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PHYLOGENETIC RELATIONSHIP BETWEEN THE GUT BACTERIAL FLORA OF HONEYBEE (*Apis Mellifera*) FROM APIARY IN OGUN STATE, NIGERIA

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ABSTACT

The present study is aimed to determine the phylogenetic relationship between honeybee (Apis mellifera) gut microbiome from Apiary in Olabisi Onabanjo University and Osoba Avenue Odo-Epo, Odogbolu Local Government. Honeybees workers (A. mellifera) totaling twenty (20) were collected into a vile having a powdery sugar from the apiary located in OOU and Osoba Avenue at Odo-Epo during rainy season in July and transported to Zoology and Environmental Biology laboratory in OOU and kept in ice-cubes (-50^{o}C) till daybreak. Before the dissection process, the bees were washed in 95% ethanol and complete alimentary canals of the bees were aseptically dissected by clipping the stinger with sterile forceps. The phylogenetic analyses based on the 16S rDNA gene were further used to characterize the organism in order to establish relationships among them. The partial 16S rDNA sequences obtained were utilized in search of reference nucleotide sequence available in NCBI GenBank database using BlastN algorithm. Maft version 7.0 was employed in the multiple alignments of nucleotide sequences while trees were drawn based on character method (Maximum Likelihood) for comparing set of data against set of models of evolution using MEGA 7.

Keywords: Phylogenetic, Pollinated, Apiary, GenBank

INTRODUCTION

Honeybee, (tribe Apini), also spelled honey bee, includes any group of insects in the family Apidae (order Hymenoptera) that makes honey. In a stricter sense, honeybee applies to any one of seven members of the genus Apisand usually only the single species, *Apis mellifera*, the domestic honeybee. This species is also called the European honeybee or the western honeybee. All honeybees are social insects and live together in nests or hives. The honeybee is remarkable for the dancing movements in the hive to communicate information to its fellow bees about the location, distance, size, and quality of a particular food source in the surrounding area (Kakumanu *et al*., 2016).

With the exception of *A.mellifera*, all other Apis species are confined to parts of Southern or Southeastern Asia. *Apis florea*, the dwarf honeybee, occurs in Southern Asia, where it builds its nests in trees and shrubs. *Apis. andreniformis*, the black dwarf honeybee, is native to forested habitats of Southeastern Asia. *Apis. dorsata*, the giant honeybee, also occurs in Southeastern Asia and sometimes builds combs nearly three metres (more than nine feet) in diameter. *Apis. cerana*, the Eastern honeybee, is native to Southern and Southeastern Asia, where it has become domesticated in some areas. It is very closely related to *A. koschevnikovi*, or Koschevnikov's bee, which is found only on Borneo and several other islands in Southeast Asia and on the Malay Peninsula. *A.*

nigrocincta is native to Indonesia and Mindanao Island in the Philippines. There are also a number of subspecies and strains of Apis (Baffoni *et al.,* 2016).

Honey is a sweet food created by bees using nectar from flowers. It is composed of 17% water and 82% carbohydrates; it has low content of fat, dietary fiber, and protein. A mixture of sugars and other carbohydrates, honey is mainly fructose (about 38%) and glucose (about 32%), with remaining sugars including maltose, sucrose, and other complex carbohydrates. Honey also contains minerals, vitamins and metals. Compared to other bee species, honey bees have been reported to increase the yield in animal pollinated crops which account for 35% of the global food production (Guo *et al*., 2015).

Muli *et al*. (2014) reported that *A. mellifera adansonii* (honeybee) is about the most common that has been given due attention in Nigeria probably because of its beneficial attributes as a natural agent of pollination and as the most important of all insect pollinators. The aim of the study was to explore the importance of species traits and species phylogenetic relationships

MATERIALS AND METHODS

The study was carried out in apiaries located in Ago-Iwoye in Ijebu North Local Government Area. Ago Iwoye has a latititude of 6.9424^{o}N and a longitude of 3.9216^{o}E respectively while Odo-Epo has a latitude of 6.7888^{o}N and a longitude of 3.9684^{o}E.

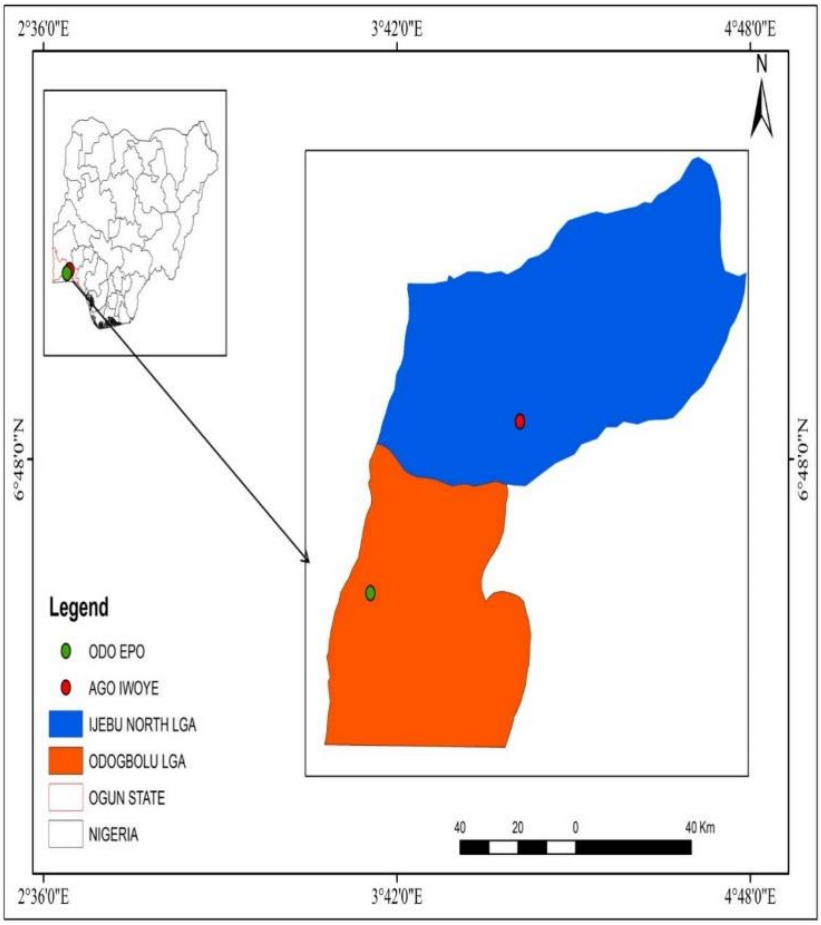

Figure 1: The study area (Source: Map Data, 2017)

Experimental Design

In order to study the cultivable honey bee gut bacteria, 20 honeybees of (*A. mellifera*) unknown age were collected each from the apiary located in OOU and Odo-Epo. Honey bee samples were collected early in the morning before sun rise. After collection, life honey bees were transported to Zoology and Environmental Biology Laboratory in OOU in a labelled vial containing sugar powder and kept at room temperature till daybreak. The collection of honeybee samples was done between July and October 2018 during the raining seasons. Before dissection, the honeybees were rinsed in 95% ethanol and complete alimentary canals of the bees were aseptically dissected by clipping the stinger with sterile forceps. The insect abdomen was open up using a sterile dissecting scissors; the guts content was taken using a sterilized swab stick into gel agar.

Isolation, Characterization and Identification of Bacterial Isolates

Isolation of Bacteria

Isolation was carried out using dilution plating method. Decimal dilutions of the soil samples were carried out by placing 1 g of the soil sample into 9 ml of distilled water. This was allowed to soak for at least 1 h and from the suspension; 1 ml was transferred to another tube containing 9 ml of distilled water and thoroughly mixed. This dilution procedure was further repeated so that there were series of five (5) tubes giving a serial dilution of 10^{-1} to 10^{-5}. Each sample collected were serially diluted, total viable count present in the soil and water samples were determined by spread plate method on nutrient agar. 0.1 ml aliquots of appropriate dilutions were spread on triplicates of sterile nutrient agar. The plates were incubated for period of 24 h in the incubator at 28 ^{o}C. Colonies that was formed during this incubation period were counted using this formula;

$$Number\ of\ colonies = dilution\ factor / volume\ of\ media\ used$$

After incubation, pure cultures were obtained from each distinct colony by sub-culturing on Nutrient agar and incubated at 37 ^{o}C for 24 h. The pure culture was then grown on Nutrient agar slant in McCartney bottles and stored in a refrigerator. The inoculants for subsequent studies were obtained by sub-culturing from the slants. Values were expressed as cfu/g for soil and cfu/ml for water.

Gram Staining

A smear of the pure isolate was made on a clean grease free slide. The smear was air – dried by waving it around for a while. The smear was heat-fixed by passed it over a bunsen burner flame. The smear was stained with crystal violet reagent for one minute. This was rinsed in slowly running tap for 5 seconds. The slide was treated with lugols iodine and allowed for 60 seconds. This was washed off in slowly running tap. Alcohol reagent was used to decolorize the primary stain, until no more dye runs off from smear. The smear was counter-stained with safranin for 30 seconds. Stained slide was slowly rinsed under running water. The slide at this point was allowed to dry on its own. The slide was observed using the oil immersion lens of the microscope. Gram positive cells stained purple/violet while gram negative cells stain pink or red.

Biochemical Characterization of the Bacterial Isolates

The following biochemical tests; indole, methyl Red, catalase, Oxidase, Coagulase, urease and sugar fermentation tests were used to confirm and identify the bacteria isolates from well water samples. Observation was recorded.

Catalase Test

Well-isolated colony was transferred into a clean slide and 1 drop of 3% H_2O_2 was added. Effort was made not reverse the order and not to mix. It was observed for immediate bubble formation.

Citrate Utilization Test
About 2.4 g of citrate agar was dissolve in 100 ml of distilled water. About ten milliliters (10 ml) of citrate medium was dispensed into each tube and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate.

Oxidase Test
Few drops of oxidase reagent were added onto Whatman filter paper. Smeared with a loop-full of organisms. Colonies from low glucose, non-selective media were used. Appearance of purple color within 30 seconds indicates a positive test.

Sugar Fermentation Tests
Sugar indicator broth was prepared using peptone water medium containing 1% fermentable sugar and 0.01% phenol red. About ten milliliters of sugar broth was dispensed into each of the test tubes, durham tube which would trap the gas if produced was inverted carefully. The test tubes were autoclaved and inoculated with a loopful of 24 h old culture of the test organisms after then incubated for 2-7 days at 36±1°C and observed daily for acid and gas production. Yellow colouration indicates acid production while gas production was indicated by displacement of the medium in the durham tube.

Indole Test
Tryptone broth (5 ml) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37 °C for 48 h. After incubation, 0.5 ml of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 mins to permit the reagent to rise. A red or red-violet colour at the top surface of the tube indicates a positive result while yellow colouration indicates a negative result.

Urease Test
The surface of a urea agar slant was streaked with a portion of a well-isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture. The cap was left loosely and incubates the tube at 35°-37°C in ambient air for 48 hours to 7 days. It was examined for the development of a pink colour after 7days.

Phylogenetic Analysis of 16SrRNA Genes of Bacterial Isolates from Well Water and Soil Sample
The phylogenetic analyses based on the 16S rDNA gene were further used to characterize the organism in order to establish relationships among them. The partial 16S rDNA sequences obtained were utilized in search of reference nucleotide sequence available in NCBI GenBank database using BlastN algorithm. Maft version 7.0 was employed in the multiple alignment of nucleotide sequences while trees were drawn based on character method (Maximum Likelihood) for comparing set of data against set of models of evolution using MEGA 7. These techniques include: distance based (Neighbour-Joining (NJ) with cluster-based algorithm) used in calculating pairwise distance between sequences and group sequences that are most similar and character based method (Maximum Likelihood) for comparing set of data against set of models of evolution to the best model for the variation pattern of sequences.

RESULTS
The results of the morphological appearances of sampled honey bees were observed to have different colonial patterns, all were flat but some were mucoid, some were wet and some were dry, also, in colour some were whitish, gold yellow, dull green, and some were colourless as presented in Table 1.

Table 1. Microbiological characteristics of sampled honey bee bacteria isolates from OOU and Odoepo

Isolation	Isolation
AGW1	Flat mucoid colourless, cream colony
AGW2	flat mucoid whitish cream colony
AGW3	Flat dry whitish cream colony
AGW4	Flat mucoid cream, gold yellow colony
AGW5	Flat dry cream colony
AWW4	Flat dry cream colony
AWW5	Flat dry whitish cream colony
IJW1	Flat dry whitish cream colony
IJW2	Flat mucoid whitish colourless colony
IJW3	Flat dry whitish cream colony
IJW4	Flat dry green colony
IJW5	Flat dry cream colony
ORW1	Flat mucoid colourless colony
ORW2	Flat dry pink colony
ORW3	Flat dry cream colony
AGW6	Flat mucoid cream colony
AGW7	Flat dry cream colony
AGW8	Flat dry cream colony
AGW9	Flat dry whitish cream colony
AGW10	Flat dry cream colony
AGW11	Flat dry colourless, creamcolony

The corresponding results for the biochemical tests carried out indicate that most of the isolates are gram positive and gram-negative cocci. Result from oxidase test showed purple colouration within 5 – 10 minutes. The result of sugar fermentation test revealed a change in colour from purple to yellow indicated acid production due to fermentation of the sugar and gas production was shown by the presence of bubble in the inverted Durham tubes. For the indole test a red ring formation was seen at the surface of the tubes as positive test. Urease positive cultures produced a purple pink color as a result of change in the colour of the indicator for urease positive isolates and are presented in Table 2.

The result of the percentage identity and closest accession of the isolates showed that *Enterobacter aerogenes* had the closest accession number on serial number 6.7. and 8 also recorded the highest percentage identity (99%) while in another study, Billiet *et al*. (2015) reported Phyla Firmicutes, Actinobacteria, and alpha-, beta-, and gamma-proteobacteria as major microbiota in the gut of Honey bee. (Table 3).

Table 2. Biochemical characterization of bacterial isolates recovered from OOU and Odoepo, Ogun State

Sample	GRM	CAT	OXI	CIT	INDO	UREA	COA	LAC	MAL	GLU	SUC	ORG
AGS1a	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
AGS1b	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
AGS1c	-ve rod	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	*E. coli* spp.
AWS2a	+ve coci	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	*Staphylococcus* spp.
AWS2b	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
AWS2c	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	*Shigella* spp.
AWS2d	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
IJS3a	-ve rod	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	*Pseudomonas* spp.
IJS3b	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
IJS3c	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	*Shigella* spp.
IJS3 d	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
ORS4a	-ve rod	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	*Proteus* spp.
ORS4b	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
ORS4c	-ve rod	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	*E. coli* spp.
AGS2a	-ve rod	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	*E. coli* spp.
AGS2b	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
AGS2c	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	*Shigella* spp.
AGS2d	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	*Shigella* spp.
AGS2e	-ve rod	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	*E. coli* spp.
AGS3a	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
AGS3b	-ve rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	*Salmonella* spp.
AGS3c	-ve rod	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	*E. coli* spp.

Key: *-ve- Negative, +ve- Positive, NA- Nutrient agar, SSA- Salmonella Shigella agar, EMB- Eosine Methylene Blue, GRAM- Gram reaction, CAT- Catalase, OXI- Oxidase, CIT- Citrate, IND- Indole, COA- Coagulase, LAT- Lactose, MAL- Maltose, GLU- Glucose, ORG- Organism*

Table 3. Percentage Identity and closest accession of Isolate from OOU and Odoepo

Bacteria Isolates	Percentage identity	Closest accession	S/No.
Aeromonas hydrophila	84%	KY938147.1	39
Cedeca davisae	88%	MF111213.1	3
Cronobacter dublinensis	84%	MK557935.1	4,5
Enterobacter aerogenes	99%	FJ976592.1	6, 7, 8
Enterobacter asburiae	94%	MK577384.1	40
Escherichia coli	89%	MK621270.1	35-38
Klebsiella michiganensis	97%	MG516135.1	19
Klebsiella oxytoca	98%	KP886828.1	11,16
Kluyvera cryocrescens	99%	MK047301.1	9,10
Providencia alcalifaciens	97%	MG754372.1	14,17
Providencia vermicola	91%	KJ833796.1	12
Pseudomonas aeruginosa	93%	MK288113.1	27-34
Pseudomonas plecoqlossicida	95%	MK467545.1	18, 20
Salmonella enteric	98%	LS483428.1	13
Serratia marcenscens	94%	LC041158.1	21-26
Serratia nematodiphila	93%	MK447122.1	15

A maximum likelihood reconstruction of the phylogenetic analysis of bacteria isolates from honey bees in the area under study is presented in Figure 1, distance phylogenetic tree for 16 bacterial isolates were presented by the neighbor-joining method. The tree showed the similarities existing between the isolates from OOU sample and the isolates from ODO EPO sample in 3 clusters of isolates.

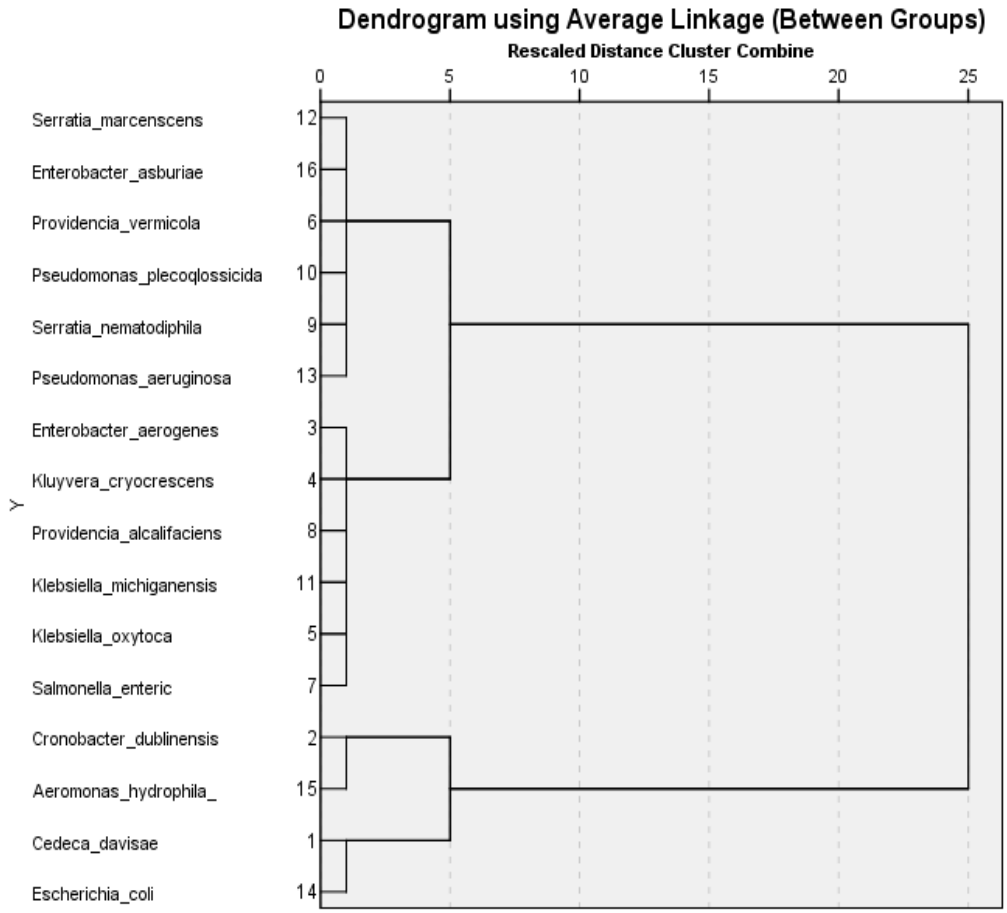

Figure 1: Dendrogram showing the reconstruction of the phylogenetic analysis of bacteria isolates from honey bees

DISCUSSION

The present study evaluated the bacterial load present in the gut of honeybee worker (*Apis mellifera*) collected from two different Apiaries in Ogun State adopting bacteriological analysis along with molecular techniques based on 16S rRNA sequences precisely characterize insects gut bacterial flora according to Prabhar *et al*. (2013). The composition of bacterial assemblage in the digestive tract of honey bee *A. mellifera* is relatively simple compared with other gut-associated communities as reported by Engel *et al.* (2013). A distinctive set of bacteria including Firmicutes, Actinobacteria, α- and γ-proteobacteria found in the honey bee alimentary canal has been assessed by using Sanger as well as next generation sequencing techniques by Li *et al*. (2012).

From the present study, the morphological appearances of sampled honey bees were observed to have different colonial patterns, all were flat but some were mucoid, some were wet and some were dry, also, in colour some were whitish, gold yellow, dull green, and some were colourless which is in general agreement with previous studies by Prabhakar *et al*. (2013).

The result of colony forming unit counts ranged from 592 cfu/mgml to 156 cfu/mgml on gram positive cocci bacterial isolates on nutrient, MacConkey and MRS agar which is similar to the result of Martinson *et al*. (2016) that reported a colony forming unit ranging

from 590 cfu/mgml to 175 cfu/mgml of gram positive bacteria on Nutrient and MRS agar.

A maximum likelihood reconstruction of the phylogenetic analysis of bacteria isolates from honey bees in the area under study is similar to one obtained from the work of Anderson *et al*. (2013), both works recorded distance phylogenetic tree for 16 bacterial isolates and 25 bacterial isolates respectively presented by the neighbor-joining method. Several actinobacterial isolates were classified in suborders of Corynebacterium and Micrococcineae. Hence culture-based method adopted during this study revealed important members of ‘‘core” bacterial community present in alimentary canals of honey bees present in apiaries located in area under study.

The phylogenetic tree of the partial 16S rDNA gene sequences of the bacterial isolates from the gut of honey bees showed relatedness among the bacteria when aligned with reference strains in GenBank, which revealed that the bacterial population in the gut of honey bee foragers in OOU and Odo-Epo were diverse, including *Cedeca davisae, Cronobacter dublinensis, Enterobacter aerogenes, Kluyvera cryocrescens, Klebsiella oxytoca, Providencia vermicola, Salmonella enteric, Providencia alcalifaciens, Serrati nematodiphila, Pseudomonas plecoqlossicida, Klebsiella michiganensis, Serratia marcenscens, Pseudomonas aeruginosa, Escherichia coli, Aeromonas hydrophila* and *Enterobacter asburiae*. *Klebsiella* spp. which were more abundant and prominent in the digestive guts of adult honeybees across all selected ecozones.

The richness of these bacterial assemblages suggests their ecological importance. For instance, the abundance of one representative of *Enterobacter aerogenes* was estimated at 29% of the total microbial gut samples analyzed. Additionally, most of the 16S rDNA gene sequences were found very comparable.

Most of these bacteria were broadly classified as facultative anaerobes, tolerant of acidic environments and ferment sugars to produce lactic or acetic acid. These bacteria are considered beneficial gut inhabitants of humans and other animals and are involved in immunomodulation, interference with enteric pathogens and the maintenance of a healthy microbiota (Anderson *et al*., 2016).

Some of the obligatory aerobic bacteria were adapted to highly acidic environments rich in sugar. As revealed by biochemical assays, many of these bacteria ferment sugars to produce lactic acid and various other end products, and several can reduce nitrate to nitrite suggesting a potential function in nitrogen metabolism within the gut. Like ants, bees also belong to hymenoptera which are eusocial insects that survive in colonies with queen and thousands of worker bees which can forage large distances for collecting nectars and pollens and return to the hive. Hence their contact with various environments acts as a vector of diverse bacterial flora. More than fifty bacterial species from 31 genera have been found associated with different ants including *Escherichia, Staphylococcus, Enterobacter, Pseudomonas, Bacillus, Streptococcus* and *Klebsiella* (Emery *et al*., 2017). A number of opportunistic pathogenic bacteria have already been reported from different insects including phytophagus insect aster leafhopper (*Macrosteles quadrilineatus*) in association with plants (Erban *et al*., 2017). Nectar has considered as an environmental bacterial reservoir. An array of bacterial species belongs to Firmicutes and Enterobacteriaceae are reported from nectar. Different *Bacillus* species were frequently reported from the nectars of Acacia and Mesquite (Anderson *et al*., 2013). Acacia

plants (*Acacia arabica*, *Acacia modesta* and *Acacia nilotica*) are major bee plants in local flora of the study area (Guo *et al*., 2015). Therefore, interaction of worker bees in the study areas nectar from the blossom of Acacia flowers catalyzed the transmission of environmental bacteria in the bee guts (Tarpy *et al*., 2015). The possible routes of bacterial contamination in honey bee and its by-products are human, hive tools, sugar feeders, wind and dust. Beekeepers skin infections, fecal contamination and sneezing can introduce pathogenic microbes into the hive environment.

Hence bee guts can act as a carrier of opportunistic bacterial pathogens. Additionally, due to political and economic disruption in the study area caused by the war against terrorism, most of the beekeepers were not able to keep their bees properly. Like in Europe, a remarkable decline in the bee hives was observed during the early 1990s caused by the Soviet collapse, rather than from widespread ecological factors (Zheng *et al*., 2017).

A number of opportunistic pathogenic bacteria have already been reported from different insects including phytophagus insect aster leafhopper (*Macrosteles quadrilineatus*) in association with plants (Erban *et al*., 2017).

Nectar has considered as an environmental bacterial reservoir. An array of bacterial species belongs to Firmicutes and Enterobacteriaceae are reported from nectar. Different *Bacillus* species were frequently reported from the nectars of Acacia and Mesquite (Anderson *et al*., 2013). Acacia plants (*Acacia arabica*, *Acacia modesta* and *Acacia nilotica*) are major bee plants in local flora of the study area (Guo *et al*., 2015). Therefore, interaction of worker bees in the study areas nectar from the blossom of Acacia flowers catalyzed the transmission of environmental bacteria in the bee guts (Tarpy *et al*., 2015). The possible routes of bacterial contamination in honey bee and its by-products are human, hive tools, sugar feeders, wind and dust. Beekeepers skin infections, fecal contamination and sneezing can introduce pathogenic microbes into the hive environment.

Hence bee guts can act as a carrier of opportunistic bacterial pathogens. Additionally, due to political and economic disruption in the study area caused by the war against terrorism, most of the beekeepers were not able to keep their bees properly. Like in Europe, a remarkable decline in the bee hives was observed during the early 1990s caused by the Soviet collapse, rather than from widespread ecological factors (Zheng *et al*., 2017).

CONCLUSION

The findings from this study showed that microbiota component community of *A. mellifera adansonii* in OOU was composed of more Gram-negative bacteria than Gram-positive bacteria in Odo Epo. From the study, dominant digestive guts' microbiota was *Cedeca davisae, Cronobacter dublinensis, Enterobacter aerogenes, Kluyvera cryocrescens, Klebsiella oxytoca, Providencia vermicola, Salmonella enteric, Providencia alcalifaciens, Serratia nematodiphila, Pseudomonas plecoqlossicida, Klebsiella michiganensis, Serratia marcenscens, Pseudomonas aeruginosa, Escherichia coli, Aeromonas hydrophila* and *Enterobacter asburiae*. *Klebsiella* spp. was more abundant and prominent in the digestive guts of adult honeybees across the two areas under study. These comparable differences in species were due to their ecological and geographical distribution which reflected on their microbiota diversity in relation to geographical distribution of associated honeybees. Geographical locations dictate

the abundance, occurrence and diversity of microbial communities and their interaction with honeybees. The study provides basic information for future genomic research aimed at understanding the evolutionary diversity and functional roles of microbiota community of honeybees and their economic benefit. The external integument and digestive guts of honeybees, *Apis mellifera adansonii* harbour significant population of microflora dependent on geographical distribution. The presence of bacterial isolates can be explore using genomics for pharmacological and fermentation purposes. Honeybees, *A. mellifera adansonii* are future potential sources of micro flora exploration through metagenomics. This will enhance discovery and development of useful products of pharmaceutical importance and microbes for bio-remediation, bio-control of pests of agricultural crops and pests of health importance

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