Original article

Evaluation of the Microorganisms in Nigeria Honey for Antagonistic Activity on Selected Bacteria causing Diarrhoea

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Abstract

Objective: The aim of this study was to determine the antagonistic activities of the isolated microorganisms from honey on the selected diarrhoeagenic bacteria, for this reason the microbiological quality of Nigerian honey was investigated. ***Materials and Methods***: Twelve (12) honey samples from different locations in Nigeria were evaluated and the microorganisms present in those samples were studied and isolated. Both clinical and typed cultures of *Escherichia coli, Salmonella typhimurium, Shigella dysenteriae, Bacillus cereus and Staphylococcus aureus* were also used for the study. ***Results***: A total of 9 bacterial and 8 fungal species were isolated from the honey samples: *Bacillus proteolyticus, Providencia rettgeri, Lysinibacillus pakiestanensis, Bacillus thuringiensis*, *Acinetobacter indicus, Bacillus cereus, Bacillus subtilis*, *Lysinibacillus fusiforms, Bacillus amyloliquefaciens* and *Aspergillus flavus, Penicillium chrysogenum, Neurospora crassa, Rhizopus stolonifer, Fusarium oxysporum, Mucor racemosus, Trichodermaviride, Saccharomyces cerevisae* respectively. Among the 9 bacteria isolated, 4 (44.44%) exerted antagonistic effect on 6 (60%) of thetest bacteria. On the other hand, 2 (25.00%)among the 8 fungi isolated had antagonistic activity against 4 (40.00%) of the test bacteria. ***Conclusion***: However, honey has anti-microbial properties that discourage the growth or persistence of many microorganisms which made them to contain low numbers and a limited variety of microbes. The bacteria isolated exerted a greater antagonistic activity than the isolated fungi on selected diarrhoeagenic bacteria, which could have been as a result of secondary metabolites like lipopeptides, polypeptides, fatty acids, isocoumarins produced by them that exhibited a wide range of biological activities such as antimicrobial.

Keywords: Honey;Microbes in honey;Diarrhoeagenic bacteria; Antagonist

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Introduction

Honey is a complex natural food substance gotten from nectar of plants and honeydew produced by bees, and it can be eaten without processing[1]. Few microorganisms have the capacity to remain in honey because of its natural properties like high osmotic pressure as a result of low water activity (average 17.2%); low pH due to the presence of organic acids, majorly gluconic acid (average 3.9); hydrogen peroxide presence produced by action of enzyme glucose oxidase; low protein content, low redox potential due to the presence of reducing sugars; and chemical agents present as lysozyme, phenolic acids, pinocembrin, terpenes, benzyl alcohol, and volatile substances[1]. High osmotic pressure results from its sugar composition, of which glucose has 28–31%, fructose 22–38%, sucrose 1–4% and maltose 1–9%[2]. Some oligosaccharides are also present in

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honey, which vary according to flowering and local production[3]. Yeasts and spore-forming bacteria are microbes of concern that are commonly found in honey, the presence of coliforms and yeasts indicate the quality of honey and they could cause human illness when they are found under certain conditions. These microorganisms are derived from primary or secondary sources of contamination, the primary sources are related to digestive tract of honeybees, which have natural microorganisms and sources of material collection such as nectar, pollen and propolis, air, flowers, and the environment inside the beehive which are very difficult to control while the secondary sources are incorporation of honey microorganisms postharvest, food handlers, cross contamination processing plants, and appliances[4]. Diarrhoeal diseases are amongst the most frequent childhood illnesses causing death in developing countries[5]. It is characterized by frequent, loose and watery stool which may result in dehydration and death. There are around 2.5 million cases of diarrhoea in children under the age of five according to the study carried out by UNICEF, in which about 1.3 million die each year and with the highest incidence being in children under 2 years of age[6-7]. Diarrhoea is the second leading cause of death among children in the world but majorly happen in India, Bangladesh, Nigeria, Afghanistan, Pakistan and Ethiopia[8]. Infective diarrhoea is a result of the activities of microorganisms or their toxins that manage to get into the gastrointestinal tract thereby causing infection. Diarrhoeagenic bacteria include *Escherichia coli, Shigella dysentery*, *Salmonella typhimurium*, *Vibrio cholerae, Bacillus cereus, Staphylococcus aureus*[9], *Campylobacter jejuni, Yersinia enterocolitica,*

Clostridium botulinum, and Enterotoxigenic *Bacteroides fragilis*[10]. So, the objective of this work is to find out the effect of microbes present in honey on bacteria causing diarrhoea disease.

Materials and Methods

Collection of honey samples

Honey Samples were sourced from twelve (12) different areas in Nigeria; Emure – Ile, Afo – Akoko, Akure, Ikakuma - Akoko in Ondo State, Enugu in Enugu State, Ibadan in Oyo State, Ikere in Ekiti State, Lagos in Lagos State, Lafia in Nasarawa State, Federal University of Agriculture Abeokuta, (FUNAAB) in Ogun State, Gusau in Zamfara State and Iree in Osun State (Table 1).

Table 1: Source of Honey Samples used in this study

S/N	LOCATION	FLORAL SOURCE
1	Emure – Ile, Owo Ondo State (Roadside)	Wildflower Honey
2	Ikere, Ekiti State	Wildflower Honey
3	Nasarawa State	Wildflower Honey
4	Ibadan, Oyo State	Wildflower Honey
5	Afo - Akoko, Ondo State	Wildflower honey
6	Iree, Osun State	Bitter leaf
7	FUNAAB, Ogun State	Wildflower Honey
8	Enugu, Enugu State (Cinomis Honey)	Wildflower Honey
9	Lagos (Kaybeck Honey)	Wildflower Honey
10	Zamfara (A & Shine Honey)	Wildflower Honey
11	Sunshine Honey, Ondo State	Wildflower Honey
12	Ikakuma – Akoko	Wildflower Honey

Diarrhoeagenic bacteria used and their sources

The test bacteria used are *Salmonella typhimurium* clinical, *Salmonella typhimurium* ATCC 14028, *Shigella dysenteriae* clinical, *Shigella dysenteriae* ATCC 11835, *Escherichia coli* clinical, *Escherichia coli* ATCC 700728, *Bacillus cereus* clinical, *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* clinical and *Staphylococcus aureus* ATCC 29213. They were obtained from Spectra Medics Laboratories Sagamu, Nigeria and Medical Microbiology Laboratory of the University College Hospital, Ibadan, Nigeria.

Isolation of microorganisms in honey samples and determination of total viable count

One millimeter of each of the honey samples was put into 9 ml of sterile water and diluted serially to obtain a dilution of 10^{-3} dilution factor. One millilitre was pipetted from 10^{-2} dilution into sterile Petri dishes. Thereafter, 20 ml of Nutrient agar, and Potato dextrose agar cooled to 45°C were poured separately onto each of the plates in triplicates and allowed to solidify. Chloramphenicol, an antibiotic was aseptically added to the Potato Dextrose Agar before inoculating to inhibit the growth of bacteria in the medium. The nutrient agar plates were incubated at 37°C for 24 hrs in aerobic and anaerobic conditions while Potato Dextrose Agar plates were incubated at 25°C for 3-5 days. After incubation, the bacterial colonies were observed and counted using a colony counter (Gallenkamp). Representative colonies of bacteria and fungi were selected and sub-cultured on fresh bacteriological media until pure cultures were obtained according to the method of [11].

Identification of the isolated bacteria from honey samples

The identification of bacteria was based on morphological characteristics and biochemical tests carried out on the isolates according to the standard method [11].

Molecular Identification of Bacteria Isolated from Honey samples

Further characterization of the isolates was done using molecular techniques for better taxonomical data and categorized into three main steps: isolation of genomic DNA, polymerase chain reaction (PCR) and DNA sequencing using 16s rRNA technique using the standard method [12].

Identification of isolated fungi from honey samples

The fungal colonies were sub-cultured on Potato Dextrose Agar (PDA). The isolates were identified based on their morphological and microscopic features using the standard method [13].

Antagonistic Effects of Microbes in Honey on Selected Diarrhoeagenic Bacteria.

a. Bacteria

The antagonistic activity of bacteria in honey against the test bacteria was done using agar well diffusion method[14]with slight modification. The standard inoculum of eachtest bacterium was prepared by inoculating a colony of 18 – 24 hrs old culture into 9ml of sterile nutrient broth and incubated at 37°C for 18 – 24 hrs, 1ml of the broth culture was serially diluted into 9ml of sterile distilled water, the dilution that matched with the turbidity of 0.5 McFarland Standard was picked for the assay and 0.1ml of each of the inoculum was spread over the Mueller – Hinton agar plates using a sterile glass spreader. Wells were bored on agar plates with sterile cork borer of 10 mm in diameter. Two hundred microliters (0.2ml) of each of the bacterial isolate isolated from honey were poured in the wells. The plates were incubated at 37°C and the zone of inhibition was observed after 24 h.

b. Fungi

Antagonistic reactions between the fungal isolates and the test bacteria were studied *in vitro*. A 100 µl of each test bacterial suspensions was introduced to cover the surface of PDA medium using a glass spreader, each of the fungal isolate was inoculated triangularly on the medium[15]. Clear zones of growth inhibition were evaluated.

Statistical Analysis

All experiments were done in triplicates. Mean and Standard deviation were calculated for all data using Descriptive Statistics and Difference between means was determined by Duncan's New Multiple Range Test at $p \leq 0.05$.

Ethical clearance

Since this study does not involve trial on animals, so no ethical approval or clearance was gotten.

Results

Types of Microorganisms present in the honey samples

Nine different types of bacterial species were isolated and studied from the honey samples. These bacterial species were *Bacillus proteolyticus* strain BHUPCV3, *Providencia rettgeri* strain IAE170, *Lysinibacillus pakiestanensis* strain NCCP – 54, *Bacillus thuringiensis* strain B116, *Acinetobacter indicus* strain SR6 -19, *Bacillus cereus* ATCC 14579, *Bacillus subtilis* strain AK4, *Lysinibacillus fusiforms* strain A1 and *Bacillus amyloliquefaciens* strain ARP23. The most frequently encountered bacterial species in the honey samples was *Lysinibacillus pakiestanensis* (58.33%) while the least frequently encountered bacterial species was *Bacillus cereus* (8.33%) (Table 2).

Eight different fungi were isolated from the honey samples. These were *Aspergillus flavus, Penicillium chrysogenum, Neurospora crassa, Rhizopus stolonifer, Fusarium oxysporum, Mucor racemosus, Trichoderma viride* and *Saccharomyces cerevisae.* The most frequently encountered fungus was *A. flavus* (50.00%) while the least encountered fungi were *Penicillium chrysogenum* (8.33%), *Neurospora crassa* (8.33%) and *Fusarium oxysporum* (8.33%) (Table 3).

Table 2: Frequency of occurrence of bacteria in the test honey samples

	Honey Samples												
Bacterial species	HEI	HIK	HN	HI	HA	HIR	HF	HE	HL	HZ	HS	HIA	Total/ %
B.proteolyticus	+	+	+	-	+	-	-	+	-	+	-	-	50.00
P. rettgeri	+	-	-	-	-	-	-	-	-	-	+	-	16.67
L. pakiestanensis	+	+	+	-	+	-	+	-	+	+	-	-	58.33
B. thuringiensis	+	-	-	-	+	+	-	-	-	+	-	-	33.33
A. indicus	+	-	-	-	+	+	-	-	-	+	-	-	333333.33 33.33
B. cereus	-	+	-	-	-	-	-	-	-	-	-	-	8.33
B. subtilis	-	+	-	-	+	-	-	+	-	-	+	-	33333333 33.33
L. fusiforms	-	+	-	-	+	-	-	+	-	-	-	-	25.00
B. amyloliquefaciens	-	-	-	-	-	-	+	-	+	+	-	+	33.33

Key: HEI = Honey from Emure – Ile, HIK = Honey from Ikere – Ekiti, HN = Honey from Nasarawa, HI = Honey from Ibadan, HA = Honey from Afo – Akoko, HIR = Honey from Iree, HF = Honey from FUNAAB, HE = Honey from Enugu, HL = Honey from Lagos, HZ = Honey from Zamfara, HS = Sunshine Honey and HIA = Honey from Ikakuma- Akoko (Mr George Honey), + = Present, - = Absent.

Table 3: Frequency of occurrence of fungi in the test honey samples

	Honey Samples												
Fungal species	HEI	HIK	HN	HI	HA	HIR	HF	HE	HL	HZ	HS	HIA	Total / %
A. flavus	-	+	+	+	+	-	+	-	-	-	-	+	50.00
P. chrysogenum	-	-	-	-	-	+	-	-	-	-	-	-	8.33
N. crassa	-	-	-	-	-	-	+	-	-	-	-	-	8.33
R. stolonifer,	-	-	-	-	-	+	-	-	+	-	-	-	16.67
F. oxysporum,	-	-	-	-	-	+	-	-	-	-	-	-	8.33
M. racemosus	+	+	-	+	-	+	-	-	+	-	-	-	41.67
Trichodermaviride	-	-	+	-	+	-	-	-	-	-	-	-	16.67
S. cerevisae	+	-	+	+	-	-	-	-	-	-	-	+	33.33

Key: HEI = Honey from Emure – Ile, HIK = Honey from Ikere – Ekiti, HN = Honey from Nasarawa, HI = Honey from Ibadan, HA = Honey from Afo – Akoko, HIR = Honey from Iree, HF = Honey from FUNAAB, HE = Honey from Enugu, HL = Honey from Lagos, HZ = Honey from Zamfara, HS = Sunshine Honey and HIA = Honey from Ikakuma- Akoko (Mr George Honey), + = Present, - = Absent.

Molecular identification of bacteria present in honey samples from different locations in Nigeria.

The 16sRNA sequencing of the bacterial isolates recovered from the honey samples revealed the identity of nine bacteria belonging to four genera; *Bacillus*, *Lysinibacillus*, *Providencia* and *Acinetobacter*. The most predominant genus was *Lysinibacillus*, the percentage identity of the identified isolates ranged from 86.52 – 100% (Table 4). The phylogenetic relatedness of the bacterial isolates highlighted a closely linked evolutionary relationship between *B. proteolyticus*, *B. thuringiensis B. subtilis, Acinetobacter indicus, Providencia rettgeri, Lysinibacillus fusiformis* and *Lysinibacillus parkistanensis* and *B.cereus* ATCC 14379. However, *B. amyloliquefaciens* showed a distinct evolutionary background compared to the other bacteria (Fig. 1). The molecular weight of the DNA of the bacterial isolates ranged from 1,300kbp to 1,500kbp (Plate 1)

Table 4: Molecular identity of the bacterial species Isolated from honey samples fromdifferent locations in Nigeria

Molecular identity of isolates	Accession No	Percentage (%)
Bacillus proteolyticus strain BHUPCV3	MN294510.1	98.19
Providencia rettgeri strain IAE170	MK414868.1	86.52
Lysinibacillus pakiestanensis strain NCCP – 54	MN396729.1	96.32
Bacillus thuringiensis strain B116	MN128540.1	96.60
Acinetobacter indicus strain SR6 -19	MN421531.1	98.10
Bacillus cereus ATCC 14579	MN326684.1	100.00
Bacillus subtilis strain AK4	KR780043.1	96.40
Lysinibacillus fusiforms strain A1	MN252063.1	100.00
Bacillus amyloliquefaciens strain ARP23	CP035899.1	99.60

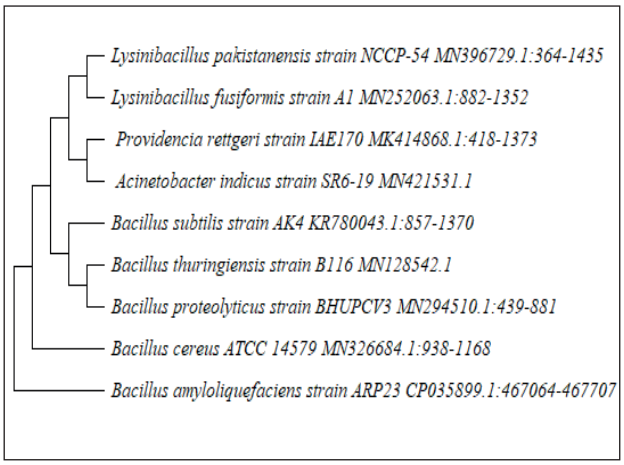

Fig. 1: Phylogenetic tree showing the evolutionary relationships of bacteria isolated from honey

Key: Legend: lane 1 - 9 are 16SrDNA bands of the isolated bacteria, L = Ladder (Marker), Kbp = Kilo base pair and Bp = Base pair

Antagonistic effects of microbes isolated from Honey on selected diarrhoeagenic bacteria

Four out of the nine bacteria isolated from the honey samples used in this study exerted antagonistic effect on six of the selected diarrhoeagenic bacteria. For example, the isolate *Bacillus thuringiensis* had the highest effect on 4 out of the 10 selected diarrhoeagenic bacteria. These four bacteria were*B. cereus* clinical, *B. cereus* ATCC 14579, *S. dysenteriae* clinical and *S. dysenteriae* ATCC 14028 followed by *B. subtilis* on 3 of the selected diarrhoeagenic bacteria; *B. cereus* ATCC 14579, *E. coli* clinical and *Salmonella typhimurium* clinical and the least were *A. indicus* and *Lysinibacillus fusiforms* having effect only on *B. cereus* and *Salmonella typhimurium* clinical respectively (Table 5). On the other hand, only 2 out of the 8 fungi isolated had antagonistic activity against 4 of the test bacteria. For example, *Aspergillus flavus* had effect on *B. cereus*ATCC 14579, *Salmonella typhimurium* ATCC 14028 and *Staph. aureus* clinical while *Penicillium chrysogenum* had effect on *Staph. aureus* ATCC 29213 (Table 6).

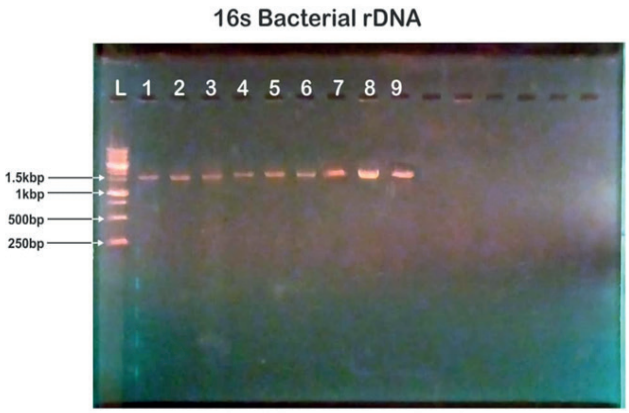

Plate 1: Gel Electrophoresis Image of bacteria isolated from different honey samples located in Nigeria

Table 5: **Antagonistic Effects of Bacteria Isolated from Honey on Selected Diarrhoeagenic Bacteria**

		Bacteria*in* Honey/ Diameter				**zone of Inhibition (mm)**			
Diarrhoeagenic Bacteria	*Providenciarett-geri*	*Bcillussubtilis*	*Bacillus thuringiensis*	*A. indicus*	*L. pakiestanensis*	*Lysinibacillusfusiforms*	*Bacillus Cereus*	*Bacillus amylo-liquefaciens*	*Bacillus proteolyticus*
B. cereus clinical	0.00 ± 0.00	0.00 ± 0.00	**18.00 ± 2.16**	**22.00 ± 5.10**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
B. cereus ATCC 14579	0.00 ± 0.00	**15.00 ± 0.82**	**15.33 ± 0.47**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
E. coli clinical	0.00 ± 0.00	**12.33 ± 0.47**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
E. coli ATCC 700728	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Salm. typhimurium clinical	0.00 ± 0.00	**12.00 ± 0.82**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**11.67 ± 1.25**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Salm. typhimurium ATCC 14028	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
*S. dysenteriae*clinical	0.00 ± 0.00	0.00 ± 0.00	**30.00 ± 5.31**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
*S. dysenteriae*ATCC 11835	0.00 ± 0.00	0.00 ± 0.00	**26.50 ± 2.62**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Staph. aureus clinical	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Staph. aureus ATCC 29213	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 6: Antagonistic Effects of Fungi Isolated from Honey on Selected Diarrhoegenic Bacteria

	Fungiin Diameter				zone of inhibition (mm)			
Diarrhoeagenic Bacteria	*Aspergillusflavus*	*Penicilliumchrysogenum*	*Neurosporacrassa*	*Fusariumoxysporum*	*Trichodermaviride*	*Mucorracemosus*	*Rhizopusstolonifera*	*Saccharomyces cerevisae*
B. cereus clinical	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
B. cereus ATCC 14579	**25.00 ± 4.08**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
E. coli clinical	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
E. coli ATCC 700728	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Salm. typhimurium clinical	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Salm. typhimurium ATCC 14028	**27.00 ± 1.41**	0.00 ± 0.00	0.00 ± 1.70	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S. dysenteriae clinical	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
*S. dysenteriae*ATCC 11835	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Staph. aureus clinical	**16.67± 2.49**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Staph. aureus ATCC 29213	0.00 ± 0.00	**11.67 ± 2.36**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Discussion

Total viable count of aerobic bacteria of honey which ranged from 1.0 – 7.33 x 10^2cfu/ml in this study was in agreement with the earlier reports[16] that it should not exceed 1.0 x 10^3cfu/ml in any honey sample. Coliforms were not detected in honey worked on in this study, this agreed with the study of[17] in which no coliform wasfound in honey they researched on. The genus *Lysinibacillus* was the most frequently encountered in the honey samples used in this study. This disagreed with earlier findings[18] that bacterial spores particularly those of the genus *Bacillus* are regularly found in honey. *Bacillus* species have heat - resistant spores and they can survive in honey at low temperatures. *Bacillus cereus*, *Bacillus subtilis* and *Bacillus licheniformis* have been associated with food poisoning[18]. Honey has not been involved in food borne outbreaks caused by *B. cereus*, though no firm evidence exists that would exclude honey as a potential vehicle of infection. The prevalent spore forming bacilli recovered from the honey samples in this study were *Lysinibacillus pakiestanensis, Bacillus subtilis, Bacillus thuringiensis*, *Bacillus proteolyticus*, *Bacillus cereus* and *Bacillus. amyloliquefaciens.* The results in this study also showed that honeys collected from different locations in Nigeria harbor arrays of fungal contamination. The fungal spores found in the honey samples were identified as *Penicillium chrysogenum, Aspergillus. flavus, Rhizopus stolonifer, Mucor racemosus, Neurospora crassa* and *Fusarium oxysporum.* The fungal arrays detected support the earlier report[19] in which eight fungal species were identified from the western Cameroun honey. It was also in conformity with the report of other researchers that fungi and spore-forming bacteria might be present in honey for a limited period of time. The occurrence of these fungi in honey could be an indication of contamination from secondary sources during handling, processing and storage or adulteration[20]. Also, the isolation of fungi such as *Aspergillus, Penicillium, Mucor* and *Saccharomyces* in honey samples in this study also supportedthe report by other researchers[21- 22] which isolated 13 species of moulds. The bacteria isolated exerted greater antagonistic activity to the test bacteria than the isolated fungi in honey. This may be as a result of lipopetidebiosurfactants produced by them.

Conclusion

This study has shown that the various microorganisms present in honey has a great antagonistic effect on the selected bacteria causing diarrhoea. For example *Bacillus thuringiensis* was the most effective in inhibiting the growth of *Shigella dysenteriae* (both typed and clinical) than the other bacteria present in the honey samples tested while on the part of fungi *Aspergillus flavus* was found to be most effective in inhibiting the growth of *Bacillus cereus* ATCC 14579, *Salmonella typhimurium* ATCC 14028 and *Shigella dysenteriae* ATCC 11835. These results clearly indicate that the microorganisms isolated in honey exerted a great antagonistic activity on the test bacteria. Our study could be exploited for microbial control on bacteria causing diarrhoea, thereby could also play a significant role as antimicrobial in treatment of diarrhoeal diseases caused by these bacteria.

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Authors,s Contributions:

Data gathering and the idea owner of this study: John-Isa JF

Study design: John-Isa JF, Adebolu TT, Oyetayo VO

Data gathering; John-Isa JF, Adebolu TT, Oyetayo VO

Writing and submitting manuscript: John-Isa JF

Editing and approval of final draft: John-Isa JF, Adebolu TT, Oyetayo VO

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