

Microbiological Examination Of Honey Marketed In Southwestern Nigeria

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ABSTRACT

Honey is valued in Nigeria both as a food and as a folk medicine. However there are growing fears from the consumers about the hygienic quality of honey being purchased from local retail outlets. Regrettably however there is a dearth of information on the microbiological status of such marketed honey in southwestern Nigeria. An attempt to fill this gap is the rationale behind the present study. Microbiological composition of samples of honey marketed in the six states of southwestern Nigeria was assessed. Seven species of heterotrophic fungi and four species of bacteria were isolated. The bacteria species were *Klebsiella edwardsii*, *K. pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The identified fungi were *Cladosporium werneckii*, *C. herbarum*, *Cephalosporium sp*; *Mucor mucedo*, *Rhizopus rubrum*, *Trichophyton rubrum*, and *Scopulariopsis brevicaulis*. Two species of pathogenic bacteria were isolated from honey samples: *S. aureus* in Ogun State and *K. pneumoniae* in Ekiti State. One pathogenic fungus, *Trichophyton rubrum* was isolated from Ondo State honey samples. This was the first record of pathogenic micro-organisms in Nigerian honey. This implies that the microbiological quality of honey in retail outlets should be properly monitored as it is an indicator of the hygienic conditions under which the product was processed, handled and stored.

KEY WORDS: Bacteria, fungi, coliforms, contamination, micro-organisms, microbes.

1. INTRODUCTION

Honey is the sugary substance produced from the nectar of flowers by the worker bees. As defined by the Codex Alimentarius Commission [1], honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects living on parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. There is a growing consumption of honey because of its high values in maintaining good health and in treatment of various diseases. With many therapeutic claims about honey, it implies that honey could play a very effective role in health care delivery in Nigeria [2], [3], and [4]). It therefore becomes necessary to monitor the hygienic quality of honey been sold to consumers in our local markets.

Micro-organisms in honey may influence the stability of the products and its hygienic quality [5]. Due to the natural properties of honey and control measures in the honey industry, honey is a product with minimal types and levels of microbes. Microbes of concern in post-harvest handling are those that are commonly found in honey (i.e., yeasts and spore-forming bacteria), those that indicate the sanitary or commercial quality of honey (i.e., coliforms and yeasts), and those that are under certain conditions could cause human illness. Primary sources of microbial contamination are likely to include pollen, the digestive tracts of honey bees, dust, air, earth and nectar sources which are very difficult to control [6]. The same secondary (after-harvest) sources that influence any food product are also sources of contamination for honey. These include air, food handlers, cross-contamination, equipment and buildings. Secondary sources of contamination are controlled by hygienic processing practice [5]. The filamentous fungi, being more spread in nature and having thermal resistant spores, with a great capacity of surviving, can be introduced in honey even by man, through dust, through the water installations or containers or even by the bees through pollen [7]. Most microbes found in honey are not dangerous for the consumer's health. However, normal honey must lack pathogenic micro-organisms that produce enteric illnesses [8]. It has been discovered that microbial contamination of honey do occur mostly in honey marketed in local markets [8], [6]. For instance in Cameroon, Tchoumboue et al. [8] isolated bacteria and fungi from honey samples collected from local markets but isolated none from honey samples collected from the Bee research farm of University of Dschang. In honey samples collected from local markets in Romania, Popa et al. [6] reported the presence of *Bacillus* sp. and eight types of fungi. While microbiological screening is going on in different parts of the world, there is dearth of information on the microbiological status of honey samples collected from local markets in Nigeria. This explains the rationale behind the present study.

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2. MATERIALS AND METHODS

Plate count was carried out to analyse the honey samples microbiologically in respect of:

Total Heterotrophic Bacteria (THB)

One millilitre of each honey sample was added to 9.0ml of sterile distilled water in a MacCarthy bottle, respectively, and vigorously agitated more uniformly (Dilution; 1/10 V/V). Then 0.1ml was diluted hundred folds, 3X, in a set of test-tubes, each containing 9.9ml sterile distilled water. One millilitre of each dilution was plated out in duplicates, employing the use of nutrient agar medium (sterile) kept in molten form. Pour plate method was adopted.

The culture plates, having allowed the agar medium to set, were incubated invertedly and aerobically at 35°C for 48hours. Thus, enumerating for only aerobes and facultative heterotrophic bacteria.

The plates were observed for growth and selected for count after the expiration of the incubational period. The culture plate in which the number of colonies was less than 300, and its duplicate, for each sample, was selected. The count so obtained was multiplied by the dilution factor at that dilution and expressed as colony forming unit (CFU) per millilitre of the original sample. This is a viable count.

Total Heterotrophic Fungi (THF)

The same procedure for the THB was repeated for the fungi count.

The differences, however, were as follows;

Malt extract agar was employed as the culture medium.

The culture plates were incubated uninvertedly and aerobically at 30 °C for 5-7 days (until the plates showed no further increase in the number of fungal colonies).

The culture plate in which the number of colonies was less than 10 was selected.

Estimation of Coliform Bacilli by Coli MPN Presumptive Test

Due to the viscosity of the honey sample, and associated condition for culturing, the sample could not be used directly but diluted. Ten millilitre of honey was therefore added to 90.0ml of sterile distilled water (Dilution: 1/10 V/V) before use.

The most probable number (MPN) of organisms was carried on each of the test samples by planting three portions in each of three dilutions in geometric series employing the use of single and double strengths of MacConkey broth.

For planting three portions in each of three dilutions in geometric series, a set of three test tubes, each containing 10ml double strength sterile broth, two sets of three test-tubes, each containing 5ml sterile single strength broth were required. Ten millilitre of the diluted honey was inoculated into each tube of double strength; 1.0ml of the sample was inoculated into each of the first set of three tubes of single strength and 0.1ml of the same sample was also inoculated into each of the other set of three tubes of the single strength.

The culture tubes were carefully agitated to mix the inoculums with the broth medium. They were then incubated at 35°C for 48hours and each tube observed for acid and gas production. A positive tube was the one in which acid and gas was produced.

The combined numbers of positive tubes in each set, arranged in order least diluted to the most diluted tubes was read out from the appropriate standard MPN table and noted. To obtain the estimated number of cells present in 100ml of the original honey, the values so obtained from the standard MPN table was then multiplied by 10. The nearest corresponding values on the same table to the calculated ones give the real estimate for conformity.

3. RESULTS

Species inventory of microbes in honey samples

The species list of bacteria and fungi isolated from honey samples from the six states of southwestern Nigeria were presented in Table I. Four species of bacteria and seven species of fungi were identified. The bacteria species were *Klebsiella edwardsii*, *K. pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The identified fungi were *Cladosporium werneckii*, *C. herbarum*, *Cephalosporium sp.*, *Mucor mucedo*, *Rhizopus japonicus*, *Trichophyton rubrum*, and *Scopulariopsis brevicaulis*.

Occurrence of Bacteria isolated from honey samples in six states of southwestern Nigeria.

The bacteria occurrences in the six states were presented in Table II. *Klebsiella edwardsii* was found in honey samples in all the six states. *Pseudomonas aeruginosa* occurred only in Lagos state. *Klebsiella pneumoniae* was found in only the Ekiti honey samples, while *Staphylococcus aureus* occurred only in Ogun state.

Occurrence of Fungi isolated from honey samples in six states of southwestern Nigeria.

The occurrences of fungi in the six states were presented in Table III. *Cladosporium werneckii* was found in Ekiti, Ondo and Oyo state honey samples. *Cephalosporium* sp. was detected in the honey samples of Ekiti, Ogun and Osun states. *Mucor mucedo* occurred in Lagos, Ondo, Osun and Oyo states' honey samples. *Rhizopus japonicus* was found in Ogun, Ondo and Oyo states' honey samples. *Cladosporium herbarum* was detected only in Ondo state honey samples. *Trichophyton rubrum* occurred only in Ondo state while *Scopulariopsis brevicaulis* occurred only in Oyo state honey samples

Mean total bacteria counts in honey samples

Table IV showed the mean total bacteria counts in honey samples from six states of southwestern Nigeria. The mean total bacteria count for Ekiti honey samples was 1.5×10^3 cfu/ml, while the mean value of 5.5×10^5 cfu/ml was obtained from the Lagos honey samples. The mean value of 1.6×10^6 cfu/ml was obtained for Ogun state honey samples and 2.0×10^5 cfu/ml for Ondo state honey samples. Osun state recorded the mean value of 3.5×10^5 cfu/ml while Oyo state had a mean value of 1.0×10^3 cfu/ml

Mean total coliform counts in honey samples

The mean total coliform counts in honey samples from six states in southwestern Nigeria showed that the mean total coliform counts for Ekiti, Lagos and Ogun states were 2.4×10^5 cells/100ml, 95.0 cells/100ml and 95.0 cells/100ml respectively (Table IV). The honey samples from Ondo, Osun and Oyo states recorded 39.0 cells/100ml, 1.1×10^3 cells/100ml and 95 cells/100ml respectively.

Mean total fungi counts in honey samples

The mean total fungi count in honey samples from the six states of southwestern Nigeria showed that the mean total fungi for Ekiti, Lagos and Ogun states were 40.0 cfu/ml, 10.0 cfu/ml, and 15.0 cfu/ml respectively. The recorded mean total fungi in honey samples from Ondo, Osun and Oyo states were 85.0 cfu/ml, 45.0 cfu/ml and 2.0×10^3 cfu/ml respectively (Table IV).

Table I : The species list of Bacteria and fungi isolated from the honey samples in six states of southwestern Nigeria.

Fungi	Bacteria
<i>Cladosporium werneckii</i>	<i>Klebsiella edwardsii</i>
<i>Cephalosporium</i> sp.	<i>K. pneumoniae</i>
<i>Mucor mucedo</i>	<i>Pseudomonas aeruginosa</i>
<i>Cladosporium herbarum</i>	<i>Staphylococcus aureus</i>
<i>Rhizopus japonicus</i>	
<i>Trichophyton rubrum</i>	
<i>Scopulariopsis brevicaulis</i>	

Table II : Occurrence of Bacteria isolated from honey samples in six states of southwestern Nigeria.

State	<i>Klebsiella pneumoniae</i>	<i>K. edwardsii</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Ekiti	+	+	-	-
Lagos	-	+	+	-
Ogun	-	+	-	+
Ondo	-	+	-	-
Osun	-	+	-	-
Oyo	-	+	-	-

+ = Presence of bacteria species

- = Absence of bacteria species

Table III : Occurrence of fungi isolated from honey samples in six states of southwestern Nigeria.

State	<i>Cladosporium werneckii</i>	<i>Cephalosporium</i> sp.	<i>Mucor mucedo</i>	<i>Rhizopus japonicus</i>	<i>Cladosporium herbarum</i>	<i>Trychophyton rubrum</i>	<i>Scopulariopsis brevicaulis</i>
Ekiti	+	+	-	-	-	-	-
Lagos	-	-	+	-	-	-	-
Ogun	-	+	-	+	-	-	-
Ondo	+	-	+	+	+	+	-
Osun	-	+	+	-	-	-	-
Oyo	+	-	+	+	-	-	+

+ = Presence of fungi species

- = Absence of fungi species

Table IV : Mean Microbial Profile of Honey samples collected from six states of southwestern Nigeria.

State	THB (cfu/ml)	TCC (cells/100ml)	THF (cfu/ml)
Ekiti	1.5×10^3	2.4×10^2	40.0
Lagos	5.5×10^5	95.0	10.0
Ogun	1.6×10^6	95.0	15.0
Ondo	2.0×10^5	39.0	85.0
Osun	3.5×10^5	1.1×10^3	45.0
Oyo	1.0×10^3	95.0	2.0×10^3

THB – Total Heterotrophic Bacteria

TCC – Total Coliform Count

THF – Total Heterotrophic Fungi

cfu - Colony forming unit

4. DISCUSSION

All the honey samples from the six states of southwestern Nigeria were contaminated with microbes. This confirms other findings that fungi and spore forming bacteria may be present in honey [9], [8]. Surprisingly however, most of the works done in Nigeria have been given conflicting reports. For example, Lawal et al. [10] reported that honey found in Akwa-Ibom, Ondo and Ogun had coliforms and total bacteria counts being absent, while honey samples from Shaki, Yola and Ibadan had some total viable counts. Other works in Nigeria did not find microbes in Nigeria honey [11], [12], and [10]. However this may be due to the fact that the workers obtained their samples from the primary sources (bee farms). According to Popa et al. [6], microbiological contamination during or after processing honey was demonstrated by the absence of the micro-organisms in the samples collected from primary sources and by the presence of bacterium (*Bacillus sp*) and eight types of fungi in the collected samples from local markets. This fact indicates the contamination from secondary sources during processing, packaging or intentional adulteration.

According to Tchoumboue et al. [8], the contamination with fungi and bacteria indicate inadequate hygienic conditions during collecting, manipulating, processing and storing.

Microbial contamination during and or post processing can also result in spoilage or persistence of some bacteria in honey [13]. Similar contaminations of other foodstuffs obtained from the local markets have been reported [14]. The microbes present in West Cameroon honey samples indicate contamination from secondary sources, during handling or adulterations. This is confirmed by their absence in honey harvested from Bee research Farm of the University of Dschang (Miel campus), Cameroon where processing and handling are always carried out in good hygienic conditions [8].

Therefore, the microbiological quality of honey may serve as an indicator of the hygienic conditions under which the product was processed, handled and stored.

The presence of micro-organisms in honey can sometimes influence the stability of the product and its hygienic quality. Normal honey must lack pathogenic micro-organisms that produce enteric illnesses. The presence of *Staphylococcus aureus* in the honey samples from Ogun state, *Klebsiella pneumoniae* from Ekiti state and *Trichophyton rubrum* (fungi) from Ondo state is a big concern for the health and well-being of consumers because of their potential health hazards. Although, the population was very low but caution must be taken to prevent such contaminations because of their reported dangers in the literatures [12], [15].

In 2003 there were 8,369 laboratory reports of bacteraemias due to *Klebsiella*, *Enterobacter*, *Serratia* or *Citrobacter spp*, of which 4,389 reports were due to *Klebsiella spp* in the United Kingdom. The majority of bacteraemias due to Klebsiellae were attributed to *Klebsiella pneumoniae* [13].

Staphylococcus aureus literally the “golden cluster seed” or “the seed gold” and also known as “golden staph” is a facultative anaerobic, gram-positive coccus, and is the most common cause of staph infections [15]. It is frequently part of the skin flora found in the nose and on skin. About 20% of the human population is long-term carriers of *S. aureus*. This bacterium can cause a range of illnesses from minor skin infections, such as pimples, boils (furuncles), scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis [16]. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing post surgical wound infections [16].

Trichophyton rubrum species are causative agents of Dermatophytosis, Onychomycosis, *Tinea barbae*, *Tinea capitis*, *Tinea corporis*, *Tinea cruris*, *Tinea faciei*, *Tinea pedis*, and *Tinea unguium*. *Trichophyton rubrum* is the most widespread among the anthropophilic dermatophytes. Anthropophilic dermatophyte is found in association with humans. *Trichophyton rubrum* is the most common agent of tinea of the feet, hands, nails, groin, and the glabrous skin, however, the scalp is rarely infected. Animals are very infrequently infected as well [17].

5.CONCLUSION

All the honey samples in the six states contained heterotrophic bacteria, heterotrophic fungi and coliform bacilli. The presence of coliforms and heterotrophic bacteria is an indication of unhygienic status of honey on sale by the retailers. This study is the first to report the presence of pathogenic micro-organisms in honey in Nigeria. The presence of pathogenic organisms like *Klebsiella pneumoniae* in Ekiti state, *Staphylococcus aureus* in Ogun state and *Trichopyton rubrum* in Ondo state necessitate an urgent need to monitor microbial status of honey marketed in different retail outlets of southwestern Nigeria.

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