

MICROBIAL AND ANTIBIOTIC CONTAMINANTS IN IMPORTED AND LOCALLY PRODUCED HONEY IN THE TAMALE METROPOLIS OF THE NORTHERN REGION OF GHANA

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ABSTRACT

Honey remains a valued natural product and has been used by humans as an important food source, disease treatment, and a healthy sugar source since ancient times. However, recent reports on the adulteration of honey and honey polluted with contaminants like pesticides, heavy metals, microorganisms as well as antibiotics have gained public attention. Thus, this study aimed to assess the quality and safety of imported and locally produced honey by specifically determining microbial and antibiotic contaminants as well as the beekeeping practices of honey producers within some locations of the Tamale metropolis. A semi-structured questionnaire was designed to gather information on the sources of honey, knowledge of diseases affecting bees, knowledge of contamination of honey, and knowledge of antibiotics use in honey production from honey producers in the study area. The procedures outlined by the Codex Alimentarius Commission were followed to ascertain the microbial quality of the honey samples. Also, the Premi® test kit was used to determine the presence of antibiotics residue in the honey samples. Only eight honey producers were identified in the study area; they all had knowledge on contamination of honey. Only two (25 %) of the honey producers had knowledge on diseases affecting bees and also the use of antibiotics in beekeeping or honey production. Concerning microbial contaminants, *Listeria* spp., *Lactobacillus* spp., *Salmonella* spp., *Escherichia coli*, *Clostridium* spp., *Campylobacter* spp., and *Staphylococcus* spp. were the microorganisms enumerated upon microbiological quality assessment of 30 honey samples. Furthermore, 27 (90 %) of the honey samples tested positive for the presence of antibiotics residue of which 6 (85.7 %) were sampled from imported source, whilst the remaining 21 (91.3 %) were locally produced. Microbial and antibiotic contaminants found in the honey sampled in the study area support the hypothesis that honey may not be as pure as might be perceived and this might be a public health concern. Again, since there is no available record on the screening or antibiotic residue in honey found on the Ghanaian market, this research is timely and necessary to provide the basis for intervention policies on the minimum limits of antibiotic residues present in honey.

Key words: Antibiotic, Campylobacter, Clostridium, Contaminants, Honey, Listeria, Microorganism, Residues, Tamale Metropolis



INTRODUCTION

Honey, like other foods, is susceptible to contamination and adulteration. Honey can be environmentally contaminated by microbes and chemicals such as heavy metals, pesticides, and antibiotics by those persons involved in all steps from honeycomb to retail market [1]. Microorganisms found in honey include bacteria, molds, and yeast, which may originate from bees, nectar, and other external sources, whilst antibiotics found in honey are attributed to its extensive application for the treatment of bacterial diseases affecting bees [2].

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 and as such does not suffer from colony collapse disorder [3]. In contrast, is the intensive use of antibiotics in professional beekeeping in developed countries for the treatment of bacterial brood diseases [4, 5].

Whereas microbial contaminants in honey could pose adverse effects on consumers' health, antibiotic residues consumed along with honey can cause modification of the intestinal flora, induce allergic reactions, cutaneous eruptions, dermatitis, gastrointestinal symptoms, anaphylaxis even at low doses and antimicrobial resistance [6, 7]. Nonetheless, in Ghana, only microbial pathogens, pesticide residues, heavy metals, and aflatoxins are extensively studied as measures to protect the safety of food for human consumption, because these hazards are perceived as the greatest threat to public health [8, 9]. Microbes such as *Listeria*, and *Campylobacter* as well as antimicrobial residues in honey from both imported and local honey has rarely been a serious concern for researchers and public health authorities, in contrast to the situation in livestock. Currently, there is no available data on *Listeria*, and *Campylobacter* nor on antibiotic residue in honey found on the Ghanaian market and this presents an avenue for potential research. The work investigated the antibiotic residues and microbial contaminants present in imported and locally produced honey as well as the beekeeping practices of honey producers within some locations of the Tamale metropolis. The results herein presented may serve as a reference to inform consumers and the public about the challenges facing the honey industry with a view to inform and instigate appropriate health responses by the relevant authorities.

MATERIALS AND METHODS

Study Design

The research was a two-phase study. The first phase was a cross-sectional survey on the production of honey within the study area. A semi-structured questionnaire was designed to gather information on the sources of honey, knowledge of diseases affecting bees, knowledge of contamination of honey, knowledge of antibiotics uses in honey production and the demographic characteristics of the eight honey producers identified in the study area. The second phase was a laboratory analysis of the honey samples that were collected from the different locations of the study area.



Sample Collection

A total of 30 honey samples were collected for this study. Of these, seven were imported from England, France, India, Spain, South Africa and United States of America. Notably, only two supermarkets in the Tamale metropolis had imported honey on their shelves at the time of sampling. Whereas the 23 locally produced honey types were from the following markets: Aboabo, Sakasaka, Tamale central, Lamashegu, Kukuo, Nyankpala, and the Nyohini, which are all locations within the Tamale metropolis. Upon collection, the honey samples were kept in an air-tight box containing ice packs and transported to the Spanish Laboratory Complex of the University for Development Studies for laboratory procedures and analyses.

Sample preparation

Sample preparation was carried out according to the procedures described in the fourth edition of the Compendium of Methods for the Microbiological Examination of Foods where 25 g of the honey sample was weighed on an electronic scale (Sartorius CP2245, USA), and homogenized with 225 ml of 0.1 % peptone water (Oxoid, Basingstoke, UK).

Enumeration of *Listeria* spp.

About 15-20 ml of the sterilized Oxford Listeria Agar Base (Alpha Biosciences, USA) was poured into sterile Petri dishes and allow to cool for solidification. Upon solidification, 100 μ l of the prepared sample was inoculated on the surface of the media. This was evenly spread across the plate using sterilized glass beads. The inoculated plates were incubated (P Selecta, Spain) at an inverted position at 37°C for 24-48 h [10]. Presumptive *Listeria* spp. appearing gold with dark centers on the agar plate were selected for pure culture and biochemical tests.

Enumeration of *Staphylococcus* spp.

For the enumeration of *Staphylococcus* spp., 100 μ l of each of the diluted samples was pipetted onto a freshly prepared Mannitol Salt Agar (MSA, Oxoid, Basingstoke, UK) plates. The inoculum was then spread uniformly on the surface of the agar plate using sterilized glass beads. After the inoculum was absorbed by the media, the agar plates were inverted and incubated at 37°C for 24-48 h. Typical colonies of *Staphylococcus* spp. appeared pinkish on the plate after incubation [10]. These were sub-cultured and pure colonies obtained for a biochemical or confirmatory test.

Enumeration of *Salmonella* spp.

For the enumeration of *Salmonella* spp., 25 g of each of the honey samples was homogenized in a 225 ml of 0.1% peptone water and incubated at 37°C for 18-24 h. Then 100 μ l of each of the pre-enriched samples was inoculated onto a freshly prepared Salmonella-Shigella Agar (SS, Oxoid, Basingstoke, UK) plates. The inoculum was then spread uniformly on the surface of the agar plate using sterilized glass beads. The agar plates were incubated at 37 °C for 24-48 h in an inverted position [10]. After incubation, agar plates with straw-colored colonies with black centers were recorded and sub-cultured for confirmation and differentiation.



Enumeration of *Campylobacter* spp.

For the enumeration of *Campylobacter* spp., 100 µl of each of the pre-enriched sample was pipetted onto a freshly prepared Charcoal Cefoperazone Deoxycholate Agar (CCDA, Oxoid, Basingstoke, UK) plates. The pipetted inoculum was spread uniformly on the surface of the media with the help of sterile glass beads. These plates were covered and incubated under micro-aerophilic conditions generated by a gas generating pack (CampyGen™ 2.5L, Oxoid) in a gas-tight container at 42 °C for 48 h [10]. Presumptive *Campylobacter* spp. appearing creamy-grey were sub-cultured to obtain pure culture for a confirmatory test.

Enumeration of *Escherichia coli*

The spread plate method of inoculation was employed in the enumeration of *E. coli*, where 100 µl of each of the diluted honey samples were pipetted onto a freshly prepared MacConkey (Oxoid, Basingstoke, UK) agar plates. The introduced inoculum was spread uniformly on the surface of the agar plate with the help of sterilized glass beads. These plates were covered and incubated at an inverted position at 37°C for 24-48 h. After incubation, colonies of bacteria with pink coloration were sub-cultured and the pure culture was obtained for the confirmatory tests for *E. coli* [10].

Enumeration of *Clostridium* spp.

For the enumeration of *Clostridium* spp., 20 g of each of the honey samples was weighed into a sterile 250 ml Schott Duran bottle (Duran Group, Germany). A sterile distilled water was added to make a final volume of 100 ml. The content was mixed uniformly and brought to boil for about 5 mins. Then 10 g of the content was homogenized in 90 ml of 0.1% peptone water (Oxoid, Basingstoke, UK) upon cooling. After that, 100 µl of each of the diluted honey sample was pipetted onto a freshly prepared Perfringens Agar Base (Oxoid, Basingstoke, UK) plates. The inoculum was then spread uniformly on the surface of the agar plate using sterilized glass beads. These plates were covered and incubated at 47 °C for 24-48 h in an anaerobic jar [11]. Presumptive *Clostridium* spp. appearing as black or dark colonies were sub-cultured to obtain pure culture for a confirmatory test.

Enumeration of *Lactobacillus* spp.

Lactobacillus MRS Agar (Alpha Bioscience, USA) was used for the enumeration of *Lactobacillus* spp. The spread plate method of inoculation was employed where 100 µl of each of the diluted honey samples was pipetted onto a freshly prepared LMRS (Alpha Bioscience, USA) agar plates. The introduced inoculum was spread uniformly on the surface of the agar plate with the help of sterilized glass beads. These plates were covered and incubated at an inverted position at 37 °C for 24-48 h. After incubation, presumptive *Lactobacillus* spp. appearing as large clear colonies were sub-cultured to obtain pure culture for confirmatory test [10].

Identification and Confirmation of Microbial isolates

Presumptive isolates of the respective microbes under the study were streaked on freshly prepared nutrient agar (Techno Pharmchem, India) and incubated at 37°C for 18-24 h to obtain pure cultures. Distinct pure colonies were selected and used for biochemical tests



such as Gram stain, catalase test, citrate test, and oxidase test. These were performed to further identify and confirm the isolates.

Antibiotic Residue Determination

Antibiotic residues in all 30 honey samples were determined using the rapid screening method. This was conducted using the Premi® test kit (R-Biopharm AG, Germany) following the manufacturer's instructions. Briefly, 100 µl of each honey sample was pipetted into an ampoule provided by the manufacturer bearing the sample's identification code. The ampoules containing the respective honey samples were pre-incubated at room temperature for 20 mins. After incubation, the ampoules were gently inverted to dispense the honey samples. Any remaining honey in the ampoules was carefully removed by filling and emptying the ampoule with demi water. The ampoules were inverted on a tissue paper to drain any residual water. All test ampoules were covered with aluminum foil supplied by the manufacturer before incubation in a water bath at 64 °C until the negative control changed color from purple to yellow.

Statistical Analysis

The data obtained from the field survey and laboratory analysis were entered into Microsoft Office Excel (2016) for processing and analysis. The data were summarized with the descriptive statistical method and the results presented using graphs and tables.

RESULTS AND DISCUSSION

Survey on Honey Production and Beekeeping Practices

A total of eight honey producers were identified and interviewed for the study. Of these, only two were women whereas the remaining six were men. Also, only two of the respondents had no formal education with the rest of the respondents passing through at least the secondary level of education. Concerning the source of honey within the study area, only two of the honey producers owned an apiary or practice beekeeping whilst the remaining six hunted and harvested honey from wild sources. Also, all the eight honey producers were aware of adulteration of honey and confessed to not adding any additive to their honey before selling. Again, only two of the honey producers had knowledge on diseases that affect honey bees as well as the use of antibiotics in honey production or beekeeping. Meanwhile, five out of the total honey producers revealed that they keep their harvested honey in used or old plastic bottles for both storages and as packaging material for selling their product, whilst the remaining three indicated otherwise (Table 1).

Even though the demand for honey is on the rise in the Northern region and the country as a whole, only few people have taken it up as a commercial business. Furthermore, the northern region of Ghana is characterized by unimodal rainfall and extreme events like drought and bush burning. Therefore, it was no surprise that only eight honey producers were identified in the study area.

The male dominance in honey production as recorded in this study was also not surprising since beekeeping has long been considered more of a masculine activity than feminine [12]. Yusuf *et al.* [13], attributes the male dominance in honey production to



the aggressive nature of the honey bee as perceived by women. Notwithstanding, the age distribution of the honey producers compare well with the findings of Oluwatosin [14] and Tijani *et al.* [15], who reported 31-40 and 31-35 as the modal age of honey producers in Ekiti and Borno state of Nigeria, respectively. This is good for the industry as this implies that honey producers within the region can actively participate in the management of the honey-production enterprise and can equally observe improved practices of ensuring productivity and quality.

Even though the sample size of the honey producers was not large enough to make a scholarly inference, the educational qualification of the honey producers captured for the present study could be said to be encouraging. Nevertheless, the educational qualification of the honey producers did not translate into their knowledge of diseases affecting bees and the subsequent antibiotic usage in honey production. Only two out of the eight producers indicated they had knowledge on diseases affecting bees. The two producers were those who owned apiaries. Again, the findings of this study revealed that the majority of the producers sampled were hunters of honey rather than beekeepers. They obtained their honey through hunting from wild sources. This is in line with the assertion of Aidoo [16], that 60 % of the locally produced honey in Ghana were from wild sources. These producers, therefore, could not have any knowledge of diseases affecting bees since they did not own apiaries nor were, they involved in the management of honeybees. It is worthy to mention that all the honey producers did not add additives to their honey before selling; however, they were aware such practices existed. However, the use of old or used plastic containers as the packaging material for honey for both sale or storage was worrying as it could serve as a secondary source of contamination.

Occurrence of Microbial Isolates from the Honey Samples

Presumptive colonies were identified and confirmed using biochemical assays. Nine bacterial genera were studied as indicators of the overall quality of the honey sampled for the study (Table 2). These genera including: *Clostridium*, *Listeria*, *Enterobacter*, *Salmonella*, *E. coli*, *Staphylococcus*, *Lactobacillus*, *Campylobacter* and *Shigella* were of concern due to reports that the intestines of bees contain 27 % of Gram-positive and 70 % of Gram-negative bacteria [17]. *Clostridium* spp. and *Lactobacillus* spp. constituted the two most predominant genera, detected in 28 (93 %) and 27 (90 %) of the 30 honey samples analyzed respectively. All seven (100 %) imported samples examined were positive for both, *Clostridium* spp. and *Lactobacillus* spp. whereas 21 (91.3 %) and 20 (87 %) of the locally produced honey samples were positive for *Clostridium* spp. and *Lactobacillus* spp. respectively. Again, of the 30 honey types analyzed in this study, *Listeria* spp., *Staphylococcus* spp, *Salmonella* spp., *E. coli*, *Campylobacter* spp. were present in 25 (83.3 %), 21 (70 %), 2 (7 %), 2 (7 %), and 1 (3 %) honey samples, respectively.

Clostridium spp. are widely distributed in the environment and are commonly found in honey because they are spore-forming bacteria that are found in the air, dust, and soils [18] and, thus, it was not surprising when it constituted the most isolated microorganism in this study. On the other hand, *Lactobacillus* forms part of the intestinal flora of honeybees [19]. Therefore, the isolation of *Lactobacillus* from the samples could be positive due to the numerous scientific reports on the synergistic effect of some strain of



Lactobacillus on foodborne pathogenic bacteria like *Campylobacter* and *Salmonella* [20], *Listeria*, *Staphylococcus* and *Clostridium*.

The occurrence of *Listeria* spp. in all seven imported samples and 18 out of the 23 local honey samples is of concern considering the recent *Listeria* outbreak in South Africa. Also, *Listeria* is not only associated with foodborne disease but meningitis and it is important to mention that the study area, the Northern region remains one of the hotspot regions for meningitis cases in Ghana. Hence, the need to further characterize the isolates. The occurrence of *Listeria* spp. in honey samples could be attributed to poor temperature control at storage and the shelf life [21]. Also, considering the much scientific evidence on the anti-staphylococcal properties of honey, it was interesting to have recorded growth in 6 (85.7 %) of the imported and 15 (65.2 %) of the local samples. The occurrence of *Staphylococcus* spp. could be attributed to the extraction, processing, or handling by the handlers since *Staphylococcus* is a normal flora of the hand [22].

Contrary to previous reports that the intestines of bees contain 27 % of Gram-positive and 70 % of Gram-negative [18], this study recorded less occurrence of Gram-negative isolates as compared to Gram-positive isolates. This could be due to the fact that Gram-negative bacteria are more susceptible to the hostile conditions of honey in comparison to Gram-positive bacteria [23]. Out of the 30 honey samples, only one of the imported samples was positive for *Campylobacter* spp. whilst *Salmonella* spp. and *E. coli* were each detected in two locally produced honey samples. Also, none of the 30 honey samples were positive for *Enterobacter* spp. and/or *Shigella* spp. Whilst some authors attribute the occurrence of *E. coli* and *Salmonella* spp. in honey to fecal and environmental contamination, the current study rather proposes the storage conditions, packaging, and storage material as the source of *E. coli* and *Salmonella* contamination. The storage room of some of the honey producers was inappropriate, which is congested with other materials. Insects like ants and flies were seen around the honey samples. Also, it is worthy to mention that the practice by some honey producers on the use of 'old or used' plastic containers as packaging material or storage containers for their harvested honey could have accounted for the overall microbial contamination of the locally produced honey samples. Adadi and Obeng [24], reported on a similar trend where honey producers in the Tamale metropolis were found transporting their harvested honey from the production sites in unhygienic plastic containers.

Antibiotics Residue Profiling

Residue of antibiotics was detected in 27 (90 %) out of the 30 honey samples. Six of these, representing 85.7 %, were from imported samples whereas the remaining 21 (91.3 %) were from locally produced honey samples (Figures 1&2).

The occurrence of antibiotic residues in honey has been reported to be a major problem that persists in honey as a result of the broad use of antibiotics for different purposes [25], especially in developed nations. However, in contrast to many global beekeepers, most beekeepers and/or honey producers in the study area have no or little knowledge of the use of antibiotics in honey production. Saleh *et al.* [26], 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 is evidence of contamination of antibiotics in honey from other sources than direct applications [27]. 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 [28]. 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 [29].

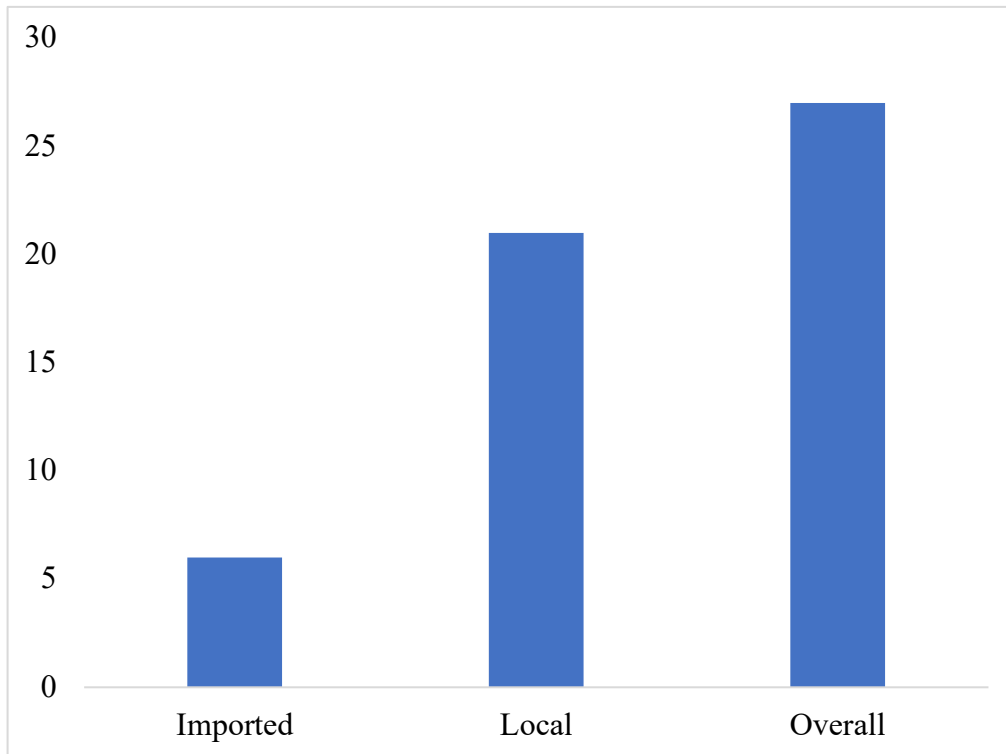


Figure 1: Frequency of occurrence of antibiotic residue in the imported and locally produced honey samples

CONCLUSION

The findings of this study provide insight on some honey contaminants: microbial and antibiotic residues. These contaminants were found in both local and imported honey samples, which make it a public health concern. Thus, requires the regulatory bodies in the country to be alert and monitor local and imported honey sold on the Ghanaian market. Further studies are needed to further characterize the isolates as pathogenic or non-pathogenic, determine the quantity and active component of the antibiotic residues detected and elucidate why some microbes grew despite the detection of antibiotic residues in those samples.

Conflict of Interest

The authors of this study declare no conflict of interest.

Table 1: Survey on Honey Production and Beekeeping Practices

Parameters	Category	Frequency	Percentage (%)
Gender	Male	6	75
	Female	2	25
Age group	20 – 29	1	12.5
	30 – 39	6	75
	40 – 49	1	12.5
Religion	Christianity	3	37.5
	Islam	5	62.5
Level of Education	Secondary	1	12.5
	Tertiary	5	62.5
	None	2	25
Sources of honey	Farm/Apiary	2	25
	Hunting/Wild	6	75
Awareness of adulteration of honey	No	0	0
	Yes	8	100
Do you add any additive before selling?	No	8	100
	Yes	-	-
Knowledge on diseases affecting bees	No	2	25
	Yes	6	75
Knowledge of antibiotics usage in beekeeping	No	2	25
	Yes	6	75
Containers for keeping the honey	New plastics	3	37.5
	Used plastics	5	62.5

Table 2: Frequency of occurrences of bacterial isolates in imported and locally sourced honey samples

Bacteria	Imported samples	Local samples
<i>Clostridium</i> spp.	7(100%)	21(91%)
<i>Lactobacillus</i> spp.	7(100%)	20(87%)
<i>Listeria</i> spp.	7(100%)	18(78%)
<i>Staphylococcus</i> spp.	6(86%)	15(65%)
<i>Salmonella</i> spp.	0	2(9%)
<i>Escherichia coli</i>	0	2(9%)
<i>Campylobacter</i> spp.	1(14%)	0
<i>Enterobacter</i> spp.	0	0
<i>Shigella</i> spp.	0	0

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