Physicochemical Properties of Imported and Locally Produced Honey did not translate into its Microbial Quality and Antibacterial Activity

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Abstract

Honey is often considered a good substitute for sugar. However, concerns about the poor quality of locally produced honey have necessitated the importation of honey to meet the domestic demand in spite of Ghana's good climatic conditions suitable for honey production. Due to the increasing level of demand for honey both locally and internationally, we highlighted here the need for an assessment of the physicochemical, microbiological quality and antibiotics resistance of microbes isolated from the imported and locally produced honey vis-à-vis international standards. Thirty honey samples which comprise 7 imported and 23 locally produced were randomly sampled from retail outlets consisting of supermarkets, marts, shops, and open markets as well as from production sites within the northern region for physicochemical analysis, microbial analysis and antibiogram. Results of the physicochemical quality analysis showed that sampled honey (both imported and locally produced) was within acceptable set standards. However, microbial quality was poor as a high incidence of bacterial contamination and resistance was recorded in the study. The 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 region.

Introduction

Honey is a natural sweetener produced by honeybees and consumed all over the world (Rao et al., 2016). In Ghana, the demand for honey exceeds supply (Akangaamkum et al., 2010) partly as a result of the latest trend of the health concern and impacts of table sugar as against the perceived health benefits of natural honey. Consumers in recent times are expressing scepticism to the consumption of honey amid news about its adulteration and contamination. This has necessitated various studies on the quality of commercialized honey from the different points of sale.

Currently, the quality of honey is dependent on its sensory, physical, chemical and microbiological characteristics (Belay et al., 2014). These factors are considered to have a high influence on the hygienic quality and stability of honey. Moisture content, pH, viscosity, hygroscopicity, ash content, crystallization, free acidity, total soluble solids, and sugar content are among the physicochemical parameters often used to characterize honey. According to Gangwar et al. (2010), these quality indices form the basis of honey's physical and chemical characterization in that they are comparatively simple to measure and provide good information value.

On the other hand, standard plate count, tests for coliforms, molds, yeasts and pathogenic bacteria like *Salmonella*, *Staphylococcus* and *Clostridium* species serves as the safety and quality indices for microbial contaminants in honey due to the inability of some producers and processors to adhere to stringent hygienic practices when handling honey (Tesfaye, 2016). Though the antimicrobial properties of honey inhibit the growth of many microorganisms some pathogenic microorganisms have been found to be resistant to the antibacterial activity of honey (Snowdon, 1999).

Antibiotic-resistant bacteria in foods of animal origin have been recognized as a threat to human health due to their possible transmission to humans via the food chain (Hilbert & Smulders, 2004; van den Honert et al., 2018). Antibiotic-resistant foodborne pathogens account for the increased morbidity and the overall 23, 000 deaths annually due to the ineffectiveness of antibiotics (CDC, 2013; Sugawara & Nikaido, 2014).

Despite standards that define honey, in Ghana, it appears the quality and safety of honey have been left in the hands of producers and sellers due to poor regulation by the appropriate institutions. Couple with this is also the limited number of studies on the antimicrobial resistance pattern of bacterial isolates in honey on Ghanaian markets. Thus, the need to investigate the physicochemical and microbiological quality as well as the antibiotic dynamics to ascertain the quality of the imported and locally produced honey vis-à-vis international standards.

Materials And Methods

Study area

The study was conducted in the Tolon district and the Tamale metropolis both in the northern region of Ghana. Geographically, the Tolon district lies between longitudes 0° 53' and 1° 25' West and latitude 9° 15' and 10° 02' North (Ghana Statistical Service, 2014a). The district is about 24 km from the Tamale metropolis which lies between latitudes 9° 16' and 9° 34' North and longitudes 0° 36' and 0° 57' West (Ghana Statistical Service, 2014b).

Sampling and preparation

A total of 30 honey comprising imported and locally produced honey were sampled for this study. Seven of these samples were imported honey found on the shelves of two supermarkets in the Tamale metropolis at the time of the study. The remaining 23 honey were locally produced honey collected from both producers and retailers in Aboabo, Sakasaka, Nyohini, Lamashegu, Kukuo, Tamale central and Nyankpala. Locally produced honey samples were categorized as producers (samples obtained directly from production sites), unbranded (samples collected from market sellers in open markets) and branded (samples collected from mini-marts, and supermarkets). In summary, 6 branded, 9 unbranded, 8 producers and 7 imported honeys were collected from the different locations and transported in an ice chest containing ice blocks to the Spanish Laboratory Complex of the University for Development Studies, Ghana for laboratory procedures and experimentation.

Physicochemical Analysis

Determination of pH

The procedure outlined by the International Honey Commission (2009) was followed to determine the pH of each of the honey samples. Briefly, 10 g of each of the honey samples was dissolved in 75 ml of distilled water in a 250 ml beaker. The solution was stirred until an even mixture was attained and the pH reading was recorded with a pH meter (Crison pH meter Basic 20, Spain).

Determination of total soluble solids

The method described by A-Rahaman et al. (2013), 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 cleaned with distilled water and then adjusted to zero at a temperature of 20 ℃. A drop of the honey sample was placed on the prism plate of the refractometer and afterward covered for the readings to be taken. Readings were directly recorded as total soluble solids in percentage.

Determination of free acidity

As outlined by the Association of Official Analytical Chemist (2000), 5 g of each of the honey sample was weighed and diluted with 37.5 ml distilled water in a 250 ml conical flask. The honey solution was titrated against 0.1 M sodium hydroxide (NaOH) whereas phenolphthalein was used as the color indicator. The endpoint was recorded and free acidity was determined as;

$$\mathrm{FreeAcidity} = \frac{\mathrm{Titre} \times \mathrm{N} \times 196}{\mathrm{WeightofSample}} \times 100\%$$

Where N = normality of the base NaOH

Determination of moisture content

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

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

Determination of ash

The determination of ash content was carried-out following the procedures outlined by the Association of Official Analytical Chemist (2000). To remove moisture that would cause foaming of the samples at the early stages of ashing, 2 g of each of the samples were kept in a previously weighed porcelain crucible and dried in an oven at 105 ℃ for 4 h. 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 h. The ash content was calculated on a dry basis according to the equation:

$$\text{Ashcontent} = \frac{\text{Weightofash} - \text{Weightofemptycrucible}}{\text{Weightofsample}} \times 100\%$$

Determination of viscosity

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

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 5 g of the sample and adding it to 100 ml 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 250 ml volumetric flask. Hundred milliliters of the solution were transferred by pipetting into a conical flask. Ten ml of 0.1 M diluted hydrogen chloride and 2–3 drops of phenolphthalein indicator was added and the solution were boiled for 3 mins.

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

Bacteriological analysis

The bacterial load of the honey samples was determined following the procedures outlined by the American Public Health Association (2001) in the compendium of methods for the microbiological examination of foods. Twenty-five grams of each of the 30 honey samples were weighed and homogenized in 225 ml of 0.1% peptone water (Oxoid, Basingstoke, UK).

Also, decimal solutions were carried out to the fifth level, that is 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} with 0.1% peptone water as the diluent.

For the enumeration of *Listeria* spp., 100 µl of each dilution was inoculated onto the surface of the freshly prepared solidified Oxford Listeria Agar Base (Alpha Biosciences, USA). Presumptive *Listeria* spp. appearing gold with dark centres on respective agar plates were selected for counting after 24 h of incubation.

On the other hand, Mannitol Salt Agar (Oxoid, Basingstoke, UK) was employed in the enumeration of *Staphylococcus* spp. A hundred microliters of each dilution were aliquoted and spread uniformly onto the surface of the agar plates. The inoculated plates were incubated in an inverted position at 37 ℃ for 24 h. Suspected colonies appearing pinkish on the MSA plate were counted as *Staphylococcus* spp.

Concerning the enumeration of *Clostridium* spp., 20 g of each honey sample was diluted to a final volume of 100 ml in a sterile 250 ml Schott Durham bottle (Durham Group, Germany). The content was mixed thoroughly and boiled for 5 mins. Upon cooling, 10 ml was drawn and homogenized in 90 ml of 0.1% peptone water. A hundred microliters of the aliquot were pipetted onto a freshly prepared Perfringens Agar Base (Oxoid, Basingstoke, UK) and incubated at 47 ℃ for 24 to 48 h in an anaerobic glass jar. Colonies appearing as black or dark on the agar plates were counted as presumptive *Clostridium* spp.

For the enumeration of *Lactobacillus* spp., 100 µl of each dilution was inoculated onto the surface of the freshly prepared solidified Lactobacillus MRS Agar Base (Alpha Biosciences, USA). Presumptive *Lactobacillus* spp. appearing as large clear colonies were counted after 24 h of incubation.

Meanwhile, to enumerate *Salmonella* spp., each honey sample was first enriched by homogenizing 25 g of the honey in 225 ml of 0.1% peptone and afterward incubated overnight at 37 ℃. A hundred microlitres of each of the pre-enriched samples were pipetted onto freshly prepared Salmonella-Shigella Agar (Oxoid, Basingstoke, UK) plates. The inoculated plates were incubated in an inverted position at 37 ℃ for 24 h. Straw-colored colonies with black centres were counted as *Salmonella* spp.

Concerning the enumeration of *Escherichia coli*, 100 µl of each of the prepared honey samples was pipetted onto freshly prepared MacConkey (Oxoid, Basingstoke, UK) agar plates. After 24 h of incubation, pink-colored colonies were counted as *Escherichia coli*.

Confirmatory tests

For identification and confirmation purpose, colonies of the respective suspected microorganisms were streaked onto a freshly prepared Nutrient Agar (Techno Pharmchem, India) and incubated at 37 ℃. After 18-24 h of incubation, pure cultures of the respective suspected bacteria were subjected to citrate test, oxidase test, coagulase test, as well as Gram stain to confirm the suspected isolates.

Antibiotic susceptibility/sensitivity test

Antibiotic sensitivity testing was carried out using Kirby-Bauer's disc diffusion method. The zones of inhibition produced by the antibiotics were measured and compared with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables for the interpretation of zone diameters. Antibiotic discs used for susceptibility/sensitivity testing were obtained from Axiom Laboratories, New Delhi, India.

Statistical analysis

Data on laboratory analysis were entered into Microsoft Office Excel (2016) for processing and further subjected to one-way analysis of variance (ANOVA) with mean comparison performed with the turkey multiple comparison test under a 95% confidence interval. The General Statistics (Genstat) edition 18 was employed in the analysis of variance.

Results And Discussion

Physicochemical properties of the honey samples

Physical and chemical properties remain one of the key indicators of assessing the quality of honey. In this study, the 30 honey sampled from the different sampling outlets differed significantly in physicochemical properties. For instance, a significant difference ($P < 0.05$) was recorded between the mean pH of imported (4.46) and locally produced honey samples (4.77). Generally, the pH of the honey samples was acidic with the pH of imported samples ranging from 3.8 to 5.20 whereas that of locally produced samples was from 3.8 to 5.89 (Table 1). These values compare well with the 3.3–6.1 reported by Aljohar et al. (2018) and also within the recommended pH (3.4 to 6.1) by the Codex Alimentarius Commission (CAC, 2001). The differences in pH can be attributed to the floral diversity and composition (Fahim et al., 2014). Adjunct to this, Sohaimy et al. (2015), mentioned that not only does the properties and composition of honey dependent on its geographical floral origin but also on the season of harvesting.

The aforementioned support the variation in free acidity recorded for the imported and locally produced samples in the present study. Free acidity of samples from imported sources ranged from 7.84 to 16.36meq/kg whilst that of locally produced honey samples was from 7.84 to 41.16meq/kg (Table 1). Also, there was a significant difference ($P < 0.05$) between the mean of 12.14meq/kg and 19.92meq/kg recorded as free acidity for imported and locally produced honey samples respectively. The findings from this study are 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. Moreover, the free acidity recorded in this study was within the maximum permissible value (50meq/kg) recommended by the Codex Alimentarius Commission (2001).

Regarding viscosity, the values ranged between 6288cP and 36000cP for imported honey samples whereas that of local samples ranged between 2112cP and 17730cP (Table 1). There was a significant difference ($P < 0.05$) between 16198cP and 7426cP recorded as the mean viscosity for imported and locally produced honey samples respectively. 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, Ghana. The variation in the values of viscosity recorded in the study could be attributed to the surrounding temperature from which they were collected (Gómez-Díaz et al., 2009).

The variations in the viscosity values are further supported by the moisture content observed in the samples from the different sources where a higher viscosity value led to lower moisture content. Conversely, 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. However, the moisture content recorded in this study was within the maximum permitted (20%) moisture content set by the Codex Alimentarius Commission (2001). It is noteworthy to emphasize that the technique employed in extracting honey could account for variation in moisture content (Adjalooc et al., 2017).

Despite Codex Alimentarius Commission regulations, that the container of which honey is kept for sale should be labelled or designated according to the floral or plant source, only one (imported) out of the thirty samples had as part of its 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 honeydew and/or its combination should be greater or equal to 1.2%. In this study, 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 (Table 1). Except

for two samples; one from branded source and the other obtained from the production site (Producer) which recorded 1.08% and 0.68% respectively, ash content for the analysed 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).

Table 1 *should be inserted here*

Table 1
Physical and Chemical Properties of the honey samples

Sample ID	Source	pH	Acidity (meq/kg)	Moisture (%)	Ash (%)	Viscosity (Cp)
JN-1	Imported	4.93^{jk}	7.84^{a}	17.69^{a}	0.07^{a}	10520^{j}
JN-2	Imported	3.95^{ab}	31.36^{def}	16.5^{a}	0.08a	35812^{n}
JN-3	Imported	3.80^{a}	33.32^{efg}	17.13^{a}	0.13ab	32070^{m}
JN-4	Imported	4.71^{i}	9.80^{ab}	17.51^{a}	0.07a	7120^{gh}
JN-5	Imported	4.52^{fgh}	7.84^{a}	15.89^{a}	0.16^{abcd}	14582^{k}
JN-6	Imported	4.10^{bc}	45.08^{g}	17.89^{a}	0.26^{abcd}	6394^{efg}
JN-7	Imported	5.03^{k}	33.32^{efg}	19.23^{a}	0.27^{abcd}	6548^{fg}
JN-8	Branded	5.89^{p}	17.64^{abc}	17.09^{a}	0.27^{abcd}	7196^{gh}
JN-9	Branded	5.37^{mn}	9.80^{ab}	17.48^{a}	0.11^{ab}	5938^{defg}
JN-10	Branded	5.67^{o}	17.64^{abc}	18.73^{a}	0.15^{abc}	9920^{ij}
JN-11	Producer	5.69^{o}	7.84^{a}	16.63^{a}	0.18^{abcd}	5732^{defg}
JN-12	Branded	4.64^{ghi}	39.20^{fg}	15.74^{a}	1.08^{f}	5028^{cdef}
JN-13	Branded	4.23^{cd}	19.60^{abcd}	16.01^{a}	0.10^{ab}	4890^{cde}
JN-14	Producer	4.10^{bc}	21.56^{bcde}	17.05^{a}	0.07^{a}	6994^{gh}
JN-15	Producer	4.07^{bc}	17.64^{abc}	15.56^{a}	0.68^{e}	15054^{k}
JN-16	Producer	4.16^{cd}	41.16^{fg}	12.41^{a}	0.43^{bcde}	9792^{ij}
JN-17	Producer	4.43^{ef}	21.56^{bcde}	12.44^{a}	0.32^{abcd}	6886^{g}
JN-18	Imported	5.21^{lm}	13.72^{ab}	13.9^{a}	0.04^{a}	9624^{ij}
JN-19	Producer	4.48^{efg}	15.68^{ab}	13.82^{a}	0.14^{ab}	17130^{l}
JN-20	Unbranded	4.94^{jk}	17.64^{abc}	15.4^{a}	0.15^{abcd}	5886^{defg}
JN-21	Unbranded	4.31^{de}	41.16^{fg}	17.69^{a}	0.37^{abcde}	4509^{bcd}
JN-22	Unbranded	4.75^{i}	9.80^{ab}	18.8^{a}	0.49^{cde}	2418^{a}
JN-23	Unbranded	5.01^{k}	21.56^{bcde}	13.93^{a}	0.50^{de}	3269^{ab}
JN-24	Unbranded	3.81^{a}	17.64^{abc}	19.55^{a}	0.44^{bcde}	2216^{a}
JN-25	Unbranded	4.52^{fgh}	9.80^{ab}	17.14^{a}	0.44^{bcde}	9742^{ij}

Values in column with the same superscript are not significantly different

Sample ID	Source	pH	Acidity (meq/kg)	Moisture (%)	Ash (%)	Viscosity (Cp)
JN-26	Unbranded	5.04^{kl}	29.40^{cdef}	17.25^{a}	0.21^{abcd}	8580^{hi}
JN-27	Unbranded	4.66^{hi}	15.68^{ab}	18.08^{a}	0.05^{a}	6362^{efg}
JN-28	Unbranded	4.81^{ij}	17.64^{abc}	16.93^{a}	0.17^{abcd}	3650^{abc}
JN-29	Producer	5.42^{n}	13.72^{ab}	17.45^{a}	0.32^{abcd}	10840^{j}
JN-30	Producer	4.65^{ghi}	21.56^{bcde}	19.11^{a}	0.31^{abcd}	9479^{ij}
LSD 5%	**0.09**	**0.09**	**6.03**	**3.53**	**0.17**	797.50
Codex	**3.4–6.1**	**-**	**≤ 50meq/kg**	**≤ 21%**	**≤ 0.6**	-
EU	**-**	**-**	**≤ 40meq/kg**	**≤ 21%**	**≤ 0.6**	-
Values in column with the same superscript are not significantly different						

Sugar content of the honey samples

The sugar content of the honey samples from the different sampling outlets was also determined (Table 2). The Codex Alimentarius Commission currently has 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 to or greater than 81.4% is categorized as high-grade honey (A or B) whereas that between 80% and 81.3% are considered 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% (Table 2). Per this, only one out of the 7 imported honey samples were in the 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 the 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. Adjunct to this, is the assertion of Nyau (2013), that the moisture content of honey can influence its sugar content, otherwise the total soluble solids. Hence, the lower moisture content will result in an increase in 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 sources recorded the least reducing and non-reducing content in comparison to local samples. Reducing sugar content ranged from 50.35–68.02% for imported samples whilst local samples recorded a range from 49.07–76.03% (Table 2). 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. With this, it can be said that both the locally produced and imported honey samples were of quality since none of the samples had their sugar content above the maximum permissible standard. Again, the CAC has 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 were 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 was below the recommended standard. The assertion of Azonwade et al. (2018), that reducing sugar content is high in arid areas than in 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 in weather, nectar conditions, extraction methods as well as storage temperatures and conditions (Elenany, 2019; Muli et al., 2007; Orina, 2012). 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.

Table 2
Sugar content of the honey samples

Sample ID	Source	TSS (%)	RS (%)	NRS (%)
JN-1	Imported	78.00^{abc}	50.35^{ab}	2.65^{ab}
JN-2	Imported	71.87^{abc}	51.69^{ab}	2.72^{ab}
JN-3	Imported	72.20^{abc}	53.87^{abc}	2.84^{abc}
JN-4	Imported	74.33^{abc}	54.60^{abcd}	2.87^{abcd}
JN-5	Imported	81.60^{abc}	56.19^{abcd}	2.96^{abcd}
JN-6	Imported	68.07^{ab}	68.02 ^{abcde}	3.58^{abcde}
JN-7	Imported	98.87^{c}	52.53^{ab}	2.77^{ab}
JN-8	Branded	97.33^{c}	73.16^{de}	2.65^{ab}
JN-9	Branded	92.00^{bc}	57.15^{abcde}	3.01^{abcde}
JN-10	Branded	76.73^{abc}	71.88^{cde}	3.78^{cde}
JN-11	Producer	62.47^{a}	68.02^{abcde}	3.58^{abcde}
JN-12	Branded	66.00^{ab}	63.56^{abcde}	3.35^{abcde}
JN-13	Branded	72.67^{abc}	56.19^{abcd}	2.96^{abcd}
JN-14	Producer	85.33^{abc}	66.91^{abcde}	3.52^{abcde}
JN-15	Producer	74.67^{abc}	76.03^{e}	4.00^{e}
JN-16	Producer	72.67^{abc}	73.37^{de}	3.86^{de}
JN-17	Producer	94.20^{bc}	66.91^{abcde}	3.52^{abcde}
JN-18	Imported	70.67^{abc}	60.42^{abcde}	3.18^{abcde}
JN-19	Producer	92.00^{bc}	76.03^{e}	4.00^{e}
JN-20	Unbranded	70.07^{abc}	50.35^{ab}	2.65^{ab}
JN-21	Unbranded	80.67^{abc}	49.07^{a}	2.58^{a}
JN-22	Unbranded	75.20^{abc}	49.69^{a}	2.62^{a}
JN-23	Unbranded	89.47^{abc}	66.17^{abcde}	3.48^{abcde}
JN-24	Unbranded	90.00^{abc}	69.30^{bcde}	3.65^{bcde}
JN-25	Unbranded	71.67^{abc}	63.56^{abcde}	3.35^{abcde}

Values in column with the same superscript are not significantly different

Sample ID	Source	TSS (%)	RS (%)	NRS (%)
JN-26	Unbranded	77.00^{abc}	54.21^{abcd}	2.85^{abcd}
JN-27	Unbranded	86.73^{abc}	51.04^{ab}	2.69^{ab}
JN-28	Unbranded	77.33^{abc}	58.17^{abcde}	3.06^{abcde}
JN-29	Producer	91.13^{abc}	51.04^{ab}	2.69^{ab}
JN-30	Producer	89.00^{abc}	51.69^{ab}	2.72^{ab}
LSD 5%	**0.09**	**14.69**	**9.52**	0.50
Codex	**3.4–6.1**	**-**	**≥ 50%**	≤ 5%
EU	**-**	**-**	**≥ 50%**	-
Values in column with the same superscript are not significantly different				

Table 2 *should be inserted here*

Bacterial Load of the honey samples

Lactobacillus spp. and *Listeria* spp. respectively emerged as the most predominant bacteria with the highest detectable load recorded in 25(83.3%) out of the total (30) honey sampled for the study. However, there was no significant difference (P = 0.26) between 1.16×10^4 CFU/ml and 8.38×10^3 CFU/ml recorded respectively as the mean *Lactobacilli* load for the imported and locally produced honey samples. This result is however less than the 3.8×10^3 to 5.5×10^7 and 6.5×10 to 6.3×10^6 CFU/ml recorded respectively for *Lactobacillus* spp. in both local and imported probiotic yoghurts sold in Accra as reported by Mahami and Odonkor (2014). Though, the biochemical test employed in the study was not enough to conclusively point out a specific strain of the bacteria. However, studies have indicated that among the lactic acid bacteria, the genus *Lactobacillus* is the most predominant species in the gut of honeybees (Tajabadi et al., 2014).

Also, there was no significant difference (P = 0.47) between the mean *Listeria* load of 1.13×10^6 CFU/ml and 8.95×10^5 CFU/ml recorded respectively for imported and locally produced honey samples that had detectable counts. Notwithstanding, *Listeria* load ranged from 3.15×10^5 to 2.93×10^6 CFU/ml for the 25(83.3%) honey samples recording detectable count. The occurrence of *Listeria* spp. in the honey samples could be attributed to post-processing contamination from the processing equipment or materials. As it was observed that honey samples particularly those from open markets (unbranded) and production sites (producer) were kept or packaged 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 the sample collection revealed that most of the market sellers have their honey in a large container which they fetch based on the quantity or amount required by the customer. Cross contaminations as a 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. Meanwhile, per 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 100 CFU/ml. Since honey can be considered in any of these categories, all 25 samples in which *Listeria* spp. was detected should be considered unwholesome for consumption.

With a detectable load of 24(80%) out of the 30 honey samples, *Clostridium* spp. emerged as the next predominant bacteria with the highest detectable load. However, there was a significant difference ($P < 0.05$) between $1.582.93\times10^6$ CFU/ml and 6.46×10^5 CFU/ml recorded respectively as the mean load of *Clostridium* for all the imported and locally produced honey samples that had detectable counts. *Clostridia* spores are widely distributed in the environment, therefore it could be assumed that the contamination of the samples could have arisen through contaminated dust particles at processing or storage or via ingestion of contaminated dust during the 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 the 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.

Concerning *Staphylococci* load, there was a significant difference ($P < 0.05$) between 1.17×10^6 CFU/ml and 5.53×10^5 CFU/ml recorded respectively as the mean *Staphylococci* load for the imported and locally produced honey samples with a detectable count. The range of *Staphylococci* load for the 21(70%) samples with detectable count was from 3.2×10^5 to 2.74×10^6 CFU/ml. 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 (2017), for honey within the Tamale metropolis. Detectable mean load of the imported samples was 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).

Concerning Gram-negative isolates, only two samples from the production sites (producers) recorded detectable counts of *E. coli* and *Salmonella* spp. out of the 30 honey samples. There was no significant difference between 4.05×10^5 CFU/ml and 3.75×10^5 CFU/ml recorded as the mean load of *E. coli* for the two samples. However, there was a significant difference ($P < 0.05$) between 9.85×10^3CFU/ml and 1.72×10^4 CFU/ml recorded as the mean load of *Salmonella* for both samples. The study recorded less contamination by gram-negative isolates despite several reports that the intestines of bees are dominated by bacteria from this group (Olaitan & Iyabo, 2007). This could be due to the inability of gram-negative bacteria to withstand the hostile conditions of honey in comparison to their counterparts, gram-positive bacteria (Erkmen & Bozoglu, 2016). The findings on the occurrence and load of *E. coli* agrees with that of Adadi and Obeng (2017), 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* was above the satisfactory load (100 CFU/ml) recommended by the Center for Food Safety (2014).

Table 3 should be inserted here

Table 3
Microbial load of the honey samples

Honey Sample	Source	Listeria spp.	Clostridium spp.	Salmonella spp.	Lactobacillus spp.	E. coli	Staphylococcus spp.
JN-1	Imported	2.78×10^{6g}	1.62×10^{6f}	ND*	2.55×10^{4l}	ND*	1.84×10^{6h}
JN-2	Imported	2.93×10^{6g}	2.34×10^{6hi}	ND*	2.2×10^{4k}	ND*	6.65×10^{5ef}
JN-3	Imported	5.3×10^{5bc}	2.53×10^{6i}	ND*	1.93×10^{4j}	ND*	1.98×10^{6i}
JN-4	Imported	3.8×10^{5abc}	1.0×10^{4a}	ND*	3.85×10^{3cd}	ND*	2.74×10^{6k}
JN-5	Imported	$6.35\times10^{5}b^{c}$	2.55×10^{5abcd}	ND*	5.4×10^{3defg}	ND*	3.8×10^{5bcd}
JN-6	Imported	3.15×10^{5ab}	2.52×10^{6i}	ND*	$5.05\times10^{3}d^{ef}$	ND*	5.8×10^{5e}
JN-7	Imported	2.88×10^{6g}	2.55×10^{6i}	ND*	2.53×10^{4l}	ND*	3.2×10^{5b}
JN-8	Branded	3.5×10^{5ab}	3.35×10^{5abcd}	ND*	1.80×10^{4j}	ND*	4.35×10^{5cd}
JN-9	Branded	3.65×10^{5ab}	1.7×10^{5ab}	ND*	3.55×10^{3cd}	ND*	ND*
JN-10	Branded	3.5×10^{5ab}	1.2×10^{5ab}	ND*	4.25×10^{3cdef}	ND*	ND*
JN-11	Producer	3.8×10^{5abc}	2.3×10^{5abc}	ND*	5.2×10^{3defg}	ND*	ND*
JN-12	Branded	ND*	ND*	ND*	ND*	ND*	ND*
JN-13	Branded	ND*	ND*	ND*	ND*	ND*	ND*
JN-14	Producer	8.45×10^{5cd}	9.45×10^{5e}	ND*	2.55×10^{3bc}	ND*	3.75×10^{5bcd}
JN-15	Producer	3.45×10^{5ab}	ND*	ND*	ND*	ND*	3.5×10^{5bc}
JN-16	Producer	ND*	ND*	ND*	ND*	ND*	ND*
JN-17	Producer	ND*	ND*	ND*	ND*	ND*	ND*
JN-18	Imported	3.5×10^{5ab}	1.79×10^{6fg}	ND*	3.0×10^{2ab}	ND*	ND*
JN-19	Producer	ND*	ND*	ND*	7.45×10^{3gh}	ND*	ND*
JN-20	Unbranded	2.9×10^{6g}	1.71×10^{6fg}	ND*	6.4×10^{3fg}	ND*	4.55×10^{5d}
JN-21	Unbranded	2.2×10^{6f}	2.93×10^{6j}	ND*	2.62×10^{4lm}	ND*	6.95×10^{5f}
JN-22	Unbranded	3.3×10^{5ab}	4.15×10^{5bcd}	ND*	3.8×10^{3cd}	ND*	2.63×10^{6j}
JN-23	Unbranded	3.2×10^{5ab}	4.25×10^{5bcd}	ND*	3.95×10^{3cde}	ND*	1.99×10^{6i}
JN-24	Unbranded	3.35×10^{5ab}	4.10×10^{5bcd}	ND*	3.35×10^{3cd}	ND*	3.95×10^{5bcd}
JN-25	Unbranded	2.56×10^{6fg}	3.95×10^{5bcd}	ND*	4.75×10^{3cdef}	ND*	2.01×10^{6i}

ND: not detected *Values in column with the same superscript are not significantly different*

Honey Sample	Source	Listeria spp.	Clostridium spp.	Salmonella spp.	Lactobacillus spp.	E. coli	Staphylococcus spp.
JN-26	Unbranded	3.25×10^{5ab}	5.00×10^{5bcd}	ND*	6.20×10^{3efg}	ND*	3.90×10^{5bcd}
JN-27	Unbranded	1.27×10^{6de}	2.08×10^{6gh}	ND*	2.39×10^{4kl}	ND*	1.04×10^{6g}
JN-28	Unbranded	1.68×10^{6e}	4.80×10^{5}bcd	ND*	1.00×10^{4i}	ND*	5.85×10^{5e}
JN-29	Producer	2.76×10^{6g}	5.55×10^{5cd}	9.85×10^{3b}	9.55×10^{3hi}	3.75×10^{5b}	4.65×10^{5d}
JN-30	Producer	4.04×10^{5abc}	6.15×10^{5de}	1.72×10^{4c}	2.85×10^{4m}	4.05×10^{5c}	5.95×10^{5ef}
LSD 5%		2.31×10^{5}	1.16×10^{3}	2.21×10^{3}	1.16×10^{3}	6.8×10^{4}	5.17×10^{4}

ND: not detected *Values in column with the same superscript are not significantly different*

Antibiotic Resistance Profile

Despite the notion that *Listeria* spp. are most prevalent in temperate regions than in 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 a 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 the resistance of *Listeria* to gentamicin was 1(6%) in this study, it however calls for public health concern since in most cases gentamicin is combined with the first choice of drugs for the 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 these bacteria have been reported to easily transfer resistance genes to other phylogenetically related Gram-positive (Lyon et al., 2008).

Table 4: 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)

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). In addition, some *Lactobacillus* spp. has the tendency of transferring antibiotic resistance gene(s) to pathogens (Preethi et al., 2017).

Table 5: Antimicrobial susceptibility test for some common antibiotics of Lactobacillus spp.

		Breakpoints (mm)			Antimicrobial Susceptibility № of isolates (%)		
Sample source	Antimicrobial	R	I	S	R	I	S
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)

Isolates of *Staphylococcus* spp. from both imported and locally produced samples were all (100%) susceptible to gentamicin and ciprofloxacin. However, whereas samples from imported sources 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 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 has been cited in numerous scientific studies and reports as the alternative option for overcoming *Methicillin-Resistant Staphylococcus aureus* (MRSA) and multidrug resistance in *Staphylococcus* (Almasaudi et al., 2017; Grecka et al., 2018; Iqbal et al., 2019; Liu et al., 2018).

Table 6: 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)

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 sources 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, 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 is 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.

Table 7: 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)

Growth of *E. coli* and *Salmonella* spp. was detected in only two samples out of the thirty (30) 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. Most of the studies are concentrated on isolates from clinical patients and few are available on food isolates. George et al. (2012), reported 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 contrasts with 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. Over time, these bacteria could assume a resistant state, thus the 2(100%) resistance was recorded for such antibiotics.

Table 8: Antimicrobial susceptibility test for some common antibiotics of E. coli.

		Breakpoints (mm)			Antimicrobial Susceptibility Nº 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)

Salmonella spp. 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. Again, Adzitey et al. (2016), reported on the 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 of 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 promoters by livestock farmers within the region (Saba, 2019).

Table 9: 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)

Conclusion

In an attempt to link the physicochemical parameters determined in this study to the microbial load, it can be said that the physicochemical qualities of the honey did not reflect 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 reported in some studies. Meanwhile, 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 witnessed from storerooms of some producers and market sellers. Lastly, honey has 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. 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.

Abbreviations

AMP: Ampicillin; AMX: Amikacin; AZM: Azithromycin; CDC; Centre for Disease Control and Prevention; CFU/ml: Colony forming unit per millilitre; CHL: Chloramphenicol; CIP: Ciprofloxacin; CTR: Ceftriaxone; CTX: Cefotaxime; CXM: Cefuroxime; E: Erythromycin; EU: European Union; GEN: Gentamycin; I: Intermediate: LSD: Least significant difference; meq/kg: milliequivalent per kilogram; NA: non-applicable; NRS: Non reducing sugar; RS: Reducing sugar; R: Resistant; RO: Roxithromycin; S: Susceptible, TSS: Total soluble solids; UK: United Kingdom

Declarations

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Availability of data materials

All necessary data supporting our findings can be found in the paper.

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Competing Interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Authors Information

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Dr. Lydia Quansah is a senior lecturer and the head of the department of Forensic Science at the faculty of biosciences of the University for Development Studies, Tamale, Ghana. Dr. Quansah holds a bachelor's degree in Agriculture Technology from the University for Development Studies, Ghana; a master's degree in Agriculture Science from the Hebrew University of Jerusalem, and a doctor of philosophy in Dessert Studies (Agriculture and Biotechnology) from the Ben-Gurion University of the Negev, Israel. Dr. Quansah's research interests are not limited to plant physiology, abiotic stress, metabolic and DNA fingerprinting, and modelling to understand metabolic pathways in seeds and mature plants but also food quality and safety.

Dr. Osman Adamu Dufailu is skilled in cell culture, molecular techniques, microscopy, and proteomics. He obtained a Ph.D. in Life Science from the University of Nottingham, UK, and is currently a lecturer in the Department of Microbiology at the Faculty of Bioscience of the University for Development Studies.

Authors contributions

Mr. Joseph Nzeh and Dr. Lydia Quansah conceptualized the research proposal and its design. Joseph Nzeh and Dr. Osman Adamu Dufailu carried out the laboratory experimentation, data analyses and its interpretation. Writing or preparation of

the manuscript was done by Joseph Nzeh whilst Dr. Lydia Quansah and Dr. Osman Adamu Dufailu peruse the manuscript to ensure it fits for publication. All authors read and approved the final manuscript.

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