Original Research Article

ANTIBACTERIAL EFFECTS OF HONEY IN NIGERIA ON SELECTED DIARRHOEAGENIC BACTERIA

ABSTRACT

Aims: This study is geared to evaluating honey as an alternative of conventional antibiotics to treat infections caused by the selected diarrhoeagenic bacteria.
Place and Duration of Study: Research laboratory of Federal University of Technology Akure (FUTA), Ondo State, Nigeria between December 2017 to May 2018.
Methodology: Honey samples from ten (10) different locations in Nigeria were screened for possible antibacterial activity on both the clinical and typed cultures of the selected diarrhoeagenic bacteria; *Escherichia coli, Salmonella typhimurium, Shigella dysenteriae, Bacillus cereus and Staphylococcus aureus* using agar well diffusion method. Conventional antibiotics were used as control. Data obtained were subjected to one way analysis of variance (ANOVA) using XL-Start, 2016 version.
Results: All the honey samples used exerted growth inhibitory activity on all the test bacteria including the ones that were resistant to the conventional antibiotics (Ofloxacin and augmentin) used as control. In some cases, the growth inhibitions mediated by the honey samples were superior to that of the conventional antibiotics.
Conclusion: This study showed that honey has antibacterial activity against the selected bacteria and therefore can be exploited as an alternative to conventional antibiotics to treat infections caused by the selected diarrhoeagenic bacteria especially the ones that were resistant to conventional antibiotics.

Keywords: Diarrhoeagenic bacteria, antibiotics, honey, antibacterial activity, infections, alternative therapy

1. INTRODUCTION

Diarrhoeal diseases are amongst the most frequent childhood illnesses and leading cause of death especially among children under five years in developing countries, in areas of inadequate water supplies, sanitation and little or no health education [1]. Loss of water and electrolytes from the body can lead to severe dehydration which can be fatal in young children, especially those already in poor health and malnourished. Diarrhoea can be caused by organisms such as certain serotypes of *Escherichia coli*, *Shigella* spp. and other organism such as *Salmonella* spp., *Campylobacter* spp., and *Yersinia enterocolitica* [2]. All sorts of diarrhoea including watery diarrhoea, invasive diarrhoea and inflammatory diarrhoea are caused by *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteriae* through infected food and *Staphylococcus aureus* and *Bacillus cereus* via food poisoning [3]. Although, diarrhoea is self-limiting, the issue of dehydration is of great concern and also when the illness is as a result of bacterial infections and antibiotic therapy is required, the problem of antibiotic resistance is also a serious problem because almost all known bacteria have developed resistant to most of the commonly employed antibiotics [4]. Also, some of these antibiotics can induce diarrhoea known as “antibiotic induced diarrhoea” [5]. Therefore, it becomes imperative to search for alternatives to conventional antibiotics to treat this disease.

Honey has been reported to exert antibacterial activity against many bacterial species [6-9]. Honey is a natural and sweet product which has a high nutritive value. It is produced when the nectar and sweet deposits from plants are brought together, modified and stored inside the honeycombs by the honeybees of the genera, *Apis* and *Meliponin* [10]. It can be classified based on the source of nectar. Honeys can either be unifloral or multifloral, depending whether the honey is produced from the nectar of only one type of flower or from nectar of flowers of various types [11]. In addition to this, honey can also be made by bees by extracting sugars from the living tissues of plants or fruits, and/or scavenge the excretions of insects (aphids) that tap the veins of higher plants. This type of honey is referred to as non-floral honey (honey dew) [12]. Honey is composed mainly of carbohydrates, smaller amount of water and a great number of minor components. Sugars are the main constituents of honey, constituting of about 95%. Honey

characterization is based on the determination of its chemical, physical or biological properties [13]. Although, there are many reports of antibacterial activity of honey against many bacterial species, this present study was carried out to investigate the antibacterial effect of local honey samples from different geographical zones of Nigeria on some selected diarrhoeagenic bacteria commonly implicated in diarrhoeal illness in the region in order to know whether the locality of source of the honey sample has any effect on its antibacterial activity on the selected bacteria.

2. MATERIAL AND METHODS

2.1 Location and duration of the research

The research was carried out in the Graduate Research Laboratory of Department of Micro biology, Federal University of Technology, Akure, Ondo State, Nigeria between February to May, 2018.

2.2 Collection of honey samples

Honey samples were collected from ten (10) different locations in Nigeria; Emure – Ile and Afo – Akoko, Ondo State, Enugu, Enugu State, Ibadan, Oyo State, Ikere- Ekiti, Ekiti State, Lagos, Lagos State, Nasarawa, Nasarawa State, FUNAAB, Abeokuta Ogun State, Zamfara, Zamfara State and Iree, Osun State. Table 1 shows the location and the floral source of the honey samples used.

Table 1: Honey samples from different locations in Nigeria.

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

2.3 Test diarrhoeagenic bacteria

The following bacteria were used in this study; *Salmonella typhimurium* ATCC 14028, *Salmonella typhimurium* clinical, *Shigella dysenteriae* ATCC 11836, *Shigella dysenteriae* clinical, *Escherichia coli*

ATCC 700728, *Escherichia coli* clinical, *Bacillus cereus* ATCC 14579, *Bacillus cereus* clinical, *Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* clinical. The test bacteria were obtained from Spectra Medics Laboratories Shagamu, Ogun State and Medical Microbiology Laboratory in University College Hospital, Ibadan, Oyo State. The isolates were further characterized in the laboratory to establish their identity based on morphological and biochemical characteristics according to the method of [3].

2.4 Antibacterial activities of honey on the test bacteria

The test bacteria were prepared and standardized to achieve the turbidity of 0.5 McFarland according to [14]. The conventional antibiotics used in this study were Ofloxacin and Augmentin and their antibiotic resistant testing was determined by testing them on the test bacteria using disk diffusion method as described by [15]. The antibacterial activity of raw unpasteurized honey on the test bacteria was determined using agar diffusion method along side with the Ofloxacin and Augmentin used as control as described by [16].

2.5 Statistical analysis

All **e**xperiments were done in triplicates. Mean, Standard deviation were calculated for all data using Descriptive Statistics, all data obtained were subjected to one way analysis of variance (ANOVA) using XL-Stat. 2016 version.

3. RESULTS AND DISCUSSION

All the ten honey samples used in this study exerted varying degrees of growth inhibition of all the test bacteria. Out of these honey samples, honey sample from Zamfara (HZ) exerted the highest growth inhibitory activity on five of the ten bacteria worked on (Table 2; values in red colour). The five bacteria are: *Bacillus cereus* ATCC 14579 (18.00mm), *E. coli* ATCC 700728 (22.67mm), *E. coli* clinical (26.00mm), *S. aureus* ATCC 29213 (21.67mm) and *S. aureus* clinical (21.43mm). This was closely followed by honey from FUNAAB (HF) which exerted the greatest growth inhibition of three of the test bacteria; *Bacillus cereus* clinical (20.33mm), *S. typhimurium* clinical (26.00mm) and *S. typhimurium* ATCC 14028 (23.00mm).

Table 2: Comparative effects of honey samples from different localities in Nigeria on the growth inhibitory activity on selected diarrhoeagenic bacteria (zone diameter in mm)

Type of bacteria	HEI	HIK	HN	HI	HA	HIR	HF	HE	HL	HZ
Bacillus cereus ATCC 14579	13.00 ± 1.00^{abc}	0.00 ± 0.00^{d}	13.33 ± 0.58^{ab}	11.00 ± 1.00^{e}	14.33 ± 2.08^{ab}	11.5 ± 0.50^{bc}	15.33 ± 1.53^{cd}	10.67 ± 1.15^{d}	10.50 ± 0.50^{c}	18.00 ± 2.65^{bc}
Bacillus cereus clinical	10.00 ± 0.00^{c}	11.67 ± 2.08^{bc}	12.33 ± 2.52^{ab}	15.00 ± 0.00^{bcd}	16.00 ± 2.65^{ab}	15.67 ± 5.13^{ab}	20.33 ± 4.51^{b}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	16.33 ± 3.79^{cd}
E. coli ATCC 700728	12.67 ± 4.62^{bc}	10.00 ± 0.00^{c}	10.33 ± 0.58^{ab}	17.33 ± 1.53^{ab}	15.67 ± 1.15^{ab}	11.5 ± 1.50^{bc}	16.00 ± 1.00^{c}	11.33 ± 0.58^{cd}	11.67 ± 2.89^{bc}	22.67 ± 3.21^{ab}
E. coli clinical	12.33 ± 2.52^{bc}	13.00 ± 2.00^{bc}	12.33 ± 0.58^{ab}	17.00 ± 3.61^{abc}	14.00 ± 1.73^{ab}	16.00 ± 2.65^{ab}	15.00 ± 0.00^{cd}	12.00 ± 2.65^{bcd}	13.33 ± 2.89^{abc}	24.33 ± 3.06^{a}
S. typhimurium ATCC 14028	10.0 ± 0.00^{c}	14.67 ± 2.52^{ab}	15.33 ± 0.58^{a}	14.33 ± 1.53^{bcde}	14.33 ± 2.52^{ab}	17.33 ± 4.04^{a}	23.67 ± 2.31^{ab}	19.00 ± 2.65^{a}	11.0 ± 1.00^{c}	10.00 ± 0.00^{e}
S. typhimurium clinical	11.33 ± 2.31^{bc}	17.67 ± 2.08^{a}	14.33 ± 4.04^{ab}	13.33 ± 1.15^{cde}	13.67 ± 1.15^{ab}	11.67 ± 1.53^{bc}	26.00 ± 1.73^{a}	11.00 ± 1.00^{d}	11.67 ± 2.89^{bc}	24.67 ± 2.08^{a}
Shigella dysenteriae clinical	12.00 ± 0.00^{bc}	12.33 ± 1.53^{bc}	13.67 ± 2.52^{ab}	13.00 ± 3.00^{de}	16.33 ± 3.21^{a}	11.33 ± 1.15^{bc}	12.00 ± 1.73^{d}	14.67 ± 0.58^{bc}	10.33 ± 0.58^{c}	12.67 ± 1.15^{de}
Shigella dysenteriae ATCC 11836	12.33 ± 1.53^{bc}	10.00 ± 0.00^{c}	13.33 ± 2.08^{ab}	14.00 ± 2.65^{bcde}	12.33 ± 2.31^{ab}	10.33 ± 0.58^{c}	16.33 ± 2.31^{c}	11.33 ± 1.53^{cd}	17.67 ± 1.53^{a}	13.00 ± 3.00^{de}
S. aureus ATCC 29213	16.67 ± 1.53^{a}	10.0 ± 0.00^{c}	11.33 ± 0.58^{ab}	20.67 ± 2.31^{a}	14.67 ± 1.53^{ab}	18.00 ± 2.65^{a}	14.67 ± 1.53^{cd}	15.33 ± 3.06^{b}	14.33 ± 4.04^{abc}	21.00 ± 4.36^{abc}
S. aureus clinical	15.33 ± 2.52^{ab}	15.33 ± 4.51^{ab}	15.67 ± 4.51^{a}	19.67 ± 0.58^{a}	12.00 ± 2.65^{a}	18.67 ± 1.15^{a}	14.00 ± 1.00^{cd}	15.33 ± 3.06^{b}	16.00 ± 3.46^{ab}	21.00 ± 1.73^{abc}

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 FUNNAB, HE= Honey from Enugu, HL= Honey from Lagos and HZ= Honey from Zamfara Data are presented as Mean ± standard deviation (SD) (n=3). Values with different alphabet as superscript along the column are significantly different at (*P*= .05).

On comparing the growth inhibition mediated by individual honey samples with the control antibiotics, it was observed that some of the honey samples exerted superior growth inhibition of the test bacteria than the antibiotics; ofloxacin and augmentin used as control. For example, honey from FUNNAB (HF) exerted superior growth inhibition of *Staph aureus* ATCC 29213, *S. typhimurium* clinical, *S. typhimurium* ATCC 14028, *Shigella dysenteriae* ATCC 11836 and *Shigella dysenteriae* clinical than that of the two conventional antibiotics used as control (Fig. 1).

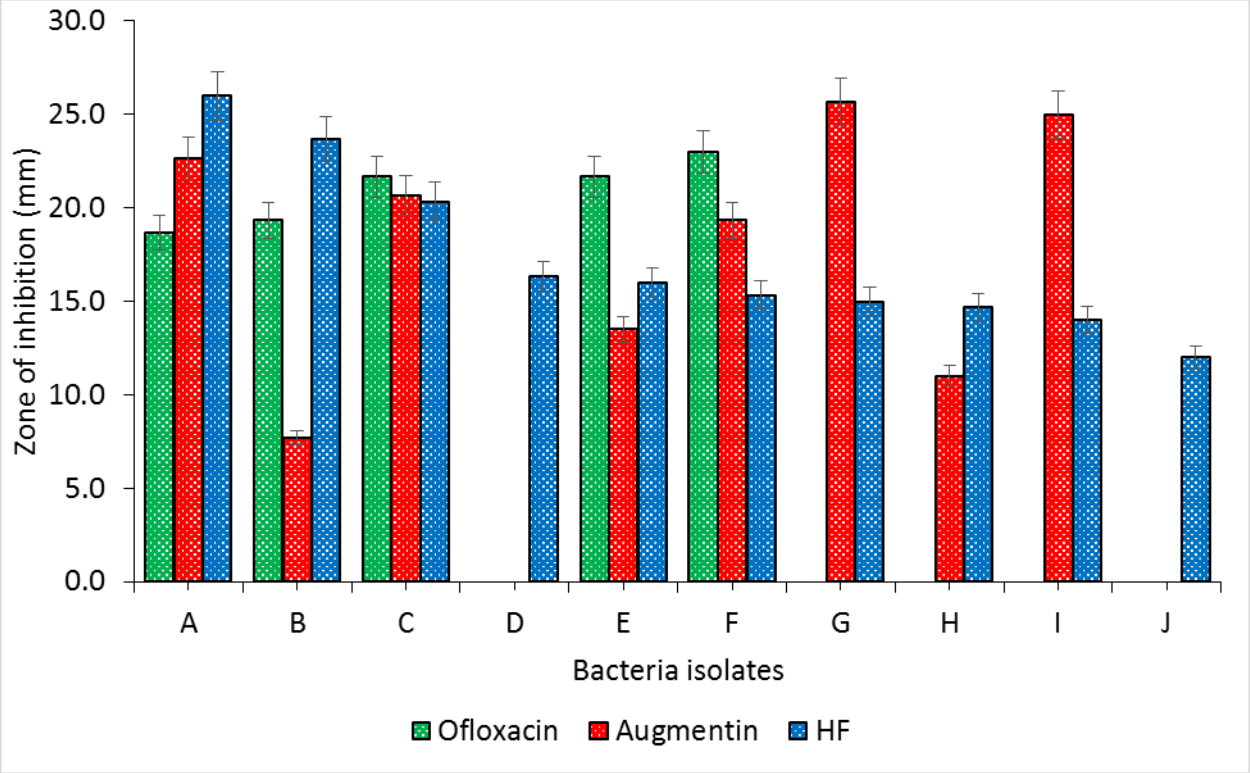

Figure 1: Antibacterial Effect of honey sample from FUNAAB on selected diarrhoeagenic bacteria.

Key: A = *Salm. typhimurium* clinical, B = *Salm. typhimurium* ATCC 14028, C = *Bacillus cereus* clinical, D = *Shigella dysenteriae* ATCC 11836, E = *E. coli* ATCC 700728, F = *Bacillus cereus* ATCC 14579 , G = *E. coli* clinical, H = *Staph. aureus* ATCC 29213, I = *Staph. aureus* clinical, J = *Shigella dysenteriae* clinical and HF = Honey from FUNAAB.

Similar trend was also observed with honey from Zamfara (HZ). This honey sample also exerted superior growth inhibitory activity on five of the test bacteria than the two antibiotics used as control. The bacterial species are *Staph aureus* ATCC 29213, *S. typhimurium* clinical, *E. coli* ATCC 700728, *Sh. dysenteriae* ATCC 11836 and *Sh. dysenteriae* clinical (Fig. 2).

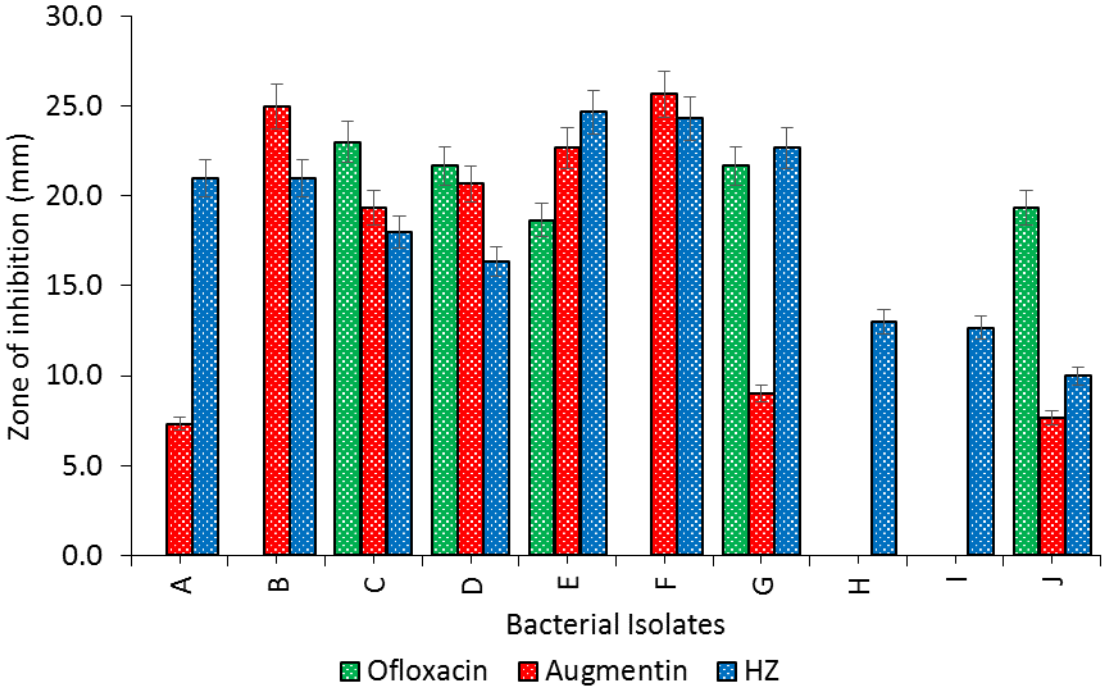

Figure 2: Antibacterial effect of honey sample from Zamfara on selected diarrhoeagenic bacteria.

Key: A = *Staph. aureus* ATCC 29213, B = *Staph. aureus* clinical, C = *Bacillus cereus* ATCC 14579 , D = *Bacillus cereus* clinical, E = *S. typhimurium* clinical, F = *E. coli* clinical, G = *E. coli* ATCC 700728, H = *Sh. dysenteriae* ATCC 11836, I = *Sh. dysenteriae* clinical, J = *S. typhimurium* ATCC 14028, and HZ = Honey from Zamfara.

Honey from Emure-Ile (HEI) on the other hand exerted superior growth inhibitory activity only on three of the test bacteria than that of the two antibiotics used as control. The bacterial species highly susceptible are *Staph aureus* ATCC 29213, *Sh. dysenteriae ATCC* 11836 and *Sh. dysenteriae* clinical (Fig. 3).

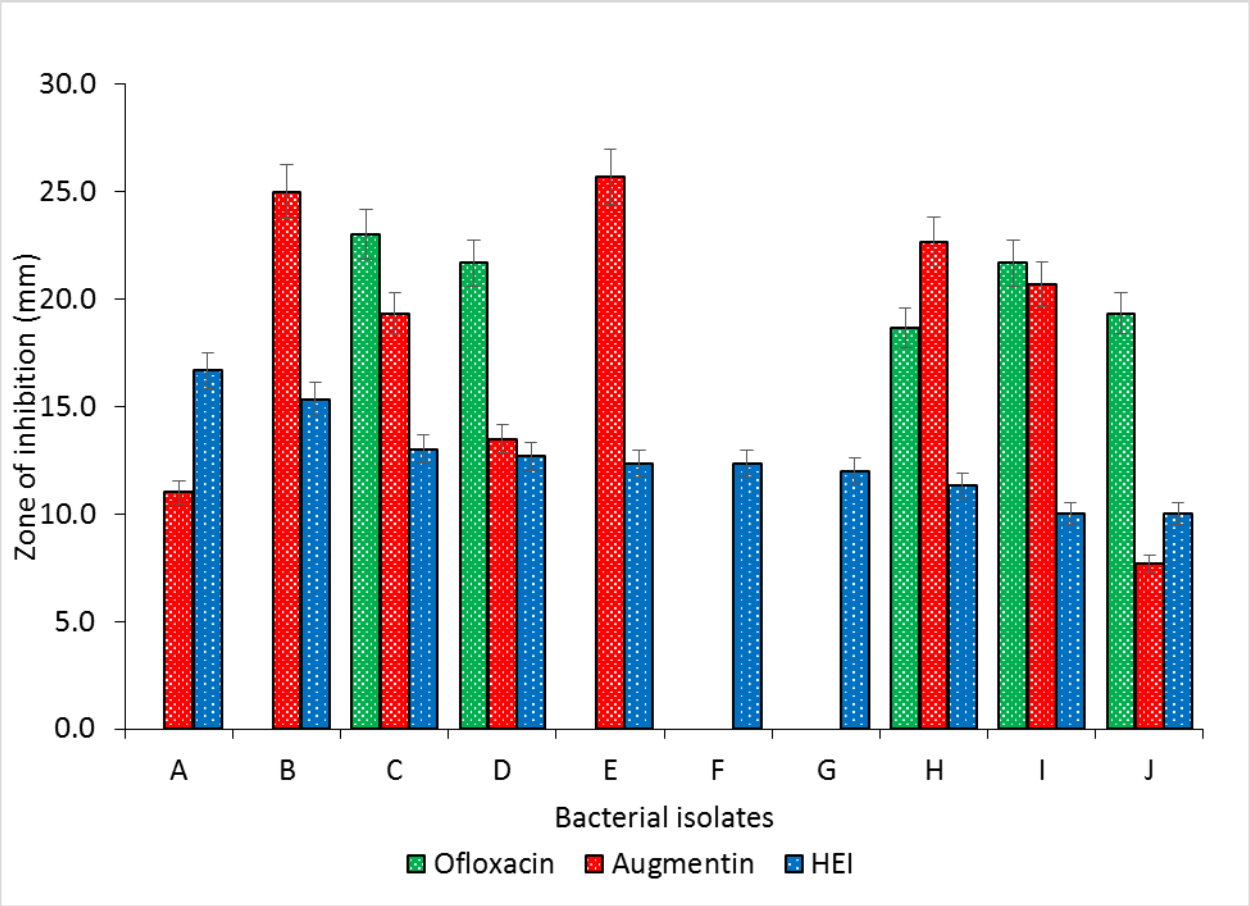

Figure 3: Antibacterial Effect of honey sample from Emure - Ile on selected diarrhoeagenic bacteria

Key: A = *Staph aureus* ATCC 29213, B = *Staph. aureus* clinical, C = *Bacillus cereus* ATCC 14579 , D = *E. coli* ATCC 700728, E= *E.coli* clinical, F = *Sh. dysenteriae* ATCC 11836, G = *Sh. dysenteriae*. clinical, H= *S. typhimurium* clinical, I = *Bacillus cereus* clinical, J = *S. typhimurium* ATCC 14028 and HEI = Honey from Emure – Ile.

Similar results were also observed with honey from Ibadan (HI) and honey from Afo-Akoko (HA), honey from Iree (HIR), honey from Enugu (HE), honey from Lagos (HL) and honey from Nasarawa (HZ) which also exerted superior growth inhibitory on exactly the same three of the test bacteria that were highly susceptible to HZ than the control antibiotics (Figures 4-9 respectively). Honey from Ikere-Ekiti (HIK) however exerted highest growth inhibition of only two of the test bacteria; *Sh. dysenteriae* ATCC 11836 and *Sh. dysenteriae* clinical (Figure 10). One unique observation however in this study is that all the honey samples used inhibited the growth of *Sh. dysenteriae ATCC* 11836 and *Sh. dysenteriae* clinical both of which were resistant to the two antibiotics used as control.

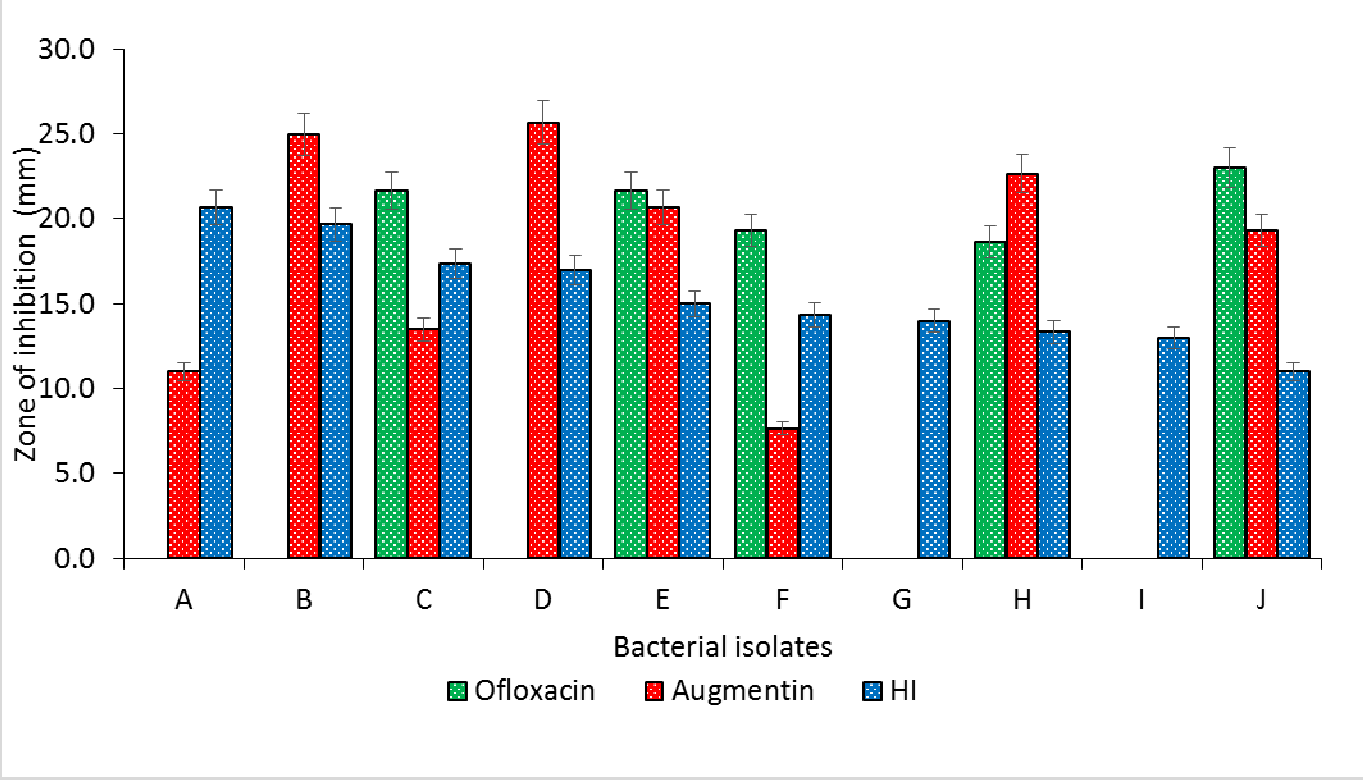

Figure 4: Antibacterial effect of honey sample from Ibadan on selected diarrhoeagenic bacteria

Key: A = *Staph*. *aureus* ATCC 29213, B = *Staph*. *aureus* clinical, C = *E. coli* ATCC 700728, D = *E. coli* clinical, E = *Bacillus cereus* clinical, F = *Salm*. *typhimurium* ATCC 14028, G = *Shigella dysenteriae* ATCC 11836, H = *Salm*. *typhimurium* clinical, I = *Shigella dysenteriae* clinical, J = *Bacillus cereus* ATCC 14579 , and HI = Honey from Ibadan.

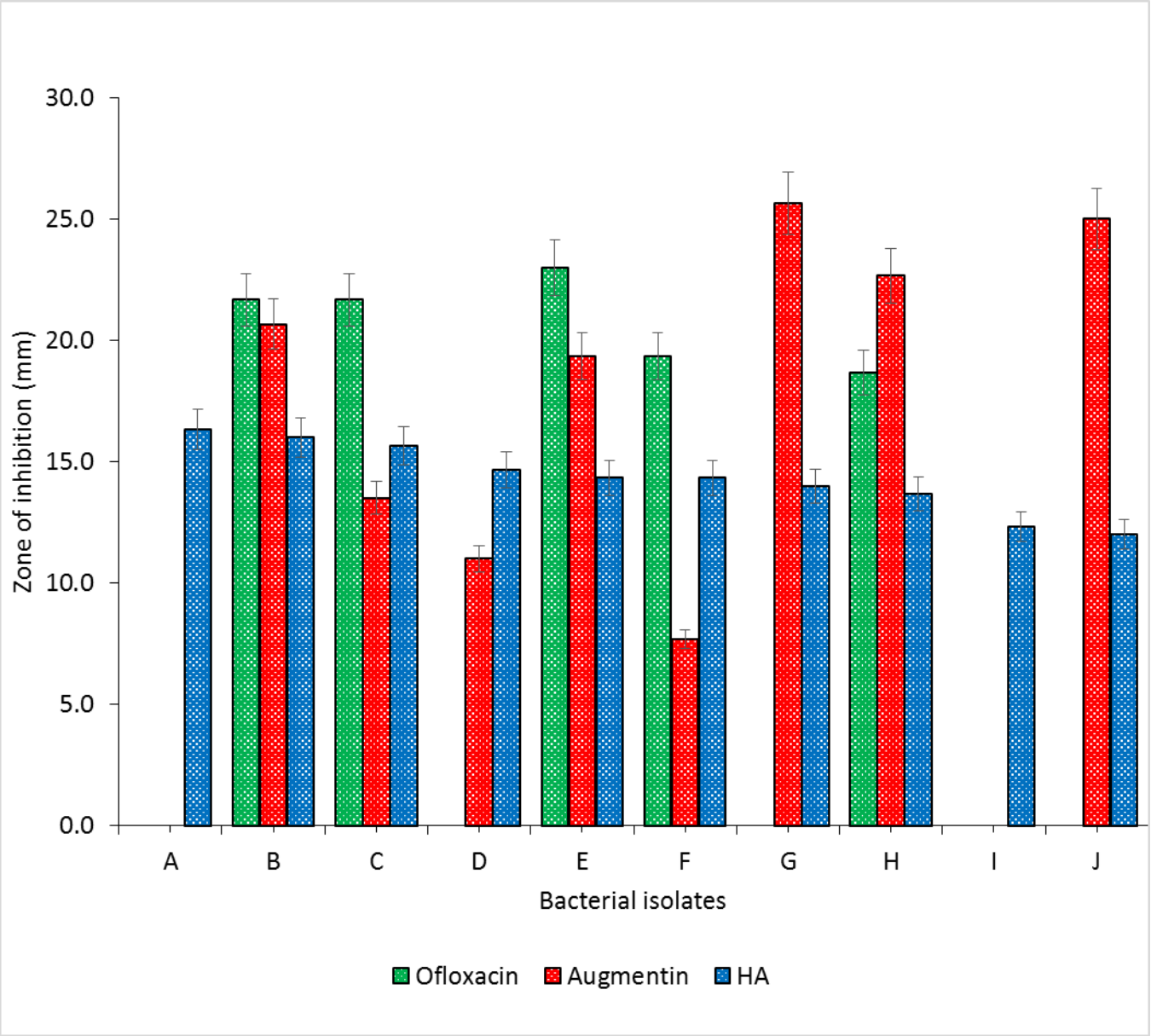

Figure 5: Antibacterial Effect of honey sample from Afo-Akoko on selected diarrhoeagenic Bacteria.

Key: A = *Shigella dysenteriae* clinical, B = *Bacillus cereus* clinical, C = *E. coli* ATCC 700728, D = *Staph. aureus* ATCC 29213, E = *Bacillus cereus* ATCC 14579 , F = *Salm. typhimurium* ATCC 14028, G = *E. coli* clinical, H = *Salm. typhimurium* clinical, I = *Shigella dysenteriae* ATCC 11836, J = *Staph. aureus* clinical and HA = Honey from Afo-Akoko.

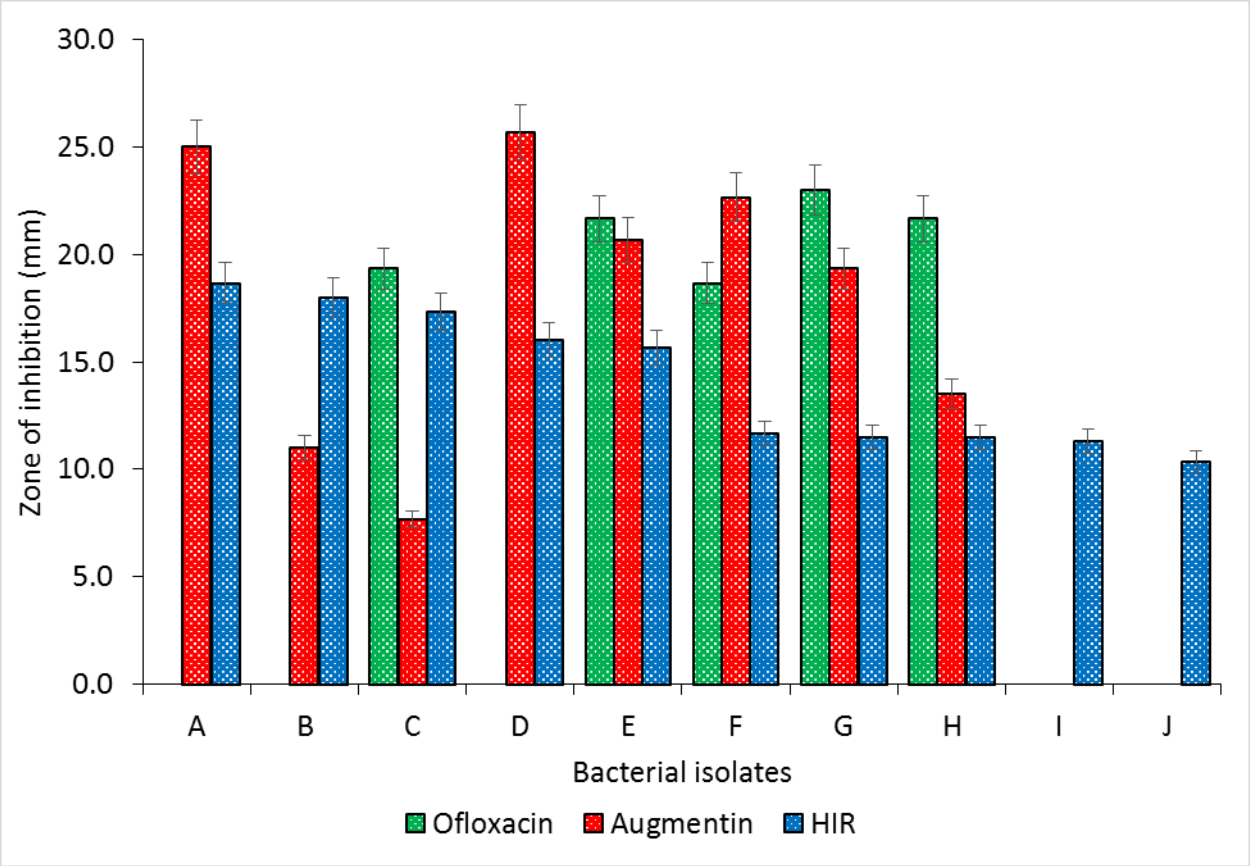

Figure 6: Antibacterial effect of honey sample from Iree on selected diarrhoeagenic bacteria.

Key: A = *Staph*. *aureus* clinical, B = *Staph*. *aureus* ATCC 29213, C = *Salm*. *typhimurium* ATCC 14028, D = *E. coli* clinical, E = *Bacillus cereus* clinical, F = *Salm*. *typhimurium* clinical, G = *Bacillus cereus* ATCC 14579 , H = *E. coli* ATCC 700728, I = *Shigella dysenteriae* clinical, J = *Shigella dysenteriae* ATCC 11836 and HIR = Honey from Iree.

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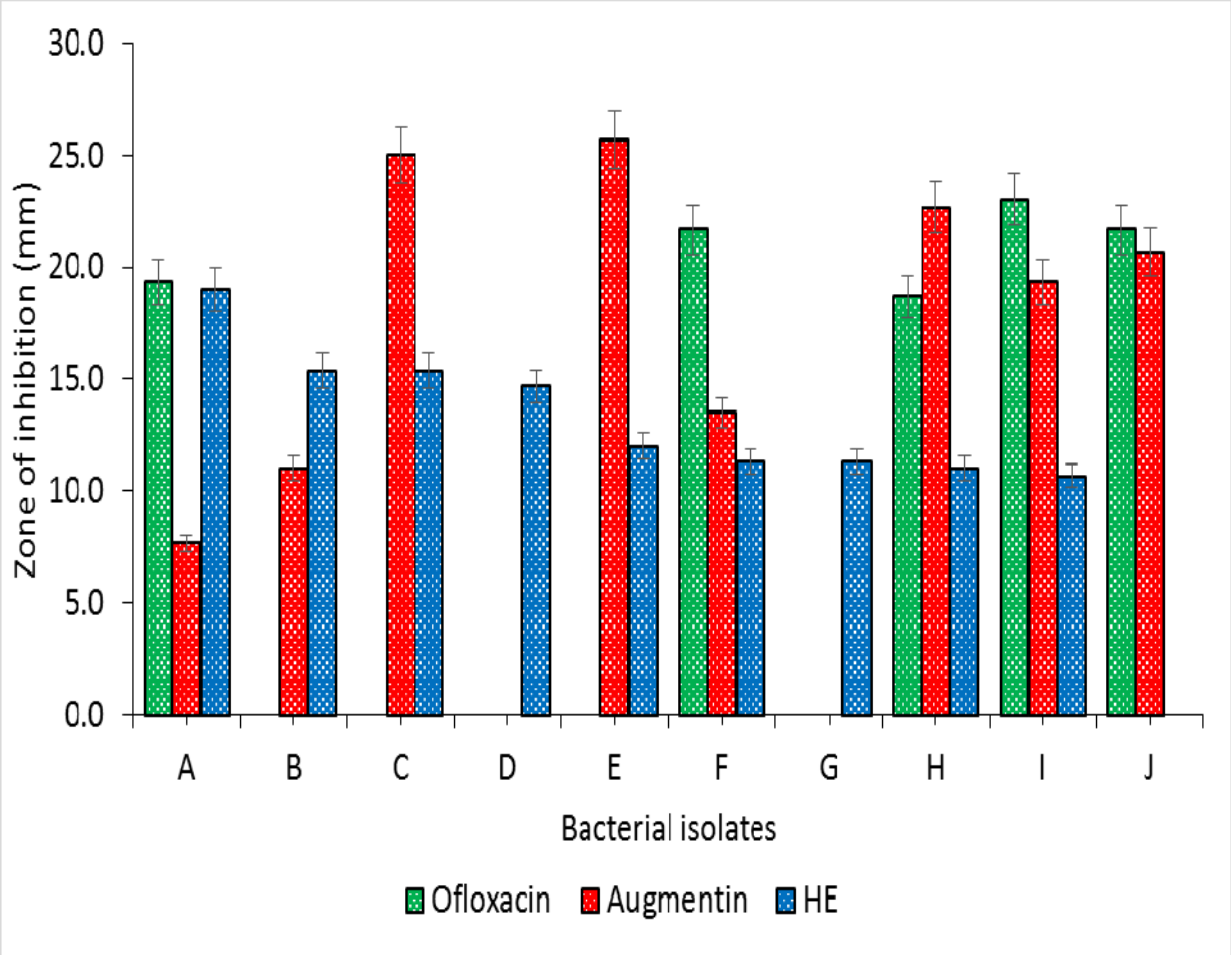

Figure 7: Antibacterial effect of honey sample from Enugu on selected diarrhoeagenic bacteria

Key: A = *Salm*. *typhimurium* ATCC 14028, B = *Staph*. *aureus* ATCC 29213, C = *Staph*. *aureus* clinical, D = *Shigella dysenteriae* clinical, E = *E*. *coli* clinical, F = *E*. *coli* ATCC 700728, G = *Shigella dysenteriae* ATCC 11836, H = *Salm*. *typhimurium* clinical, I = *Bacillus cereus* ATCC 14579 , J = *Bacillus cereus* clinical and HE = Honey from Enugu.

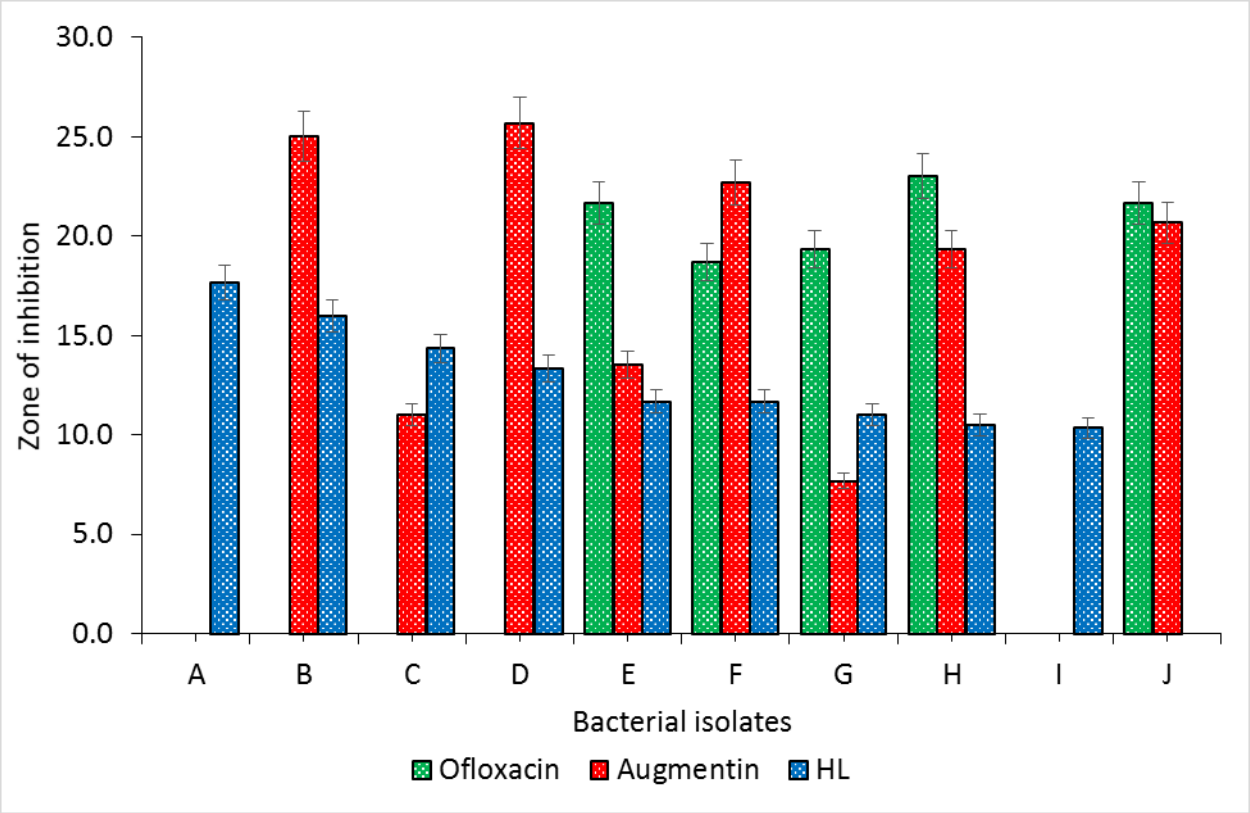

Figure 8: Antibacterial effect of honey sample from Lagos on selected diarrhoeagenic bacteria.

Key: A = *Shigella dysenteriae* ATCC 11836, B = *Staph*. *aureus* clinical, C = *Staph*. *aureus* ATCC 29213, D = *E*. *coli* clinical, E = *E*. *coli* ATCC 700728, F = *Salm*. *typhimurium* clinical, G = *Salm*. *typhimurium* ATCC 14028, H = *Bacillus cereus* ATCC 14579 , I = *Shigella dysenteriae* clinical, J = *Bacillus cereus* clinical and HL = Honey from Lagos.

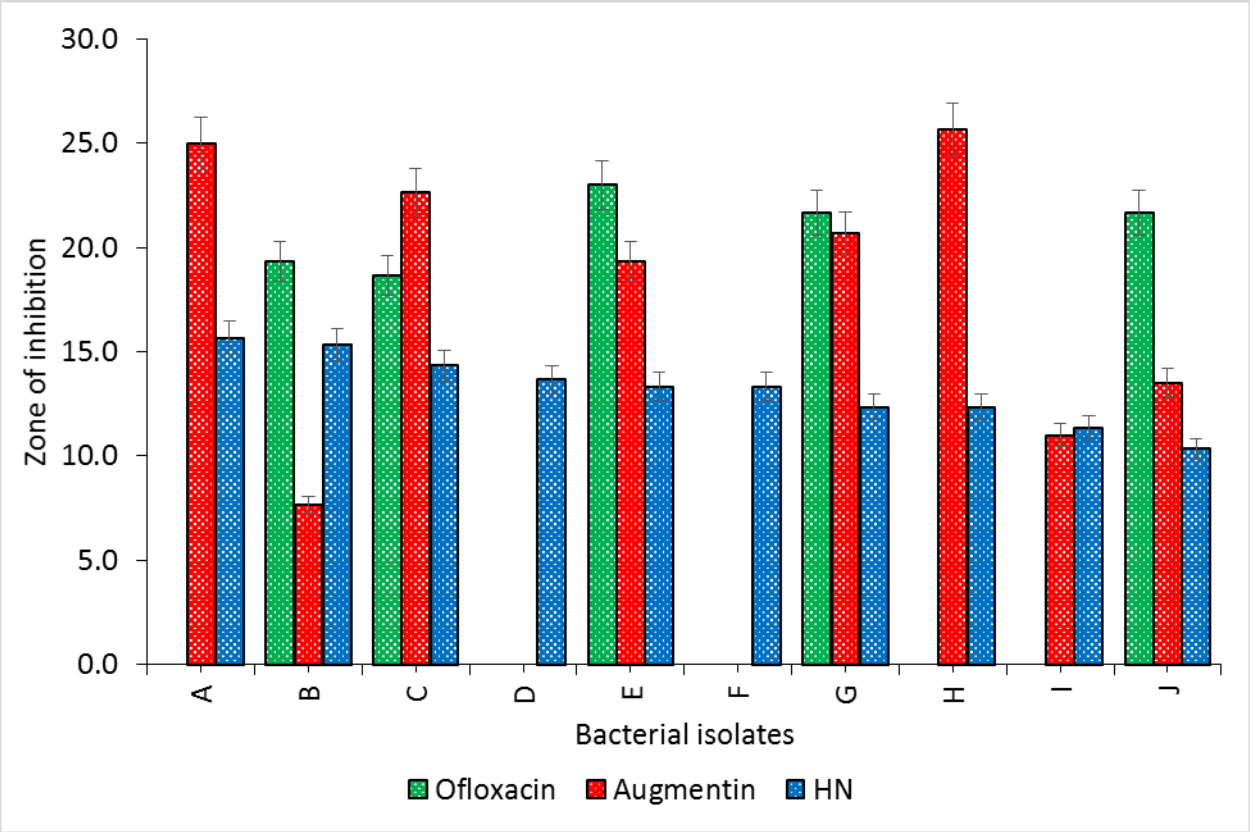

Figure 9: Antibacterial effect of Honey Sample from Nasarawa State on selected diarrhoeagenic bacteria

Key: A = *Staph. aureus* clinical, B = *Salm. typhimurium* ATCC 14028, C = *Salm. typhimurium* clinical, D = *Shigella dysenteriae* clinical, E = *Bacillus cereus* ATCC 14579, F = *Shigella dysenteriae* ATCC 11836, G = *Bacillus cereus* clinical, H = *E. coli* clinical, I = *Staph. aureus* ATCC 29213, J = *E. coli* ATCC 700728 and HN = Honey from Nasarawa

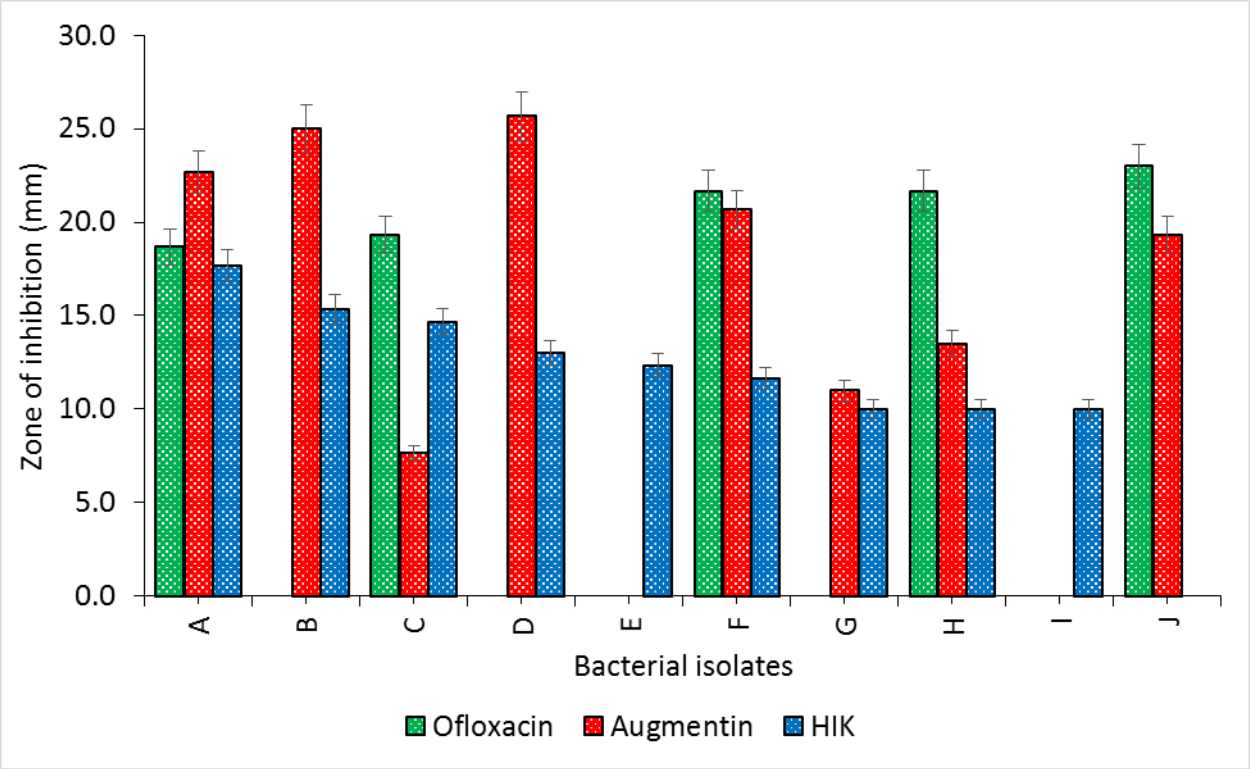

Figure 10: Antibacterial Effect of honey sample from Ikere- Ekiti on selected diarrhoeagenic bacteria.

Key: A = *Salm*. *typhimurium* clinical, B = *Staph*. *aureus* clinical, C = *Salm*. *typhimurium* ATCC 14028, D = *E*. *coli clinical*, E = *Shigella dysenteriae* clinical, F = *Bacillus cereus* clinical, G = *Staph*. *aureus* ATCC 29213, H = *E*. *coli* ATCC 700728, I = *Shigella dysenteriae* ATCC 11836, J = *Bacillus cereus* ATCC 14579 , and HIK = Honey from Ikere-Ekiti.

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The results of this study agrees with previous report on the antibacterial activity of honey against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* [17] although in this present study, *Salmonella typhimurium* was used instead of *Salmonella typhi.* This work however disagrees with the report of Mohapatra [18] that *Staph aureus* was the most sensitive to all the honey samples they worked on among the test bacterial strains they used. It is also in disagreement with the report of Sohaimy *et al*. [19] and Almasaudi *et al.* [20] that *S. aureus* is the most susceptible bacterial species to honey collected in Iraq and Egypt. Omafuvbe and Akanbi [21] found similar results through well diffusion method that Nigerian honey showed activity against *Salmonella typhimurium*. (12 – 22 mm), *B. cereus* (12 – 29 mm), and *E. coli* (19 – 38 mm). In contrast, the same author reported that honeys from different regions in Nigeria were not active against *S. aureus*, Also in Nigeria, Omoya *et al*. [22] reported a similar result on antimicrobial activity of honeys against *E. coli* (13 – 20 mm) and *Salmonella typhimurium* (8 – 18 mm). The antibacterial activity observed in this study was bactericidal more than bacteriostatic. This goes

contrary to the work of Laallam *et al*. [23] which reported that the antibacterial action of honey is essentially bacteriostatic but is in agreement with the report of Lusby *et al*. [24] that it is bactericidal. Comparison of the results in the different figures showed that some of the honey samples were more efficient in inhibiting the growth of the studied pathogenic bacteria than the other. Literature has shown that different honey types possess different efficacies against the same type of bacteria [10,20]. The differences might be due to origin, composition and the harvest period of the honeys that are used.

4. CONCLUSION

This study has shown that the antibacterial activity of honey samples used vary from one locality to the other. For example, honey samples from FUNAAB (HF) was the most effective in inhibiting the growth of *S. typhimurium* (both typed and clinical) than the other honey samples tested. All the honey samples used however were effective against *Shigella dysenteriae* (both typed and clinical isolates used) that were resistant to the two antibiotics used as control. These results clearly indicate that local honey samples in Nigeria are endowed with a broad spectrum antibacterial activity on the test bacteria. These findings therefore could be exploited in the treatment of diarrhoeal diseases caused by these bacteria as an alternative to conventional antibiotics to which some of the test bacteria have developed resistance especially *Shigella dysenteriae,* the aetiological agent of shigellosis.

ETHICAL APPROVAL

Scientific and ethical permit/clearance were obtained from the head of Department of Microbiology, Federal University of Technology Akure (FUTA) to Medical Microbiology Laboratory in University College Hospital, Ibadan, Oyo State before the release of test bacteria and commencement of the research.

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