, 2007). However, the lack of legislation or restrictions in some countries in obtaining antibiotics plays a key factor on the rise in misuse of antibiotics (Morgan et al., 2011). In Saudi Arabia and some other Middle East countries, patients bypasses health care system and obtain antibiotics from private pharmacies without prescription from a doctor (Khalil et al., 2013). A recent survey conducted to assess the prevalence of misuse of antibiotics in Saudi Arabia, the study reported 38.7% and 57.8% among

Saudi adults and paediatric respectively (Alanazi et al., 2015). Nevertheless, antibiotics are not restricted to the treatment of bacterial infections even in countries with strict regulations and firm controls. In some of such countries, antibiotics that are not used to treat human infections are still found in large concentrations in the environment due to their use for farming purposes (M. L. Cohen, 2000; Martinez, 2009). Another cause of the emergence of superbugs or multi-drug resistant bacteria is the non-prescription use of antibiotics. Non-prescription is when there is little or no informed education on the appropriate use of antibiotics as well as appropriate regulations to minimize its adverse effect Guzman et al., 2007; Llor & Cots, 2009). Non- prescription use of antibiotics has been assessed to play a critical role in the misuse of antibiotics among individuals (Hawkey, 2008; Kumarasamy et al., 2010; Rossolini et al., 2008). Nonetheless, inappropriate prescription of antibiotics by healthcare professionals has also been studied to contribute to public misuse of antibiotics which in the long term, results in antibiotic resistance (Kotwani et al., 2010; Perz et al., 2002). Furthermore, self- medication has been found as an important behavioural aspect that contributes significantly to the misuse of antibiotics. Sarahroodi et al. (2010), opined that misuse of antibiotics as a result of lack of education is common in both low, middle- and high-income households as well as low and high educated societies. Misuse and overuse of antibiotics vary by geographical region; this practice is higher in Asia-Pacific countries (Lee et al., 2013). Furthermore, Van Boeckel (2014), argues that antibiotic consumption per person is high in middle income countries. They added that the extensive use and misuse of antimicrobial drugs in these countries have influenced the rapid increase in the frequency of resistant pathogens. Also, the intensive use of antibiotics in agriculture both as a veterinary drug for treatment purposes and in small doses as growth factors have resulted in an increased in the development of resistant bacteria. An example is the increase of a particular strain of salmonella resistance to five antibiotics from

0.6% in 1979 to 34% in 1996 (Gustafson & Bowen, 1997). Findings from the consumer tips association (CSPI) revealed that the continuous use of antibiotics for sub-therapeutic purpose in livestock resulted in a greater number of resistant bacteria (CSPI, 2007). This findings was affirmed in a research where of the 78 *Campylobacter jejuni* isolates in an Irish poultry industry, the resistance to ampicillin, tetracycline, naladixic acid, kanamycin, erythromycin, streptomycin and ciprofloxacin was 35.9%, 20.5%, 20.5%, 1.2%, 10.5% ,2.5% , and 17.9% respectively (Fallon, Osullivan, Maher, & Carrol, 2003). In Africa, country's like Kenya is reported to have about 14,600kg of active antimicrobial agents used in animal food production. Of these, cotrimoxazole and tetracycline is investigated to account for nearly 78% of the overall antibiotics use (Mitema et al., 2001).

2.11.2 Antibiotics used as Growth Promoters

The use of antibiotics varies in purpose across different geographical regions. However, included in this list though not exhaustive are some examples of antibiotics commonly used as growth promoters; Aminoglycosides, Bacitracin, Avoparcin, Nitrofurans, Virginiamycin, Avilamycin, Synercid, Fluoroquinolones, Sulphonamides, Flovomycin, Macrolides, Trimethoprine, Spiramycin and Bioprine (Ellerbroek et al., 2004). Lee (2001), opined that approximately 80% of all food producing animals received treatment of antibiotics either directly to combat bacterial infection or as a component of their feed for growth promotion. The purpose of antibiotics in animal rearing was for the control and treatment of diseases. Antibiotics have been helpful in the control or treatment of arthritis, gastrointestinal infections, mastitis, respiratory and other infections in farm animals (Draisci et al., 2001).

2.12 Antibiotic Resistance

Bacteria resistance to antibiotics has become a global concern which has necessitated the search for new antimicrobial compounds (Dixon 2003; Laxminarayan et al., 2013). Indeed, one of the most pressing problems pose to healthcare professionals is the rise in antibiotic resistance as a result of inappropriate prescription (Iyalomhe et al., 2011). Antibiotic resistance is described as bacteria ability to withstand or resist the bacteriostatic and/or bactericidal potency of an antibiotic (Lashley & Durham, 2007). The US Centre for Disease Control and Prevention reported that, at least 23, 000 people die each year due to the ineffectiveness of existing antibiotics (CDC, 2013). Health care professionals over the years have been working to associate antibiotic use and its resistance. Barbosa and Levy (2000), opines that establishing the amount of antibiotics use to its emerging resistance is not easy to investigate. This is because there is the general perception that antibiotics are confined to modern therapy. Wright and Poinar (2012), explain that since antibiotics are of ancient origin the evolution of resistance conferring genes and antibiotic biosynthetic genes started some million years ago. The emergence of antibiotic resistance was recognized soon after its discovery but less attention was given to it since there was the availability of several different classes. Aminov (2009), emphasized on how people were misled in thinking that the efficacy or the potency of antibiotic against its target organism would make the incidence of infectious diseases a thing of the past. This was actually confirmed when General William H. Stewart, a US surgeon publicly makes the declaration that, *"it is time to close the book on infectious diseases and declare the war against pestilence won"* (Sengupta, Chattopadhyay, & Grossart, 2013).

2.12.1 Antibiotic Resistance Mechanism

The diverse ways by which microorganisms particularly bacteria escape the potent effect of an antimicrobial agent are what is termed as resistance mechanisms. However, to appreciate the

mechanisms of resistance, it is imperative to take a closer look at the different mechanisms of action of the varying antibiotics and this has been presented as a snapshot in the figure below.

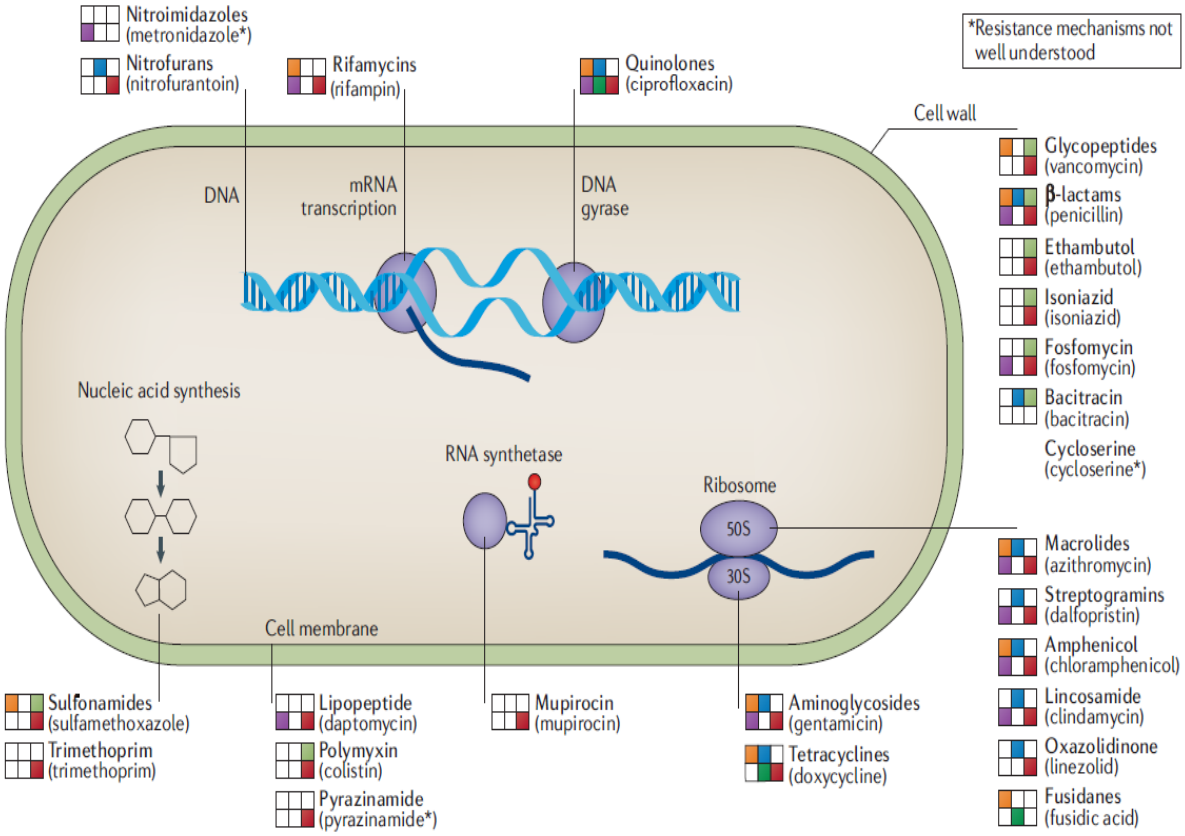


Figure 1: Mechanisms of actions of Antibiotics

Source: (Boolchandani, D'Souza, & Dantas, 2019)

Until recently, microorganisms particularly bacteria are known as one of the smartest creatures on earth and thus the phenomena *"Microbial Intelligence"* (Ford, 2017). As intelligent creatures there is no doubt that they will be armoring themselves against agents that threatens their survival. Therefore, as shown in (Figure 1) above bacteria have developed the following mechanisms to counteract the actions of antibiotics;

- The formation of bacterial cell interactions,
- Changes within the proteome,
- Ability to change within the existing genome of a bacteria cell and
- Acquisition of plasmids by horizontal gene transfer

The above mechanisms have been described as the four (4) major ways by which bacteria counteract the mechanisms of actions of antibiotics (Boerlin & Reid-Smith, 2008; Mah, 2012; Shousha et al., 2015; Woodford & Ellington, 2007). Millan (2018), opined that due to the possession of plasmid which is able to replicate independently of the chromosome some bacteria are able to transfer horizontally resistant genes between other bacteria by conjugation. The transfer of antibiotic resistance from lactic acid bacteria to Listeria in a food matrix has been reported by (Toomey et al., 2009). In an in vitro study, they observed the transfer of erythromycin resistance from lactic acid bacteria to Listeria. Also, Charpentier et al. (1999), were able to carry out the transfer of an antibiotic resistance gene (Pip823) from a *Listeria monocytogenes* to *E. coli*. Nevertheless, the human gut microbiota has been found as another important factor to the development of antibiotic resistance particularly in hospitalized patients (Millan, 2018). There is the possibility of the transfer of antibiotic resistance genes present in the gut microbiome to be horizontally transferred from these resident species to pathogenic species (Francino, 2016). *Salmonella* spp., *Campylobacter* spp., and *E. coli* are among the most known antibiotic resistant strains (Prakash, 2008).

On the other hand, some bacteria both Gram positive and Gram negative are known to secrete the enzyme B-lactamase which gives them the inherent ability of escaping the action of some antibiotics. Way back in the 1989, *Staphylococcus aureus* was reported to secrete large quantities

of B-lactamases which enables it to confer resistance to the antibiotic penicillin (Medeiro, 1997; Philippon et al., 1989). It is sad to know that over 1,000 β-lactamases have been described and reported and the list would not be dropping as far as evolution of bacterial is concerned.

Furthermore, the inactivation of antibiotics due to its modification by some bacteria through the production of enzymes are well documented. It is interesting to know that, majority of the antibiotics that are rendered ineffective as a result of enzymatic modifications works by inhibiting protein synthesis of the target bacteria at the ribosome level (Wilson, 2014).

Moreover, decreased antibiotic penetration and efflux activity can decrease permeability, an antibiotic resistance mechanism employed mostly by Gram negative. Efflux pump system was first observed in *E. coli*, in the year 1980, where the organism was able to pump tetracycline out of its cytoplasm (McMurry et al., 1980). Many classes of efflux pumps have been characterized since then in both Gram-positive and negative pathogens. Not only is this mechanism common among most bacteria but it also affects broad spectrum of antibiotic classes comprising of fluoroquinolones, polymyxins, β-lactams, carbapenems and protein synthesis (Munita & Arias, 2016).

2.12.2 Consequences of Antibiotic Resistance

One of the consequent failures of antibiotics due to bacteria resistance is the prolonged stay in hospitals and also the associated high mortality and morbidity (Davey et al., 2013). Also, residues of antibiotics consumed along animal foods have been found to cause hypersensitivity, gastrointestinal disturbance, tissue damage and neurological disorders (Lee et al., 2000). Occurrence of antibiotic residues in foods has been linked to the development of resistant strains of bacteria as a result of ingesting sub therapeutic doses of antibiotics (Hardman & Limbird, 2007).

In the United States, at least two (2) million people suffer infections with bacteria that are resistant to common antibiotics used in treatment of such infections. The consequences of antibiotic resistance are not only limited to health but have a great impact on the economy. The United States government spends $20 billion each year as a treatment cost on cases of resistant bacteria (Lee et al., 2013).

2.12.3 Public Health Hazards and Harmful Effects of Antimicrobial Residues

According to Crosby (1991), the harmful effects of the consumption of any animal product or products containing traces of antibiotics is exactly the same as the equivalent dose administered directly. Antimicrobial residues has been found to results in adverse effects such as allergic reactions, direct toxicity and development of antimicrobial resistance among bacterial pathogens (Dupont & Steel, 1987). This necessitates public health concerns since according to Booth (1988),these side effects are potential carcinogenicity, mutagenicity as well as causes teratogenic effects on human health.

2.12.4 Methods used to detect antimicrobial residues in food of animal origin

According to EEC (1986), residue is any substance possessing pharmacological action and of conversion product as well as other substances that can be transmitted to animal products and are likely to cause adverse effects on human health. In other words, residues are either parent compounds or their metabolites that may be stored, get deposited or otherwise accumulates within the cells, tissues, organs or edible products of animals following its use

Residue analysis methods are mostly classified as quantitative, semi quantitative and qualitative. These classifications include enzymatic calorimetric assay, microbial inhibition assay, receptor binding assay, immunoassay and chromatographic methods. Residue analysis has been found to

include both screening and confirmatory tests. Aerts et al. (1995), described screening methods as the first hand analysis of sample in order to establish the presence or absence of residues. The different screening methods described for the detection of antibiotics in foods of animal origin are enzyme-linked immunosorbent assay (ELISA), thin layer chromatography (TLC), the Nouws antibiotic test (NAT), a commercial ampoule test, the Premi® Test and others (Bansal, 2010; Gaurav et al., 2014; Shalaby et al., 2011). Even though, confirmatory methods give negligible false positive results but screening methods has been found to be easier, faster and the cheapest method for screening of any antibiotic residue in food from animal sources (Ebrahimpour et al., 2015; Igarashi et al., 1961).

Premi® test is a microbial inhibition screening test developed by DSM, Netherlands for the rapid detection of antimicrobials in foods of animal (Reybroeck, 2000). Stead et al. (2004), vividly described the test as a microbiological test which is based on the principle of inhibition of growth of microorganisms (*Bacillus stearothermophilus*); a thermophile sensitive to many antimicrobials. The Premi® test antibiotic residue screening can detect a broad spectrum of most veterinary used drugs (antimicrobials) in honey, fish, eggs, shrimps, feeds, urine and meat like beef, pork, poultry (Ezenduka et al., 2011; Popelka et al., 2005; Reybroeck, 2000). It employs the principle of agar diffusion test in that bromocresol purple the colour indicator changes to yellow when imbedded microorganism undergoes active metabolism. However, colour remains same if there is an inhibition of growth by the imbedded organisms as a result of the presence of an antibiotic.

Generally assessing microbial contamination is key to promoting health. However, there is little or no work on microbial profile in honey samples in the Ghanaian market. Therefore, there is the need for a study to access the microbial load in honey samples in the Ghanaian market. Antimicrobial resistance remains a global canker. Food safety, especially as a result of

antimicrobial residues in foods such as honey is increasingly becoming alarming. Although, there has been work on antimicrobial resistance and antimicrobial residues in food, the data in Africa, particularly in Ghana remains inadequate. Hence, the need to study the *Antibiotic Residues and Resistance Profile of Bacteria Isolates in Imported and Local Produced Honey in the Ghanaian Markets.*

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study Area

All honey samples were collected from locations within the Tamale metropolis of the northern region of Ghana. Tamale Metropolis is located in northern Ghana and lies between latitude 9^0 24'2.84''N and longitude 0^0 50'21.48''E. Laboratory procedures and experimentation were performed at the Spanish laboratory complex in the Nyankpala campus of the University for Development Studies.

3.1 Study Design

Antibiotic residues and resistance profile of bacteria isolates in locally produced and imported honey in locations within the Tamale metropolis was a two (2) phase study.

The first phase was a cross-sectional survey on the production and consumption of honey within the study area. Two (2) different sets of semi-structured questionnaires were administered to the producers and consumers of honey. Data was gathered on consumers preference for local and/or imported honey; consumers perception on the quality of locally and imported honey; honey producers knowledge on bee diseases; treating diseased bees with antibiotics and among others depending on the public knowledge of the use of antibiotics in honey production or beekeeping.

The second phase was on sampling and laboratory analyses of the randomly sampled honey from the different locations within the Tamale metropolis. Physicochemical analyses, microbiological quality, antibiotic susceptibility/sensitivity test and antibiotic residue determination were carried out.

In all 30 honey samples were collected for the study. The 30 honey samples were categorized as; Imported (honey from foreign sources); Branded (locally produced honey that are packaged and found on shelves of supermarkets and/or other shops), Unbranded (local samples obtained from open or local markets and sold by market sellers); and Producers (samples obtained directly from the production sites or from honey producers). Table 1 shows the distribution of the samples.

Table 1: Distribution of total samples collected for the study

Sample Category	№ of Samples	Percentage (%)
Imported	7	23
Branded	6	20
Unbranded	9	30
Producers	8	27
Total	30	100

3.2.1 Sampling

All honey samples were obtained from locations within the Tamale metropolis of the northern region of Ghana. The first set of samples which were those from imported sources were all obtained from supermarkets, mini-marts and shops within the Tamale metropolis. The second set of samples were local samples that were found in supermarkets, mini-marts and shops. These were designated as “Branded.”

Plate 1: Imported and Branded honey on shelves of a supermarket

The third batch of samples were those collected from market sellers from the various markets within the metropolis. These samples were obtained from the Aboabo market, Sakasaka market, Tamale central market, Lamashegu market, Fuo market and the Nyohini market. Honey samples from these sources were designated as "Unbranded." The last set of samples were those obtained from honey producers identified within the metropolis and those close to the metropolis like the Tolon District, Kumbungu District and Sagnarigu District.

Plate 2: Local samples from market sellers **Plate 3: A honey producing area**

All collected samples were wrapped with cling film to prevent as much dust particles as possible and packed into an ice chest containing ice packs and then transported to the laboratory.

Plate 4: Samples of honey in the laboratory

3.2 Analysis of Honey samples

The flow diagram (Figure 2) gives an overview of the laboratory analyses performed on the obtained honey samples.

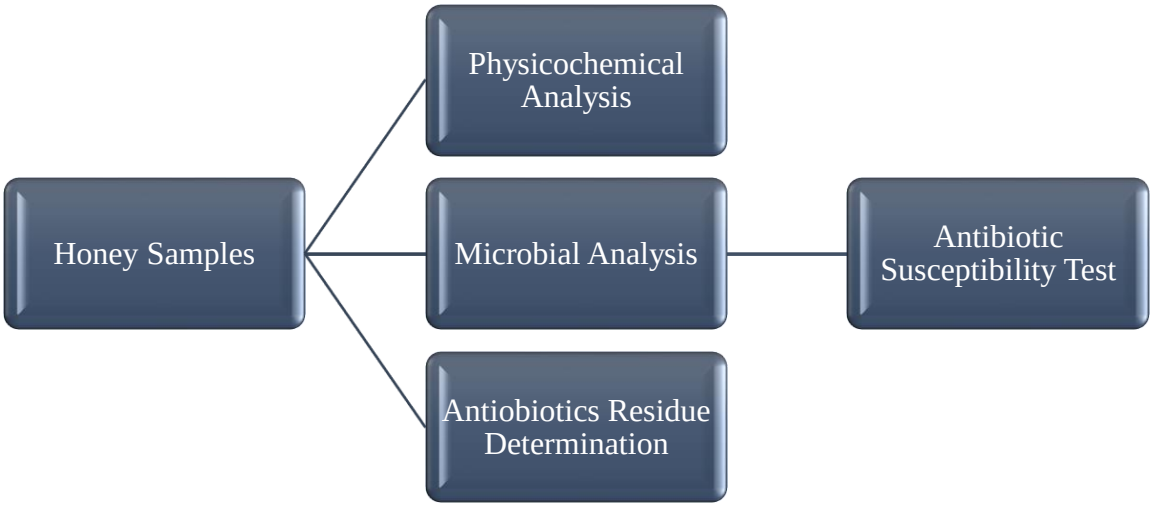


Figure 2: Flow chart of laboratory analysis

3.2.1 Physicochemical Analysis

The purity/quality of honey depends on its physical and chemical attributes. As such quality indices such as pH, total soluble solids, acidity, moisture, ash, viscosity, total sugars, reducing sugars and non-reducing sugar content of all the sampled honey were determined to assess their quality. All analysis was done in triplicates using standard protocols. However, results obtained for the physicochemical parameters of the 30 honey samples were compared to the International Food Standards for honey stated by the Codex Alimentarius for the quality of honey.

3.2.1.1 Determination of pH

pH of the honey samples was determined according to the method described by the International Honey Commission (2002). This was done by measuring five (5) grams of the sample and diluted

in a 50ml distilled water to make a 10% solution. The pH meter (pH meter Basic 20, Crison Instruments S. A., Barcelona, Spain) was first calibrated at a room temperature with buffers at pH 4 and 7 respectively. The electrodes were washed with distilled water and allowed to dry up with the use of a tissue paper upon each successive reading.

3.2.1.2 Determination of Total Soluble Solids

The method described by Mazumdar and Majumder (2003), was employed in the determination of total soluble solids of the 30 honey samples using the portable refractometer (Labolan S.L, Spain). The instrument was cleansed with distilled water and then adjusted to zero at a temperature of 20℃. A reasonable quantity of the honey sample was placed on the prism-plate of the refractometer and afterwards covered for the readings to be taken. Readings were directly recorded as total soluble solids in percentage.

3.2.1.3 Determination of Free Acidity

Free acidity of all the honey samples were measured according to the method outlined by Association of Official Analytical Chemist (AOAC, 2000). One gram (1g) of the sample was weighed and diluted with 20ml of distilled water in a 250ml conical flask. Two drops of phenolphthalein indicator were added and titrated against 0.1M sodium hydroxide (NAOH). The values obtained were subjected to the formula below.

$$\text{Free Acidity} = \frac{\text{Titre} \times \text{N} \times 196}{\text{Weight of Sample}} \times 100\%$$

Where N = normality of the base NaOH

3.2.1.4 Determination of Moisture Content

Five (5) grams of the samples were accurately weighed and dried in a hot air oven at 105℃ for 4 hours to a constant weight. This is according to the method described by AOAC (2000). The percent moisture content was calculated on dry basis as;

$$\text{Moisture content} = \frac{\text{Weight of wet sample-weight of dry sample}}{\text{Weight of wet sample}} \times 100\%$$

3.2.1.5 Determination of Ash

The determination of ash content was done according to the procedures outlined by AOAC (2000). To remove moisture that would cause foaming of the samples at early stages of ashing; two (2) grams of each of the samples were kept in a previously weighed porcelain crucible and dried in an oven at 105℃ for 4 hours. Upon removing the crucibles from the oven, they were then cooled in a desiccator. The materials were then ashed and dried in a hot muffle furnace at a temperature of 600℃ for 2 hours. The ash content was calculated on dry basis according to the equation:

$$\text{Ash content} = \frac{\text{Weight of ash} - \text{Weight of empty crucible}}{\text{Weight of sample}} \times 100\%$$

3.2.1.6 Determination of Viscosity

The viscosity of all 30 honey samples were determined according to the methods given by AOAC (2000) using the viscometer. Spindle number 4 was used and the results were recorded in centipoises (cP).

3.2.1.7 Determination of Sugars

Determination of sugars, that is total sugars, reducing sugar and non-reducing sugar were carried out through Lane and Eynon method as described by Shahnawaz et al. (2013).

Total sugars and reducing sugars were determined by weighing 5g of the sample and adding it to a 100ml of warm distilled water in a beaker. This solution was mixed by stirring until all the soluble matters got dissolved and then filtered through a Whatman filter paper into a 250ml volumetric flask. Hundred (100) milliliters of the solution was transferred by pipetting into a conical flask. Ten (10) ml of 0.1Mdiluted hydrogen chloride and 2-3 drops of phenolphthalein indicator was added and the solution was boiled for 3mins.

On cooling, 100 ml of the sample solution was pipetted and prepared into a burette. This solution was used for titration against Fehling's solution and the interpretation was calculated:

$$\text{Reducing sugar} = \frac{\text{Factor} \times \text{Dilution}}{\text{Titre} \times \text{Weight of sample} \times 10} \times 100\%$$

$$\text{Total sugar} = \frac{\text{Factor} \times \text{Dilution} \times 2.5}{\text{Titre} \times \text{Weight of sample} \times 10} \times 100\%$$

Non- Reducing sugar (NRS) was estimated by subtracting reducing sugar from total sugar,

That is, NRS% = Total sugar – Reducing sugar.

3.2.2 Microbiological Analyses

Microbial analysis of all honey samples was carried out in the Biotechnology/Microbiology at the Spanish Laboratory complex of the University for Development Studies. Enumeration of microorganisms were carried out following international rules and standards particularly that

described in the Compendium of methods for the Microbiological Examination of Foods (APHA, 1992). To determine the microbiological quality of the honey samples, mesophilic microorganisms like *Listeria spp*., *Staphylococcus spp*. which are commercial quality parameters; the indicators of sanitary quality (*Escherichia coli* and *Enterobacter*) and the indicators of safety *Campylobacter spp*., *Shigella spp*. and sulphite-reducing *Clostridium spp.* and *Salmonella spp.* were analyzed.

Glasswares such as borosilicate petri dishes, pipettes (Pobel), conical flasks (Pyrex), measuring cylinders (Pobel) and beakers (Borosilicate) were sterilized using the hot air oven (P Selecta, Spain) at 250℃ for 2hrs. All media except *Salmonella-Shigella* agar and Pipette tips were sterilized by autoclaving at 121℃ at 1atm pressure for 15mins. Inoculating loops and forceps were sterilized by flaming over the gas flame (blue flame) while laboratory benches were disinfected with 70% alcohol.

Sample preparation was done according to the procedures described by Instituto Português da Qualidade (2002) where 10g of the honey sample was weighed on an electronic scale (Sartorius CP224S, Germany), and homogenized with 90ml of peptone water (Oxoid, Basingstoke, UK). Also, for each honey sample, serial dilutions were carried out to the fifth (5^{th}) level, that is 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} using 0.1 % peptone water as diluents.

Microbial load was carried out according to the methods described by Speck (1992), with modifications. All media used were prepared according to the instructions of the manufacturers. About 15- 20 ml of the sterilized media were poured into each sterilized petri dishes and allow to cool for solidification. Upon solidification, 100ul of the prepared sample was inoculated on the surface of the solidified media. This was evenly spread across the plate by the use of sterilized glass beads. Inoculated plates were incubated in the incubator (P Selecta, Spain) at an inverted position and according to the growth condition/requirement of the microorganism.

3.2.2.1 Enumeration of Microbes

Escherichia coli counts were determined for all the 30 honey samples according to the procedure described by the Center for Food Safety and Applied Nutrition using MacConkey agar medium (Oxoid, Basingstoke, UK). Inoculated samples on MacConkey agar plates were incubated at 44.5℃ for 48hrs. Plates that showed pinkish colonies were selected and counted using colony counter (P Selecta Digital S, Spain).

Listeria spp. counts were determined following the procedures of International Organization for Standardization (2015) using Oxford *Listeria* Agar base (Alpha Biosciences). Presumptive *Listeria spp.* appearing gold with dark centers on agar plate were selected for count after 24 hours of incubation.

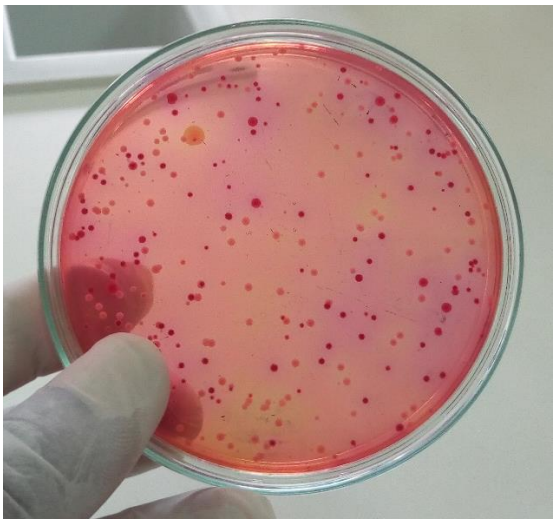


Plate 5: *E. coli* on MacConkey Agar Plate

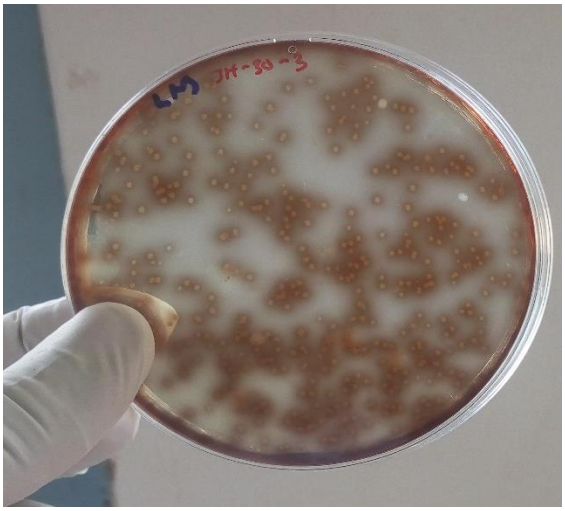


Plate 6: *Listeria* spp. on Oxford Agar Plate

Load of *Staphylococcus* spp. in all 30 honey samples were determined according to the Standards set by the Food and Drug Administration Bacteriological Analytical Manual (2001) using the

Mannitol Salt Agar (MSA, Oxoid, Basingstoke, UK). Suspected colonies of *Staphylococcus* spp. appearing pinkish on the MSA plate were selected for counting after 24 hours of incubation.

Load of *Salmonella* spp. and *Shigella* spp. were determined using Salmonella Shigella Agar base medium (SS Agar; Oxoid, Basingstoke, UK). The agar plates were inoculated heavily with the prepared sample whilst the spread plating method was employed to ensure an even distribution of the inoculum on the surface of the medium. The agar plates were incubated at 35℃ for 24hrs in an inverted style. After 24 hours of incubation plates with straw colored colonies with black centers and those without black centers were selected for count of *Salmonella* spp. and *Shigella* spp. respectively.

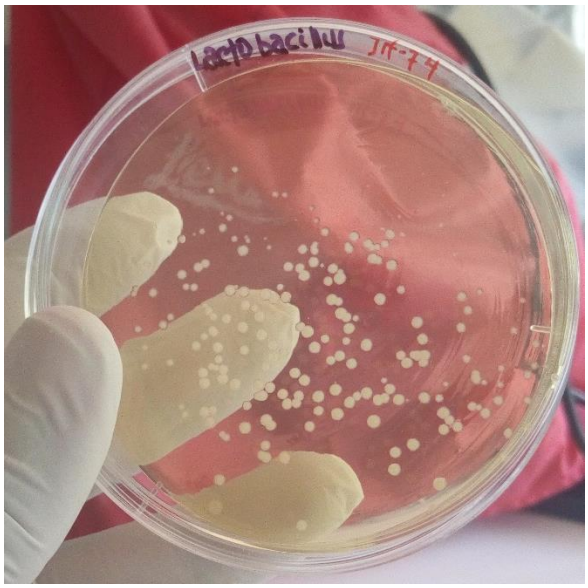
For the enumeration of *Campylobacter spp.* prepared samples were inoculated on Charcoal Cefoperazone Deoxycholate Agar (CCDA, Oxoid, UK) agar plates and incubated under micro-aerophilic condition generated by a gas generating pack (CampyGen™ 2.5L, Oxoid) in gas-tight containers at 42 °C for 48 hours. Presumptive Campylobacter spp. were selected for count after 48hours of incubation.

Lactobacillus MRS Agar (Alpha Bioscience) was used for the determination of load of *Lactobacillus spp.* in all thirty (30) honey samples whereas Perfringens Agar base (Oxoid, UK) was employed in the determination of counts of *Clostridium spp*.

3.3.3.1 Identification and Confirmation of Enumerated Microorganisms

After 24-48 hours of incubation, distinct colonies were streaked on a freshly prepared nutrient agar (Techno Pharmchem, India) and incubated at 37℃. This was carried out to obtain pure cultures

for identification and confirmation purposes. Biochemical tests including Gram stain, catalase test, citrate test and oxidase test were carried out to identify and confirm the isolates.



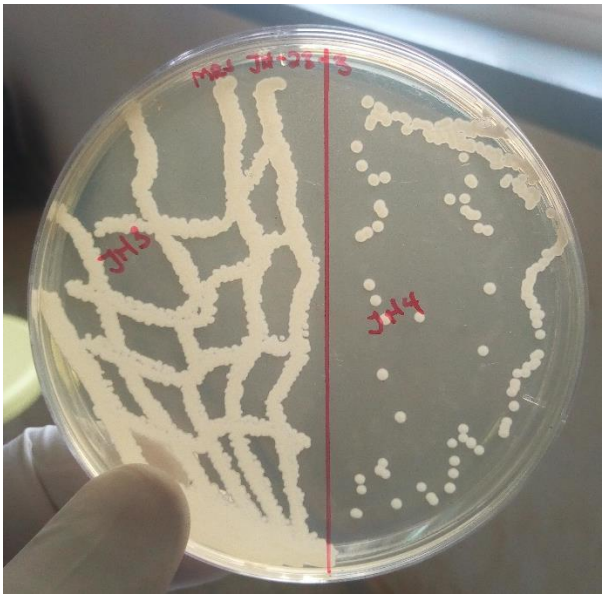


Plate 7: Colony after 24hrs of incubation **Plate 8: Distinct pure culture colonies**

3.3.3.2 Gram staining

Pure culture isolates were smeared on clean slides, air dried and heat fixed by passing over a flame. The slides were flooded with crystal violet solution for one (1) minute, washed with water and flooded with gram's iodine for one (1) minute. The slides were washed with water and decolorized with 95 % ethyl alcohol dropped from a dropping bottle until no violet color was visible from drain off solution. The slides were then washed with water and counter stained with Safranin stain for about 30 seconds and washed with water. The slides were air dried and examined under a microscope (Leica DMLS2 ×100, Wetzlar, Germany) using 100 X. Gram staining was performed according to the procedures described by Gonzelez-Zorn et al. (2015).

3.3.3.3 Catalase test

Catalase test was carried out for all the bacteria isolates according to the procedures described by Gonzalez-Zorn *et al*. (2015). A drop of Hydrogen per Oxide was put on a glass slide. Colonies were picked with a sterile loop and added to the drop. Observation was made for bubbles production. Colonies that have catalase are able to break down Hydrogen per oxide into water and Oxygen, which can be seen in the form of air bubbles leaving the solution.

Plate 9: Set up for catalase test

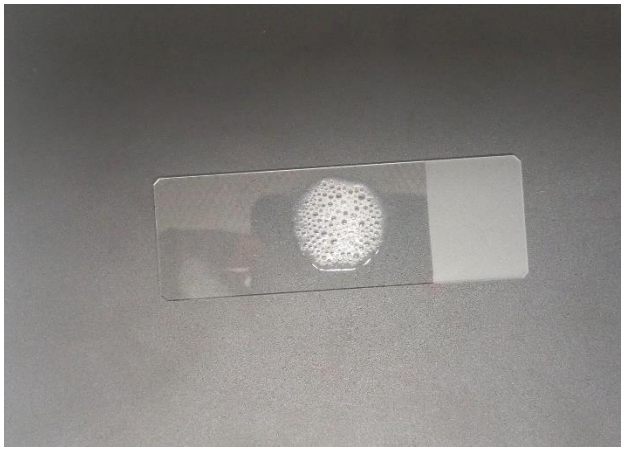


Plate 10: Catalase positive isolate

3.3.3.4 Citrate Test

Citrate test narrows down the choice of bacteria when making identification. Citrate test assesses the bacterial capacity to utilize citrate as the sole source of carbon and this was done according to the procedures described by Gonzalez-Zorn *et al* (2015). Distinct colony was picked with a sterile loop and streaked across the plate containing citrate media without breaking the agar and then incubated for 24 hours. Citrate positive isolates were determined by color change of the Simmons Citrate agar.

3.3.3.5 Oxidase test

Oxidase strips (Oxoid, UK) were used in confirmation of isolates from all the honey samples by randomly scooping colonies with sterilized loop onto the oxidase strips. It was then left for some few minutes to indicate a change in color from colorless to dark blue.

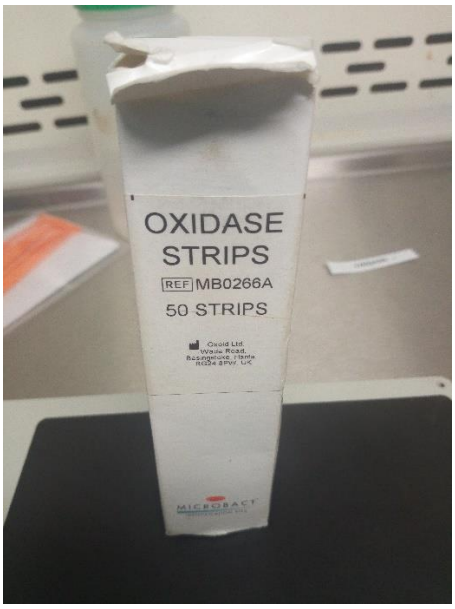


Plate 11: Oxidase test set up

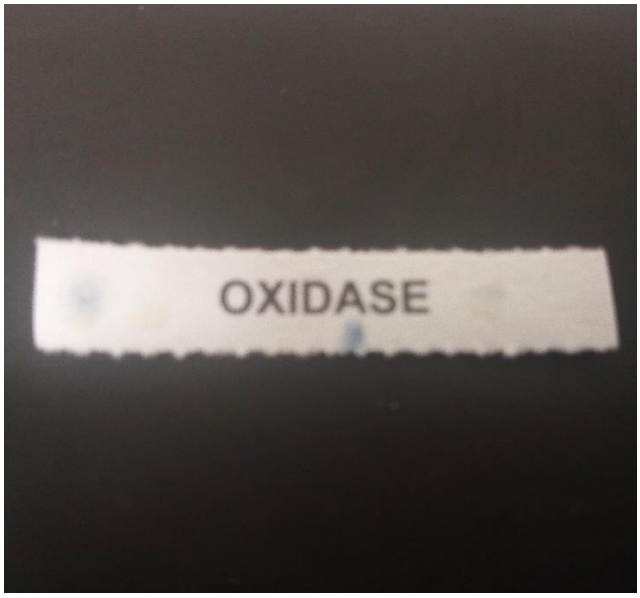


Plate 12: Oxidase positive isolate

3.3.3 Antibiotic Susceptibility/Sensitivity Test

Antibiotic sensitivity testing was carried out using Kirby-Bauer's disc diffusion method. Bacteria isolates were maintained on fresh nutrient agar plates and incubated overnight. Antibiotic sensitivity testing was carried out using Mueller-Hinton (MH) agar (Oxoid, UK). Fresh overnight pure cultures on nutrient agar plates were suspended in 0.9 ml sterile saline solution to attain a concentration commensurate with a 0.5 McFarland's standard. Sterile cotton swabs were dipped into the 0.5 McFarland's standard saline suspension, excess liquid from the swab was removed by pressing it against the inner side of the tube and three lines drawn on the MH plate with the swab in three different directions. The plate was swabbed in the same three directions to cover the entire

plate by streaking back and forth from edge to edge. The plate was rotated approximately 45° and the swabbing procedure was repeated the third time to ensure that the inoculum is evenly distributed (Hudzicki, 2010). A sterile forcep was used to transfer each antibiotic disc onto the inoculated MH agar plates and incubated for 24 hours at 37°C. The zones of inhibition produced by the antibiotics were measured and compared with the EUCAST tables (The European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of zone diameters. Version 8.0, 2018) to determine the susceptibility/sensitivity levels of the various bacterial isolates.

The antibiotic discs used for susceptibility/sensitivity testing was obtained from Axiom laboratories, New Delhi, India.

Table 2: Antibiotics discs for gram negative isolates

Antimicrobial Agent	Code	Content (mcg)
Ampicillin	AMP	10
Ceftriaxone	CTR	30
Ciprofloxacin	CIP	5
Cefotaxime	CTX	30
Amikacin	AMK	30
Cefuroxime	CXM	30
Gentamicin	GEN	10
Chloramphenicol	CHL	30

Table 3: Antibiotic discs for gram positive isolates

Antimicrobial Agent	Code	Content (mcg)
Azithromycin	AZM	30
Ciprofloxacin	CIP	5
Erythromycin	E	15
Gentamicin	GEN	10
Amikacin	AMK	30
Roxithromycin	RO	15

3.3.4 Antibiotic Residue Determination

Antibiotics residues in all thirty (30) honey samples were determined using the rapid screening method. This was done using the Premi® test kit (R-Biopharm AG, Germany). Hundred microliters (100µl) of each honey sample was pipetted into an ampoule bearing the sample's identification code. The ampoules containing the respective honey samples were pre-incubated at room temperature for 20 minutes. The ampoules were gently inverted to dispense the honey samples after the 20 minutes of incubation. Remains of honey were carefully removed by filling and emptying the ampoule with demi water whilst droplets of water were removed by putting the ampoule inverted on a tissue paper. All ampoules were covered with aluminum foil supply by the manufacturer to prevent evaporation at water bath's temperature. Finally, the tests ampoules were incubated in a water bath at 64℃ until the negative control changed color from purple to yellow.

3.3.5 Statistical Analysis

Responses from respondents on the production and consumption of honey and data on laboratory analysis were entered into Microsoft office Excel (2013) for processing and further analysis.

Whereas data from survey was analyzed using descriptive statistics and results presented on graphs and tables; data obtained from laboratory analysis was subjected to one-way analysis of variance (ANOVA) with mean comparison performed with the turkey multiple comparison test under 95% confidence interval. The General Statistics (Genstat) edition 18 was used in conducting both analyses.

CHAPTER FOUR

RESULTS

4.1 Survey on Honey Consumers

4.1.1 Background Information on Honey Consumers

A total of one hundred and thirty-two (132) respondents views were solicited for the present study (Table 4). Out of the 132 respondents, 92(69.7%) were males whilst 40(30.3%) were females. Also, the age group 20-29 constituted the greater percentage (85.6%) of respondents. Only 17(12.9%) of the respondents were married. Individuals with tertiary education dominated in terms of level of education of respondents.

Table 4: Demographic Characteristics of Consumers of Honey

Parameters	Category	Frequency	Percentage (%)
Gender	Male	92	69.7
	Female	40	30.3
Age group	Less than 20	2	1.5
	20 – 29	113	85.6
	30 – 39	16	12.1
	40 – 49	0	0
	50 – 59	0	0
	60 and above	1	0.8
Marital Status	Single	115	87.1
	Married	17	12.9
	Divorced	0	0
	Separated	0	0
Level of Education	Basic	0	0
	JHS	0	0
	Secondary	4	3
	Tertiary	128	97

	None	0	0

Source; Fieldwork, 2019

4.1.2 Consumption of Honey

Hundred and twenty-nine of the respondents representing 97.7% of the were consumers of honey out of the 132 respondents (Table 5). Out of these, 79(59.8%) were occasional consumers whereas 27(20.5%) rarely consumes honey. However, 3(2.3%) indicated they do not consume honey at all.

Table 5: Consumption Pattern of Honey

Parameter	Category	Frequency	Percentage (%)
Do you eat honey?	No	3	2.3
	Yes	129	97.7
How often do you eat honey?	Not at all	3	2.3
	Regularly/Daily basis	13	9.8
	Weekly	5	3.8
	Monthly	5	3.8
	Occasionally	79	59.8
	Rarely	27	20.5

Source: Fieldwork, 2019

4.1.3 Honey Purchasing Preferences of Consumers

Most of the respondents 35(27%) buy honey on yearly basis; 33(25%) buys honey on monthly basis; 6(4%) buy honey on occasionally; 4(3%) buy honey on weekly basis; and 1(1%) buy honey on a daily basis. Some respondents 44(33%) indicated they have family and/or friends who they obtained honey from; thus, they do not buy at all.

Also, on the preferred type of honey, 115(87%) of the respondents otherwise, referred here as consumers prefer the local honey to the foreign honey. Only 8(6%) of the respondents prefer the foreign honey to the local honey whilst 9(7%) prefer both the imported and locally produced honey.

According to 50(38%) of the respondents, reliability was their reason for their preferred honey whereas 25(19), 20(15%) and 17(12%) gave reasons as traceability, accessibility and patriotism, respectively for their preferred honey.

Meanwhile, 54(41%) prefer to purchase honey directly from the honey producers whilst 32(24%) and 24(18%) prefer to purchase from hawkers and supermarkets, respectively. However, summary of all the responses have been presented on Table 6 below.

Table 6: Honey purchasing preferences of consumers

Parameter	Category	Frequency	Percentage (%)
How often do you buy honey?	Daily	1	1
	Weekly	4	3
	Monthly	33	25
	Yearly	35	27
	Occasionally	6	4
	Rarely	9	7
	Not at all	44	33
Preferred honey Type?	Local honey	115	87
	Imported honey	8	6
	Both Local & Imported	9	7
Reasons for preferred honey	Pricing	5	4
	Accessibility	20	15
	Traceability	25	19
	Quality	9	7
	Taste	5	4
	Patriotism	17	12
	Reliability	50	38
	No reason	1	1
Preferred Source of Purchase	Distributors	12	9
	Hawkers	32	24
	Producers	54	41
	Retail Markets	10	8
	Supermarkets	24	18

Source: Fieldwork, 2019

4.1.4 Consumers knowledge on contamination of honey

The questionnaire inquired from respondent whether they have an idea concerning honey contamination. Here, 102(77%) respondents indicated they are aware that the quality of honey can be hampered, 25(19%) revealed that they had no knowledge on honey serving as a reservoir of contaminants whereas the remaining 5(4%) opined that they are not very sure about honey serving as a medium of contamination (Figure 3).

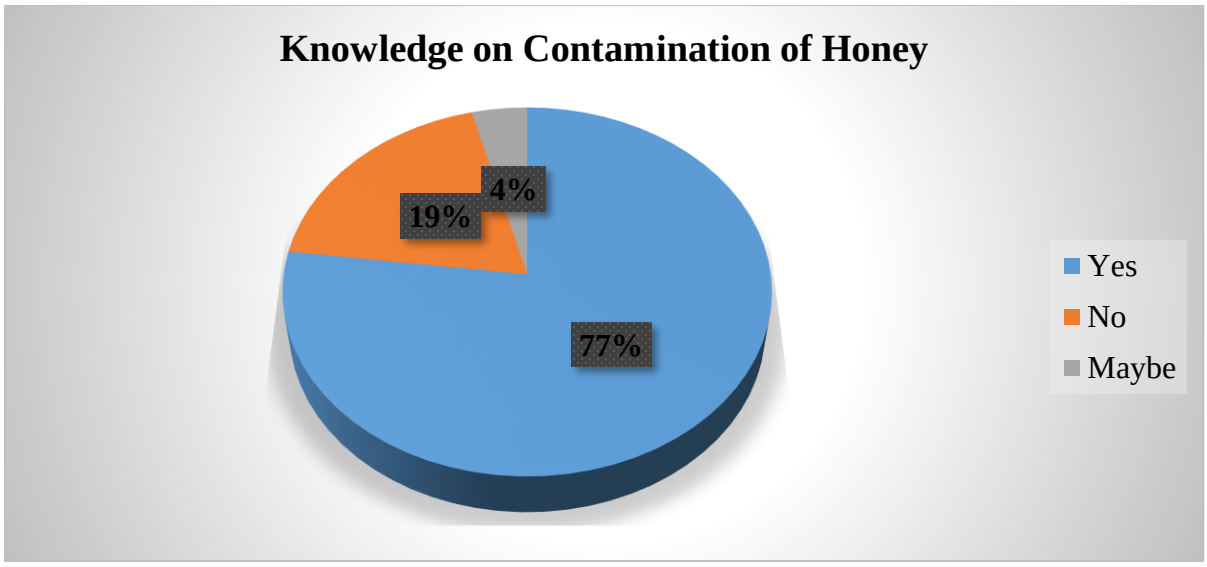


Figure 3: Consumers knowledge on contamination of honey

Source: Fieldwork, 2019

4.1.5 Consumers knowledge on antibiotics usage in beekeeping

To assess consumers' knowledge on the use of antibiotics in beekeeping, their knowledge on diseases affecting bees was sought. Out of the 132 respondents, 37(28%) of them indicated they are abreast with diseases affecting bees, 93(70%) of them revealed that they had no knowledge on diseases affecting bees whereas 2 confess they are not sure of diseases affecting bees.

Only 30(23%) out of the total respondents affirmed that they are aware of the use of antibiotics in beekeeping by beekeepers whereas 95(72%) and 7(5%) revealed that they are not aware and sure of the use of antibiotics in beekeeping respectively.

Concerning health implications associated with the consumption of honey with antibiotics residues, 16(12%) of the respondents, otherwise referred here as consumers, revealed that they are conscious of the risks of consuming honey contaminated with residues of antibiotics. The remaining 76(58%) and 40(30%) indicated they are not aware of the health implications of consuming honey with antibiotics residues.

Table 7: Knowledge of Antibiotic usage in Beekeeping

Parameter	Category	Frequency	Percentage (%)
Awareness of Diseases of bees	Maybe	2	2
	No	93	70
	Yes	37	28
Awareness of Antibiotic usage in beekeeping	Maybe	7	5
	No	95	72
	Yes	30	23
Awareness of dangers associated with consuming honey with antibiotic residues	Maybe	40	30
	No	76	58
	Yes	16	12

Source: Fieldwork, 2019

4.2 Survey on Honey Producers

4.2.1 Demographic Characteristics of Honey Producers

Table 8 gives an information on the demographic characteristics of honey producers identified from some locations within the region. A total of 8 honey producers were identified and interviewed for the study. On gender, it was observed that honey production was dominated by males 6(75%) as compared to their female 2(25%) counterparts. The active age group involved in the production of honey was 30-39 (75%). It happened that in terms of religion, respondents were either Muslim 5(62.5) or Christian 5(37.5%). Only two (2) of the respondents happens to have no formal education with the rest of the respondents passing through at least the secondary level of education.

Table 8: Demographic Characteristics of Honey Producers

Parameters	Category	Frequency	Percentage (%)
Gender	Male	6	75
	Female	2	25
Age group	Less than 20	0	0
	20 – 29	1	12.5
	30 – 39	6	75
	40 – 49	1	12.5
	50 – 59	0	0
	60 and above	0	0
Religion	Christianity	3	37.5
	Islam	5	62.5
Level of Education	Basic	0	0
	JHS	0	0
	Secondary	1	12.5
	Tertiary	5	62.5
	None	2	25

Source: Fieldwork, 2019

4.2.2 Sources of Honey

Honey producers were interviewed on the sources of their honey. Out of the 8 producers, only 2(25%) harvest honey from their apiary through beekeeping. The remaining 6(75%) producers confessed that they collect their honey from wild sources (Table 5).

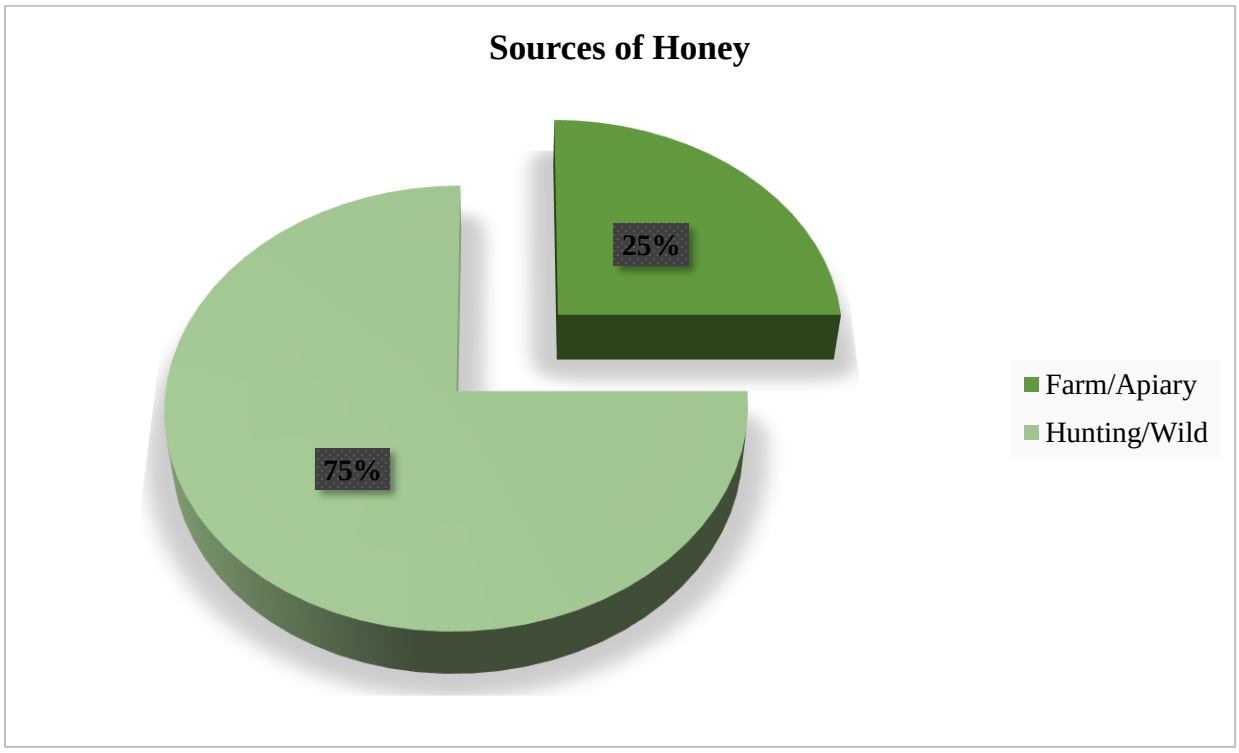


Figure 4: Sources of Honey

Source: Fieldwork, 2019

4.2.3 Producers knowledge on contamination of honey

Producers were assessed on their level of knowledge of contamination of honey using some key determinants. All eight (8) honey producers interviewed for the study expressed their awareness of adulteration of honey. Three (3) of the producers confessed that customers have been making complains about honey's adulteration. Four (4) of the producers opined that they have no information about customers complaining about the adulteration of honey whereas the remaining

two (2) producers asserted that customers do sometimes complain about honey adulteration (Table 9).

On the awareness of some producers adding additives to their honey before selling, 5(62.5%) of the producers answered yes whilst 2(37.5%) of the producers indicated there is the likelihood of some producers adding additives before sale of their products. All eight (8) honey producers indicated that they do not add additives to their honey before selling.

Five representing 62.5% of the honey producers keep their harvested honey in used plastic bottles for selling whilst 3(37.5%) of them uses new plastic bottles for the sale of their products.

Table 9: Perceptions of honey producers on contamination of honey

Parameters	Category	Frequency	Percentage (%)
Awareness of adulteration of honey	No	0	0
	Yes	8	100
Do customers complain of adulteration?	Sometimes	1	12.5
	No	4	50
	Yes	3	37.5
Are you aware some producers add additives before selling?	Maybe	3	37.5
	No	0	0
	Yes	5	62.5
Do you add any additive before selling?	Maybe	-	-
	No	8	100
	Yes	-	-

Containers for keeping the honey	Glass Bottles	-	-
	Metal containers	-	-
	New plastics	3	37.5
	Used plastics	5	62.5

Source: Fieldwork, 2019

4.2.4 Honey producer's knowledge on diseases affecting bees

Out of the eight (8) honey producers sampled for the study, only two (2) of them have knowledge on diseases affecting bees whilst the remaining six (6) have no knowledge on diseases that affect bees.

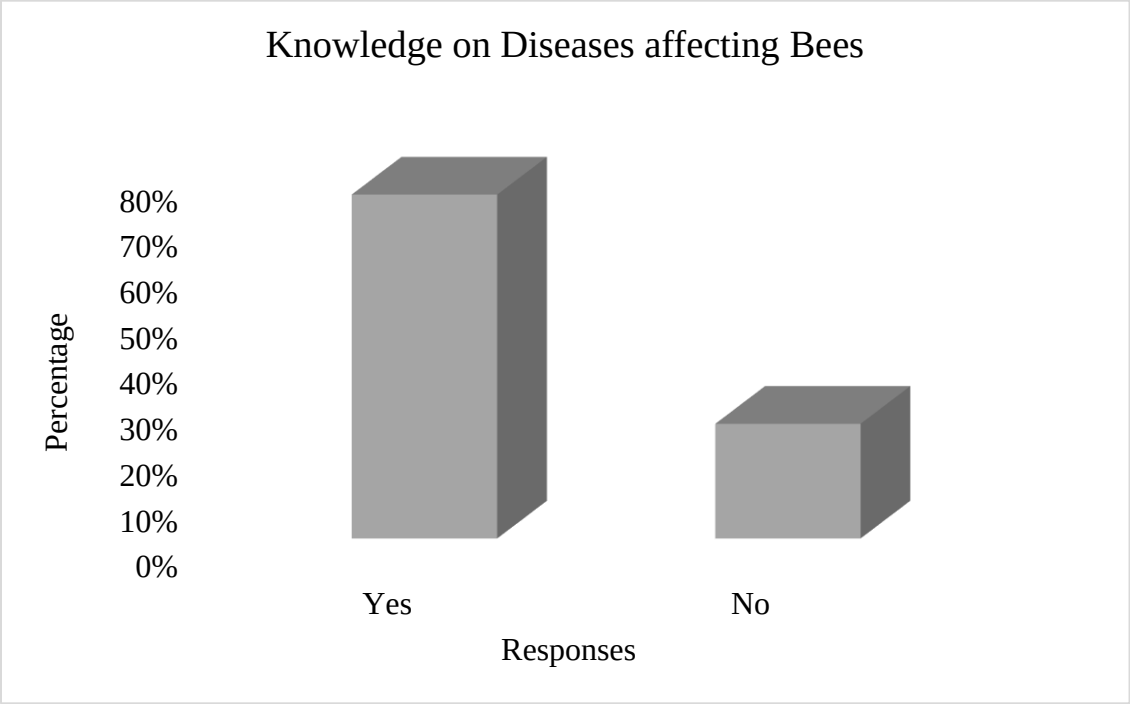


Figure 5: Honey producers' knowledge of diseases affecting bees

Source: Fieldwork, 2019

4.2.3 Honey producer's level of knowledge of antibiotics usage in beekeeping

Two representing 25% of the honey producers within the Tamale metropolis were aware or have knowledge on the use of antibiotics in beekeeping whilst the remaining 6(75%) have no knowledge on the use of antibiotics in beekeeping (Figure 7).

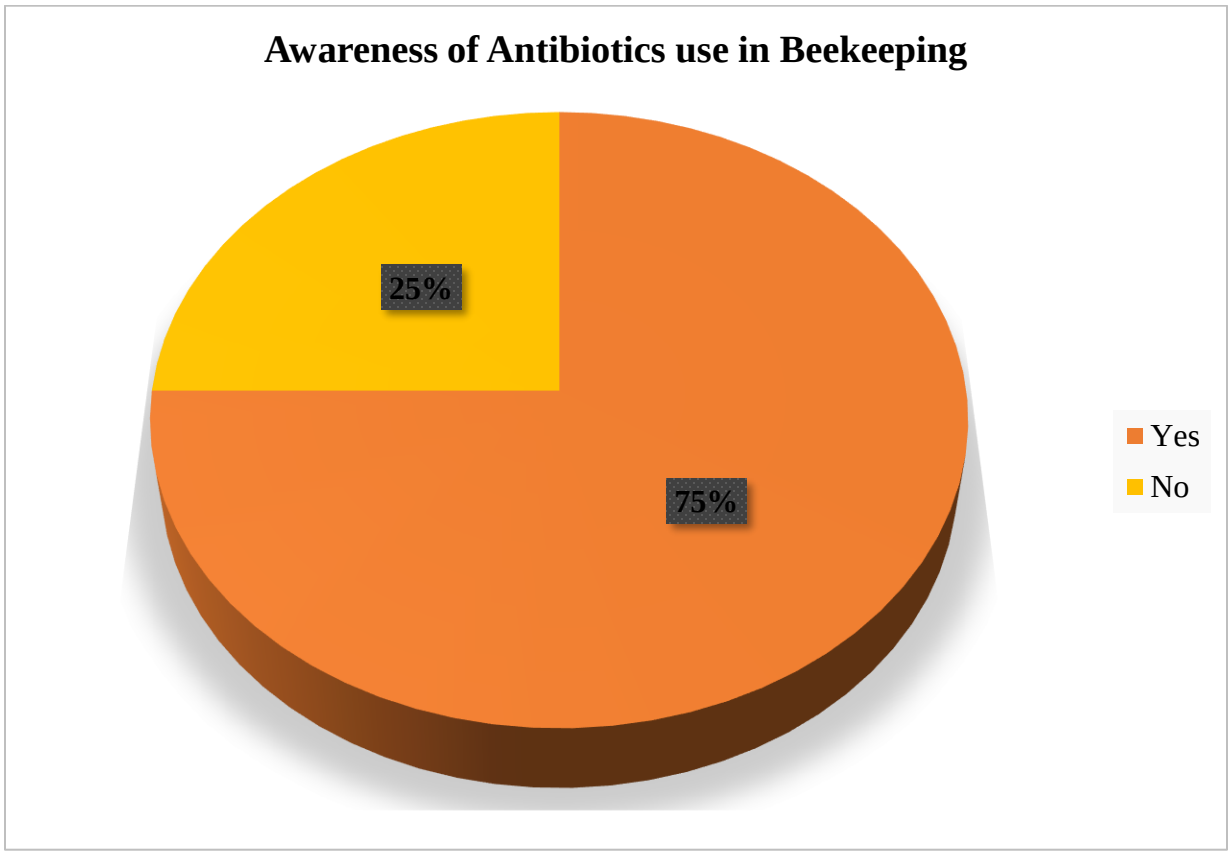


Figure 6: Awareness of antibiotics uses in beekeeping by the honey producers

Source: Fieldwork, 2019

4.3 Physicochemical Parameters of the Honey Samples

The pH of all the 30 honey samples ranged from 3.8 to 5.89. The mean pH of all the imported honey samples was 4.46 whereas locally produced honey samples recorded a mean pH of 4.77. There was significant difference ($P<0.05$) between the mean pH of imported and locally produced honey samples. The pH of samples from imported source ranged from 3.8 to 5.20 whereas that of locally produced samples was from 3.8 to 5.89. Also, the mean pH for the four categorizations were as follows, 5.14 for local samples obtained from supermarkets (branded), 4.65 for local samples collected from open markets (unbranded), 4.62 for samples obtained from production sites (producers), and 4.46 for samples from imported source.

However, free acidity for all the 30 honey samples ranged between 7.84 and 43.12 meq/kg with a mean of 18.11 meq/kq. There was significant difference ($P<0.05$) difference between the mean of 12.14 meq/kq and 19.92meq/kg recorded as free acidity for imported and locally produced honey samples respectively. Free acidity of samples from imported source ranged from 7.84 to 16.36 meq/kg whilst that of locally produced honey samples was from 7.84 to 41.16 meq/kg. Also, the mean of free acidity according to the four categorizations was 12.14meq/kg for samples from imported source, 15.71 for samples obtained from the production sites (producers), 21.15meq/kg for local samples obtained from supermarkets (branded), and 23.70 for local samples collected from open markets (unbranded).

For the thirty (30) honey samples, moisture content ranged from 12.41 to 19.55%. There was no significant difference ($P=0.97$) between 16.65% and 16.68% recorded as the moisture content for imported and locally produced honey samples, respectively. However, the moisture content of all 7 imported honey samples was from the range 13.9 to 17.89% whilst that of locally produced honey samples ranged from 12.41 to 19.55%. Also, the mean moisture content per the four

categorizations were as follows; 15.56% for samples obtained from the production sites (producers); 16.65% for imported samples; 17.20% for samples obtained from open markets (unbranded); and 17.38% for local samples obtained from the supermarkets (branded).

Viscosity values for the 30 analyzed honey samples ranged between 2112cP and 36000cP with an overall mean of 9473cP. There was significant difference ($P<0.05$) between 16198cP and 7426cP recorded as the mean viscosity for all imported and locally produced honey samples, respectively. However, viscosity of the imported honey samples ranged between 6288cP and 36000cP whereas that of local samples ranged between 2112cP and 17730cP. Also, the mean of viscosity per the four categorizations were as follows, 5182cP for samples from the open markets (unbranded), 6678cP for local samples obtained from supermarkets (branded), 10512cP for samples obtained from the production sites (producers), and 16198cP for imported honey samples.

The ash content of the 30 honey samples ranged between 0.03% and 1.31% with a mean of 0.27%. There was significant difference ($P<0.05$) between 0.12% and 0.32% recorded as the mean for all imported and locally produced honey samples, respectively. However, the ash content of the imported samples ranged from 0.04 to 0.26% whereas a range of 0.05 to 1.08% was recorded for locally produced honey samples. The means of ash content for the four categorizations were as follows, 0.33% for local samples from supermarkets (branded), 0.12% for samples from imported source, 0.31% for both samples from the production sites (producers) and open markets (unbranded), respectively.

Total soluble solids ranged from 62.47 to 98.87% for all the analyzed honey samples with a mean of 80%. There was significance difference ($P<0.05$) between 73.8% and 81.9% recorded as the overall mean for samples from imported source and locally produced samples respectively. Total soluble solids of the imported samples ranged from 68.07 to 81.6% whilst the range of 62.47 to

98.87% was recorded for locally produced honey samples. The means of total soluble solids per the four categorizations were as follows, 73.82% for imported samples, 79.79% for local samples obtained from open markets (unbranded), 82.68% for samples obtained from production sites (producers), and 83.93% for local samples obtained from the supermarkets (branded).

Also, total sugar content ranged from 51.65 to 80.03% with a mean of 60.37% for all the 30 honey samples. There was significant difference among some of the samples. Total sugar content ranged from 53.0 to 71.6% in imported samples whilst that of locally produced honey samples was from 51.65 to 80.03%. However, mean total sugars per the four categorizations were as follows, 59.42% for samples from imported source, 59.83% for samples from open markets (unbranded), 65.69% for local samples from supermarkets (branded), and 69.73% for samples from production sites (producers).

Meanwhile, 49.07 to 76.03% with a mean of 60.37% was the range of reducing sugar content for all the 30 honey samples. There was significant difference among some of the samples. Reducing sugar content ranged from 50.35 to 68.02% for samples from imported source whereas the range of 49.07 to 76.03% was recorded for locally produced honey samples. However, means of reducing sugar content according to the four categorizations were as follows, 56.45% for imported samples; 56.84% for local samples from open markets (unbranded), 62.41% for local samples obtained from supermarket (branded), and 62.25% for samples obtained from the production sites (producers).

Non-reducing sugar content was from the range 2.58 to 4% with a mean of 3.18% for all the thirty different honey samples. There was significant difference among some of the samples. The mean of non-reducing sugars ranged from 2.65 to 3.58% for imported honey samples and 2.58 to 4% for locally produced honey samples. However, means of non-reducing sugars per the four categorizations were as follows, 2.97% for samples from imported source, 2.99% for samples from

open markets (unbranded), 3.29%% for local samples from supermarkets (branded), and 3.49% for samples from production sites (producers).

Table 10: Physicochemical Parameters of Honey Samples

Sample ID	pH	TSS (%)	Acidity (meq/kg)	Moisture (%)	Ash (%)	Viscosity (Cp)	TS (%)	RS (%)	NRS (%)
JN-1	4.93^{jk}	78.00^{abc}	7.84^{a}	17.69^{a}	0.07^{a}	10520^{j}	53.00^{ab}	50.35^{ab}	2.65^{ab}
JN-2	3.95^{ab}	71.87^{abc}	31.36^{def}	16.5^{a}	0.08a	35812^{n}	54.41^{ab}	51.69^{ab}	2.72^{ab}
JN-3	3.80^{a}	72.20^{abc}	33.32^{efg}	17.13^{a}	0.13ab	32070^{m}	56.71^{abcd}	53.87^{abc}	2.84^{abc}
JN-4	4.71^{i}	74.33^{abc}	9.80^{ab}	17.51^{a}	0.07a	7120^{gh}	57.48^{abcd}	54.60^{abcd}	2.87^{abcd}
JN-5	4.52^{fgh}	81.60^{abc}	7.84^{a}	15.89^{a}	0.16^{abcd}	14582^{k}	59.14^{abcd}	56.19^{abcd}	2.96^{abcd}
JN-6	4.10^{bc}	68.07^{ab}	45.08^{g}	17.89^{a}	0.26^{abcd}	6394^{efg}	71.60^{abcde}	68.02 ^{abcde}	3.58^{abcde}
JN-7	5.03^{k}	98.87^{c}	33.32^{efg}	19.23^{a}	0.27^{abcd}	6548^{fg}	55.30^{ab}	52.53^{ab}	2.77^{ab}
JN-8	5.89^{p}	97.33^{c}	17.64^{abc}	17.09^{a}	0.27^{abcd}	7196^{gh}	77.01^{de}	73.16^{de}	2.65^{ab}
JN-9	5.37^{mn}	92.00^{bc}	9.80^{ab}	17.48^{a}	0.11^{ab}	5938^{defg}	60.15^{abcde}	57.15^{abcde}	3.01^{abcde}
JN-10	5.67^{o}	76.73^{abc}	17.64^{abc}	18.73^{a}	0.15^{abc}	9920^{ij}	75.66^{cde}	71.88^{cde}	3.78^{cde}
JN-11	5.69^{o}	62.47^{a}	7.84^{a}	16.63^{a}	0.18^{abcd}	5732^{defg}	71.60^{abcde}	68.02^{abcde}	3.58^{abcde}
JN-12	4.64^{ghi}	66.00^{ab}	39.20^{fg}	15.74^{a}	1.08^{f}	5028^{cdef}	66.90^{abcde}	63.56^{abcde}	3.35^{abcde}
JN-13	4.23^{cd}	72.67^{abc}	19.60^{abcd}	16.01^{a}	0.10^{ab}	4890^{cde}	59.14^{abcd}	56.19^{abcd}	2.96^{abcd}
JN-14	4.10^{bc}	85.33^{abc}	21.56^{bcde}	17.05^{a}	0.07^{a}	6994^{gh}	70.43^{abcde}	66.91^{abcde}	3.52^{abcde}
JN-15	4.07^{bc}	74.67^{abc}	17.64^{abc}	15.56^{a}	0.68^{e}	15054^{k}	80.03^{e}	76.03^{e}	4.00^{e}
JN-16	4.16^{cd}	72.67^{abc}	41.16^{fg}	12.41^{a}	0.43^{bcde}	9792^{ij}	77.23^{de}	73.37^{de}	3.86^{de}
JN-17	4.43^{ef}	94.20^{bc}	21.56^{bcde}	12.44^{a}	0.32^{abcd}	6886^{g}	70.43^{abcde}	66.91^{abcde}	3.52^{abcde}
JN-18	5.21^{lm}	70.67^{abc}	13.72^{ab}	13.9^{a}	0.04^{a}	9624^{ij}	63.60^{abcde}	60.42^{abcde}	3.18^{abcde}
JN-19	4.48^{efg}	92.00^{bc}	15.68^{ab}	13.82^{a}	0.14^{ab}	17130^{l}	80.03^{e}	76.03^{e}	4.00^{e}

JN-20	4.94^{jk}	70.07^{abc}	17.64^{abc}	15.4^{a}	0.15^{abcd}	5886^{defg}	53.00^{ab}	50.35^{ab}	2.65^{ab}
JN-21	4.31^{de}	80.67^{abc}	41.16^{fg}	17.69^{a}	0.37^{abcde}	4509^{bcd}	51.65^{a}	49.07^{a}	2.58^{a}
JN-22	4.75^{i}	75.20^{abc}	9.80^{ab}	18.8^{a}	0.49^{cde}	2418^{a}	52.31^{a}	49.69^{a}	2.62^{a}
JN-23	5.01^{k}	89.47^{abc}	21.56^{bcde}	13.93^{a}	0.50^{de}	3269^{ab}	69.65^{abcde}	66.17^{abcde}	3.48^{abcde}
JN-24	3.81^{a}	90.00^{abc}	17.64^{abc}	19.55^{a}	0.44^{bcde}	2216^{a}	72.95^{bcde}	69.30^{bcde}	3.65^{bcde}
JN-25	4.52^{fgh}	71.67^{abc}	9.80^{ab}	17.14^{a}	0.44^{bcde}	9742^{ij}	66.90^{abcde}	63.56^{abcde}	3.35^{abcde}
JN-26	5.04^{kl}	77.00^{abc}	29.40^{cdef}	17.25^{a}	0.21^{abcd}	8580^{hi}	57.06^{abcd}	54.21^{abcd}	2.85^{abcd}
JN-27	4.66^{hi}	86.73^{abc}	15.68^{ab}	18.08^{a}	0.05^{a}	6362^{efg}	53.72^{ab}	51.04^{ab}	2.69^{ab}
JN-28	4.81^{ij}	77.33^{abc}	17.64^{abc}	16.93^{a}	0.17^{abcd}	3650^{abc}	61.24^{abcde}	58.17^{abcde}	3.06^{abcde}
JN-29	5.42^{n}	91.13^{abc}	13.72^{ab}	17.45^{a}	0.32^{abcd}	10840^{j}	53.72^{ab}	51.04^{ab}	2.69^{ab}
JN-30	4.65^{ghi}	89.00^{abc}	21.56^{bcde}	19.11^{a}	0.31^{abcd}	9479^{ij}	54.41^{ab}	51.69^{ab}	2.72^{ab}
LSD 5%	**0.09**	**14.69**	**6.03**	**3.53**	**0.17**	**797.50**	**10.02**	**9.52**	**0.50**
Codex	**3.4 - 6.1**	**-**	**≤50meq/kg**	**≤21%**	**≤0.6**	**-**	**≥50%**	**≥50%**	**≤5%**
EU	**-**	**-**	**≤40meq/kg**	**≤21%**	**≤0.6**	**-**	**≥50%**	**≥50%**	**-**

Source: Laboratory Analysis, 2019 *Values in column with the same superscript are not significantly different*

*TSS – Total Soluble Solids *CAS – Codex Alimentarius Standard *EU – European Union Standard

*TS - Total Sugars *RS – Reducing Sugars *NRS – Non-Reducing Sugars

4.4 Occurrence of Bacteria Isolates in Honey

Listeria spp., *Lactobacillus* spp., *Salmonella* spp., *Shigella* spp., *Escherichia coli, Clostridium* spp., *Campylobacter* spp., *Enterobacter* spp., and *Staphylococcus* spp., were the microorganisms enumerated upon microbiological quality assessment of the thirty (30) honey sampled for the study.

Clostridium spp. were the most predominant bacteria enumerated from the analyzed honey samples. It was detected in 28(93.3%) of the honey samples. Sample JN-16 and JN-17 which had no growth of *Clostridium* spp. were samples obtained from the production sites (producers).

Lactobacillus spp. proceeded isolates of *Clostridium* spp. as the second most predominant bacteria and was detected in 27(90%) of the total honey samples. Again, the three (3) samples which showed no growth of *Lactobacillus* were samples obtained from the production sites (producers).

Listeria spp. was detected in 25(83.3%) of the total honey samples which was analyzed for the presence of *Listeria*. The five (5) samples which had no growth of *Listeria* were locally produced honey collected from the supermarkets (2) and the production site (3) respectively.

Growth of *Staphylococcus* spp. was detected in 21(70%) of the total honey samples. Only one (1) of the samples from imported source had no growth of *Staphylococcus* whilst eight (8) of the locally produced honey samples were free from *Staphylococcal* growth. Of the eight local samples that detected no growth of *Staphylococcus*, four was from local samples collected from supermarkets (branded) and the other four from samples obtained from the production sites (producers).

All thirty (30) honey samples were free from contamination of *Shigella* spp. However, two (2) of the samples obtained directly from local producers were not spared from contamination of *Salmonella* spp. and *E. coli* which are in the same family as *Shigella*. Meanwhile, sample JN-3 which was from an imported source was the only sample that recorded growth of *Campylobacter* spp.

Table 11: Occurrence of Bacteria Isolates in the Honey Samples

Bacteria	No. of Samples (+)	Percentage	No. of Samples (-)	Percentage	Total
Listeria spp.	25	83.3%	5	16.7%	30(100%)
Lactobacillus spp.	27	90%	3	10%	30(100%)
Shigella spp.	0	0%	30	100%	30(100%)
Salmonella spp.	2	7%	28	93%	30(100%)
Escherichia coli	2	7%	28	93%	30(100%)
Enterobacter spp.	0	0	30	100	30(100)
Campylobacter spp.	1	3%	29	97%	30(100%)
Clostridium spp.	28	93%	2	3%	30(100%)
Staphylococcus spp.	21	70%	9	30%	30(100%)

** Positive occurrence (+) ** Negative occurrence (-)

4.3 Microbial Load Profile of the Honey Samples

In determining the original cell density of each of the sample in colony forming unit per milliliter (CFU/ml), the range 30-300 colonies were adopted. With this, any plate that recorded colony count below 30 was considered statistically not significant thus non-detectable.

Out of the 30 honey samples, 24(80%) had detectable count for *Clostridium* spp. The load of *Clostridium* for the 24 samples ranged from 1.0×10^4 to 2.93×10^6CFU/ml with a mean of $8.642.93\times10^5$CFU/ml. There was significant difference ($P<0.05$) between $1.582.93\times10^6$CFU/ml and 6.46×10^5CFU/ml recorded respectively as the mean load of *Clostridium* for all the imported and locally produced honey samples that had detectable count. The mean load of *Clostridium* per the four categorization were as follows, 2.93×10^5CFU/ml for samples collected from the production sites (producers), 5.28×10^5CFU/ml for local samples collected from the supermarkets (branded), 1.04×10^6CFU/ml for local samples obtained from open markets (unbranded), and 1.59×10^6CFU/ml for samples from imported source.

Meanwhile, there was no significant difference ($P=0.26$) between 1.16×10^4CFU/ml and 8.38×10^3CFU/ml recorded respectively as the mean *Lactobacilli* load for the imported and locally produced honey samples. However, *Lactobacilli* load for the 25(83.3%) honey samples that had detectable count ranged from 3.0×10^2 to 2.84×10^4CFU/ml. The mean *Lactobacilli* load per the four categorizations were as follows, 6.65×10^3CFU/ml for samples from the production sites (producers), 8.52×10^3CFU/ml for local samples collected from the supermarket (branded), 9.8×10^3CFU/ml for local samples collected from open markets (unbranded), and 1.16×10^4CFU/ml for samples from imported source.

Also, there was no significant difference (P=0.47) between the mean *Listeria* load of 1.13×10^6CFU/ml and 8.95×10^5CFU/ml recorded respectively for imported and locally produced honey samples that had detectable count. Notwithstanding, *Listeria* load ranged from 3.15×10^5 to 2.93×10^6CFU/ml for the 25(83.3%) honey samples recording detectable count. Meanwhile, the mean *Listeria* load per the four categorizations was 5.92×10^5CFU/ml for samples collected from the production site (producers), 6.57×10^5CFU/ml for local samples from the supermarket (branded), 1.13×10^6CFU/ml for imported samples, and cCFU/ml for local samples from open markets (unbranded).

Conversely, there was significant difference (P<0.05) between 1.17×10^6CFU/ml and 5.53×10^5CFU/ml recorded respectively as the mean *Staphylococci* load for the imported and locally produced honey samples with detectable count. The range of *Staphylococci* load for the 21(70%) samples with detectable count was from 3.2×10^5 to 2.74×10^6CFU/ml. However, the mean *Staphylococci* load per the four categorizations was 1.26×10^5CFU/ml for local samples from the supermarkets (branded), 2.23×10^6CFU/ml for samples from the production sites (producers), 1.13×10^6CFU/ml for local samples from open markets (unbranded), and 1.17×10^6CFU/ml for imported samples.

However, only two samples (JN-29 & 30) from the production sites (producers) recorded detectable count of *E. coli* and *Salmonella* spp. out of the 30 honey samples. There was no significant difference between 4.05×10^5CFU/ml and 3.75×10^5CFU/ml recorded as the mean load of *E. coli* for sample JN-29 and JN-30 respectively. However, there was significant difference (P<0.05) between 9.85×10^3CFU/ml and 1.72×10^4CFU/ml recorded as the mean load of *Salmonella* for sample JN-29 and JN-30 respectively. Notwithstanding, details of the microbial load for the 30 honey samples have been presented in Table 12 below.

Table 12: Microbial load of the honey samples

Honey Sample	Source	*Listeria spp.*	*Clostridium spp.*	*Salmonella spp.*	*Lactobacillus spp.*	*E. coli*	*Staphylococcus spp.*
JN-1	Imported	2.78E+06^{g}	1.62E+06^{f}	ND*	2.55E+04^{l}	ND*	1.84E+06^{h}
JN-2	Imported	2.93E+06^{g}	2.34E+06^{hi}	ND*	2.20E+04^{k}	ND*	6.65E+05^{ef}
JN-3	Imported	5.30E+05^{bc}	2.53E+06^{i}	ND*	1.93E+04^{j}	ND*	1.98E+06^{i}
JN-4	Imported	3.80E+05^{abc}	1.00E+04^{a}	ND*	3.85E+03^{cd}	ND*	2.74E+06^{k}
JN-5	Imported	6.35E+05b^{c}	2.55E+05^{abcd}	ND*	5.40E+03^{defg}	ND*	3.80E+05^{bcd}
JN-6	Imported	3.15E+05^{ab}	2.52E+06^{i}	ND*	5.05E+03d^{ef}	ND*	5.80E+05^{e}
JN-7	Imported	2.88E+06^{g}	2.55E+06^{i}	ND*	2.53E+04^{l}	ND*	3.20E+05^{b}
JN-8	Branded	3.50E+05^{ab}	3.35E+05^{abcd}	ND*	1.80E+04^{j}	ND*	4.35E+05^{cd}
JN-9	Branded	3.65E+05^{ab}	1.70E+05^{ab}	ND*	3.55E+03^{cd}	ND*	ND*
JN-10	Branded	3.50E+05^{ab}	1.20E+05^{ab}	ND*	4.25E+03^{cdef}	ND*	ND*
JN-11	Producer	3.80E+05^{abc}	2.30E+05^{abc}	ND*	5.20E+03^{defg}	ND*	ND*
JN-12	Branded	ND*	ND*	ND*	ND*	ND*	ND*
JN-13	Branded	ND*	ND*	ND*	ND*	ND*	ND*
JN-14	Producer	8.45E+05^{cd}	9.45E+05^{e}	ND*	2.55E+03^{bc}	ND*	3.75E+05^{bcd}
JN-15	Producer	3.45E+05^{ab}	ND*	ND*	ND*	ND*	3.50E+05^{bc}
JN-16	Producer	ND*	ND*	ND*	ND*	ND*	ND*
JN-17	Producer	ND*	ND*	ND*	ND*	ND*	ND*
JN-18	Imported	3.50E+05^{ab}	1.79E+06^{fg}	ND*	3.00E+02^{ab}	ND*	ND*

JN-19	Producer	ND*	ND*	ND*	7.45E+03^{gh}	ND*	ND*
JN-20	Unbranded	2.90E+06^{g}	1.71E+06^{fg}	ND*	6.40E+03^{fg}	ND*	4.55E+05^{d}
JN-21	Unbranded	2.20E+06^{f}	2.93E+06^{j}	ND*	2.62E+04^{lm}	ND*	6.95E+05^{f}
JN-22	Unbranded	3.30E+05^{ab}	4.15E+05^{bcd}	ND*	3.80E+03^{cd}	ND*	2.63E+06^{j}
JN-23	Unbranded	3.20E+05^{ab}	4.25E+05^{bcd}	ND*	3.95E+03^{cde}	ND*	1.99E+06^{i}
JN-24	Unbranded	3.35E+05^{ab}	4.10E+05^{bcd}	ND*	3.35E+03^{cd}	ND*	3.95E+05^{bcd}
JN-25	Unbranded	2.56E+06^{fg}	3.95E+05^{bcd}	ND*	4.75E+03^{cdef}	ND*	2.01E+06^{i}
JN-26	Unbranded	3.25E+05^{ab}	5.00E+05^{bcd}	ND*	6.20E+03^{efg}	ND*	3.90E+05^{bcd}
JN-27	Unbranded	1.27E+06de	2.08E+06^{gh}	ND*	2.39E+04^{kl}	ND*	1.04E+06^{g}
JN-28	Unbranded	1.68E+06^{e}	4.80E+05bcd	ND*	1.00E+04^{i}	ND*	5.85E+05^{e}
JN-29	Producer	2.76E+06^{g}	5.55E+05^{cd}	9.85E+03^{b}	9.55E+03^{hi}	3.75E+05^{b}	4.65E+05^{d}
JN-30	Producer	4.04E+05^{abc}	6.15E+05de	1.72E+04^{c}	2.85E+04^{m}	4.05E+05^{c}	5.95E+05^{ef}
LSD 5%		***2.31E+05***	***1.16E+03***	***2.21E+03***	***1.16E+03***	***6.8E+04***	***5.17E+04***

ND: not detected

Values in column with the same superscript are not significantly different

4.6 Antibiotic Sensitivity Testing

4.6.1 Antibiotic sensitivity and resistant pattern of *Listeria spp*.

Antibiotic test was performed on all *Listeria* isolates enumerated from both the imported and locally produced honey samples. *Listeria* isolates from the seven (7) imported honey samples were all 100% susceptible to gentamicin and ciprofloxacin. In contrast, 1 out of the 18 isolates of *Listeria* from the locally produced honey samples showed 11% resistance to both gentamicin and ciprofloxacin. Also, whereas isolates from imported samples showed 28.6% and 71.4% intermediate and sensitivity responses to roxithromycin; isolates from local samples were 5.6% intermediate, 27.8% resistance and 66.7% susceptible to same antibiotic. However, isolates of Listeria from both imported and locally produced honey showed some level of resistance to amikacin, azithromycin and erythromycin. Notwithstanding, details of the antibiotic sensitivity and resistance pattern of all isolates of Listeria has been shown in Table 13 below.

Table 13: Antimicrobial susceptibility test for some common antibiotics of *Listeria* spp.

		Breakpoints (mm)			Antimicrobial Susceptibility № of isolates (%)		
Sample source	**Antimicrobial**	R	I	S	R	I	S
Imported	RO	NA	NA	NA		2 (28.6)	5(71.4)
	AMX	NA	NA	NA	5(71.4)	1(14.3)	1(14.3)
	E	NA	NA	NA	3(42.9)	3(42.9)	1(14.3)
	AZM	NA	NA	NA	1(14.3)		6(85.7)
	GEN	NA	NA	NA			7(100)
	CIP	NA	NA	NA			7(100)
Producer	RO	NA	NA	NA			5(100)
	AMX	NA	NA	NA	5(100)		
	E	NA	NA	NA	1(20)	2(40)	2(40)
	AZM	NA	NA	NA	1(20)		4(80)
	GEN	NA	NA	NA			5(100)
	CIP	NA	NA	NA			5(100)
Branded	RO	NA	NA	NA	1(25)		3(75)
	AMX	NA	NA	NA	2(50)		2(50)
	E	NA	NA	NA	2(50)	1(25)	1(25)
	AZM	NA	NA	NA			4(100)
	GEN	NA	NA	NA			4(100)
	CIP	NA	NA	NA			4(100)
Unbranded	RO	NA	NA	NA	4(44.5)	1(11)	4(44.5)
	AMX	NA	NA	NA	5(56)	2(22)	2(22)
	E	NA	NA	NA	8(89)	1(11)	
	AZM	NA	NA	NA	2(22)	1(11)	6(67)
	GEN	NA	NA	NA	1(11)		8(89)
	CIP	NA	NA	NA	1(11)		8(89)

NA: non-applicable **R**: Resistant **I**: Intermediate **S**: Susceptible **RO**: Roxithromycin **AMX**: Amikacin **E**: Erythromycin **AZM**: Azithromycin **GEN:** Gentamycin **CIP**: Ciprofloxacin

4.6.2 Antibiotic sensitivity and resistant pattern of *Clostridium* spp.

Antibiotic test was performed on all *Clostridium* isolates enumerated from both the imported (7) and locally produced honey samples (21). *Listeria* isolates from the seven (7) imported honey samples were all 100% susceptible to gentamicin and ciprofloxacin. In contrast, 5(23.8%) and 6(28.6%) out of the 21 isolates of *Listeria* from the locally produced honey samples showed resistance to gentamicin and ciprofloxacin respectively. Also, isolates of *Clostridium* from both the imported and locally produced honey samples showed some level of resistance to roxithromycin, amikacin, erythromycin and azithromycin. Table 14; present details of the antibiotic test of isolates of *Clostridium* to some commonly used antibiotics.

Table 14: Antimicrobial susceptibility test of some common antibiotics of *Clostridium* spp.

		Breakpoints (mm)			**Antimicrobial Susceptibility № of isolates (%)**		
Sample source	**Antimicrobial**	R	I	S	R	I	S
Imported	RO	NA	NA	NA	1(14)	2(29)	4(57)
	AMX	NA	NA	NA	7(100)		
	E	NA	NA	NA	3(43)	4(57)	
	AZM	NA	NA	NA		3(43)	4(57)
	GEN	NA	NA	NA			7(100)
	CIP	NA	NA	NA			7(100)
Producer	RO	NA	NA	NA	2(33)		4(67)
	AMX	NA	NA	NA	3(50)		3(50)
	E	NA	NA	NA	3(50)	3(50)	
	AZM	NA	NA	NA	2(33)	4(67)	
	GEN	NA	NA	NA	2(33)		4(67)
	CIP	NA	NA	NA	2(33)		4(67)
Branded	RO	NA	NA	NA	3(50)		3(50)
	AMX	NA	NA	NA	3(50)		3(50)
	E	NA	NA	NA	1(17)	4(66)	1(17)
	AZM	NA	NA	NA	1(17)	1(17)	4(66)
	GEN	NA	NA	NA	3(50)		3(50)
	CIP	NA	NA	NA	2(33)		4(67)

Unbranded	RO	NA	NA	NA	3(33.3)	3(33.3)	3(33.3)
	AMX	NA	NA	NA	3(33.3)		6(66.7)
	E	NA	NA	NA	4(44.4)	2(22.2)	3(33.3)
	AZM	NA	NA	NA	2(22.2)	2(22.2)	5(55.6)
	GEN	NA	NA	NA	1(11)		8(89)
	CIP	NA	NA	NA	1(11)		8(89)

4.6.2 Antibiotic sensitivity and resistant pattern of *Lactobacillus* spp.

All *Lactobacillus spp*. isolated from samples from imported sources were 7(100%) susceptible to roxithromycin, azithromycin, gentamicin and ciprofloxacin. Eighty-six percent 6(86%) of the isolates were resistant to amikacin whilst only 2(29%) were susceptible to erythromycin.

However, isolates of Lactobacillus from locally produced honey samples were 2(10%) resistant to roxithromycin; 3(15%) resistant to ciprofloxacin; and 1(5%) resistant to azithromycin and gentamicin respectively. Table 15 gives a summary of the sensitivity and resistance profile of isolates from the different sources.

Table 15: Antimicrobial susceptibility test for some common antibiotics of *Lactobacillus* spp.

		Breakpoints (mm)			Antimicrobial Susceptibility № of isolates (%)		
		R	I	S	R	I	S
Sample source	**Antimicrobial**						
Imported	RO	NA	NA	NA			7(100)
	AMX	NA	NA	NA	6(86)		1(14)
	E	NA	NA	NA		5(71)	2(29)
	AZM	NA	NA	NA			7(100)
	GEN	NA	NA	NA			7(100)
	CIP	NA	NA	NA			7(100)
Producer	RO	NA	NA	NA	2(40)		3(60)
	AMX	NA	NA	NA		5(100)	
	E	NA	NA	NA	2(40)	2(40)	1(20)
	AZM	NA	NA	NA			5(100)
	GEN	NA	NA	NA			5(100)
	CIP	NA	NA	NA	1(20)		4(80)
Branded	RO	NA	NA	NA			6(100)
	AMX	NA	NA	NA	5(83)		1(17)
	E	NA	NA	NA		3(50)	3(50)
	AZM	NA	NA	NA			6(100)
	GEN	NA	NA	NA			6(100)
	CIP	NA	NA	NA	1(17)		5(83)
Unbranded	RO	NA	NA	NA		5(56)	4(44)
	AMX	NA	NA	NA	7(78)		2(22)
	E	NA	NA	NA	5(56)	2(22)	2(22)
	AZM	NA	NA	NA		1(11)	8(89)
	GEN	NA	NA	NA	1(11)		8(89)
	CIP	NA	NA	NA	1(11)		8(89)

4.6.2 Antibiotic sensitivity and resistant pattern of *Staphylococcus spp*.

Isolates of *Staphylococcus* from the six (6) imported and fifteen (15) locally produced honey samples were both 21(100%) susceptible to gentamicin and ciprofloxacin. Nonetheless, whereas isolates from imported samples were 6(100%) susceptible to roxithromycin; local samples showed 1(6.7%) resistance to same antibiotic. Isolates of *Staphylococcus* from local honey samples recorded 1(6.7%) to azithromycin in contrast to 6(100%) sensitivity recorded for same antibiotic for isolates from imported honey samples. Notwithstanding, isolates of *Staphylococcus* from the different sources showed some level of resistance to erythromycin. Table 16 gives a summary of the antibiotic test for *Staphylococci* isolates from the imported and locally produced honey samples.

Table 16: Antimicrobial susceptibility test for some common antibiotics of *Staphylococcus* spp.

		Breakpoints (mm)			Antimicrobial Susceptibility № of isolates (%)		
Sample source	**Antimicrobial**	R<	I	S≥	R	I	S
Imported	RO	15	16-20	21			6(100)
	AMX	16	17	18	5(83)		1(17)
	E	18	19-20	21	5(83)		1(17)
	AZM	18	19-20	21			6(100)
	GEN	18	-	18			6(100)
	CIP	20	-	20			6(100)
Producer	RO	15	16-20	21			4(100)
	AMX	16	17	18	1(25)		3(75)
	E	18	19-20	21	2(50)		2(50)

	AZM	18	19-20	21		4(100)
	GEN	18	-	18		4(100)
	CIP	20	-	20		4(100)
Branded	RO	15	16-20	21		2(100)
	AMX	16	17	18		2(100)
	E	18	19-20	21		2(100)
	AZM	18	19-20	21		2(100)
	GEN	18	-	18		2(100)
	CIP	20	-	20		2(100)
Unbranded	RO	15	16-20	21	1(11)	8(89)
	AMX	16	17	18		9(100)
	E	18	19-20	21	6(67)	3(33)
	AZM	18	19-20	21	1(11)	8(89)
	GEN	18	-	18		9(100)
	CIP	20	-	20		9(100)

4.6.2 Antibiotic sensitivity and resistant pattern of *Salmonella spp*.

Out of the 30 honey sampled, only 2 which were from the production site recorded growth of *Salmonella spp*. All isolates were 100% resistant to ampicillin, cefuroxime, ceftriaxone and cefotaxime. Table 17; gives a summary of the antibiotic test performed on the *Salmonella* isolates.

Table 17: Antimicrobial susceptibility test for some common antibiotics of *Salmonella* spp.

		Breakpoints (mm)			**Antimicrobial Susceptibility № of isolates (%)**		
Sample source	**Antimicrobial**	R<	I	S≥	R	I	S
Producers	AMP	14		14	2(100)		
	CXM	16	17	18	2(100)		
	CTX	17	18-19	20	2(100)		
	CTR	20	21-22	23	2(100)		
	CHL	17	-	17			2(100)
	CIP	19	20-21	22		1(50)	1(50)
	GEN	14	15-16	17		1(50)	1(50)

AMP: Ampicillin, CXM: Cefuroxime, CTX: Cefotaxime, CTR: Ceftriaxone
CHL: Chloramphenicol

4.6.2 Antibiotic sensitivity and resistant pattern of *E. coli*

Out of the 30 honey sampled, only 2 which are from the production site recorded growth of *E. coli*. All isolates were 100% resistant to ampicillin, cefuroxime, ceftriaxone, cefotaxime, chloramphenicol and ciprofloxacin. Table 18; gives a summary of the antibiotic test performed on the *E. coli* isolates.

Table 18: Antimicrobial susceptibility test for some common antibiotics of *E. coli*.

		Breakpoints (mm)			**Antimicrobial Susceptibility № of isolates (%)**		
Sample source	**Antimicrobial**	R<	I	S≥	R	I	S
Producers	AMP	14		14	2(100)		
	CXM	16	17	18	2(100)		
	CTX	17	18-19	20	2(100)		
	CTR	20	21-22	23	2(100)		
	CHL	17	-	17	2(100)		
	CIP	19	20-21	22	2(100)		
	GEN	14	15-16	17			2(100)

AMP: Ampicillin, CXM: Cefuroxime, CTX: Cefotaxime, CTR: Ceftriaxone
CHL: Chloramphenicol

4.7 Antibiotics Residue Profiling

Antibiotics residue determination was performed for all the thirty (30) honey samples using the Premi® test kit (R-Biopharm, Germany). Six out of the seven honey samples from imported source were found to contain antibiotics residue.

However, twenty (21) out of the locally produced honey samples were also found to contain antibiotics residue. In general, 27(90%) of the honey samples from the different sources were contaminated with residues of antibiotics.

Table 19: Antibiotics Residue Profiling of the Honey Samples

Sample ID	Source	Present (+)	Absent (-)	Sample ID	Source	Present (+)	Absent (-)
JN-1	Imported	+		JN-16	Producer	+	
JN-2	Imported	+		JN-17	Producer	+	
JN-3	Imported	+		JN-18	Imported	+	
JN-4	Imported	+		JN-19	Producer	+	
JN-5	Imported	+		JN-20	Unbranded	+	
JN-6	Imported		-	JN-21	Unbranded	+	
JN-7	Branded	+		JN-22	Unbranded	+	
JN-8	Branded	+		JN-23	Unbranded	+	
JN-9	Branded		-	JN-24	Unbranded	+	
JN-10	Branded		-	JN-25	Unbranded	+	
JN-11	Producer	+		JN-26	Unbranded	+	
JN-12	Branded	+		JN-27	Unbranded	+	
JN-13	Branded	+		JN-28	Unbranded	+	
JN-14	Producer	+		JN-29	Producer	+	
JN-15	Producer	+		JN-30	Producer	+	

Source: Field Work, 2019 **+ Samples tested positive for antibiotic residues **- Samples tested negative for antibiotic residues

CHAPTER FIVE

DISCUSSION

5.1 The Production and Consumption of Honey from Locations within the Northern Region of Ghana

Of all the products of the honeybee, honey is the most commercialized in Ghana. It is utilized by all age groups for purposes like prevention of diabetes and/or developing of retentive memory (Akpabli-Tsigbe, 2015). Generally, the demand for honey in the country is on the rise (Akangaamkum et al., 2010). However, there are reports that suggest that the demand for honey in the country is met by foreign importations (Abdul-Malik & Mohammed, 2012).

In this study, we report that males (6) are more involved in honey production than females (2). This is not surprising since beekeeping has long been considered more of a masculine job than feminine (Ogaba & Akongo, 2002). This is similar to the 68% males and 32% female honey producers reported by Akangaamkum et al. (2010), in Ghana. Yusuf et al. (2014), attributed the 93% male dominance in honey production to the aggressive nature of the honeybee as perceived by women. Moreover, the age distribution of most of the honey producers reveal that they were within the active age of 20-40 years. This is quite good for the industry as this implies that honey producers within the region can actively participate in the management of the honey producing enterprise and can equally observe improved practices of ensuring productivity and quality. The age distribution of the honey producers compares well with the findings of Oluwatusin (2008) and Tijani et al. (2011), who reported 31-40 and 31-35 as the modal age of honey producers in Ekiti and Borno state of Nigeria, respectively. Furthermore, the educational qualification of the honey producers captured for the present study was encouraging with the majority 5(62.5%) having tertiary education. This is in line with the findings of Ezekiel et al. (2013), where 90% of the

respondents were said to have a good educational background. Notwithstanding, the educational qualification of the honey producers did not translate into their knowledge on diseases affecting bees and the subsequent antibiotic usage in honey production. Only two (2) out of the eight (8) producers indicated they have knowledge on diseases affecting bees. The two producers were those who own apiaries. Again, the findings of this study revealed that majority 6(75%) of the producers sampled are hunters of honey rather than beekeepers. They obtained their honey through hunting from wild sources. This is in line with the assertion of Aidoo (2005), that 60% of the locally produced honey in Ghana are from wild sources. These producers will therefore have no knowledge on diseases affecting bees since they do not own apiaries. Furthermore, the two beekeepers that had knowledge on antibiotics usage in beekeeping, confessed that they are not into such practices themselves.

Concerning the level of knowledge on contamination of honey, all eight (8) producers expressed their concern on contamination of honey. Three (3) of them mentioned that consumers have been buying from them directly because of the issue of adulteration at the retail outlets. All the honey producers stated that they do not add additives to their honey before selling, however, they are aware such practices exist. Notwithstanding, in terms of packaging honey for sale and/storage, 5(62.5%) of the producers indicated they use old/used plastic containers whilst the remaining 3(37.5%) said they keep or store their honey in new plastic containers. This practice does confirm why honey collected even directly from the production sites were contaminated with microorganisms. Adding to this, Adadi and Obeng (2016), reported that honey producers carried harvested honey in unhygienic plastic containers from production sites within the Tamale Metropolis of the Northern Region of Ghana

It was observed that honey consumption was popular among all age but higher 113(85.6%) with the younger age (20-29). This could be attributed to the fact that individuals of this age range are more conscious of their health and well-being as asserted by Wen et al. (2015) and Botchway et al. (2015). Furthermore, 128(97%) of these respondents have tertiary educational background and there is the possibility that they were aware of the nutritional and physiological benefits of honey. However, this is not to dispute the fact that the older generation are also aware of the benefits of honey. Cantarelli et al. (2008), opined that individuals particularly students have the general belief that the consumption of honey as a food or as part of diet is linked to brain development and retentive memory.

Although 129(97.7%) of the respondents indicated that they are consumers of honey. However, 79(59.8%) consumes honey occasionally, with 9.8%, 3.8% and 3.8% indicating that they consume honey on daily, weekly and monthly basis, respectively. A similar observation was made by Cosmina et al. (2015), where more than 90% of the respondents indicated that they consumed honey of which the majority (35%) stated that they consumed honey occasionally. In the present study, personal interview with some of the occasional consumers revealed that they do not resort to daily intake of honey because of the availability of an alternative source like table sugar. This is obvious since the study of consumer behaviors reveals that there is a likelihood of a consumer to depend more often on a substitute product if it is readily or easily available in the market.

Also, consumers' preference for imported and/or locally produced honey as well as the point of purchase was sought. Purchasing preference of honey by consumers indicated that few 8(6%) of the respondents have taste for foreign honey. They mentioned adulteration and non-labelling of locally produced honey as the reason for their preference for imported honey. Labelling, they said gives them information concerning the source and nutritional composition of the honey and allows

for the consumer to have a choice in what to purchase depending on their needs. This is somewhat affirmed by Roman et al. (2013), where labelling was emphasize to give customers knowledge of the quality of the product and also to make choices among brands.

However, the 115(87%) respondents indicating they prefer locally produced honey could be described as a good attitude and a key determinant towards the economic growth and development of the nation. Nonetheless, this could further be supported by the findings of the present study where 17(12%) of the respondents opined 'patriotism' as the reasons why they prefer locally produced honey. Notwithstanding, the results compares well with other findings where respondents tended to favor products from their home country (Troiano et al., 2014; Zulail et al., 2014).

On the preferred point of purchase of honey; 54(41%) preferred buying directly from the producers; 32(24%) buy from hawkers; 24(18%) purchase from the supermarkets whiles 10(8%) prefer buying from the open markets from stationary vendors. The 54(41%) consumers who preferred buying directly from the honey producers indicated they prefer to know the source of the food they consumed thus their decision to purchase directly from the producers. Others indicated that the fear of adulteration in the hands of market sellers will make them go for honey purchased directly from the production sites.

"I know supermarkets stocks their shelves with quality products"- this was a reason of a respondent who prefers to obtain honey for consumption from the supermarkets. The issue of adulteration and labelling were among the reasons why some consumers tend to purchase honey from supermarkets and/or shops.

Ease of accessibility, conveniences and availability were some of the given reasons why some consumers prefer to purchase from hawkers and from open markets. The findings on the consumers preference on the point of purchase corresponds well with Meng et al. (2014), who reported that a large number of Ghanaians obtains their food supply from hawkers but will resort to supermarkets in terms of quality products.

Regarding consumers knowledge on contamination of honey, 102(77%) indicated that they are aware honey can be contaminated from different sources whereas 25(19%) confessed they have no idea honey can be contaminated. *"…had it not been this research I have never heard of contamination of honey…"* –response from a respondent pursuing postgraduate studies in Agricultural Economics. This probably might be due to the general notion that honey is pure and free from contaminants (Wacker, 2012)

Also, 95(72%) of the respondents confessed they have no idea on the use of antibiotics in beekeeping or honey production. This is in tandem with the 93(70%) who indicated they have no knowledge on the diseases affecting bees. Surprisingly, five (5) members interviewed from the Nyankpala campus chapter of Apiculture Students Association of Ghana (APSAG) also confessed they are not aware of diseases affecting bees left alone its treatment with antibiotics. Tantamount to this, only 16(12%) of the respondents affirmed that they are aware of the dangers associated with the consumption of honey with antibiotics residue.

Physicochemical characteristics of imported and locally produced honey

The increasing consumption of honey have necessitated various studies on the quality of honey from the different points of sale. Physical and chemical qualities remain one of the key indicators of assessing the quality of honey. In this study, the pH, acidity, moisture content, viscosity, ash content, total soluble solids and sugar contents were determined to ascertain the overall quality of imported and locally produced honey obtained from the different outlets within the study area.

The pH of all the 30 honey samples was acidic and ranged between 3.80 and 6.0. Of these; imported samples ranged from 3.8 to 5.20 whereas the locally produced honey samples ranged from 3.8 to 5. 89. There was significant ($P<0.05$) difference in pH between the imported and locally produced honey samples. pH values observed in the samples from the different sources compares well with the 3.3 – 6.1 reported by Aljohar et al. (2018). Nonetheless, they were within the recommended pH (3.4 to 6.1) by the Codex Alimentarius Commission (CAC, 2001). pH is an important quality of honey as it influences greatly microbial growth and thereby contributing to the longer shelf life of the honey product (Gomes et al., 2010). The pH of the imported and locally produced honey samples were low enough to prevent microbial growth since most microorganism grow an optimum pH of 7 (Abdulkhaliq & Swaileh, 2017). However, the differences in pH can be attributed to the floral diversity and composition from which the honey samples were made since different floral sources and composition constitutes different pH (Fahim et al., 2014). Also, the findings on pH of the imported and locally produced honey samples were contrary to the assertion of Aljohar et al. (2018), that honey from tropical countries generally have a lower pH characteristics compared to those from the temperate countries. The season of harvesting honey within the region could have accounted for the higher pH values in the locally produced honey as most of the honey producers interviewed mentioned the rainy season as the ideal season of

harvesting. Since relative humidity will be high during the rainy season, there is the possibility of these samples pulling moisture from the surrounding environment and eventually causing dilution (Molan, 1996). Adding to this, Sohaimy et al. (2015), mentioned that not only does the properties and composition of honey dependent on its geographical floral origin but on the season of harvesting. This can further be supported by the free acidity that was recorded for the imported and locally produced samples in the present study.

In this study, locally produced honey samples recorded a free acidity mean value of 19.92meq/kg in comparison to 12.14meq/kg recorded for samples from imported source. There was significant ($P<0.05$) difference in the free acidity of the imported and locally produced honey samples. The findings from this study is in line with the findings of Akhtar et al. (2014), who reported a variation in free acidity (20.7-43.1meq/kg) in imported and locally produced honey sampled from markets in Peshawar, Pakistan. Notwithstanding, the ranged of free acidity recorded for imported (7.84 to 16.36meq/kg) and locally produced honey (7.84 to 41.16meq/kg) were within the maximum permissible value of 50meq/kg recommended by the Codex Alimentarius Commission. In terms of the variation in free acidity of the imported and locally produced honey, Prica et al. (2014), argue strongly that free acidity of honey depends on the floral source and that some acids are introduced into the honey through the nectar. Therefore, since both imported and locally produced honey samples were from different geographical origin, it is possible they will differ in free acidity content.

Shehat et al. (2010), mentioned viscosity as an important parameter to look out for in honey as it has a great influence on its physical and sensory characteristics. The viscosity recorded for the 30 analyzed honey samples was between 2112cP and 36000cP. Locally produced honey samples recorded a relatively lower mean viscosity (7426cP) in comparison to the mean viscosity of

16198cP recorded for samples from imported source. The mean of viscosity for the four different categorizations were as follows; 16198cP for imported samples; 10512cP for local samples collected from the production sites (producer); 6678cP for local samples collected from supermarkets (branded); and 5182cP for local samples collected from open markets (unbranded). The imported honey samples are anticipated to have less incidence of microbial growth due to the high viscosity in comparison to the locally produced honey samples. This is because high viscosity of honey helps to provide barrier against growth of microorganisms (Jantakee & Tragoolpua, 2015). However, there are no international standard set for the minimum or maximum value of viscosity, thus the result on viscosity was compared to that of other researchers. Even though, the viscosity of the locally produced honey samples was relatively lower in comparison with their imported counterparts; it was however higher in comparison to the 6575.89cP recorded by Boateng and Ofosu (2018) for 20 honey sampled in Tema metropolis. Ramzi et al. (2015), recorded a maximum viscosity of 30000cP whereas a maximum of 270500cP was reported by Abu-Jdayil et al. (2002) for 6 honey samples. Lullah-deh et al. (2018), recorded a similar trend where most of the samples collected from open markets were of lesser viscosity values as compared to samples from other sources. Gómez-díaz et al. (2009), pointed out that the temperature of a particular environment will have an effect on honey viscosity, as such the variation in viscosity values could be attributed to the surrounding temperature from which they were collected. Since samples collected from open markets (unbranded) were seen mostly under the hot or scorch sun, it was no surprising that these samples had the least mean value of viscosity. In contrast, imported and local samples obtained from the supermarkets (branded) and from the production sites (producers) were found in places of relatively cold atmosphere like the supermarkets, mini-marts, shops and storage rooms. The variations in the viscosity values is further supported by the moisture content observed

in the samples from the different sources where a higher viscosity value led to a lower moisture content.

There are several reports that suggest that the viscosity of honey is directly dependent on its moisture content. Conversely, it was surprising to observe that the variation in moisture content of the imported and locally produced honey samples was not significantly (P=0.97) different from each other despite variations in viscosity. The mean moisture content for samples from imported source was 16.65% in comparison to 16.68% recorded for locally produced honey samples. The relatively high viscosity of the samples from imported source (16198cP) could have accounted for the lower moisture content. On the other hand, it was observed from the results on viscosity of locally produced honey samples that samples from supermarkets (branded) and open markets (unbranded) recorded a relatively lower viscosity values thus resulting in an overall lower viscosity of the locally produced samples despite the lower moisture content. This could have accounted for the variation that was not observed in the moisture content of both samples.

However, for the different categorizations, honey from the production sites (producers) recorded the least moisture content (15.56%) in comparison to the 16.65%, 17.20% and 17.38% recorded for samples from imported source, open markets (unbranded) and supermarkets (branded) respectively. In the findings of Akhtar et al. (2014), on the physicochemical properties of imported and locally produced honey in Pakistan, the moisture content of the local honey samples were relatively lower compared to the imported samples. The findings of this study agree with the aforementioned researchers when the moisture content of the samples obtained directly from the production sites (producers) was lower than that of the imported honey samples. Regarding comparison of the moisture content of the imported honey to that collected from open markets (unbranded) and supermarkets (branded), there was no significant difference. The lower moisture

content of the locally produced honey samples can be attributed to the solar extraction technique practiced by the honey producers as interviewed for the study. Adjaloo et al. (2017), affirmed this in their study where solar extraction technique of honey recorded the least moisture content as compared to hand, press, and cold extraction techniques. Notwithstanding, the moisture content of all the analyzed honey samples fall within the maximum permitted moisture content of 20% set by the Codex Alimentarius Commission (2001). The moisture content recorded for both the imported and locally produced honey samples in this study should be low enough to inhibit the survival of microorganisms. Also, both honey samples should have a longer shelf life due to the relatively low moisture content (Namini, 2018).

According to the Codex Alimentarius Commission regulation's, the container of which honey is kept for sale should be labelled or designated according to the floral or plant source. This is to aid consumers/customers to make choices among brands of honey. For all the 30 honey samples, only sample JN-3 (imported) had as part of it's labelling the floral source. Nevertheless, the ash content of honey has been used as an indicator of the floral source of honey. Per the set standard, the ash content of blossom honey should be less or equal to 0.6% whilst that of honey dew and/or its combination should be greater or equal to 1.2%. There was significant ($P < 0.05$) difference between the mean value of 0.116% and 0.315% recorded as the moisture content for imported and locally produced samples respectively. Except for sample JN-12 (Branded) and JN-15 (Producer) which recorded 1.08% and 0.68% respectively, ash content for the analyzed honey samples were within the maximum set for blossoms honey. The variations in the ash content of the honey samples from the different sources may be attributed to many factors like the physiology of the different plants, atmospheric conditions and the soil condition of the geographic location of each honey sample (Shahnawaz et al., 2013).

To further assess the authenticity of the honey samples, the dissolved solids were determined. Total soluble solids is a reliable index of adulteration and a critical factor in considering the glycemic index which is of a great concern for diabetic patients (Viuda-Martos et al., 2010). Also, the total soluble solids of honey are the different sugars found in it which accounts for about 80% or more solids by weight (Nyau et al., 2013). The Codex Alimentarius Commission currently have no standard for the maximum or minimum permissible total soluble solids for honey, thus the Honey Judging and Standard as reported by Sanford (2003), was adopted. According to the grading system of the Honey Judging and Standards, honey with a total soluble solid equal or a greater than 81.4% is categorized as a high-grade honey (A or B) whereas that between 80% and 81.3% are considered of a lower grade C. There was a significant ($P<0.05$) difference between the mean percentage of total soluble solids recorded for samples from imported source (73.8%) and local samples (81.9%).

Total soluble solids of imported samples ranged between 62.4% and 90.6% whereas local samples ranged between 60% and 108%. Per this, only one (1) out of the 7 imported honey samples was of Grade 'A' category. The remaining six (6) fall below Grade 'C.' For the local samples; 11 were of Grade 'A'; 11 were below Grade 'C' whilst only 1 was in Grade 'C' category. Akhtar et al. (2014), reported a lower total soluble solid content of imported honey in Pakistan as against locally produced honey samples. However, they failed to give an account of their observation. Notwithstanding, Lakhanpal (2010), mentioned that storage temperatures may either contribute to increasing or decreasing the total soluble solid content of honey. Nyau (2013), added that the moisture content of honey can influence its sugar content, otherwise the total soluble solids. Hence, a lower moisture content will result in an increase total soluble solid as observed in some samples. This however is reflected in the reducing and non-reducing sugar content of both honey samples

where samples from imported source recorded the least reducing and non-reducing content in comparison to local samples.

Reducing sugar content ranged from 50.35 to 68.02% for imported samples whilst local samples recorded a range from 49.07 to 76.03%. There was no significant (P=0.07) difference between the mean reducing sugar content for imported and locally produced samples. Similarly, there was no significant difference between the mean non-reducing sugar content recorded for both imported samples (2.65 to 3.58%) and local samples (2.58 to 4%). According to Krishnasree and Ukkuru (2017), the non-reducing sugar content of honey generally indicates its sucrose content. The aforementioned researchers pointed out that a high sucrose content of honey is an indication of adulteration with sugar or could be due to the inability of the bees to convert the sucrose content in the honey. Nonetheless, the Codex Alimentarius Commission (2001), stated 5% as the maximum permissible non-reducing sugar content of honey. Going by this, it can be said that both the locally produced and imported honey samples were of quality since none of the samples had its sugar content above the maximum permissible standard. Meanwhile, Aazza et al. (2013), mentioned that the reducing sugar content of honey consist mainly of fructose and glucose of which fructose is the most abundant. Again, the CAC have stated that the reducing sugar content of honey should be greater than or equal to 60% (≥60%). Inferring from the standard, only 2 out of the 7 samples from the imported sources and 12 out of the 23 locally produced samples were within the permissible range. Of the 23 locally produced honey samples 3 out of the 6 samples from supermarkets (branded); 3 out of the 9 samples from open markets (unbranded); and 6 out of the 8 samples from the production sites (producers) were within the permissible range. These findings are in line with that of Namini (2018), where reducing sugar content of some of the honey samples were below the recommended standard. The assertion of Azonwade et al. (2018), that

reducing sugar content are high in arid areas than humid areas could form the basis of the variation in the reducing sugar content of the imported and locally produced honey samples.

Generally, the variation observed among the physicochemical parameters in this study could be attributed to the geographical differences of weather, nectar conditions, extraction methods as well as storage temperatures and conditions (Elenany, 2019; Muli, Munguti, & Raina, 2007; Orina, 2012). Also, it was realized from the study on the physicochemical parameters that most honey samples from the different sources were within the recommended standards. Therefore, it is anticipated that this will be translated into its microbial quality.

5.2 Microbial quality of imported and locally produced honey

Generally, honey in its pure state presents a hostile environment for microorganisms to thrive. Though, the term "quality" is often used commonly, but its definition is quite complex. Unnevehr and Jensen (1999), opine that quality is a blend of features that makes a product acceptable. Despite standards that define honey (Codex Alimentarius Commission, 2001), in Ghana, it appears the quality and safety of honey has been left in the hands of producers and sellers due to poor regulation by the appropriate institutions.

In the current study, nine (9) bacterial genera were enumerated for a possible indication of the overall quality of the honey sampled for the study. These genera; *Clostridium*, *Listeria*, *Enterobacter*, *Salmonella*, *E. coli*, *Staphylococcus*, *Lactobacillus*, *Campylobacter* and *Shigella* were of concerned due to reports that the intestines of bees contain 27% of Gram-positives and 70% of Gram-negatives (Olaitan & Iyabo, 2007). Notwithstanding, it should be noted that most of these bacteria are the leading cause of foodborne diseases.

Clostridium spp. are commonly found in honey because they are spore-forming bacteria which are common in air, dust and soils (Snowdon & Cliver, 1996). It was no surprising that *Clostridium* spp. constituted the most 28(93%) occurring genera of bacteria in the 30 honey samples analyzed. Samples from imported source recorded 7(100%) growth for *Clostridium* spp. whereas locally produced samples showed 21(91.3%) growth for *Clostridium* spp. Except sample JN-15 and JN-16 which were from the production sites (producers), all the other local samples from the different sources recorded growth for *Clostridium* spp. Also, with a mean of 1.58×10^{6}CFU/ml, samples from imported sources recorded the highest detectable load of *Clostridium* spp. compared to 6.46×10^{5}CFU/ml recorded for local samples. However, among the local samples, the highest detectable load of 1.04×10^{6}CFU/ml was recorded for samples collected from open markets (unbranded) whilst 5.28×10^{5}CFU/ml and 2.93×10^{5}CFU/ml was recorded for samples obtained from supermarkets (branded) and that from production sites (producers) respectively. The finding on the frequency of occurrence of *Clostridium* spp. between the imported and local samples is somewhat in line with the findings of Nevas et al. (2002), where the occurrence of *Clostridium* spp. in imported honey samples was as twice as that of locally produced samples in Finland.

As mentioned earlier *Clostridia* spores are widely distributed in the environment, therefore it could be assumed that the contamination of the samples could have arose through contaminated dust particles at processing or storage or via ingestion of contaminated dust during foraging of bees (Mustafina et al., 2015). This is further supported by the Food Standards Australia New Zealand (FSANZ, 2016), where it has been mentioned that spores of *Clostridium* spp. are widely spread in the environment and are also part of intestinal flora of most food producing animals and as such should be considered potentially hazardous in a food sample only when it exceeds 10^{3}CFU/ml. Per

this, only 2 out of the 6 local samples obtained from supermarkets (branded) and 4 out of the 8 samples from the production sites (producers) could be said to be wholesome.

Aside *Clostridium* spp., the other organism of interest as far as the microbiological quality was of concerned was *Lactobacillus*. *Lactobacillus* spp. preceded *Clostridium* spp. as the second predominant 27(90%) isolates of bacteria in this study. It occurred in 7(100%) of all samples from imported source but in 20(87%) of locally produced samples. Growth of *Lactobacillus* spp. was not detected in 3 of the samples obtained from the production sites (producers) but was detected (100%) in all local samples obtained from the supermarkets (branded) and open markets (unbranded) respectively. Again, samples from imported source recorded a higher detectable mean load of 1.16×10^4CFU/ml as against 8.38×10^3CFU/ml recorded for locally produced samples. The mean detectable load recorded for the various local samples were 6.65×10^3CFU/ml (producers); 8.52×10^3CFU/ml (branded); and 9.83×10^3CFU/ml (unbranded). This result is however less than the 3.8×10^3 to 5.5×10^7 and 6.5×10 to 6.3×10^6CFU/ml recorded respectively for *Lactobacillus* spp. in both local and imported probiotic yoghurts sold in Accra as reported by Mahami and Odonkor (2014). Even though, the specific strain of the isolates of *Lactobacillus* spp. has not been established since biochemical test performed on the isolates was not enough to conclusively point out to a specific strain of the bacteria. However, studies have indicated that among the lactic acid bacteria, the genus *Lactobacillus* are the most predominant species in the gut of honeybees (Tajabadi et al., 2014). Also, it could be said that the isolation of *Lactobacillus* from the samples should be of less concern since there are several scientific reports on the synergistic effect of some strain of *Lactobacillus* on foodborne pathogenic bacteria like *Campylobacter* and *Salmonella* (Ivanovic & Baltic, 2010); *Listeria*, *Staphylococcus* and *Clostridium* (Bahlol, 2015).

Due to the high temperature coupled with the solar extraction method by most honey producers in the region, it was anticipated that contamination of *Listeria* spp. would have been at its barest minimum. However, results on the occurrence of *Listeria* in the analyzed honey samples suggested otherwise. All the seven (7) samples from imported source showed growth of *Listeria* spp. whilst 18 out of the 23 local honey samples showed growth of *Listeria* spp. Also, all the samples from open markets (unbranded) showed growth of *Listeria* but 4 out of the 6 local samples collected from the supermarket (branded) and 5 out of the 8 samples collected from the production sites (producers) showed growth of *Listeri*a. With a detectable mean load of 1.13×10^{6}CFU/ml, samples from imported source was higher than local samples which recorded 8.95×10^{5}CFU/ml. However, there was no significant difference between these means of load. The occurrence of *Listeria* spp. in the honey samples could be attributed to post-processing contamination from the processing equipment or materials. As mentioned earlier most of the honey producers keeps or packaged their honey in used containers. Among the locally produced honey, samples from the open markets (unbranded) recorded the highest mean load of 1.32×10^{6}CFU/ml, as against 6.57×10^{5}CFU/ml and 5.92×10^{5}CFU/ml recorded for samples from supermarkets (branded) and production sites (producers) respectively. The observation made as part of sample collection revealed that most of the market sellers have their honey in a large container which they fetch from based on the quantity or amount required by the customer. Cross contaminations as result of this could have accounted for this load since most of them sell other products. This is supported by the assertion of Vorst et al. (2016), that there is the possibility of cross contaminations of *Listeria* contaminated food at the retail level. Aside post-harvest or post-processing contamination, the occurrence and load of *Listeria* spp. in both imported and locally produced honey could be attributed to poor temperature control at storage and the length of shelf life (FSANZ, 2016). Particularly for the imported samples,

some have been on the shelves since 2014. An interview with a shop attendant in one of the supermarkets revealed that only expatriates found in the region request or comes for such honey. The reason given was that these foreigners have no trust for the local honey whereas the price alone scares some local consumers. According to the Center for Food Safety (2014), refrigerated foods, foods intended for infants or ready to eat foods should be devoid of *Listeria* spp. and if present should not exceed 100CFU/ml. Since honey can be considered in any of these category, all 25 samples of which *Listeria* spp. was detected should be considered unwholesome for consumption.

The occurrence of *Staphylococcus* spp. was of less concern to this study due to the many scientific reports on the anti-staphylococcal potential of honey. However, it was interesting when growth occurred in imported 6(85.7%) and local samples 15(65.2%). The detectable mean load of *Staphylococcus* spp. was high in imported source (1.17×10^6CFU/ml) compared to 5.53×10^5CFU/ml recorded for local samples. However, among the local honey, samples from open markets (Unbranded) also recorded growth in all 9(100%) samples with a detectable mean of 1.13×10^6CFU/ml. The detectable mean load of the 2 samples from supermarket (branded) was 1.26×10^5CFU/ml whilst 2.23×10^5CFU/ml was the detectable mean load for the 4 samples obtained from the production site (producers) which showed growth of *Staphylococcus* spp. Results on *Staphylococci* load of local samples were higher than 7.0×10^4 CFU/ml and 9.0×10^4CFU/ml reported by Adadi and Obeng (2016), for honey within the Tamale metropolis. Detectable mean load of the imported samples were also higher than the range of 10^2-10^4CFU/g reported by Uran et al. (2017), for honey samples from different manufacturers in Turkey. Since *Staphylococcus* is a normal flora of skin surfaces it could be possible that the handlers might have introduced it into the honey during extraction, processing or handling (Voula et al., 2013). The presence of

Staphylococcus spp. in 21(70%) out of the total samples calls for concern taking into consideration the health and well-being of consumers of honey due to the reported dangers available in literatures (Adebiyi, Akpan, Obiajunwa, & Olaniyi, 2004).

This study recorded less occurrence of Gram-negative isolates as compared to Gram-positive isolates despite the assertion that intestines of bees are found to contain only 27% of Gram-positives but 70% of Gram-negatives (Olaitan & Iyabo, 2007). Out of the 30 honey samples, *Campylobacter* spp. was detected in only sample JN-3 which was from an imported source. There was no detection of *Enterobacter* spp. and *Shigella* spp. in all the 30 samples from the different sources. Detectable load of *E. coli* and *Salmonella* spp. occurred only in sample JN-29 and JN-30 which were collected from the production sites (producers). *Salmonella* load of 9.85×10^{3}CFU/ml and 1.72×10^{4}CFU/ml was recorded for sample JN-29 and JN-30 respectively whereas 3.75×10^{5}CFU/ml and 4.05×10^{5}CFU/ml was recorded as *E. coli* load for JN-29 and JN-30 respectively.

The findings on the occurrence and load of *E. coli* agrees with that of Adadi and Obeng (2016), who recorded a mean count of 6.0×10^{4}, 7.0×10^{4} and 1.1×10^{5} CFU/ml in 3 out of 6 honey samples obtained from producers directly from their production sites in the Tamale metropolis. However, results from the aforementioned study did not record any growth of *Salmonella* spp. as observed in this study. Nonetheless, the detectable load of *E. coli* and *Salmonella* were above the satisfactory load (100CFU/ml) recommended by the Center for Food Safety (2014). Whereas other authors attribute the occurrence of *E. coli* and *Salmonella* spp. in honey to fecal and environmental contamination, the current study propose the storage room, packaging and storage material as the source of *E. coli* and *Salmonella* contamination. The storage room of some of the honey producers were inappropriate, that is congested with other materials. Insects like ants and flies were seen

around the honey samples. Also, sample JN-29 and JN-30 were among the samples taken from honey producers who confessed to store and/or package their honey in used plastic bottles. Adzitey et al. (2016), reported on high incidence of *Salmonella* contamination in reuse containers in the Tamale metropolis of the Northern region of Ghana. Also, in the survey of production of honey, it was realized that most of the honey producers were hunters of honey rather than honey producers as they claimed. Therefore, the transportation of the harvested honey from the harvesting location to their homes could have accounted for the contamination. The study of Adadi and Obeng (2016), gave an account of how transporting honey from production sites on motorbikes contributed to its contamination by bacteria from the family enterobacteriaceae.

In an attempt to link the physicochemical parameters determined in this study to the microbial load and occurrence, it can be said that physicochemical qualities of the honey did not reflected on its microbial quality. Since most of the physicochemical parameters were within the permissible standard, bacteriological quality was expected to be high. However, the lower moisture content and pH as well as the high viscosity and sugar content did not translate to inhibiting some bacteria as suggested by some authors (Albaridi, 2019; Roslan et al., 2015). Also, the study recorded high incidence of contamination by Gram-positives despite report that the intestines of bees are found to contain 70% of Gram-negatives (Olaitan et al., 2007). This could be due to the fact that Gram-negative bacteria are more susceptible to a lower moisture content in comparison to Gram-positive bacteria (Erkmen & Bozoglu, 2016; Ross & Nichols, 2014). Again, the response of Gram-positive bacteria to low pH has been well studied. Cotter et al. (2001), have made it known that Gram positive bacteria like *Listeria*, *Clostridium* and *Lactobacillus* possesses the glutamate carboxylases (GAD) system that offers them the ability of pH control. This therefore could have accounted for the high occurrence of *Clostridium* spp, *Lactobacillus* spp. and *Listeria* spp. in the analyzed honey

samples. Nevertheless, the occurrence and multiplication of the bacteria isolates could also be attributed to certain conditions like high temperature storage, smash lid as observed in an imported sample and accessibility of either air or water particularly uncovered honey witness from store rooms of some producers and market sellers (Rózańska & Osek, 2012).

In most cases honey have been used as an antibacterial agent when antibiotics have failed, thus the detection and isolation of bacteria from the imported and locally produced honey gives a possible indication of the presence of resistant bacteria.

5.3 Antibiotic susceptibility pattern of bacteria isolates in imported and locally produced honey

The emergence of antibiotic resistant strains of bacteria in foods is a potential public health hazard considering how antibiotic resistance can be shared among bacteria. The uncontrolled and intensive use of antibiotics in food producing animals like in beekeeping has resulted in the increase of resistant strains, be it from the environment or clinical sources (Baquero et al., 2008). Due to the mechanisms of action of antibiotics, antibiogram was performed with different antibiotics for Gram-positive isolates and Gram-negative isolates enumerated from both imported and locally produced honey sampled for the study.

Growth of *E. coli* and *Salmonella* spp. was detected in only two samples out of the thirty honey sampled for the study. These two samples were from locally produced honey samples that were obtained from the production sites (producers). *E. coli* detected in these two samples showed 2(100%) resistance to ampicillin, cefuroxime, ceftriaxone, cefotaxime, chloramphenicol and ciprofloxacin. However, these isolates were susceptible to gentamicin. Nevertheless, *Salmonella* spp. which is in the family as *E. coli* was 2(100%) susceptible to chloramphenicol but 1(50%) intermediate to ciprofloxacin and gentamicin respectively. Also, all the isolates of *Salmonella* were 2(100%) resistant to ampicillin, cefuroxime, ceftriaxone and cefotaxime. Studies on antibiotic

resistance of *Salmonella* spp. and *E. coli* isolates from honey are limited particularly in Ghana. Most of the studies are concentrated on isolates from clinical patients and few available on food isolates. George et al. (2012), reported on a 28.6-46.4% resistance of *E. coli* isolates from clinical patients in some hospitals in Kumasi to gentamicin, ciprofloxacin and ceftriaxone whereas 14.4-47.4% was for isolates which showed intermediate responses. In the same region as the present study, Adzitey et al. (2015), recorded a high susceptibility of *E. coli* isolates from drinking water to ciprofloxacin (94.64%), ceftriaxone (89.29%) and gentamicin (89.29%). This contrast the findings of this study where *E. coli* isolates were all resistant to ciprofloxacin and ceftriaxone. However, the aforementioned researchers did not fail in mentioning that intermediate responses were observed. Intermediate resistance refers to the condition where an isolate of a bacteria neither shows resistance or sensitivity to a particular antibiotic. Overtime, these bacteria could assume resistant state, thus the 2(100%) resistance recorded for such antibiotics.

Again, Adzitey et al. (2016), reported on high incidence of intermediate responses of 34 *Salmonella* isolates from water sources in Tamale to ceftriaxone(17.65%), gentamicin (17.65%) and ciprofloxacin (2.94%). Most of the honey producers interviewed in this study had no or little knowledge on the use of antibiotics in beekeeping. Therefore, the emergence of antibiotic resistant *E. coli* and *Salmonella* isolates from the honey samples could be attributed to the indiscriminate use of antibiotics in feeds as growth promoter by livestock farmers within the region (Saba, 2019). On the hand, for Gram-positives, isolates of *Lactobacillus* spp. recorded the least incidence of resistance for the tested antibiotics. All isolates of *Lactobacillus* from the imported samples were 6(100%) susceptible to azithromycin, gentamicin, ciprofloxacin and roxithromycin. However, for the local samples susceptibility to azithromycin was 19(95%); gentamicin 19(95%); ciprofloxacin 18(90%); and 13(65%) for roxithromycin. Both imported and local samples recorded above 50%

resistance to amikacin. The use of ciprofloxacin and gentamicin in livestock production could be a link to the resistance of the isolates of *Lactobacillus* in the local samples (Boamah et al., 2017; Ministry of Health, 2017). Also, most *Lactobacillus* spp. are said to be intrinsically resistant to several antibiotics (Álvarez-Cisneros & Ponce-Alquicira, 2018). Nonetheless, the detection of antibiotic resistant *Lactobacillus* isolates from honey is a cause for concern. As mentioned above, despite biochemical tests performed in this study not being enough to point out to the specific strain(s) enumerated from the honey samples. There are available literatures that suggest the synergistic effects of some strains of *Lactobacillus* on pathogenic organisms. In addition, they have the tendency of transferring antibiotic resistance gene(s) to pathogens in the gastrointestinal tract of humans and animals (Preethi et al., 2017).

Staphylococcus spp. which was the least predominant bacteria in terms of the occurrence of Gram-positives showed 21(100%) susceptibility to gentamicin and ciprofloxacin. That is isolates from both imported and local samples. However, whereas samples from imported source recorded 5(83%) resistance to amikacin, only 1(7%) of isolates from local samples was resistant to same antibiotic. Also, 5(83%) of the isolates from imported source was resistant to erythromycin as against 8(53%) of the isolates from the local samples. Saba et al. (2017), reported on 13% resistance to erythromycin for 47 isolates of *Staphylococcus* spp. from hospital settings in the Tamale metropolis. The detection of antibiotic resistant *Staphylococcus* isolates from honey should be of a public health concern. This is because honey have been cited in numerous scientific studies and reports as the alternative option of overcoming *Methicillin Resistant Staphylococcus aureus* (MRSA) and multi drug resistance in *Staphylococcus* (Almasaudi et al., 2017; Grecka et al., 2018; Iqbal et al., 2019; Liu et al., 2018).

As the highest occurring bacteria in this study, isolates of *Clostridium* spp. from both imported and local samples showed some level of resistance to at least three (3) of the tested antibiotics. Isolates of *Clostridium* from imported source showed 7(100%) susceptibility to gentamicin and ciprofloxacin. For same antibiotics, the susceptibility pattern was 15(71%), and 16(76%) for isolates from local samples. In the study of Koluman et al. (2013), isolates of *Clostridium* from 5 out of the 19 honey samples were resistant to gentamicin. However, whereas isolates from imported samples showed 7(100%) resistance to amikacin, isolates of local samples were 9(43%) resistant to the same antibiotic. According to the European Medicines Agency (2018), amikacin are among the aminoglycosides used extensively as veterinary drugs. This could have accounted for the high resistance in isolates from imported sources. On the other hand the expensive price of amikacin as well as its uncommonness' in Ghana could contribute to its low patronage by most livestock farmers (Newman et al., 2011). This could be an influence on the relatively low resistance recorded for the isolates in the local samples. Nonetheless, there are limited data on the antimicrobial resistance of *Clostridium* spp. in honey due to the several reports of antibacterial activity of honey on *Clostridium* (Ahmed et al., 2014; Oinaala et al., 2015). However, the occurrence of antibiotic resistant *Clostridium* spp. in the honey samples should be of clinical importance due to the number of reported cases of botulism.

Despite the notion that *Listeria* spp. are most prevalent in temperate regions than the tropics (Amene & Firesbhat, 2016), this study recorded a significant level of contamination of *Listeria* even in the local samples. Surprisingly, isolates of *Listeria* from the local samples recorded 1(6%) resistance for both gentamicin and ciprofloxacin in comparison to 7(100%) susceptibility recorded for isolates in imported samples. Also, there was high incidence of resistance to amikacin recorded for both imported 5(71%) and local 12(67%) samples. Bezirtzoglou et al. (2016), reported on the

resistance of *Listeria* to ciprofloxacin in one sample that recorded growth of *Listeria*. Even though, resistance of *Listeria* to gentamicin was 1(6%) in this study, it however calls for public health concern since in most cases gentamicin are combined with the first choice of drugs for treatment of listeriosis (Chen et al., 2010). Resistance to gentamicin recorded for isolates of *Listeria* from the local samples could be attributed to its common use in the country (Labi et al., 2018). Nonetheless, the resistance of isolates of *Listeria* to most of the tested antibiotics should be a cause for concern since this bacteria have been reported to easily transfer resistance gene to other phylogenetically related Gram-positives (Lyon et al., 2008).

Unlike many other global beekeepers, most honey producers in the region had little or no knowledge of the treatment of bees with antibiotics. In contrast, is the intensive use of antibiotics in professional beekeeping in the developed countries (Carrillo, 2014) for the treatment of bacterial brood diseases (Mutinelli, 2003). This could have accounted for the enumeration of resistant bacteria from the imported samples. However, the enumeration of resistant bacteria in the locally produced honey samples could be attributed to the indiscriminate use of antibiotics as veterinary drugs for therapeutic treatment and for growth promoting by other livestock farmers within the region.

5.4 High Incidence of Antibiotics Residue in Locally Produced Honey Samples

The occurrence of antibiotic residues in honey has been mentioned as the major problem which persist in honey as a result of the broad use of antibiotics for the different purposes (Ullah et al., 2013). However, in contrast to many global beekeepers, most beekeepers and/or honey producers in the region have no or little knowledge of the use of antibiotics in honey production.

In this study, thirty (30) honey consisting of 7 imported and 23 locally produced honey sampled from different locations within the region were analyzed for the presence of antibiotics residue

using the Premi® rapid screening method. Using the Premi® test kit, Addo et al. (2015), reported an overall 36(18%) of the presence of antibiotics residues in liver and kidney of 200 cattle carcasses from selected abattoirs in Accra, Ghana. Also, Agmas and Adugna (2018), opined that the Premi® test kit as a suitable initial screening for antibiotics residue is very sensitive and would only detect residues when their level is high.

Residue of antibiotics was detected in 27 out of the 30 honey samples. Six (6) of these were from imported samples whereas the remaining 21 was from locally produced honey samples. Saleh et al. (2017), reported on the detection of antibiotic residue in 5 out of 9 imported samples and 2 out of 7 in local honey samples in Yemen. The detection of antibiotics residue in the locally produced honey samples is alarming because of the lack or little knowledge of the use of antibiotics in honey production by these producers. However, there are evidences of contamination of antibiotics in honey from other sources than direct application (FAO, 2009). The presence of antibiotics in the locally produced honey samples could be attributed to the available antibiotics in the environment through human applications and the various agricultural uses (CDC, 2013; OECD, 2008). The aforementioned factors coupled with the intensive use of antibiotics by foreign beekeepers could have accounted for the presence of antibiotics in the imported honey samples (Al-Waili et al., 2012; Reybroeck et al., 2012; Underwood et al., 2019).

Allergic reactions, teratogenic effects, nephrotoxicity, renal dysfunctions and damage to central nervous system are among some of the reported cases associated with the consumption of honey with antibiotics residues (Barrasso et al., 2018; Korkmaz et al., 2017; Wilmart et al., 2016). As such the detection of antibiotics residue in the honey samples from the region should be a cause for alarm. Notwithstanding, per the Codex Alimentarius Commission, antibiotics of any form should not be detected in a sample of honey meant for consumption.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion;

The consumption of honey spanned through all age range with most of the consumers having a preference for the locally produced honey. Notwithstanding, most of the producers and consumers of honey interviewed in this study have little knowledge of antibiotics use in honey production and its related health implications.

Also, the physicochemical parameters of most of the imported and locally produced honey sampled for the study met the various international set standards. However, this did not translate into its bacteriological quality as the bacteria load of most of the honey samples were above the recommended limits. Also, the study recorded a high incidence of antibiotics residues in both the imported and locally produced honey. In conjunction, was the high prevalence of antibiotic-resistant bacteria isolates from the honey samples.

In general, the locally produced honey samples (particularly samples from the production sites) could be said to be of quality in comparison to the imported honey as some were of minimum or free from bacteria contamination.

Nonetheless, the presence of contaminants (microbes and antibiotics residue) in some of the imported and locally produced honey samples is a public health concern and might be dangerous to the consumers' health.

6.2 Recommendation;

It is recommended that studies should be conducted on the molecular characterization of the isolates of bacteria from the honey samples since biochemical tests were not enough to point out to specific strains.

Also, studies should be carried out on fungi, yeast or mold in both the imported and locally produced honey samples for a comprehensive microbial study since the current study focused on bacteriological quality.

Furthermore, the specific antibiotics and their levels should be determined in subsequent studies using HPLC or LC/MS or GC/MS.

Lastly, due to the current unavailability of data on antibiotics residues in honey in the region and the country as a whole, the study should serve as reconnaissance for a national assessment of antibiotics in honey.

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